Central American Institute for Studies on Toxic Substances (IRET-UNA)
Program on Work, Environment and Health in Central America (SALTRA)

SALTRA

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MESOAMERICAN NEPHROPATHY

Report from the First International Research Workshop on MeN

Programa Financiado por la Unión Europea

IRET

UNA UNIVERSIDAD NACIONAL COSTA RICA

MESOAMERICAN NEPHROPATHY

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MESOAMERICAN NEPHROPATHY

Report from the First International Research Workshop on MeN

Bringing together knowledge, research questions, and initiatives related to Chronic Kidney Disease of unknown origin in Mesoamerica

NOVEMBER 28-30, 2012 HOTEL BARCELÓ PALMA REAL, SAN JOSÉ, COSTA RICA

ORGANIZED BY
Central American Program for Work, Environment and Health - SALTRA-
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MAIN MESSAGE

There is an epidemic of chronic kidney disease of unknown etiology (CKDu) in several parts of Mesoamerica. This public health problem is of such magnitude and severity that urgent, exhaustive and collaborative actions must be put into place to elucidate the cause(s), act on available information to prevent further disease and find permanent solutions for prevention and mitigation.

The general consensus of the workshop was that the strongest causal hypothesis for the epidemic is repeated episodes of heat stress and dehydration during heavy work in hot climates. Co-factors to consider interacting with heat stress or influencing the progression of CKDu, include excess use of non-steroidal anti-inflammatory drugs (NSAIDs) and fructose consumption in rehydration fluids. Contributing factors for the epidemic could include inorganic arsenic, leptospirosis, pesticides, or hard water. Interventions to reduce heat stress and improve hydration with controlled trials are recommended.

The need for well-designed, interdisciplinary collaborative research is emphasized, with priority attention on biopsy studies to elucidate pathophysiology, prevalence studies to further measure magnitude and identify new population groups at risk, and prospective cohort studies to advance etiologic research. Attention to treatment of existing CKD is also a priority.

A **Declaration** was signed by workshop participants emphasizing the public health problem and the immediate need for prevention. Support is offered to governments to act now on existing knowledge and to local researchers and multisector actors to advance research through collaborative efforts.

An international research consortium of investigators was established.

DECLARACIÓN MeN

30 noviembre, 2012
San José, Costa Rica

Reconociendo que la enfermedad renal crónica está devastando trabajadores y sus familias en múltiples partes de Centroamérica,

Apoyando la declaración de COMISCA del 2011, "*Unidos para Detener La Epidemia de Las Enfermedades Crónicas No Transmisibles (ECNT) en Centroamérica y la República Dominicana*", la cual incluye la enfermedad renal crónica como prioridad entre la enfermedades no-transmisibles sujetos a vigilancia,

Nosotros, los participantes en el taller de la Nefropatía Mesoamericana (MeN) organizado por SALTRA, del 28 al 30 de noviembre del 2012, declaramos que existe prueba de una enfermedad renal crónica de origen no-determinado (MeNu) afectando a Mesoamérica.

Esta es una enfermedad silenciosa que está sobresaturando a los sistemas de salud y resulta en muertes prematuras. Por eso, acciones de prevención y promoción de la salud son de primordial importancia. Los participantes de esta reunión han suministrado evidencia basada en sus investigaciones y están activamente buscando la etiología de esta enfermedad. Es fundamental realizar acciones interdisciplinarias, al nivel global y local, con el fin de dar respuesta a este problema urgente y trágico de salud pública.

Considerando lo anterior, ofrecemos nuestro apoyo a los gobiernos para asistirles en reconocer esta enfermedad en América Central y para colaborar con investigadores locales y con actores multisectoriales para:

1. enfrentar este problema uniendo recursos y utilizando evidencia científica existente

2. prevenir muertes prematuras en las poblaciones más afectadas mediante intervenciones ahí donde sea posible.

Alejandro Riefkohl
Andrés Robles
Annika Östman-Wernerson
Aurora Aragón
Carl-Gustaf Elinder
Carlos Manuel Orantes
Carolina Guzmán
Catharina Wesseling
Channa Jayasumana
Christer Hogstedt
Cinthya Bonilla
Clemens Ruepert
Daniel Brooks
David Friedman

MeN DECLARATION

November 30, 2012
San José, Costa Rica

Recognizing that chronic kidney disease is devastating to workers and families in many parts of Central America, and

Supporting COMISCA's 2011 declaration "Unidos para Detener Ia Epidemia de Ias Enfermedades Crónicas No Transmisibles (ECNT) en Centroamérica y la República Dominicana", which includes chronic kidney disease as a priority among the non-transmittable chronic diseases subject to surveillance

We, the participants in the Mesoamerican Nephropathy (MeN) workshop organized by SALTRA, November 28-30, 2012, declare that there is sufficient proof of a chronic kidney disease of undetermined origin (MeNu) affecting Mesoamerica.

This is a silent disease that is overwhelming public health systems, leading to premature deaths. Therefore, prevention and health promotion are essential. The participants of this meeting have provided substantial evidence describing the problem and are actively seeking the etiology of the disease. Global and local interdisciplinary action and research are essential to address this urgent and tragic public health problem.

As such, we offer our support to governments to aid them in recognizing the disease in Central America and to collaborate with local researchers and multi-sector actors to:

1. address this issue by pooling resources and using existing scientific evidence,

2. prevent premature deaths in the most affected populations by intervening where possible.

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PALABRAS DE BIENVENIDA

M.Sc. Marianela Rojas
Coordinadora Regional Programa SALTRA

En nombre del Programa Salud, Trabajo y Ambiente, les doy la más cordial bienvenida a Costa Rica y al Primer Taller Internacional de Investigación en MEN, la epidemia de nefropatía mesoamericana.

Hoy nos hemos dado cita en Costa Rica científicos y profesionales de 14 países diferentes: Suecia, Estados Unidos, México, Sri Lanka, Holanda, España, Canadá, , Dinamarca, Guatemala, El Salvador, Honduras, Nicaragua, Panamá y Costa Rica. Muchos de los que estamos aquí presentes hoy, ya han dedicado importantes esfuerzos durante su vida laboral para esclarecer el enigma de la enfermedad renal crónica de causa desconocida. El programa SALTRA tiene 8 añ os de trabajar en este grave problema de salud pública en Centroamérica, especialmente nuestros colegas de SALTRA en Nicaragua, El Salvador y Costa Rica.

Quisiera destacar la presencia de la delegación de investigadores suecos en este taller, en especial el Dr. Christer Hogstedt, quien fue co-director y co-fundador del Programa SALTRA en su primera Fase I, así como la Dra. Kristina Jakobsson, quienes desde el 2005 han apoyado incansablemente a nuestro grupo de investigadores centroamericanos. También asisten a este taller varios de los colegas que hace tres años establecieron junto con SALTRA la Red para el Estudio de la Enfermedad Renal Crónica en Mesoamérica, RERCEM, específicamente el Dr. Correa-Rotter de México. Hoy está aquí la Dra. Agnes Soares, representando a la sede central de la OPS / Washington. Y, aunque por razones de tiempo no puedo mencionar a todos con su nombre, igualmente quiero destacar la presencia de investigadores centroamericanos y estadounidenses de importantes estudios en Nicaragua y en El Salvador incluyendo los colegas del Ministerio de Salud

WELCOME WORDS

M.Sc. Marianela Rojas
Coordinadora Regional Programa SALTRA

On behalf of the Program Work, Environment and Health (SALTRA), I give you the warmest welcome to Costa Rica and the Ist International Research Workshop on MeN, the epidemic of Mesoamerican Nephropathy.

Today we have gathered in Costa Rica scientists and professionals from 14 different countries: Sweden, United States, Mexico, Sri Lanka, The Netherlands, Spain, Canada, Denmark, Guatemala, El Salvador, Honduras, Nicaragua, Panama and Costa Rica. Many of those who are present here today, have already devoted important efforts during their working life to elucidate the enigma of chronic kidney disease of unknown cause. The SALTRA program has worked now for 8 years with this serious public health problem in Central America, especially our colleagues from SALTRA in Nicaragua, El Salvador and Costa Rica.

I would like to highlight the presence of the delegation of Swedish researchers in this workshop, especially Dr. Christer Hogstedt, who was co-director and co-founder of the SALTRA program in its first phase, as well as Dr. Kristina Jakobsson, who since 2005 have tirelessly supported our Central American research group. Also attending this workshop are several colleagues who three years ago, established together with SALTRA the Network for the Study of Chronic Kidney Disease in Mesoamerica, RERCEM, specifically Dr. Correa-Rotter of México. Today, here is also Dr. Agnes Soares, representing PAHO Headquarters in Washington, DC. And, although due to time constraints, I cannot mention everyone by name, I would also like bring to your attention the presence of Central American and U.S. investigators of important studies in Nicaragua and El Salvador including members of the

de el Salvador, UNAN-León, la Universidad de Boston y la Fundación La Isla. Estamos muy contentos de contar con nuestro colega de Sri Lanka quien demostró gran persistencia y paciencia en obtener una visa para Costa Rica. Agradezco la presencia de todos que ya han jugado un papel o que están en una posición para jugar un papel en el futuro en la búsqueda de causas y soluciones respecto a la enfermedad renal crónica de etiología desconocida. Es un honor para SALTRA ser el anfitrión de cada uno de los aproximadamente 50 expertos e investigadores que participan en este evento.

Finalmente quisiera enfatizar el objetivo del taller. Estamos hoy aquí con el propósito de colaborar, para lograr una diferencia para los miles de personas afectadas en este momento por la epidemia de enfermedad renal crónica de causa desconocida, para todas aquellas personas que caerán víctimas de esta grave enfermedad en el futuro, y también pensando en los miles de trabajadores ya fallecidos, en Centroamérica, Asia y posiblemente otras partes del mundo. Tenemos una oportunidad única de revisar, reflexionar y planificar como grupo unido, de integrar eficiencia y eficacia en los futuros planes de investigación y de acción. Espero de todo corazón que juntos aprovechemos esta oportunidad con madurez y sabiduría.

Les deseo, en nombre de todo el Programa SALTRA, una linda y muy productiva estancia en nuestro país. Gracias por venir y estar aquí hoy. Que sean todos muy bienvenidos.

LIC. SANDRA LEÓN
Rectora Universidad Nacional

El desarrollo de la ciencia, y en especial de las ciencias aplicadas, como la medicina, deberá ser siempre una de las prioridades principales de todo Estado y nación. Y en particular, en aquellos países que se encuentran aún en una fase de despegue económico y social, como es el caso de todas las naciones hermanas que integramos la gran región mesoamericana.

El Estado costarricense y la Universidad Nacional siempre han sido enfáticos en promover una agenda de impulso al desarrollo de la ciencia y la tecnología, y vemos con entusiasmo la realización de este evento de tanta utilidad y significado para avanzar en el campo de la medicina y en el mejoramiento de la calidad de vida de nuestros pueblos. La importancia de este taller queda bien establecida a partir de los datos que informan sobre la realidad concreta de nuestros países:

Sabemos, por ejemplo, que desde hace varias décadas se han incrementado los casos de fallo renal en ciertas regiones en Centroamérica, a lo largo de la costa del Pacífico, en El Salvador, Nicaragua y el norte de Costa Rica. En la ac-

Ministry of Health of El Salvador, UNAN-León, Boston University and La Isla Foundation. We are very pleased with our colleague from Sri Lanka who showed great persistence and patience in getting a visa for Costa Rica. I appreciate the presence of all that have already played a role, or that are in a position to play a role in the future in the search for causes and solutions regarding chronic kidney disease of unknown etiology. It is an honor for SALTRA to host each of the approximately 50 experts and researchers participating in this event.

Finally, I would like to emphasize the purpose of the workshop. We are here today in order to work together, to make a difference for the thousands of people affected by the epidemic of chronic kidney disease of unknown cause, for all those people who will become victims of this serious disease in the future, and also thinking of the thousands of workers already deceased, in Central America, Asia and possibly other parts of the world. We have a unique opportunity to review, reflect and plan as a united group, to integrate efficiency and effectiveness in future research and action plans. I hope with all my heart that together we will make use of this opportunity with maturity and wisdom.

I wish you, on behalf of the entire SALTRA program, a nice and very productive stay in our country. Thank you for coming and being here today. You are all most welcome.

LIC. SANDRA LEÓN
Rectora Universidad Nacional

The development of science, and especially of applied sciences such as medicine, should always be one of the main priorities of any state and nation. And this is particularly so in those countries which are still in a phase of economic and social take-off, as it is the case of all sister nations that integrate the great Mesoamerican region.

The State of Costa Rica and our Universidad Nacional have always been emphatic in promoting an agenda towards the development of science and technology, and it is therefore with enthusiasm that we witness this valuable and meaningful event to achieve advances in the field of medicine and in the improvement of the quality of life of our peoples. The importance of this workshop is well established from the data that inform about the concrete reality of our countries.

We know, for example, that cases of kidney failure have increased for several decades in certain regions in Central America along the coast of the Pacific, in El Salvador, Nicaragua and Northern Costa Rica. Today, the

tualidad, los gobiernos de Centroamérica y la Organización Panamericana de la Salud (OPS) reconocen que existe una verdadera epidemia de enfermedad renal crónica, o ERC. También en otras partes del mundo se ha identificado brotes de ERC, entre ellos en Sri Lanka y en India.

Este taller, que tenemos el gusto de inaugurar el día de hoy, es organizado por el Programa Salud, Trabajo y Ambiente en América Central (SALTRA), el cual es coordinado desde el Instituto Regional de Estudios en Sustancias Tóxicas (IRET) de la Universidad Nacional. El taller aborda la epidemia de enfermedad renal crónica, como un problema de salud pública y de orden económico de primordial importancia, porque afecta a muchos trabajadores quienes sin tratamiento fallecen en edades jóvenes y porque, por otro lado, la atención médica de las víctimas ha desbordado la capacidad de los sistemas de salud en varios países de la región.

Es la misión de la Universidad Nacional investigar problemas sociales y de salud pública y proponer soluciones. La UNA está comprometida con el desarrollo de toda la sociedad y en particular con la integración, la potenciación y la ampliación de oportunidades de los sectores sociales menos favorecidos o excluidos de los beneficios del desarrollo. Esta enfermedad aflige de forma desigual a los sectores más pobres.

En Costa Rica, por ejemplo, la mortalidad por ERC es cinco veces mayor en la provincia de Guanacaste que en el resto del país, y los afectados son principalmente trabajadores de la caña de azúcar. Los cortadores de la caña constituyen una población altamente vulnerable de trabajadores, muchos de los cuales migran desde Nicaragua por razones de desempleo y otros pertenecen al sector trabajador más pobre de Costa Rica.

Ya en noviembre del 2005, SALTRA organizó el primer taller centroamericano sobre ERC, en León-Nicaragua, para discutir con socios de la región y de Suecia este grave problema de salud pública. Desde entonces, una serie de estudios de distintos grupos de investigadores han avanzado sobre el conocimiento de la epidemia y han generado una serie de hipótesis. Sin embargo, en este momento, la causa o las causas de la epidemia siguen siendo desconocidas y tampoco se conoce con certeza la gravedad de la epidemia en su verdadera extensión.

Es una obligación del sector académico centroamericano atender este problema y apoyar a los gobiernos para buscar soluciones y definir sus políticas de salud, sociales y económicas. No obstante, ante la complejidad de este enigma son absolutamente necesarias las alianzas internacionales con científicos y expertos de distintas disciplinas de otras partes del mundo.

governments of Central America and the Pan American Health Organization (PAHO) recognize that there is a real epidemic of chronic kidney disease, or CKD. Also in other parts of the world outbreaks of CKD have been identified, including Sri Lanka and India.

This workshop, which we have the pleasure to inaugurate today, is organized by the Program Health Work, Environment in Central America (SALTRA), which is coordinated from the Central American Institute for Studies on Toxic Substances (IRET) of the Universidad Nacional. The workshop addresses the epidemic of chronic kidney disease as a public health as well as an economic problem of paramount importance, because it affects many workers who without treatment die at young age, and, because the medical care of the victims has overwhelmed the capacity of the health systems in several countries in the region.

It is the mission of the Universidad Nacional to investigate social and public health problems and propose solutions. Universidad Nacional is committed to the development of the whole society and in particular with the integration of, empowerment of, and the expansion of opportunities for the social sectors less favored or excluded from the benefits of development. This disease disproportionately afflicts the poorest sectors. In Costa Rica, for example, CKD mortality is five times higher in the province of Guanacaste as compared to the rest of the country, and those affected are mainly sugarcane workers. The cane cutters are a highly vulnerable population of workers, many of whom migrate from Nicaragua for reasons of unemployment and others belong to the poorest worker sector of Costa Rica.

InNovember 2005, SALTRA organized the first Central American workshop on CKD, in Leon-Nicaragua, to discuss this serious public health problem with partners in the region and Sweden. Since then, a series of studies by different groups of researchers have advanced knowledge regarding the epidemic and have generated a series of hypotheses. However, at this time, the cause or causes of the epidemic remain unknown and the seriousness of the epidemic is not known with certainty to its true extent.

It is an obligation of the Central American academic sector to address this problem and to support governments to seek solutions and to define their health, social and economic policies. However, given the complexity of this puzzle, it is absolutely necessary to build international partnerships with scientists and experts with different disciplines from other parts of the world.

Exactly seven years after the first workshop, SALTRA

Exactamente siete años después del primer taller, SALTRA hoy convoca nuevamente a sus socios y aliados para evaluar el problema y buscar en qué dirección orientar los esfuerzos para llegar a soluciones eficaces. Esta vez participan más de 50 expertos provenientes de 15 países, de disciplinas y áreas de experiencia muy distintas. El objetivo del taller es revisar todo lo que se conoce sobre la epidemia de ERC hoy día y priorizar cómo avanzar en dilucidar el problema.

Espero que el espíritu de colaboración entre investigadores y profesionales de los países centroamericanos y expertos de países de todo el continente de América, Europa y Asia signifique un gran paso para adelante en el camino hacia políticas eficaces de prevención y mitigación.

Les extiendo, por lo tanto, a todas y a todos ustedes, mi más calurosa bienvenida, y les deseo el mayor de los éxitos en su trabajo orientado a encontrar respuestas científicas y a definir los mejores métodos para prevenir y reducir la incidencia del ERC, que tanto daño está ocasionando en nuestros países, y en particular entre sus sectores más vulnerables.

¡Gracias por su gentil invitación y muy buenos días a todas y todos ustedes!

today convenes again its partners and allies to assess the problem and to determine find in which way to direct the efforts to arrive at effective solutions. This time, there are more than 50 experts are involved from 15 countries with different disciplines and different areas of expertise involved. The workshop aims to review all that is currently known about the epidemic of CKD and prioritize how to progress in elucidating the problem.

I hope that the spirit of collaboration between researchers and professionals from the Central American countries and experts from countries of the continents of America, Europe and Asia means a great step forward on the road to effective prevention and mitigation policies.

Therefore, I extend to all of you, my warmest welcome and wish you the greatest success in your work to find scientific answers and to define best practices to prevent and reduce the incidence of CKD, that is causing so much damage in our countries, and in particular among their most vulnerable sectors.

Thanks for your kind invitation and very good morning to all you!

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PROLOGUE

There is an epidemic of chronic kidney disease in several parts of Mesoamerica. The causes of the disease remain basically unknown and policy makers lack data for informed decisions. However, it is known with certainty that chronic kidney disease of unknown etiology (CKDu) in Mesoamerica is a public health problem of such a magnitude and severity that from an ethical perspective, the most urgent, exhaustive and collaborative actions must be put in place to elucidate the causes and find solutions for prevention and mitigation. Details of the situation of CKDu in Mesoamerica and hypotheses are discussed at length in this report. There is no way around it: we must join efforts to confront this epidemic.

HISTORY

In November 2005, SALTRA[1] convened a Central American workshop in León, Nicaragua about CKD (www.saltra.una.ac.cr). Since then, Central American, Swedish and US researchers have been involved in characterizing CKD occurrence in Nicaragua and El Salvador and examining different hypotheses, from pesticides and metals to heat stress among sugarcane cutters, a work population identified to be at very high risk for CKD. These studies provided new evidence about the CKD epidemic in Mesoamerica, but progress to find the etiology is slow and research efforts have not been sufficiently integrated.

Then, in November 2010, SALTRA joined with the Autonomous University of Mexico (UNAM) to

1. In 2005 SALTRA was the Program on Work and Health, Phase I, a Central American-Swedish collaborative university-based research and action program to improve occupational health in the region. Phase I was funded by Sida and received also US and other international support.

hold a second workshop specifically to promote research collaboration within the Mesoamerican region. This workshop (in Heredia, Costa Rica) resulted in the creation of RERCEM, a Network for the Study on the Mesoamerican Nephropathy.

Nonetheless, with the epidemic advancing and workers dying, it was also clear that broader international collaborations and funding would be needed to accelerate the unraveling of this complex public health problem. Communications started with scientists in other parts of the world with abnormal CKDu occurrence. In addition to those already active in Mesoamerica, other scientists around the world started expressing willingness to provide their expertise and become involved. Therefore, SALTRA[2], accelerated its efforts to boost research collaboration for Mesoamerican CKDu and, in November 2012, organized the workshop upon which this report is based.

OBJECTIVES OF THE WORKSHOP

The objectives of this research workshop were:

1. To describe the state of the art of the Mesoamerican CKDu, i.e. to examine the knowledge both about conventional CKD and about the CKDu epidemic from all possible angles including clinical, environmental and social aspects;
2. To identify gaps in knowledge and define what knowledge needs to be generated in order to elucidate the causes of the disease and to be able to propose specific and effective preventive and mitigation measures;
3. To prioritize a research agenda and identify methods to obtain the needed knowledge;
4. To facilitate and promote collaboration between different research groups and institutions aiming to improve coherence between the research initiatives, use scarce resources more effectively, and provide a platform for raising international awareness and increasing funding possibilities.

WORKSHOP METHODS AND OUTCOMES

With these objectives in mind, an organizing technical committee was formed. Several of its members had already been involved in the organization of the two previous workshops. It was decided that this workshop should become a 3-day discussion forum with invitations to a relatively small group of experts from a range of disciplines responsible for their own expenses. A total of 51 researchers from 15 different countries attended the workshop November 28-30, 2012, representing the desired diversity of disciplines and expertise (see Appendix 2).

Several months before the workshop took place, the organizing committee invited experts to write short review papers on specific CKD topics, covering epidemiology of CKDu, pathology of CKDu, biomarkers, environmental and life style risk factors, heavy work in hot climate, genetic susceptibility, and policies and socio-economic drivers. The papers summarized the state of the art and identified knowledge gaps. The papers were available to all participants in advance.

These reviews were presented by the experts in the first part of the workshop, thus reflecting 'What do we know?' and introducing 'What do we need to know?' to continue on the correct road to finding causes and solutions. After the workshop, all papers were revised and amended by the authors as needed, based on the discussions and new information that emerged during the workshop. In addition to the advance reviews a poster session at the workshop provided the participants with an opportunity to present additional data available in the region and elsewhere that could shed light on issues around CKDu. The expert papers are presented staring on page 37 of this report. Poster abstracts are presented starting on page 185. Complete posters are included in the digital version.

During the workshop all participants contributed opinions, information, and data regarding knowledge and knowledge gaps for a number of central issues. These inputs were provided in two ways. First, during a session of 'Rotating Table Discussions', groups consisting of 6-8 participants visited each of 7 topic tables (hosted by a facilitator and rapporteur) for 15 minutes; thus, almost all but table leaders had input into the 7 different themes. After all rounds, the facilitator and rapporteur selected 4-6 main messages from the discussions, which were presented and commented on in plenary. The final versions of the reports from the Rotating Table Discussions' are presented starting on page 131.

In the second part of the workshop working groups were organized in two sessions. In the first working groups discussed issues related to different study designs. Participants signed up for one of seven Working Groups on: cross-sectional, case-control, cohort, intervention, experimental, and ecohealth/spatial epidemiology studies. A second round of Working Groups was organized for the final workshop day, which addressed important methodological issues. The final versions of the Working Groups are presented starting on page 149.

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2. In 2012, SALTRA initiated Phase II as the Program on Work, Environment and Health in Central America, consisting of a network of university based Centers in Occupational and Environmental Health located in the six Central American countries. Phase II is funded by the EU.

The last part of the workshop focused on how to set priorities for a policy agenda, with presentations on interventions and treatment options, health system responses, and application of research and policies to social and environmental improvements. A final panel discussion completed the workshop's efforts with an agreement for the establishment of a research consortium and an official workshop declaration acknowledging the Mesoamerican Nephropathy and urging international collaboration and support to find the causes and alleviate the human suffering in the region.

We hope this workshop will mark a turn in the history of CKDu in Mesoamerica (the Mesoamerican nephropathy), this puzzling and expensive public health problem that ends the lives of the poorest workers of our region, prematurely and inequitably. Furthermore, the contributions made it clear that this is probably not an isolated Mesoamerican problem and that similar epidemics or endemics are occurring also elsewhere. We therefore hope that this report will reach beyond Mesoamerica and increase international awareness about a regional problem with a possibly global scope and, thus, contribute to effective attention from the research and public health communities.

Heredia, March 31, 2013

THE ORGANIZING COMMITTEE

Catharina Wesseling
Jennifer Crowe
Christer Hogstedt
Kristina Jakobsson
Rebekah Lucas
David Wegman

NOTES:

- The workshop title used the term 'Mesoamerican Epidemic Nephropathy'. In fact, its acronym 'MEN' has been used in several publications and international forums previous to the workshop. However, it was pointed out during the workshop, that 'MEN' is an acronym that might appear to exclude attention to women as victims of the disease and that another name should be proposed.

  There was no consensus regarding the epidemic or endemic nature of CKDu in Mesoamerica. Although in the opinion of the Organizing Committee 'epidemic' is justified considering there is not just a continuous low occurrence of CKDu in the area but rather a clearly increasing frequency of the disease, not everyone present at the workshop agreed with this. It may be possible that we face the situation where an endemic disease has acquired epidemic proportions. Among alternative terms proposed was 'Mesoamerican Nephropathy' and for the moment we have changed 'MEN' to MeN in all documents (although we are aware that the gender issue is still to be addressed).
- Throughout the document there is an inconsistent use of the terms Mesoamerica and Central America. There are different definitions for delimitations regarding Middle America, depending on whether the focus is geographic, geological, historical-cultural, political or economic. Panama (the most southern country), Belize (the only ex-British colony of the isthmus), or the south of Mexico (Mexico is considered part of North-America) may be included or excluded. We must keep in mind that for the study of the MeN epidemic, we target from south to north: Panama, Costa Rica, Nicaragua, El Salvador, Honduras, Belize, Guatemala, and the south of Mexico.
- We are most grateful for all the hard work done by the authors contributing the pre-workshop papers, the rapporteurs and facilitators from all the discussion groups and the abstract authors. We are also deeply grateful for the work done by those who worked behind the scenes to help assure the success of the workshop and the publication of this document (see Acknowledgements).
- Short summaries of the workshop results will be presented in international, scientific journals and professional meetings as well as to political and social institutions for urgent actions. The workshop organizers are very pleased with the decision to form a Research Consortium on MeN.
- The contents of all papers and posters remain the opinion and responsibility of the authors and, unlike the summaries of rotating table discussions and working group discussions, are not to be considered consented workshop conclusions.
- This publication has been finished with financial assistance of the European Union. The content of the publication is the responsibility of the authors and the SALTRA program. The content does not necessarily reflect the opinion of the European Union.
- This report has been published in English since the workshop was held in English to facilitate participation of as many countries as possible. Funding is being sought to translate this report to Spanish.

EXTENDED SUMMARY

INTRODUCTION AND WORKSHOP GOALS

The international SALTRA workshop on the Mesoamerican Nephropathy (MeN) was designed to review present knowledge of MeN and similar epidemics of chronic kidney disease of unknown origin (CKDu) elsewhere, set research priorities, bring together researchers, and establish international collaborations.

A dozen experts had been asked to write and delivered short *state-of-the-art papers* of relevant topics in advance of the meeting as the basis of known knowledge. Those summarizing papers were presented at the beginning of the three-day meeting, covering broad fields of epidemiology of MeN and CKDu elsewhere, pathology of CKDu, biomarkers, environmental and life style risk factors, heavy work in hot climate, genetic susceptibility, and policies and socioeconomic drivers. The papers summarized the state of the art and identified research gaps. This section of the workshop was complemented with *poster contributions* by many workshop participants.

The next session consisted of a series of *rotating table discussions*, where all participants contributed with their knowledge and with identification of data gaps regarding key topics. These topic were, specifically, prevalence and incidence of CKDu; biomarkers for early detection of CKDu; diagnostic practices for early and late detection of CKDu; exposure levels of environmental and human risk factors and validity aspects; social and working conditions in affected populations; hard work, heat, dehydration and access to water; and beliefs, disbeliefs, political and legal acceptance of the epidemic.

These first two sections set the stage for the second half of the workshop, consisting of *working groups* on different themes with reflections on how to move forward. The objectives of the different working group themes were to summarize our understanding of the international scope of CKDu; consider how best to establish the extent and severity of MeN; examine relevant causal hypotheses; examine socioeconomic drivers; evaluate possible prevention measures and policies; recommend optimal research designs; and focus efforts to control or eliminate the disease burden. The working groups examined the key aspects of different study designs: cross-sectional, case-control, cohort, intervention, experimental, and ecohealth/spatial epidemiology studies. The working groups also scrutinized methodological issues important for most MeN studies: clinical and epidemiological case definition of MeN; measuring exposures to work load, heat stress and dehydration; characterizing environmental and occupational exposure to nephrotoxic agents; and measuring personal risk factors. One additional working group reviewed all hypotheses that emerged during the workshop and made a proposal to prioritize them. And, finally, one working group discussed collaboration and the forms this can take in the near future.. The workshop finalized with a series of presentations and plenary discussions about how to move forward, focusing on prevention and treatment, health system responses, and linking research to policy making.

The state-of-the-art papers and posters, the summaries of the rotating table discussions, the results from the working groups and the policy papers are published in this report. ***This extensive summary emphasizes the working group results, since these results derived from the previous workshop sections on existing knowledge and knowledge gaps regarding the key topics of MeN, and integrated the discussions that took place around these topics during the workshop.***

GENERAL CONSIDERATIONS FOR THE NEED OF STUDIES ON MEN

Much work has been done in the eight years since the first SALTRA workshop on MeN with studies published from Central America along with suggestive evidence of MeN-like nephropathies in several Asian countries. This workshop considered whether there is still work to be done in describing the extent of the problem in countries already reporting the condition as well as countries where it is suspect or unexamined.

Prevalence studies are needed because identification of geographic locations and demographic/occupational groups with a higher or lower prevalence can continue to provide clues to the etiology of the disease. The disease is not well characterized in many areas such as Guatemala, Panama, Honduras, and even in regions within of Nicaragua, El Salvador and Costa Rica. Outside of Central America, it might be useful to conduct prevalence studies in areas where this disease might be going unnoticed but where various hypotheses suggest that there should be an excess of CKD with characteristics similar to those reported in Central America (e.g., Brazil, Ecuador, Egypt, India). We still need to understand pat-

terns and trends, and also better understand prevalence in populations that are not necessarily high risk, such as adolescents.

Incidence studies, though requiring more time and resources, are needed to study the natural history and progression of this disease. One proposal was to identify a population that best represents a particular geographic area/country rather than studying a convenience sample. It was suggested that we study incidence as a part of a surveillance system possibly using a *sentinel surveillance system on a regional level* (i.e. Central America).

Further studies of any type, however, should require agreement on a case definition for CKDu and common biomarkers along with study of novel biomarkers of disease/markers of progression. There was a general agreement that it was most important to identify *early disease*.

It was also noted that it is essential to use *standardized methods*. The methods for the different analyses should be clearly specified to allow comparison between regions.

MOVING FORWARD WITH POPULATION STUDIES

Defining a case: There are two different objectives to defining a case, one for clinical diagnosis and the other for use in population studies of early or pre-disease. Both were discussed with some preliminary proposals for how best to manage each objective.

A **basic clinical definition** for MeN patients was reasonably agreed on: persons with abnormal kidney function by internationally-accepted standards, living in Mesoamerica and with no other known causes for CKD, i.e. diabetes, hypertension, polycystic kidney disease (PKD), and other known causes. MeN patients have low renal function with typically no hypertension and no edema on physical examination.

There was general agreement on potential methods to identify the best and cheapest method for a clinical diagnosis:

- Clinical history, family history, information about work, liquid intake, medication including intake of NSAIDs
- Imaging - e.g. to exclude polycystic kidney
- Blood tests - e.g. electrolytes, hemoglobin, eGFR (creatinine, cystatin C), uric acid, glucose
- Urine samples - degree of proteinuria, hematuria, different biomarkers for tubular damage (NAG, protein HC, β2 microglobulin)
- Kidney biopsy - including light microscopy, immunofluorescence and electron microscopy

Patients included in clinical studies should be followed longitudinally to identify the natural course of the disease. If families with an increased risk of being affected by MeN could be identified in these pilot studies, genetic studies could be performed.

A **case definition in epidemiological studies** based on criteria emanating from a composite clinical case definition may be valuable for descriptive purposes, n.b. to be used for calculation of the need for medical care in the community. However, if the aim of an epidemiological study is to explore risk and susceptibility factors, such a definition of outcome should not be used as a single outcome. Instead, several different components should be investigated, and reported separately, with focus both on early and more advanced signs of adverse effects. Thus, not only kidney disease, but also renal function should be assessed. Following are some recommendations on basic methods that can be used in small-scale investigations by local researchers with limited funding.

- *Use of a creatinine-assay which is calibrated to reference methods.* The Jaffe method for serum creatinine determination is simple and low cost. Regardless, all creatinine methods should be traceable to a reference method based on isotope dilution-mass spectrometry (IDMS).
- *Use of the CKD-EPI formula for estimation of glomerular filtration rate (eGFR).* The Cockcroft-Gault and the MDRD equations were developed for clinical purposes. These equations perform poorly in obese persons. In contrast, the CKD-EPI formula was developed from many research studies, including population based and among multiple ethnicities (not yet in Central America).
- *Use of a semi-quantitative dipstick for proteinuria.* Dipstick testing is valuable for rapid and simple screening but results should be reported for all concentration levels. Also the nature of the samples must be described to interpret the findings in their context.
- *Dip-stick proteinuria as the only screening tool for case-finding is inadequate.* Clinical observations and epidemiological findings of MeN indicate that glomerular filtration may be affected without signs of proteinuria.
- *The clinical definition of chronic kidney disease as the only outcome reported is not sufficient.* Proteinuria, serum creatinine and eGFR should always be reported, as multiple categories, for the purpose of assessing more sensitive endpoints.

Measuring exposures: The characterization of exposure for individuals or populations is essential to understanding potential causes of MeN. It be-

came clear that there is no single study that will allow us to investigate the many different exposures that are hypothesized to be associated with MeN for the following reasons:

- There are many different agents of interest, including pesticides, metals, infectious agents, medications, and fructose;
- There is potential for both occupational and non-occupational (i.e. environmental and/or behavioral) exposures to these agents, including work load, heat stress & dehydration;
- There are different objectives that could be addressed (e.g. characterizing exposure pathways versus evaluating exposure-disease relationships).

Since all of these factors will influence the design of a study a conceptual model was developed to summarize the different ways in which exposure could be characterized and related to disease status.

Exposures that affect the kidney can originate as biological, chemical, physical, ergonomic and even psychosocial ones (Figure 1). These exposures can occur in both occupational and non-occupational environments. Exposure indicates that an individual has come into contact with an agent. In the case of direct contact, routes of uptake include inhalation, ingestion and dermal contact, but exposures can also occur through psychological/mental means. Only a portion of total exposure is absorbed leading to the internal dose or total absorbed dose. Biomarkers of total absorbed dose, when available, can provide information about dose across multiple exposure routes from both occupational and non-occupational settings. Similarly, only a portion of the total absorbed dose will result in an effective dose - the amount of the agent that reaches the critical target sites of interest. Individual susceptibility factors will influence uptake, transformation and ultimately the disease. For example, genetic differences may account for differences in the ability to resist the impact of dehydration or to metabolize certain chemicals, which could either increase or decrease susceptibility to CKDu depending on whether the original agent or its metabolite is more toxic. Similarly, individuals who are severely dehydrated may be more susceptible to the effects of exposure to low levels of nephrotoxic agents. Finally, to understand and examine any part of this process consideration must be given to the social determinants that influence the entire continuum.

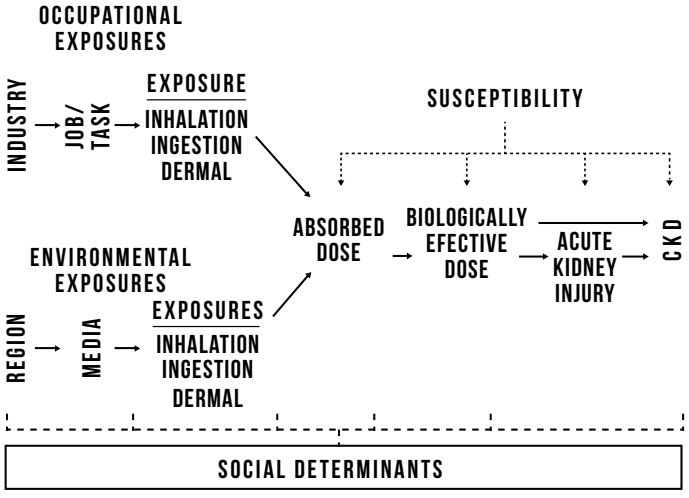


Figure 1. Conceptual model for exposure-related disease: A variety of exposure types from both work and general environments are received and transformed into an effective dose leading to chronic kidney disease (CKD). Pathophysiologic changes that result in CKD can occur directly, as a slow continuum, or follow from a series of repeated clinical or subclinical kidney disease episodes. The route to CKD is affected by social determinants across the spectrum and by individual susceptibility factors when exposure is experienced.

In addition, consideration was given to characterizing and measuring levels of nephrotoxic risk factors, different environmental media, using questionnaires to characterize personal exposure, and using biomarkers to assess total absorbed dose. These are described in the sections below.

Workload - Two types of workload measurements were discussed, physiological and qualitative. Physiological measures of workload include heart rate and oxygen consumption. Heart rate can be used as a measure of workload as well as relative intensity and is relatively inexpensive and convenient for field-testing. Importantly, there is a linear relationship between oxygen consumption and heart rate and subsequently, heart rate can be used to indicate metabolic workload. Use of a simple equation (220 – age) enables the researcher to estimate the relative intensity of the workload and therefore, comparisons can be more accurately made across age ranges, fitness levels and environmental conditions.

Qualitative measures of workload are also valuable tools in assessing workload in the field. Such measures included productivity measures and metabolic assessment. Productivity assesses the rate of production (i.e., quantity of material produced in a given time), whereas qualitative metabolic assessments describe a movement and assigns a level of intensity to it. To assess workload, qualitative metabolic assessments are an acceptable initial

assessment, though researcher bias and experience must be recognized. Heart rate is a reliable and valid physiological measure of cardiovascular workload and intensity. The combination of heart rate and qualitative assessment are the most accessible, reliable and informative measures of workload. It is important to note that workload assessment perhaps has the most meaning or significance to intervention studies in workplace settings.

Heat Stress - Workplace heat stress or heat exposure can be measured at three levels: 1) immediate ambient conditions, 2) general ambient conditions, and 3) internal body core temperature. The immediate worksite ambient environment can be accurately assessed and interpreted using specialized equipment and validated heat stress indexes. Wet Bulb Globe Temperature (WBGT) is an example of such an index that is well-established and widely used. Special equipment, however, is needed to measure WBGT according to the international standard. General environmental conditions can be assessed using data from existing local weather stations. With the use of mathematical formulas temperature and humidity data can be converted, with some assumptions, to the WBGT heat stress index or to other heat stress indices. Internal body core temperature can be assessed via a number of measurement methods each with its own advantage and limitation. The majority of such measurements are only practical in laboratory settings. However, some measures can be used in the field. Gastrointestinal temperature (measured via a telemetric pill) is the most reliable and valid measure available for field-testing to date, though there are limitations with this measure that include transmission range (approximately 1 meter) and participant consent/exclusion. Auditory canal temperature measurement is an alternative means of assessing body core temperature in the field, however there are notable validity and reliability issues with this measurement that must be taken into account and addressed.

Dehydration - Markers of dehydration or the fluid balance range from urine and blood markers to changes in body weight. Measurement of urine and serum sodium and osmolality may be useful markers of volume depletion and dehydration. Urine density or urine specific gravity is a common measure of hydration state as it is cheap, non-invasive and easy to measure. It is important to note that urinary measures will provide only a rough index of dehydration or renal concentrating ability as the kidney responds to changes in the osmolality of body fluids. Indeed, urinary measures are only sensitive to large acute changes in fluid balance (i.e., 3% or 5% body mass deficit) and lag behind plasma osmolality measures. Therefore, it is recommended that urinary measures be used to assess hydration status on a day-to-day basis (assuming the same time of day and behavioral pattern is followed).

Venous blood samples can be use to indicate changes in plasma volume or plasma osmolality. Changes in plasma volume can be calculated from changes in hemoglobin concentration and hematocrit ratio and this equation reliably reflects changes in plasma volume during acute bouts of exercise-induced dehydration when the change in plasma osmolality is less than 13 mosmol/kg. However, despite the validity of blood measures to acute changes in hydration state, it is unclear if the same validity remains with chronic exposure. Plus, the invasive nature of venous blood draws and the relatively difficult and expense can make these measures prohibitive for some studies.

Changes in body mass are perhaps the cheapest and most reliable means of determining an individual's fluid balance. A minimum of three early morning semi-nude weigh-ins should be averaged to determine an individual's baseline body mass. Changes in body mass over time can then be easily calculated. Difficulties can arise with this measure in finding: a flat, solid surface for the scales to rest on; privacy for participants; and scales working under high ambient temperatures.

Environmental Media - Of the various types of environmental media, characterizing drinking water should be the highest priority. There was general consensus that the highest priority agents should be inorganic arsenic and pesticides (particularly those that are known to cause AKI). The quality of drinking water, in particular the potential for pesticide contamination, is a leading concern in the affected communities and should be addressed.

The characterization of agents in drinking water could be used as part of an exposure-focused study to explore the relationship with total absorbed dose, or as part of an epidemiologic study as a way to characterize exposure and explore the relationship with AKI and/or CKD. Additionally, such an investigation could be done solely for surveillance purposes. Even if these agents are not risk factors for CKDu, ensuring that these communities have access to clean drinking water is certainly a worthwhile endeavor from a broader public health perspective. With regard to hardness of water, most workshop participants agreed on that hard water is not a risk factor for CKD, but given widespread community concerns in several affected areas, it is an important issue to address.

In addition to drinking water, characterizing agents in soil and/or ambient air could be useful depending on the research objective. Dust levels in agricultural areas can be high during dry seasons, likely resulting in exposure to any agents present in soil via inhalation and/or incidental ingestion. If biomarkers of the agent of interest are not available, then measuring contaminants in soil or air may be the best option for characterizing exposure. Or, if a particular agent is found to be elevated in biomarkers of total absorbed dose, analysis of soil and/or ambient air may provide important information about the source.

Similarly, analysis of targeted types of food could provide useful information about the role of diet. Since there are many types of food, each from different sources, it would be most efficient to either target samples of commonly consumed food, or allow results from epidemiologic studies to guide selection. For example, if a food frequency questionnaire is used to characterize consumption of different food types, and some are associated with higher risk of CKDu, then these food types could be targeted for chemical analysis.

As a less traditional and potentially innovative approach, it could be useful to consider whether studies of livestock or other animals could provide useful information. Studying exposure and/or disease in animals from the affected region could represent an opportunity to obtain data that would be more difficult or impossible to obtain from humans.

Finally, it is important to consider both spatial and temporal variability when measuring agents in any other media described above. The agents of interest are not uniformly distributed over time and space, so each study must be designed with that in mind. For example, if we think the levels of a particular agent in drinking water might vary over time due to periodic events (e.g. agent only used at certain times of week, month or growing season; or increased levels after severe weather events), then a cross-sectional study will not sufficiently characterize levels and a repeated measures study would be preferable.

Questionnaires - Carefully constructed (and when possible validated) questionnaires are important tools for characterizing personal exposure, particularly when attempting to characterize exposure that occurred in the past. One of the most useful approaches is to obtain work histories and/or residential histories, which can be used to develop exposure matrices for specific agents. Individuals can also be asked directly to self-report exposure to specific agents, though often individuals either don't know the names of agents/products/chemicals or were never aware they were using them in the first place. Assessing exposure via questionnaire can be especially useful when biomarkers of chronic exposure are not available.

Questionnaires can also be used to gather information about personal life style factors, such as diet, medication use, smoking and alcohol habits. Food frequency questionnaires are designed to collect information about the frequency and amount of consumption for many different types of food. Diet could represent an important exposure pathway because the food/drink is contaminated with nephrotoxic agents or because the food/drink contains specific ingredients of potential concern (e.g. fructose). As for medications, aminoglycoside antibiotics and chronic use of non-steroidal anti-inflammatory drugs (NSAIDs) are associated with acute kidney injury in a dose- and duration-dependent manner. Additionally, 'traditional' medicines based on herbs or other natural ingredients are also used in the region. Ideally, questionnaires could be used to assess the types of medications, amount of use, and frequency of use. However, since subjects may not be able to readily identify the medications that they use, specialized questionnaires designed to enhance recall of such information should be utilized.

Biomarkers of Exposure - As described above, biomarkers can be used to provide a useful measure of total absorbed dose. A biomarker of total absorbed dose (often obtained in biological material such as blood, urine, hair, or nails) can provide information about exposures across multiple exposure routes in both occupational and non-occupational settings.

However, temporal variability – also referred to as within-subject variability – is a critically important consideration during study design. The agents of interest vary in terms of their biological half-lives as well as in terms of their exposure patterns. Several metals, as well as some pesticides like DDT, have long biological half-lives such that a single measurement in blood provides a measure of cumulative exposure over a period of time that can range from months to years depending on the agent. However, many other pesticides have very short biological half-lives, such that a single measurement in urine only provides information about exposure over a period of time that can range from hours to days depending on the agent.

There is one situation in which a biomarker with a short half-life may provide information about exposure over a longer period of time: if the exposure is routine, such as through drinking water or as the result of another daily task. In such case, the biomarker still only measures the most recent expo-

sure, but because of the consistent exposure pattern over time, it may still provide a useful way to classify cumulative exposure.

The most common approach to utilizing biomarkers is to measure specific agents (or metabolites) of interest, which always comes with the risk of analyzing for the wrong agents. However, emerging technologies may provide alternatives to this one-agent-at-a-time approach. For example, a metabolomics-driven approach provides a global analysis of biochemicals that can be used to explore profile differences between populations with and without disease. If certain biochemicals occur more frequently (or exclusively) in the population with disease, it is possible that they are the result of the disease itself and may represent a novel biomarker of CKD. However, it is also possible that such biochemicals may represent important exposures or metabolites that are key risk factors for the disease.

Finally, there is the need to consider whether the concentrations of agents measured in blood or urine may be increased as the result of decreased kidney function, which could lead to an artificial conclusion that increased exposure resulted in decreased kidney function (i.e. reverse causation).

Social and Working Conditions: An essential part of understanding the drivers of the CKDu epidemic is to understand the contributing macro-level factors. Social determinants have been described as underlying disease risk factors. Furthermore, in the absence of known causality, an effort to focus resources on interventions less proximal to the cause may have bigger impacts on incidence and prevalence. Therefore, we need systematic identification of potential social factors and working conditions that might be contributing causal factors to the epidemic.

An important component of the social determinants of this disease is who makes up the workforce and where they are from. For example, it is common for cane workers to work the *zafra* then move somewhere else to work in a different field, so understanding where they go and what they do could provide important information. Further, studying the different working conditions for contracted and sub-contracted workers could shed light on the distribution of contributing factors of the disease. Using appropriate statistical analyses and stratification we can more accurately determine which social determinants are risk factors of the disease.

Another important step to take is to standardize data collection across countries. Creating a database of comparable data in all countries where there is an excess of MeN cases would be invaluable. Some variables to consider assessing: information on education, access to health care, unemployment, length of workday, alternative employment/activities, unemployment and, in general, poverty. Standardization is evidently also important for data collection on diet, water intake, and agrochemical use to assess exposure to nephrotoxic agents.

LIMITING HYPOTHESES

One of the most difficult tasks facing those concerned about understanding the causes and the opportunities for prevention of MeN is the number of plausible hypotheses that have been proposed and considered. First and foremost, it is absolutely crucial that we endeavor to create a means to bring together existing knowledge on each hypothesis in a single location so that researchers are able to understand what is known and what investigations have been conducted. We propose the creation of a matrix, detailing the current studies addressing each hypothesis and their findings, as well as an endnote file containing these studies to include the grey literature, abstracts and protocols for ongoing research pre-publication. This should be accomplished through the Consortium on MeN established at this workshop (see below). Having this information in a centrally accessible location will allow us to better evaluate which hypotheses have been sufficiently investigated with which techniques, and will help researchers direct their efforts towards the areas with the greatest need for further investigation.

In limiting hypotheses regarding the etiology of MeN, it is also crucial to see whether the geographic and demographic extent of the disease might be greater than what we have identified so far, so that we can be confident that our investigations encompass the full scope of the disease. We suggest a useful area of investigation may be targeted small-scale prevalence studies outside well-investigated regions (in addition to Mesoamerica) and demographic groups (in addition to male agricultural workers). We also suggest that a better characterization of geographic distribution within known areas would be useful. La Isla Foundation is in the process of compiling GIS data into a regional map of MeN, and incorporating further information to enhance this resource would be highly valuable.

One group of participants considered the information presented at the workshop and to some extent that available from the published literature. This resulted in a proposal (Table 1) to organize these hypotheses in a priority order to urge attention to the most compelling without closing the door to any hypothesis.

Table 1 – Proposed Priorities for Exploring Hypotheses for Causes of MeN
Highly Likely, High Priority to Investigate Further
Heat stress and dehydration (including electrolyte imbalances)
Non-steroidal anti-inflammatory drugs (NSAIDS)
Possible, High Priority to Investigate Further
Arsenic
Fructose intake
Nephrotoxic medications, including homeopathic medications
Leptospirosis and other endemic infections
Possible, High Priority but Logistically Difficult at this Time
Genetic susceptibility and epigenetics
Low birth weight and other prenatal, perinatal, and childhood exposures that increase susceptibility
Unlikely but strongly believed, Medium Priority to Investigate Further
Pesticides
Urinary tract diseases and sexually transmitted diseases (STDs)
Little Information, Medium Priority to Investigate Further
Calcium in drinking water, or water 'hardness'
Medication contamination and use of homeopathic medicines and non-approved drugs
Unlikely, Low Priority for Further Investigation
Lead
Mercury
Cadmium
Uranium
Aristolochic acid

This table represents only a preliminary assessment of the etiological hypotheses, and should be considered modifiable as new evidence and hypotheses arise. Monetary and time costs for methodologies necessary to investigate each hypothesis are also crucial to consider in establishing priorities for hypothesis investigation.

Regardless of which hypothesis or group of hypotheses is being considered in current or future studies there are a number of covariates that should be considered. These include those noted in Table 2.

Table 2 - Necessary covariates to consider
Drug, tobacco, and alcohol use
Diet and nutrition
Genetics, using ethnic subpopulation categorization as a proxy
Poverty and socioeconomic status (necessary also because it can emerge as a confounding or obscuring variable)
Co-morbid conditions – diabetes, hypertension, kidney stones

A number of techniques were put forward for investigating and narrowing down hypotheses on the etiology of MeN for future research. The most effective approach proposed is to focus on MeN hotspot areas for hypothesis testing initially, subsequently moving to non-hotspot areas once the plausibility of the hypothesis is established. Environmental sampling might be a useful technique for comparing hotspots to non-hotspots, with a particular focus on the evaluation of drinking water. Finally, with respect to hypotheses that are widely believed by affected populations, community-based participatory research should be an important tool in achieving community buy-in and acceptance of conclusions.

CONSIDERATIONS IN SELECTING THE DESIGNS OF POPULATION STUDIES

Population studies offer great promise for elucidating the nature, natural history and opportunities for prevention of MeN. Each of the approaches available brings advantages and disadvantages so the workshop reviewed all approaches and summarized what each approach has to offer. We considered each of the well-established study designs: Cross-Sectional, Cohort, Case-Control, and Intervention studies. But we also considered an evolving approach that offers promise - working with an ecosystem perspective.

Cross-Sectional Studies: Cross-sectional studies are usually the most convenient study type and sometimes the only possibility. They characterize the present situation and prevalence of disease and could also suggest hypotheses of causality but are hardly ever conclusive. With a cross-sectional study design we cannot find those who might have died from the disease and the disease might have forced the victims out of an occupational group. We cannot study the progress of the disease in a cross-sectional study and there are also recall bias problems.

Cohort Studies: A number of design and logistical issues regarding the implementation of a cohort study to investigate the causes of MeN were explored leading to the conclusion that, despite the efficiencies of a retrospective cohort study, a prospective study would be necessary to capture the necessary exposure and covariate information. While it was acknowledged that an occupationally-based cohort would be best for studying occupational hypotheses, such as heat exposure/strenuous work, the discussion focused primarily on a population-based cohort. Furthermore, for the present it is proposed that that the primary outcome should be CKD. In order to lay the groundwork for a cohort study of this magnitude, it would be necessary to conduct preliminary studies to: (1) accurately and efficiently measure exposure factors of interest and identify populations that would provide variation in exposure, and (2) demonstrate that it is possible to retain a sufficient proportion of subjects over the follow-up period.

Exposures and outcomes - Since there are a variety of potential causes that have been proposed for MeN not all fit well into a single cohort

study design, so an important step would be to identify the key exposures of interest and design the study around them. At the same time, many researchers have suggested that the cause of MeN may involve more than one factor. Therefore, it would be important to be able to consider multiple exposures within one study. A study that used intermediate biomarkers as outcomes would be smaller and less expensive. However, given the uncertainty regarding the predictive value of biomarkers for the endpoint of CKD, it is probably necessary to use CKD itself as the primary outcome. Intermediate outcomes should, of course, be measured as well.

Nature of cohort - A *specific* cohort, based on the identification of a group of people who are considered exposed as well as a group not exposed would ensure that the targeted exposure(s) were well represented and require a smaller number of subjects, if a group with high exposures can be identified. For an occupational hypothesis such as heat exposure/strenuous work or agrichemical exposure, groups with high and low exposure to these conditions could be identified and enrolled. If it is difficult to obtain access to an occupational based cohort, targeting of higher and lower risk geographic areas could serve as a substitute. Such an approach has been used for prevalence studies in Nicaragua and El Salvador, where certain geographic locations were used to represent different types of industries, crops, and altitude (climate).

A *prospective cohort study* can collect information on exposure and disease in real time but is more expensive and takes more time to complete, while a *retrospective cohort study* must rely on records or other sources of information to retroactively determine exposure and disease status but is less expensive and can be completed much more quickly. Since it is very unlikely that companies in the region have records from the past of sufficient detail and duration to properly assess exposure status, the most appropriate cohort studies will need to be prospective in design. However, should the opportunity present itself, a retrospective study should be considered because of the efficiencies in time and cost.

Potential sources of records for retrospective cohort studies might include registries of workers from unions, social security, or labor insurance programs, census data collection in different localities, general health surveys, prior prevalence studies, and health center records of CKD patients (this last source perhaps most appropriate for a study of progression of the disease).

Study population - Given the prominence of occupational heat exposure/strenuous work as a hypothesis, occupationally based cohorts that reflected a range of exposure levels might be an advantage. However, concern was raised about the feasibility of obtaining access to these populations. Alternatively, selection of populations from different areas where a particular industry or type of work is the main source of employment could also provide the variation in exposure levels needed for an effective study. The study population should be relatively young (e.g., upper age limit 65 years or younger) to increase the likelihood that cases of CKD represented MeN rather than more typical forms of the disease. It would also be best if the study population was drawn from different countries to reduce the possibility that results reflected unique local conditions rather than factors that are driving the broader epidemic.

Outcomes - The primary outcome would be CKD except for individuals who die prior to testing the outcome would be cause of death. Secondary outcomes would be biomarkers of kidney injury with specific biomarkers to be determined based on circumstances. Testing would occur every year, which would allow for early diagnosis, information on natural history, maintenance of contact to reduce loss to follow-up (LTF), and updating of exposure status.

Major determinants and exposures - Potential factors that could be assessed in such a study, whether occupationally- or residentially-based, include life-long residential and occupational history, including climate, ergonomic, and chemical exposures; amount, frequency, and type of fluid consumption at work and at home; inorganic arsenic exposure; pharmaceuticals (particularly NSAIDS) and natural remedies; infectious diseases; fructose intake; and possibly others. Current occupational exposure to heat/strenuous work could be measured directly in an occupational cohort. Current exposure in a residential cohort and past exposure in either cohort would need to rely on a job-exposure matrix, based to the extent possible on actual observation and measurement that could be developed as part of preparatory studies (see below). In addition to exposure assessment of individual subjects, environmental analyses could be conducted to evaluate potential nephrotoxic agents in water, sediments, soil, air, and/or food. Also determinants of exposures should be examined in an historical context such as work organization, contractual issues, child labor, migrancy, and economic and agricultural policies.

Pre-studies to assess potential - Follow-up studies of prior (and in most cases published) cross-sectional studies would clarify disease progression as individuals could be followed for 3-5 years allowing indications of the number of cases and time for converting from e.g. glomerular filtration rate, GFR, stage 2 to 3. Potentially new CKD markers could also be validated and tested in these participants. Investigations in high and low prevalence areas of hypothesized risk exposures would provide necessary information for power calculations and for choosing study populations for a prospective study on kidney disorders. Attention could be directed to exploring feasibility for assessing targeted exposures such as for heat and exertion investigations creating an appropriate job-exposure matrix.

Case-Control Studies: Case-control studies were judged to have limited value for understanding etiology due to the difficulty in collecting historical information on specific exposures and/or the low prevalence of specific exposures among cases and controls. At present, methods for identifying cases in an otherwise healthy population are not conducive to identifying cases of early or subclinical disease, limiting the utility of the case-control design in investigating exposures leading to the initial development of subclinical nephropathy.

Because a case-control study has considerably lower time and monetary costs, it can be helpful as a tool in contributing to the decision on which hypotheses merit further exploration through cohort designs. Under these circumstances case-control studies may be more useful for population-based rather than industry-based studies, potentially as part of a multi-center, cross-national study to evaluate risk factors in a population not restricted to a specific occupational group. Recruitment of cases could be conducted through hospitals or health clinics, or through whole population census or random sampling. Women and younger populations would be useful groups to focus on, or at least endeavor to represent well, in future case-control designs since less is known about the disease in these groups. With respect to case-control investigation, it remains crucial that case definitions are consistent and clear across different studies in different settings and countries.

Generally, case-control designs appear useful tools where the study is directed at the role of risk factors that are present early in life or exposures that can be assessed long after they are experienced. For example, the case-control design could be effective in studies of genetic risk factors for MeN, both in the investigation of specific genes and combinations of genetic characteristics. Other areas of investigation include antibody markers of prior infection, metals and chemicals that produce long-term biomarkers, and factors included in hospital records such as prescription of antibiotic use. The study of occupational exposures may be appropriate, particularly with people who have done the same work for a long time and where measuring current exposures is representative of past exposures. Such exposures, however, would need to be of at least moderate prevalence in the study base. Factors like personal behaviors can be investigated through case-control, although assessment of these needs to be well standardized to address recall bias.

Exposures are best assessed through measurement of biomarkers, application of validated questionnaires, and review of accurate historical records where available. Biomarkers of long-term exposure are ideal but not essential; biomarkers of short-term exposure can also be useful if the exposures are routine and we have additional data on the duration of that exposure. Carefully constructed and validated questionnaires can be useful tools for characterizing both occupational and non-occupational exposures when direct measures or historical records are not available. Diet and medication can also be evaluated with questionnaires again requiring careful construction and validation.

Intervention studies: Interventions designed to eliminate only one risk factor associated with MeN are difficult to design when the cause is so poorly understood. Nonetheless, it is clear that working in hot conditions, dehydration and use of NSAIDs are reasonably established risks for those in early stages of CKD. Therefore, avoiding those risks is sound advice for at risk working populations. The workshop participants suggested small, well-conducted controlled studies comparing ordinary/present with optimal/adjusted intake of water and salt during heavy hot work such as during *zafra*. These studies could ideally be replicated in multiple worksites and countries. This appears to be a good time to do such studies as there is political support in Central America as well as support from a number of the sugarcane companies who are seeking concrete advice that can be given to employers and workers. El Salvador may be a good place to start intervention studies as the harvesters are usually landowners, so access to workers for intervention studies and alternative solutions (such as working early in the morning and late in the afternoon) are easier to achieve.

In order to carry out even these limited studies expert advice and studies are needed to determine practical work/rest regimes that can be field-tested. Related issues include: examination of hydration

methods to maintain balance of water and salts during heat exposure and heavy work.

Ideally, an experimental and a control group could be compared for peak workload achieved and decline in productivity both before and after an intervention. Pilot testing is essential to assure an accurate measure of hydration since there is debate about the usefulness of urine data (including urine specific gravity) and since weighing harvesters is logistically challenging.

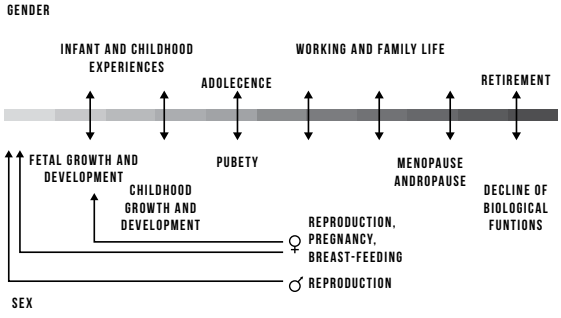
Intervention studies have the additional complication of addressing community beliefs about causality such as pesticides or unsafe water. It is generally a good idea to test water in both affected areas and non-affected areas. Participatory research (i.e. involving participants in the sampling of water and deciding together with experts where to sample from and at what time) is one way to help address this challenge. Communication strategies need to involve trust building with the researchers. At the same time, researchers also need to be aware that participating in a study can sometimes put a worker at risk for losing his job.

Intervention studies involving screening must be sure there is healthcare available for workers before screening takes place. Additionally, researchers must be acutely aware that workers may risk being fired and blacklisted as a result of creatinine screening and that those who are fired may need to work illegally due to the lack of other work options. Therefore, it is essential to guarantee data protection i.e., that the results of the tests will not be linked directly to the person in any database (should be coded), and that only the person being tested should be allowed to have access to the results directly. Additionally, since harvesters are usually paid by the amount of cane they cut, provisions must be made to assure workers participating in the study do not lose income.

Working with an Ecosystem Perspective: Since neither risk factors nor protective factors are fully understood, an ecosystem approach to human health, coupled to spatial epidemiology and a life-cycle perspective, was introduced as a methodology that could assist in better understanding the environmental, biological and social factors that contribute to and/or influence the development of CKD.

An ecosystem approach to human health provides a framework to examine the pathways and complex interactions between the social and physical environments and health outcomes. It is intervention-driven and builds on the convergence of expertise in the health, social and natural sciences to conceptually map out a trans-disciplinary understanding of the clinical, sub-clinical and infra-clinical[1] disease patterns within a particular geo-spatially defined ecosystem, considering not only the physical aspects, but also social, cultural and economic factors that may influence the development of the disease.

From a life-cycle perspective, it is important to consider possible fetal exposures, parents' health status, childhood living conditions and age at which one started working, all of which may contribute to the development of the disease. Figure 1 shows a schematic representation of the lifecycle, with an emphasis on gender (social construct) and sex (biological differences). At each stage in this lifecycle, social, environmental and biological factors may contribute to the development of CKD. In this life-cycle perspective, it may be useful to identify protective factors as well as potential risks for kidney dysfunction.



Taken from Mergler D. 2012[2].

The undertaking of an ecosystem lifecycle approach will require scientists, communities and decision-makers to work together with open minds, and a degree of humility, flexibility and solidarity. Two other practical issues were discussed. Discussants believed that the network of researchers created at this meeting could be useful to access professionals with different backgrounds and fields of expertise (e.g. nephrologists, epidemiologists, physiologists, toxicologists, gender experts, geographers, sociologists, bio-geo-chemists, etc) to get their input when designing a research project regarding this topic. Furthermore, a practical need is to develop a conceptual framework with an eco-health perspective around MeN and its determinants, risk factors, causal pathways and hypotheses in this region.

1. Infra-clinical refers to early physiological alterations which occur in an apparently healthy individual to maintain homeostasis, but if prolonged, may lead to a disease state.

2. Mergler D. Neurotoxic exposures and effects: gender and sex matter! Hänninen Lecture 2011. Neurotoxicology.

In order to implement an ecosystem lifecycle approach the following need to be undertaken:

- Identify an area of concern (illness, toxins, type of industry), particularly where there is previous information;
- Delimit the spatial boundaries of this ecosystem and the populations living within its boundaries;
- Examine, within this ecosystem and with these populations, the physical, health, economic and social main drivers;
- Initiate studies to examine the pathways between potential exposures throughout the lifecycle, the factors that influence these exposures and health outcomes. Involve communities and other stakeholders both in the design and the solutions

EXPERIMENTAL, MECHANISTIC AND METHODS RESEARCH

It is important that a continued open dialogue exists between basic science and clinical developments as new knowledge develops about MeN. In this way we can better characterize the disease and ensure more effective patient care as well as insure a better-informed experimental design. How best to achieve this requires consideration and action.

Genetic and epigenetic studies: Genetic and epigenetic studies may provide important information with respect to genetic susceptibility of Mesoamericans to different environmental factors. A geno-wide, non-hypothesis driven research model may be most successful in identifying such a gene or gene complex. Should a genetic susceptibility be identified in Mesoamericans, this genotype could be introduced into an animal model to assess the impact of different environmental risk factors on the pathogenesis of CKD.

Studies in animal models: Animal models can provide important information concerning the synergistic interactions of different environmental risk factors. Such interactions might include, for example: dehydration in combination with arsenic, fructose, nonsteroidal anti-inflammatory drugs (NSAIDS), or hard water. In this manner we could better isolate environmental effects that would inform clinical practice and experimental design. A limitation with animal models is that they replicate acute exposure, however, CKD is a chronic disease. This limitation must be considered when interpreting result and translating findings from the animal model to the diseased population. The alternative of developing a chronic disease state in an animal model is not an option in the foreseeable future.

Kidney biopsy analysis: With the exception of results published in this report there has been an extremely limited number of CKDu kidney biopsies analyzed from the region. Such analyses are most important for characterizing the disorder and should be promoted, under safe conditions.

A common registry and repository: A multi-lateral bio-bank that could store urine samples, serum samples and DNA would be extremely beneficial. This would enable sample storage for future analysis with advanced techniques and understanding, thus, maximizing the impact of samples collected in the field today. Similarly, a common database or registry would promote collaboration and advancement in the field of CKD. However, specimen collection needs to be carefully considered and uniformly followed. Also, ethical consideration of participant consent to prospective sample testing has to be adhered to. This would be an expensive undertaking, requiring a large amount of funding. The value of such a registry and repository merits consideration however.

Other areas of potential CKD experimental research: Studies should be conducted in females to identify an underlying factor potentially increasing the susceptibility of Mesoamericans of both genders to dehydration and heat exposure. Other situations where CKD may be present without hypertension or diabetes could be studied to improve understanding of MeN. For example, in elite athletes who consume high volumes of fluid and sugar and are exposed to high heat load repeatedly. Another example would be to examine the role of uric acid. Data seem to indicate that there is a disproportionate level of uric acid in early stages of renal failure. Mechanistic insight into the proliferation of uric acid could elucidate the CKD disease pathway.

Advances in Biomarkers: Biomarkers might be useful to define the pathophysiology, natural history, early clinical detection of CKD and possible treatment, and as surrogates in intervention studies. There is also a need to develop biomarkers for better detection of exposures such as agrichemicals and infections. Caution was raised about use of currently available commercial biomarkers. While these may prove useful there is always a concern that financial considerations may outweigh careful evaluation of these. Concern was also raised that if studies showed abnormalities in

some biomarkers before their sensitivity and specificity were well established, they might be employed as screening tools for employment resulting in denying employment to workers without actual kidney disease.

In addition to albumin, several small proteins or peptides have been proposed as early markers of acute and chronic renal tubular injury, i.e. 2-microglobulin, clusterin, cystatin C, kidney injury molecule-1 (Kim-1), trefoil factor 3, neutrophil gelatinase-associated lipocalin (NGAL) and others. Cystatin-C may also provide a more accurate measure for estimating GFR.

More information is needed regarding the operational characteristics of these markers such as accuracy for diagnosis of acute kidney injury and/or CKD, stability on storage and varying collection conditions, and assay validity. A number of emerging technologies have made it possible to measure several proteins in a single urine sample in a reliable and rapid way such that large numbers of samples can be analyzed and run in a high throughput manner. It would be of value to explore such potential early markers in well-designed and focused studies, as sensitive injury biomarkers may indicate disease before the development of elevated creatinine or proteinuria. However, it has to be kept in mind that the long-term prognostic value of most of these markers is not yet well characterized.

Genetic studies should be undertaken to identify genes that may alter the risk of the disease and possibly to identify the environmental trigger. Because of the ease of collection, development should focus on urine biomarkers.

ORGANIZING FOR THE FUTURE

The participants in the working group identified their primary objective as one of capitalization. Namely, participants aimed to capitalize on the momentum and progress of workshop discussions in order to improve international collaboration on MeN epidemiologic, medical and policy research initiatives, as well as to consolidate as a group. To that end, the workshop participants agreed on the following stated objective: *To develop collaboration that builds upon the ongoing work in the region and the progress that we have made at this meeting.* To work towards the identified common objective, working group participants proposed two key initiatives.

Terminology Adjustment: Throughout the course of the workshop, during both the presentations and at the roundtables, there emerged a vigorous debate on the endemic/ epidemic nature of the disease. It is doubtful that this debate will be resolved soon, and certainly not without further research. Conference participants expressed concern that, in the interim, failure to correctly define the disease could lead to alienation of affected communities and underreporting. In order to fully understand the disease, participants argued that universally agreed on terminology for accurate capture was critical. To that end, working group participants proposed that we use the term Mesoamerican Nephropathy (MeN) instead of Mesoamerican Epidemic Nephropathy or Mesoamerican Endemic Nephropathy.

Consortium Development: Participants proposed that the development of a unified standing body would be the most effective way to address previously identified issues related to consolidation and coordination. The proposed consortium model would consist of a network of affiliated scientific researchers across fields working together to increase understanding and public awareness of the disease. The working group participants hope that the proposed consortium would be in a position to host a similar conference for MeN in 2014.

The consortium structure would be as follows:

- **Coordinated by SALTRA** - Roundtable participants agreed that SALTRA's geographic situation, academic affiliation, pool of existing resources, international contacts, and organizational experience/capacities place the organization in an ideal position to coordinate the consortium's activities. Upon obtaining funding, SALTRA would hire a part-time paid administrator who would exclusively focus on administrative matters, communications, and planning activities related to the consortium.
- **Board** - Roundtable participants agreed that the consortium should be governed by a rotating board, comprised of 6 to 8 members, representing different geographical locations and areas of expertise. Participants also agreed that a temporary board should be selected at the workshop to serve until a permanent board is formed.
- **Membership**
  -Full Members - Researchers and clinicians actively working to combat MeN in Mesoamerica would be eligible for full membership.
  -Associate/ Observer Members - Associate or observer members would not have all of the privileges of full membership, however they would be invited to participate in selected meetings, serve on working groups, and access research resources. Intergovernmental and regional or-

ganizations (e.g. PAHO, WHO, ILO, COMISCA), government representatives, NGO representatives, and researchers focusing on the epidemic in other parts of the world, would all be invited to participate.

- **Working Groups** - Consortium working groups could be formed on an ad hoc basis to address specific issues such as methodology, public policy, and public education.

Consortium activities would include:

- I**nformation sharing** – Consortium meetings and publications would serve as a forum for information sharing, allowing its membership a mechanism through which they can quickly disseminate new ideas, share recent developments in the field, and collaborate on future initiatives.
- **Compile and disseminate research** – Working group participants agreed that the consortium would provide an appropriate forum for gathering and sharing completed research results and new research from a central location.
- **Funding seeking collaboratio**n – Though consortium members would apply for individual funding, the working group participants suggested that the consortium would be ideal for facilitating collaboration on grant proposals.
- **Serve as a bridge for translating results to policy makers** – Working group participants believed that the consortium should act as a mechanism through which research findings are compiled, distilled, and translated into actionable, informed policy recommendations.
- **Inform other initiatives** – Working group participants suggested that consortium members could play a key role in informing or supporting outreach, advocacy and education initiatives of other similarly-concerned organizations for communities at risk.

The following steps were identified as crucial towards implementing the proposed strategy:

- **Solicit interest in the consortium** – An email will be sent out to all workshop participants gauging their interest and willingness to participate in the proposed consortium.
- **Develop a webpage announcing the formation of the consortium** – SALTRA will develop a webpage which will announce the formation of the consortium, share workshop developments, and detail next steps.
- **Seek funding for the research administrator position** - With the aid of the temporary board, SALTRA will seek funding for the part-time research administrator position. Possible funding sources to be targeted include ISN, LASN, and the Spanish government.
- **Seek endorsements** – SALTRA and the temporary board will seek endorsements from pertinent and interested organizations and institutions.
- **Paper request** – SALTRA and the temporary board will issue a call for published and unpublished papers on MeN and related topics to be stored in a central registry available to all consortium members, which will be continually updated
- **Formation of a temporary board** – the working group participants proposed the formation of a temporary board to:
  - Formulate statement of purpose for the consortium ;
  - Finalize the structure of the consortium; and
  - Develop procedural mechanisms and terms of reference for consortium leadership, partnership, and membership.

Proposed members temporary board members, included:

- Jennifer Crowe (SALTRA, Universidad Nacional, Costa Rica)
- Aurora Aragón (CISTA, SALTRA, UNAN-León, Nicaragua)
- Sandra Peraza (SALTRA, University of El Salvador)
- Ricardo Correa Rotter (National Medical Science and Nutrition Institute Salvador Zubiran, Mexico)
- Manuel Cerdas (Social Security of Costa Rica)
- Dan Brooks (Boston University)
- Kristina Jakobsson (Lund University)
- Y-Vonne Hutchinson (advisory role, La Isla Foundation, Nicaragua)

EPIDEMIOLOGY OF CKD OF UNKNOWN CAUSES IN MESOAMERICA

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An epidemic of chronic kidney disease of unknown causes (CKDu) that appears to mainly affect young male manual workers has been reported in the media, unpublished scientific literature and, more recently, peer-reviewed published manuscripts. The aim of this summary is to describe the current knowledge on the distribution and epidemiology of CKDu in Mesoamerica, identify the main data gaps, and propose activities to address them.

1. WHAT WE KNOW

Extent of the problem

Reports of excess cases of CKDu are concentrated in the Pacific coast lowlands to the west of the mountain range that extends throughout Southern Mexico and Central American countries. Areas where studies have been conducted are shown in the map below and will be discussed later in this summary. The only exception would be Aguascalientes (located in north central Mexico), where an excess of CKD has been reported among children (Góngora-Ortega, 2008)

SOURCE: AMADOR, J.J. 2012

Mortality statistics

Mortality data is the only nationally based system of surveillance for CKD. Furthermore, to the extent that the case fatality rate for CKD is high, it serves as a lagging indicator for incidence. There is variation in the extent of ascertainment of mortality data at national Ministries of Health by country within Mesoamerica and Panama. One study in Chichigalpa compared the availability of mortality data from the national ministry of health register with the civil registration at the municipality and found that 21% of deaths in the latter were not recorded in the national register (Narváez-Caballero & Morales-Mairena, 2008). The benefit of mortality data depends on the absence of substantial bias by geography, occupation, sex, and age in extent of reporting of death certificates or attribution bias in cause of death. To our knowledge, no studies have assessed the potential for bias.

The overall number of deaths due to CKDu in Mesoamerica as a whole or in any of the individual countries is uncertain, primarily because there is no estimate of the percentage of deaths from CKD that could be classified as CKDu, a figure that likely varies substantially by country. However, El Salvador has the highest overall mortality rate from kidney disease in the world with 51.8 deaths per 100,000. Nicaragua and Honduras are also included in the top 10 countries with highest kidney mortality worldwide (25.5 and 24.6 deaths per 100,000 respectively) (WHO, 2008), and CKD appears as the second leading cause of mortality among men of working age in El Salvador (El Salvador Ministry of Public Health, 2010).

Mortality figures also show that the epidemic seems to concentrate in certain regions within countries. In Costa Rica, the province of Guanacaste appears to be the main province affected (Cerdas, 2005). In El Salvador, most CKD cases in the end stage of the disease referred to a national hospital came from coastal areas of the country (Trabanino, 2002). In Nicaragua, mortality due to kidney disease during 2009-2011 was almost 9 times as high in León and Chinandega as the median mortality rate in the country (11 deaths per 100,000), and more than twice as high as the rate in Granada, the next

highest department (Figure 1). On the other hand, other regions of the country, such as in the east, do not appear to be affected. Mortality due to kidney disease in León and Chinandega has also increased over time, increasing twofold between 2000-2009 (Figure 2). Mortality data at the municipal level indicates that Chichigalpa (Chinandega department, sugarcane area) and Larreynaga (León department, mining area) have similar rates of mortality and are the highest in Nicaragua. In Chichigalpa, 20-25% of deaths among males between the ages of 30-59 is due to CKD (Figure 4).

The mortality rate among men is much higher than among women. In 2002, the male:female ratio across Nicaragua was approximately 4:1 (Figure 3). In León and Chinandega, the male:female ratio was approximately 6:1, while there was little or no male excess in many other departments with low mortality rates from CKD. Departments with a male excess tend to be in the Pacific region. Although mortality rates among women were much lower than among men, in 2002, rates for women were still highest in León and Chinandega, and were also elevated in Granada and Rivas, all departments in the Pacific region.

Another key demographic characteristic of the epidemic is the young age at which victims are affected. In Chichigalpa, for example, deaths were concentrated in young-middle age groups among men, while deaths among women did not show a particular pattern (Figure 4).

▸ *Prevalence data and key risk factors*

There have been a number of studies (most of them unpublished) conducted in Nicaragua and other Central American countries in the past ten years. For a more exhaustive list, one can access reviews by Cuadra et al (2006) and Brooks et al (2010). Some of these studies were carried out using proteinuria measured by dipstick as the only measure of kidney damage. However, as diabetes and hypertension are unlikely to be important causes of CKDu in Mesoamerica, measures of glomerular proteinuria may be less informative. Nevertheless, virtually all of these studies detected elevated rates of proteinuria (mostly in the macroalbuminuria range). The study conducted by Dominguez and colleagues (2003) is of particular interest because it is the only study to our knowledge that has produced prevalence estimates in Mexico, Guatemala, and Honduras (El Salvador was also included). 40 % of the men in the coastal areas showed some degree of proteinuria, compared to 14% of women in the coastal areas and 10% of men living at >500 masl. 28% of men with proteinuria living in the coastal zone were <45 years old, and only 12% of them reported pre-existing hypertension or diabetes.

Table 1 summarizes prevalence studies published in the peer-reviewed literature. All studies analyzed serum creatinine and reported the glomerular filtration rate (GFR). In addition, they all assessed medical, occupational, environmental and behavioral risk factors by questionnaire. One limitation common to all studies was that creatinine was tested only at a single point in time. Because the case definition for CKD requires two consecutive GFR values <60ml/min per 1.73m^2, a single measurement overestimates the prevalence, but there is little information on its magnitude (Bottomley, 2011; de Lusignan, 2011). It should be noted that the U.S. National Health and Nutrition Examination Survey (NHANES), which serves as the primary source for prevalence of reduced GFR in the U.S. population, likewise uses only a single measurement; therefore, comparisons of prevalence in Mesoamerica and the United States are based on the same testing frequency (O'Donnell, 2011).

The limitations of these studies, which resulted primarily from a lack of resources, have been described in earlier reviews (Cuadra, 2006; Brooks, 2010). The limitations include cross-sectional design, reliance on prevalent rather than incident cases, questionnaire-based assessments with potential for exposure misclassification in general and recall bias in particular, and a lack of information on quality control measures, among other problems. Nevertheless, they have served an important role in moving beyond a reliance on mortality data, providing additional quantification to the magnitude and regional variation of MEN, and adding evidence for certain hypotheses about the causes of MEN.

Four main conclusions have emerged from these studies (Brooks, 2012). First, there is substantial variation in risk of CKD according to type of industry and occupation. Workers in the sugarcane cultivation, mining, and fishing or shipping industries have higher prevalence rates, while areas in which coffee growing and services dominated show no evidence of excess disease. Second, persons living at low altitudes are more likely to have CKD than those living at higher elevations. Peraza et al (2012) have demonstrated how this pattern persisted even when comparing two sugarcane communities at different sea levels. Third, males appear to be much more affected than women. Fourth, age groups affected by CKD are much younger than the age distribution that one would expect for a chronic disease. To illustrate this point, O´Donnell (2011) compared the distribution of eGFR among

age and sex groups in Quezalguaque (a municipality located in León, Nicaragua) with the same groups in the USA based on data from NHANES (Figure 5). The overall prevalence of decreased eGFR was 1.8 times greater in Quezalguaque. However, the relative differences were much greater at younger ages: men ages 30–41 in Quezalguaque had rates 16 times as high and also were much more likely to have Stage 4 disease compared to their counterparts in the USA,. Women in Quezalguaque also had elevated age-specific prevalence compared to women in the USA, but the differences were more modest.

We are aware of one other prevalence study for which data recently became available. The Pan-American Health Organization (PAHO) sponsored a study in the city of Managua in 2003 to assess prevalence of and risk factors for chronic disease, particularly diabetes and hypertension. At the request of the Ministry of Health, blood samples were also tested for serum creatinine. A total of 1,704 respondents (85% response rate) were randomly selected using a stratified neighborhood sampling plan. Fewer than 1% of subjects between the ages of 20-59 had eGFR values <60ml/min per 1.73m^2, and there were no differences in prevalence between males and females [J Amador, unpublished data].

2. KNOWLEDGE GAPS

As described above, epidemiological information about CKDu is still very limited:

- The available information is based mainly on records of deaths attributable to CKD and a few community prevalence studies. For the most part, these studies have been conducted in areas where large numbers of cases of CKD have been reported; the extent of disease in other areas is not well understood.
- There are as yet no official or unofficial estimates of the numbers of new cases of CKDu, people living with the disease, or deaths attributable to CKDu in Mesoamerica and Panama.
- Data on incidence would benefit efforts to determine cause(s) of MEN, as well as provide information on whether incidence is increasing, stable, or decreasing, which would be important for public health planning and evaluation of interventions. Data on the incidence of CKD are lacking since it is not usually included in the set of diseases included in surveillance systems in the region, which are focused almost exclusively on infectious disease.
- Systematic data are also lacking on the natural history of the disease: time between early injury and occurrence of disease, latency time from occurrence to diagnosis, and survival time from diagnosis to death. A better understanding of these components might help focus on certain hypotheses by providing a framework for timing of exposure, along with an understanding of factors that might slow the progression of the disease.

In addition, there are a number of methodological questions that need to be discussed and evaluated as to their potential importance:

- In Sri Lanka, a case definition for CKDu was developed: (1) no past history of or current treatment for diabetes mellitus or chronic and/or severe hypertension, snake bite, urological disease of known aetiology or glomerulonephritis; (2) normal HbA1C (<6.5%); (3) BP <160/100 mm Hg untreated or <140/90 mm Hg on up to two antihypertensive agents (World Health Organization, 2010). No case definition has been established for CKDu in Mesoamerica[1].
- Handling of serum creatinine in data analysis:
- - Some studies relied primarily on a serum creatinine value above the laboratory reference value for their main analysis, while others used a value of eGFR<60ml/min per 1.73m^2;
- - All studies listed in the table used the Modification of Diet in Renal Disease (MDRD) study equation. However, in a community-based study where the great majority of subjects will have eGFR≥60ml/min per 1.73m^2, the CKD-EPI equation appears to be more accurate (Stevens, 2010);
- - Use of a single measure of creatinine (Bottomley, 2011; de Lusignan, 2011);
- With few exceptions, data on risk factors have been collected exclusively by questionnaire instead of environmental or biological testing. Questions have not been validated, nor have they been standardized across research groups.

3. RESEARCH NEEDS

Priorities to address these gaps in knowledge include:

- Active surveillance of CKD incidence and its social and demographic determinants at the population level. A case definition of this particular presentation of CKD is needed as well as strengthened surveillance systems capable of monitoring new cases arising from clinical practice and current active surveillance systems

1. Case definitions (clinical and epidemiological) were proposed as a part of the workshop and resulting collaborations and can be found in this report.

in workplaces and research interventions.

- Prevalence studies in additional areas selected on a systematic basis.
- Longitudinal studies capable of assessing incidence and progression
- Burden of disease, quality of life, and social determinants (social inequalities and poverty) studies.

Figure 1

Ckd Mortality, Nicaragua,2009-2011

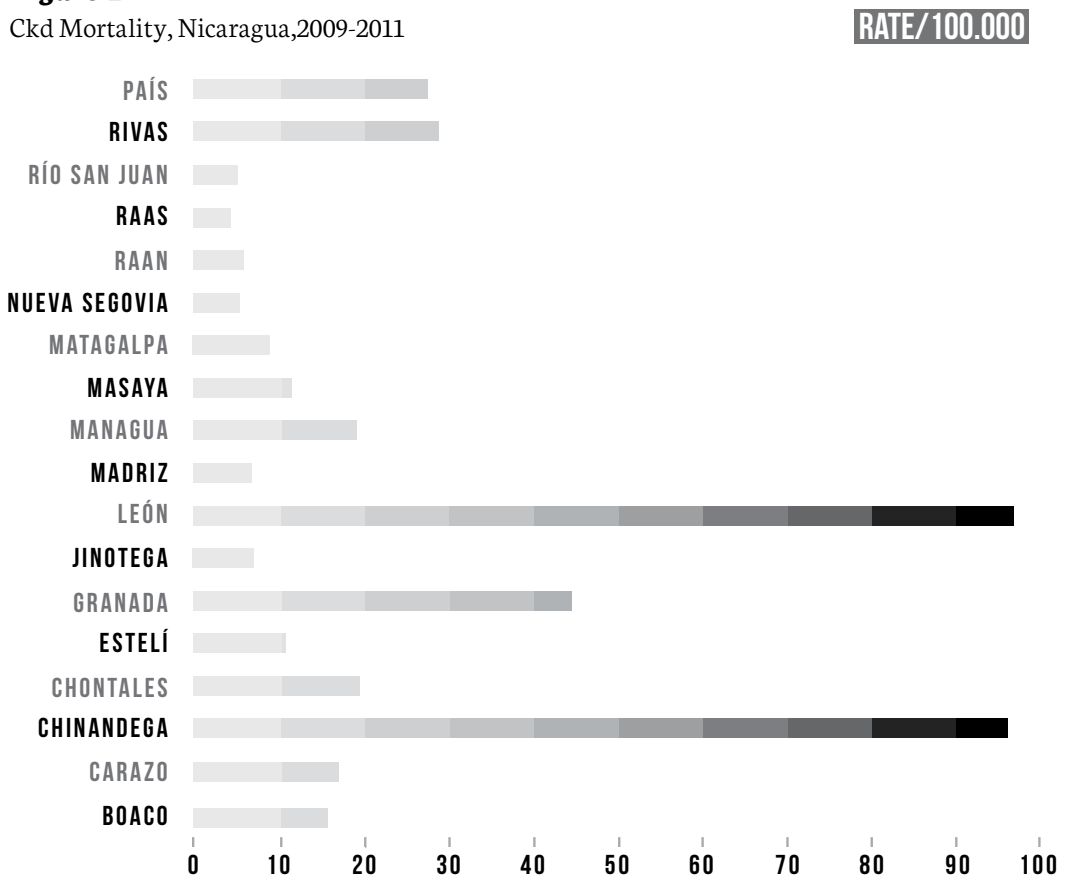
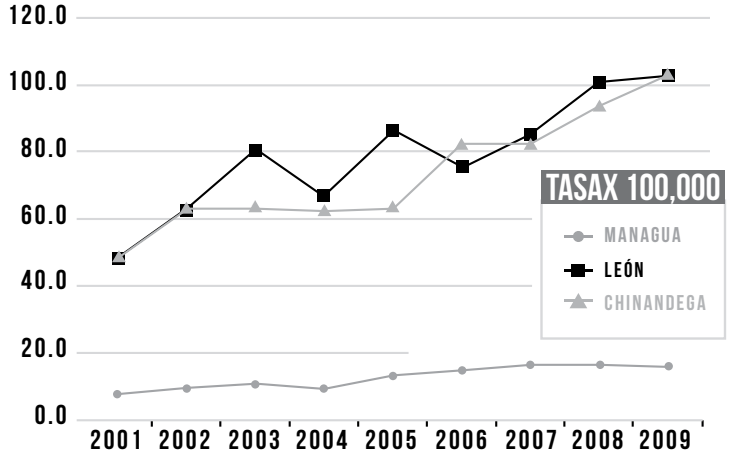


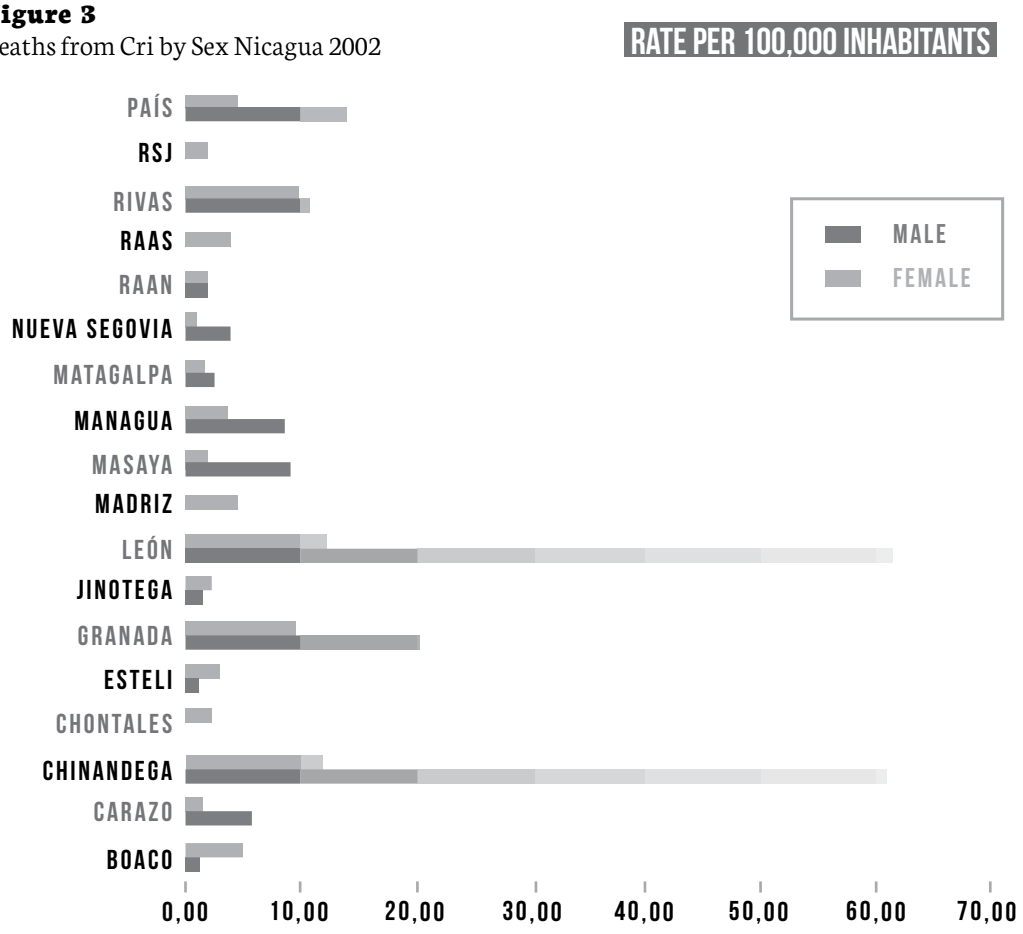
Figure 2

Mortalidad por ERC, Nicaragua 2001-2009



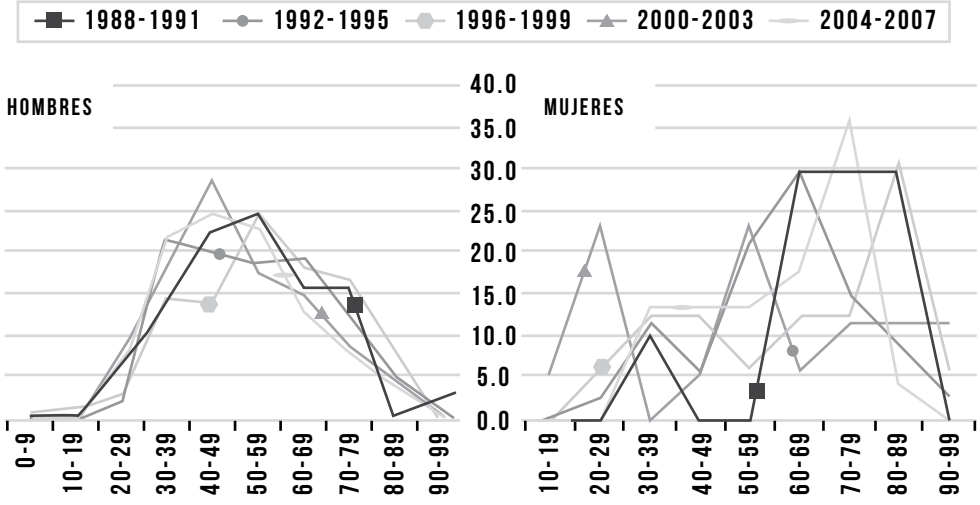
Source: Nicaragua Ministry of Health. Dirección General de Planificación y Desarrollo. Oficina Nacional de Estadísticas.

Figure 3
Deaths from Cri by Sex Nicagua 2002



Source: Nicaragua Ministry of Health. Dirección General de Planificación y Desarrollo. OficinaNacional de Estadísticas.

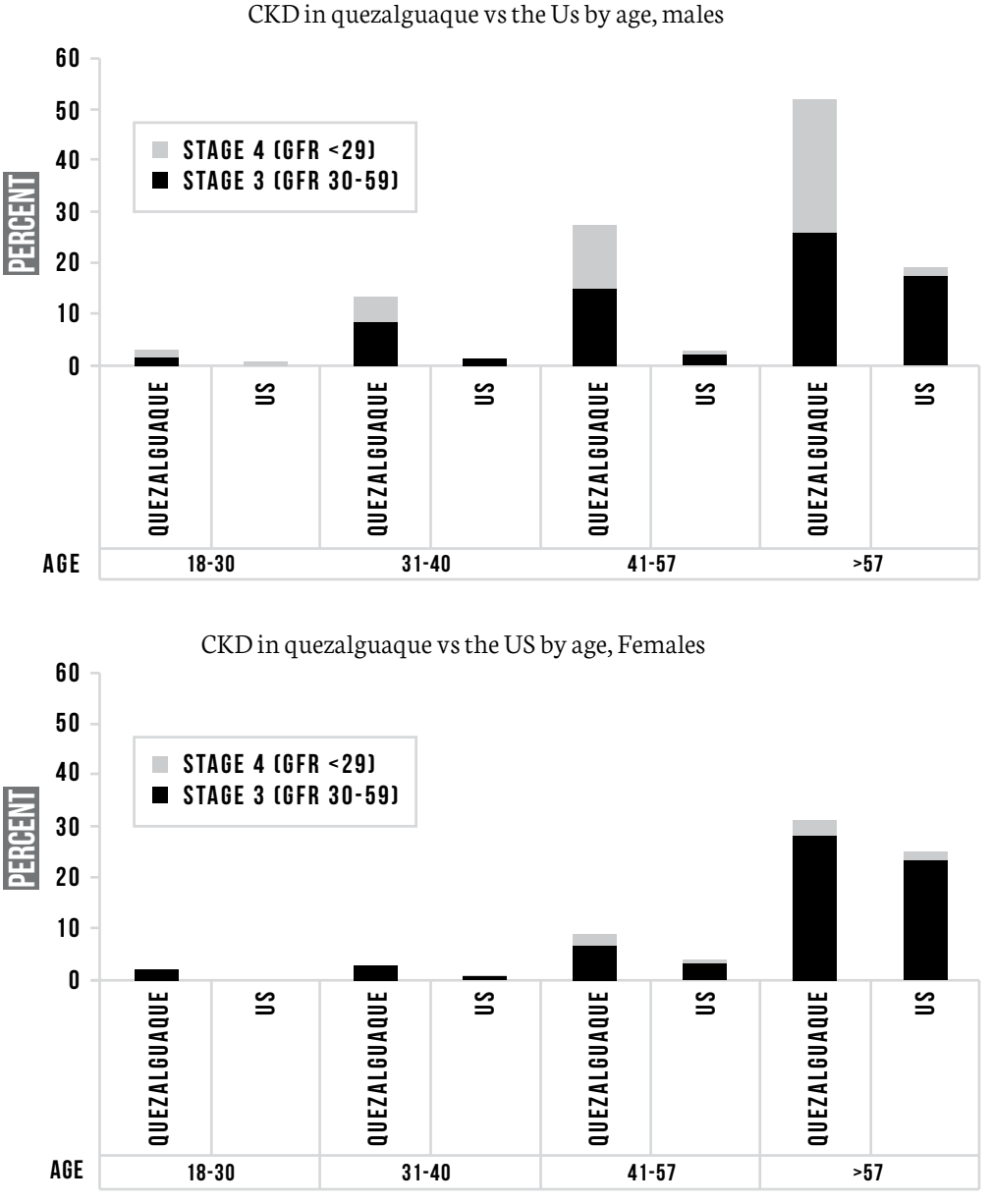
Figure 4
Distribution of CKD deaths (1988-2007) in Chichigalpa (Nicaragua) by sex and age.



Source: Narváez-Caballero & Morales-Mairena, 2008.

Figure 5

Prevalence of kidney disease in Quezalguaque compared with the USA using the NHANES 1999–2006 data



Source: Adapted from O´Donnell et al. (2011).

Table 1. Summary of regional community-based studies of chronic kidney disease that used serum creatinine and eGFR as disease estimates (adapted from Weiner, 2012)

LOCALE(S) STUDIED	OCCUPATION *	N	ALTITUDE †	PREVALENCE AND POPULATION	RISK FACTORS
EL SALVADOR: 2 COMMUNITIES JIQUILISCO, USULUTÁN AND SESORI, SAN MIGUEL (GARCÍA-TRABANINO, 2005)	Agriculture	291	Low	·Study included men only ·Sampling pattern only tested serum creatinine in male coastline residents with 1+ or greater proteinuria	·Dipstick proteinuria (1+ or greater noted in 46% in low altitude coastal community vs. 13% in high-altitude inland community · 37 of 80 men with lab measurements had creatinine>1.5 mg/dL; of these 37, 81% had 1+ proteinuria and only 19% 2+ or greater proteinuria
		62	High		
NICARAGUA: 9 COMMUNITIES LEÓN AND CHINANDEGA (SANOFF, 2010)	Varied	997	Not stated in article but likely varied elevations	·Men only after too few cases found in women ·12.4% of population with eGFR below 60	·Agricultural field labor, consumption of "lija" (alcohol available in a form susceptible to contamination by toxins), and consumption of >5L of water per day independently associated with presenceof reduced eGFR ·Hypertension and diabetes not more common in CKD
NICARAGUA: 5 COMMUNITIES LEÓN AND CHINANDEGA (TORRES, 2010)	Subsistence Farming + Mining	445	Low	·High prevalence of reduced eGFR, with 14% of men but only 3% of women with eGFR below 60 ·Abnormal creatinine levels in 31% and 24% of male and female agricultural workers, respectively, at 100 - 300 m above sea level, but not occurring at higher altitudes	·Abnormal creatinine levels not common in higher altitude villages ·Banana/sugarcane independently associated with higher creatinine in men and mining/subsistence farming independently associated in both men and women
	Banana/Sugar	384	Low		
	Fishing	216	Low		
	Coffee	92	High		
EL SALVADOR: BAJOLEMPA REGION (ORANTES, 2011)	Agriculture	775	Low	· eGFR<60 ml/min/1.73 m2 in 17% of men vs 4% of women	·Non-steroidal anti-inflammatory drug use common ·Most affected individuals with neither diabetes nor hypertension

NICARAGUA: QUEZALGUAQUE, LEÓN (O´DONNELL 2011)	Inland Varied, agriculture	771	Mostly Low	·High prevalence of reduced eGFR, with 20% of men with eGFR<60 ml/min per 1.73m2 vs 8% of women ·13.4% of men age 30-41 with low eGFR	·Residence at lower altitude associated with lower eGFR
NICARAGUA: MATAGALPA (LAUX, 2012)	Coffee	267	Very High	·Very low prevalence of reduced eGFR, with no individuals below 40 years old having reduced eGFR	
EL SALVADOR: 5 COMMUNITIES (PERAZA, 2012)	Sugar	129	Low	·High overall prevalence of reduced eGFR	·Discrepant findings in sugar communities with CKD common at low altitude sugar communities but rare in the high altitude sugar community; 18% of men had eGFR below 60 in low altitude sugar communities vs 0-2% in high altitude sugar communities ·Women less affected than men (8% in low altitude sugarcane vs 1-3% in other communities) ·Proteinuria uncommon and low-grade
	Sugar/Services	159	Low		
	Sugar	120	High		
	Coffee	124	Very High		

* Sugarcane cutting is considered the most intensive in terms of physical labor, with services considered the least physically intensive job.

† Temperatures are significantly lower at higher altitudes; low altitude suggests elevations from sea level to 100m while high altitude suggests 500m to 1000m above sea level, and very high 1000+m above sea level eGFR, estimated glomerular filtration rate

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SPATIALLY CLUSTERED CHRONIC KIDNEY DISEASE

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Countries in Mesoamerica, in particular El Salvador and Nicaragua, have reported an unprecedented increase in the number of patients at the end stage of renal disease that burdens health care systems, and poor agriculture or mining based communities. The concentration of cases of chronic kidney diseases – CKD in Mesoamerica has been reported in scientific and lay literature and in all mass media. According to these reports, it appears that there are geographical clusters of CKD, and a high concentration of CKD of unknown etiology documented in multiple study sites, or captured by analysis of national and regional registries systems for end stage renal disease – ESRD.

It is worth noting that CKD was the 19th leading cause of death in 1990, and in 2010 became the 7th in Central America and the Dominican Republic. This sharp change has yet to be explained. CKD is also estimated to be the 7th cause of Disability Adjusted Life Years (DALYs) in Nicaragua and, the 9th in El Salvador, representing a heavier burden for these countries when compared to other countries of Central America[1].

The objectives of this paper are to provide an overview of CKD and ESRD distribution in the population in different parts of the world outside Mesoamerica, and to present an analysis of the CKD-related mortality rates in Central America and other selected countries of the Region of the Americas. The information is not exhaustive; it is an attempt to contextualize the core discussions of the MeN workshop on causality of CKD in Mesoamerica.

1. WHAT WE KNOW

It is estimated that for every case of ERSD in the USA, there are 200 in stages 3 and 4, and 5000 in stages 1 and 2 of CKD (Barsoum RS, 2006). The number of patients with ESRD being treated with renal replacement therapy varies with the level of affluence of the country. In 2010, the highest rates of ERSD were found in Taiwan, with 2584 per million, and in Japan and the USA with 2260 and 1870 per million respectively. In the Americas, Argentina reports rates of 795 per million population of ESRD in replacement therapy, Brazil 479, Canada 1144, Chile 1161, Colombia 544, Jalisco from Mexico 1402, and Uruguay 1033 per million (US Renal Data System, 2012).

The majority of the countries do not have reliable registries for CKD, or regular surveillance systems capable of detecting patterns of disease distribution in the population that could facilitate the identification of trends and clusters of CKD. Most estimates of incidence or prevalence rates are based on registered patients in treatment for ESRD, or on community based surveys.

1.1 Distribution of cases of CKD or ESRD in populations outside Mesoamerica

Brazil

In Brazil, based on data extracted from the registry of procedures of high complexity of the Unified Health System (SUS) for the period 2001 to 2011, Northeast (21.2%) and Southeast (51.6%) regions concentrated the majority of the cases of patients in ESRD. More than 72% of the patients were younger than 64 years, and 30.4% were younger than 44 years. A total of 42.2% of all cases were of CKD of unknown etiology. The prevalence increased 50% in 10 years, with approximately 5% increase per year, from 341 to 513 patients per million population between 2001 and 2010, and 10% increase in the incidence among older ages (>64 years of age). Lethality, however, remained unchanged in the same period. Information on race/ethnicity has being collected since 2008. In this period, while representing only 0.2% of the cases, 80% of the indigenous population that initiated replacement therapy had a diagnosis of CKD with unknown etiology, a

1. Source: Institute for Health Metrics and Evaluation – IHME – University of Washington. Results presented at the COMISCA meeting, Santo Domingo, 2012 by Professor Rafael Lozano

number that is much higher than average for all other race/ethnicity groups (Moura L. et al., unpublished).

Canada

Incidence of ESRD is significantly higher among Aboriginal Canadians aged <22 years than among age-matched white individuals, with an incidence rate ratio 1.82 for Aboriginal boys and 3.24 for Aboriginal girls. A number of studies explore the causes of these differences (Samuel SM et al., 2012).

China

A cross-sectional survey of a nationally representative sample of Chinese adults estimated the prevalence of CKD, and documented geographic differences, demonstrating high prevalence of chronic kidney disease in the north (16.9% [15.1–18.7]) and southwest (18.3% [16.4–20.4]) regions when compared with other regions. No estimates of CKD of unknown etiology were presented (Zhang et al., 2012).

El-Minia Governorate, Upper Egypt

A high prevalence of CKD in El-Minia Governorate was observed, estimated at 308 per million population. Most (76%) of the patients lived in rural areas, compared to 57% of the controls ($p<0.001$). Possible environmental causes have been investigated (Kamel & El-Minshawe, 2010).

India

In India, the distribution of the disease varies geographically, with the largest differences seen in CKD of unknown etiology; national average of 16% (range between 10% in the East, and 20% in the South). The ratio men/women is 2.2/1 for undetermined CKD, and 2.5/1 for diabetic nephropathy (Rajapurkar et al., 2012).

Pakistan

Prevalence estimates in the general population are based on registered patients with ESRD. CKD with unknown etiology is estimated in 7% (Dialysis Registry of Pakistan 2009).

Sri Lanka

Endemic occurrence of chronic kidney disease with unknown etiology is reported to be concentrated in certain parts of the north central dry zone of Sri Lanka. The disease occurs in settlements where groundwater is the main source of drinking water and is more common among low socio-economic groups, particularly among the farming community in cascade irrigation systems (Bandara et al., 2007; Wanigasuriya et al., 2011; Chandrajith et al., 2011).

Thailand

A Thai study showed that the prevalence of CKD increases progressively from urban areas to urban slums to rural areas, suggesting the presence of unique risk factors in rural populations (Perkovic V et al., 2009).

Tunisia

Chronic Insterstitial Nephropaty (CIN) of unknown etiology similar to BEN (Balkan Endemic Nephropaty) is found in Tunisia. Ochratoxin A (OTA) is reported in multiple studies. One study found higher levels of exposure to OTA in the general population when compared to reference values, and a higher (76%) prevalence of any level of OTA in the group of patients with CIN of unknown etiology; this group also had the highest mean blood concentration levels of OTA when compared to 4 different matched control groups (Zaied et al., 2011).

USA Zuni Indians

The prevalence of end-stage renal disease among the Zuni Indians was estimated as being 18.4-fold and 7.4-fold higher than among European Americans and American Indians/Alaskan Natives, respectively. One population based study documented prevalence estimates of 26% for incipient albuminuria, and 24% for overt albuminuria in the Zuni Indians, which are similar to those in an Australian Aboriginal community (Sha VO et al., 2003).

1.2 Exploratory mortality analysis of Chronic Kidney Disease in Central America, 2000-2009[2]

Mortality is the most reliable indicator available for the majority of countries. The analysis presented here uses mortality for the period from 2000 to 2009 and it is based on the availability of data in each country. The quality of mortality data mainly the underlying cause of death varies from country to country in the Latin America and the Caribbean Region. For instance, Honduras does not report coded causes of death. PAHO estimates a mortality under-registration of 35% and 25% in Nicaragua and El Salvador respectively, having a 2.1% and 16% of ill-defined causes of death in each country.

2. All mortality analysis were performed by professionals of Surveillance and Disease Prevention and Control (HSD), PAHO/WHO

Costa Rica, Guatemala and Panama have better quality of mortality data, but still deal with an under-registration of death around 10% . No data from Belize is included in this report because of reliability issues of small numbers (PAHO, 2012).

Diseases of the genitourinary system (N00-N99, ICD-10) is among the ten leading causes of deaths in most of the Central American countries, being the second leading cause of death in El Salvador, the fourth in Nicaragua, sixth in Panama, ninth in Costa Rica, and the tenth in Guatemala. Furthermore, it is the fifth leading cause of death in Puerto Rico and Peru, and the eighth in the United States and Argentina. According to empiric evidence, most of deaths in this group have renal failure as underlying cause of death (N17-N19, ICD-10).

In general, more than 90% of the deaths are due to chronic kidney disease (N18, ICD-10) and unspecified kidney failure (N19, ICD-10), and the contribution from acute renal failure (N17, ICD-10) varies substantially between the countries, being 4% in El Salvador and Nicaragua, 9% in Costa Rica, 19% in Guatemala and Panama. The proportion of unspecified kidney failure is in general added to the number with the code for chronic kidney disease, but there are also large differences between the countries regarding the contribution of this category, being 3% in Nicaragua, 28% in El Salvador, 8% in Costa Rica and Panama, and 33% in Guatemala.

Figure 1 shows the trends of mortality due to chronic kidney disease and unspecified kidney failure in the period from 2000 to 2009. The age-standardized mortality rate increases on average 2.2 deaths/100,000 per year in males in El Salvador (65 deaths/year), 2.2 deaths/100,000 per year in males in Nicaragua (64 deaths/year), 1 death/100,000 per year in females in El Salvador (33 deaths/year), and 0.5 death/100,000 per year in females in Nicaragua (15 deaths/year). No increasing trends in CKD mortality was observed in the other countries.

Figures 2A and 2B show a sharp increase with age in El Salvador and Nicaragua, starting at a very young age. In both countries, mortality rates among men are significantly higher, and the male/female ratios have not changed from 2001 to 2009.

Compared to Cuba, which has the lowest mortality rates due to CKD and was used as a control, El Salvador has a mortality rate ratio for male 30 times higher, and Nicaragua, more than 20 times higher. (Figure 3).

Figure 2
Chronic Kidney Disease & Unspecified Renal Failure Mortality Rates by Age & Sex (x 100,000 pop.) in selected countries. 2001 (A) and 2008 (B)

Figure 2A, year 2008

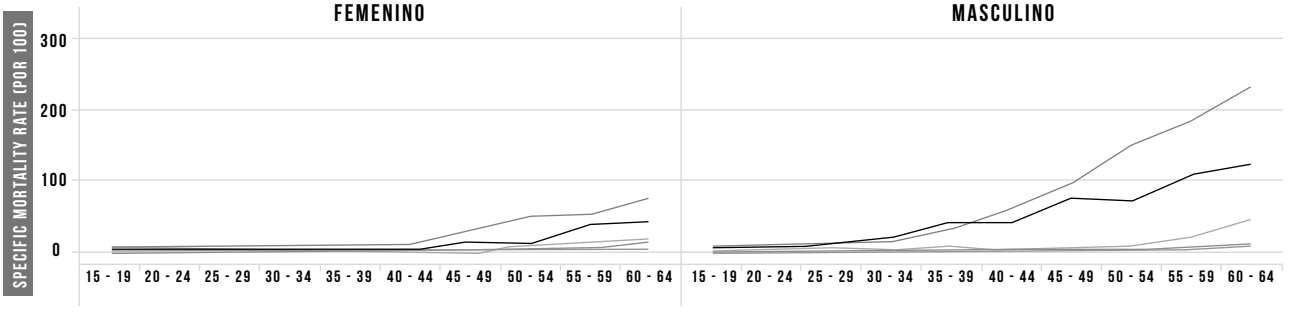


Figure 2b, year 2008

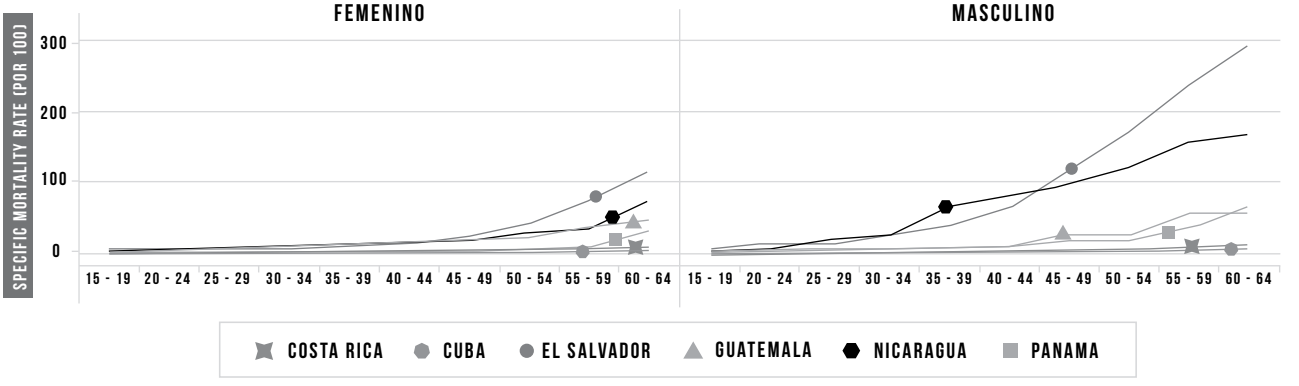


Figure 1
SMR of Chronic Kidney Disease & Unspecified Renal Failure in selected countries 2000-2009 (x 100,000 pop.)

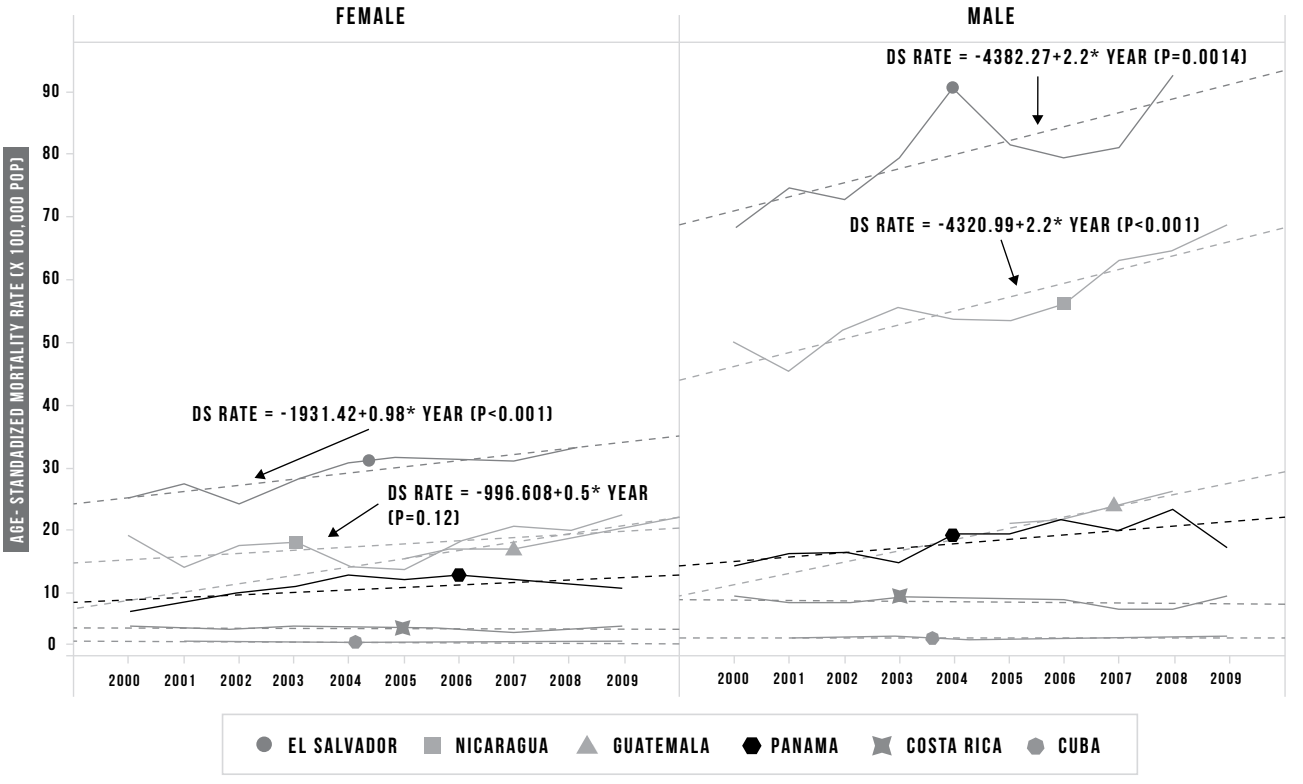
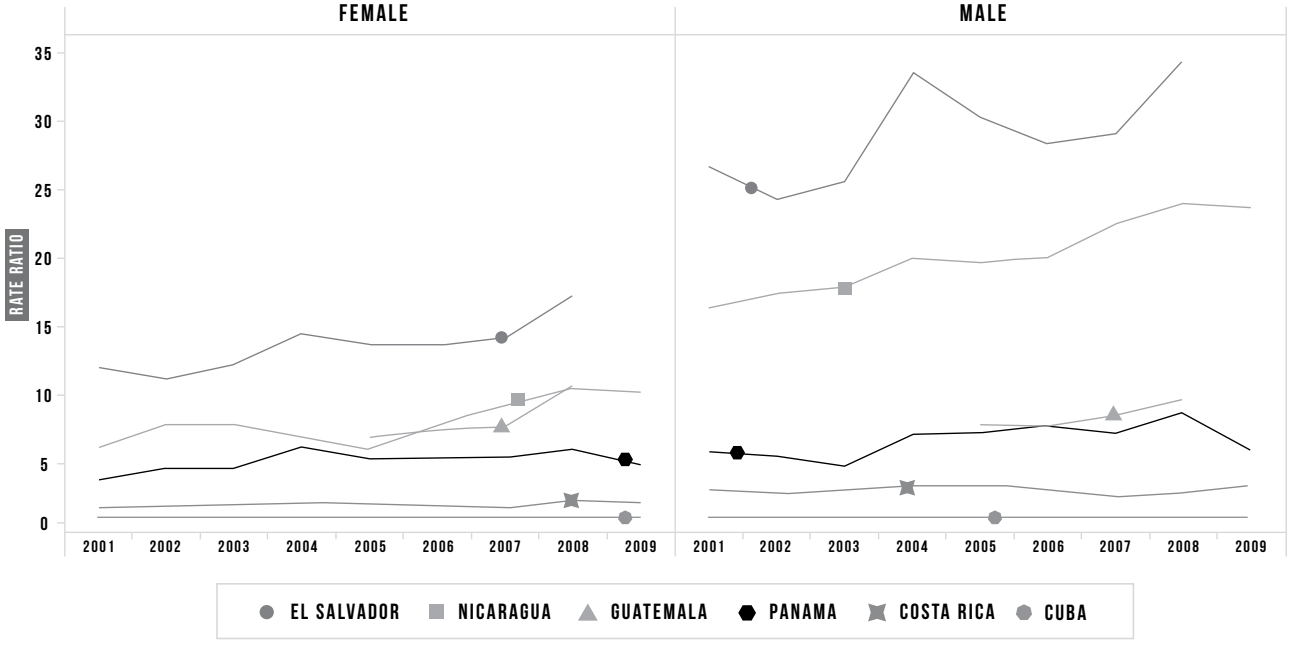


Figure 3
Chronic Kidney Disease & Unspecified Renal Failure Standardized Mortality Rate Ratios of Selected Countries vs. Better Situation (Cuba) 2001-2009



2. KNOWLEDGE GAPS, AND RESEARCH NEEDS TO IMPROVE OUR KNOWLEDGE OF THE EPIDEMIOLOGY OF CKD IN MESOAMERICA

- Population-based estimates of ESRD and CKD in general and of unknown etiology; Is incidence of CKD increasing?
- Are there higher prevalence rates at national level in all countries of Mesoamerica? Is the social gradient and geographical distribution of CKDu different than that of the CKD in general in these countries?
- How does each country define CKD and ESRD for clinical practice / epidemiological research / clinical research? (There is a need to harmonize case definition). Are there any known factors intervening on how doctors fill out the death certificate?
- Is the number of patients with CKD of unknown etiology related to lack of diagnostic capacity or to entry at the health services at later stages of the disease? There is a need to provide better estimates of people with CKD and ESRD not having access to health services.
- Are there estimates of age / sex / geographical location in each country regarding higher under-registration of deaths? Are these locations in any case coincident with the geographical concentration of CKD cases or deaths?
- Is higher CKD mortality rate in El Salvador and Nicaragua related to higher lethality of the disease in Mesoamerica? (Is higher mortality pathognomonic of MeN, or the result of the frailty of the health services? Or the result of increased individual vulnerability?)
- A time series analysis of the mortality due to CKD including a large period of time and environmental data and policies should be done in order to provide additional information related to the starting time frame of CKD in Central America.
- Are the planned, ongoing and just finished research studies in Mesoamerica seeking the *determinants* of the individual cases of CKD (and CKDu) or the determinants of incidence rates of CKD (and CKDu)?

Using Geofrey Rose's approach to the question of aetiology, "*if exposure to a necessary agent is homogenous within a population, then case/control and cohort methods will fail to detect it, as they will only identify markers of susceptibility*". The consequence for policy-making is that the corresponding strategies in controlling the disease will be the "high risk" approach that seeks to protect the susceptible individuals (screening for certain characteristics or biomarkers for example). Seeking to answer the second question, "why are the incidence rates of CKDu (or CKD) higher in this population", could inform the strategy attempts to shift the whole distribution of exposure in the population (changes in social protection, norms or legislation, for instance).

Although policies do not need to follow only one approach, Rose's questions remain relevant and are "*a challenge for those implementing public health programmes nationally and locally to consider which approach they are taking, being clear about its appropriateness and drawbacks.*"

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CHRONIC KIDNEY DISEASE OF UNKNOWN ORIGIN IN SRI LANKA

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SRI LANKA

An increase of chronic kidney disease not related to common risk factors like diabetes and hypertension, hereafter called CKDu, was first identified in the 1990s in the North Central Province (NCP) of Sri Lanka. The disease is now reported as endemic in certain geographical areas, predominantly observed in low-income male agricultural labourers and paddy farmers, and with a relatively young age at detection (1,2). Prevalence estimates of persistent proteinuria in 5-9% and up to 15% in the adult population have been published (3,4). In certain areas CKDu is claimed to be the most common cause of death (5).

Extensive national research activities have been dedicated to CKDu in coordinated programs in collaboration with the World Health Organization (WHO) (4). This includes population-based prevalence studies using proteinuria for case finding, establishment of a hospital-based CKD registry, collection of environmental samples and post-mortem studies of CKDu. A final report has recently been published. A large population-based survey performed between 2010-2012 reported a similar prevalence of persistent proteinuria in men and women, while more severe CKDu (grade 3: persistent albuminuria and eGFR<60 ml/min/1.73 m^2) was more common in men, 20% , vs 5% in women in the endemic areas (4).

A recent population-based study included subjects from an endemic area in the North Central Province and from the central and southern region (6). A majority of the cases from NCP had no known traditional risk factors, and most of them were farmers. In contrast to cases from the NCP area, almost all identified cases from the other areas presented with traditional risk factors, mainly diabetes and long standing hypertension.

Based on hospital case observations from NCP, the disease is characterized by insidious onset and tubular proteinuria, usually alpha-1 and beta-2 microglobulinuria, high urine NGal levels (>300 ng/mg creatinine and the absence of edema (7,8). The dominant histopathological observation in a renal biopsy study, including 64 cases of CKDu, was interstitial fibrosis and tubular atrophy with or without nonspecific interstitial mononuclear cells infiltration (9). Glomerular sclerosis, glomerular collapse, and features of vascular pathology such as fibrous intimal thickening and arteriolar hyalinosis were also common.

AN ENDEMIC DISEASE OF ENVIRONMENTAL ORIGIN?

The endemic occurrence and the socioeconomic pattern of disease have prompted a search for environmental risk factors, mainly focusing on heavy metals, biological risk factors, and agrochemicals.

Fluoride exposure through drinking water and tea

In several regions of the dry zone of Sri Lanka, excessive quantities of fluoride in groundwater have affected the water quality significantly and the incidence of dental and skeletal fluorosis is widespread (10). However, high fluoride levels are found in the endemic region as well as the non-endemic regions. Thus, fluoride exposure cannot explain the geographical and gender distribution of CKDu.

Cadmium exposure through drinking water and food

Cadmium (Cd) in drinking water and food was tentatively suggested as a potential cause of CKDu (11-13). The WHO research team analyzed urine Cd excretion in healthy people in the endemic area (mean 0.6, median 0.18 µg/g creatinine) and in a non-endemic area (mean 0.3, median 0.3 µg/g creatinine) (4). People with CKDu excreted significantly higher levels of Cd compared to healthy people both in the endemic and non-endemic areas. In contrast, another study did not report a difference in U-Cd between cases and unaffected relatives (9).

Hitherto no high cadmium levels have been detected in drinking water samples from the CKDu endemic areas (4,14). Cd in surface soil was higher in endemic than in non-endemic areas, and cadmium levels in tobacco and lotus were higher in endemic than in non-endemic areas (4). It is well known that rice is the most important dietary source of cadmium. However, monitoring of Cd in rice and vegetables from both endemic and non-endemic areas showed levels below allowable limits (4).

In summary, Cd exposure has not emerged as a likely explanation for endemic CKDu. It should however be noted that early signs of tubular dam-

age and effects on bone metabolism have been indicated at U-Cd levels between 0.5 and 1 µg/g creatinine (15,16). Thus, the observed Cd levels in segments of the population give small or no margins as to early effect levels.

Arsenic exposure

Arsenic (As) is a well-known toxic metal for several organ systems, but CKD has hitherto not been recognized as an adverse effect (17). Observations on abnormal pigmentation observed in palms and soles of CKDu patients were basis for the hypothesis that chronic arsenic toxicity could be a cause of CKDu (7). The study included CKDu patients (case definition however not given) and a control group. A high prevalence of hyperpigmentation and keratosis in palms and soles in the patients, but such manifestations were also found among controls, albeit less common. The levels of total arsenic in hair were higher in patients than in the control group. Arsenic in hair is mainly of the inorganic (toxic) form; thus the clinical observations and biomarker results are congruent. Arsenic levels in hair, but not in nails, were slightly higher in CKDu cases than in non-cases in the endemic area, according to results in the WHO study population (4).

U-As was also determined in both studies. The finding of higher U-As in healthy subjects in the endemic area, compared to the non-endemic area, is difficult to interpret since no separation of organic and inorganic arsenic was performed. Also CKDu patients had lowerurinary As levels. Thus these observations should be viewed with caution, as no data were provided about potential differences with regard to dietary organic arsenic compounds, which are non-toxic and may explain the findings in urine.

Water samples from 234 different sources in the endemic and non-endemic area did not reveal As above stipulated levels, i.e. <10 ug/l (4). The soil and bedrock in disease endemic areas in Sri Lanka is not rich in arsenic. The potential source of arsenic exposure has thus been attributed to agrochemicals (18).

Studies exploring not only total As but also inorganic As in urine as a biomarker are warranted to further elucidate the possible role of As. Also, the possibility of genetic susceptibility should be explored. Furthermore, analyses of As in hair and nails are notoriously problematic if external contamination is present, which is likely if occupational handling of agrochemicals (in contrast to dietary exposure) would be the source of exposure.

A new hypothesis on a possible interaction between hardness of water and As has been brought forward (19) based on observations that a vast majority of the CKDu patients had consumed hard or very hard water at least for 5 years before diagnosis. Hardness was hypothesised as a carrier of arsenic to victim's body and enhancing the toxicity by antagonistic mechanisms at cellular levels. This is a hypothesis which needs further elucidation.

Lead and mercury exposure

Lead (Pb) is a well-known nephrotoxic metal, causing acute kidney damage at high doses, and also chronic renal disease after long-term low-dose exposures (20). U-Pb levels in CKDu patients and healthy subjects in the endemic and non-endemic areas were similar (4). Information on B-Pb was not available. Similarly, no studies on mercury exposure were found.

Plant toxins

Aristolochia has been commonly used as an herb ingredient in Ayurvedic medicine. Thus, there remains a possibility of involvement of aristolochic acids in CKDu. However, if such remedies are common in the rural population and use is not differential with respect to geography or sex, an endemic disease pattern cannot be explained. The use of herbal medicines with aristolochia has been investigated, indicating limited concern for widespread oral exposure (21). Moreover, *Aristolochia indica* does not grow in abundance in NCP and contamination of paddy cultivations is reported to be unlikely (14, 22).

Mould toxins

Preliminary studies on urinary mycotoxins have shown higher levels of Ochratoxin A in CKD_u patients, but also in their non-diseased relatives compared to Japanese controls (23). This is a finding which indicates exposure, but the study sheds no light on disease etiology. Ochratoxin is a nephrotoxicant in swine (24), but the human evidence is still limited. A study on levels of ochratoxin A in food samples from the endemic area reported levels below the recommended statutory maximum limit, and concluded ochratoxin to be an unlikely potential risk factor for nephropathy in the North Central Province (25).

Ingestion of surface water contaminated by cyanobacteria

It has been hypothesized that consumption of water from surface freshwater reservoirs with pres-

ence of toxins produced by cyanobacteria could be a risk factor (26). This was based on observations of high prevalent areas clustered around reservoirs/tanks of irrigation systems, with presence of blooms of cyanobacteria, and low prevalence of the disease in communities with water from natural springs. However, the nephrotoxocity of cyanobacterial toxins in humans is not well established and it cannot explain the high prevalence of disease among groundwater consumers.

▸ *Pesticides*

The use of pesticides is widespread in agriculture, resulting not only in occupational but also para-occupational and environmental exposures in the population (4). Acute nephrotoxicity of certain pesticides after occupational or other intoxication events is well known. In contrast, there is little evidence for chronic (low)-dose nephrotoxicity of pesticides.

ONGOING ACTIVITIES

There are several groups working on CKDu in Sri Lanka. Heavy metal toxicity, genetic susceptibility and the possible role of repeated dehydration are key topics. In addition to that, two separate randomized clinical trials are being conducted to assess the renal effects of enalapril and plant-based medication in adults with CKDu grade I,II and III.

CONCLUSION

There is still little scientific evidence of the determinants of CKDu or its aetiology (27). Population-based cross-sectional studies may have underestimated the true prevalence of CKD as a positive single dip-stick test for proteinuria was used as a first step for case finding. Following the recent WHO report a thorough scientific reporting is needed for an understanding of the results of the investigations that have tried to unravel the enigmatic endemic CKDu.

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CHRONIC KIDNEY DISEASE OF UNKNOWN ORIGIN IN INDIA

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There are no comprehensive reports on the magnitude and pattern of chronic kidney disease (CKD) in India. Thus, a CKD workgroup, initially comprising of nephrologists from a few centres but gradually rising to include 188 centeres all over the country, have created an Indian CKD registry, which documents demographics, etiological spectrum, practice patterns, variations and special characteristics of CKD (1). At the end of September 2010, the Registry had received 54,813 submissions, with data collected in a standardized format according to predetermined criteria. The first report from 52,273 adult patients from the south (35%), north (28%), west (25%) and east (11%) zones, respectively confirms the emergence of diabetic nephropathy as the pre-eminent cause of CKD in India. A significant proportion (16%) had CKD of undertermined etiology. These patients were younger, had a lower income and more advanced CKD. Patients presenting to public sector hospitals were poorer, younger, and more likely to have CKD of unknown etiology. Patients in this category presented more frequently with advanced CKD, relatively short history, few symptoms until late in the disease, absent or mild hypertension and little or no proteinuria.

A few cross-sectional population-based studies have also been performed (2-5).

ENDEMIC CKD IN INDIA

There are many reports of endemic CKD in rural districts of the province Andhra Pradesh, especially the Uddanam region (6-9).

In brief, reports in media have stated that

- From 2007 to 2012, 1,520 patients from Uddanam received care for CKD from a state health insurance program for the poor. This number significantly understates the burden of a disease that is latent until it reaches its advanced, deadly stages.
- Unpublished results from a study by Harvard Medical School found that 37% of the population in the hardest hit village, Akkupalli, had the disease.
- Unlike Sri Lanka and Central America, the illness affects men and women roughly equally, according to separate findings by researchers from Harvard and Stony Brook University.
- The gender equality and geographic concentration of the illness have focused concentration on potential contamination, particularly in the drinking water.

Few scientific publications are available. We have found only one published scientific paper (10). A conference abstract with a detailed poster presentation reports a very high prevalence of decreased renal function, defined as <60 ml/min//1.73 m^2 (MRDR equation) in 188 adult men, 62% and 255women, 49 % in a cross-sectional study performed in a village with known CKDu excess in the Srikakulam district, Andhra Pradesh (11). The prevalence of proteinuria (1+ or more) was 20% in men, and 15% in women. Renal biopsies from selected patients revealed essentially normal glomeruli, variable lymphocytic peritubulitis, tubular atrophy and interstitial fibrosis, most conspicuous in the deep cortex near the junction, and nonspecific cytopathic changes in proximal and distal tubules. In three nearby farming and fishing villages, the prevalence of decreased renal function was much lower. Hypertension or diabetes did not explain the findings, and trace element analyses of water from all villages did not exceed reference values.

Thus, in contrast to findings in Mesoamerica, the prevalence of renal disease of unknown origin in the endemic area was not markedly different between men and women. The biopsy findings indicated tubular disease.

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MORPHOLOGICAL EXAMINATION OF RENAL BIOPSIES TO ASSESS AND EVALUATE NEPHROTOXICITY, AND IN PARTICULAR MESOAMERICAN NEPHROPATHY

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1. WHAT WE KNOW

In order to really understand, comprehend and diagnose kidney disease, examination of the kidney morphology is usually needed. This is usually done by microscopical examination of renal biopsies, or occasionally from tissue samples obtained at autopsy. Albeit renal biopsies are done on a routine basis in most hospitals having renal departments, it is a bit complicated, takes about one day in the hospital, is relatively expensive and most importantly, carries a small but significant risk for complications, mainly in the form of bleeding. Therefore renal biopsies are mainly taken in the clinical setting where the cause of the kidney disease is not evident and/or it is important to assess the degree of acute changes that may be treated. In the elucidation of the common cause of the infamous Balkan Nephropathy and Chinese Herb Nephropathy renal biopsies were crucial [1-4]. Relatively few renal biopsies have been obtained from persons exposed to environmental toxins such as lead [5] [6] or in cases of nephrotic syndrome in connection with exposure to inorganic mercury [7-11]. When such data are available, and have been published, it contains very important information on the nature of the kidney damage. Renal biopsies may reveal glomerular inflammation, damage with sclerosis and degeneration of the normal glomerulus, interstitial inflammation and/or fibrosis, different types of acute and chronic tubular changes or vascular lesions. A vast number of different specific types of kidney diagnoses can be set from examinations of renal biopsies, and in the clinical setting, findings often have important consequences for the treatment and long term prognosis [12].

Patients with Mesoamerican Nephropathy (MeN) usually present with low range of proteinuria and therefore a tubulointerstitial renal disease has been suspected [13-16]. However, only a limited number of renal biopsies from affected individuals have been examined, and no publications about the renal pathology of MeN have yet been published.

Endemic outbreaks of CDK of uncertain etiology in hot farming areas have also been reported from Sri Lanka [17]. In a screening program the prevalence of proteinuria was examined in 6,153 individuals from three regions; it varied from 2.3 to 9.5% . A subsequent analyses of serum creatinine from persons with proteinuria showed that a high proportion had lowered eGFR (<60 mL/min/1.73m^2) [18]. Twenty-six patients underwent a kidney biopsy procedure and they reported microscopic findings indicative of tubulointerstitial disease. In another morphological study on 64 biopsies from people in the same region a tubulointerstitial injury was suggested as well [19]. Also in Sri Lanka various environmental exposures have been suggested as causative factors, e.g. chronic dehydration and environmental pollutants [18]. However, the cases for biopsy included patients with proteinuria and hypertension and the renal biopsy findings from Sri Lanka are not readily comparable with biopsies taken from cases with MeN in Central America.

2. BRIEF OUTLINE OF IMPORTANT NEW KNOWLEDGE

To investigate the renal morphology and laboratory findings of MeNpatients, a team of clinical nephrologists, researchers and a renal pathologist from El Salvador and Karolinska Institutet in Sweden have gathered renal biopsies, urine and blood samples from eight patients with suspected MeN in El Salvador. The examined patients have all been working on sugarcane plantations. The renal biopsies were examined with light microscopy, electron microscopy and immunofluorescence, and the results show a similar morphological picture with glomerulosclerosis (29-78% global glomerular sclerosis) and changes indicating glomerular ischemia, but only minor vascular changes. In addition to these glomerular changes, chronic tubulointerstitial damage was observed. Laboratory findings show eGFR between 27-79 ml/min/1,73m² and hypokalemia was present in 6 of the 8 included patients. Urine tests show low levels of urine albumin but elevated levels of biomarkers for tubular injury [20].

The findings indicate, in contrast to what has been suggested previously, that MeN is a renal disease af-

fecting both glomeruli and tubuli/interstitium and not only a tubulointerstitial disease as it has been looked upon until now.

3. HOW TO ADVANCE? TOP PRIORITY RESEARCH AND POLICY INITIATIVES

A detailed presentation of the eight cases, with occupational and history, information on other exposures such as from NSAID, biochemical and in particular, renal morphological findings has been prepared and submitted to a peer-review journal [20]. It is important to ensure a very high quality of the report and to furnish for increased awareness and concern within the scientific community aiming at attaining more interest and funding for unraveling the cause of MeN.

However, more biopsies are needed. The patients examined so far all come from El Salvador. Do patients suffering from MeN in other countries (i.e. Nicaragua), display the same clinical and morphological pattern? All cases examined from El Salvador had chronic kidney disease (CKD) with a lowered glomerular filtration rate (eGFR varied from 24 to 60 ml/min/1.73m²). It would be of value to examine cases of suspected MeN earlier in the process as morphological abnormalities usually are more specific in the early phase of renal diseases.

A clinical description of MeN is highly desirable. At present we are in principal looking for unknown causes of a disease (MeN) which has, as yet, not been satisfactorily defined. If a clear-cut diagnosis could be set and agreed upon this would clearly enhance the further work on elucidating the etiologies.

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HOW TO ASSESS RENAL EFFECTS

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INTRODUCTION

Humans may become exposed to environmental toxic substances in many different ways. Depending on exposure type, intensity, duration and population, environmental or occupational exposures may give rise to different types of renal effects. Acute renal effects are occasionally seen, but far more commonly reported are the chronic, or long-term, renal effects. The chronic renal effects vary from discrete urinary changes with increased excretion of tubular enzymes to a pronounced proteinuria, affected glomerular filtration rate and eventually in rare cases end stage renal disease with uremia. Secondary effects on the mineral metabolism and blood pressure may also occur. Parallel with functional changes, different types of morphological changes have been observed in renal biopsies.

FUNCTION OF THE KIDNEYS AND INDICATORS OF INJURY

The kidneys have a vital role in all vertebrates controlling and adjusting the internal environment. The kidneys regulate the extracellular fluid pH, and thus indirectly also affects the intracellular pH regulation, salt and electrolyte balance, excretion of waste products and elimination of exogenous substances from food, certain toxic substances and drugs. The kidneys also have key role in the mineral metabolism, regulation of blood pressure and also have many endocrine functions including the formation of hormones necessary for the production of red blood cells. Without renal function an individual dies within a few days to a week due to accumulation of fluid, waste products and disturbed electrolyte balance.

THE GLOMERULAR FILTRATION

In humans, about 20 percent of the blood circulation, cardiac output, is via the renal arteries diverted to the kidneys. Each kidney has about a million nephrons, the kidney's functional unit, with the glomerulus (renal corpuscle) containing a tuft of specialized arteries surrounded by Bowman's capsule and tubules opening into the collecting ducts. Through the basement membrane, that connects the glomerular capillaries endothelium with capsule epithelium, a plasma ultrafiltrate (primary urine) is produced by hydrostatic pressure. The fluid flow goes from the capillaries into the Bowman's capsule and on to the proximal tubule. In proximal tubules, followed by Henles loop, distal tubules and the connecting tubule 98-99 percent of all water, salts, sugars, amino acids, etc., is reabsorbed by an active process while waste endogenous substances (such as urea and creatinine) is retained in the more and more concentrated urine. In this way water-soluble substances and proteins in plasma with a molecular size less than about 60 thousand Daltons (Da) are filtered through the glomerulus, are reabsorbed, or retained in the urine that concentrates close to 100 times in its flow from the glomerulus to the collecting ducts that finally empties into the papillary ducts and the renal pelvis.

As the amount of primary urine formed is large, about 180 L/24h or 125 mL/min, in an adult human the capacity of the kidneys to eliminate water soluble substances is impressive. This is called the glomerular filtration rate (GFR) and comprises the renal clearance for small water soluble substances such as inulin, an inert small polysaccharide molecule. For substances that are actively secreted by the tubules into the urine, for example several types of commonly prescribed antibiotics, the renal clearance may be higher than 125 mL/min whereas it for substances that are bound to large proteins in plasma, that are not filtered through the glomerulus, it may be much slower. The glomerular filtration can be directly measured with great precision after infusion of inulin or several other water-soluble small molecules that are freely filtered through the glomerulus such as ^{125}I-Iothalamate, ^{51}Cr-EDTA, and Iohexol.

There are also endogenous substances that can be used to estimate the glomerular filtration rate. The most commonly used is creatinine which is formed in muscle cells from the degradation of creatinine, but also from consumption of meat. Creatinine, which has a molecular size of 113 Da, is formed at relatively constant rate and freely filtered through the glomerulus. In the case of lowered GFR the plasma (or serum) concentration creatinine increases. Straight forward measurement and presentation of plasma creatinine is the most common way to make a crude

assessment of the renal function. If urine is collected for specified period of time (usually 24 h) the endogenous creatinine clearance can be calculated from *U-volume * U-creatinine concentration/ plasma creatinine*. This measurement of GFR has been widely used, but is not without problems, the most important is the circumstance that creatinine is not merely filtered but also actively secreted into the urine. Thus GFR calculated from endogenous creatinine clearance will thus overestimate the GFR, in particular at relatively low values of GFR.

An alternative way to estimate the GFR from creatinine is to use some sort of formula based on plasma creatinine, age, gender and ethnicity [1, 2] or age, gender and weight [3]. The latter, the Cockcroft Gault formula, has been used extensively in clinical practice since it was presented in 1976 but thorough validations have shown that the more recently developed formulas are more accurate. eGFR may become grossly erroneous in certain patients and situations, for example in the case of starvation or muscle depletion when the endogenous creatinine production is much lower than in an average person of the same age, gender and ethnicity (black or non-black). Cystatin C as another endogenous marker easy to measure can be used to estimate the GFR. In contrast to creatinine cystatin C is not influenced by muscle mass and there is no need for incorporation of age, gender and ethnicity in the formulas for estimating GFR [4] [5]. Although assessment of GFR using cystatin C has been used for many years now and increasingly so, few publications of renal effects have used cystatin C to monitor GFR.

INDICATORS OF KIDNEY DAMAGE

The most important functions of the kidneys are related to the GFR, and thus this variable is most important to assess when discussing or presenting renal effects from noxious substances such as metals. It has been shown in series of prospective population cohort studies that a decrease in the GFR translates into increased risk for not merely renal disease but also cardiovascular disease and overall mortality [6]. According to a US follow-up of more than 4,500 persons aged over 65, the risk of death (from any cause, but especially cardiovascular death), myocardial infarction and stroke increased by 10-50 percent for every decrease in eGFR of approximately 10 ml/min/1.73 m^2 and that after adjustment for a range of other risk factors such as age , sex and diabetes [7]. An increased mortality is seen already at a relatively small, and asymptomatic, decrease in the GFR. Very similar results have also been reported by other investigators [8-10]. The presence of proteinuria (measured as U-albumin creatinine index) simultaneously with reduced eGFR based on cystatin C or creatinine, increases the risk of mortality and end stage renal disease requiring treatment even more [11].

URINARY MARKERS OF KIDNEY INJURY

Apart from a change in the GFR, the most important and early marker of a glomerular damage is proteinuria, which is the hallmark of a glomerular disease. The normal urine protein excretion is less than 150 mg protein/24h of which albumin comprises about 20-30 mg. However, if the proteinuria increase and becomes abnormal it is usually the albumin proportion that increases most. In clinical practice therefore, one often measures and monitors the urinary albumin excretion. This can be measured in different ways, in a semi-quantitative way using a paper indicator stick, by quantification of the 24h urinary excretion or, which is most practical, by measuring the albumin/creatinine ratio. In normal well-functioning kidneys, the urinary excretion of albumin is less than 30 mg/g creatinine, which corresponds to a 24h excretion of about the same amount. Micro-albuminuria is defined as an albumin excretion from 30-300 mg per day (or g of creatinine) and macro-albuminuria >300 mg per day (or g of creatinine). In the case of a nephrotic syndrome, proteinuria exceeds 3.5 g/24h. Albumin has a molecular size of about 65 kDa which is above to the threshold size of the pores in the basement barrier in glomerulus. Thus elevated albuminuria indicates a damage of the basement barrier.

If the glomerular membrane damage becomes more severe, larger plasma-proteins, such as immunoglobulins, are filtered through the membrane into the primary urine. Extensive glomerular damage, with albuminuria in the order of, or exceeding 1 g/g creatinine, and often parallel occurrence of inflammatory cell cast and red blood cells in the urine, is typical for acute glomerulonephritis. The red and inflammatory cells in urine are indicative of inflammation. Often acute glomerulonephritis is also accompanied with a drop in the glomerular filtration rate. If left untreated, acute glomerulonephritis of different types may develop chronic glomerulonephritis and progress to chronic renal failure. In order to set a specific diagnose of glomerulonephritis, a renal biopsy is necessary.

Increased excretion of certain proteins in the urine may also occur from causes other than increased glomerular permeability to large proteins, having a molecular size of albumin or larger. Several smaller plasma-proteins, that have a molecular size less than albumin, are normally filtered through the

glomerular basement membrane but subsequently efficiently reabsorbed by the proximal tubular cells, degraded and brought back to the circulation. In the case of a tubular damage the tubular cells are incapable of reabsorbing the total amount of small plasma proteins and these proteins will occur in the urine and can be used as sensitive indicators of renal tubular effects. Two small plasmaproteins commonly measured in urine to detect tubular damage are β_2-microglobulin (11.8 kDa), retinol binding protein, RBP (21 kDa) and β1-microglobulin also called protein HC. As the normal reabsorption is very efficient monitoring these proteins enables detection of relatively small changes in the normal tubular function. Measuring β2-microglobulin may serve as an example; The normal plasma concentration of β2-microglobulin is around 1.5 mg/L and this is freely filterable through the glomerulus. 180 L of primary urine is formed per 24 hours, corresponding to a filtered amount of β_2-microglobulin of 270 mg. In urine the concentration normally is around 0.1 mg/L indicating that the reabsorption normally is well above 99.9% of the filtered amount. A decreased capacity in the proximal tubules from 99.0 to 99.9% will bring about a tenfold in the urinary concentration of β_2-microglobulin.

There are also others proteins that may serve as sensitive indicators of tubular dysfunction or damage. Proximal tubular enzymes and proteins, such as a-glutathione, S-transferase (a-GST) and N-acetyl-β-d-glucosamidase (NAG) and several others may be released into the urine as a direct consequence of cell injury. Measuring tubular proteins and enzymes have frequently been used to identify possible early effects from exposure to metals and in the diagnosis of acute kidney injury [12, 13].

ACUTE AND CHRONIC EFFECTS ON KIDNEYS

Environmental and occupational exposures may cause acute as well chronic renal damage. Acute kidney injury is characterized by a rapid decrease in the glomerular filtration rate (GFR) and a decreased urinary production. This is reflected by increasing plasma (or serum) creatinine. The more pronounced and prolonged, the more severe the damage. According to the **RIFLE** criteria **R**isk is defined as an increase of creatinine from the patient's baseline with 50% , **I**njury an increase by 100% and **F**ailure by 200% . This roughly corresponds to drop in the glomerular filtration rate of 25, 50 and 75% respectively. **L**oss indicates a total loss of renal function for > 4 weeks and **E**nd Stage Renal Disease (ESRD) means need for renal replacement therapy [14]. If the precipitating cause of acute injury is withdrawn, for example by reconstituting the normal renal blood flow or eliminating the noxious exposure from nephrotoxic substances such as pharmaceutical drugs or, which is the theme of this text, metals, the renal function in most cases normalizes and leave few chronic changes. However, if acute kidney injury occurs repeatedly, it has, in animal experiments been shown that it may progress to chronic effects with glomerular damage as well [15].

A system to classify the degree of renal impairment, or chronic kidney disease (CKD) has been introduced and gained general acceptance [16]. It is based on the estimated glomerular filtration rate, eGFR (ml/min/1.73 m^2), and abnormalities or markers of damage, including abnormalities in blood or urine tests (persistent albuminuria and/or hematuria) or imaging studies.

CKD stage 1 Kidney damage with normal or increased GFR, GFR > 90 mL/min/1.73 m^2

CKD stage 2 Kidney damage with mildly decreased GFR, GFR 60-89 mL/min/1.73 m^2

CKD stage 3 Moderately decreased GFR 30-59 mL/min/1.73 m^2

CKD stage 4 Severely decreased GFR 15-29 mL/min/1.73 m^2

CKD stage 5 Kidney failure GFR <15 mL/min/1.73 m^2

In clinical practice, chronic kidney disease is defined as either kidney damage or GFR less than 60 mL/min per 1.73 m^2 for 3 or more months. At stage 4 and 5 decreasing GFR will may result in a variety of metabolic changes and disturbances of fluid and mineral balance, and after some time anemia and progressive symptoms of uremia will develop. Individuals having CKD stage 3 or more have a clearly increased risk for mortality, which holds true in all age categories.

MORPHOLOGICAL CHANGES IN THE KIDNEYS

In order to really understand, comprehend and diagnose kidney disease, examination of the kidney morphology is usually needed. This is usually done by microscopical examination of renal biopsies, or occasionally from tissue samples obtained at autopsy. Albeit renal biopsies are done on a routine basis in most hospitals having renal departments it is a bit complicated, takes about one day in the hospital, relatively expensive and, most importantly, carries a small but significant risk for complications mainly in the form of bleeding. Therefore renal biopsies are mainly taken in the clinical setting where the cause of the kidney disease is not evident and/or it is important to assess the degree of acute changes that may be treated.

In the elucidation of the common cause of the infamous Balkan Nephropathy and Chinese Herb Nephropathy, renal biopsies were crucial [17-20]. Relatively few renal biopsies have been obtained

from persons exposed to metals other than lead [21] [22]. Nevertheless data from those that are available, and have been published, contain very important information on the nature of the kidney damage. Renal biopsies may reveal glomerular inflammation, damage with sclerosis and degeneration of the normal glomerulus, interstitial inflammation and or sclerosis and different types of acute and chronic tubular changes. A vast number of different specific types of kidney diagnoses can be set from examinations of renal biopsies, and in the clinical setting findings often have important consequences for the treatment and long term prognosis [23].

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ACUTE KIDNEY INJURY AND THE DEVELOPMENT OF CHRONIC KIDNEY DISEASE. ROLE OF NOVEL BIOMARKERS.

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The development of acute kidney injury (AKI) is very common in the critically ill patient and AKI may have a mortality of up to 80% in the context of multiple organ failure (1), yet it was until recently believed that most patients that had a good outcome in relation to their main cause of morbidity, would fully recover from AKI and that it was very unlikely that such a patient would progress to chronic kidney disease (CKD).

In contrast, recent observational studies have demonstrated that AKI can be implicated in the development of CKD and it is likely that this association represents causality (2,3,4). As AKI represents in general a discrete event, this relation with progression to CKD needs to be confirmed. Randomized and controlled rigorously planned studies evaluating renal function in hospitalized patient (with and without AKI) will clarify the subsequent incidence of CKD in this population and will help elucidate the potential relationship between AKI and CKD.

During the last decades, there has been a major increase of CKD worldwide, mainly due to diabetes mellitus, hypertension, and obesity, which in itself has become a major health burden. These risk factors are present in Mesoamerica in a similar way to what is seen worldwide. In addition, it was not until recently that a devastating epidemic of CKD among agricultural workers living in rural Nicaragua and other Central American countries, including El Salvador, Guatemala, and Costa Rica was described. In a few studies performed in Mesoamerica, there is mention of a wide range of "potential risk factors", including exposure to nephrotoxic pesticides, heavy metals as lead, cadmium, arsenic, ingestion of artisanal alcohol and others (5,6,7). There has been no systematic research directed to understanding the pathophysiological mechanisms behind this disease.

To date there is no conclusive evidence of any specific risk factor being the cause of this epidemic. However, the demographic characteristics of those affected allow us to emphasize that the etiology of this specific epidemic is not due to diabetes, hypertension or obesity, as the vast majority of those affected are young, normotensive, and lean male agricultural workers, mainly from the coastal, humid and very hot zones, exposed to profound repeated daily dehydration during their labor and without a background of diabetes and hypertension.

Dehydration and volume depletion is one of the major causes of AKI worldwide. As stated above, agricultural workers in the Mesoamerican region are subjected to daily severe dehydration (loss of up to 3 kg of weight after a day of labor) (8). We hypothesize that the CKD seen in this population, sometimes called Mesoamerican nephropathy, is initiated by severe recurrent dehydration and that recurrent dehydration may trigger AKI.

BIOMARKERS OF RENAL INJURY

For more than half a century, the biochemical evaluation of renal function has been based on measurements of serum creatinine and blood urea nitrogen (9). These traditional biomarkers, however, have limitations in terms of sensitivity, in particular in their ability to detect early renal injury. Serum creatinine concentration is modified by multiple factors, many of them not associated with renal injury, including gender, age, race, muscle mass, metabolism, medications, hydration status, among others. Additionally, elevation of serum creatinine is usually assessed with a significant delay after renal injury has occurred. Up to 50% of kidney function may be lost before serum creatinine begins to rise.

The identification of biomarkers for early diagnosis is of relevance to improve our capability to offer timely and effective therapeutic strategies (10). An ideal biomarker would have to provide the capability of early prediction and diagnosis of AKI (before increase in serum creatinine), identification of the primary location of injury (proximal tubule, distal tubule, interstitium, vasculature), the ability to estimate the duration (prerenal, AKI, CKD), severity, and finally the capability to identify the etiology of AKI (ischemic, septic, toxic, combination) (11). In addition, it is of great relevance to find biomarkers that can correctly stratify the extent of AKI experienced by each patient, as these individuals that suffer AKI are assumed to be at high risk of developing CKD.

When a new biomarker is identified, a process has to be performed to determine its sensitivity and reliability as a marker of kidney injury. This re-

quires several steps that include: a) animal experimental studies designed to identify those molecules overexpressed in AKI and the identification of a reliable and reproducible method to quantify the biomarker in urine samples; b) clinical studies to determine if the proposed biomarker may be measured in samples of patients with AKI earlier than what serum creatinine elevation may establish; c) clinical validation studies to determine specificity and sensitivity, and; d) population studies in different stages of the disease.

During the past decade, multiple efforts have been made to develop appropriate renal injury markers that can predict in an early and sensitive manner the development of AKI. The development of new genomic and proteomic technologies and procedures has allowed the identification of molecules in which serum and urinary concentration are modified in thepresence of AKI. These "new biomarkers" are expected to be more sensitive to detect early development of AKI. A large number of these molecules have been identified, being some of the most relevant ones: cistatin C, interleukin 18 (IL18), neutrophil gelatinase-associated lipocalin-2 (NGAL), kidney injury molecule-1 (Kim-1), N-acetyl-β-D-glucosaminidase

(NAG), liver fatty-acid binding protein (L-FABP), and heat shock protein 72 (Hsp72). (11).

Further discussion of these biomarkers is provided in the review (11).

The table below presents a comparison of biomarker performance in: early detection of AKI, renal injury stratification, pharmacological intervention monitoring, recovery of kidney injury and prognosis prediction.

References:

Table 1. Comparison of biomarkers performance in: early detection of AK, renal injury stratification, pharmacological intervention monitoring, recovery of kidney injury and prognosis prediction.

BIOMARKER	EARLY DETECTION	INJURY SEVERITY	PHARMACOLOGICAL INTERVENTION	RECOVERY	PROGNOSIS
NGAL	✓	✓	N.D.	✓	✓
Kim-1	x	✓	✓	x	✓
IL-18	✓	✓	N.D.	N.D.	✓
Cystatin C	✓/x	N.D.	N.D.	N.D.	✓
NAG	✓	N.D.	N.D.	N.D.	✓
L-FABP	✓	✓	N.D.	✓	✓
Hsp72	✓	✓	✓	✓	N.D.

Hsp, heat shock protein; IL, interleukin; Kim, Kidney injury molecule; L-FABP, liver fatty-acid binding protein; NAG, N-acetyl-β-D-glucosaminidase; N.D., not determined; NGAL, neutrophil gelatinase - associated lipocalin - 2.

Taken from: Barrera-Chimal J, Bobadilla NA. Biomarkers. 2012 Aug;17(5):385-93

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RENAL EFFECTS FROM EXPOSURE TO LEAD, CADMIUM AND MERCURY

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1. WHAT WE KNOW

In this text we will discuss documented renal effects in humans from lead, cadmium and mercury. Humans may become exposed to metals, metalloids, and their compounds in different ways. Some metals and metal compounds have been and are still used in human medicine. More often, however, exposure to metals occur from environmental and occupational exposure, which depending on exposure type, intensity, duration and population may illicit different types of renal effects. Acute effects occasionally are seen, but far more commonly reported are the chronic, or long-term, renal effects. The chronic renal effects vary from discrete urinary changes with increased excretion of tubular enzymes to pronounced proteinuria, affected glomerular filtration rate and eventually, in rare cases, end stage renal disease with uremia. Secondary effects on the mineral metabolism and blood pressure may also occur. Parallel with functional changes different types of morphological changes have been observed in renal biopsies. The number of renal biopsies from humans exposed to metals other than lead is, however, relatively few.

LEAD

Lead poisoning (plumbism) has been known for centuries. However, in order to give rise to renal functional changes, at least in adults, it appears that the exposure to lead need to be very high, high enough to cause other and severe signs and symptoms of lead poisoning. The typical morphological findings in renal biopsies, with lead intranuclear inclusion bodies, seen also in experiments in rats[1], have only been seen in humans at high, possibly ongoing, lead exposure and often in combination with concurrent high consumption of alcoholic beverages.

Goyer 1971 [1] provided an extensive review on lead and the kidney based on observations in humans and experimentally exposed animals and concludes that intranuclear inclusion bodies in the tubular cells comprise a hallmark of renal lead toxicity. These inclusion bodies are composed of a lead-protein complex containing about 5% of lead. Increased concentrations of amino acids in urine are found. Emmerson in Kidney International (1973) reviewed chronic lead nephropathy in humans [2] and summarized that granular contracted kidneys were recognized as regular features of chronic lead intoxication during the late nineteenth and early twentieth century. Renal biopsies obtained from five men with heavy occupational exposure were examined by Cramer et al 1974 [3]. Two men had lead exposure of less than one year while three men had been exposed for from four to more than 30 years. At the time of the examination blood lead levels ranged from 71 to 129 ug/dL. Renal function tests were normal in all except for a reduced glomerular filtration rate (GFR) in one worker.

It appears, however, that the diagnosis of lead nephropathy often is given based on a history of previous heavy lead exposure, often assessed by an EDTA test, in the combination of deteriorated renal function and non-specific morphological histopathological findings of chronic tubulointerstitial nephropathy and nephrosclerosis [4] also in the absence of the typical intranuclear inclusions. This inherits a clear risk for incorrectly diagnosing lead nephropathy as both lead exposure and chronic kidney disease with unspecific morphological findings are common. However, it is evident from the cited papers that intranuclear inclusion bodies are not always seen in lead nephropathy.

Baker et al 1979 [5] analyzed dose-response relationships between blood lead levels and toxic effects in 160 lead workers in two smelters and a chemicals plant. Blood lead levels ranged from 16-280 ug/dL. Clinical evidence of toxic exposure was found in 70 workers (44%), including colic in 33, wrist or ankle extensor muscle weakness in 12, and anemia in 27 and possible encephalopathy in two. Elevated blood urea nitrogen, suggestive of an effect of the GFR was noted in some of the long-term lead workers, but more specific measurement using endogenous creatinine clearance in 17 of the men with high BUN gave GFR values in the normal range, from 65 to 142 mL/min/1.73 m^2 in all but one of the examined. Urinary levels of β_2-microglobulin were elevated in two of these workers.

The same year Wedeen et al [6] presented detailed data from renal function tests and biopsies in 57 of 113 lead exposed workers with positive EDTA lead-mobilization test The GFR was measured by the clearance of iothalamate. A GFR less than 90 mL/

min/1.73 m^2 was considered evidence of renal disease. This is a relatively high cut-off value as the normal range of GFR in adults usually is considered to be in the order of 60 to 80 mL/min/1.73 m^2. Of the 21 men that displayed GFR <90 mL/min/1.73 m^2fifteen men (having GFR ranging from 52-88 mL/min/1.73 m^2) were diagnosed as having lead nephropathy as 'The diagnosis of lead nephropathy was firmly established in the cases of 15 men in which no other possible cause of reduced renal function could be identified'. Only three of the 21 patients had increased serum creatinine or BUN concentrations, and all but one excreted less than 100 mg of urinary protein in 24 hours. Renal cortex was examined from biopsy specimens obtained from 12 patients. Light microscopy was normal in six of these cases. In six, focal tubular atrophy and interstitial disease were present. The typical intranuclear inclusion bodies were not seen in these biopsies with generally rather unspecific findings of chronic nephropathy which may well have other causes than lead.

From the mid 1980-ies more than 20 cross-sectional studies on renal effects from occupational exposure to lead have been published, and remarkably few of them report any, or very limited, effects on the renal function as assessed by plasma or serum creatinine. Evans and Elinder [7] compiled and commented on all cross-sectional studies available at that time with data on GFR (or estimated GFR) from occupationally exposed individuals since 1985 and summarized that the majority of the studies had used imprecise measures of renal function, such as serum- or plasma creatinine, blood urea nitrogen, or creatinine clearance. In spite of all that has been reported on the effects from acute or ongoing excessive exposure, there were only a few studies in which the GFR was significantly reduced in any of the examined individuals. The approximate frequency of Chronic Renal Failure (GFR <60 ml/min/1.73m^2) from the observations was 1.5% , a percentage almost identical to the population prevalence of stage 3 chronic kidney disease (1.4%) among 40- to 59-year-old subjects [8].

Urinary excretion of tubular proteins and enzymes in lead exposed individuals have also been examined repeatedly during the last twenty years. Albeit there is evidence of some subtle changes, or differences as compared to controls examined at the same time, most of the studies are negative, and provide no or limited evidence that lead exposure, at the exposure levels usually encountered nowadays, with blood-lead levels around or less than 40 ug/dL, cause any renal effects [7, 9, 10]

Thus, as I see it after scrutinizing all available data, CKD is neither a common or prominent effect in adults after lead exposure. This is in contrast to what is sometimes claimed, e.g. 'lead nephropathy is a not uncommon cause of renal failure' [11] or that 'Chronic lead nephropathy is a progressive and irreversible disease'[12]. Indeed in Europe, the number of individuals with 'lead nephropathy' as a reported cause of ESRD is very low; 7 reported cases out of 143,733 patients with end stage renal disease ESRD (Kramer A. ERA-EDTA Registry, cited by Evans and Elinder 2011 [7]).

▸ *Children are possibly more susceptible to lead than adults!*

For children there may however be another story. In 1929, Nye first described a high incidence of young people with chronic nephritis in Queensland, Australia, and linked it to the high frequency of childhood plumbism in the same area [13]. In later follow-up studies, these children treated for lead intoxication showed a much higher age-adjusted mortality rate caused by the chronic nephritis compared with the general population in other areas of Australia [14]. However, the relationship between childhood lead intoxication and chronic nephritis has been difficult to confirm. A follow-up in the US of 62 children treated for plumbism in 1983 revealed no significant difference in elevation of serum creatinine, proteinuria, or blood pressure after 17–23 years compared with sibling controls [15]. Yet another follow-up study of children treated for lead intoxication during 1932–1945 showed no significant difference in serum creatinine compared with controls, but the exposed subjects had higher creatinine clearance and higher blood pressure [16]. Bernardbet al [17] reported on renal effects in 144 children aged 12 to 15 years living in a lead contaminated area in Belgium and 51 controls. The average blood lead levels in the group with highest values were 14.9 ug/dL which was almost twice that in the controls (8.7ug/dL). Several urinary variables were examined (□2-microglobulin, CC16, RBP, albumin and NAG) and RBP fell out in a dose response related manner.

From Poland Osman et al [18, 19] report on significant associations between blood lead and serum creatinine and cystatin C, indicative of a lowered GFR, and increased urinary excretion of NAG in environmentally lead exposed children. Blood lead levels ranged from 1.9 to 28.1 ug/dL. Two recent studies on adolescents show positive cross-sectional relationship between PbB and serum-cystatin C or eGFR from serum creatinine [20, 21]. Even though it has been difficult to establish that childhood lead intoxication causes renal failure, there is reason to believe that lead exposure may be more hazardous to children compared with adults, and possibly affecting the renal function.

CADMIUM

In the beginning of the 1950s it was recognized the occupational cadmium exposure may cause kidney injury with proteinuria [22]. This was shown to be of a tubular type [23, 24]. The early cadmium induced tubular damage is characterized by an increased excretion of low molecular weight plasma proteins (such as ß2-microglobulin, retinol binding protein, RBP and protein HC or alpha-1-microglobulin). Other urinary markers indicative of a tubulotoxic effect are increased excretion N-acetyl-ß-D-glucosaminidase (NAG), an enzyme localized in lysosomes of the tubular cells.

Only very few human renal biopsies from cadmium poisoned humans have been published. Bonell et al [25] described gross tubular atrophy, ischemic and degenerative glomerular lesions, interstitial fibrosis, and marked subcapsular lymphocytic infiltration in kidneys from men who died from cadmium induced uremia.

Apart from occupational exposure to cadmium also environmental exposure, usually via contaminated food, may cause the same type of tubular damage, and this has been studied extensively in Japan [26-28], but also in other cadmium polluted areas of Belgium, China, and Sweden [29-31] [.29-32]

A large number of studies have been presented showing that cadmium may cause tubular dysfunction or damage [27]. What has been, and still is, debated is the clinical significance of this tubular proteinuria, whether it aggravates when exposure ceases, if other functions of the kidney may become affected and at what exposure levels these tubular effects develop.

In almost all cases, tubular proteinuria is irreversible even if exposure has ceased [33-36]. In severe cases of cadmium toxicity, tubular injury can also lead to one or more of the following; renal glucosuria, aminoaciduria, hypercalciuria, polyuria due to decreased concentration capacity and diminished ability to handle an acid (NH_4Cl) load [26].

Glomerular effects from cadmium

Although research on the renal effects of cadmium has focused on tubular injury, such toxicity rarely gives rise to symptoms or clinical disease. More important is glomerular injury which may occur if the tubular damage is pronounced, which can lead to a fall in glomerular filtration rate (GFR) [37, 38] and eventually in some cases end-stage renal disease [39] [26]. Data from Japan shows that individuals environmentally exposed to cadmium may have a decreased GFR [40]. Uremia was a common cause of death among Japanese farmers suffering from Itai-Itai disease and has been observed in workers previously heavily exposed to cadmium ([26, 27].

A large cohort study from a cadmium polluted area of Sweden indicates an increased risk for end stage renal disease from cadmium exposure. The age standardized incidence rate ratio (SIR) of requiring renal replacement therapy over a 18 year period was significantly higher among those with increased exposure to cadmium [41]. The SIRs among those with low, moderate, and high cadmium exposure were 1.4, 1.9, and 2.3, respectively.

Renal stones

A few studies have demonstrated an increased prevalence of kidney stones among individuals occupationally exposed to cadmium [42, 43].

Dose response assessment

The relationship between the urinary excretion of cadmium and the prevalence of renal effects has been examined in several studies. Often cadmium in urine has been used as a measure of exposure. This has been practical and reasonable in most situations as cadmium accumulates in the body and in the kidney and cadmium in urine has been shown to mirror the kidney, or body, burden of cadmium. Based upon cross-sectional studies of occupationally exposed workers, it was previously suggested that 10 ug Cd/g creatinine would be a safe level below which kidney dysfunction would rarely develop [44]. A urinary cadmium concentration of 10 ug/g creatinine roughly corresponds to a kidney cortex concentration of 200 mg Cd/kg, a level long considered to be the "critical level" above which kidney damage may first be seen. Realizing the variation in individual susceptibility to cadmium, a document from the World Health Organization stated that renal tubular proteinuria would develop in about 10 and 50 percent of individuals with kidney cortex cadmium concentrations of 200 and 300 mg/kg, respectively [27].

Subsequent reports however, indicate that this approach represents a serious underestimation of the risk and an overestimate of the critical concentration of cadmium in the kidney [45]. An important and frequently cited study of the general population in Belgium revealed that a significant (up to 10 percent) proportion of the environmentally-exposed population displayed evidence of cadmium induced renal dysfunction at urine cadmium con-

centrations exceeding a level of only 2 to 3 ug Cd/g creatinine [29]. A US study of workers occupationally exposed to cadmium reported a 10 percent prevalence of tubular proteinuria among patients with urinary cadmium concentrations of 5 to 7 ug Cd/g creatinine [46]. Results from Swedish battery workers support the findings of the US study and suggest that significant renal effects (e.g. tubular proteinuria) are detected if cadmium levels are above a critical level of approximately 3 ug Cd/g creatinine [47]. Depending upon age, the prevalence of ß2-microglobulinuria was 5 to 15 percent at urinary cadmium concentrations of 3 ug Cd/g creatinine.

A review of the literature combined with an analysis of the risks from environmental cadmium exposure concluded that there is an extremely low margin of safety between current levels of exposure to cadmium and that which may cause renal toxicity [45]. If the average daily intake of cadmium is increased from 15 to 30 mcg, approximately 1 percent of the adult population would be expected to develop renal tubular damage (as detected by tubular proteinuria). In certain high risk groups, such as women with poor iron stores, the percentage affected would be even higher.

Age influences the dose-response relationship between renal cadmium levels and renal dysfunction; older individuals are more likely to suffer from renal toxicity at a given level of cadmium exposure [47].

Increased mortality among Japanese farmers with proteinuria due to environmental exposure to cadmium has been observed in several epidemiologic studies [48-50]. A 15-year follow-up of 3,178 inhabitants in a cadmium polluted area of Japan revealed a dose-related increase in the overall age adjusted mortality with increasing urinary excretion of ß2-microglobulin [51]. Persons with an initial examination showing urine ß2-microglobulin exceeding 1 to 10 mg/g creatinine had more than twice the mortality compared to those with normal values (<0.3 mg/g creatinine) [51] [52]. The elevated mortality rate, in particular, is associated with cardiovascular and renal diseases [49] [51].

Taken together, available epidemiological evidence indicates that the initial rather subtle renal effects from cadmium in the form of a tubular proteinuria eventually may develop, or at least contribute, to more severe renal diseases and increased mortality [41, 52].

MERCURY

It was recognized as early as 1818 that mercury caused proteinuria in humans [53]. Mercury is now recognized to cause the nephrotic syndrome and/or tubular injury with tubular dysfunction, with membranous nephropathy being the typical lesion seen with renal biopsy. Proteinuria disappears as exposure to mercury ceases.

Nephrotic syndrome

The use of mercurial diuretics [53] or other forms of mercury exposure [54] [55] may result in the nephrotic syndrome with varying pathologic features. Most cases that were examined pathologically revealed membranous nephropathy [53] [56, 57]. Protein excretion rates as high as 44 g/day have been reported. Many of these cases in the past were due to the use of injections of organic mercury as diuretics in patients with generalized edema. This use has now been supplanted by loop diuretics such as furosemide.

However, mercury exposure may result from certain Chinese traditional medicines and in skin lightening creams or hair dyes, and exposure may be high enough to precipitate renal effects with proteinuria [58]As an example, 11 patients (10 women and 1 man) with normal renal function had mercury exposure from such products for 2 to 60 months [58]. Urinary mercury concentrations ranged between 12 and >400 mcg/L. Three patients had nephrotic syndrome. Light microscopy revealed thickened glomerular basement membrane and mildly proliferative mesangial cells.

Renal lesions other than membranous nephropathy may result from mercury exposure. Some cases of Kenyan women who used mercury-containing skin lightening cream, for example, revealed lesions of minimal change disease rather than membranous nephropathy [59]. Two cases of nephrotic syndrome from minimal change glomerulonephritis were also reported in Hong Kong after use of a skin lightening cream containing 3 percent mercury [60]. Initially, blood and urine concentrations of mercury were in the order of 30 to 60 ug/L but decreased to normal levels after cessation of cream use and treatment with D-penicillamine.

The nephrotic syndrome may also occur in association with acrodynia or occupational exposure to mercury. In two reports, for example, the nephrotic syndrome developed in 2 of 50 workers and 4 of 72 workers exposed to mercury compounds ([61] [55]. All of these patients completely recovered within a few months after cessation of the exposure. Two cases of membranous nephropathy and nephrotic syndrome were reported from a fluorescent-tube recycling industry in Germany [62]. Heavy occupational exposure to mercury was evident from markedly elevated urinary excretion of mercury (118 and 158 mcg Hg/L, respectively). Two years af-

ter withdrawal from exposure to mercury, urinary excretion of mercury and protein was almost normalized in one patient. The second patient was treated but lost to follow-up.

Tubular dysfunction and acute kidney toxicity.

Occupational exposure to mercury is also associated with subtle abnormalities in tubular function. One study, for example, found a slightly higher prevalence of elevated urinary excretion of albumin, transferrin, retinol binding protein, and the tubular enzyme ß-galactosidase in chloralkali workers with a urinary excretion of mercury exceeding 50 ug/g creatinine [63].

These changes presumably reflected tubular injury, leading to the excretion of smaller proteins that are normally filtered and then largely reabsorbed. The tubular lesions are probably dose-related (with large doses [grams] causing acute tubular necrosis) [64]. However, mercury in a fluid metallic form does not appear to have any markedly renal effects. Winker et al [65] report on a case with IV injection of 8 g metallic Hg. No biochemical renal abnormalities were recorded in spite of extremely high urinary levels, up to 12 mg/l(sic) after treatment with DMPS (50 mg IV daily).

Analysis of the tubular enzyme N-acetyl-ß-D-glucosaminidase (NAG) appears to be particularly effective in unraveling early evidence of nephrotoxicity from mercury [66-68]; other early tubular markers have also been identified [69, 70]. In a thorough cross-sectional examination of chloralkali workers exposed to mercury at air concentrations of approximately25 ug/m^3, a significant correlation and dose-response relationship was observed between the urinary excretion of mercury and NAG [68]. No significant differences were observed between exposed and control patients with respect to other renal parameters, including urinary excretion of albumin, orosomucoid, ß2-microglobulin, copper, or in the serum creatinine concentration.

These more subtle tubular effects from mercury may be reversible. As an example, evidence of nephrotoxicity (or other adverse effects) was not observed, after extensive examination, among workers previously (average of six years) exposed to relatively low levels of mercury [71].

2. WHAT DO WE NEED TO KNOW AND UNDERSTAND, I.E. A BRIEF OUTLINE IDENTIFYING IMPORTANT KNOWLEDGE GAPS

The Boston University group has made extensive studies on exposure to several potentially toxic metals in CKD affected areas of Western Nicaragua [72]. Cadmium, arsenic and uranium were measured in urine and lead in whole blood obtained from 100 workers. Exposure as assessed by biological monitoring was generally low and well below those where nephrotoxic effects may be anticipated [73].

Thus the populations in the CKD affected areas of Western Nicaragua do not appear to have either the type of metal exposure or the exposure levels of metals necessary to induce renal effects or injury.

Furthermore, the clinical characteristics of individuals affected by Mesoamerican Endemic Nephropathy (MEN) with lowered GFR, none or limited proteinuria and history of agricultural work in hot environments [74, 75], are not similar to those reported to have renal effects from cadmium, lead or mercury [73], as discussed above.

3. HOW TO ADVANCE?

It is highly desirable to get more information on the clinical features of MEN, including renal biopsies from affected individuals, and to define an operational clinical diagnose of MEN.

Clinical findings, including possible morphological changes in the kidneys, may suggest or indicate a typical type of exposure or suggest pathophysiological pathways for the development of MEN.

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THE POSSIBLE ROLE OF ALCOHOL AND SMOKING IN RELATION TO THE CKD EPIDEMIC IN CENTRAL AMERICA

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1. WHAT WE KNOW

There is a tight association between Cardiovascular Disease (CVD) and Chronic Kidney Disease (CKD). CVD patients often develop CKD and CVD accounts for the majority of morbidity and mortality in patients with CKD[1].

It is therefore not surprising the smoking is an important risk factor for the development of CKD and mortality in CKD patients[2].

Ejerblad et al [3] analyzed smoking habits in relation to the development of CKD in a large case-control study in Sweden. Eligible as cases were native 18- to 74-yr-old Swedes whose serum creatinine for the first time and permanently exceeded 3.4 mg/dl (men) or 2.8 mg/dl (women). A total of 926 cases and 998 control subjects frequency matched to the cases by gender and age within 10 yr, were included. A face-to-face interview and a self-administered questionnaire provided information about smoking habits and other lifestyle factors. The risk for CKD increased with high daily doses of cigarettes (OR among smokers of >20 cigarettes/d, 1.5; 95% CI, 1.1 to 2.2), long duration (OR among smokers for >40 yr, 1.4; 95% CI, 1.0 to 2.1), and a high cumulative dose (OR among smokers with >30 pack-years, 1.5; 95% CI, 1.1 to 2.1). Smoking increased risk most strongly for CKD classified as nephrosclerosis , but significant positive associations were also noted with glomerulonephritis.

Orth et al (1998) in another case-control study examined to what extent smoking influenced the renal deterioration rate in 582 patients with prevalent kidney diseasewith IgA glomerulonephritis , as a model of inflammatory renal disease, and autosomal dominant polycystic kidney disease. Cases were those patients that had progressed to end stage renal disease (n=102) and controls were patients who were not in end stage renal disease or those whose serum-creatinine remained stable during the observation period. Matching for renal disease, gender, age at renal death and region of residence resulted in 102 individually matched pairs. In men a significant dose-dependent increase of the risk to progress to end stage renal disease with smoking was found; When "<5 pack-years" served as reference, 5 to 15 pack-years gave an odds ratio of 3.5 (95% CI, 1.3 to 9.6), and >15 pack-years 5.8 (95% CI 2.0 to 17). In women no effect from smoking was seen in this study. However, as was later pointed out, smoking has deleterious effects on all facets of the emergence and development of CKD; from the appearance of microalbuminuria in type 1 diabetes, the development of type 2 diabetes, progress of CKD to more severe renal failure, and survival in dialysis or after renal transplantation [2, 4].

Alcohol consumption on the other hand, and in accordance with what is the case for CVD, rather seems to be preventive with regard to the development of CKD, at least if the exposure is not excessive. Schaeffner et al 2005 [5] conducted a prospective cohort study of 11,023 healthy men. Alcohol consumption was categorized into 1 or fewer, 2 to 4, 5 to 6, and 7 or more drinks per week. The main outcome measures were elevated creatinine levels (defined as > or = 1.5 mg/dL) and reduced estimated eGFR (defined as < 55 mL/min). After 14 years, 473 men (4.3%) had elevated creatinine levels and 1296 (11.8%) had eGFR<55 mL/min/1.73 m^2. Compared with men who consumed no more than 1 drink per week, men who consumed 2 to 4 drinks weekly had a multivariable-adjusted OR of 1.04 (95% CI, 0.81-1.32), men who consumed 5 to 6 drinks per week had an OR of 0.92 (95% CI, 0.68-1.25), and men who consumed at least 7 drinks weekly had an OR of 0.71 (95% CI, 0.55-0.92). Similar associations were observed between alcohol consumption and a decreased eGFR.

An inverse association was found between frequency of drinking alcohol and CKD in apparently healthy men in a cross-sectional study from Japan [6]. 9,196 men underwent a health check-up. CKD was defined as eGFR <60 mL/min/1.73 m^2, estimated from serum creatinine. Frequency of alcohol drinking was obtained from a questionnaire and divided into five categories: nondrinkers, once or twice a week, three or four times a week, five or six times a week, and everyday drinkers. Multivariable-adjusted [age, body mass index, hypertension, diabetes, hyper-low-density lipoprotein (LDL) cholesterolemia, smoking, and physical activity] odds ratios and 95% confidence intervals (CIs) were calculated using logistic regres-

sion analysis. Compared with the results for the nondrinkers, the multivariable-adjusted odds ratios of CKD were as follows: 0.8 (95% CI 0.6-0.95) for 1-2 drinks per week, 0.7 (95% CI 0.6-0.9) for 3-4 drinks per week, 0.8 (95% CI 0.6-0.97) for 5-6 drinks per week, and 0.6 (95% CI 0.5-0.7) for everyday drinkers.

The influence of moderate alcohol consumption on renal function in elderly people was examined in an elderly Italian population [7]. The renal function, measured as eGFR from creatinine, was assessed in 3,404 persons, aged 65-84 years. The results indicate that moderate quantities of alcohol are not injurious to renal function in elderly men. Prevalence and incidence of CKD (defined as eGFR < 60 ml/min/1.73m^2) during a follow-up of 3.5 years risk of impaired renal function were estimated by sex and alcohol consumption groups. In men, both prevalence and incidence results suggest an inverse linear relationship between moderate alcohol consumption and the risk of mild renal impairment. A U-shaped association was shown for women at the incidence phase, suggesting a higher risk of developing renal impairment for women who drink more than 24 g alcohol/d.

A criticism that may be applied to all of these studies is that a lowered GFR is defined based on measurements of plasma creatinine and that the creatinine concentration is not merely a function of GFR but also the muscle mass and intake of protein rich food. One may assume that individuals with a higher intake of alcohol have less muscle mass and perhaps consume less protein rich foodstuffs, thereby forming spurious associations between alcohol and low plasma creatinine, and subsequent higher eGFR. It is nevertheless unlikely that this possible association between alcohol consumption on the one hand and plasma creatinine on the other would be able to explain the whole difference in plasma creatinine concentration in those who consume less or more alcohol.

In the context of alcohol and CKD the possible association with lead exposure from illicit 'moon-shine' liquor should be mentioned. In several reports dealing with the possible association between lead exposure and CKD there has been simultaneous exposure to alcohol and lead [8]. In fact, many of the men reported as having lead nephropathy have possibly been exposed to considerable amounts of alcohol over extended periods of time as well.

2. WHAT DO WE NEED TO KNOW AND UNDERSTAND, I.E A BRIEF OUTLINE IDENTIFYING IMPORTANT KNOWLEDGE GAPS

A nested case-control study from Nicaragua suggest that a locally brewed alcoholic drink '*Lija*' may contribute to the development of CKD in farmers living in Western Nicaragua [9]. A cross-sectional survey was conducted with assessment of medical, social, and occupational history and exposures in conjunction with measurement of serum creatinine. Cases were defined by eGFR <60 mL/min/1.73 m^2. 124 cases were compared to 873 persons without eGFR >60 mL/min/1.73 m^2. Agricultural labor was associated with an increased risk for low eGFR (OR = 2.5, 95% CI, 1.6-3.9, p < 0.0001). Consumption of unregulated alcohol '*lija*' was associated with CKD (OR = 2.10, 95% CI, 1.3-3.4), and drinking 5 L or more of water per day (OR = 3.6 vs. 1 L 95% CI, 1.5-4.5,). It was suggested that some sort of contamination of the '*lija*' could be involved in development of CKD in the area, but no specifications with regard to the content of '*lija*' was given. There may be a possibility that high reported intake of '*lija*', in the same way as a high intake of water, is an indicator of frequent and physically demanding agricultural work and heat stress rather than specific exposures from liquids.

3. HOW TO ADVANCE? TOP PRIORITY RESEARCH AND POLICY INITIATIVES

It is clear that smoking, and possibly alcohol, may affect and influence the development of CKD, and in different directions. These exposures may act as effect modifiers but cannot be the cause of MEN. In future studies information on current and previous smoking and drinking habits are useful.

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HYPOTHESIS SUMMARY· PESTICIDES

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1. WHAT WE KNOW

Exposure to pesticides (here used as a general term to include insecticides, herbicides, fungicides, and rodenticides) is one of the possible etiologic factors contributing to the epidemic of chronic kidney disease (CKD) in Central America. In fact, because agricultural workers appear to be one of the main groups affected by this disease, exposure to pesticides is a common hypothesis. However, due to the lack of quantitative exposure data, it has not been possible to evaluate the possible link between specific pesticides and CKD. This summary will therefore focus on the human studies conducted in the region that indirectly address the potential role of pesticides (using qualitative surrogates of exposure), as well as a systematic review of the published literature for 36 pesticides that have reportedly been used in the region.

› *Summary of Evidence from Studies in Central America*

The human studies conducted in Central America to date have relied on qualitative surrogate measures such as: employment in the agricultural industry (in general), employment in a particular sector of the agricultural industry (*e.g.* sugarcane, corn, coffee, etc.), employment as a pesticide applicator, and/or self-reported pesticide use.

- Torres et al. (2010) evaluated the prevalence of reduced estimated glomerular filtration rate (*ie* eGFR<60 mL/min/1.73 m^2) among 1,096 residents of five communities in Northwest Nicaragua. Among men, the prevalence was highest in the communities of miners/subsistence farmers (19%) and banana/sugarcane workers (17%) as compared to the communities where the dominant industries were fishing (10%), coffee (7%), and services (0%). Self-reported exposure to pesticides was not evaluated, though it is important to note that prevalence of CKD was higher than expected in the mining and fishing communities as well.
- Sanoff et al (2010) evaluated the prevalence of reduced eGFR among 997 residents of communities in Western Nicaragua. A significantly higher risk of reduced eGFR was observed among participants who reported a history of agricultural fieldwork (OR=2.5, p<0.0001), with significant industry-specific ORs of 1.6 (corn, p=0.03), 2.3 (sugar, p=0.0003), 3.1 (rice, p<0.0001), and 3.2 (banana, p=0.0009). Self-reported history of pesticide exposure was not a significant predictor of reduced eGFR in multivariate models, where the OR of 1.4 (p=0.14) was weaker than for history of agricultural fieldwork.
- Peraza et al (2012) evaluated the prevalence of reduced eGFR among 664 residents of five communities in El Salvador, two at sea level (predominantly sugarcane and cotton industries) and three at elevations ranging from 500-1,650 meters above sea level (predominantly sugarcane, coffee, and service industries). Among men, the prevalence of reduced eGFR was 18% in the lowland communities and 1% in the high-altitude communities. Among men in the coastal communities, the adjusted OR for 10-year increments of sugarcane or cotton plantation work was 1.9 (95% CI, 1.2-3.3). Because the work practices and pesticide use were similar in the two regions, it is important to note that decreased kidney function was observed in the sugarcane community located at sea level but not in the sugarcane community located at high altitude.
- McClean et al (2012) evaluated biomarkers of kidney injury and eGFR among 284 sugarcane workers in Western Nicaragua. Cane cutters and seed cutters were observed to have the lowest eGFR at both pre-zafra and late-zafra and experienced the largest decreases in eGFR during the zafra, as compared to workers in the other five sugarcane jobs (*i.e.* factory workers, drivers, seeders, irrigators, pesticide applicators). An increase in urinary neutrophil gelatinase-associated lipocalin (NGAL), a biomarker of kidney injury, during the zafra was primarily evident in the cane cutters, with an increase that was 19.2 µg/g creatinine higher than among factory workers (p=0.04). Biomarkers of kidney injury and CKD were consistently low among pesticide applicators as compared to the other sugarcane jobs.
- McClean et al (2012) also evaluated reduced eGFR among 51 miners, 60 construction workers, and 53 port workers in Western Nicaragua who had never worked in the sugarcane industry. In men between the ages of 20 and 59, the prevalence of

reduced eGFR was 6% among miners, 3% among construction workers, and 8% among port workers, higher than expected in a population of relatively young men. Self-reported exposure to pesticides was not evaluated as a risk factor.

- Laux et al (2012) evaluated the prevalence of reduced eGFR among 267 residents of a coffee-growing village in Nicaragua. None of the 120 men (0%) and 2 of the 147 women (1.4%) were found to have reduced eGFR. Among the men, 98.3% reported that they "work preparing/applying pesticides" and 92.5% reported that they "primarily work in the agricultural industry."
- O'Donnell et al. (2010) evaluated the prevalence of reduced eGFR among 771 residents of Quezalguaque, Nicaragua. Though the overall prevalence of reduced eGFR was high (12.7%), neither self-reported pesticide use nor occupational history were found to be significant predictors.
- Orantes et al (2011) evaluated the prevalence of reduced eGFR among 775 residents of three rural communities in El Salvador. Though the overall prevalence of reduced eGFR was high (17.9%), neither 'self-reported contact with agrochemicals' (p=0.5) nor 'history as an agricultural worker' (p=0.4) were found to be significant predictors.

These results provide evidence that the prevalence of reduced eGFR is elevated among workers in the agricultural industry. Agricultural workers likely have higher exposures to pesticides compared to non-agricultural workers, but there are many other differences that could also be important risk factors (*e.g.* exposure to other agents, heat stress, demographic characteristics, etc.). Additionally, the studies summarized above provide evidence that the prevalence of reduced eGFR is elevated among workers in non-agricultural industries as well, such as fishing, mining, construction, and port workers. Though exposure to pesticides may in fact be a risk factor, or part of a combination of risk factors, it is not possible to draw that conclusion based on the data available at this time.

▸ *Summary of Evidence for Specific Pesticides*

McClean et al (2010) conducted a review of 36 pesticides that have reportedly been used in Western Nicaragua. The report provides a detailed description of the methods and results, which are briefly summarized here. For each pesticide, information was obtained from Material Safety Data Sheets (MSDSs) and from United States government agencies such the Environmental Protection Agency (EPA), National Institutes for Occupational Safety and Health (NIOSH), Agency for Toxic Substances and Disease Registry (ATSDR), and other comparable international agencies. Information about each pesticide was also obtained via an extensive literature search conducted using the United States National Library of Medicine's PubMed electronic database. This search was designed to be broad in scope (i.e. as inclusive as possible) and was carried out in a consistent manner for each of the 36 pesticides.

Based on this review, there was no evidence that exposure to any of the 36 pesticides is associated with CKD. However, regarding the potential for an association with *acute* kidney damage, there was strong evidence of an association for two pesticides (2,4-D and paraquat dichloride) and good evidence of an association for four pesticides (captan, cypermethrin, glyphosate, and 1,2-dibromo-3-chloropropane). The herbicides 2,4-D and glyphosate are notable as they are two of the most widely used herbicides in commercial agriculture as in non-commercial applications (i.e. home and garden). The remaining 30 agrichemicals were determined to have limited or no evidence of an association.

Most of the available literature addresses acute kidney damage and not CKD. Acute kidney damage can be followed by recovery back to levels of prior function, although recent data suggest that acute kidney injury is associated with worse long term (chronic) kidney and mortality outcomes. This finding raises the possibility that, although clinical recovery has occurred, subclinical injury may remain unresolved and may predispose those affected to the progressive decline in kidney function. In other words, it is possible that repeated subclinical acute kidney injury could lead to progressive kidney fibrosis and ultimately to reduced eGFR, but this pattern of development of CKD has not been proven.

2. WHAT DO WE NEED TO KNOW AND UNDERSTAND?

Future studies that aim to investigate the possible link between pesticides and CKD should focus on improving the methods for assessing exposure to specific pesticides. Biomarkers of exposure have the advantage of quantifying total absorbed dose of specific pesticides for each individual. However, an important limitation of using biomarkers is that many of the pesticides of interest have short biological half-lives and only provide information about recent exposure. Additionally, biomarkers of exposure to pesticides are expensive to analyze.

The qualitative surrogate measures required to assess the relationship between long-term exposure and CKD are either not specific to pesticide expo-

sure (i.e. history of work in agricultural industry) or associated with extensive measurement error (i.e. self-reported pesticide use). Since we are better able to quantify short-term exposure as compared to long-term exposure, future studies of pesticide exposure should include biomarkers of acute kidney injury. Furthermore, the unproven hypothesis that repeated subclinical acute kidney injury could progress to CKD is an important area of research with respect to the epidemic of CKD in Central America, particularly with respect to investigating the potential role of pesticide exposure. Prospective studies in which exposure is measured and changes in kidney function assessed, particularly long-term studies, would be particularly valuable but expensive to undertake. Additionally, such studies would possibly be subject to Hawthorne effects, a concern that should be considered during the design phase.

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POSSIBLE ASSOCIATION BETWEEN *LEPTOSPIRA* INFECTION AND CHRONIC KIDNEY DISEASE OF UNKNOWN ORIGIN IN CENTRAL AMERICA

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Chronic Kidney Disease of Unknown Origin (CKDu) has emerged as a major public health issue in several Central American countries. It is characterized by chronic kidney disease (CKD) apparently of non-glomerular origin that disproportionately affects young male agricultural workers. Although its cause has yet to be determined, several hypotheses exist, including heat stress, agrichemicals, heavy metals, aristolochic acid, nephrotoxic medications, contaminants in locally distilled alcohols, genetic susceptibility factors and infectious diseases.[1-3]

There are multiple infectious diseases that are associated with some form of renal involvement. Nevertheless, for an infectious disease to be associated with CKDu, several factors have to be present simultaneously, including a high incidence of the disease in affected regions; similar demographic characteristics in high risk groups; there should not be a discrepancy between the overt clinical findings caused by the infectious disease and those seen in CKDu; a pathophysiologic mechanism must be shared, that is the infectious agent must cause tubulointerstitial disease like the one seen in CKDu; and the infectious agent must cause or increase the risk of developing CKD. For instance, post-streptoccoccal glomerulonephritis, subacute bacterial endocarditis, human immunodeficiency virus (HIV), hepatitis B and C, syphilis, leprosy, malaria and schistosomiasis can cause renal dysfunction; while the pathophysiological mechanism varies, it usually involves an immune response that injures the renal glomeruli.[4-14] Chronic pyelonephritis is associated with renal insufficiency, but urinary tract infections in men before the age of sixty are extremely rare unless there is an underlying anatomic abnormality.[15] *Streptococcus, Legionella, Salmonella, Yersinia,* Epstein-Barr virus, cytomegalovirus, HIV, and *Mycoplasma* infection can cause acute interstitial nephritis (AIN); however, it's a rare manifestation of disease, extrarenal findings are usually present, and renal function usually recovers.[16-22] Reactivation of polyomavirus BK often causes AIN, but this mainly occurs in immunocompromised patients, particularly renal transplant recipients.[23] Hantaviruses are rodent-borne viruses that cause two important clinical syndromes: hemorrhagic fever with renal syndrome (HFRS) in Europe and Asia, and hantavirus cardiopulmonary syndrome (HCPS) in the Americas. Nephropatia Epidemica is a milder form of HFRS caused by a particular hantavirus named Puumala virus, and manifests as AIN. Its association with CKD is controversial. Puumala virus is found in Scandinavia and in parts of Europe west of the Ural Mountains.[24-34] Even though the possibility of a non-identified strain of hantavirus in Central America that produces asymptomatic infection and renal disease exists, there is currently no evidence to support this hypothesis. Therefore, the disease entities mentioned above are unlikely to be associated with CKDu. *Leptospira* is not thought to cause CKD, however it merits further discussion because it is common in regions affected by CKDu, it disproportionately affects male agricultural workers, subclinical infection is common, it causes AIN in humans, and can cause chronic kidney injury in other mammals.

CURRENT KNOWLEDGE

Leptospirosis is considered a re-emerging infectious disease, and is probably the most common bacterial zoonosis worldwide. The global burden of disease is difficult to quantify due to the non-specific clinical symptoms and the lack of efficient confirmatory testing. It is caused by pathogenic spirochetes of the *Leptospira spp.* genus. Typically, leptospires are classified into serovars based on their antigenic determinants. More recently, a classification based on molecular phylogenetic analysis divides *Leptospira* genus into several species. Human infection occurs after contact with water or soil contaminated by the urine of animal reservoirs. Although a variety of mammals can acquire infection, the most important sources of transmissions to humans are rats, dogs, cattle, and pigs. Human-to-human transmission is only thought to occur rarely. Endemic infection is common in tropical humid environments with poor sanitation, with the occurrence of epidemic outbreaks during the seasonal rains. Risk of transmission is highest among individuals with occupations that put them in contact with surface water, moist soil, farm and domestic animals; inhabitants of poorly hygienic urban centers subject to seasonal flooding; recreational

outdoor activities; and military training in endemic regions.[35-45]

Leptospires infect humans through the mucosa or non-intact skin, proliferate and disseminate hematogenously to all organs. The incubation period ranges from 2 to 30 days. Some species and serovars are thought to be more pathogenic than others. The clinical manifestations of disease vary in intensity, and include subclinical infection, an undifferentiated febrile illness, or the most severe form characterized by jaundice, acute kidney injury (AKI), and potentially lethal pulmonary hemorrhage.[35-37, 40] Men are affected more often by clinical disease than women. Due to the protean manifestations, the diagnosis of leptospirosis demands a high clinical suspicion. Definitive diagnosis by laboratory testing requires demonstrating the presence of the organism by culture isolation, detection of nucleic acids or antigens in body fluids, or immunohistochemical visualization in tissue. However, since cultures do not become positive for weeks and polymerase chain reaction based assays are expensive limiting their availability in resource constrained settings, serologic assays have remained the mainstay in leptospirosis diagnosis. Among these tests, the gold standard is the microscopic agglutination test; in which live antigen suspensions are titrated with patient's sera and then inspected microscopically for agglutination.[35, 37] It's generally believed that after infection, antibodies are protective as long as the concentrations are high enough, so reinfections can occur after antibody titers decline. Furthermore, antibodies provoked by an infection with a particular serovar do not necessarily protect against infection with other serovars.[36, 37]

Data from epidemiologic studies, national health ministries and other public health organizations have shown the Caribbean, Central and South America, as well as Southeast Asia and Oceania to be highly endemic for the disease. Central American countries with high incidence of leptospirosis include Costa Rica, particularly in the central-south Pacific region of Brunca, and in the Huetar Atlantica and Huetar Norte regions; Nicaragua, particularly León and Chinandega in the Pacific region, the southern regions of Río San Juan, the northern regions of Jinotega and Nueva Segovia, as well as Estelí; and El Salvador. The lower incidence of the disease in Panama, Guatemala, and Honduras, may represent inadequate surveillance systems.[46, 47] Due to underreporting, the common occurrence of subclinical infection that goes unrecognized, and the undifferentiated febrile illness that is frequently misdiagnosed, accurate data on the incidence, mortality and morbidity is limited, and is often based on hospital series. Epidemiologic studies suggest that exposure to leptospires among the population in Central America is significant, particularly in rural areas. For instance, in Nicaragua, an unpublished study conducted during a non-outbreak setting in the municipalities of El Sauce and Achuapa by the Centers for Disease Control and Prevention (CDC) and the Ministry of Health in Nicaragua, showed a high exposure to leptospires with seroprevalence as high as 42% (32.3% among women and 55.2% among men), and asymptomatic acute infections in 4 to 7% .[48]

Renal involvement is a prominent feature of both mild and severe cases of leptospirosis. The life cycle of the bacteria is completed as leptospires traverse the interstitial spaces of the kidney, penetrate the basement membrane of the proximal renal tubules, cross through proximal renal tubule epithelial cells, and become adherent to the proximal renal tubular brush border. Interstitial nephritis is the primary renal pathophysiologic lesion in acute leptospirosis. There is increased distal tubular potassium excretion, hypokalemia, hypomagnesemia and polyuria due to abnormal regulation of fluid and electrolyte transporters. Acute tubular necrosis can develop in severe cases. Generally, normalization of plasma creatinine and blood urea nitrogen occurs in the second week of disease.[49-51]

Infection of a variety of mammalian hosts, including dogs, rats, cattle, pigs and raccoons, can lead to a chronic disease process when leptospires successfully evade the immune response and colonize renal tubules. Tubulointerstitial nephritis as seen in chronically infected animals may progress to tubular atrophy and renal fibrosis.[52] Although an association between leptospirosis and chronic renal colonization or development of CKD in humans has not been shown, this is an area where research remains scant. In a study conducted in Brazil in 1963, renal biopsy specimens obtained between the eleventh and fourty-fifth day of illness in 13 patients with leptospirosis showed evidence of tubulointerstitial damage even in cases with mild to moderate renal impairment. It is worth mentioning that in this study, there was evidence of recurrent interstitital nephritis as indicated by the fact that active and regenerative aspects were seen side by side.[53] Renal colonization by leptospires can last for weeks to months, possibly even years, with unknown pathophysiologic consequences. A study exploring the use of a polymerase chain reaction assay in urine as a diagnostic tool for leptospirosis, found long-term shedding in samples collected more than one year after acute infection.[54] Another recent study in a rural Amazonian community in Peru screened urine samples for the presence of leptospiral nucleic acids, finding that almost 5% of healthy people were urinary shedders but did

not have serological evidence of recent infection, suggesting chronic, asymptomatic renal colonization by leptospires.[55] Furthermore, only a few small studies have evaluated renal recovery after leptospirosis. In one study that followed 35 patients with acute infection hospitalized with AKI, renal recovery was complete at six months except for urinary concentration capacity.[56] Another study followed patients hospitalized with AKI for three months; among the 58 patients included in the study, at 90 days of follow-up, 10.3% had persisting mild renal insufficiency, and 19% , despite normalization of their serum creatinine had continued tubular dysfunction.[57] These studies suggest that although normalization of serum creatinine occurs in the vast majority of patients, tubular dysfunction can persist at least for several months after infection.

KNOWLEDGE GAPS

A link between leptospirosis and CKD represents an evidence gap, mainly stemming from either lack of research or study design flaws, primarily the small number of subjects studied and the relatively short follow-up of cases considering the long latency expected for CKD to develop. The epidemiology of leptospirosis and CKDu have similarities, which include the population at risk, mainly young male agricultural workers, and the geographic distribution,[46-48, 58, 59] making the association between leptospirosis and CKD a limitation in the research base that needs to be addressed. Since leptospirosis usually presents as a subclinical disease, or an undifferentiated febrile illness that is frequently unrecognized or misdiagnosed,[35, 38, 61] and exposure to the organism begins at an early age, with several studies showing large number of cases occurring in children,[40, 60] it is very likely that a large number of individuals living in endemic areas have experienced repeated infections. Therefore, it is of particular importance to explore whether recurring mild or subclinical leptospirosis could lead to multiple episodes of AIN, resulting in progressive kidney fibrosis and ultimately CKD.

PRIORITY RESEARCH INITIATIVES

Stakeholders have to determine after a prioritization process if leptospirosis is one of the main future research needs regarding CKDu. This includes the appropriateness of the research when viewed against competing hypothesis, and the likelihood of there being an association between CKDu and leptospirosis. Data from Nicaragua show that the departments of León and Chinandega are the most affected by both CKDu and leptospirosis.[47] Although caution is warranted in interpreting the data since no national surveillance system for CKD exists, and information comes from CKD mortality ascertained by death certificates; and leptospirosis incidence is biased towards areas with the most dramatic presentation of disease -pulmonary hemorrhage-; the similarity between the departments affected by CKDu and leptospirosis is striking. Currently, there is a pilot study being conducted in Nicaragua by Boston University School of Public Health in conjunction with the CDC, exploring if an association exists between leptospirosis and markers of renal tubular injury among workers in sugarcane and other industries. From a total of 489 workers included in the study, 282 had serial samples drawn at baseline and six months later, allowing for comparisons between exposure to leptospires and markers of renal tubular injury before and during the harvest season. Preliminary results show a high exposure to leptospires among sugarcane workers overall, particularly sugarcane cutters, and individuals that applied for a job but were not initially hired due to an abnormal creatinine upon screening. Both of these groups show a high percentage of acute and recent infections. Other job categories with high exposure to leptospires include irrigators and pesticide applicators. Among other industries, miners are the most affected group. Correlation between exposure to leptsopires and renal tubular markers is still not available. Hopefully, once the study is finalized, results will help determine the appropriateness of leptospirosis research.

Another area of importance in the prioritization process is that of feasibility. The design of a high-quality study to explore the association between leptospirosis and CKDu has to overcome several obstacles. Seropositivity among the population at risk is high given the common exposure to leptospires, demanding serial testing to determine if repeated infections are occurring. In addition, long term monitoring of renal function and markers of tubular injury would be needed in order to allow sufficient time for chronic sequelae to arise. A prospective study of this nature would be resource-intensive, demand several years of follow-up, and would be difficult to conduct. It is important to determine if a study with these characteristics is justified, as well as analyzing alternative study designs -like a case-control study- based on their strengths, limitations, potential to reduce bias, and feasibility.

In summary, leptospirosis is a common infectious disease with epidemiologic characteristics similar to CKDu. It is well known to cause renal involvement, and although it has not been proven to cause CKD in humans, biologic plausibility exists. A link between leptospirosis and CKD represents an

evidence-gap due to lack of research and limited data on long-term outcomes in existing studies. An association between leptospirosis and CKDu is possible, and a prioritization process is required to determine the appropriateness and feasibility of conducting research in this area, which is important, desirable and with high potential impact given the major public health problem that CKDu represents.

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BALKAN NEPHROPATHY, CHINESE HERBS NEPHROPATHY, ARISTOLOCHIC ACID NEPHROPATHY AND NEPHROTOXICITY FROM MYCOTOXINS

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1. WHAT WE KNOW

Balkan endemic nephropathy (BEN) is a chronic tubulointerstitial nephritis affecting residents of rural villages located in valleys near tributaries of the Danube River. Although this endemic nephropathy was first described in the literature in the late 1950s, anecdotal reports and church records suggest that the disease was present in these countries many decades before (Djukanovic and Radovanovic 2008).

Fig 1: Map depicting discrete pockets of endemic nephropathy and associated upper urinary tract carcinoma in rural farming villages in Croatia, Bosnia-Herzegovina, Serbia, and Romania

Taken from: Grollman 2013

The most striking aspect of the endemic is its discrete, stable geographic distribution. For the past 50 years, no new endemic foci have emerged, nor have any known endemic areas become non-endemic (Batuman 2006). There is a marked focal occurrence, with differences not only between but also within farming villages, with unaffected and affected families living in close proximity. Men and women are equally affected, and BEN is nowadays usually not manifest before age 60. The familial pattern of disease does not follow a Mendelian heritage. There is a strong association with upper urinary tract transitional cell (urothelial) cancer, which affects up to 50% of BEN patients (Batuman 2006; Grollman and Jelakovic 2007; Djukanovic and Radovanovic 2008).

The clinical characteristics of Balkan nephropathy include tubular proteinuria (e.g. β_2-microglobulinuria), glucosuria, tubular acidosis, impaired concentrating capacity, aseptic leucocyturia, a decreased kidney size, anaemia and progression to end-stage renal disease. In contrast to many other chronic kidney diseases, there is an absence of hypertension and oedema. The histopathologic findings from renal biopsies are typical and include hypocellular interstitial fibrosis, tubular atrophy, limited interstitial infiltrates and relatively mild glomerular changes (de Jonge and Vanrenterghem 2008).

A variety of environmental factors that may be causative have been explored during the past 50 years, including heavy metals, polyaromatic hydrocarbons, viruses, and trace elements, withexposure to the mycotoxin ochratoxin A (OTA) being the focus of research for many years up to 2007, when it was shown that aristolochic acid (AA) indeed is the causative toxin (Grollman, Shibutani et al. 2007) (de Jonge and Vanrenterghem 2008) (De Broe 2012) (Grollman 2013). The hypothesis that a nephrotoxic and carcinogenic plant alkaloid derived from Aristolochia species could be a potential aetiologic factor for BEN was formulated as early as the 1970s, based on epidemiologic, environmental and agricultural research. At that time observed that the *Aristolochia clematitis*, a plant native to the endemic region, often grows in cultivated fields where its seeds containing AA co-exist with wheat grain during the annual harvest. As bread, the dietary staple of the region, is prepared traditionally from

flour made from locally-grown wheat, residents of the endemic region consuming homemade bread, may over time, be exposed to toxic amounts of AA.

The unraveling of the enigma of Balkan nephropathy came from a thorough exam of another epidemic of chronic kidney disease that co-occurred with urothelial malignancies; the **'Chinese herbs nephropathy.'** In 1993, there was an outbreak of a rapidly progressive tubulointerstital kidney disease in Belgian women who had followed a slimming regimen in a medical clinic in Brussels (Vanherweghem, Depierreux et al. 1993). Since 1990, the prescribed slimming pills contained two herbs imported from China, and therefore the disease was initially named as 'Chinese herbs nephropathy'. Later investigations clearly showed that the slimming pills contained AA, probably because one of the prescribed herbs had been inadvertently substituted by *Aristolochia fangchi*. The detection of AA-specific DNA adducts in kidney and urinary tract tissues from patients with 'Chinese herbs nephropathy' and Balkan nephropathy unambiguously demonstrated exposure to AA (Grollman 2013). Balkan nephropathy and Chinese herbs nephropathy are now properly being referred to as **aristolochic acid nephropathy**.

Aristolochia species have been used widely for more than 2000 years in Chinese and Ayurvedic medicine (*traditional Indian medicine*) as well as in Europe, Latin America, and the United States. The earliest mention of Aristolochia in China dates to the fifth century. Although banned in many countries, Aristolochia species continue to be used as a component in traditional herbal remedies; as a result, aristolochic acid nephropathy represents a global health problem of considerable magnitude.

Recently Grollman (Grollman 2013) adopted the molecular epidemiologic approach that had been used in elucidating the cause of Balkan and Chinese herbs nepropathy, with aristolactam (AL)-DNA adducts in the renal cortex as a biomarker of exposure, in patients with upper urinary tract (UUC) cancer in Taiwan, the country with the highest recorded incidence of urinary tract cancer in the world. The analyses showed that a majority of the patients had similar mutations and AL-DNA adducts as observed in patients with Balkan and Chinese herbs nephropathy. Aristolochia herbal remedies are extensively used in Taiwan, and it has been estimated that approximately one-third of the population have been exposed to herbs containing, or likely to contain, AA.

Parallel with increasing, and eventually, totally convincing evidence that aristolochic acid is the cause of Balkan and Chinese Herbs nephropathy, the evidence for **mycotoxins** as a cause of nephropathy has decreased. Nevertheless the mycotoxin ochratoxin A (OTA) has been linked to the genesis of several disease states in both animals and humans. It has been described as nephrotoxic, carcinogenic, teratogenic, immunotoxic, and hepatotoxic in laboratory and domestic animals (O'Brien and Dietrich 2005). OTA and other mycotoxins may be formed by moulds, mainly by *Aspergillus ochraceus* and *Penicillium verrucosum*, when corn and other mainly animal feeds/foods are kept for long periods of time in humid and warm storage. In particular, pigs and chickens appear to become affected, but large species- and sex-specific differences exist. The kidneys taken from pigs at slaughter with mycotoxic porcine nephropathy are enlarged and mottled, or enlarged and pale. (Stoev, Dutton et al. 2010). Microscopical examination of kidneys from slaughtered pigs with suspected mycotoxin nephropathy in Bulgaria showed 'degenerative or atrophic changes affecting the proximal convulated tubulus and proliferative changes in the interstitium'(Stoev, Hald et al. 1998).

In North African countries, the most suspected food susceptible to be contaminated by OTA is domestic cereals like sorghum and wheat, olives, spices and imported cereals. In Tunisia, the presence of OTA in a large number of frequently consumed foods have been found, and a chronic interstitial nephropathy (CIN) of unknown etiology with many similarities to BEN has been described. The interstitial nephropathy is slowly progressing and is a leading cause of terminal renal failure and death in the fourth or the fifth decade of life. Studies have repeatedly shown high rates of relatively higher blood OTA contamination among CIN of unknown etiology nephropathy patients compared to controls, and likewise in their typical food. (Hassen, Abid et al. 2004). (Hmaissia Khlifa, Ghali et al. 2012). However, a recent study did not find any association between OTA in serum and kidney-effect markers in 150 women from rural, industrial and urban areas in Morocco (Zizi et al 2010) cited in (Skerfving 2006-2011).

Although there are large differences between species in the susceptibility to OTA and also the analytical measures used, it is worth noting that the levels of OTA recorded in blood from humans with suspected nephrotoxicity from OTA are similar to those reported from pigs with mycotoxin nephropathy (Stoev, Dutton et al. 2010). However, the studies of OTA nephrotoxicity in humans are mainly based on cross-sectional studies of patients and healthy controls. Assessment of exposure to OTA has been crude, and mainly based on measured OTA in blood which may be confounded by the renal function; thus a reverse causality cannot be ex-

cluded. Therefore, in spite of well-established nephrotoxicity from OTA in pigs and chickens (Krogh, Axelsen et al. 1974), the possible role from OTA in the development of CKD in humans is, as yet, not very well established.

2. WHAT DO WE NEED TO KNOW AND UNDERSTAND?

Is there any evidence that exposure aristolochic acid (AA), or ochratoxin A (OTA), are in any way involved in the development of MeN in Central America? The absence of urotelial cancers speaks against AA as a main risk factor in MeN. Likewise, the marked gender difference in MeN clearly speaks against dietary exposure from AA contamination of food as a risk factor. However, a gendered use of herbal medications with AA, predominantly in certain occupational segments could, in theory, be consistent with the MeN observations, alone or in combination with other risk factors.

3. TOP PRIORITY RESEARCH AND POLICY INITIATIVES?

Although it does not seem likely that MeN is caused by AA or mycotoxins, there are some research questions that might be worth exploration, although not of top priority.

It would be of value to assess whether inhabitants living in MeN-affected villages are exposed to aristolochic acid from herbal medicines or contamination of food from the plant *Aristolochia clematitis* or similar local varieties. If this is commonly occurring, there is a clear reason to give advice against such use, which may aggravate or potentiate other risk factors.

If AA unexpectedly would emerge as a potential risk factor, renal biopsies from individuals suffering from MeN would be very informative as individuals with aristolochic acid nephropathy (AAN) display a typical histopathological pattern. Moreover, aristolactam (AL)-DNA adducts in the renal cortex can be used as a biomarker of exposure.

Exposure to OTA, or other types of potentially nephrotoxic mycotoxins being involved in the pathogenesis of MeN, might be more difficult to completely rule out. First, it should be explored whether food contamination with mycotoxins is commonly occurring in regions where MeN is prevalent, but not in regions with low prevalence of MeN. Renal biopsies in patients with MeN would also be informative, if these display pronounced tubular cell damage and interstitial inflammation it would be compatible with nephrotoxicity from mycotoxins.

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IS HARD WATER AN ETIOLOGICAL FACTOR FOR CHRONIC KIDNEY DISEASE OF UNKNOWN ORIGIN?

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WATER HARDNESS

Hardness of water is caused mainly due to presence of cations, Ca^{2+} and Mg^{2+} together with anions carbonates, bicarbonates, sulphates and chlorides. Temporary hardness is caused by the presence of dissolved calcium and magnesium bicarbonate minerals whereas permanent hardness is caused by sulfate and chloride compounds. Temporary hardness can be reduced either by boiling the water or by the addition of lime but permanent hardness cannot be removed by boiling. According to WHO apparently there is no convincing evidence to the effect that water hardness causes adverse health effects in humans. Some studies have shown a weak inverse relationship between water hardness and cardiovascular disease in men, when water contains up to a level of 170 mg calcium carbonate per litre of water. According to the WHO guidelines given in 2009, water hardness classified as soft 0-60mg/L, moderately hard 61-120 mg/L, hard 121-180 mg/L and very hard >181 mg/L.

SRI LANKAN AGRICULTURAL NEPHROPATHY (SAN)

SAN has emerged as an important public health problem in rice cultivating areas in the dry zone of the Sri Lanka especially in north central province. (Fig 01) One common finding is that villagers who happen to drink water from wells with very hard water have been affected with SAN. Information on groundwater hardness obtained from the Water Resources Board (Fig.02) presents extends of hardness in groundwater resources of Sri Lanka. Comparison of distribution of prevalence of SAN with groundwater hardness appears to have a strong positive correlation with each other. Nevertheless it was also observed that areas with hard groundwater such as in Jaffna peninsula which is also an agricultural area did not report to have SAN patients.

SOIL

Soils differ in their physical, chemical and biological properties and particularly in capacity to retain cations and anions. Arsenic retention by soils has widely been studied (Smith et al.1998) and secondary data available on soil types of Sri Lanka were examined from the data obtained from the Survey Department (Fig.3). SAN endemic areas are predominantly covered with Reddish brown earths and low humic gley soils and they are inherently with high capacity of retaining ions, metals and metalloids, especially arsenic (Smith et al.1998,Violante and Pigna 2002). Visual comparison of distribution maps of soil types, groundwater hardness and SAN prevalence provide strong evidence that these three factors show significant correlation among them. Difference of soil types in the SAN endemic areas and the ones mentioned above was conspicuous and the metal/ ion holding capacity of soils in SAN endemic area is significantly greater than the soils in other areas of the country, indicating metals/ions are retained well in soil of the SAN endemic areas.

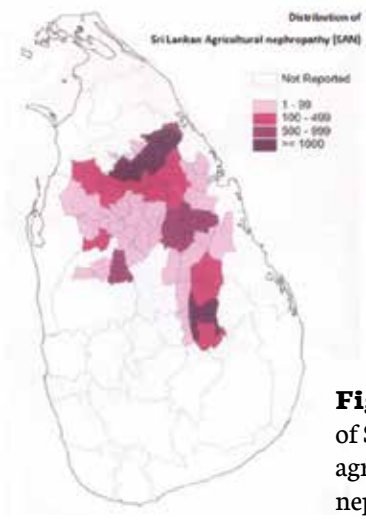

Fig. 1: Distribution of Sri Lankan agricultural nephropathy (SAN)

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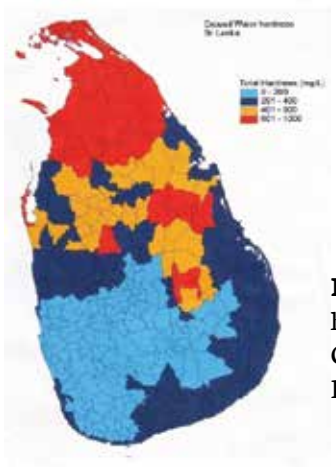

Fig. 2: Distribution of hard water in Sri Lanka Courtesy: Water Resources Board, Sri Lanka

Fig. 3: Distribution of Soil types in Sri Lanka Courtesy:Survey department,Sri Lanka

A study was conducted during 2011, to determine the hardness of drinking water source of SAN patients in Padavi-Sripura (n=78), Kebithigollawa (n=38), Mahawilachchiya (n=33) Polpithigama (n=48) in the dry climatic zone and compare them with that of the drinking water sources in Gampaha (n=35), Kandy (n=32) and Deniyaya (n=28) ,which are located in wet zone and where SAN is not prevalent. Total hardness of the water samples was measured using EDTA titration (EPA 130.2) method and calcium content was measured using flame AAS.

Highest average hardness (496±34 mg /L) was observed at Padavi-Sripura and the values ranged from 110±54–1120±62 mg/L. The second hardest water was found from Polpithigama area which ranged from 70±8 – 715±47 mg/L. The lowest hardness among the test sites was observed at Kandy (n=38), where the hardness ranged from 08±2 –220±21 mg/L. Calcium levels in the water samples taken from wells that CKDu patients have used for drinking purposes were detected at an average concentration of 183.2 mg/L, range of 32±4 - 398±28 mg/L. Average concentration of 13.6 mg/L in the range of 2.1±0.8 - 42±14 mg/L has been detected from the water samples collected from Gampaha, Kandy and Deniyaya. Hence the results revealed that most of the water samples tested in SAN prevalence areas contain very hard water.

Our finding also revealed that hardness of bottom layer of water in the wells was gradually increased with depth and presence of significantly high levels of Fe^{3+} and Mn^{2+} ions in hard water samples. These levels exceed WHO prescribed values for drinking water. The results substantiate the general complaint by the villagers in the study area that the quality of water is unacceptable for drinking.

A statistically significant positive correlation ($P < 0.008$) was revealed between occurrence of SAN and hard water consumption. 96% of the SAN patients have consumed hard or very hard water at least for 5 years before diagnosis of the disease.

KNOWLEDGE GAPS

Though we have noticed a link between ground water hardness and prevalence of CKDu current scientific knowledge does not explain the situation well.

Possible explanations are,

- Hardness may retain comparatively high amount of nephrotoxic substances and may act as a carrier of these toxic substances to victim's body.
- Hardness may improve the absorption of heavy metals in GI tract.
- Hardness may enhance the heavy metal toxicity by various antagonistic mechanisms in cellular levels.

Although it is believed that hardness alone does not cause any damage to kidney, it may become hazardous in the presence of nephrotoxic metals.

TOP PRIORITIES

It is essential to divert more resources to consolidate these findings and to plan and implement strategies to analyze drinking water sources and soil especially in other part of the world where epidemics of nephropathies have been observed. If hard water is identified as a common risk factor the exact mechanism of action should be explored.

POLICY LEVEL

In Sri Lanka, the government has already decided to supply soft water to the people who are living in SAN endemic areas as a precautionary measure.

This decision followed a statement made in the 3rd progress report submitted by WHO expert panel on CKDu in Sri Lanka headed by Prof. Shanthi Mendis (WHO-Geneva) to the ministry of health Sri Lanka.

"Water from 98 water sources used by patients with CKDu was analyzed for hardness. 99% are hard to very hard. Hardness of water is known to affect heavy metal toxicity through antagonistic mechanisms and this may play a role in renal toxicity caused by heavy metals in the north central province."

Central American region is sharing similar geo environmental conditions with Sri Lanka which are common to tropical countries. Hence findings from Sri Lanka may help to improve knowledge, plan research and take policy decisions related to Mesoamerican Epidemic of Nephropathy.

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HYPOTHESIS SUMMARY· ARSENIC

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1. WHAT WE KNOW

Exposure to heavy metals is one of the possible etiologic factors contributing to the epidemic of chronic kidney disease (CKD) in Central America. This summary will focus specifically on arsenic as a possible cause. Arsenic (As) is a naturally occurring metalloid in the earth's crust that can exist in the inorganic or organic forms, with inorganic arsenic generally recognized as being far more toxic to humans than organic arsenic. Arsenic can also exist in different valence or oxidization states, with the most common environmental states to which humans are exposed being trivalent (III) or pentavalent (V) arsenic. Trivalent arsenic is typically more toxic than the pentavalent form due to its ability to generate reactive oxygen species (ROS).

Arsenic in Central America

Contamination of arsenic in surface and groundwater has been detected in many Latin American countries over the past 10-15 years, including some regions of Nicaragua (Bundschuh et al. 2011a; Lacayo et al. 1992; Lopez et al. 2012). It has been estimated that approximately 4.5 million people in Latin America are chronically exposed to high levels of As (>50ppb) in drinking water (McClintock et al. 2012). Numerous studies in Latin America have demonstrated links between high levels of As exposure and adverse health effects, including dermatological effects, cancers, reproductive outcomes, and childhood cognitive function (McClintock et al. 2012). Dating back to 1996, there is evidence of human exposure in Nicaragua from a documented history of arsenic-induced skin lesions in some regions (personal communication, Juan Jose Amador).

The Pacific Ring of Fire, a series of active volcanoes and trenches, runs directly through the western region of Nicaragua. High arsenic concentrations are often found in the hot springs and geothermal wells of volcanic systems, making this an area of concern for possible arsenic contamination of drinking water. In fact, one of several possible mechanisms of drinking water contamination occurs when As-rich geothermal groundwater mixes with cold aquifers (Lopez et al. 2012). Together, PAHO and UNICEF have a global initiative for assessing As in drinking water, in which Nicaragua participated between 2002-2004. A UK NGO based in Leon (Nuevas Esperanzas) has done an extensive sampling of wells in Telica, a municipality of Leon. Analyses demonstrated that arsenic levels were alarmingly high in some wells, with the highest recorded concentration being 900ppb, which is 90 times the WHO exposure guideline of 10ppb. Many of wells sampled had arsenic levels exceeding the WHO exposure guideline (Longley 2010).

Arsenic and Chronic Kidney Disease

Heavy metals are among the most common environmental agents associated with CKD. Chronic exposure to most notably lead and cadmium, but other metals as well, is associated with tubular disease (Sabolic 2006). It is believed that these metals accumulate in the proximal tubule cells, causing damage leading to problems with reabsorption and secretion. Though exact mechanisms remain largely unknown, it is generally recognized that the mechanism of effect is due to generation of reactive oxygen species (ROS). Arsenic, though not a heavy metal and not recognized as a cause of CKD, may cause kidney damage in animal models and humans. The kidney is one of the major target organs for arsenic toxicity (Jomova et al. 2011; Majhi et al. 2011). After exposure, arsenic is distributed throughout the body and then concentrates in the kidneys, where it is excreted (Prabu and Muthumani 2012). Arsenic can generate ROS, including free radicals and peroxides, which have the potential to damage membrane lipids, proteins or enzymes in the tissues, and DNA (Banerjee et al. 2011; Jomova et al. 2011; Majhi et al. 2011). The resulting oxidative stress may be a mechanism for As-induced nephrotoxicity, and this mechanism, though poorly understood, may be consistent with that of heavy metals. Therefore, arsenic-induced nephrotoxicity and more specifically, CKD, is biologically plausible.

Several epidemiological studies have demonstrated an association between chronic arsenic exposure and kidney damage, though most have been cross-sectional in nature, which limits causal conclusions, and most have primarily examined markers of subclinical kidney injury, limiting conclu-

sions about CKD. One study indicated significantly increased standardized mortality ratios (SMRs) due to kidney disease in a population living in a region with low-to-moderate levels of arsenic (10-100 ppb) in drinking water (Meliker et al. 2007). Similarly, another study showed that mortality from renal disease declined gradually after improvement of the drinking-water supply system to eliminate arsenic from artesian well water (Chiu and Yang 2005). Several studies in various human populations have found a significant correlation between increasing levels of urinary arsenic and increasing biomarkers of kidney damage (retinol binding protein (RBP), β2-microglobulin (β2M), and N-acetyl-b-D-glucosaminidase (NAG)) (JW Chen et al. 2011; Eom et al. 2011; Halatek et al. 2009) and decreasing estimated glomerular filtration rate (eGFR) (JW Chen et al. 2011; Huang et al. 2011). A longitudinal cohort study in Bangladesh suggests that exposure to arsenic from well water was positively correlated with proteinuria in a dose-response manner (Y Chen et al. 2011). When examining combined exposure to cadmium and arsenic, increased levels of both As and Cd in urine caused considerably higher biomarker values of renal tubular damage, measured as increased urinary levels of β2M or NAG, than each of the exposures alone (Nordberg 2010). Similarly, urinary concentrations of Cd and As were positively correlated both with NAG and with urinary oxidative stress indices (malondialdehyde (MDA) and 8-hydroxy-2¢-deoxyguanosine (8-OHdG)), suggesting that chronic exposure to low levels of Cd and/or As might produce tubular damage in the kidney through oxidative stress in humans (Huang et al. 2009).

In the biological sampling of workers in Leon and Chinandega conducted by the Boston University School of Public Health (BUSPH), we found that concentrations of total arsenic in urine exceeded the World Health Organization's guideline of 100 µg/L for 3 workers out of 99 sampled. Though there were not significant differences in urinary arsenic by job category, workers with the highest arsenic exposures were found to have significantly higher serum creatinine (p=0.04) and significantly lower eGFR (p=0.01) while controlling for age and sex. On average, the eGFR among workers with high arsenic was 9.0 mL/min/1.73 m^2 lower than workers with low arsenic (McClean et al. 2012). In Sri Lanka, where there is a similar epidemic of chronic kidney disease, the WHO released a report stating that "the majority of men and women suffering from this disease excrete raised levels of arsenic and/or cadmium in the urine." They report that "about 63% of CKDu patients had urine arsenic levels above 21 ug/g creatinine" and that "urine arsenic levels above 21ug/g creatinine have been shown to cause changes in kidney tissue that lead to chronic kidney disease" (WHO 2012).

Finally, CKD has recently gained attention as being a health problem in arsenic-endemic areas such as Bangladesh and parts of Asia. Due to a lack of kidney registries, it is difficult to ascertain the prevalence and true burden of CKD in these low- and middle-income countries (Ayodele and Alebiosu 2010). Nevertheless, it has been estimated that in Bangladesh, the prevalence of CKD is 17% (Tsukamoto et al. 2009); a study in one city estimated that between 13% and 16% of the population had CKD (Huda et al. 2012). While causal associations cannot be made from these data, the observations are interesting. Of note, the prevalence of other common CKD risk factors such as diabetes is not known.

2. WHAT DO WE NEED TO KNOW AND UNDERSTAND?

- *Are individuals living in this area being exposed to high levels of arsenic (and if so, how)?*

As of yet, there is no evidence of high levels of environmental arsenic in the areas of Chinandega and Leon thought to be most affected by the CKD epidemic. Though high arsenic concentrations in water have been detected in many regions of Central American and in nearby Telica, we cannot conclude that other areas in Chinandega and Leon are similarly affected. The ENACAL Director of the Chinandega region has claimed that there is no arsenic contamination in this region. In the BUSPH biological sampling study of workers in this region, urinary arsenic concentrations among miners (GM=26 µg/dL) were higher than in the US general population (GM=8.4 µg/dL), but arsenic concentrations among all other workers were generally consistent with concentrations in the US general population (McClean et al. 2012). As such, there is no evidence of increased exposure to arsenic (and specifically inorganic arsenic) in this population, which is one major data gap when trying to link this CKD epidemic to arsenic exposure.

In this region, the most likely source of exposure would be through ingestion of drinking water contaminated with arsenic. Diet is another potential major source of exposure in the region; because arsenic is ubiquitous in the environment, it can contaminate the food supply if crops uptake As contaminated soils and irrigation water. Rice, in particular, selectively uptakes As due to physiology and paddy growing conditions, though the amount and form of arsenic varies by region, soil type, and

cultivar of rice (Adomako et al. 2011; Bundschuh et al. 2011b; Gilbert-Diamond et al. 2011; Ye et al. 2012; Zavala and Duxbury 2008). Furthermore, these foods can be contaminated if treated with arsenical pesticides, but it is unknown if these pesticides are currently used in this region or have been used in the past.

It is also important to recognize that the type of arsenic (inorganic vs. organic) to which an individual is exposed is important in determining subsequent health effects. Seafood often contains high amounts of arsenic that is usually harmless but that can confound total urinary arsenic levels. In the worker population sampled by BUSPH, we analyzed for total urinary arsenic; we were most interested in inorganic arsenic, but it is unknown what the relative concentration was in these samples.

▸ *Could arsenic exposure be an etiologic factor in this epidemic?*

Arsenic alone is unlikely to be the sole etiologic agent responsible for this epidemic. Exposure to arsenic via water or food would likely affect men and women equally, but in Central America, CKD affects men more than women, often at ratios of 3:1 or greater. However, exposure to arsenic could be a contributory cause, and it is possible that some other occupational exposure is modifying this relationship. Additionally, it is important to consider that workers working in hot conditions may be at greater risk of increased exposure to arsenic via drinking water. Even though they may be dehydrated, they likely still consume more water (and therefore more arsenic, if the water is contaminated) than the general Nicaraguan population.

We have demonstrated that arsenic is ubiquitous in Central America (though we do not know the extent of contamination in those regions most affected by CKD). Though we do not fully understand the mechanism of effect that arsenic has on the kidneys, arsenic-induced nephrotoxicity is biologically plausible. There is an increasing body of literature demonstrating an association between chronic exposure to arsenic and subclinical kidney damage, but it is not known whether repeated incidents of acute kidney damage caused by arsenic exposure could lead to CKD, which represents a major data gap.

3. HOW TO ADVANCE? TOP PRIORITY RESEARCH AND POLICY INITIATIVES.

To determine if there is the potential for exposure in this area from drinking water, one necessary step is to comprehensively sample drinking water wells in the area and analyze for arsenic. In future studies of workers in the region, it will be important to speciate urinary arsenic to rule out contribution of organic species from seafood consumption to total arsenic levels. Nails are other biomarkers that might represent a better measure of chronic exposure to arsenic. Though arsenic is still not well understood in the context of CKD, it is important to recognize that arsenic as an independent risk factor has not been well-studied with biomarkers of CKD in a prospective study.

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DRUG-INDUCED NEPHROTOXICITY IN THE CENTRAL AMERICAN CKD EPIDEMIC

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1. WHAT WE KNOW

Drug nephrotoxicity has been considered among the possible etiologic factors contributing to the epidemic of chronic kidney disease (CKD) in Central America. Although there is evidence that the use of non-steroidal anti-inflammatory drugs (NSAID), antibiotics and diuretics is common in the population at risk, it is likely that these agents are contributory rather than a primary cause, based on the features of the CKD which contrast with the usual presentation of kidney disease due to these drugs. These agents are not uncommon causative agents in acute kidney injury (AKI), but this injury is usually reversible and has only rarely been associated with chronic kidney disease. Fored et al (1991) in a large case control study on the role of previous exposure of analgesic drugs for the development of CKD saw no significant effect from self-reported use of NSAID [1].

NSAID cause two types of acute kidney injury, hemodynamically-mediated and acute interstitial nephritis[2]. Hemodynamically mediated AKI from NSAID use is due to their inhibition of the production of vasodilator prostaglandins. These vasodilator prostaglandins are particularly important in maintaining renal blood flow and glomerular filtration rate in states of decreased effective circulating volume, such as volume depletion and heart failure. Typically the renal function is affected in kidneys with compromised function with reduced arterial blood flow. This scenario is more common in the elderly and in the case of arteriosclerotic kidneys. Case-control studies have shown that, albeit the over-all risk from acute kidney injury is low, individuals with risk factors in the form of increased age, cardiovascular co-morbidity, or exposure to other nephrotoxic drugs have a two to fourfold increased risk for hospitalization because of acute renal failure[3]. Using the General Practice Research Database in the United Kingdom 103 patients were confirmed as idiopathic cases of acute renal failure (ARF) and were compared with 5,000 controls frequency matched by age and sex [4]. Current users of NSAIDs had a relative risk (RR) for ARF of 3.2 (95% confidence interval [CI], 1.8 to 5.8), and the risk declined after treatment was discontinued. Increased risk was present with both short- and long-term therapy and was slightly greater among users of high doses. Risk increased with concomitant use of NSAIDs and diuretics (RR, 11.6; 95% CI, 4.2 to 32.2). This form of AKI is, as far as we currently know, reversible on stopping the NSAIDs since it is not associated with structural injury. However, NSAID induced vasoconstriction may increase the risk of acute tubular necrosis caused by ischemia or other nephrotoxins. This process may be relevant in the Central American CKD epidemic, although one might expect to see more cases of clinically manifest AKI if it was an important cause.

Acute interstitial nephritis from NSAIDs is thought to be an allergic reaction to the drug. It often occurs together with a nephrotic syndrome having the pathologic features of minimal change disease. Patients typically present with an acute to subacute rise in serum creatinine associated with pyuria, white blood cell casts, proteinuria and frequently hematuria. It occurs within weeks to months of starting NSAIDs and usually recovers spontaneously once the offending agent is stopped. The association with nephrotic syndrome is in contrast to the presentation of CKD in Central America, which occurs in the absence or with only low grade proteinuria. Whether CKD ever occurs with NSAIDs in the absence of AKI is uncertain, although these agents are thought to be one of the causes of analgesic nephropathy[5], a tubulointerstitial disease similar to that seen in Central America.

There have been several community-based prevalence studies of CKD in Nicaragua and El Salvador with prevalences of CKD as high as 19% in some communities [6-9]. NSAID use in these communities ranged from 10% to as high as 75% , but in none of these studies were NSAIDs found to be a risk factor for CKD, although the confidence intervals were wide and included point estimates consistent with an adverse effect.

Medication use has been a particular focus of the BU team's studies in Nicaragua. In a pilot study utilizing medical chart reviews of 243 workers

[10], it was found that medications were commonly prescribed. Medical visits occurred for a wide variety of reasons including mandatory pre-employment physicals (22%), infectious diseases other than urinary tract infections (14%), urinary tract infections (12%) and disorders of the joints and adjacent tissues (9%). Given the frequency of the diagnosis of infection it is not surprising that antibiotics were the most commonly prescribed class of agents, accounting for 34% of all prescriptions. NSAIDs were also commonly prescribed accounting for 25% of all prescriptions. In personal interviews with 10 workers, three of them confirmed prior use of gentamicin and nine had used NSAIDs.

The BU team also conducted qualitative interviews with ten physicians and nine retail pharmacists to gather information on the use of nephrotoxic medications [11]. The physicians stated aminoglycosides were rarely used except in severe UTI or sepsis patients who were unable to take oral medications and it was only given in the hospital or in health center beds. Although eight of the physicians recognized the possible nephrotoxic effects of chronic NSAID ingestion, they indicated that NSAIDs were often used for back pain but not if there was known CKD. All the pharmacists believed that UTIs and chistata were related to CKD, either as an early form of the disease or as being on the causal pathway. The pharmacists listed a variety of antibiotics that were used to treat chistata or UTIs, including quinolones, penicillins, sulfas and aminoglycosides. Some pharmacists acknowledged that they sometimes sell medications without a prescription because patients demand treatment.

Although antibiotics are frequently used to treat "chistata", penicillins and quinalones are most frequently used and these are rare causes of kidney disease, usually manifest as AKI in the setting of allergic interstitial nephritis. However, aminoglycosides, which must be administered parenterally, are sometimes used and more frequently result in nonoliguric acute kidney injury which may have few clinical manifestations unless renal function is severely impaired. As in most forms of AKI, complete recovery usually occurs, although irreversible kidney damage has been reported, particularly with prolonged therapy. It is believed that aminoglycoside nephrotoxicity requires at least five days of continuous exposure. It has been reported that repeated courses separated by days or weeks may have increased toxicity because of the accumulation of drug in the kidney[12]. Other risk factors for aminoglycoside nephrotoxicity include advanced age and number of co-morbid conditions. Neither of these risk factors is prevalent in the CKD population of Central America, making aminoglycoside an unlikely primary cause of the CKD epidemic. However, it may function as a sensitizing factor in some patients, particularly given its wide use.

2. WHAT DO WE NEED TO KNOW AND UNDERSTAND, I.E. A BRIEF OUTLINE IDENTIFYING IMPORTANT KNOWLEDGE GAPS

1 Available studies have not quantitated the duration and dose of analgesic use. In future survey studies, validated methods of quantitation should be employed, since toxicity may be dose and duration related. It would be useful to calculate the recent as well the cumulative dose in similar way as was done for acetaminophen exposure by Fored et al [1].

2 Similar population based survey studies on the frequency of aminoglycoside and diuretic use might be performed, including information on dose and duration.

3. HOW TO ADVANCE? TOP PRIORITY RESEARCH AND POLICY INITIATIVES

Drug-induced nephrotoxicity is unlikely to be the sole cause of CKD in Central America. However drug induced nephrotoxicity may well be an important contributing factor. If the normal renal blood flow is compromised by dehydration, NSAID use might worsen the already reduced renal perfusion. Even if acute kidney injury from NSAID and dehydration is considered to be reversible in most cases, it has recently been show experimentally that repeated acute tubular damage may proceed to the development of chronic glomerular changes[13].

The possibility for a conjoined effect from the prevalent use of NSAID and concomitant repeated dehydration should be assessed in future cohort studies.

Animal experiments examining the chronic renal effects of the combination of repeated dehydration and exposure to NSAID should be encouraged.

As a policy initiative, one might consider a program to caution on the chronic use of NSAIDs and that such use should only be done under the supervision of a physician. Similarly the non-prescription use of antibiotics should be discouraged.

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HYPOTHESIS SUMMARY· HEAT EXPOSURE

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CURRENT KNOWLEDGE ABOUT THE POTENTIAL ROLE OF HEAT EXPOSURE IN MEN

The areas of high prevalence of Mesoamerican Epidemic Nephropathy (MEN) are classified as tropical zones with alternating wet and dry seasons.[1] Thus, outdoor workers labor in hot conditions, often with high humidity. The climates in Sri Lanka and the east coast of India, where two other similar epidemics have been identified, are similar. It is unclear whether the ambient temperature in Mesoamerica has increased over time.[2] A preliminary, unpublished analysis of data from a weather station in northwest Nicaragua from 1973-2009 suggests that during the months of January-April, maximum daily temperature may have increased by approximately 2˚C from 1973-1989 and then remained relatively constant since that time.

The most widely accepted measure of heat exposure is the wet bulb globe temperature (WGBT) index, which incorporates temperature, humidity, solar radiation, and wind speed. The American Conference of Governmental Industrial Hygienists (ACGIH) heat exposure threshold limit value for acclimatized individuals continuously working with no breaks is 26.7˚C WBGT under moderate work load and 25.0˚C WGBT under heavy work load.[3] The international standard, ISO, recommends 26˚ C as the level above which, hourly rest periods are required in heavy labor jobs (metabolic rate > 500 W).[4] In Central America, individuals commonly work in conditions that exceed these levels. In an assessment of sugarcane field workers during the month of April, WBGT readings ranged from 26.9˚C to 33.2˚C with a mean of 30.6˚C.[5] The U.S. Occupational Safety and Health Administration (OSHA) standard requires workers performing heavy work under these conditions to work for 15 minutes and rest for 45 minutes out of each hour.[6] It should be noted that these recommendations and mandates are specifically based on prevention of acute health effects from excess heat exposure and not on risk of chronic kidney disease (CKD).

A large proportion of the labor force in Central America works in industries at high risk of excess heat exposure, including agriculture (26% of the economically active population) and construction (7%).[7,8] Other outdoor occupations identified as at risk of excess heat exposure include fishing, mining, and port work, among others. Workers in factories also often labor in conditions of high heat and humidity, though the lack of direct exposure to solar radiation does have an important impact on the overall level of heat exposure.[2,8] The ILO conducted two studies of labor processes in sugarcane and rice production in 1998 and 2004, respectively, and identified excess heat exposure as a concern in each industry.[9,10] Based on observations conducted among sugarcane workers in Costa Rica and Nicaragua, both field and mill workers appear to be exposed to heat in excess of ISO and OSHA standards.[5,11,12] A study of workers in other industries is currently in progress in Nicaragua (A Aragón, personal communication).

Two intervention studies designed to increase hydration have been conducted among sugarcane workers. In one study, workers who drank more water or rehydration solution had increased productivity;[13] in the second, workers who were assigned to drink an electrolyte-balanced rehydration solution had a smaller decrease in GFR from beginning to end of the day compared to those assigned to drink water.[14] At least three sugarcane companies in Central America have modified their practices to address heat exposure. Whether these changes have affected the occurrence of CKD is unknown.

The fact that heat exposure can have harmful effects on kidney function is well known. Agricultural workers, miners, athletes, and soldiers are among the occupations with particularly high risk. High temperature, high humidity, strenuous work, and insufficient hydration can cause the mechanisms used to release heat from the body to become overtaxed, leading to a rise in core body temperature and heat stress. The extent of heat stress can range from minor heat illness including heat cramps and heat rash, to heat exhaustion, to major heat illness such as heat stroke, where core body temperature increases to critical levels (40˚C). Under these conditions, renal vasoconstriction frequently ensues, resulting in dramatic reductions in renal blood flow and oxygen delivery. Exertion can also damage the skeletal muscle tissue, leading to the release of myoglobin (a condition called *rhabdomyolysis*), which can directly damage the kidney. The combination of rhabdomyolysis with direct tubular toxicity and dehydration with reduced renal blood flow and renal ischemia leads to kidney injury.[15,16]

The damage suffered by the kidney in this sce-

nario of heat stress with the pathologic picture of acute tubular necrosis is termed acute kidney injury (AKI). In the past, the occurrence of AKI was considered to be unrelated to the risk of developing CKD. However, recent studies suggest that individuals who recover from AKI are at increased risk for CKD, likely as a result of underlying subclinical damage to the kidney.[17] Although overt manifestations of AKI are known to occur in Mesoamerica, their extent is unknown, and it is doubtful that this mechanism on its own can explain a substantial proportion of the excess cases of CKD occurring in Mesoamerica.

A second mechanism by which excess heat exposure and/or dehydration might lead to CKD is through the development of kidney stones (nephrolithiasis), which cause scarring and other damage to the kidney and can lead to CKD.[18,19] Kidney stones occur more frequently in areas of higher ambient temperature.[20,21] An excess occurrence of nephrolithiasis has been demonstrated among workers in hot conditions (e.g., smelter workers, workers in outdoor occupations).[22-24] In addition, males are at greater risk than women, independent of occupation. The prevalence of kidney stones in Mesoamerica is unknown; physicians interviewed in northwest Nicaragua had differing opinions regarding their frequency and their possible relationship with CKD in the region.[25] Of possible significance, *chistata* (pain on urination) is a frequent complaint in Nicaragua among both men and women.[25] Although frequently clinically diagnosed as urinary tract infections (with resulting antibiotic treatment), urine cultures among 50 male sugarcane workers with current symptoms or white blood cells in their urine were uniformly negative.[26] An alternative explanation is that the condition could be a symptom of volume depletion, leading to supersaturation of the urine and crystal formation causing irritation of the urinary tract. Interestingly, some physicians have reported the appearance of the urine as "sandy", which could be consistent with the presence of micro-crystals in the urine, with the potential to cause trauma as they pass through the kidney tubules.[25]

The third, and most commonly proposed, mechanism by which heat exposure combined with dehydration might cause CKD is based on the theory that repeated, chronic volume depletion, even in the absence of overt heat stress symptoms, leads to subclinical injury to the kidney, which accumulates over time and overwhelms renal repair mechanisms, gradually reducing kidney function until it is sufficiently impaired to be diagnosed as CKD.[27] Unlike the previous two mechanisms, there has been almost no research on this mechanism, so it is unclear whether CKD can be caused in this manner. Nevertheless, this hypothesis would appear to have a biologically plausible basis, given the known effects of heat exposure on the kidney and the developing evidence of a connection between acute kidney injury and CKD.

It is widely believed that the causes of Mesoamerican nephropathy are multifactorial, acting in either a synergistic or additive manner. Thus, heat exposure is usually, though not always, described as one component of a broader causal constellation. Within this framework, two basic mechanisms have been proposed:

1 Volume depletion increases the susceptibility of the kidneys to other nephrotoxic exposures. Such exposures include widespread use of pain medications (particularly nonsteroidal anti-inflammatory drugs, or NSAIDs) or overconsumption of fructose, though other exposures that have nephrotoxic potential (e.g., agrichemicals, heavy metals) could also be involved. Alcohol has been mentioned, but usually as a factor that accentuates volume depletion, rather than as a nephrotoxic exposure potentiated in the context of a volume depleted kidney.

2 Subclinical kidney damage is initiated at a younger age, and heat exposure speeds the progression to clinical disease. Examples of initiating factors that have been proposed include childhood infectious diseases, arsenic, and low birth weight. Although not an initiator of kidney damage, genetic factors might also increase susceptibility to the renal effects of heat exposure.

▸ *Epidemiological Data*

Although limited, the epidemiological evidence to date supports treating heat exposure as an important hypothesis:

1. EPIDEMIOLOGICAL DATA ON HEAT EXPOSURE AND CKD. Several studies have demonstrated an increased rate of mortality and hospitalization from kidney disease during heat waves.[28-30] However, most of the effect has occurred among those with pre-existing kidney disease and is of uncertain relevance for a population of younger workers. We are aware of only one study of heat exposure as a causal factor for CKD among a population of healthy workers. Tawatsupa et al. analyzed self-reported data on job-related heat exposure and subsequent self-reported diagnosis of kidney disease among participants in the Thai Cohort Study.[31] Men with physical jobs and exposed to excess heat were 2.6 times as likely to report physician-diagnosed kidney disease (not further specified) as men with no excess heat exposure. Frequency and duration of excess heat exposure were also related to increased risk.

2. EPIDEMIOLOGICAL DATA FROM MESO-

AMERICA. As described in the **article Epidemiology of CKD of unknown causes in Mesoamerica** (Brooks, Ramirez in this report) both mortality and prevalence data suggest that MEN is highest along the Pacific Coast in El Salvador, Nicaragua, and Costa Rica, all lowland areas with high temperatures. At the same time, it should be noted that other lowland areas (e.g., eastern region of Nicaragua) do not appear to have elevated mortality rates. Departments at higher elevation, with lower temperatures, have lower mortality rates. To our knowledge, the only reported excess of CKD in a high altitude region is a cluster of cases in the state of Hidalgo in Mexico.[32]

Differences in mortality and prevalence between and within occupations are also consistent with heat exposure. The two municipalities with the highest mortality rates in Nicaragua are Chichigalpa (sugarcane) and Larreynaga (mining). Among residents living at low altitude, a high prevalence of elevated creatinine has been found among agricultural workers, miners, fishermen, stevedores, and construction workers,[26,33,34] while workers in the service industry and coffee workers, the latter of whom live and work at higher elevations, have had low prevalence of elevated creatinine.[33,35,36] The most obvious shared exposure between the worker groups with higher prevalence is manual labor in a hot environment.

Among agricultural workers, Sanoff et al. found similar creatinine elevations among workers in sugarcane, rice, corn, and bananas.[34] In that study, workers who drank more water during the day were more likely to have elevated creatinine. This counterintuitive result could be interpreted either as evidence that the water is contaminated with a nephrotoxic agent or that greater consumption of water is a proxy measure of heat exposure but is not sufficient to prevent CKD. Peraza et al. found higher rates among sugarcane workers at lower elevation compared to higher elevation.[37,38] Cane cutters, who are generally considered to have the most strenuous job among sugarcane workers, had the highest prevalence of elevated creatinine, as well as the highest incidence of new cases, compared to other jobs in sugarcane over the course of a harvest season.[26]

KEY GAPS IN KNOWLEDGE

Heat exposure is currently characterized mainly by occupation and temperature (altitude), which are at best indirect and easily confounded proxies. Measurement of the components of the actual exposure (heat, work intensity, hydration) would lead to more refined classification of exposure by occupation. It would also provide information on the extent of interindividual variability among workers in the same occupation. Comparisons of disease incidence based on these measurements would be less susceptible to exposure misclassification and confounding than simple comparisons by type of occupation. It would be ideal if sufficient information could be obtained to characterize exposure according to different metrics, such as peak, time-weighted average, or cumulative exposure.

Characterization of heat exposure is a necessary step, but it would not answer the fundamental question: can chronic exposure in the absence of overt severe heat stress cause CKD? A simple model for the effect of heat exposure on CKD can be expressed as follows:

Exposure > early effects > subclinical kidney injury > CKD

Research is needed to characterize and tie together each of these steps, including:

- *Early effects*: changes in core temperature, weight, blood pressure, urine concentration, heart rate, serum creatinine, creatinine phosphokinase
- *Kidney injury*: relevant biomarkers of injury

Different groups use different biomarkers of kidney injury. It would be helpful to be able to define and settle on a standard group of biomarkers that have the following characteristics:

- Identify injury at an earlier stage than serum creatinine;
- Predictive of eventual development of CKD of unknown cause in Mesoamerica;
- Sensitive and specific for kidney injury in community as well as clinical settings;
- Provide information regarding the possible anatomical location of injury.

PRIORITY RESEARCH INITIATIVES

Both in-depth occupational exposure assessments and exposure assessments combined with assessment of subsequent steps on the disease pathway are needed. These studies, either on their own or taken as a group, should include:

- Longitudinal assessment, at a minimum pre- and post-shift but also over calendar time;
- Assessment of workers in occupations at higher and lower risk for heat exposure (including indoor workers exposed to hot conditions), based on both the nature of work and climatological conditions;
- Hydration should be measured by the quantity and type of fluids consumed at work, away from work, and during different times of year;
- Employ additional measures of kidney damage and function other than serum creatinine.
- More detailed urinalysis related to urinary signs and symptoms, including complaints of *chistata*,

and review of ultrasounds for evidence of kidney stones.

- Collection of extensive and accurate data on other types of exposures, both to control for confounding and to identify potential synergistic factors

In addition to the focus on specific groups of workers, many components of this research could also be conducted in the wider community among adults and children. These efforts would help evaluate the possible impact of non-occupational heat exposure and hydration practices, as well as address the hypothesis that kidney damage may be initiated prior to beginning to work.

Finally, because occupational heat exposure is a concern regardless of whether it causes CKD, research on the effectiveness and the unintended consequences of different interventions to reduce the impact of heat exposure should proceed without waiting for causally-focused research to be completed. For example, before making recommendations to increase hydration on the job, there should be a systematic assessment of water quality to determine whether safe sources of drinking water are available. Increased consumption of water that contains nephrotoxic contaminants (e.g., arsenic) might in theory increase the risk of CKD. There may also be additional barriers to increasing water consumption among workers and in the population more generally. Lack of time or access, competition from soda and other flavored drinks, concerns about biological and toxicological contamination of the water, and cultural factors have been suggested. Combined qualitative and quantitative research to assess these potential barriers would also be worthwhile.

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EMPIRICAL DATA FROM COSTA RICA ON HEAT STRESS AND HYDRATION

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INTRODUCTION

Guanacaste is a hot (average maximum daily temperature 36˚C in April), northwestern coastal province at low altitude in Costa Rica. Based on preliminary analyses of the country's mortality statistics between 1997 and 2011, the mortality rate of chronic kidney disease (ICD10 N18, N19) was 5 times higher in Guanacaste than the rest of the country.

A three year study in the Guanacaste region of Costa Rica was undertaken between 2009 and 2012 to evaluate the risk of heat stress and possible health outcomes in sugarcane harvesters. International standards (Technical Prevention Norms, NTP) from Spain (INSHT, 2012) were used to determine the Wet Bulb Globe Temperature (WBGT), the metabolic load for harvesting sugarcane and the corresponding Instituto Nacional de Seguridad e Higiene en el Trabajo,(INSHT 2012), and OSHA limit values (OSHA 1999) for avoiding heat stress (Crowe et al, forthcoming). Symptom questionnaires and urine analyses were also conducted.

CURRENT KNOWLEDGE

Description of work and heat exposure

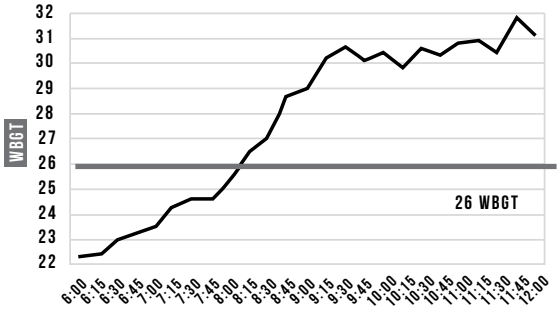
Cutting starts between 5:00 and 6:30 am (depending upon how far the workers have to travel). The workers stop cutting when they are tired (usually up to a maximum of 12:00 pm), but are paid according to how much they cut.

Using the NTP 323, we determined the approximate metabolic load for harvesting sugarcane to be 261 W/m^2 (405 kcal/hr), which corresponds to a threshold limit value of 26˚C WBGT for work at 100% effort (NTP 322). Workers are at risk for heat stress while working at full capacity when the WBGT is above 26 ˚C.

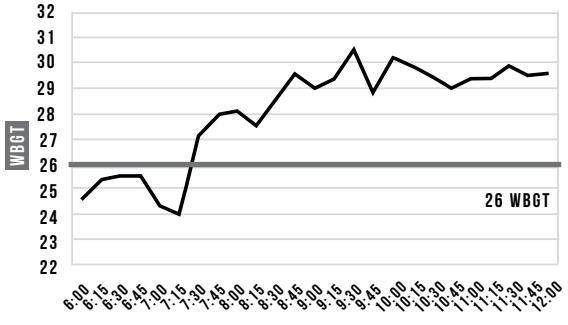
WBGTs were measured every fifteen minutes between 6:00am and 12:00pm for six days in February 2011. The threshold limit was surpassed as early as 7:15 am. By 9:00 am, all measurements were clearly above the limit value, a fact that, under OSHA recommendations, would require that workers rest 25% of each hour under these conditions to avoid risk of heat stress. After 10:00 am, conditions on all days were such that OSHA recommendations would require working only 25% of each hour. Figure 1 shows WBGT conditions compared to the limit value of 26˚C on two typical days (Days 3 and 4). Days 3 and 4 differed with Day 3 starting cooler and finishing hotter than Day 4, and Day 4 passing the threshold limit value earlier than Day 3. Both days, however, like all of the days measured, demonstrated that workers who cut sugarcane all morning are under heat stress conditions for the majority of the shift.

Figure 1. WBGT measurements on two distinct days and the corresponding OSHA limit value (26˚C) for work at 100% effort (February 2011).

a. Day 3



b. Day 4



Symptoms

A symptom questionnaire was conducted in February 2011 with harvesters (n=112) and non-harvesters (n=63) from the same company. Non-harvesters had jobs in offices, the processing plant or, in a few cases, in the field but in work that did not require heavy labor. Harvesters and non-harvesters

were similar in age and in the number of harvests worked, but non-harvesters had more education (median 10 vs 5 years) and more often reported consuming alcohol (76% vs 45%). Participants were asked about a range of symptoms (heat-related and non-heat related) and the frequency of the symptoms. Preliminary analyses using chi-squared or Fisher's Exact test to compare harvesters to non-harvesters demonstrated significant differences ($p \leq 0.05$) in the following potentially heat-related or dehydration-related symptoms experienced at least once a week during the harvest season: pain/burning while urinating, nausea, headache, fever, difficulty breathing, swelling of the hands or feet, muscle twitches, and dizziness.

Liquid consumption

Harvesters carry their own water to the field. We asked each worker how much he consumed during the work shift and visually verified the data according to the amount left in the jugs. Harvesters drank an average of 5 L of water (range 1-10L). Most workers additionally drank 0.25-0.5 L of coffee or "fresco" (fruit juice with water and sugar) or a powdered drink mixed with water during the shift.

Urine data - preliminary analysis

In 2012, the season after the data collection of symptoms, the company provided 40 grams of a brand-name hydration powder that workers mixed with their own water. Urine specific gravity was performed for 53 sugarcane cutters, pre and post shift for three days in January at the same time that the hydration powder was provided by the company. Concentrated urine, as an indicator of dehydration (specific gravity >1.020), was observed among 24% of the workers at least one of the three mornings and in 54% of the workers at least one of the three afternoons. In addition, frequent alterations in urinary sediment (hematuria, leukocyturia, proteinuria among others) were found.

Conclusions

This study is the first in Costa Rica to examine the metabolic load of sugarcane harvesters and the corresponding limit values for avoiding heat stress under international standards. It is clear that sugarcane harvesters are under heat stress for much of their work shift. Significant differences between harvester and non-harvesters in the occurrence of most of the potentially heat-related or dehydration-related symptoms were observed. Though many workers consume considerable amounts of water, 54% of the workers who provided samples for urine specific gravity were considered to be dehydrated (urine specific gravity >1.020) at least one of the three afternoons they were tested. It is imperative that conditions be improved allowing for better hydration and decreased risk for heat stress through planned rest breaks and task rotation.

KNOWLEDGE GAPS (WHAT DO WE NEED TO KNOW AND UNDERSTAND?)

General

- Very little is known about the effects of heat exposure on health and productivity in the medium and long term.
- International norms/standards are meant to protect workers but not to quantify heat stress risk. Standardized methodologies should be developed to quantify heat stress risk.
- NTP, OSHA, WBGT all have been criticized as inaccurate, over-protective and difficult to use. WBGT is expensive. Other standards exist, but have not been widely accepted. Agreement on the best index to use for heat stress quantification would help standardize heat stress quantification for CKD studies between countries.

In Costa Rica

- Although weight was measured pre and post shift in the study described above, the heat affected the scales and data are unusable. We do not know whether workers experience a weight loss due to fluid loss during the shift.
- Little is known about the heat stress in Costa Rica in occupations outside of harvesting.
- Creatinine levels of sugarcane harvesters have not been studied in Costa Rica.
- It is difficult to quantify the delayed effects of heat exposure. For example, the effects of a high WBGT on a Monday might be seen in health and productivity outcomes on Tuesday or Wednesday. More information about how to account for this lag would be helpful.

PRIORITIES FOR RESEARCH INITIATIVES

- New standards for heat-stress evaluation and corresponding protection measures.
- Kidney function tests pre and post-harvest and pre and post shift at different times during the harvest.

- Physiological data (heart rate, core body temperature, weight,) pre and post shift. These data could help provide the basis to evaluate intervention measures including planned hydration and work rotation.
- Long term health/symptom study that could be analyzed together with long term estimated WBGT data (Kjellstrom, Crowe 2011).

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THERMOREGULATION· PHYSIOLOGY AND MEASURES

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PHYSIOLOGICAL CHANGES DURING WORK IN A HOT CLIMATE

Exercising in the heat, for any population, increases thermoregulatory and cardiovascular demand. Heat production during exercise is 15–20 times greater than at rest (Nadel, Wenger et al. 1977). This endogenous heat generation, in addition to exogenous heat from the external environment, must be offset by the body's heat loss mechanisms (skin blood flow and sweating) in order to avoid significant hyperthermia. These mechanisms rely upon the principles of conduction, convection, evaporation and radiation to dissipate heat to the environment. When ambient temperature rises above 20°C, the contributions of conduction, convection, and particularly radiation, become increasingly insignificant, with the bulk of heat dissipation in the exercising human resulting from sweat evaporation (Armstrong and Maresh 1993). For example, in hot dry conditions evaporation can account for as much as 98% of dissipated heat (Armstrong and Maresh 1993).

Humans are capable of producing a tremendous amount of sweat (upwards to ~2.0 L/h) during prolonged heat exposure (Gerking and Robinson 1946, Sawka, Burke et al. 2007). When sweat loss exceeds fluid replacement, total body water content is reduced, increasing plasma tonicity (osmolarity) and decreasing blood volume (hyperosmotic-hypovolaemia) (Sawka 1992, Cheuvront, Carter et al. 2004). This state of dehydration (hypohydration) increases cardiovascular strain during heat stress, as evidenced by augmented increases in body core temperature and heart rate while reducing cardiac filling (Montain and Coyle 1992). Appropriate fluid replacement during heat exposure reduces cardiovascular strain and prevents hypohydration-related decrements in cardiovascular performance (Sawka, Burke et al. 2007).

During heat exposure, skin blood flow can increase from ~300 to ~7500 mL·min^{-1}, which results in upwards to 50% of cardiac output being diverted to the skin to enable heat transfer (Rowell, Brengelmann et al. 1969, Rowell 1986). To accommodate increases in skin blood flow and sweating, blood is diverted away from the visceral vascular beds to the periphery (Rowell 1983). This is due to an increase in sympathetic activity and vagal withdrawal that reduced splanchnic and renal blood flow as well as increasing the inotropic (force of contraction) and chronotropic (rate of contraction) modulation of the heart (Rowell, Detry et al. 1971, Frey and Kenney 1979). Therefore, this heat-induced shift of blood volume to the periphery increases cardiovascular strain and limits cardiovascular response capacity.

When environmental heat stress is combined with muscular exercise, the cardiovascular system must simultaneous meet thermoregulatory and metabolic needs. Essentially, this dual demand can be distill down to blood flow delivery/demand, which is ultimately limited by an individual's available cardiac output (Rowell 1974, Gonzalez-Alonso, Crandall et al. 2008). In an effort to compensate, blood flow to visceral vascular beds is reduced even further during exercise in the heat. For example, during exercise in a hot (50°C) environment renal blood flow and glomerular filtration rate is further reduced when compared to resting values in the heat or exercise values in the cool (21°C) and notably these reductions occur even when fluid balance is maintained (Radigan and Robinson 1949). Thus, exercise in the heat can pose a severe challenge to human cardiovascular control, and thus the provision of oxygen to exercising muscles and vital organs (Gonzalez-Alonso, Crandall et al. 2008).

With prolonged exercise and exposure to hyperthermia, exertional heat illness can occur. The pathophysiology of exertional heat stroke involves complex interactions at the cellular and large systems level, including factors such as cellular damage, cardiac abnormalities, proinflammatory cytokines, and circulatory dysfunction (Adams, Stacey et al. 2012). To date, there is a relatively large body of knowledge concerning the basic science of heat illness, however, there remain significant difficulties in diagnosing milder forms of heat illness. Furthermore, the prevalence of heat illness in high-risk populations is not well established.

› *Current knowledge gaps*

- Quantitative assessment of the thermoregulatory challenges experienced by workers.
- Quantitative measurement of body temperature

and oxygen consumption in workers exposed to high ambient temperatures.
- Changes in renal blood flow during a work day/week/season
  - How solar load (i.e., radiant heat) influences exertional physiological strain.

METHODS OF QUANTIFICATION

When describing body temperature, body core temperature describes the temperature of the internal structures deep within the body (i.e., the heart, splanchnic regions and brain) as opposed to peripheral structures such as the skin. Ideally, body temperature measurements should be convent, unaffected by environmental conditions, have a rapid response and quantitatively reflect small changes in body temperatures (Byrne and Lim 2007).

Pulmonary artery temperature

Pulmonary artery blood temperature is considered the gold standard for body core temperature measurement as it is a composite of blood temperatures returning to the heart from all regions of the body (Pearson, Ganio et al. 2012). However, the invasive nature of this measurement makes it expensive and impractical for most research setting and subsequently, other approaches are preferable.

Oesophageal temperature

Oesophageal temperature is commonly used in laboratory-based research because of its location near the left ventricle, aorta and consequently, blood exiting the heart to be delivered to the body (Moran and Mendal 2002). To measure oesophageal temperature, a thermistor is inserted through the nose, and placed approximately at the level of the right atrial. Oesophageal readings respond rapidly to changes in body core temperature (Shiraki, Konda et al. 1986), while being less invasive than pulmonary artery temperature measures. A disadvantage associated with oesophageal temperature measurement is an intolerance or irritability to the placement of the thermistor. Additionally, ingestion of fluid temporarily affects temperature measurements (Moran and Mendal 2002).

Rectal temperature

Rectal temperature has also been commonly used in the laboratory setting. Though, accurate under steady-state conditions, a participant's cultural or personal beliefs can make rectal measurements unfeasible. Furthermore, during rapid changes in body core temperature, rectal measures can lag behind other core sites (i.e., oesophageal) (Moran and Mendal 2002). It should be noted however that there is no physical discomfort with rectal measurements.

Gastrointestinal temperature

Gastrointestinal temperature is measured by swallowing a telemetric pill that transmits a temperature signal, relative to the surrounding gastrointestinal temperature, by radio wave to an external receiver for data logging or instant display. Use of this measure has become increasingly popular (particularly in field studies) given its convenient nature and validity with respect to oesophageal and pulmonary blood temperature measures under a variety of environmental conditions (Byrne and Lim 2007, Pearson, Ganio et al. 2012). Though, it should be noted that gastrointestinal temperature readings lag slightly during rapid changes in body core temperature (Pearson, Ganio et al. 2012). Other considerations include the sensor's transmission range (i.e., 1 meter) and participants' exclusion criteria (individuals with any prior gastrointestinal surgery or condition should be excluded).

Tympanic thermistor

This thermistor is placed in the canal near the tympanic membrane. The advantage of this method is that the tympanic membrane receives blood directly from the internal carotid artery, which also supplies the hypothalamus (Moran and Mendal 2002). Placement of the auditory canal thermistor is also easy accessible and non-invasive. Of note, auditory canal temperature can accurately measure body core temperature when a thermocouple probe is used (Amoateng-Adjepong, Del Mundo et al. 1999, Cattaneo, Frank et al. 2000), which is insulated (i.e., with cotton wool) against the confounding effects of ambient temperature (Marcus 1973).

Furthermore, auditory canal temperature readings are closer to pulmonary artery measures than either rectal or axilla methods when participants are **supine** and exposed to passive heat stress (+0.5˚C; (Robinson, Charlton et al. 1998). However, this method is problematic during physical work in the heat, errors resulting from dirt, inaccurate placement, technician error or ambient interference (Briner 1996, Moran and Mendal 2002).

Infrared auditory canal thermometers are now commonly used within hospitals and clinical settings, as they are convenient, hygienic and quick to use. However, even new generation thermometers have error limits of up to 0.76˚C (despite manufacturers specifying error limits of 0.2˚C) in ICU patients resting in controlled environments (Haugan, Langerud et al. 2012). Thus, the reliability of such devices in moving humans outside is questionable. Added to this, the reproducibility of infrared measurements is poor (Amoateng-Adjepong, Del Mundo et al. 1999).

Sublingual oral temperature

Though easy to use and non-invasive, sublingual oral measurements can be affected by breathing rate or environmental factors (Moran & Mendal, 2002). Subsequently, sublingual temperatures do not correlate well with more accurate core measurements.

Axilla temperature

Axilla measures are highly variable between participants and record significantly lower body core temperature than other core measures (Waterhouse *et al.*, 2005). Furthermore, axilla measurements are slow or sluggish to register acute changes in body core temperature (Robinson, Charlton et al. 1998).

Skin temperature

Skin temperature provides important information with respect to the temperature gradient between the body's interface (the skin) and the environment. Skin temperature can be measured wirelessly using an iButton surface thermistor placed on the skin. Typically skin temperature is measured at a number of sites, with measures weighted to calculate mean skin temperature.

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FRUCTOSE AND KIDNEY DISEASE

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1. WHAT DO WE ALREADY KNOW?

Intense exercise under hot conditions can lead to water depletion (dehydration) and an increase in serum osmolarity (1). In the proximal tubule there is increased reabsorption of glucose-rich fluid, leading to increased glucose flux. In addition, the rise in serum osmolarity stimulates aldose reductase (AR), which converts glucose to sorbitol, and which is then converted to fructose by sorbitol dehydrogenase. The combination of increased AR and increased substrate should result in increased fructose levels in the renal cortex. This would be further amplified if rehydration consists of sweetened drinks that contain sugar (which contains 50 percent fructose and 50 percent glucose). Indeed, studies in both laboratory animals and humans have shown that the ingestion of fructose-rich drinks is associated with an increase in urinary fructose excretion despite minimal changes in serum fructose (2,3). Furthermore, a loss of extracellular volume could lead to a decrease in blood pressure that could reduce renal blood flow, induce mild ischemia and further enhance aldose reductase expression. Thus, the combination of dehydration (loss of both water and sodium), eventually coupled with hydration with fructose-enriched fluids, will result in increased fructose levels in the proximal tubule.

On the other hand, we reported that fructose is injurious to proximal tubular cells (4). Specifically, the proximal tubular cells are one of the major sites where fructokinase (KHK) is expressed. In turn, the metabolism of fructose by fructokinase is distinct from other sugars in that the metabolism is extremely rapid and leads to ATP depletion. This does not occur when glucose is metabolized as glucose has a feedback mechanism that prevents excessive phosphorylation. In turn, the reduction in ATP causes an ischemic-like shock to the cell, and generates uric acid from the degradation of ADP and AMP. We have found that the uric acid drives oxidative stress and inflammation in the cell, and at renal levels those effects result in vasoconstriction, glomerular hypertrophy, microvascular lesions, tubulointerstitial inflammation and fibrosis, and in long term albuminuria and glomerulosclerosis (5).

2. WHAT DO WE NEED TO KNOW AND UNDERSTAND?

To better study this, we need to first show that chronic dehydration can cause chronic kidney disease via this mechanism in laboratory animals. To then determine if this is the cause of Mesoamerican nephropathy, we need to obtain epidemiological and clinical evidence that this mechanism is a strong candidate.

3. HOW TO ADVANCE?

We are already performing animal studies to address this, including using mice that genetically lack fructokinase. We are also planning an epidemiological study to determine if chronic dehydration among sugarcane workers is a key risk factor for chronic kidney disease in this population, and whether it is modulated by the type of drink or exposure to fructose in these workers. Additionally, we are performing a descriptive study to see if markers of renal injury correlate with the severity and timing of dehydration in these workers. Ultimately, if evidence for this mechanism is shown, a clinical study involving better hydration, with or without the use of newly developed fructokinase inhibitors, could be given to see if this disease can be prevented.

4. HOW CAN RESEARCH RESULTS AND POLICIES BE APPLIED TO CONTRIBUTE TO SOCIAL AND ENVIRONMENTAL IMPROVEMENTS?

If the disease can be shown to be due to activation of this mechanism, it could lead to a better approach for preventing this condition by altering and improving hydration practices, including the choice of hydration solution, and possibly by implementing specific therapies.

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GENETIC SUSCEPTIBILITY TO KIDNEY DISEASE IN MESOAMERICA· A DEVELOPING HYPOTHESIS

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There are few, if any, common diseases that can be attributed to either genes or environment alone. Instead, common diseases typically result from environmental influences acting on a susceptible genetic background.(1) When both the environmental and genetic factors are unknown, identifying either component independently is difficult. In the case of Mesoamerican Nephropathy, understanding genetic susceptibility may inform the search for the exposure, and vice versa.

CURRENT KNOWLEDGE

How local gene pools are shaped

High local incidence of any disease immediately suggests environmental factors as the clearest explanation for the geographic distribution. However, the frequencies of specific genetic variants can also become highly concentrated in particular geographic distributions through clear mechanisms. First, when local populations are established by a small number of individuals, the "founder effect" can lead to certain gene variants carried by those founders being markedly overrepresented in subsequent generations. The high frequency of these gene variants can increase even further by chance ("genetic drift") or because these genetic variants are beneficial ("selection"). Even gene variants that are not initially overrepresented by a founder effect can become surprisingly common through either drift or selection. So a high local incidence of disease may also be explained, at least in part, by local population genetics. The most probable scenario is that only a subset of the local population is at high risk of disease when exposed to some injurious factor.

Central American populations

Genetic variants causing disease tend to be easiest to find in homogeneous populations (such as Icelanders). Until recently, very few genetic studies looking for disease-causing variants have been attempted in admixed population. In admixed populations, it is difficult to determine if a variant is enriched in cases because it actually causes disease or because cases and controls have different ancestry. For example, if kidney disease cases have more African ancestry than controls, many genetic variants more common in Africans will be enriched in cases even though these variants are entirely unrelated to kidney disease. Since Central American populations tend to have significant European, African, and Native American ancestry, the search for disease-causing variants is especially complex. In the case of Mesoamerican Nephropathy, the difficulty posed by mixed ancestry may be partly offset by the fact that the disease-causing variants are likely to be very powerful (i.e. have a large effect size). Recently, a better understanding of the genetic architecture of ancestral populations (e.g. Africans, Caucasians, Native Americans) and advances in statistical techniques have made the problems of identifying disease-causing genes in mixed populations manageable, but by no means easy.(2, 3)

Lessons from kidney disease in other populations

African Americans suffer from chronic kidney failure at rates of 4-5 times higher than European (Caucasian) Americans. Much of this difference had traditionally been attributed to a variety of environmental and socioeconomic factors. Recently, taking advantage of special tools to study admixed populations, we found that genetic variants in a gene called APOL1 accounted for essentially all of this risk difference in non-diabetic kidney disease.(4) Two different coding mutations were identified that cause amino acid changes in ApoL1 protein and greatly increase risk of kidney disease. The 3.5 million African Americans with two APOL1 risk variants have a 7-30 fold increased risk of several different types of chronic kidney disease. We believe these risk variants became extremely common because they protected sub-Saharan Africans against the trypanosomes that cause African Sleeping Sickness.(5) Environment still remains a critical factor, as the majority of these individuals with the

high-risk genotype will not ultimately develop disease. Now, we can begin studying high-risk individuals with and without kidney disease with a hope of finding the relevant environmental factors, a task that would have been essentially impossible previous to the discovery of these unusually powerful gene variants. Since immunity against infectious diseases is one of the most powerful driving forces in human evolution, similar stories have likely occurred throughout the world in specific populations.(6) An understanding of the major pathogens in the Mesoamerican region, past and present, may help illuminate the nature of the current kidney disease epidemic.

Examples of gene-environment interactions in human disease

Adverse effects from drugs are generally unpredictable and often extremely severe. Recently, several genetic variants have been discovered that greatly predispose individuals to complications. Unlike genetic variants for disease such as hypertension or diabetes that confer small changes in risk, these gene/exposure interactions impart huge increases in risk. In isolation, neither the genetic variants nor the drugs lead to harm, but together the chances of severe side effects increases dramatically. Examples are Stevens-Johnson Syndrome with specific HLA-B types and anti-epileptic drugs, myopathies with SLCO1B1 variants and statin drugs, and bone marrow suppression with certain TPMT genotypes and azathioprine.(7) The key point is that during human evolution there has not been any selection pressure to eliminate these variants, which appear to be harmful only under special circumstances. Similarly, some Central American populations may have high frequency of genetic variants that only become problematic in the setting of new exposures.

Conversely, natural selection can exacerbate harmful combinations. For example, individuals with certain mutations in the G6PD gene inherit some resistance to malaria. These genetic variants can become more common where malaria is endemic. However, individuals with G6PD deficiency are predisposed to severe side effects (caused by hemolysis—or lysis of red blood cells) in the setting of treatment with many drugs (such as some quinine derivatives) or consumption of some foods such as broad beans ("favism").(8)

KNOWLEDGE GAPS

Some reasons to consider a genetic component for Mesoamerican Nephropathy

Untangling the effects of genes and environment can be very challenging when their interaction is required to cause disease. Genetic contribution to disease needs to be considered when environmental causes are not apparent despite intensive investigation. Ultimately, we may need to re-evaluate many different environmental exposures in light of specific genetic backgrounds. The statistical signals from environmental factors may only become apparent when non-susceptible individuals are removed from the analysis. Here are a few of the many observations in Mesoamerican Nephropathy compatible with a genetic component:

1 High local rates of a relatively uncommon disease suggest a very powerful risk factor. In theory, very powerful risk factors should be more readily identifiable than weaker ones. Powerful disease-causing genetic variants can increase in frequency (due to founder effects, selection, or drift) but remain "invisible" unless tested for. Local differences in disease-causing variant frequency may help explain regional differences in disease prevalence.

2 Anecdotal evidence suggests that kidney failure among agricultural workers may cluster in families. This clustering may be explained by shared environment such as occupation, but does not explain why other families in the same occupation do not develop disease. Familial clustering of the disease suggests shared gene variants.

3 Men develop kidney failure at rates many times higher than women in the same communities. Occupational exposure seems a likely explanation for part of the difference. However, genetic explanations such as disease-causing variants on the X-chromosome should also be considered (G6PD deficiency is an example).

4 Microenvironment influences genetics. For example, in Nicaragua kidney disease tends to follow a coastal pattern and become much less common as altitude increases. The likely explanation is heat or local industry. However, gene variants that protect against coastal pathogens (e.g. malaria, yellow fever, dengue fever, etc.) not present at higher altitudes may be enriched in coastal areas through selection, and may contribute to disease.

Questions we can ask that might support a genetic contribution to kidney disease.

1 Is there evidence for statistically significant familial clustering? For instance, are agricultural workers with kidney disease more likely to have a

first-degree relative with kidney disease than healthy workers with similar work histories?

2. Does disease susceptibility travel with individuals as they migrate to and from high incidence areas?
3. Are there enough twins with similar exposures to show differences in hereditability between identical and fraternal twins? (probably not)

RESEARCH PRIORITIES

› *Possible types of genetic study*

1. Genetic Association Study. We compare the frequency of genetic variants in cases and controls. This can be done for variants in "candidate" genes that we suspect may cause the type of kidney disease we see in the epidemic. Alternatively, it can be performed with an unbiased, "genome-wide" approach. In genome-wide studies, we test hundreds of thousands of variants simultaneously and then make statistical corrections to account for differences in ancestry. We also need to adjust our results based on the number of variants we test in order to reduce false positives.
2. Family-based studies. In this type of study, we look for variants that tend to be inherited together with disease in groups of related individuals.
3. Gene-environment study. We can analyze how specific exposures strengthen associations between variants and disease.

› *Why finding disease-causing genetic variants could be helpful*

We may be able to identify individuals who are at high risk for developing kidney disease. Of equal importance, we may be able to use the genetic findings to learn about the nature of the environmental exposure. As an example, if a strongly causal variant is discovered in a liver detoxifying enzyme, we may be able to identify a toxic compound that cannot be metabolized in susceptible individuals. Similarly, a mutation in a molecular transporter or channel could also point to the etiology of the kidney disease. For example, finding a mutation in a gene important for tubular handling of sodium, calcium, uric acid, oxalate, or other compound may help us better understand the nature of the causal environmental factor.

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EXPERIENCES OF LA ISLA FOUNDATION

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In the absence of a known and accepted etiology of the devastating epidemic of chronic kidney disease of unknown cause (CKDu) in Central America, the macro level socio-economic factors that can contribute to the onset of the disease or exacerbate its effects become especially important. They represent our only potential points of intervention and serve as the context for all of us moving forward in our research.

La Isla Foundation (LIF) is in the unique position to evaluate the macro causes of the CKDu epidemic. We are active in the sugarcane producing communities of Northwestern Nicaragua where we witness the realities of daily life and where we hear the testimonies of workers and their families. We are also active in the research community where we are privy to the latest hypothesis on causality as well as findings. Lastly, we are tapped into the international media circuit where we are first to receive and participate in the development of the latest news stories and analysis of the epidemic.

The following is a summary of La Isla Foundation's experience in Nicaragua regarding the socio-economic factors and policies that are connected to the CKDu epidemic. We will provide a general overview of the situation and subsequently speak more specifically towards the legal framework that could serve as a guide for future policy pressure points in amelioration of the epidemic.

We will explore the socio-political factors that exacerbate and perpetuate the disease as well as potential legal avenues toward increased protection of workers and their families:

- The cycle of poverty that leads to the cycle of death
- Workers' access to healthcare and pension benefits
- International policies/Expansion of sugar biofuel production/Market prices for sugar
- Corporate noncompliance to local and regional laws and regulations
- Development of a legal framework for protection

THE CYCLE OF POVERTY THAT LEADS TO THE CYCLE OF DEATH

Work in the sugar plantations is largely viewed as the only job available for people in the communities of Guanacasatal Sur, Chichigalpa. People, mostly men, work under grueling conditions and are diagnosed with CKDu after as little as 2 or 3 cutting seasons. Before the beginning of the harvest, all employees have mandatory creatinine level screenings. If creatinine levels are higher than 1.3 mg/dl, they are not allowed to work and those with contracts are fired. Since nearly all of the people who work in the sugarcane fields live in abject poverty on a roughly $5/day salary, not working means no food for their families. Because they are forced to make this impossible choice, sick men will often use the ID number of someone else and work via subcontractors. The harsh working conditions that incentivize physical overexertion speed up the progression of CKDu. When men become too sick to work or die of CKDu, their young sons often are forced to leave school and start working to support the family. When they get sick after a few seasons, they are presented with the same choice as their fathers.

La Isla has seen this cycle first-hand in our work with affected communities. Low salaries and lack of career and educational opportunities severely limit the choices that affected men and their families have. Many families find themselves forced into the situation that we have described. The effects of the cycle of poverty and death ripple through society, creating a culture of fatalism and a community where people watch their loved ones die in a painful and traumatic way.

In the past, research has tiptoed around the concept of bad work practices as a contributing factor to the epidemic. It is important to emphasize that no epidemic exists in a bubble. Though CKDu does not affect sugarcane workers exclusively, we should not lose focus on communities of sugarcane workers where prevalence is as high as 68% and where 46% of all male deaths in the last 10 years were due to CKDu. Losing sight of this would be a huge disservice to the communities and to research. Digging deeper into the social context surrounding the epidemic is essential to moving research forward.

WORKERS' ACCESS TO HEALTHCARE AND PENSION BENEFITS

When workers' blood tests show high creatinine levels, they are fired and lose access to the plantation hospital. Guanacastal Sur has a small health clinic with limited supplies and the nearby town of Chichigalpa (about a 45 minute to 1 hour walk) has a larger health

clinic, also with limited supplies. The nearest hospital is about 30 km away and means either walking an hour into town to catch a bus, or taking a taxi. It is often an all day ordeal and people cannot afford a day off work. Lost wages and the cost of transportation back and forth is a deterrent from seeking healthcare.

For affected workers, difficulties resulting from inability to physically access healthcare are further compounded by the inability to access the financial benefits of rightfully earned pensions. A new social security law heavily favors the interests of the Nicaraguan government and the industry over those of the worker. The result is that many workers believe they are not eligible for their pension benefits. La Isla has partnered with a lawyer in Chichigalpa to develop outreach materials that explain the ways in which workers may qualify for benefits, how to apply for benefits, and how to file a complaint in cases where benefits have been wrongfully withheld. Additionally, LIF legal staff and local counsel have offered free services for those seeking advice about their pension or social security benefits.

INTERNATIONAL POLICIES/EXPANSION OF SUGAR FOR BIOFUEL PRODUCTION/MARKET PRICES FOR SUGAR

The world's desire for renewable energy with an emphasis on biofuels was a catalyst for a large-scale expansion of sugar production in Nicaragua. In the European Union alone, each member state is required to have 20% of its energy sources and 10% of its transport fuels to be from renewable sources by 2020. This has opened the door for international and multilateral organizations to participate in the expansion of the market, i.e. The World Bank Group. This also led to the displacement of communities previously located among the cane fields and incentivized small-landowners and subsistence farmers to sell their land to the expanding sugar plantations. Finally, this increased demand necessitates higher levels of production, which in turn pressures sugarcane workers to increase their output under already harsh conditions.

Recently, La Isla was asked to join the Bonsucro roundtable, an international certification body, which has developed the first ever metric-based standard for sustainable sugarcane production. The Bonsucro standard requires participating companies to observe core ILO standards, provide a safe working environment, apply Bonsucro human rights standards to suppliers and contractors, pay a national minimum wage, and offer their employees fair contracts. In the absence of fully functioning legal systems, certification regimes such as Bonsucro provide a unique alternative for private sector regulation.

CORPORATE NONCOMPLIANCE WITH LAWS AND REGULATIONS

As researchers, scientists, and medical practitioners work to determine causality; the law provides another entry point in reducing occurrences of the disease. Knowledge of the law and their rights can empower workers to advocate for better working conditions on their own behalf. It can be an avenue towards obtaining compensatory damages or strengthening regulatory and oversight mechanisms. And, once causality is determined, outside of medical interventions, the law provides a platform for measures that need to be taken to reduce instances of the disease.

However, when thinking of the legal mechanisms applicable to those suffering from CKDu, it is important to note that the law, particularly on the international level has not caught up with the realities of the disease. Though international law has long recognized health as a fundamental human right, it has yet to develop a full body of laws that address occupational illness. Traditionally the right to health implies that the law recognizes the right of every member of a country's populace to "enjoy the highest attainable standard of physical and mental health" without discrimination based on race, gender, socio-economic status, religion, or political belief. More specifically, the right to health is interpreted to include the right to freely access adequate medical facilities and services, to be protected from non-consensual medical activities, and the right to access the necessary basic resources such as clean water and food to maintain a healthy lifestyle. Though some of these legal provisions apply broadly to those suffering from CKDu, they fail to account for the unique characteristics of the epidemic. For instance, under international law, CKDu is not recognized as an occupational illness. Furthermore, because causality has yet to be determined, assignment of legal liability under international law in CKDu cases is very difficult. This is why it is often necessary to look at national laws when thinking about legal redress for CKDu within the occupational illness framework. However, where national laws do not exist or are not enforced, international law may be the only source for recourse.

Given the state of the law and the specificity of the issue, which is one of an occupationally associated disease for which causality has yet to be determined; rather than looking for an explicit protection of those suffering from occupational illness within international law, a more instructive approach would be to examine laws related to other instances of state and corporate non-compliance with the law that could precipitate or exacerbate negative health outcomes.

DEVELOPING A LEGAL FRAMEWORK FOR WORKERS' PROTECTION

When developing a general framework for protection, it is important to first address questions of liability. Legally, there are at least two avenues towards assigning responsibility for worker protection - 1) a state can be held responsible for willfully refusing to enforce the law, not allowing fair use of its legal systems, or knowingly permitting violations to occur - this analysis is particular useful in areas of regional and international human rights, 2) a corporate entity may, of course, be held responsible for a violation of state/national law. Most likely a corporate entity will not be held liable under international law.

The LIF legal team is currently in the process of developing a legal framework that can be used to aid in the protection of sugarcane workers suffering from CKDu and their families. Below are a few of the legal sources that the team is in the process of examining for the purposes of framework construction:

National / Domestic Laws - Each country's set of laws and systems for regulation will be different. However, domestic laws often provide the most direct and certain avenue for enforcing protections. In our case, examples of relevant domestic law can be found in the Nicaraguan Constitution, the 1982 Social Security Act and its amendments, the addition of CRI to the official list of occupational illnesses, and the Labor Code.

Though Nicaragua's protections appear extensive on paper, they are often more difficult to access in practice. Rule of law is weak in Nicaragua and the country experiences relatively high levels of corruption at all levels of government. For the purposes of this section, it is important to note that where domestic legal systems fail other avenues for protection should be explored.

Trade Law - One such alternative to domestic law is trade law, particularly bi-lateral trade agreements. Trade law arguably has more enforcement power than other transnational / international legal regimes because of the possibility of imposing sanctions. Though the WTO does not incorporate international labor rights analysis in its rulings, several bilateral agreements have incorporated international labor standards into their trade framework. Examples of bilateral trade agreements include:

- **Free Trade Agreement between the US, Central America, and the Dominican Republic (DR-CAFTA)** - Article 16 of the DR-CAFTA requires that state parties "strive" to ensure that labor rights such as those outlined in the ILO declaration of Fundamental Principles and Rights at Work, including: the freedom of association, the right to organization and collective bargaining, a prohibition against forced labor, elimination of child labor, and ensuring adequate working conditions. However, this is not a firm binding obligation. In fact, parties may only utilize dispute settlement mechanisms under the CAFTA regime for labor issues where a state has repeatedly failed to enforce labor laws in a manner which affects trade between two states.
- **EU Association Agreement with Central America -** This is essentially the EU's free trade agreement with Central America. Similar to the DR CAFTA trade regime, the EU's association agreement recognizes the importance of fundamental principles for worker protection. It also provides mechanisms for civil society participation and a binding process for arbitration if labor standards are violated. This is a relatively new agreement. Time will tell how these provisions work in practice.

Regional Human Rights Law - Regional human rights law is an interesting option since it can carry the force of international law but in some cases may have greater cultural resonance for legal practitioners, advocates, judicial officials, and affected citizens. Mesoamerican examples include:

- **Declaration on the Rights and Duties of Man** - Adopted in 1948 in Bogotá, this declaration concerns the basic rights and duties for individuals. It does not discuss the specific obligations of the state to protect and respect these rights. As a declaration, it is not legally binding
- **American Convention on Human Rights** - This binding convention reflects several of the human rights principles mentioned in across bodies of law. These include freedom of association, the right to assembly, right to a fair trial or due process and the right to privacy.

International Law - International Law is more difficult to enforce than both domestic and trade law. However, it is useful as a norm setting mechanism. Relevant international treaties include:

- **International Labor Organization Treaties -** ILO's core labor standards include: freedom of association, the right to organize, the right to collectively bargain, and the abolition of the worst forms of child labor
- **The International Convenant on Civil and Political Rights and its First Optional Protocol (ICCPR)** - The International Covenant on Civil and Political Rights is for the most part, a self-executing treaty that guarantees the rights to freedom of association, peaceful assembly, due process, and equality before the law. Its optional protocol establishes a committee to hear cases where rights violations occur.
- **The International Convention on Eco-**

nomic Social and Cultural Rights (ICESCR)- The International Convention on Economic Social and Cultural Rights is an instrument that promotes the progressive realization of economic, social, and cultural rights. For our purposes, relevant rights include - the rights to favorable conditions of work, to work in general, to form trade unions, to social security, to an adequate standard of living, and to health.

- **Convention on the Rights of the Disabled** - This relatively new treaty protects the rights of disabled persons to equality before the law, freedom from exploitation, to the enjoyment of the highest attainable standard of health without discrimination based on disability, to work, and to maintain an adequate standard of living.
- **WHO Declaration on Occupation Illness** - This non-binding instrument encourages governments to develop national programs and policies to address occupational health issues. It also encourages employers to plan and design safe and healthy work and workplace environments.

ORGANIZATIONAL CHALLENGES

As the LIF increases its organizational focus on rights protection as means by which to reduce the prevalence of CKDu in its communities of operation, it has faced challenges, particularly in areas related to rule of law and worker intimidation.

Weak judicial mechanisms and lack of rule of law- Transparency International, which has links with local organization "Etica y Transparencia", ranked Nicaragua 134th of 183 countries in its 2011 Corruption Perceptions Index. The World Bank's Governance Matters study ranked Nicaragua in the 24th percentile for rule of law in 2010. The World Economic Forum's Competitive Index Rankings ranked Nicaragua 132nd of 139 countries for judicial independence in 2010-2011. To us, access to justice is a very real issue. Corruption is endemic. In our experience, we have seen fair trial issues do arise for laborers and community advocates at the local level when they pursue claims against larger entities.

Worker Intimidation –According to the US State Department 2012 Investment Climate Statement, labor activists and NGOs alleged that employers routinely violated collective bargaining agreements and labor laws with impunity. Although employers are legally required to reinstate workers fired for union activity, formal reinstatement requires a judicial order which can be difficult to obtain. In practice, employers often do not reinstate workers because of lack of legal enforcement. Labor leaders also complain that employers use company unions to disrupt the organization of independent unions. Discussions that we have had with local community members confirm this phenomenon. Worker intimidation and threats of unfair dismissal have impeded our research efforts on the ground, affecting participation in our studies because of perceived threats to economic and job security. We cannot emphasize the importance of this issue enough. As the CKDu epidemic increases in severity, workers find themselves further at risk for retaliation if they speak out.

LIF LEGAL TEAM PROGRAMMING

Given, the climate in which we work, our relatively new legal team is in the process of evaluating the best ways that the law can be used to increase worker protections. Programming includes:

- Provision of Legal Advisory Services
- Public Education and Outreach
- Legal Research and Advocacy

LESSONS LEARNED

The biggest lesson that we, as an organization, have learned is how much we don't know. Most of the data that we have about the frequency and severity of rights violations is anecdotal. Though researchers have studied working conditions of sugarcane workers, the nature and characteristics of the disease, possible contributing factors, and demographic characteristics; there really hasn't been an interdisciplinary study that looks at socio-economic factors, work place conditions and living conditions against the back drop of legal compliance.

Going forward, we would encourage us all to look ahead not just medically or scientifically but holistically. It may be years before we fully understand CKDu. In the meantime, people will continue to die. Alternative points of intervention for reducing occurrences need to be explored. Even after the disease becomes better understood, informed policy decisions will still need to be made to ensure that this hard earned knowledge is put to good use.

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SUMMARIES OF
ROTATING TABLE DISCUSSIONS

PREVALENCES AND INCIDENCES OF CKD IN GENERAL POPULATIONS AND OCCUPATIONAL GROUPS

Facilitator: Dan Brooks
Rapporteur: Rebecca Laws

This discussion was focused on two fundamental questions:

1. Do we need more prevalence studies? Why or why not? What populations are important to include (i.e. communities, occupational groups)?
2. Do we need incidence studies? What do we gain from them? What populations are important to include (i.e. communities, occupational groups)?

In response to question (1), there were three general views:

- **a.** There is no need for more prevalence studies. We have learned what we can from these types of studies and conducting more would be a diversion of time and resources.
- **b.** We need to perform more prevalence studies because identification of geographic locations and demographic/occupational groups that are higher or lower prevalence can continue to provide clues to the etiology of the disease. The disease is not well characterized in many areas such as Guatemala, Panama, and even the Caribbean coast of Nicaragua and Costa Rica. Outside of Central America, it might be useful to conduct prevalence studies in areas where this disease might be going unnoticed (e.g., Brazil, Egypt, India) but where various hypotheses suggest that there should be an excess of CKD with characteristics similar to what we are seeing in Central America. We still need to understand patterns and trends, and also better understand prevalence in populations that are not necessarily high risk, such as adolescents. Furthermore, we need to better characterize the baseline rate of disease for future research and to provide further ammunition for funding opportunities.
- **c.** From a public health perspective, we need prevalence studies in order to involve government (i.e. Ministries of Health) and focus interventions.

There were several suggestions on how to focus future prevalence studies. First, we can further analyze already existing studies to more closely examine patterns and trends, which will save time and resources. We can also integrate prevalence studies into already existing serial approaches and surveys, such as the Demographic and Health Survey (DHS) and reproductive health surveys of women and children, in those countries in which they are currently administered. It was also suggested that we create a report that pulls together all the information we already have about prevalence in this region to serve as a common reference for everyone moving forward.

In response to question (2), everyone generally felt that there is a need for incidence studies, though it is recognized that they require time and resources. Nearly everyone agreed that we must study the natural history and progression of this disease, and a prospective study will be the best way to accomplish this. It was discussed, however, that this should be done in a determined way, i.e. we should identify a population that best represents a particular geographic area/country, and not just choose a convenience sample. Everyone agreed that we need better surveillance to establish the burden of disease. It was suggested that we study incidence as a part of a surveillance system. In particular, we could use a sentinel surveillance system, which could use a regional approach (i.e. Central America) so that it is not necessarily up to each individual country to implement. Before starting incidence studies, it will be important to identify a case definition for CKDu and discuss novel biomarkers of disease/markers of progression.

BIOMARKERS FOR EARLY DETECTION OF CKD, AND HOW/IF BIOMARKERS CAN BE USED FOR ELUCIDATIONS OF PATHOPHYSIOLOGY

Facilitator: Ricardo Correa Rotter
Rapporteur: James Kaufman

Several common themes arose during the biomarker round tables, including:

1 The need to develop biomarkers for early disease, prior to the progression to stage 3 CKD. These biomarkers might be useful to define the pathophysiology, for early clinical detection of CKD and possible treatment, and as surrogates in intervention studies.

2 Biomarkers for better detection of exposures such as agrichemicals and infections should be developed.

3 Genetic studies should be undertaken to identify genes that may alter the risk of the disease and possibly to identify the environmental trigger.

4 Because of the ease of collection, development should focus on urine biomarkers.

5 Although several biomarkers such as NGAL, IL-18, KIM-1, Hsp72, β2-microglobulin, β1-microglobulin, and NAG have been studied, more information is needed regarding their operational characteristics such as accuracy for diagnosis of acute kidney injury and/or CKD, stability on storage and varying collection conditions, and assay validity.

6 A biorepository should be established with samples from existing studies and future studies to provide a library to a wide group of investigators. Samples should include DNA, urine, serum, and plasma.

In addition to these major themes, other issues raised by the participants included (in no particular order):

1 Cystatin-C may provide a more accurate measure of eGFR.

2 Measurement of urine and serum sodium and osmolality may be useful markers of volume depletion and dehydration.

3 Biomarkers should be developed that predict the susceptibility to the disease.

4 Studies should be done during the work day to identify subclinical renal injury and biomarkers may be an important component of this research effort.

5 Currently available commercial biomarkers may receive marketing hype because of financial considerations. Researchers may have patents and published studies may not be free of bias.

6 It would be useful to have biomarkers that differentiate pre-renal azotemia from actual renal injury. Some suggested that cases defined as pre-renal azotemia may actually involve parenchymal injury.

7 Albuminuria is not considered a reliable biomarker of the disease, although changes within the normal range or even in the range of microalbuminuria have not been carefully explored.

8 Concern was raised that if studies showed abnormalities in some biomarkers before their sensitivity and specificity were well established, they might be employed as screening tools for employment resulting in denying employment to workers without actual kidney disease.

9 Biomarkers will need to be tested in longitudinal studies of more than one zafra season.

10 Uric acid should be studied since it is a good predictor of CKD.

11 Anemia might be useful as a marker for CKD, although it was noted that anemia is a late manifestation of the disease occurring after a significant reduction in eGFR.

12 If we understood the etiology, we could develop better biomarkers.

13 Based on the pathology showing significant glomerular sclerosis, perhaps biomarkers of glomerular disease should be explored. The absence of significant albuminuria, tempered the enthusiasm for this approach.

14 Biomarkers predicting progression would be useful.

15 The cost of biomarkers is a consideration.

16 Heat shock protein in the urine should be investigated.

17 Former workers want research in patients who already have CKD.

18 Need biomarkers to detect pesticides in urine or blood, particularly organophosphate which may be detected for two weeks in the urine.

19 Measurement of serum creatinine in Central America is not always IDMS traceable, but should be.

20 Develop a screening algorithm for biomarkers from a public health perspective.

DIAGNOSTIC PRACTICES FOR EARLY AND LATE DETECTION OF CKD IN DIFFERENT REGIONS

Facilitator: Manuel Cerdas
Rapporteur: Annika Östman Wernerson

There was a general opinion that it was most important to identify **early disease**. We identified **two diagnostic levels**: an *epidemiological* to allow screening for the disease and a *clinical l*evel in order to confirm that the cases identified seem to be suffering from MEN. The objective at this first stage should be to identify the best and cheapest analyzes or methods to be used at each level.

It was pointed out that is essential to use **standardized methods**. The methods for the different analyses in the described pilot studies should be specified to allow comparison of the different regions.

FOR SCREENING – POSSIBLE MEN

The screening should be done community based (not company based).

Potential methods to identify the best and cheapest method for an epidemiological diagnosis:

- Standardized methods for measuring S-creatinine and/or cystatin C to identify persons with impaired renal function
- Blood pressure
- Dip slide to identify the presence or absence of proteinuria. Patients with low grade proteinuria should not be excluded for further diagnosis.
- Exclude diabetes

FOR CONFIRMING THE DIAGNOSIS BY NEPHROLOGISTS

In small cohorts as described above.

Potential methods to identify the best and cheapest method for a clinical diagnosis:

- Clinical history; family history, information about work, liquid intake, medication including intake of NSAIDs
- Imaging - eg to exclude polycystic kidney
- Blood tests- eg electrolytes, hemoglobin, eGFR (creatinine, cystatin C), uric acid, glucose
- Urine samples - degree of proteinuria, hematuria, different biomarkers for tubular damage (NAG, protein HC, β2 microglobulin)
- Kidney biopsy - including light microscopy, immunofluorescence and electron microscopy

The patients included in these pilot studies should then be **followed longitudinally** to identify the natural course of the disease.

If families with an increased risk of being affected by MEN could be identified in these pilot studies, **genetic studies** could be performed.

EXPOSURE LEVELS OF NEPHROTOXINS AND RISK FACTORS FOR CKD IN HUMANS AND THE ENVIRONMENT WITH VALIDITY ASPECTS

Facilitator: Donna Mergler
Rapporteur: Michael McClean

This discussion focused on attempting to prioritize which nephrotoxic agents, or potentially nephrotoxic agents, should be the highest priority as the subject of future research studies. The agents under consideration were those addressed in the presentations during the first day of the meeting, including pesticides, metals, infectious agents, medications, and fructose.

PESTICIDES:

There was general agreement that the evidence in support of pesticides as a casual factor in the epidemic of chronic kidney disease is quite limited. However, it was also recognized that the affected communities strongly believe that pesticides are the cause of the epidemic. Given the limitations of the available information, as well as the concerns within the community, there was general agreement that the potential role of pesticides should be explored in future studies. The investigations should focus on characterizing exposure to specific pesticides using biomarkers whenever possible. Since many of the pesticides of interest have short biological half-lives, biomarkers may only be useful for characterizing acute exposures whereas carefully designed questionnaires may be useful for characterizing chronic exposures.

METALS:

Metals such as mercury, lead, cadmium, and uranium are known to be nephrotoxic but, given the lack of evidence gathered to date, were felt to be a low priority for future studies. However, the consensus of the group was that arsenic should not be downgraded to the same degree, and that speciated arsenic in particular should be evaluated in future studies so that exposure to the inorganic fraction (rather than total arsenic) can be explored. Inorganic arsenic is best measured in urine or nail samples, though each approach has methodological issues that should be considered during study design.

MEDICATIONS:

Interviews with physicians and pharmacists in Central America indicate that nephrotoxic drugs are sold over the counter and widely used to treat pain and other symptoms. In particular, aminoglycoside antibiotics and chronic use of non-steroidal anti-inflammatory drugs (NSAIDs) are associated with acute kidney injury in a dose- and duration-dependent manner. Additionally, 'traditional' medicines based on herbs or other natural ingredients are also used in the region. There was general agreement that the types of medications, amount of use, and frequency of use should be characterized in future studies. If such markers exist, biomarkers of medication use may be preferable to assessment via questionnaire since subjects are unlikely to be able to identify the names of the medications that they use.

INFECTIOUS AGENTS:

Of the various infectious agents that were discussed, the consensus of the group was that leptospirosis should be highest priority to address in future studies. Serological analyses are not well suited to provide information about intensity of exposure or cumulative exposure, but can provide evidence of acute, recent, and/or past infection.

FRUCTOSE:

Fructose consumption has emerged as in interesting hypothesis and there was general support for further investigation as a potential risk factor. A possible biological mechanism was described during the first day of the meeting, and there have been reports that consumption of soft drinks is extremely high in the region.

METHODOLOGICAL ISSUES

In addition to the discussion of special agents, the groups also raised several methodological concerns. First, several groups mentioned the need to consider whether the concentrations of agents measured in blood or urine may be increased as the result of decreased kidney function, which could lead to an artificial conclusion that increased exposure resulted in decreased kidney function (i.e. reverse causation).

Second, there was general consensus that there is a need to characterize the quality of drinking water in the affected regions (i.e. metals, pesticides, hardness, etc), which is important from a general public health perspective even if contaminants in drinking water are not specifically linked to the CKD epidemic.

Third, it was noted that spatial analyses of exposure and/or CKD (i.e. using geographic information systems) would be a potentially useful approach that has not yet been utilized in the region.

Fourth, future studies should consider developing exposure matrices as a way to characterize exposure to specific agents in occupational and/or non-occupational settings. And finally, following an intriguing report that kidney problems have been observed among livestock in the affected regions, it could be useful to consider whether studies of cows or other animals could provide useful information that would be more difficult or impossible to obtain from humans.

SOCIAL AND WORKING CONDITIONS IN AFFECTED POPULATIONS

Facilitator: Aurora Aragon
Rapporteur: Ilana Weiss

Each of the seven groups had lively conversations about the social and working conditions of affected populations. Below is a summary of some of the points the groups had in common as well as highlights from each discussion.

FOCUS ON THE MACRO

An essential part of understanding the drivers of this epidemic is to understand the contributing macro-level factors. For example, asking how the macro-economic demands influence the local working conditions, or how pressure from international development agencies or ethanol and sugar prices impact daily sugar quotas. The answers to these questions can provide some insight on the epidemic and serve as an important context for all our research.

THIRD PARTY CERTIFICATION

Many of the groups identified third-party certifications as having the most potential for an immediate impact on the amelioration of harsh working conditions. However, all agreed that more research needed to be done. Before the scientific community can support 3rd-party certification we need to know what makes for a successful experience and what is merely a bought-and-paid-for certification? What requirements are in place and how are they guaranteed to be true and effective? What surveillance is in place and how are the organizations monitored? These questions must be answered to ensure that certifications do not merely lead to aesthetic improvements.

SYSTEMATIC, STANDARDIZED COLLECTION OF SOCIAL DATA IN STUDIES

We need systematic identification of potential social factors and working conditions that might be contributing causal factors to the epidemic. Using stratification and statistical analyses we can more accurately determine which social determinants are risk factors of the disease. Another important step to take is to standardize data collection across countries. Creating a database of comparable data in all countries where there is an excess of MeN cases would be an enormous tool. Some examples of interesting data points that came up at the tables were: information on education, access to health care, unemployment, length of workday, alternative employment/activities, unemployment, diet, water intake, exposure to pesticides, and poverty.

MAKE THIS VISIBLE TO POLICY MAKERS

The groups determined that in order to break the cycle of poverty, policy makers must be involved. The reticence of government involvement till now focused the conversations on how to engage populations so that the people will go to their governments asking for change. One idea was to further engage the media to get images and stories to the public. The idea is that by going through "the people", special interests can be sidestepped. Another idea was to petition the WHO or the International Kidney Society for an official MeN awareness day. This can engage the larger scientific community as well as the general population and disseminate information about the epidemic on a larger scale.

MIGRANT WORKERS

An important component of the social determinants of this disease is who the workforce is and where they are from. Many groups recognized the importance studying migrant workers, looking at both international and internal migration. Since it is common for cane workers to work the *zafra* then move somewhere else to work in a different field, understanding where they go and what they do could provide important information. Further, studying the different working conditions for contracted and sub-contracted workers could shed light on the distribution of contributing factors of the disease.

FOCUSED RESOURCES/INTERRUPT THE CYCLE OF POVERTY

Social determinants have been described as the causes of the causes of disease. The tables spoke of the importance of integrating social determinants into our research, both to explain aspects of causality and to show to policy makers. The point was also made that in the absence of known causality, an effort to focus resources on interventions less proximal to the cause may have bigger impacts on incidence and prevalence.

HARD WORK, HEAT, DEHYDRATION AND ACCESS TO WATER AND OTHER LIQUIDS

Facilitator: Jennifer Crowe
Rapporteur: Rebekah Lucas

The seven discussions at this table focused on identifying "what we know," "what we need to know" and how to find out what we need to know" regarding hard work, heat, dehydration and access to hydration as they relate to the epidemic of CKD in the region. For the purposes of focusing the discussion, the group discussed sugarcane workers. It was clear that, although similarities exist, there are also important differences in the first-hand experiences participants from different countries report. A summary of the discussions and general conclusions is provided below.

WHAT DO WE KNOW?

Hydration

In both sugarcane and non-sugarcane communities from different countries, there is some quantitative and some anecdotal evidence regarding the drinking habits and the water access of susceptible populations. Common themes included:

- Water is accessed from a variety of sources including wells, city water or company-provided water.
- In many communities, there is a fear of local water sources being contaminated that prevents workers from drinking adequate water.

The amount of water consumed by sugarcane workers tends to vary from one country to the next, and there are reports that range from 1-10 liters per shift. For example, in Costa Rica, workers drink an average of 5 L per shift and 500 ml of rehydration fluid, but before 2012, those with access to hydration fluid were minimal. One person reported an example from Panama where workers consume 4-5 L water and 1 L of rehydration in 500 ml doses. The use of rehydration solution there has reportedly reduced worker desertion by approximately 40% .

Qualitative, qualitative and anecdotal evidence from various countries suggest that sugarcane workers may be working in conditions that promote dehydration both as a result of company practices as well as personal beliefs/behaviors. For example:

- Some workers also report that that drinking high volumes slows them down and prevents them from meeting their quota or goal.
- Some workers perceive that drinking Coca Cola or other soft drinks makes them feel better and that they work for longer when they consume it. (Particularly in El Salvador).
- In many cases, workers have to carry the water they wish to consume with them to the field. As a result, water heats up rapidly making it unpleasant to drink, is often too far from the workers to motivate frequent consumption, and is limited by the worker's desire to carry water in addition to the other things he must take to the field.
- In cases where workers carry their own water to the field, the containers used are of questionable origin and may themselves be sources of toxins. The example was given from El Salvador of old pesticide containers being used.

Hard work and heat

According to international standards harvesting sugarcane is classified as hard manual labor. Harvesters are exposed to sun and heat as evidenced by WBGT data from Costa Rica. In interviews and anecdotal evidence from multiple countries, workers report being "over sunned." Work conditions can differ between companies, occupations and countries. For example, length of the shift, payment scheme (fixed quota versus fixed timeframe), water access, rehydration supplementation (i.e., "bolis"), and ambient temperatures can differ depending on the time of year, geographical location or the employer.

Generally, men over the age of 18 are employed, though women cut sugarcane in some countries, notably Nicaragua. Most sugarcane harvesters have other jobs during the non-harvest season and some tend to their own farms during the harvest season, and are therefore exposed to a number of risk factors.

Sugarcane harvesters are not the only vulnerable occupation, though they tend to have a higher heat exposure than most other jobs when taking into account metabolic load and ambient conditions.

WHAT DO WE NEED TO KNOW?

On the one hand, it is clear that sugarcane har-

vesters are often under heat stress and that dehydration can easily happen due to the logistics present in many workplaces. This means that intervention is necessary based on already existing knowledge to protect worker health and safety. However, in the context of examining the hypothesis that heat exposure and/or dehydration are linked to the development of chronic kidney disease in Mesoamerica and Panama, there are a number of research questions that need to be answered. The areas discussed at the table are summarized below.

The types of fluids workers are consuming and how they are consuming it

How does this relate to the maintenance of their fluid balance? What's the best practice? In the case that workers carry their own water to the field:

- Does this limit how much they choose to drink? Does this cause a meaningful increase in metabolic load?
- Are old pesticide containers sometimes used as water containers and if so, what chemical exposures does this cause?

Do workers drink in response to thirst or to prevent thirst?

This timing of drinking affects the renin-angiotensin system and may impact kidney function.

Water quality: Is it safe to drink local water sources in the volumes recommended?

It is essential to guarantee water safety in any study that aims to improve (likely through increasing consumption) the hydration of workers.

- What deterrents are there to drinking from local water sources (i.e., lack of access or fear of possible contamination)?
- Can these deterrents be confirmed or discounted? Can they be circumvented?
- Do such deterrents lead to a higher consumption of soft drinks?

Dietary considerations

- Should workers take salt tablets to aid the rehydration process?
- What is the salt content of their existing diet?

Heat illness history

- Is prior heat strain (i.e., recent exposure to excessive heat stress) recognized as significantly increasing the risk of heat illness and heat stroke? (i.e. Workers who go to the field in the day(s) following heat strain are at higher risk for further heat illness.)
- Which heat illness symptoms do workers frequently experience? Could such information could be important in the identification of suitable bio-markers?

Exposure levels

- The level of heat exposure has yet to be fully quantified across an entire harvest season.
- What is the degree of heat exposure (ambient and metabolic combined) in each country or at different altitudes/microclimates within countries?
- What degree of dehydration do workers experience? How frequently?
- Does heat stress and/or dehydration exacerbate underlying causes of the disease?
- Is heat a contributing factor or a primary cause?

Historical data

- What were work conditions like 10, 20, 30 years ago?
- Have work conditions (particularly related to heat exposure and hydration) changed? If so, in what way and when?
- Has the climate in the different countries changed significantly?

Childhood exposure

- Could exposure to certain risk factors during childhood initiate the disease process? What risk factors are present during childhood?
- Are children working in subsistence farming?
- What were the childhood exposures of harvesters who are currently in the field or diagnosed with CKD?
- Could childhood or adolescent exposures be affecting workers in later life?

Anti-inflammatory drugs (NSAIDs)

- Who takes them? How often are they taken? Why are they taken?

- Could improving working conditions lessen the consumption of NSAIDs? (i.e. Are workers who are less heat exposed and better hydrated less likely to take NSAIDs?

Alcohol consumption

Since alcohol consumption can be directly related to hydration and can increase the probability of heat illnesses, it is important to know:

- What and how much is consumed (current and past exposures)?
- How is homemade alcohol made? (Could this be a source of inorganic phosphate consumption?)

Intervention studies

- Which interventions are the most beneficial and applicable to the work environment?
- It was mentioned that, although research on different hydration methods is necessary to protect workers, that researchers must also be careful not to fall into the trap of "making a business out of hydrating."

Can we standardize hydration recommendations in the region (through policy recommendations)?

HOW DO WE FIND OUT WHAT WE NEED TO KNOW?

Qualitative research is very important and has a vital role in focusing quantitative research, i.e., what cultural traditions influence work conditions or work habits or what interventions are feasible for the community in question.

Quantitative methods could be used to address the following issues:

Heat and dehydration exposure:

It is necessary to achieve accurate measurement of fluid balance and sweat loss when working. Table participants who have attempted this in the field confirmed that it is very difficult to do well. For example, scales don't function properly in the heat and uneven terrain, scales have to be carried for more than a kilometer in some cases to get to the worksite and it is impossible to get workers to weight in the nude. There was some disagreement on the accuracy and use of urinary biomarkers for hydration, but it was agreed that it would be helpful to assess potential heat and fluid balance biomarkers (i.e., urine sodium and potassium levels or heat shock protein) and it was stressed that obtaining blood samples from participants is often difficult.

In assessing heat exposure, it is important to obtain achieve accurate measurement of metabolic heat generation when working. International standards can generally be used successfully for this purpose, but new, simpler methodologies would be welcome.

It would be extremely helpful to develop an exposure matrix for heat, hydration status and metabolic load for different occupations and job titles.

Intervention studies – developing and testing intervention protocols:

It was clear that intervention studies need to be done. Improvements need to be made in working conditions, but those improvements need to be evaluated to determine which are the most beneficial and relevant. Quantitative and qualitative methods should be used, but the studies (at least at first should be small scale (pilot) and should use repeat measures design (pre and post intervention).

Men, women and children should be included in studies where possible to compare exposures and outcomes between groups.

Historical data

Historical data could be used to investigate possible changes in behavior, work and/or living conditions potentially contributing to CKD. Possible options for obtaining such data include:

- Conduct interviews to gain historical perspectives from workers.
- Data mining.
- Investigate time trends with respect to disease incidence (i.e., seasonality or mortality trends and mortality mapping).

BELIEFS, DISBELIEFS, POLITICAL AND LEGAL ACCEPTANCE OF CKD EPIDEMIC IN MESOAMERICA AND ELSEWHERE

Facilitator: Agnes Soares da Silva
Rapporteur: Y-Vonne Hutchinson

There were seven rounds of group discussions; each presented some specific insights to the topic. This report does not reproduce the full range of the discussions and the richness of the debate. It is rather a summary of the salient points that were touched by all the groups.

AS WE TRY STRUGGLE WITH HOW THE DISEASE IS DEFINED (EPIDEMIC OR ENDEMIC, OCCUPATIONAL?), DO WE RISK ALIENATING PATIENTS OR THOSE AT RISK WHO DO NOT FIT CERTAIN PROFILES (CHILDREN, INFORMAL WORKERS)? SHOULD WE RENAME THE DISEASE TO ENCOURAGE ACCEPTANCE?

Roundtable participants considered the effects of classification efforts on public acceptance of the disease. An ongoing point of debate that arose both during the conference presentations and in our roundtable discussions was whether the disease should be considered endemic or epidemic, or both. Additionally several roundtable participants mentioned that a narrow focus on the occupational nature of the disease could unintentionally alienate certain groups of people such as rice or corn farmers, child laborers, or participants in the informal workforce, who do not fit into the category of sugarcane or mining laborers. It was suggested that as we learn more about the disease, we should consider changing the name to avoid narrowing the focus, as this could mistakenly lead to investigations and policy actions being done only to cover the groups mentioned. However, in subsequent discussions, it was countered that by drawing the attention away from the clearly high risk group of sugarcane cutters towards other groups not yet identified as at very high risk, we allow urgently needed policy actions to be delayed for this most affected group. This has happened in Nicaragua. For etiologic research, all groups are important, but for prioritized and focused policy actions, those at highest risk now should be targeted first.

LEGAL ACCEPTANCE IS NOT THE SAME AS POLITICAL ACCEPTANCE

In Nicaragua, the disease is recognized as occupational, in El Salvador it is not. However, this does not mean that the workers in Nicaragua are better off than in El Salvador. Additionally, the focus of the international health community is on NCDs (non communicable diseases) with the strategy of "four diseases, four risks", which does not include CKD. This happened despite the fact that CKD was included in the Regional Mexican Declaration on NCDs, a proposal made by the Minister of Health of El Salvador. This means that the governments do not receive external aid to deal with the disease as well, as donors tend to follow the global health agenda.

PERHAPS WE SHOULD LEARN FROM THE HIV EXPERIENCE IN WHICH A CROSS-SECTORAL APPROACH CONTRIBUTED TO TREATMENT AND PREVENTION EFFORTS

Roundtable participants also mentioned that we should look at cross-sectoral models of interventions, as an integrated public health approach that incorporates all sectors may be more effective. A comparison was made with HIV/AIDS that received a lot of attention when it started affecting high income groups. CKD primarily affects lower-income agricultural laborers, and it will not reach that stage. However, it was the concerted actions that halted the AIDS epidemics in most countries, not isolated interventions.

THERE IS GOVERNMENT INTEREST AND AWARENESS OF THE ISSUE; HOWEVER THIS DOES NOT TRANSLATE INTO POLICY OR ACTION. PERHAPS, BECAUSE SUCH ACTIONS COULD GO AGAINST A GOVERNMENT'S ECONOMIC INTERESTS

Many participants noted that across regions there is government awareness of the issue. However, this awareness has not translated into political or legal action. For example, it was mentioned that in Nicaragua protection exists only on paper. In fact, recent changes to the social security law made it harder for workers to receive benefits. This is perhaps be-

cause these countries are highly dependent on the agro-industry, and the governments are cautious in proposing actions that could negatively impact sugarcane production, for instance.

Moreover, in the absence of political pressure, companies have little incentive to change their policies. However, there are other ways to influence company practices. The example was given, also in Nicaragua, where sugarcane producers claimed to not have funds for hydration initiatives for field workers. However, once articles were published on the BBC website and in The Guardian, the companies suddenly found funds for hydration for thousands of workers. Therefore public influence can be an important tool in promoting legal and political acceptance.

WHERE THE GOVERNMENT DOES ACT, IT OFTEN HAPPENS IN THE ABSENCE OF COORDINATION WITH ACADEMIC RESEARCHERS

It was mentioned that the universities do not have access to sufficient funds to do all the research that they feel is needed. Governments devote resources to their own identified research priorities, making it more difficult for independent research and academic institutions to obtain funding. Furthermore, there is not much cross-institutional collaboration or cooperation, resulting in work mostly done in isolation. Research priorities are not agreed upon in any forum, and therefore each group (universities, NGOs, governments) work each on their own priority agenda. Researchers complain that sometimes they feel that government priorities do not fall in line with what they see on the ground.

SOME PATIENTS BELIEVE THE ONLY CAUSE OF THE DISEASE IS THEIR CONTACT WITH PESTICIDES. OTHERS RESIST DIAGNOSIS AND TREATMENT, BECAUSE THEY PERCEIVE THE DISEASE AS A DEATH SENTENCE OR A PUNISHMENT. ALSO, THEY RELATE GOING TO THE HOSPITAL FOR TREATMENT AS ACCELERATING DEATH, AS MOST PATIENTS DIE SHORTLY AFTER STARTING DIALYSIS

Because risk communication is often a neglected aspect of the work done in the most affected communities, and scientific uncertainties are difficult to understand and communicate, communities at risk have, in some cases, latched on to disputable causes of the disease. It was mentioned that in El Salvador and Sri Lanka most patients believe that the disease is solely due to pesticide use. In both cases the populations tend to believe their own hypotheses than those offered by the scientific community, which prevent them from drinking enough water during the work time. Although dehydration may not be the sole cause of CKD, it certainly increases the risk of kidney damage.

Additionally, patients will resist diagnosis and treatment because of personal beliefs. For instance, in El Salvador, many people believe that sickness is a punishment from God. They prefer healing by spiritual means over medical assistance. In the case of Sri Lanka, after diagnosis patients do not want to return for follow up. They believe that death is imminent or equate treatment with death and would rather die at home. It was suggested that medical practitioners should consider incorporating patients' beliefs about their illness into treatment and that more concerted public education efforts should be undertaken.

SUMMARIES OF
WORKING GROUP DISCUSSIONS

CROSS SECTIONAL STUDIES WITH ATTENTION TO TARGET GROUPS AND EXPOSURE MEASURES

Facilitator/Rapporteur: Aurora Aragón
Participants: Ricardo Leiva, Zulma Trujillo, Manuel Cerdas, Desmond Williams

The first point discussed was whether this type of nephropathy is a problem faced by the whole general population or specific occupational and/or geographically related groups. We also discussed whether it is likely that there is only "one cause," or if there are several factors involved. To respond to such questions, the group found it important to perform more cross sectional studies.

The group further discussed the following: It is important to characterize prevalence of traditional and non traditional risk factors in Mesoamerica to make sure that we are facing a new entity characteristic of Mesoamerican countries.

To better understand the magnitude of the problem we propose to perform cross sectional surveys at least in four countries in Mesoamerica.

We recommend that the sampling frame should represent geographical, sex and occupational distribution, rural and urban populations. Emphasis should be made on hot working conditions and it is important to start including women doing strenuous jobs working in hot environments.

The questionnaires applied should contain core questions for all countries in addition to specific questions by country. Concerning occupational history, it is important to investigate those occupations that have mostly been associated with the disease as well as other occupations.

Medical history should emphasize the history of transmittable diseases and NSAIDS use,

Physical examination should include anthropometric measures and blood pressure.

Single urine and blood samples should at least be performed with early morning samples and, if feasible, include measures at the end of work shift.

Questions related to amounts of drinking water, (including sources and quality), fructose drinks, heat stress and recent history of signs and symptoms of dehydration should be included.

NATIONAL AND INTERNATIONAL COLLABORATORS

In all countries, the natural counterpart should be the Ministries of Health as COMISCA has stated this disease as priority among the chronic diseases in Central American countries and Dominican Republic.

Other collaborators should be water institutions (for information and quality of water determination), social security systems, statistics and census national institutes, societies of nephrology, employers of the main companies in the countries and universities.. We should also look for institutions related to agriculture, environment and mining and to include other local health-related organizations, and community organizations.

International organizations that should be involved are PAHO and CDC.

Concerning potential funding options we propose to look at:

1. LATINAMERICAN SOCIETY OF NEPHROLOGY
2. EU/PAHO/CDC/GLOBAL FUND/NIH,

To include the study of other chronic diseases such as diabetes, hypertension, and metabolic syndrome might make a funding proposal more attractive.

We propose two options for naming the initiative:

* **MENRY**
  (**M**ESOAMERICAN ENDEMIC NEPHROPATY **R**ESEARCH **I**NITIATIVE)
* **IPINEM**
  (**I**NICIATIVA **P**ARA LA **I**NVESTIGACION DE LA **N**EFROPATIA **E**NDEMICA **M**ESOAMERICANA)

CASE-CONTROL STUDIES

Facilitators: David Wegman, Michael McClean
Rapporteur: Nate Raines
Participants: Hildaura Acosta, Juan Jose Amador, Olaf Jensen, Carlos Orantes, Annika Östman-Wernerson, Reina Turcios Ruiz

Case-control studies can be a powerful tool in investigating epidemics, particularly during the initial identification of risk factors. Our working group discussed their potential utility in the continuing investigation of the Mesoamerican Endemic Nephropathy (MEN) epidemic. In general, we concluded that case-control designs have limited value for understanding etiology due to the difficulty in collecting historical information on specific exposures and/or the low prevalence of specific exposures among cases and controls. Prospective studies are preferable for studying etiology due to the need for quantitative exposure assessment and the apparent multifactor etiology of the disease. Because a case-control study has considerably lower time and monetary costs, it can be helpful as a tool in contributing to the decision on which hypotheses merit further exploration through prospective designs.

Our group discussed a number of specific suggestions for new case-control studies. We concluded that they may be more useful for population-based rather than industry-based studies, potentially as part of a multi-center, cross-national study to evaluate risk factors in a population not restricted to a specific occupational group. Recruitment of cases could be conducted through hospitals or health clinics, or through whole population census or random sampling. Women and younger populations would be useful groups to focus on, or at least endeavor to represent well, in future case-control designs since less is known about the disease in these groups. We also noted that most current surveillance in MEN-affected regions, as well as our current methods for identifying cases in an otherwise healthy population, are not conducive to identifying cases of early or subclinical disease, limiting the utility of the case-control design in investigating exposures leading to the initial development of subclinical nephropathy.

There are a number of areas of investigation where case-control designs appear useful tools in future examination of the MEN epidemic. Generally, these are areas where the study is directed at the role of risk factors that are present early in life or exposures that can be measured long after they are experienced. For example, the case-control design could be effective in studies of genetic risk factors for MEN, both in the investigation of specific genes and combinations of genetic characteristics. Other areas of investigation include antibody markers of prior infection, metals and chemicals that produce long-term biomarkers, and factors included in hospital records such as prescription antibiotic use. The study of occupational exposures may be appropriate, particularly with people who have done the same work for a long time and with whom we think measuring current exposures is representative of past exposures. Such exposures, however, would need to be of at least moderate prevalence in the study base. Factors like personal behaviors can be investigated through case-control, although assessment of these needs to be well standardized to address recall bias.

Case definition is crucial when designing case-control studies. The central conclusions of this workshop regarding case definition are discussed in the Case Definition working group. With respect to case-control investigation, it remains crucial that case definitions are consistent and clear across different studies in different settings and countries. Furthermore, it is highly desirable that the methods for identifying cases be relatively easy, non-invasive (if possible) and low-cost.

Exposure assessment was also addressed in greater depth in a separate working group. In general, we concluded that exposures are best assessed through measurement of biomarkers, application of validated questionnaires, and review of accurate historical records where available. Biomarkers of long-term exposure (ie antibodies, or metals and chemicals and their metabolites which remain in the body for long periods of time) are ideal but not essential; biomarkers of short-term exposure can also be useful if the exposures are routine and we have additional data on the duration of that exposure. Carefully constructed and validated questionnaires can be useful tools for characterizing both occupational and nonoccupational exposures when direct measures or historical records are not available. Diet and medication can also be evaluated with questionnaires again requiring careful construction and validation. Accurate records do not exist for many exposures of interest, but may be available in some instances; for example, reviewing prescriptions obtained from a dispensary can indicate intake of nephrotoxic medications, or payment received during a working season might be used as a proxy for tons of crop cut.

Prospective cohort studies and experimental

studies will be preferable designs for addressing particular research questions. However, case-control studies should not be discounted as a useful tool, particularly where effective means of evaluating past exposures exist.

COHORT STUDY DESIGN FOR STUDYING INCIDENCE AND CAUSES OF CKD IN MESOAMERICA

Reported by Daniel Brooks and Christer Hogstedt
Participants: Ineke Wesseling, Ingvar Lundberg, Alejandro Riefkohl, Rebecca Laws, Marvin González, Cinthya Bonilla, Ramón Vanegas

SUMMARY

The working group discussed a number of design and logistical issues regarding the implementation of a cohort study to study the causes of MEN. The group first concluded that despite the efficiencies of a retrospective cohort study, a prospective study would be necessary to capture the necessary exposure and covariate information. While it was acknowledged that an occupationally-based cohort would be best for studying occupational hypotheses, such as heat exposure/strenuous work, the discussion focused primarily on a population-based cohort. The workgroup also thought that the primary outcome should be CKD rather than intermediate markers. The size of the cohort was estimated to range between 2500 and 9000, depending in part on whether sex-specific risks among men or women were deemed necessary. A rough estimate of cost for a 5-year study of both men and women ranged from $6.3 to $10 million USD, depending on cohort size and frequency of follow-up. In order to lay the groundwork for a cohort study of this magnitude, it would be necessary to conduct preliminary studies to: (1) accurately and efficiently measure exposure factors of interest and identify populations that would provide variation in exposure, and (2) demonstrate that it is possible to retain a sufficient proportion of subjects over the follow-up period.

BACKGROUND

A cohort study generally begins with identification of a defined, fixed population in which exposure status is assessed at baseline (and may be updated over time) and follows it for development of one or more outcomes. Cohort studies can be designed in different ways: of most relevance in the context of CKD in Central America is the nature of the cohort (specific or general) and the timing (prospective or retrospective). The appropriateness of these designs is affected by the nature and rarity of the exposure(s), frequency of the outcome, duration between exposure and outcome, and availability of information.

Exposures and outcomes

There are a variety of potential causes that have been proposed for MEN; not all fit well into a single cohort study design, so an important step would be to identify the key exposures of interest and design the study around them. At the same time, many researchers have suggested that the cause of MEN may involve more than one factor. Therefore, it would be important to be able to consider multiple exposures within one study.

A study that used intermediate biomarkers as outcomes would be smaller and less expensive. However, given the uncertainty regarding the predictive value of biomarkers for the endpoint of CKD, it is probably necessary to use CKD itself as the primary outcome. Intermediate outcomes should, of course, be measured as well.

Is a specific or general cohort better for study of MEN?

Nature of cohort: A *specific* cohort is based on the identification of a group of people who are considered exposed to some agent or condition, as well as a group not exposed. The hallmark of a specific cohort is that subjects are selected based on whether or not they are exposed to a specific factor. A *general* cohort is based on identification of a large group of people who share a common characteristic (e.g., geography, sex, age, etc.), but not directly based on their exposure status. Because of this less direct selection based on exposure, general cohorts generally are larger and/or focus on more common exposures than do specific cohorts.

A specific cohort would ensure that the targeted exposure(s) were well represented and require a smaller number of subjects, if a group with high exposures can be identified. For an occupational hypothesis such as heat exposure/strenuous work or agrichemical exposure, groups with high and low exposure to these conditions could be identified and enrolled. If it is difficult to obtain access to an occupational based cohort, targeting of higher and lower risk geographic areas could serve as a substitute. The statistical power of the study would be maximized if the key exposure(s) were present in almost 50% of

subjects. Such a high figure would be difficult to reach in a general cohort unless the exposures were naturally that common in the population. A general cohort would therefore require many more subjects.

▸ *Is a prospective or retrospective study better for study of MEN?*

A *prospective* cohort study begins with subjects without the disease, while a *retrospective* study is established after some of the subjects have developed disease. A prospective study can collect information on exposure and disease in real time but is more expensive and takes more time to complete, while a retrospective study must rely on records or other sources of information to retroactively determine exposure and disease status but is less expensive and can be completed much more quickly. There are also ambispective studies combining the retro- and prospective designs.

The working group briefly considered the possibility of a retrospective cohort study, which is frequently conducted in occupational settings where there are good employment records, but quickly agreed that it was very unlikely that companies in the region had records from the past of sufficient detail and duration to properly assess exposure status, and that it could be quite difficult to locate people to determine whether they had developed CKD. However, should the opportunity present itself, such a study should be considered because of the efficiencies in time and cost.

In addition to companies, other potential sources of records for retrospective cohort studies might include registries of workers from unions, social security, or labor insurance programs, Census data collection in different localities, general health surveys, prior prevalence studies, and health center records of CKD patients (this last source perhaps appropriate for a study of progression).

There must be a sufficient number of incident cases of CKD in the study base. There are no good data on incidence of new cases in Mesoamerica.

PROSPECTIVE MULTINATIONAL COHORT STUDY AND WARRANTED PRE-STUDIES

▸ *Realistic study base*

Study population: Given the prominence of occupational heat exposure/strenuous work as a hypothesis, occupationally based cohorts that reflected a range of exposure levels might be an advantage. However, concern was raised about the feasibility of obtaining access to these populations. Alternatively, selection of populations from different areas where a particular industry or type of work is the main source of employment could also provide the variation in exposure levels needed for an effective study. Such an approach has been used for prevalence studies in Nicaragua and El Salvador, where certain geographic locations were used to represent different types of industries, crops, etc. The study population should be relatively young (e.g., upper age limit 65 years or younger) to increase the likelihood that cases of CKD represented Mesoamerican nephropathy rather than more typical forms of the disease. It would also be best if the study population was drawn from different countries to reduce the possibility that results reflected unique local conditions rather than factors that are driving the broader epidemic.

Timeframe: For purposes of discussion, we assume a five-year grant period, with a first year of planning and preparation, three years of recruitment and follow-up, and one year of analysis and report preparation.

Outcomes: The primary outcome would be CKD, as determined by suitable kidney function tests, e.g. Serum Creatinine (SCr) and estimated glomerular filtration rate (eGFR). For individuals who die prior to testing, mortality data and medical records could be accessed for cause of death. Secondary outcomes would be biomarkers of kidney injury with specific biomarkers to be determined based on consultation with experts in the field. Testing would occur every year, which would allow for early diagnosis, information on natural history, maintenance of contact to reduce loss to follow-up (LTF), and updating of exposure status.

Major determinants and exposures: Potential factors that could be assessed in such a study, whether occupationally- or residentially-based, include life-long residential and occupational history, including climate, ergonomic, and chemical exposures; amount, frequency, and type of fluid consumption at work and at home; inorganic arsenic exposure; pharmaceuticals (particularly NSAIDS) and natural remedies; infectious diseases; fructose intake; and possibly others.

Current occupational exposure to heat/strenuous work could be measured directly in an occupational cohort. Current exposure in a residential cohort and past exposure in either cohort would need to rely on a job-exposure matrix, based to the extent possible on actual observation and measurement that could be developed as part of preparatory studies (see below).

In addition to exposure assessment of individual subjects, environmental analyses could be conducted to evaluate potential factors in water (e.g., hardness, arsenic and other heavy metals, agrichemicals, etc.), sediments, soil, air, and/or food. Also determinants of exposures should be examined in an historical context such as work-organization, contractual issues, child labor, migrancy, and economic and agricultural policies. **Cohort size:** A major factor in the feasibility of such a study is the required number of subjects. During the workgroup discussion, a "guesstimate" resulted in a sample of 10 000 men and 5 000 women. A more careful post-workshop analysis resulted in lower estimates for men and higher for women. A set of tables with a range of assumptions specified and additional caveats noted is attached as appendix 1.

Assuming the assumptions are within a reasonable range, we estimate that a sample size of approximately 2500 men and 6630 women would be appropriate if the goal was to assess factors causing CKD in both men and women separately. From the perspective of cost efficiency, one might choose to first only study men, which would require only about one-fourth as many subjects. If the goal was to assess these factors in a combined group, then a combined total of 3480 men and women equally represented in the cohort would be sufficient. However, there would be insufficient power to draw conclusions about either group separately, which would seem to be problematic given the stress on how the disease disproportionately affects men. Unless it were possible to identify a specific cohort of highly exposed women, some consideration must be given to the idea that it may not be realistically possible to study women in a cohort design, at least not until the basic causes are determined and a more targeted cohort could perhaps be identified.

These figures should still be considered as very rough estimates, but they are at least based on a more systematic analysis than the figures presented by this workgroup at the workshop. We made some guessing during the workshop on the costs of such a study and gave an interval of 5-10 million US dollars. After the workshop we have made some still very crude estimates with many assumptions and come within that range – c.f. appendix 2. The costs could easily be recalculated for e.g. a men only study base.

➤ *Pre-studies to motivate the potential and realism in an application of this size*

a) Exper*iences from follow-up of performed cross-sectional studies in Nicaragua and El Salvador*

Follow-up studies of already performed (and in most cases published) cross-sectional studies would clarify the problems and potentials for tracing investigated persons over 3-5 years as well as give indications of the number of and time for converting from e.g. GFR stage 2 to 3 and the possibility to validate and test new CKD markers.

b) *Investigations in high and low prevalence areas of hypothesized risk exposures for power calculations and for choosing study populations for a prospective study on kidney disorders*

- Heat and exertion investigations (e.g. creating a job-exposure matrix concerning those exposures)
- Environmental analyses of water hardness, metals, pesticides, fertilizer residues, etc. in pertinent substrates
- Biological sampling/monitoring of metals (especially inorganic arsenic), leptospirosis and more
- Diet, water consumption and other beverages
- Medicine consumption, particularly NSAIDS, in general and at different periods of job activities
- Kidney biopsies (from early stage CKD as well as ESRD and deceased patients)
- Genotyping

APPENDICES

Appendix1. Cohort study sample size estimates

Men only

SCENARIO	INCIDENCE RATE EXPOSED	INCIDENCE RATE UNEXPOSED	RR	PERCENT EXPOSED	RATIO EXP : UNEXP	N EXPOSED	N UNEXPOSED	N TOTAL
1	.03	.01	3	25%	1:3	243	729	972
2	.03	.015	2	25%	1:3	501	1504	2005
3	.03	.015	2	33%	1:2	576	1151	1727
4	.03	.015	2	50%	1:1	823	823	1646
5	.02	.067	3	50%	1:1	614	614	1228
6	.02	.01	2	50%	1:1	1223	1223	2446
7	.01	.005	2	50%	1:1	2506	2506	5012

Women only (assuming that unexposed women have same rate as unexposed men, but that women are less likely to be exposed and/or exposed women have less extreme exposure resulting in lower RR).

Constants: 85% power, 3-year follow-up, 20% LTF

Scenario 1-7 represents same base rate as men but exposure ranges from 10-20% compared to 25-50% among men

Scenario 1a-7a represents the same conditions as the above scenarios + a reduction in the RR compared to men (3->2, 2->1.5)

SCENARIO	IR_E	IR_U	RR	E:U RATIO	NE	NU	NT
1	.03	.01	3	1:9	410	3690	4100
2	.03	.015	2	1:9	880	7920	8800
3	.03	.015	2	1:6	936	5616	6552
4	.03	.015	2	1:4	1020	4080	5100
5	.02	.0067	3	1:4	733	2932	3665
6	.02	.01	2	1:4	1542	6168	7710
7	.01	.005	2	1:4	3106	12424	15530
1a	.02	.01	2	1:9	1330	11970	13300
2a	.022	.015	1.5	1:9	3086	27774	30860
3a	.022	.015	1.5	1:6	3262	19572	22834
4a	.022	.015	1.5	1:4	3526	14104	17630
5a	.013	.0067	2	1:4	2312	9248	11560
6a	.015	.01	1.5	1:4	5321	21284	26605
7a	.0075	.005	1.5	1:4	10708	42832	53540

Additional caveats beyond the stated assumptions used to produce the above estimates:

1 For the mixed group, we would only have power for a mixed group in general, not for men or women alone.

2 The estimates are based on the cohorts being constructed in such a way that the exposure of interest is relatively common (range 25-50%). This might not be too difficult for a more common exposure such as heat/strenuous work among men in the areas of concern, but would be difficult if we want to calculate power on a less common exposure.

3 The estimates are based on the assumption that we can dichotomize between high exposure and little/no exposure. If we have a more continuous distribution instead and we categorize exposure, we will have 3-4 categories. This might or might not reduce our power to compare the highest to lowest categories, depending on assumptions, though it would allow us to make a dose-response assessment.

4 Many researchers have suggested that there might be an interaction. Again, given different assumptions, this might increase the necessary sample size.

Appendix 2. Crudely estimated costs for a follow-up study during 1+ 4 + 1 years

a) *1 year of planning and preparations for a 3 year follow-up study*

One full-time senior researcher salary (could be 2-4 persons sharing) á $ 130 000/year incl personal overheads plus 25% university overheads = $ 162 500

b) *Field study: first-time interview and clinical investigation*

During one year after establishing a study base of 9130 persons with 45 persons investigated per day (5 days/week) by 3 teams of two persons per team, lab costs, field living costs and a program center staff of 3 full-time persons for tracing persons, organizing the field study and checking the data flow could crudely be estimated to be 9 persons paid on average US$30 000/year (incl personal overhead) = $ 270 000

Lab costs, job loss substitution etc US $100/ study persons = $913 000

Per diems and hotel rooms for 200 days of field work for 6 persons at $100/day = $ 120 000

Car costs, office, computers, mobile phones, consultants and unforeseen = $ 117 000

University overheads, incl. accountants and audit experts, 25% = $ 355 000

Crude Sub-total of b) = $ 1 775 000

c) *Exposure measurements during a 4year follow-up time*

Estimated cost = $ 400 000 (300 000 plus university over-head)

d) *Interviews and lab tests after 3 years follow-up (during 4 years from the first to the last study person)*

Could perhaps be estimated as the same as for the base-line study. Fewer will be investigated due to loss to follow-up but the efforts to find people will probably cost as much = $ 1775 000

e) *Compilation of collected data during five years and analyses after data collection*

One statistician half-time during 4 years at $ 65 000/year and full-time during one year at $ 130 000,

One PI half-time during 4 years at $ 65 000/year and full-time during one year at $ 130 000, two doctoral student salaries during five years at $ 100 000/year and student

Subtotal for e) $ 1700 000 and with 25% university overheads: $ 2 225 000

Crude Grand Total: $ 6 337 500 ($ 162 500 + 1 775 000 + 400 000 + 1 775 000 + 2 225 000)

COMMENT

If the study persons would be called for interviews and tests also after 1 and 2 years this would probably increase the cost with 2 x $ 1 775 000, i.e. a total of the order of $ 10 000 000 (9 887 500)

INTERVENTION STUDIES

Facilitators: Agnes Soares and Carl-Gustaf Elinder
Rapporteur: Jennifer Crowe
Participants:Carolina Guzmán, Andrés Robles, Ramón Antonio García-Trabanino

THE NEED FOR INTERVENTION STUDIES

Working group participants agreed that intervention studies are necessary but challenging since we cannot propose interventions designed to eliminate only one risk factor associated with chronic kidney disease in workers in Mesoamerica, as there is great uncertainty related to the cause of the disease. Nonetheless, it is clear that working in hot conditions, dehydration and use of NSAIDs are well-established risks for those in early stages of chronic kidney disease (CKD).Therefore, avoiding those risks is a sound advice for working populations known to be at risk.

Specifically, recommendations include:

- Give time for rest and rehydration
- Pay should be by the hour rather than by the amount of work achieved
- Guarantee of the quality of the water (free of harmful levels of physical, biological or chemical contaminants)
- Access to primary health care services, and prevention of the use of NSAIDS;
- Do not exclude any possibility of causal relationship a priori, unless there is sufficient scientific evidence of no causal relationship. (No evidence of causal relationship does not mean there is evidence of no causal relationship.)

SMALL, WELL CONDUCTED STUDIES NEEDED

We recommend small, well-conducted controlled studies comparing ordinary/present with optimal/adjusted intake of water and salt during heavy hot work such as during sugarcane harvest (zafra). These studies could ideally be replicated in multiple worksites and countries. This appears to be a good time to do such studies as there is political support in Central America as well as support from a number of the sugarcane companies. There is a need for concrete advice that can be given to employers and workers.

RESEARCH NEEDS

Expert advice and studies are needed to:

- Determine practical work/rest regimen that can be field tested. (This is particularly important since OSHA standards applied in the tropics lead to very little working time in the field).
- Evaluate hydration methods to maintain balance of water and salts during heat exposure and heavy work.
  - There is some concern that some workers might need more salts during worktime hydration. We should enlist the help of exercise physiologists for determining these needs and evaluating intervention plans to meet those needs.
- Ideally, an experimental and a control group could be compared for their peak of work and decline in productivity both before and after an intervention.
- Pilot testing is essential to assure an accurate measure of hydration since there is debate about the usefulness of urine data (including urine specific gravity) and since weighing harvesters is logistically challenging and virtually impossible to do in the nude.

IMPORTANCE OF WATER TESTING AND PARTICIPATORY METHODOLOGIES

Some of the researchers in the group shared their experiences (specifically in El Salvador) that their research indicating that pesticides are not likely a direct cause of CKD in sugarcane harvesters were not well received by the public. One researcher has been pressured by the mayor of a town to not present evidence that did not link pesticides to CKD. Other populations in other countries reportedly do not trust the water (due to fear of water hardness, heavy metals or other contaminants) and therefore do not drink as much water as they should. This has several implications for intervention research:

- Water testing should always accompany intervention studies in which researchers will recommend drinking more water. It is generally a good idea to test water in both affected areas and non-affected areas.
- It is likely that community members will distrust their water source, even if there is no evidence of contaminants. Participatory research (i.e. involving participants in the sampling of water and deciding where to sample from and at what time) is one way to help address this challenge. Communication strategies need to involve trust building with the researchers. At the same time, researchers also need to be aware that participating in a study can sometimes put a worker at risk for losing his job.

ETHICAL CONSIDERATIONS

Intervention studies involving screening must be sure there is healthcare available for workers before screening takes place. Additionally, researchers must be acutely aware that workers may risk being fired and blacklisted as a result of creatinine screening and that those who are fired may need to work illegally due to the lack of other work options. Therefore, it is essential to guarantee data protection i.e., that the results of the tests will not be linked directly to the person in any database (should be coded), and that only the person being tested should be allowed to have access to the results directly. Additionally, since harvesters are usually paid by the amount of cane they cut, provisions must be made to assure workers participating in the study do not lose income.

LOGISTICAL CONSIDERATIONS

El Salvador may be an ideal place to start intervention studies as the harvesters are usually landowners, so access to workers for intervention studies and alternative solutions (such as working early in the morning and late in the afternoon) are easier to achieve.

There remains a need for intervention studies in working populations in addition to sugarcane harvesting. In El Salvador, the payment mechanisms are different and harvesters are only allowed to cut to a maximum amount, therefore creating an easier scenario for intervention.

EXPERIMENTAL STUDIES & MECHANISTIC RESEARCH

Facilitators: Richard Johnson and Ricardo Correa Rotter
Rapporteur: Rebekah Lucas
Participants: David Friedman, Joe Yracheta, Gaby Sánchez-Lozada, Hildaura Acosta, Rebekah Lucas

MAIN POINTS

1. Simultaneous clinical & animal research
2. Genetic and epigenetic studies
3. Synergistic studies in animal models
4. Animal model limitations
5. Increased kidney biopsy analysis
6. Consider registry and repository
7. Other areas of potential CKD experimental research

Simultaneous clinical & animal research

It is vital that a continual and open relationship/dialogue exists between basic science and clinical developments. In this way we can better characterize the disease and ensure more effective patient care as well as a better-informed experimental design. How best to achieve this requires consideration and action.

It is also important that an open mind be maintained in experimental research with respect to the significance of current risk factors and the advent of new risk factors.

Genetic and epigenetic studies

Genetic and epigenetic studies may provide important information with respect to genetic susceptibility of Mesoamericans to different environmental factors. A geno-wide, non-hypothesis driven research model maybe most successful in identifying such a gene.

Genetics and animal model research: If a genetic susceptibility can be identified in Mesoamericans, this genotype could be introduced into an animal model to assess the impact of different environmental risk factors on the pathogenesis of CKD.

Synergistic studies in animal models

Animal models can provide important information concerning the synergistic interactions of different environmental risk factors. Such interactions might include: arsenic + dehydration; fructose + dehydration; nonsteroidal anti-inflammatory drugs (NSAIDS) + dehydration; and phosphate + dehydration. In this manner we can isolate compounding environmental effects that would inform clinical practice and experimental design.

Phosphate should be considered as a potential risk factor as other research indicates that phosphate exposure causes acute and chronic kidney disease. To date, the potential role of phosphate in Mesoamerica CKD has not been investigated. This needs to be addressed.

Animal model limitations

A limitation with animal models is that they replicate acute exposure, however, CKD is a chronic disease. This limitation must be considered when interpreting result and translating findings from the animal model to the diseased population. The alternative of developing a chronic disease state in an animal model is not an option in the foreseeable future. Though, with further clinical information regarding disease development this may become possible.

Increased kidney biopsy analysis

To date there has been an extremely limited number of CKD kidney biopsies analyzed. Biopsy results show interesting preliminary findings. However, there is considerable debate within the group as to the benefit/cost of kidney biopsies in this patient group.

A common registry and repository

A multi-lateral bio-bank that could store urine samples, serum samples and DNA would be extremely beneficial. This would enable sample storage for future analysis with advanced techniques and understanding, thus, maximizing the impact of samples collected in the field today. Similarly, a common database or registry would promote collaboration and advancement in the field of CKD. However, specimen collection needs to be careful consideration and uniformly followed. Also, ethi-

cal consideration of participant consent to prospective sample testing has to be adhered to. This would be an expensive undertaking, requiring a large amount of funding. The value of such a registry and repository merits consideration however.

Other areas of potential CKD experimental research

Other situations where CKD may be present, for example in elite athletes who consume high volumes of fluid and sugar and are exposed to high heat load repeatedly.

The role of uric acid. Data seems to indicate that there is a disproportionate level of uric acid in early stages of renal failure. Mechanistic insight into the proliferation of uric acid could elucidate CKD disease pathway.

Studies should be conducted in females to identify an underlying factor potentially increasing the susceptibility of Mesoamerican men to dehydration and heat exposure.

IN SUMMARY:

- Mechanistic insight into CKD has an important role in identifying the relevant impact and possible synergistic reaction of identified risk factors.
- Identifying the pathogenesis of CKD requires current and sound understanding of clinical and scientific developments.
- Genetics may play an important role in identifying the population's susceptibility to different risk factors
- Experimental limitations must be understood for correct interpretation and application.

WORKING WITH AN ECOSYSTEM PERSPECTIVE

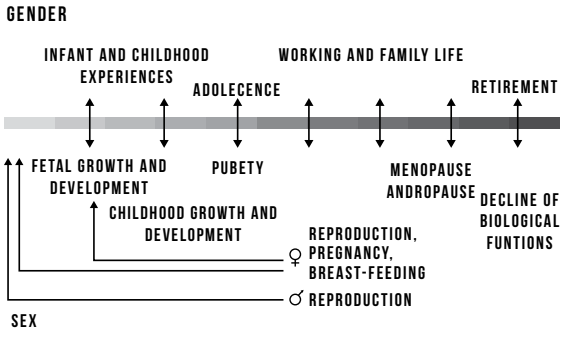
Facilitators: Donna Mergler, Kristina Jakobsson
Rapporteur: Oriana Ramírez Rubio
Participants: Roberto Ruíz, Eugenio Vilanova

Although male sugar cane workers have been in focus as a group at high risk, it is obvious that other populations also may be affected, albeit inadequately investigated. Neither risk factors nor protective factors are yet understood. Therefore an ecosystem approach to human health, coupled to spatial epidemiology and a life-cycle perspective, was introduced as a methodology that could assist in better understanding the environmental, biological and social factors that contribute to and/or influence the development of CKD.

An ecosystem approach to human health provides a framework to examine the pathways and complex interactions between the social and physical environments and health outcomes. It is intervention-driven and builds on the convergence of expertise in the health, social and natural sciences to conceptually map out a trans-disciplinary understanding of the clinical, sub-clinical disease and infra-clinical[1] disease patterns within a particular geo-spatially defined ecosystem, considering not only the physical aspects, but also social, cultural and economic factors that may influence the development of the disease

CKD is a chronic disease, resulting from physiological alterations that can occur over a long period of time prior to the apparition of the clinical disease. In an adverse situation, there is initial physiological adaptation, which when prolonged can lead to a slow breaking down of the mechanisms that maintain homeostasis and adequate kidney functioning. Biomarkers of early alterations to kidney functions may prove particularly useful to consider with respect to the possible factors that are contributing to this imbalance or change. For example, biomarkers of early tubular changes provide data on a continuous scale, rather than dichotomous (sick/not sick), offering the possibility of examining changes within communities with potential risk factors, but with fewer persons with the clinically defined disease. These biomarkers usually have high sensitivity with low specificity, compared to clinical diagnoses, which requires high specificity. In an ecosystem approach, biomarkers of early alterations can be useful in exploring the relations between various exposures, possible interactions and effects.

From a life-cycle perspective, it is important to consider possible fetal exposures, parents' health status, childhood living conditions and age at which one started working, all of which may contribute to the development of the disease. Figure 1 shows a schematic representation of the life-cycle, with an emphasis on gender (social construct) and sex (biological differences). At each stage in this life-cycle, social, environmental and biological factors may contribute to the development of renal insufficiency and CKD. In this life-cycle perspective, it may be useful to identify protective factors as well as potential risks for kidney dysfunction.



Taken from Mergler D. 2012[2].

The working group concluded with a recommendation for applying an ecosystem life-cycle approach, emphasizing that it requires scientists, communities and decision-makers to work together with open minds, and a degree of humility, flexibility and solidarity:

- Identify an area of concern (illness, toxins, type of industry), particularly where there is previous information;
- Delimit the spatial boundaries of this ecosystem and the populations living within its boundaries;
- Examine, within this ecosystem and with these populations, the physical, health, economic and social main drivers;

1. Infra-clinical refers to early physiological alterations which occur in an apparently healthy individual to maintain homeostasis, but if prolonged, may lead to a disease state.

2. Mergler D. Neurotoxic exposures and effects: gender and sex matter! Hänninen Lecture 2011. Neurotoxicology.

- Initiate studies to examine the pathways between potential exposures throughout the life-cycle, the factors that influence these exposures and health outcomes;
- Involve communities and other stakeholders both in the design and the solutions.

Also, two other practical issues discussed were:

- Use the network of researchers created at this meeting to access professionals with different backgrounds and fields of expertise (e.g. nephrologists, epidemiologists, physiologists, toxicologists, gender experts, geographers, sociologists, bio-geo-chemists, etc) to get their input when designing a research project regarding this topic.
- Develop and produce a conceptual framework with an eco-health perspective around CKD and its determinants, risk factors, causal pathways and hypotheses in this region.

DEFINING THE DISEASE, WHAT IS MESOAMERICAN NEPHROPATHY (MeN)?

Facilitator and rapporteur: Ricardo Leiva
Participants: Reina Turcios-Ruiz, Marvin González, Ramón Vanegas, Ingvar Lundberg, Zulma Trujillo, Roberto Ruiz, Ramón García Trabanino, Annika Östman-Wernerson, Manuel Cerdas

The group discussed the demographic, clinical and laboratory characteristics of MeN patients. Case definitions for epidemiologic studies were further discussed after the workshop within a subgroup of workshop participants and the results of these discussions are presented in the paper by Jakobsson et al.

WHAT IS CKDU IN MESOAMERICA, THE MESOAMERICAN NEPHROPATHY (MEN)?

A basic definition for MeN patients is that they are persons with abnormal kidney function, by internationally-accepted standards, living in Mesoamerica and with no other known causes for CKD, i.e. diabetes, hypertension, polycystic kidney disease (PKD), and others.

EPIDEMIOLOGIC PROFILE OF MEN PATIENTS

MeN patients live in low-land rural areas of Mesoamerica, are predominantly men and relatively young. They have low-level education, low income, and limited access to health care. They are often agricultural workers, in particular sugarcane workers. They work in physical strenuous jobs and in hot climatic conditions. They may be in contact with nephrotoxic substances, environmentally or through personal habits.

CLINICAL CHARACTERISTICS OF MEN PATIENTS

MeN patients have a low renal function with typically no hypertension and no edema at physical examination. Laboratory findings in blood include low renal function, early anemia, low sodium and potassium, and hyperuricemia. Findings in urine include benign sediment, low density, and non- nephrotic proteinuria. The histology has been described for the first time by Wernerson-Östman et al as a combination of tubulointerstitial and glomerular damage and needs to be further defined. Imaging (ultrasound) shows normal or reduced kidney size.

RECOMMENDATIONS FOR METHODS OF DEFINING OUTCOMES IN EPIDEMIOLOGICAL STUDIES ON RENAL FUNCTION AND KIDNEY DISEASE

Editor's note: During the working group on "defining the disease", the discussion on epidemiological case definitions was not finalized. As such, the following document was prepared post-workshop by Kristina Jakobsson, Dan Brooks and Ricardo Correa-Rotter and revised by the Organizing Committee and the Temporary Consortium Board members.

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A working definition for a clinical case of MeN is abnormal kidney function, by internationally-accepted standards, living in Mesoamerica and with no other known causes for CKD, i.e. diabetes, hypertension, polycystic kidney disease, and others. The clinical presentation (*see the working group report on Defining the disease, What is Mesoamerican Nephropathy (MeN)? This volume page 165*) and the epidemiology of the disease (*see Brooks et al. Epidemiology of unknown causes in Mesoamerica. This volume, page 37*) have been described in other sections of this report. However, MeN is not an accepted disease entity, and there is a profound lack of knowledge regarding its pathophysiology. Only a few renal biopsies have been performed, indicating that MeN is a disease affecting not only tubuli/interstitium but also glomeruli (*See:* Östman-Wernerson *A, Wijkström J et al. Morphological examination of renal biopsies to assess and evaluate nephrotoxicity, and in particular Mesoamerican Nephropathy. This volume, page 59).*

The aim of this paper is not to suggest a single definition of MeN for use in epidemiological studies, but to give some recommendations on basic methods that can be used in epidemiological studies with limited funding. Such epidemiological studies are generally concerned with the occurrence and development of CKD rather than its consequences. Outcome measures which capture both early and more advanced signs of adverse effects are needed. Moreover, whereas individual-level data are in focus in clinical settings, it should be kept in mind that epidemiological studies are focused on outcomes on population level.

USE A CREATININE ASSAY CALIBRATED TO REFERENCE METHODS

The Jaffe method for creatinine determination, elaborated in the late 1880s, is still used in many laboratories due to its simplicity and low cost. As there is no standard recipe for the Jaffe method, much methodological variation has occurred over time. This lack of methodological standardization implies that interchangeability of Jaffe results is still an issue (Delanghe and Speechkaert 2011).

Most modern creatinine assays are based on enzymatic determination. Regardless of the method used, all creatinine methods should be traceable to a reference method based on isotope dilution-mass spectrometry (IDMS) (Peake and Whiting 2006).

For the MDRD and CKD-EPI equations for GFR estimation, it is recommended that creatinine assays be calibrated to the IDMS standard; however, the earliest MDRD Study equation, with a co-efficient of 186, was based on a non-calibrated assay, with which measured serum creatinine typically overestimated true serum creatinine due to the presence of non-creatinine chromogens. Accordingly, the restated MDRD Study equation uses a constant of 175, instead of the original 186, i.e. a 6% reduction (Levey et al, 2005). A critical issue is that specific assays may either overpredict or underpredict actual serum creatinine (SCr), making calibration of creatinine assays critical for GFR estimation (Miller et al 2005).

When the CKD-EPI equation was developed, all creatinine values were recalculated to standardized creatinine measurements using the Roche enzymatic method. Thus, if non- calibrated creatinine measures are used, recalibration of SCr prior to use in the CKD-EPI equation is necessary.

USE THE CKD-EPI FORMULA TO CALCULATE THE ESTIMATED GLOMERULAR FILTRATION RATE (EGFR)

An overview of different equations of GFR and their performance is given in the Appendix to this paper.

In short, the Cockcroft-Gault and the MDRD

equations were developed for clinical purposes, that is, for grading of disease, and for within-patient assessment of kidney function deterioration (Florkowski et al 2011, Stevens et al 2006). These equations perform poorly in obese persons, and may underestimate GFR, especially in the upper normal or mild deterioration range.

In contrast, the CKD-EPI formula was developed from many research studies, including population-based studies. No equations have been validated in Central American populations. Nevertheless, the CKD-EPI formula is recommended, as its performance has been evaluated in multiple ethnicities (Stevens et al 2011).

The use of serum cystatin C for determination of GFR as an alternative to SCr-based estimation could also be considered (see Appendix for rationale and a brief description). While equations that incorporate both SCr and cystatin C perform best, particularly in individuals with GFR levels above 60 mL/min, eGFR based on SCr is far less expensive and clearly sufficient in younger individuals with normal expected muscle mass for their demographic characteristics. However, this may not be the case in underprivileged and malnourished populations in developing countries, for which reference equations never have been evaluated.

USE A SEMI-QUANTITATIVE DIPSTICK, AND REPORT RESULTS FOR ALL CONCENTRATION LEVELS

In epidemiological studies, proteinuria can be detected with a standard semi-quantitative dipstick. A common proteinuria dipstick grading for "trace" may approximate urine protein under 30 mg/dL, 1+ from 30 to 100 mg/dL, 2+ from 100 to 300 mg/dL, 3+ from 300 to 1000 mg/dl and 4+ over 1000 mg/dL. Microalbuminuria-specific dipsticks, with higher sensitivity and specificity, could provide more reliable semi-quantitative evaluations of low grade urine albumin concentrations, from 20 mg/dL and up. In epidemiologic studies on MeN, standard proteinuria dipsticks may be sufficient, since cases of MeN have very little or no proteinuria and the key factor for defining CKD in MeN is through eGFR.

Critically, urine dipsticks cannot normalize for whether the urine is concentrated or dilute, such that very concentrated urine specimens may overestimate albuminuria while very dilute specimens may underestimate albuminuria.

There is a basal level of albuminuria below 10mg/g creatinine which is considered non-pathologic, while values between 10 and 30 mg/g are associated with a higher risk of cardiovascular events and death, although these values are of uncertain pathologic significance beyond their ability to affect prognostication. Values between 30-300mg/day are termed *microalbuminuria*, and these levels are associated with a clear increase in cardiovascular and kidney disease events. Microalbuminuria is not equal to a dipstick-assessed concentration in a spot sample, as the dipstick measures the concentration in the sample, not the daily excretion. However, dipstick testing is valuable for rapid and simple screening. As normal urine contains small amounts of protein, negative to trace reactions are usual in concentrated urine. In contrast, a trace to 1+ reaction in much diluted urine is suggestive of significant proteinuria. The presence of albumin in the urine, particularly at higher levels within the upper range of 'microalbuminuria' and above, typically indicates a glomerular process.

A morning spot sample is the best option, as it is more concentrated, but is usually not possible to obtain in an epidemiological study. Accordingly, it is important to record the time of collection of the samples, along with other factors that could affect the concentration of the urine, such as the extent of physical activity prior to the collection of the sample, and to interpret the findings in that context.

OTHER SENSITIVE URINARY MARKERS OF EARLY RENAL INJURY MIGHT BE USEFUL

The albumin/creatinine ratio is a well-established marker of renal function. In normal well-functioning kidneys, the urinary excretion of albumin is less than 30 mg/g creatinine, which corresponds to a 24h excretion of about the same amount

In addition to albumin, several small proteins or peptides have been proposed as early markers of acute and chronic renal tubular injury, i.e. b2-microglobulin, clusterin, cystatin C, kidney injury molecule-1 (Kim-1), trefoil factor 3, neutrophil gelatinase-associated lipocalin (NGAL), N-acetyl--D-glucosaminidase (NAG), Interleukin-18 and others (Lock 2010, Devarajan 2010). A number of emerging technologies have made it possible to measure several proteins in a single urine sample in a reliable and rapid way such that large numbers of samples can be analyzed and run with high throughput. It may be of value to explore such potential early markers in well-designed and focused studies, as sensitive urine biomarkers of kidney injury may indicate disease before the development of elevated SCr serum creatinine or significant proteinuria. However, it has to be kept in mind that the long-term prognostic value of most of these markers is not yet well characterized.

See also papers "How to assess renal effects"

(page 61) and "Acute kidney injury and the development of chronic kidney disease. Role of novel biomarkers" (page 65) in this volume.

DO NOT USE DIPSTICK PROTEINURIA AS THE ONLY SCREENING TOOL FOR CASE-FINDING.

The clinical observations on MeN, as well as findings in epidemiological studies indicate that glomerular filtration may be affected without signs of proteinuria. Thus, case-finding using dipstick screening as a first step may seriously underestimate disease prevalence. An illustration of the magnitude of underestimation is given, using unpublished data from a population-based study in Nicaragua (Torres et al 2010).

MALES			ALBUMINURIA [B] WITHIN EACH LEVEL OF EGFR		
$eGFR_{MDRD}$ [a]		prevalence	<30	30-299	300+
	n	%	n (%)	n (%)	n (%)
>90	324	70	300 (92)	25 (8)	1 (0.3)
60-89	87	19	62 (71)	24 (28)	1 (1)
30-59	45	10	31 (69)	10 (22)	4 (9)
15-29	10	3	3 (30)	5 (50)	2 (20)
<15	5	1	3 (60)	1 (20)	1 (20)
Total	*465*		*399 (85)*	*65 (14)*	*9(2)*

FEMALES			ALBUMINURIA [B] WITHIN EACH LEVEL OF EGFR		
$eGFR_{MDRD}$ [a]		prevalence	<30	30-299	300+
	n	%	n (%)	n (%)	n (%)
>90	539	87	481 (89)	55 (10)	3 (0.6)
60-89	62	10	52 (82)	10 (16)	0 (0)
30-59	13	2	11 (85)	1 (8)	1 (8)
15-29	4	0.6	1 (25)	3 (75)	0 (0)
<15	2	0.3	1 (50)	1 (50)	0 0)
Total	*620*		*546 (88)*	*70 (11)*	*4 (0.6)*

[a] estimated according to the MDRD equation
[b] albuminuria in a single spot urine sample

In this Nicaraguan population-based sample, 62% of men with eGFR <60 ml/min per 1.73m^2 would not have been detected, if a dipstick test for proteinuria (one single spot sample) with the cut-off <30 mg/dL had been used as a screening for case-finding. On the other hand, 69% of the men with proteinuria 30+ mg/dL had eGFR > 60 ml/min per 1.73m^2.

Similarly, 68 % of the women with eGFR <60 ml/min per 1.73m^2 would not have been detected with a dipstick screening, and 93% of women with proteinuria had eGFR > 60 ml/min per 1.73m^2.

It therefore seems clear that MeN does not generally follow the pattern of renal injury observed in diseases such as diabetic nephropathy, which is the most prevalent kidney disease worldwide, and has a strong correlation between reduced eGFR and presence of proteinuria. The absence of proteinuria does not preclude the presence of MeN and it can even be stated that proteinuria, if present is apparently of low grade.

DO NOT USE THE CLINICAL DEFINITION OF CHRONIC KIDNEY DISEASE AS THE ONLY OUTCOME REPORTED

In 2002, the Kidney Disease Outcome Quality Initiative (KDOQI) set an internationally-recognized definition for CKD. That definition of CKD was recently reviewed and updated by the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines, but the previously defined thresholds of glomeular filtration rate (GFR, eGFR<60 ml/min per 1.73m^2) was endorsed (Levey, 2011). The KDIGO CKD classification is based on the association of eGFR, levels of albuminuria and/or other markers of kidney disease, and is intended primarily for clinical use. The staging of CKD requires investigations of kidney function twice, at least 90 days apart.

THE KIDNEY DISEASE: IMPROVING GLOBAL OUTCOMES (KDIGO) STAGES OF CHRONIC KIDNEY DISEASE

STAGE	GFR*	*DESCRIPTION*
1	*90+*	*Normal kidney function but urine findings** or structural abnormalities or genetic trait point to kidney disease*
2	*60-89*	*Mildly reduced kidney function, and other findings (as for stage 1) point to kidney disease*
3A *3B*	*45-59* *30-44*	*Moderately reduced kidney function*
4	*15-29*	*Severely reduced kidney function*
5	*<15 or on dialysis*	*Very severe, or end-stage kidney failure (sometimes called established renal failure)*

*All GFR values are normalized to an average surface area (size) of 1.73m2

** Albuminuria > 30 mg/day, hematuria, or other imaging or pathologic evidence of kidney damage

A similar "quasi staging" for case definition can reasonably also be applied to epidemiological studies aimed at assessing risk and susceptibility factors. However, because such studies are generally concerned with the development of CKD rather than its consequences, a staging expressed as a binary outcome (n.b. eGFR<60 ml/min per 1.73m^2) should never be the only outcome reported, and additional measures with focus both on early and more advanced signs of adverse effects should always be given. However, it has to be kept in mind that, in epidemiologic studies, determination of early stage, especially CKD Stage 1, is prone to important misclassifications, except in the presence of overt albuminuria without reduced eGFR.

Such a "quasi staging" follows the international KDIGO scheme but is based on a single measurement of S-Cr and a dipstick for proteinuria. Due to creatinine fluctuation, there will almost certainly be some false positive as well as false negative results from using a single measure (Bottomley et al 2011, deLusignan et al 2011), though the extent is unknown.

BIOBANK BLOOD AND URINE SAMPLES

Usually, the budget for an epidemiological study is tight, with limited resources for extended biochemical analyses of markers of exposure and early effects. Still, taking into consideration the substantial effort that lies behind most epidemiological studies and the rather limited marginal costs for a few additional samples from each individual, the additional cost for secure storage is well worth considering. Biological samples from well-characterized individuals can be invaluable for future research.

REPORTING OF RESULTS – THE STROBE GUIDELINES

A well-designed study with stringent analysis and reporting is of value, even if it is small. Good advice on how to report an observational study (and thus also on how to design the study) is given in the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) recommendations to improve the quality of reporting of observational studies (Vandenbroucke et al 2007). The STROBE Statement consists of a checklist which relate to the title, abstract, introduction, methods, results and discussion sections of articles. The aim is to provide guidance to authors about how to improve the reporting of observational studies and facilitates critical appraisal and interpretation of studies.

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1. *Leiva et al. Defining the disease, What is Mesoamerican Nephropathy (MeN)? This volume page 165*)

2. Bottomley MJ, Kalachik A et al. Single estimated glomerular filtration rate and albuminuria measurement substantially overestimates prevalence of chronic kidney disease. Nephron Clin Pract 2011; 117:c348-352.

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Appendix: A brief description of widely used equations for estimations of glomerular filtration rate

This text is to a large extent based on a Mini-Review by Christopher M Florkowski and Janice SC Chew-Harris, Methods of Estimating GFR – Different Equations Including CKD-EPI. Clin Biochem Rev Vol 32 May 2011 p 75-79.

COCKCROFT - GAULT FORMULA

Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. Nephron 1976;16: 31-41.

Participants: Hospitalized patients with CKD

Men: ≈24

Women: 9

$$eCCr = \frac{(140\text{-}age) \times Mass\ (kg) \times Constant}{S\text{-}Cr\ (\mu mol/L)}$$ Constant is 1.23 for men and 1.04 for women

Several authors, especially in recent publications, have corrected the Cockcroft-Gault result for body surface area (BSA).

The fact that weight does appear in the Cockcroft-Gault equation may be viewed as logical because weight is strongly related to muscular mass and, thus, to serum creatinine. This relationship, however, is less evident in an obese patient because weight also reflects fat mass. Thus, the Cockcroft-Gault equation is not accurate in obese patients when actual weight is used in the formula.

MDRD FORMULA

Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D et al. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med 1999;130:461-70.

Participants: Patients with CKD (364 polycystic, 525 glomerular, 121 tubulointerstitial, 618 other or unknown)

Men: 983

Women: 645

Country of origin: USA

Diabetes: 6 %

Mean weight: 79.6 ± 16.8 kg

The MDRD study was based on a multicentre trial to evaluate the effect of dietary protein restriction and blood pressure control on progression of renal disease in 1628 patients with CKD, with the added objective of developing an equation that could improve the prediction of GFR from plasma creatinine. GFR was measured as the renal clearance of ^{125}I-iothalamate, and creatinine clearance was computed from creatinine excretion in a 24 h urine collection and a single measurement of plasma creatinine. Stepwise multiple logistic regression analysis was employed to determine the set of variables that best predicted GFR. A 6-variable equation was first derived. Subsequently a simplified 4-variable version, which included age, gender, plasma creatinine value and race differentiation as white or black was published. Results are expressed as per 1.73 m^2 of body surface area.

Table. eGFR prediction equations based on plasma creatinine concentration

MDRD e GFR =

186 x [Plasma Creatinine (µmol/L) x 0.0011312]$^{-1.154}$
x [age(years)]$^{-0.203}$
x [0.742 if female] x [1.212 if black] [equation1]

MDRD e GFR (IDMS aligned) =

175 x [Plasma Creatinine (µmol/L) x 0.0011312]$^{-1.154}$
x [age(years)]$^{-0.203}$
x [0.742 if female] x [1.212 if black] [equation2]

The MDRD study equation was subsequently validated in patients with diabetic kidney disease, renal transplant recipients, and African-Americans with non-diabetic kidney disease. Given that the MDRD equation was originally derived from a group of CKD patients, its utility for healthy individuals remains unclear. Its accuracy in predicting GFR is best reflected in those with mild kidney impairment. It is recognized that MDRD tends to underestimate renal function in those with a normal eGFR >90 mL/min/1.73m^2.

It has not been validated in children under 18 years of age, in pregnant women, in patients above 70 years of age, and in ethnic groups other than African-American. More importantly, given the rise in the epidemic proportions of global obesity, the MDRD equation has not yet been validated at extremes of body weight, further limiting its usefulness in targeting individuals at higher risks of developing CKD.

CKD-EPI EQUATION

Levey AS, Stevens LA, Schmid CH, Zhang Y, Castro AF, Feldman HI, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med 2009;150:604-12.

Lesley A. Stevens, Marcie A. Claybon, Christopher H. Schmid, et al Evaluation of the Chronic Kidney Disease Epidemiology Collaboration equation for estimating the glomerular filtration rate in multiple ethnicities. Kidney International; Mar2011, Vol. 79 Issue 5, p555-562

Participants: 8254 participants in 10 studies (equation development data set) and 3896 participants in 16 studies (validation data set). Prevalence estimates were based on 16 032 participants in NHANES

Women: 44%
Diabetes: 29%
Mean BMI: 28 (SD 6)
Hispanics: 5%
Asians: 1%

CKD-EPI eGFR

Female with Creatinine < 62 µmol/L; use e GFR = $144x (Cr/61.6)^{-0.329} x (0.993)^{age}$
Female with Creatinine > 62 µmol/L; use e GFR = $144x (Cr/61.6)^{-1.209} x (0.993)^{age}$
Male with Creatinine < 62 µmol/L; use e GFR = $144x (Cr/61.6)^{-0.411} x (0.993)^{age}$
Male with Creatinine > 62 µmol/L; use e GFR = $144x (Cr/61.6)^{-1.209} x (0.993)^{age}$

where Cr is the plasma creatinine (µmol/L)

For -African-Americans the corresponding coefficients are 166 (females) and 163 (males)

The CKD-EPI equation was developed based on research studies and clinical populations ("studies") with measured GFR, and NHANES (National Health and Nutrition Examination Survey), 1999 to 2006. The original serum creatinine values were recalibrated to the Roche enzymatic method.

The CKD-EPI equation includes log serum creatinine (modeled as a 2-slope linear spline with sex specific knots at 62 µmol/L in women and 80 µmol/L in men), with gender, race and age on the natural scale. It is therefore effectively four different equations for whites (men, women, above the knot value, below the knot value) and another four for African-Americans in whom a different factor is used.

The CKD-EPI equation is somewhat more precise and accurate than the MDRD Study equation, especially at higher GFRs. However, the sample used to develop and validate the CKD-EPI equation included few elderly and nonwhite persons.

A recent study (Stevens et al 2011) has suggested that modification of the CKD-EPI equation may not be necessary for GFR estimation in Native Americans and Hispanics.

CYSTATIN C IN SERUM FOR ESTIMATION OF GFR

Weinert LS, Camargo EG, Soares AA, Silveiro SP. Glomerular filtration rate estimation: performance of serum cystatin C-based prediction equations. Clin Chem Lab Med. 2011 Nov;49(11):1761-71. doi: 10.1515/CCLM.2011.670

Two analytical methods for determination of cystatin C are available commercially, i.e. nephelometry and turbidimetry, with nephelometry reported to be the more accurate.

Many studies have compared cystatin C concentrations or cystatin C-derived equations with gold standard methods. Most studies have found cystatin C or the reciprocal of cystatin C to be superior, or at least equivalent to serum creatinine for the detection of decreased GFR. Several equations based on cystatin C have been reviewed (Weinert et al 2011).

The advantage of cystatin C equations is that they depend less on anthropometric data, allowing the development of simpler equations than those based upon creatinine. Muscle mass has no or little influence on serum cystatin C and dietary protein intake has no significant effect on it.

MEASURING EXPOSURE TO WORK LOAD, HEAT STRESS & DEHYDRATION

Facilitator y Rapporteur: Rebekah Lucas
Participants: Pedro Vinda, Andrés Robles, James Kaufman

MAIN POINTS

1. Work load
- Physiological measures
- Qualitative measures

2. Heat stress
- General ambient conditions - Weather stations
- Immediate ambient conditions - WGBT (dry & wet bulb temp, wind and humidity)
- Internal body temperature & skin temperature
- Heat shock proteins

3. Dehydration
- Urine – density & osmolality
  - A crude measure?
- Venous blood samples – Hct, Hb & osmolality
  - Indicates fluid distribution within the body
  - Important for hydration interventions
- Nude or semi-nude changes in body weight
  - Informative if done correctly
  - Very difficult to do correctly in most Central American work settings

› *Workload*

Two general types of workload measurements were discussed, physiological measures and qualitative. The physiological measures of workload discussed included heart rate and oxygen consumption. Although oxygen consumption accurately describes the metabolic cost of exercise or work, with mobile metabolic units available for field-testing, it was felt that such units would be cumbersome for an individual to wear for long durations. As an alternative, heart rate was discussed as a preferable physiological measurement of workload as heart rate monitors that log beat-to-beat data can be worn with little or no discomfort. Importantly, there is a linear relationship between oxygen consumption and heart rate and subsequently, heart rate can be used to indicate metabolic workload and to ascertain the relative intensity of a workload for an individual. To do so, maximum heart rate (the maximum number of beats per min an individual's heart can pump) must be determined. This can be done by performing a relatively short (7-12 min) exercise stress test (i.e., incremental treadmill or step test) *or* can be estimated using a simple equation; 220 minus the individual's age. Once maximum heart rate is known or calculated, the relative intensity of the workload can be calculated. For example, if a worker has a maximum heart rate of 190 beats per minute and when working their heart rate reaches 150 beats per minute, by dividing 150 by 190 and multiplying it by 100 we can determine that the worker is working at a relative intensity of 79% of their heart rate max. Use of this simple equation would enable the researcher to estimate the relative intensity of the workload and therefore, more accurate comparisons could be made across age ranges, fitness levels and environmental conditions.

Qualitative measures of workload were also discussed as a valuable tool in assessing workload in the field. Such measures included productivity measures and metabolic assessment. Productivity assesses the rate of production (i.e., quantity of material produced in a given time), whereas qualitative metabolic assessments describe a movement and assigns a level of intensity to it. The latter can provide excellent basic information with respect to the type of work and work conditions. However, qualitative metabolic assessments are subjective and therefore vulnerable to researcher basis and experience. Productivity assessments provide important information regarding performance, however external factors can influence outcomes (such as machine malfunctions or crop density). Also, company opposition to productivity assessments may make such measures impossible.

To assess workload, qualitative metabolic assessments are an acceptable initial assessment, though researcher bias and experience must be recognized. Heart rate is a reliable and valid physiological measure of cardiovascular workload and intensity. The combination of heart rate and qualitative assessment are the most accessible, reliable and informative measures of workload. It is important to note that workload assessment perhaps has the most meaning or significance to intervention studies in workplace settings.

▸ *Heat Stress*

Workplace heat stress or heat exposure measurements were categorized into three different levels:

1. Immediate ambient environment of the workplace
2. General ambient environment of the geographical location
3. Internal body core temperature of the worker

To assess the immediate worksite environment, thermometers should be placed where the heat exposure is similar to that experienced by the worker(s) and data collection should ideally occur at regular intervals over a number of days. Validated heat stress indexes should be used to quantify the level of heat exposure, an example being Wet Bulb Globe Temperature (WBGT). It is important that radiant heat load, evaporative capacity and internal heat production be factored into such calculations. It was noted however, that the validity of heat stress indexes have not been established for certain work conditions or populations and therefore should be used cautiously.

General environmental conditions can be assessed using data from existing local weather stations. The benefit of using such data is that it is often freely accessible and can provide historical environmental data. A limitation is that local weather station weather conditions may differ from that at the actual worksite (i.e., often weather stations are located at airfields where there is a high radiant heat load if the sun is out).

Internal body core temperature can be assessed via a number of measurement methods each with its own advantage and limitation. The majority of such measurements are only practical in laboratory settings. However, some measures can be used in the field. Gastrointestinal temperature (measured via a telemetric pill) is the most reliable and valid measure available for field-testing to date, though there are limitations with this measure that include transmission range (approximately 1 meter) and participant consent/exclusion. Auditory canal temperature measurement is an alternative means of assessing body core temperature in the field, however there are notable validity and reliability issues with this measurement that must be taken into account and addressed.

Other physiological markers such as heat shock proteins (HSP) may also indicate heat exposure or tolerance.

▸ *Dehydration*

Several markers of dehydration or the fluid balance were discussed.

Urine density or urine specific gravity is a common measure of hydration state as it is cheap, non-invasive and easy to measure. Urine osmolality can also be used interchangeably or in addition to urine density. However, it was felt that urinary measures provided only a rough index of dehydration, being sensitive only to large acute changes in fluid balance. Therefore, it was recommended that urinary measures be used to assess hydration status on a day-to-day basis (assuming the same time of day and behavioral pattern is followed).

Venous blood samples can be used to indicate changes in plasma volume or plasma osmolality. Changes in plasma volume can be calculated from changes in hemoglobin concentration and hematocrit ratio (Dill and Costill 1974). Plasma osmolality can also be used to assess acute changes in hydration state (Popowski, Oppliger et al. 2001). Although, it was noted that plasma osmolality reflects extracellular fluid osmolality and not extracellular fluid volume per se. Therefore, considering the invasive nature of venous blood draws and the relatively difficult and expense, venous blood samples seemed less suitable than other basic measures.

Changes in body mass was considered the cheapest and most reliable means of determining an individual's fluid balance. Fluid and food intake may need to be recorded depending on the question being addressed. Furthermore, difficulties can arise with this measure in finding: a flat, solid surface for the scales to rest on; privacy for participants; and scales that work under high ambient temperatures.

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CHARACTERIZING ENVIRONMENTAL AND OCCUPATIONAL EXPOSURE TO NEPHROTOXIC AGENTS

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Rapporteur: Alejandro Riefkohl
Participants: Hildaura Acosta, Carolina Guzman, Carlos Orantes, Eugenio Vilanova, Joe Yracheta

INTRODUCTION

Our working group focused on discussing strategies for characterizing environmental and occupational exposure to nephrotoxic agents. There is no single study that will allow us to investigate the many different exposures that are hypothesized to be associated with CKDu for the following reasons:

- there are many different agents of interest, including pesticides, metals, infectious agents, medications, and fructose;
- there is potential for both occupational and non-occupational (i.e. environmental and/or behavioral) exposures to these agents;
- there are different objectives that could be addressed (e.g. characterizing exposure pathways versus evaluating exposure-disease relationships).

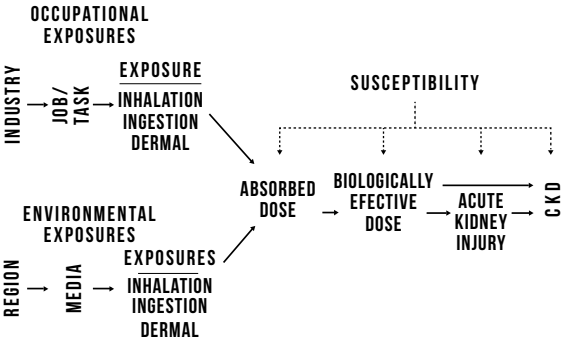
All of these factors will influence the design of a study. So rather than attempt to design a single study, our group discussed a conceptual model that may provide a useful way to summarize the different ways in which exposure could be characterized and related to disease status.

CONCEPTUAL MODEL FOR EXPOSURE-RELATED DISEASE

Figure 1 reflects the fact that exposures can occur in both occupational and non-occupational environments. In occupational settings, individuals are employed in different industries, in which they perform different jobs or tasks, such that they may have exposure to nephrotoxic agents via inhalation, ingestion, and/or dermal contact. In non-occupational settings, individuals live in particular regions/communities in which environmental media may be contaminated (e.g. drinking water, food supply, soil, ambient air/dust), such that they may have exposure to nephrotoxic agents via inhalation, ingestion, and/or dermal contact. Importantly, the same individuals may be exposed to nephrotoxic agents in both occupational and non-occupational settings.

The total amount of exposure to a particular agent is the result of inhalation, ingestion, and dermal contact in all settings. Exposure means that an individual has come into contact with an agent, but that does not necessarily mean that the agent was absorbed. Only a portion of the total exposure will be absorbed and result in internal dose or total absorbed dose, which can be characterized using biomarkers. A biomarker of total absorbed dose (often obtained in biological material such as blood, urine, hair, or nails) can provide information about exposures across multiple exposure routes in both occupational and non-occupational settings. Similarly, only a portion of the total absorbed dose will result in biologically effective dose, which is the amount of the agent - or a metabolite of the agent - that reaches the critical target sites of interest. Biomarkers of biologically effective dose are more difficult to obtain and rarely available, since we would ideally like to know how much of an agent is reaching the kidney.

Figure 1.
Conceptual model for exposure-related disease



It may be useful to consider a hypothetical example in which we describe potential exposure pathways for cadmium. An individual may be employed in the agricultural industry, where s/he works in the field, and has exposure to cadmium via ingestion (e.g. ingestion of drinking water from source at work, incidental ingestion of dust from fields) and inhalation (e.g. smoking). The same individual may also live in a region/community where the home has a soil floor and uses well water, where s/he has exposure to cadmium via ingestion (e.g. ingestion of drinking water from home well, incidental ingestion of dust within home) and inhalation (e.g. smok-

ing). The total absorbed dose of cadmium would result from all of these exposures and could be estimated by quantifying cadmium in a urine sample. The biologically effective dose would be the portion of total absorbed dose that reached the kidney and could be quantified if kidney tissue was available (unlikely).

The biologically effective dose of a particular agent (or metabolite) may first result in acute kidney injury (AKI), which over time could progress to chronic kidney disease; or the agent may directly cause CKDu. Importantly, individuals have different susceptibility at each one of these steps due to potentially modifying factors such as heat exposure, genetic characteristics, co-exposures to other agents, or pre-existing health conditions. For example, genetic differences may account for differences in the ability to metabolize certain chemicals, which could either increase or decrease susceptibility to CKDu depending on whether the original agent or its metabolite is more toxic. Similarly, individuals who are severely dehydrated may be more susceptible to the effects of exposure to a low level of nephrotoxic agent.

In addition to the above discussion of exposure pathways, we also had more focused discussions of characterizing levels of agents in environmental media, using questionnaires to characterize personal exposure, and using biomarkers to characterize total absorbed dose. These are described in the sections below.

ENVIRONMENTAL MEDIA

Of the various types of environmental media, the working group agreed that improving the characterizing of drinking water should be the highest priority. There was general consensus that the highest priority agents should be inorganic arsenic and pesticides (particularly those that are known to cause AKI). The quality of drinking water, in particular the potential for pesticide contamination, is a leading concern in the affected communities and should be addressed.

The characterization of agents in drinking water could be used as part of an exposure-focused study to explore the relationship with total absorbed dose, or as part of an epidemiologic study as a way to characterize exposure and explore the relationship with AKI and/or CKD. Additionally, such an investigation could be done solely for surveillance purposes. Even if these agents are not risk factors for CKDu, ensuring that these communities have access to clean drinking water is certainly a worthwhile endeavor from a broader public health perspective.

In addition to drinking water, characterizing agents in soil and/or ambient air could be useful depending on the research objective. Dust levels in agricultural areas can be high during dry seasons, likely resulting in exposure to any agents present in soil via inhalation and/or incidental ingestion. If biomarkers of the agent of interest are not available, then measuring contaminants in soil or air may be the best option for characterizing exposure. Or, if a particular agent is found to be elevated in biomarkers of total absorbed dose, analysis of soil and/or ambient air may provide important information about the source.

Similarly, analysis of targeted types of food could provide useful information about the role of diet. Since there are many types of food, each from different sources, it would be most efficient to either target samples of commonly consumed food, or allow results from epidemiologic studies to guide selection. For example, if a food frequency questionnaire is used to characterize consumption of different food types, and some are associated with higher risk of CKDu, then these food types could be targeted for chemical analysis.

As a less traditional and potentially innovative approach, it could be useful to consider whether studies of livestock or other animals could provide useful information. There was an intriguing report at the Workshop that kidney problems have been observed among livestock in the affected regions. Studying exposure and/or disease in animals from the affected region could represent an opportunity to obtain data that would be more difficult or impossible to obtain from humans.

Finally, it is important to consider both spatial and temporal variability when measuring agents in any other media described above. The agents of interest are not uniformly distributed over time and space, so each study must be designed with that in mind. For example, if we think the levels of a particular agent in drinking water might vary over time due to periodic events (e.g. agent only used at certain times of week, month or growing season; or increased levels after severe weather events), then a cross-sectional study will not sufficiently characterize levels and a repeated measures study would be preferable.

QUESTIONNAIRES

Carefully constructed (and when possible validated) questionnaires are important tools for characterizing personal exposure, particularly when attempting to characterize exposure that occurred in the past. One of the most useful approaches is to obtain work histories and/or residential histories, which can be used to develop exposure matrices for specific agents. Individuals can also be asked directly to self-report exposure to specific agents, though often individuals either don't know the names of agents/products/chemicals or were never

aware they were using them in the first place. Assessing exposure via questionnaire can be especially useful when biomarkers of chronic exposure are not available.

Questionnaires can also be used to gather information about diet and medication use. Food frequency questionnaires are designed to collect information about the frequency and amount of consumption for many different types of food. Diet could represent an important exposure pathway because the food/drink is contaminated with nephrotoxic agents or because the food/drink contains specific ingredients of potential concern (e.g. fructose). As for medications, aminoglycoside antibiotics and chronic use of non-steroidal anti-inflammatory drugs (NSAIDs) are associated with acute kidney injury in a dose- and duration-dependent manner. Additionally, 'traditional' medicines based on herbs or other natural ingredients are also used in the region. Ideally, questionnaires could be used to assess the types of medications, amount of use, and frequency of use. However, since subjects may not be able to readily identify the medications that they use, specialized questionnaires designed to enhance recall of such information should be utilized.

BIOMARKERS OF EXPOSURE

As described above, biomarkers can be used to provide a useful measure of total absorbed dose. A biomarker of total absorbed dose (often obtained in biological material such as blood, urine, hair, or nails) can provide information about exposures across multiple exposure routes in both occupational and non-occupational settings.

However, temporal variability – also referred to as within-subject variability – is a critically important consideration during study design. The agents of interest vary in terms of their biological half-lives as well as in terms of their exposure patterns. Several metals, as well as some pesticides like DDT, have long biological half-lives such that a single measurement in blood provides a measure of cumulative exposure over a period of time that can range from months to years depending on the agent. However, many other pesticides have very short biological half-lives, such that a single measurement in urine only provides information about exposure over a period of time that can range from hours to days depending on the agent.

There is one situation in which a biomarker with a short half-life may provide information about exposure over a longer period of time: if the exposure is fairly routine, such as through drinking water or as the result of another daily task. In such case, the biomarker still only measures the most recent exposure, but because of the consistent exposure pattern over time, it may still provide a useful way to classify cumulative exposure.

The most common approach to utilizing biomarkers is to measure specific agents (or metabolites) of interest, which always comes with the risk of analyzing for the wrong agents. However, emerging technologies may provide alternatives to this one-agent-at-a-time approach. For example, a metabolomics-driven approach provides a global analysis of biochemicals that can be used to explore profile differences between populations with and without disease. If certain biochemicals occur more frequently (or exclusively) in the population with disease, it is possible that they are the result of the disease itself and may represent a novel biomarker of CKD. However, it is also possible that such biochemicals may represent important exposures or metabolites that are key risk factors for the disease.

Finally, the working group discussed the need to consider whether the concentrations of agents measured in blood or urine may be increased as the result of decreased kidney function, which could lead to an artificial conclusion that increased exposure resulted in decreased kidney function (i.e. reverse causation). The working group lacked the expertise necessary to evaluate these potential methodological concerns, but mention them here as worthy of further consideration.

MEASURING PERSONAL FACTORS

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Rapporteur: Ilana Weiss
Participants: Sandra Peraza, Laura Gabriela Sánchez, Cinthya Bonilla, Oriana Ramírez, Roberto Antonio Ruiz

Although measuring personal factors seems easy and self-explanatory, precision is essential for good quality data. A well-defined protocol with specific roles designated to the same individual during data collection, training, supervision and quality control for everyone involved in taking measurements is necessary when measuring any personal factors. This group has discussed the most important factors to measure in CKDu research with specific recommendations to strengthen data quality.

WEIGHT AND HEIGHT

Measuring weight and height in the field in previous studies has proven to be a bigger challenge than expected. Generally speaking, since every field situation is different, researchers should try to create the best possible conditions for privacy and comfort of the workers, while assuring the best possible accuracy and comparability in the measurements.

With regard to weight, the first recommendation is to invest in the most reliable scales, well known brands mostly used for research, which are usually more expensive. They will last longer and be more reliable in the field. If multiple scales will be used in the study, they should all be the same brand and model to make sure that comparable results can be obtained. The scales should be calibrated before and at the site of study. A standardized protocol should be established and understood by the field workers to properly instruct the participants, for example, to remove shoes, empty pockets but keep clothing on, or take certain clothing off depending on privacy circumstances.

With regard to height, since the measuring is usually done in the field, it is important to make sure that the surface where the device is located is flat and close to a wall when possible. For the most accurate height measurements, researchers should use a measuring device with a sliding arm that rests on the participant´s head. Participants should remove their shoes, and stand straight with their feet together. Those taking measurements should make sure that the arm of the measuring device rests on the skull and not on the hair. In the field, some workers are hesitant to take off their shoes or boots due to fear of bad odors; however the research team should make clear to the workers that it does not matter. One way of avoiding uncomfortable situations for the participant is to locate both scales and height meters outdoors.

NUTRITIONAL STATUS AND EATING/DRINKING HABITS

Before designing a system by which to measure nutrition and eating/drinking habits, it is important to recognize that a wealth of resources and data already exist. There has been a great deal of research into the nutritional value of specific foods and existing databases are available with detailed information. This could save time and resources as well as ensure that data collected adequately captures dietary realities. In questionnaires that ask about current nutrition, it is important to provide training to interviewers agreeing on the meaning of portion size. Previous experiences have shown that bringing examples with utensils commonly used together with a measuring cup will lead to the best approximation of amounts of food and liquids consumed by participants. When specialized or regional food are to be registered (local food like “tamales”), researchers should take advantage of existing mechanisms for calculating nutritional value. A food sample can be dehydrated and subsequently frozen, turning it into a powder. The freeze-dried powder can be sent to a lab for analysis.

SMOKING, ALCOHOL AND DRUG CONSUMPTION

There are standard well-tested questions regarding smoking, drinking and drug use habits. However, in particular when these questions are suspected to be connected with the disease under study, there is a tendency towards underreporting. Due to the sensitive nature of asking people about their habits, special steps can be taken to help bolster data quality. It may be important to find colloquial ways of asking these questions, and complement with qualitative methods to get more information about local traditions. With questions about smoking, alcohol, and drug consumption, the researcher should avoid using yes or no questions (i.e. Do you drink? y/n). Instead they should frame the questions to make honest responses easier (i.e. How many beers do you drink per week?).

In addition, before the study population is given the questionnaire, it is valuable to explain to them

why having accurate information about the use of these substances is important. If participants understand that having accurate information could lead to a causal link or an association, they may be more inclined to tell the truth despite the embarrassment or discomfort of the question. Second, interviewers should be chosen carefully and strategically. In the case of CKDu, young and middle aged men are the target population; therefore the interviewer should preferably also be a young or middle-aged man. Training people from neighboring or similar communities to perform the questionnaire interviews may be considered. They may have better opportunity to get more accurate answers than an outsider would. A third suggestion is that when asking about drug use, questions could be framed as self-medication in a way that could capture illegal druguse as well.

MEDICATION/SELF-MEDICATION

For information on medication use in communities, investigators should research medical drug use in areas of interest prior to the start of the study, such as nephrotoxic antibiotics and NSAIDS. Researchers can speak with drug-reps that serve the area, medical personnel in clinics, hold focus groups, etc. to determine what kinds of medications of relevance for the study are most commonly used. The researcher can then bring samples of the most commonly used medications and ask people to pick the ones they use and then ask about quantity. Including herbal medications is important in kidney disease studies. When exploring herbal medication, researchers must get help from an herbalist who is familiar with local use of herbal medications.

CO-MORBIDITY

Information on co-morbidity should preferably be accessed from medical records or through healthcare providers at the local clinic rather than from self-reporting. Although ideal, it is difficult in rural areas in Central America where records are not kept and means for laboratory analyses are not readily available.

With regard to diabetes, HbA1C is the most important measurement for diagnosis of diabetes to include in CKDu research since this test serves as a marker for average blood glucose levels over the previous months prior to the measurement. Particularly for males, who are not used to seeking healthcare, the diagnosis of diabetes could be a new finding. Additionally, questions related to three big symptoms of type 2 diabetes, polyuria (need of urinating frequently), polydipsia (increased thirst and fluid intake) and poliphagia (increased hunger), should be asked and urine chemical analysis (urine dipsticks) should be performed in search of glucose and ketone levels.

With regard to measuring hypertension, steps should be followed to ensure the best environment, device, and techniques. First of all, the person will be asked about having been diagnosed with hypertension, and, whether any medication is taken. He or she must be relaxed and sitting comfortably in a chair with a backrest and feet flat on the floor, legs uncrossed. The right arm is the preferred arm for measurement. The arm should be supported and resting at heart level, for example on a table. The device to be used for blood pressure measurement must to be tested previously if it is an automated device, batteries should be checked and spare batteries should be kept. An appropriately sized cuff must be used since a cuff that is too small will overestimate the blood pressure and if it is too big, will underestimate it. The best option is to have two types of cuffs. Ideally, three measurements should be taken. High blood pressure could also be a new finding during the study. Some participants experience what is known as "white coat hypertension". For that reason it is advisable that, if a person has a high blood pressure with the first measurement ($\geq$140/90) and no previous diagnosis, to wait some minutes and repeat the measurement of blood pressure two times more with an interval of at least 20 minutes between each measurement. The first one should be discarded and an average of the two following measurements should be made. However with normal blood pressure and no history of high blood pressure, one measurement should be enough.

With regard to urinary tract infections (UTI), if the participant states that he/she has been to a doctor and received antibiotics, UTI is usually accepted as a fact. However, in Central America it has been observed that dysuria (so called 'chistate') can be a consequence of dehydration but misinterpreted as UTI both by the patient and the physician. It would be important to ask more questions to better define the origin of the symptoms. For example, questions on dry mouth, cramps, and dizziness as well as on type of work/activities performed and amount of liquid intake before the symptoms appeared could help to clarify if the person is experiencing the effects of dehydration or UTI. In addition, it is important to test the urine with urine dipsticks and, if conditions of field work allow it, send the urine sample for culture to further examine the presence of UTI.

Along the same line, questions about kidney stones should include inquiries of acute episodes of low back pain and having attended an emergency room at the hospital for diagnosis of such pains. Ultrasound examination could provide evidence of lithiasis if it is still present.

LIMITING HYPOTHESES REGARDING THE ETIOLOGY OF MeN· SUMMARY OF RECOMMENDATIONS

Facilitator: Carl Gustaf Elinder
Rapporteur: Nate Raines
Participants: Rebecca Laws, David Wegman

This working group addresses the variety of different hypotheses proposed explaining the etiology of Mesoamerican Endemic Nephropathy.

First and foremost, it is absolutely crucial that we endeavor to create a means to bring together existing knowledge on each hypothesis in a single location so that researchers are able to understand what is known and what investigations have been conducted. We propose the creation of a matrix, detailing the studies addressing each hypothesis and their findings, as well as an endnote file containing these studies which includes the grey literature and abstracts and protocols for ongoing research pre-publication. This should be accomplished through the consortium on MeN established at the First International Conference on MeN. Having this information in a centrally accessible location will allow us to better evaluate which hypotheses have been sufficiently investigated with which techniques, and will help researchers direct their efforts towards areas with greatest need for further investigation.

In limiting hypotheses regarding the etiology of MeN, it is also crucial to see whether the geographic and demographic extent of the disease might be greater than what we have identified so far, so that we can be confident that our investigations encompass the full scope of the disease. We suggest a useful area of investigation may be targeted small-scale prevalence studies outside well-investigated regions (in addition to Mesoamerica) and demographic groups (in addition to male agricultural workers). We also suggest that a better characterization of geographic distribution within known areas would be useful. La Isla Foundation is in the process of compiling GIS data into a regional map of MeN, and incorporating further information to enhance this resource would be highly valuable.

CURRENT HYPOTHESES

With respect to the current hypotheses on the etiology of MeN, using the information present in the literature and presented at the First International Conference on MeN, we have proposed the following categorizations based on the viability of each:

Highly Likely, High Priority to Investigate Further

- Heat stress and dehydration (including electrolyte imbalances)
- Non-steroidal anti-inflammatory drugs (NSAIDS)

Possible, High Priority to Investigate Further

- Arsenic
- Fructose intake
- Nephrotoxic medications, including homeopathic medications
- Leptospirosis and other endemic infections

Possible, High Priority but Logistically Difficult at this Time

- Genetic susceptibility and epigenetics
- Low birthweight and other prenatal, perinatal, and childhood exposures that increase susceptibility

Unlikely but strongly believed, Medium Priority to Investigate Further

- Pesticides
- Urinary tract diseases and sexually transmitted diseases (STDs)

Little Information, Medium Priority to Investigate Further

- Medication contamination and use of homeopathic medicines and non-approved drugs

Unlikely, Low Priority for Further Investigation

- Lead
- Mercury
- Cadmium
- Uranium
- Aristolochic acid
- Phosphorous
- Hard water

Necessary covariates to consider

- Drug, tobacco, and alcohol use
- Diet and nutrition
- Genetics, using racial subpopulation categorization as a proxy
- Poverty and socioeconomic status (necessary also because it can emerge as a confounding or obscuring variable)
- Co-morbid conditions – diabetes, hypertension

This list represents only a preliminary assessment of the etiological hypotheses, and should be considered modifiable as new evidence and hypotheses arise. Monetary and time costs for methodologies necessary to investigate each hypothesis are also crucial to consider in establishing priorities for hypothesis investigation.

TECHNIQUES

We discussed a number of techniques that would be useful in investigating and narrowing down hypotheses on the etiology of MeN going forward. We feel an effective approach would be to focus on MeN hotspot areas for hypothesis testing initially, subsequently moving to non-hotspot areas once the plausibility of the hypothesis is established. We also suggest that environmental sampling might be a useful technique for comparing hotspots to non-hotspots, with a particular focus on the evaluation of drinking water. Finally, with respect to hypotheses that are widely believed by populations affected by the disease, we suggest that community-based participatory research should be an important tool to achieve community buy-in and acceptance of conclusions.

FUTURE COLLABORATION

Facilitator: Catharina Wesseling
Rapporteur: Y-Vonne Hutchinson
Participants: Christer Hogstedt, Jennifer Crowe, Ricardo Correa Rotter, Kristina Jakobsson, Agnes Soares, Daniel Brooks, Channa Jayasumana

OBJECTIVE

The participants in the working group identified their primary objective as one of capitalization. Namely, participants aimed to capitalize on the momentum and progress of workshop discussions in order to improve international collaboration on MeN epidemiologic, medical and policy research initiatives, as well as to consolidate as a group. To that end, workshop participants of this working group agreed on the following stated objective:

To formalize institutionalized collaboration that builds upon the ongoing work in the region and the progress that we have made at this meeting.

IDENTIFIED QUESTIONS

Once participants identified this objective, several foundational concerns emerged. This workshop set a precedent as the first international meeting of MeN. However, though enthused by the hypotheses, opportunities, and analyses presented, working group participants felt that some distillation of principles was needed to facilitate united progress. Participants focused on several key questions:

- How should we organize going forward?
- What is our mission?
- What are our values?
- What do we want?

PROPOSED STRATEGY

To answer the questions posed above and to work towards the identified common objective, working group participants proposed two key initiatives.

Terminology Adjustment - Throughout the course of the workshop, during both the presentations and at the roundtables, there emerged a vigorous debate on the endemic/ epidemic nature of the disease. It is doubtful that this debate will be resolved soon, and certainly not without further research. Conference participants expressed concern that, in the interim, failure to correctly define the disease could lead to alienation of affected communities and underreporting. In order to fully understand the disease, participants argued that universally agreed on terminology for accurate capture was critical. To that end, working group participants proposed that we use the term Mesoamerican Nephropathy (MeN) instead of Mesoamerican Epidemic of Nephropathy (MEN) or Chronic Kidney Disease of Unknown Origin (CKDu).

Consortium Development - Participants proposed that the development of unified standing body would be the most effective way to address previously identified issues related to consolidation and coordination. The proposed consortium model would consist of a network of affiliated scientific researchers across fields working together to increase understanding and public awareness of the disease. The working group participants hope that the proposed consortium would be in a position to host a similar conference for MeN in 2014.

The consortium structure would be as follows:

- Coordinated by SALTRA - Roundtable participants agreed that SALTRA's geographic situation, academic affiliation, pool of existing resources, international contacts, and organizational experience/capacities place the organization in an ideal position to coordinate the consortium's activities. Upon obtaining funding, SALTRA would hire a part-time paid administrator who would exclusively focus on administrative matters, communications, and planning activities related to the consortium.
- Board - Roundtable participants agreed that the consortium should be governed by a rotating, board comprised of 6 to 8 members, representing different geographical locations and areas of expertise. Participants also agreed that a temporary board should be selected at the workshop to serve until a permanent board is formed.
- Membership
  - Full Members - Researchers and clinicians actively working to combat MeN in Mesoamerica would be eligible for full membership.
  - Associate/ Observer Members - Associate or observer members would not have all of the privileges of full membership, however they would be invited to participate in selected meetings, serve on working groups, and access research resources. Intergovernmental and regional organizations (e.g. PAHO, WHO, ILO, COMISCA), government representatives, NGO representatives, and researchers focusing on the epidemic

in other parts of the world, would all be invited to participate.
- Working Groups - Consortium working groups could be formed on an ad hoc basis to address specific issues such as methodology, public policy, and public education.

Consortium activities would include:

- Information sharing – Consortium meetings and publications would serve as a forum for information sharing, allowing its membership a mechanism through which they can quickly disseminate new ideas, share recent developments in the field, and collaborate on future initiatives.
- Compile and disseminate research – Working group participants agreed that the consortium would provide an appropriate forum for gathering and sharing completed research results and new research from a central location.
- Funding seeking collaboration – Though consortium members would be free to apply for individual funding, the working group participants suggested that the consortium would also be ideal for collaboration on grant proposals. SALTRA's established reputation and university resources combined with the collective force of members' accomplishments would lend the consortium members the kind of institutional legitimacy that can often be quite difficult to obtain individually.
- Serve as a bridge for translating results to policy makers – Working group participants believed that the consortium should act as a mechanism through which research findings are compiled, distilled, and translated into actionable, informed policy recommendations.
- Inform other initiatives – Working group participants suggested that consortium members could play a key role in informing or supporting outreach, advocacy and education initiatives of other similarly-concerned organizations for communities at risk.

NEXT STEPS

The following steps were identified by the working group participants as crucial towards implementing the aforementioned proposed strategy:

- Solicit interest in the consortium – An email will be sent out to all workshop participants gauging their interest and willingness to participate in the proposed consortium.
- Develop a webpage announcing the formation of the consortium – SALTRA will develop a webpage which will announce the formation of the consortium, share workshop developments, and detail next steps.
- Seek funding for the research administrator position - With the aid of the temporary board, SALTRA will seek funding for the part-time research administrator position. Possible funding sources to be targeted include ISN, LASN, and the Spanish government.
- Seek endorsements – SALTRA and the temporary board will seek endorsements from pertinent and interested organizations and institutions.
- Paper request – SALTRA and the temporary board will issue a call for published and unpublished papers on MeN and related topics to be stored in a central registry. available to all consortium members, which will be continually updated
- Formation of a temporary board – the working group participants proposed the formation of a temporary board to:
  - Formulate statement of purpose for the consortium ;
  - Finalize the structure of the consortium; and
  - Develop procedural mechanisms and terms of reference for consortium leadership, partnership, and membership.

  Proposed members temporary board members, included:
- Jennifer Crowe (SALTRA, Universidad Nacional, CR)
- Aurora Aragón (CISTA, SALTRA, UNAN-León, Nicaragua)
- Sandra Peraza (SALTRA, University of El Salvador)
- Ricardo Correa Rotter (National Medical Science and Nutrition Institute Salvador Zubiran, Mexico)
- Manuel Cerdas (Social Security of Costa Rica)
- Dan Brooks (Boston University)
- Kristina Jakobsson (Lund University)
- Y-Vonne Hutchinson (advisory role, La Isla Foundation)

HEALTH SERVICES FOR INDIVIDUALS WITH CHRONIC KIDNEY DISEASE, MUNICIPALITY OF CHICHIGALPA, NICARAGUA 2012-2013

Dr. Juan José Amador and Lic. Damaris López,
Boston University

Chronic Kidney Disease (CKD) has attained epidemic proportions in the western region of Nicaragua. The highest prevalence and mortality rates occur in the departments of Chinandega and León, with more than 70 deaths for every 100,000 inhabitants per year. CKD mortality in the municipality of Chichigalpa continues to rise, and a record high of 100 deaths per year is expected to occur in 2012. CKD patients are typically young male agricultural workers in their economically productive life, with mortality occurring in all age groups, including young individuals. Ongoing research to determine the cause of this public health problem is being conducted by Boston University and UNAN León. Another aspect of this public health problem is the quality and type of medical services offered to patients in the department of Chinandega and the municipality of Chichigalpa, which until now have remained insufficient. The Ministry of Health has destined funds from the national treasury equivalent to $3.25 million USD, and as of October 2012, has begun the construction of a primary care hospital in the municipality of Chichigalpa, which includes a nephrology clinic to improve medical management of CKD, and will offer services to patients with end stage renal disease, including hemodialysis and peritoneal dialysis. The Ingenio San Antonio (ISA) and the German Bank of investment and Development (DEG) have donated $320,000 USD to the Ministry of Health in Nicaragua for the construction and equipment of the nephrology clinic in Chichigalpa, starting in 2013. Additionally, ISA has donated the five hectares of land where the hospital and nephrology clinic are being built.

INVESTIGATING OCCUPATIONAL FACTORS AND BIOMARKERS OF KIDNEY FUNCTION AMONG NICARAGUAN WORKERS

Rebecca Laws, Daniel Brooks, Juan José Amador, Daniel Weiner, James Kaufman, Oriana Ramirez Rubio, Jose Marcell Sánchez Rodríguez, Michael McClean, Boston University

INTRODUCTION:

In Nicaragua, an excess prevalence of chronic kidney disease (CKD) with unknown etiology has been described in young, male agricultural workers. Though the majority of recorded cases are sugarcane workers, it is unknown whether other industries are affected. Our goals were to characterize the type of kidney damage, evaluate occupational factors, and investigate the role of metals exposure.

METHODS:

Our study population included 284 sugarcane workers, 51 miners, 60 construction workers, and 53 port workers. Blood and urine samples were collected during two rounds of sampling (beginning and end of harvest season (zafra)) for sugarcane workers and only during the second round for other workers. We analyzed biological samples for metals and biomarkers of kidney injury and CKD. We used linear regression models to investigate predictors of kidney injury and/or CKD.

RESULTS:

Estimated glomerular filtration rate (eGFR) was significantly different by sugarcane job and decreased by 6.4 mL/min/1.73 m^2 in cane cutters as compared to factory workers (p=0.006). Similarly, NGAL was significantly different by job and increased most among cane cutters, by 19.2 mg/g creatinine, compared to factory workers (p=0.04). More workers than expected in other industries had eGFR <60 mL/min/1.73 m^2, indicating CKD. Generally, urine albumin was low in all workers. Heavy metals were not associated with markers of kidney function, with the exception of arsenic; workers with the highest arsenic exposures had significantly lower eGFR (p=0.01).

CONCLUSIONS:

In sugarcane workers, biomarkers of kidney injury and CKD were highest among field workers and lowest among factory workers, supporting the hypothesis that workers with the greatest heat exposure are at greater risk of developing disease. These data provide evidence of CKD among workers in other industries and indicate a tubulointerstitial disease. Finally, there is some evidence that high exposure to arsenic is associated with biomarkers of CKD.

END STAGE RENAL DISEASE IN EL SALVADOR: EVIDENCE OF NON-TRADITIONAL RISK FACTORS

Ricardo Leiva, MD, Silvia Leiva, MD, Luis Trujillo, MD Zulma Trujillo, MD. Servicio de Nefrología, Hospital Nacional Rosales, El Salvador.

INTRODUCTION

Hospital Nacional Rosales in San Salvador is the main safety net hospital for the uninsured in El Salvador. Outpatient hemodialysis and peritoneal dialysis are offered but due to the high incidence of end stage renal disease (ESRD), most new patients can only be offered intermittent peritoneal dialysis with a rigid peritoneal dialysis catheter (weekly treatment). Hospital Nacional Rosales is a 450 bed hospital and the most common admission diagnosis is renal failure with 300-400 new patients per year. Our previous work revealed that 67% of the incident patients with ESRD did not have traditional risk factors for chronic kidney disease such as diabetes or hypertension, and that the sub-group without traditional risk factors tended to be younger and predominately male (87%). Furthermore, this subgroup tended to be farm workers reporting contact with pesticides.

Hospital Nacional Rosales is a referral hospital and to better understand the geographic distribution of the incident patients with ESRD we designed a study to estimate the incident rates of ESRD per department (the country is divided into 14 departments).

METHODOLOGY

El Salvador does not maintain a precise ESRD registry. In cooperation with the Pan American Health Organization, we developed an epidemiological survey in which ESRD patients (Hemodialysis, Peritoneal Dialysis or Kidney Transplant) were interviewed. The probable causes of ESRD and demographic information was collected.

RESULTS

The prevalence of ESRD was 12.5 cases per 100,000 habitants, and the most affected ages where between 20 and 70 years. The highest prevalence of ESRD was in the Department of La Paz with a prevalence rate of 25.3 cases per 100,000 habitants and with a male predominance of three times greater than expected.

67 % of the cases did not have traditional risk factors for chronic kidney disease. Potential risk factors were identified and included exposure to chemicals, mainly pesticides or fertilizers and drinking form wells.

CONCLUSIONS

Like many countries, the rate of ESRD is rising and the treatment costs are prohibitive in El Salvador. Initial epidemiologic research suggests that non-traditional risk factors for chronic kidney disease could be responsible for some portion of the rampant rise in ESRD rates in El Salvador. There is an urgent need for further research into the possible causative role of pesticides, heavy metals, contaminated water, dehydration or other non-prescribed medications or herbs.

Keywords: End Stage Renal Disease, Risk factors

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SUGAR-CANE HARVESTING AND KIDNEY DISEASE IN ECUADOR

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INTRODUCTION

Sugar-cane harvesting requires extremely strenuous physical labor in hot climates. Risks to workers include acute traumatic injury, musculoskeletal disorders (MSDs), heat stress, and dust inhalation. This project represents an international collaboration with the union (SCIV/FENACLE) of 1200 workers who perform all of the tasks involved in harvesting. Mixed methods (qualitative/quantitative) are being used to characterize these jobs and risks, with the goal of developing ergonomics and safety controls that are feasible, effective, and acceptable to the workers.

METHODS

Job groups were selected for study on the basis of physical and organizational characteristics (exposures). A standardized questionnaire was developed to cover medical and injury history, work history, physical and psychosocial job features, MSDs, and demographics. The questionnaire was pilot-tested in one-on-one interviews and revised as appropriate for administration to union members in face-to-face interviews during the harvest season. Group interviews, followed by videotaping of work in the cane field, provided detailed information about work organization and job content (tasks by season, pay basis, etc.).

RESULTS

Kidney disease, diagnosed by a physician, was reported by 23% of participating workers and reported much more frequently by cane cutters than by other workers. Less than half of those with self-reported history of renal disease also reported diabetes or hypertension, two of the most common causes of kidney disease in working-age people. Despite being paid on the basis of production, interviewed cane cutters reported regularly being too fatigued to be able to work more than 6 hours per day even when more work was offered. The fields are burned before harvest, to reduce weeds and to drive away snakes. Often they are still hot – even smoking - when the cane cutters enter. Dehydration and lack of potable water are common complaints. Air-borne ash was a frequent exposure for 73% of study respondents.

DISCUSSION

Cane cutting is a relatively well-paid job compared to others in this region of Ecuador but involves a exposure to a number of work stressors. The combination of quantitative and qualitative findings indicated that kidney disease may be excessive in this population. A follow-up investigation of kidney disease biomarkers and work practices is warranted in this work setting.

HISTOPATHOLOGICAL FEATURES OF SRI LANKAN AGRICULTURAL NEPHROPATHY

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Sri Lankan Agricultural Nephropathy has become a key public health concern in certain agricultural areas in Sri Lanka due to the dramatic rise in its prevalence and mortality among young farmers for last two decades. Clinically the disease is characterized by tubular proteinuria, usually alpha-1 and beta 2-microglobulinuria, and the absence of hypertension and edema.

Histopathological observations of 34 renal tissues obtained at Padavi Sripura Hospital from December 2010 to July 2012 were scored according to Banff 97 Working Classification of Renal Allograft pathology.

Tubular Interstitial nephritis with or without nonspecific interstitial mononuclear cell infiltration was the dominant histopathological observation. Glomerular sclerosis and glomerular collapse were also common.

Study concludes that tubulointerstitial damage which is commonly seen in toxic nephropathies is the major pathological lesion in SAN. The disease process appears to mainly affect the proximal tubules and the interstitium giving rise to characteristic, recognizable histopathological and clinical features. Presences of alpha-1 and beta-2 microglobulin in patient's urine samples are compatible with the histopathological observation.

SRI LANKAN AGRICULTURAL NEPHROPATHY AND HIGH GROUND WATER HARDNESS – POSSIBLE LINK

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Hardness of water is caused mainly due to presence of cations, Ca^{2+} and Mg^{2+} together with anions carbonates, bicarbonates, sulphates and chlorides. Temporary hardness is caused by the presence of dissolved calcium and magnesium bicarbonate minerals whereas permanent hardness is caused by sulfate and chloride compounds. Temporary hardness can be reduced either by boiling the water or by the addition of lime but permanent hardness cannot be removed by boiling. According to WHO apparently there is no convincing evidence to the effect that water hardness causes adverse health effects in humans. Some studies have shown a weak inverse relationship between water hardness and cardiovascular disease in men, when water contains up to a level of 170 mg calcium carbonate per litre of water. According to the WHO guidelines given in 2009, water hardness classified as Soft 0-60mg/L, moderately hard 61-120 mg/L,hard 121-180 mg/L and very hard >181 mg/L.

Sri Lankan Agricultural Nephroathy (SAN) emerged as an important public health problem in rice cultivating areas in the dry zone of the Sri Lanka. A frequent complaint of the inhabitants of the SAN endemic areas in Sri Lanka is the increasing hardness in dug wells and tube wells that receive water from shallow regolith aquifers.

Present study was conducted to determine the hardness of drinking water source of SAN patients in Padavi-Sripura (n=78), Kebithigollawa (n=38), Mahawilachchiya (n=33) Polpithigama (n=48) in the dry climatic zone and compare them with that of the drinking water sources in Gampaha (n=35), Kandy (n=32) and Deniyaya (n=28) ,which are located in wet zone and SAN is not prevalent. Total hardness of the water samples was measured using EDTA titration (EPA 130.2) method and calcium content was measured using flame AAS.

Highest average hardness (496±34 mg /L) was observed at Padavi-Sripura and the values ranged from 110±54–1120±62 mg/L. The second hardest water was found from Polpithigama area which ranged from 70±8 – 715±47 mg/L. The lowest hardness among the test sites was observed at Kandy (n=38), where the hardness ranged from 08±2 –220±21 mg/L. Calcium levels in the water samples taken from wells that SAN patients have been used for drinking purposes were detected at an average concentration of 83.2 mg/L, range of 32±4 - 398±28 mg/L. Average concentration of 13.6 mg/L in the range of 2.1±0.8 - 42±14 mg/L has been detected from the water samples collected from Gampaha, Kandy and Deniyaya. Hence the results revealed that most of the water samples tested in SAN prevalence areas contains very hard water.

Our finding also revealed that hardness of bottom layer of water in the wells was gradually increases with depth and presence of significantly high levels of Fe^{3+} and Mn^{2+} ions in hard water samples. These levels exceed WHO prescribed values for drinking water. The results substantiate the general complaint by the villagers in the study area that the quality of water is unacceptable for drinking.

A statistically significant positive correlation ($P < 0.008$) was revealed between occurrence of SAN and hard water consumption. 96% of the SAN patients have consumed hard or very hard water at least for 5 years before diagnosis of the disease. We have already published data on presence of Arsenic(As), Cadmium (Cd) and Uranium (U) in agrochemicals used in Sri Lanka that have a possible link to SAN. It was found that most of the pesticides and chemical fertilizers containing phosphate were contaminated with As,Cd,U and our findings revealed that As and Cd content in soil gradually decreases with depth, particularly in the agricultural areas implying that it is not present naturally in soils nevertheless has been introduced from the surface, most probably due to anthropogenic activities. Hardness may act through several mechanisms to carry these toxic substances to victim's body and enhance the toxicity. Although it is believed that hardness alone does not cause any damage to human health it may become hazardous in the presence of arsenic, cadmium and other pollutants.

Keyword:
Hard water, Arsenic, Cadmium, Sri Lankan Agricultural Nephropathy

CHRONIC KIDNEY DISEASE AND ASSOCIATED RISK FACTORS IN BAJO LEMPA, EL SALVADOR. THE NEFROLEMPA STUDY 2009-2010

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INTRODUCTION AND OBJECTIVES:

In El Salvador chronic kidney disease (CKD) is the leading cause of hospital death. The Nefrolempa project aims to determine the prevalence of the CKD, urinary markers of renovascular damage, and associated risk factors.

METHODS:

Nefrolempa is a study epidemiological-clinical cross-sectional and analytical study conducted during the years 2009-2010, based on the active search of CKD and the associated risk factors in communities of the Bajo Lempa, Usulután, Jiquilisco, and El Salvador. Communities, social organizations, and institutions of higher education and health actively participated. Doctors, nurses, clinical laboratory technicians, medical health technician students, and health promoters were selected and trained. Prior consent of the participants was obtained, and after a pilot study to assess the methodology, social determinants and clinical and epidemiological data were collected through the application of a family and personal medical history prepared for the purpose. House to house visits were performed with technically trained and certified personnel for medical consultations: anamnesis, physical measurements, urine markers of reno-vascular damage through test strips; determinations in blood of creatinine with estimation of glomerular filtration rate by means of the MDRD formula, glucose, total cholesterol, LDLc, HDLc, and triglycerides.

RESULTS:

554 families and 1215 persons ≥ 18 years, both sexes, were studied. The prevalence of CKD was 15.4% ; males: 22.8% and females: 9.5% . The prevalence of renal insufficiency was 8.8% ; males: 15.9% and females: 3.2% . Prevalence by stage was: stage 1: 4.2% ; stage 2: 2.4% ; stage 3a: 3.5% ; stage 3b: 2.5% ; stage 4: 2.3% ; stage 5: 0.5% . The prevalence of markers of kidney damage was: 12.1% ; males (17%) and female (8.1%). Prevalence by markers: microalbuminuria (A2) 5.9% ; macroalbuminuria (A3): 0.3% ; proteinuria: (3.5%); hematuria: 2.2% ; proteinuria-hematuria: 0.2% . The prevalence of traditional risk factors was high: DM: 8.9% ; HT: 19.2% ; family history of CKD: 21.2% ; dyslipidemia: 49.9% ; overweight: 29.5% ; obesity: 22.1% ; metabolic syndrome: 15.2% . Also the prevalence of exposure to nephrotoxic agents was high: consumption of NSAIDs: 80.9% ; consumption of medicinal plants: 62.3% ; contact with chemicals: 52.1% .

CONCLUSIONS:

A high prevalence of risk factors for CKD in the studied population was shown. Predominated the CKD of unknown cause (CKDu) not associated with DM, hypertension or proteinuria > 1 g/l. Univariate and multivariate analysis using multiple logistic regression (MLR) with CI 95% were performed. Associated risk factors in MLR were age (OR 4.630), being male (OR 1.874), farmer occupation (OR 1.761), hypertension (OR 1.750) hypertriglyceridemia (OR 1.733) and family history of CKD (OR 1.712). The presence of a double burden was observed, of traditional vascular atherosclerotic risk factors and non-traditional occupational and toxic environmental factors, which may act synergistically to produce kidney damage. The results of this study, and others conducted by the Salvadoran nephrology community, demonstrate the presence of a new disease entity, not yet characterized at depth: "nefropathy of Salvadoran agricultural communities". The similarity with reports of CKD in other Central American countries and in the south of Mexico could justify the hypothesis of a "Regional Central American or Mesoamerican Nephropathy". To corroborate these hypotheses require further epidemiological, clinical and toxicological studies. Finally, the information obtained has been useful to plan resources for health care for the affected population and has constituted the basis for the design of a Kidney Health Unit in Bajo Lempa, integrated by a multi-

disciplinary team for preventive and curative intervention actions in the detected patients, implemented by the Ministry of health of El Salvador. This allows continuity of the work in the rest of the communities of the region studied, as well as the expansion of new health studies and actions to other Salvadoran rural communities.

Key words:
chronic kidney disease /epidemiology, risk factors, prevalence, occupational health, environmental health, agrochemicals, El Salvador.

RESEARCH IN KIDNEY HEALTH

Carlos Manuel Orantes, Instituto Nacional de Salud, Ministerio de Salud, El Salvador.

The National Institute of Health has carried out population-based epidemiological investigations. After 2 years, 11 communities, 1306 families and 5018 individuals of all the ages had been studied in rural communities of the municipalities of Jiquilisco (Bajo Lempa), San Miguel and Guayapa Abajo. The communities had high prevalences between 16 to 20 % and decreased kidney function was found from early age, including in persons under the age of 20.

A very particular epidemiological pattern has been found in the agricultural communities, with both sexes affected, adults and adolescents, predominantly males (a male-female relation of 2:1 ages between 20 and 59). With regard to the cause, in the majority of diagnosed patients there was no association with diabetes mellitus, hypertension or other primary kidney disease, i.e., its cause is unknown.

As preliminary results, a high coexistence of risk factors suggests the presence of a double burden of causal as well as progression factors; i.e. traditional risk factors of diabetes mellitus, hypertension, obesity, dyslipidemia - and nontraditional risk factors of occupational and environmental toxicity that may act synergistically in relation to the kidney damage.

Investigations have started to determine environmental and occupational toxicity. Studies of the content of pesticides and heavy metals in surface and ground waters, soils and sediments have started in Bajo Lempa, with the collaboration of researchers from Ohio University, the Ministry of Health, Ministry of Environment, and the Administration of Water and Aquaducts (ANDA). Soil samples have been collected taking into account areas were people who have kidney disease have been working.

Preliminary research results show the presence of heavy metals in soil; cadmium and arsenic levels are the highest in the area where the largest number of diseased workers has been working. These results suggest a possible connection between the presence of cadmium and arsenic (a highly toxic heavy metal that affects the kidney) and the presence of the disease.

We have implemented a new paradigm to manage kidney health, through clinical-epidemiologic research for the development of integrated and cross-sectoral actions of health promotion and prevention of chronic kidney disease, and through comprehensive and integrated networks of health services with regard to risk factors at the community level. Interventions benefit individuals, families and communities in the health-areas where they are carried out; the model is included in a program integrating prevention and medical attention of chronic kidney disease which is in the process of implementation at national level, as part of the actions framed within the health reform process.

KIDNEY DAMAGE MARKERS IN NICARAGUAN ADOLESCENTS IN A REGION OF AN EPIDEMIC OF CKD OF UNKNOWN ETIOLOGY

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BACKGROUND

An epidemic of CKD is occurring across Central America with over 20,000 deaths, mainly among younger men. Most studies have focused on occupational factors, but the large number of cases at a young age suggests that initial damage may begin in childhood.

METHODS

We studied markers of kidney damage in 200 students (age 12-18) with no prior work history from 4 schools in Nicaragua (1 Jinotega, 2 Masaya, 3 North Chichigalpa, 4 South Chichigalpa). Schools were selected to represent a range of risk based on adult CKD mortality data. Urine was tested by dipstick and analyzed for ACR, NGAL, NAG, and IL-18.

RESULTS

Dipstick proteinuria (3%) or glucosuria (1%) were rare, and only 8% had ACR >30 mg/g. The median IL-18 level (pg/ml) was higher than in healthy controls identified from other studies (45, IQR 21-115 vs. 15, IQR 7-28). The ratio of mean levels for NGAL, NAG, and IL-18 by sex and school are shown in the table.

Males had lower levels for all markers, particularly NGAL and IL-18. The results by school were consistent with their *a priori* risk: among males, the highest mean levels of NAG, NGAL, and IL-18 were at the highest risk school. Females at the two highest risk schools had elevated levels of NAG. Results were the same regardless of whether measurements were normalized for urine creatinine.

Mean ratio (95% CI)

	NAG	NGAL	IL-18
SEX			
F	Ref	Ref	Ref
M	0.78 (0.58-1.05)	0.25 (0.20-0.32)	0.29 (0.23-0.37)
SCHOOL (MALES)			
1 (LOWEST RISK)	Ref	Ref	Ref
2	1.43 (0.70-2.92)	1.36 (0.87-2.13)	0.58 (0.35-0.96)
3	2.15 (1.13-4.07)	0.77 (0.52-1.16)	0.85 (0.54-1.33)
4 (HIGHEST RISK)	3.25 (1.61-6.55)	1.53 (0.98-2.38)	1.62 (0.99-2.66)
SCHOOL (FEMALES)			
1 (LOWEST RISK)	Ref	Ref	Ref
2	1.28 (0.86-1.90)	0.76 (0.45-1.27)	0.65 (0.40-1.05)
3	2.00 (1.36-2.94)	1.28 (0.77-2.13)	0.64 (0.40-1.04)
4 (HIGHEST RISK)	2.27 (1.46-3.52)	1.40 (0.79-2.50)	0.57 (0.33-0.98)

CONCLUSIONS

The results suggest that tubular kidney damage may be present among children in an area of epidemic CKD. If confirmed, factors in addition to occupational exposure should be studied as possible causes of CKD.

CHRONIC KIDNEY DISEASE IN NICARAGUA· A QUALITATIVE ANALYSIS OF SEMI-STRUCTURED INTERVIEWS WITH PHYSICIANS AND PHARMACISTS

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4. Boston University School of Medicine, Boston, MA, United States

BACKGROUND:

Northwestern Nicaragua has a high prevalence of chronic kidney disease (CKD) of unknown etiology in young adult men. Interestingly, a high frequency of urinary tract infections (UTI) is also reported among men along with a dysuria syndrome described by manual laborers as "chistata." This study examines health professionals' perceptions regarding etiology of UTI and chistata and their treatment approaches, including use of potentially nephrotoxic medications.

METHODS:

Nineteen in-person semi-structured interviews were conducted in November 2010 among ten randomly selected physicians and nine pharmacists in the region. Using qualitative methodology, responses were coded and analyzed for patterns of agreement or disagreement and the occurrence of themes.

RESULTS:

Health professionals perceived CKD as a serious and growing problem in the region, primarily affecting young men working as manual laborers without traditional risk factors for CKD. All interviewees regarded occupational and environmental exposure to sun, heat, and dehydration as critical factors associated with the occurrence of CKD, and noted that hydration efforts may be influenced by perceived water contamination. These factors were also considered to play a role in the occurrence of chistata in the region, the symptoms of which were often treated with non-steroidal anti-inflammatory drugs (NSAIDs), diuretics and antibiotics, including quinolones and aminoglycosides. Physicians acknowledged that the diagnosis of UTI was usually not based on microbial culture, and opined that the use of potentially nephrotoxic medications may be contributing to CKD.

CONCLUSIONS:

Interviews provided evidence suggesting that diuretics, antibiotics and NSAIDs are widely used and sold over the counter for symptoms that may be related to volume depletion. Acute kidney damage coupled with volume depletion and exposures including medications and should be further evaluated as causal factors for CKD in this region.

POSSIBLE ASSOCIATION BETWEEN LEPTOSPIRA INFECTION AND CHRONIC KIDNEY DISEASE OF UNKNOWN ORIGIN IN CENTRAL AMERICA

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INTRODUCTION

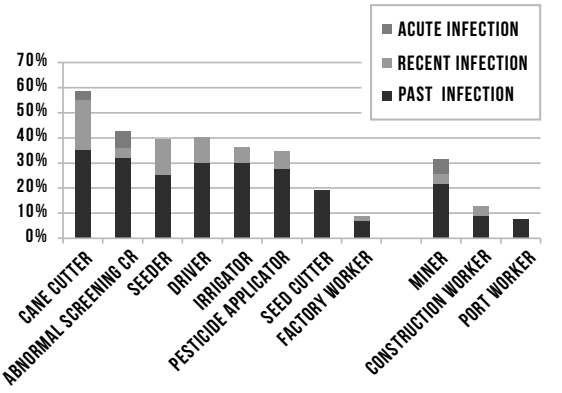
Chronic kidney disease of unknown origin (CKDu) in Central America is a major public health problem. It is characterized by chronic kidney disease of non- glomerular origin that disproportionately affects young male agricultural workers.[1-3] Leptospirosis, a bacterial zoonosis with epidemiologic characteristics similar to CKDu is among the causal hypothesis.[4-6] It causes renal injury, and although it is not known to cause chronic kidney disease in humans, biologic plausibility exists.[7] A link between leptospirosis and chronic kidney disease represents an evidence-gap due to lack of research and limited data on long-term outcomes in existing studies.[8,9] It is important to explore whether recurring mild or asymptomatic leptospirosis can lead to multiple episodes of subclinical acute interstitial nephritis, resulting in progressive kidney fibrosis and ultimately CKD. Boston University with collaboration from the Centers for Disease Control and Prevention (CDC) is conducting a study in Nicaragua among workers in sugarcane and other industries to determine if leptospirosis is associated with urine markers of kidney injury.

STUDY DESIGN

Study population includes 282 sugarcane workers representing seven different job tasks within the industry; 47 individuals that had abnormal serum creatinine during initial screening for hire; and 160 workers from non-sugarcane industries including mining, construction and port workers, who have never worked in sugarcane. Exposure to *Leptospira* was determined by using the microscopic agglutination test, ELISA and urine PCR. Serum creatinine and an estimated glomerular filtration rate were used to determine kidney function. Urinary markers of kidney injury include urine albumin, neutrophil gelatinase-associated lipocalin, N-acetyl-beta- D-glucosaminidase, and interleukin-18.

PRELIMINARY RESULTS

Leptospirosis among workers in sugarcane and other industries



CONCLUSIONS

Past exposure to *Leptospira* is high, particularly among cane cutters (35.3%). The high percentage of recent infections among workers in the sugarcane industry suggests that many exposures occurred in a non-occupational setting or in the previous harvest season. Acute infection is common among cane cutters (4%), subjects with an abnormal screening creatinine (6.4%), and miners (6%). These acute infections may represent mild or subclinical disease that would have otherwise gone unrecognized. Urine PCR was negative in all subjects, arguing against chronic colonization of renal tubules by leptospires.

FUTURE WORK

Correlation between *Leptospira* exposure and urine markers of kidney injury is still not available, and will be important to determine if leptospirosis is associated with subclinical kidney injury.

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ASSESSMENT OF THE POTENTIAL INFLUENCE OF WATER QUALITY IN THE INCIDENCE OF CHRONIC KIDNEY DISEASE (CKD) IN NICARAGUA

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This action is under a Project aiming to identify the need for improving integrated water quality in two Departments in Nicaragua (See Poster presentation), supported by AECID (*) by collaboration of CISTAS-UNAN Leon (Nicaragua) and the Toxicology Unit of IB-UMH Elche (Spain). After a contact with the local situation it was confirmed that the Chronic Kidney Disease is a serious public health problem with high social susceptibility. Therefore we focus the main activity of the project in evaluating the potential relation with water quality and to identify the what can be done to understand the problem, doing some analytical work and establish an Action Plan for a further detail evaluation of the potential role of water quality in CKD

In a First stage a visit of the Spanish team to Nicaragua the existing epidemiological information performed by the CISCA researchers (Nicaragua) in collaboration with other institution were discussed and evaluated. Specific representative communities were visited, contact with population were done for detecting the social filling, observation in situ of the several systems of getting water from underground water resources. Then a planning was done for getting representative water samples in representative communities. It was planned to do analytical work of inorganic elements (28 elements, heavy metals and other elements), pesticides and microbiological parameters. In order to test the viability of doing in the future human non-invasive biomonitoring, it was designed getting also a number of hair samples in a reduced number of humans in one specific area.

The program of sampling was later performed by the staff of CISTAS (Edipcia Roque) with help of voluntary collaborators. In a Second Stage, a visit of two researcher of CISTAS were done to Spain (Aurora Aragon and Edipcia Roque). During this visit further analysis of the epidemiological data were done, the analysis of elements in water were performed in the Spain laboratory, and discussion with other Spanish Toxicologists were done to evaluate other factors to be analyse and in particular potential biomarker of exposure and early biomarker of kidney (and other pathologies) were discussed.

The analysis of element in water and hair have been done, data are under quantitative evaluation. The microbiological analysis have been also performed and the pesticide analysis in going on. For getting final Conclusion detail evaluation of the data is going on. Some provisional conclusion on the analysis of element, suggest that no spectacular high level of con inorganic contaminant can be deduced globally. Therefore in principle it seems that the presence of heavy metal does not seems t be of high concern. Although level of some element, like AS, Se, V, Mn, Mg, cases are observed higher to recommended guidelines, they does not seem to be of high concern by itself. However it is still needed a detail evaluation in relationship to geographical distribution and to evaluate not only individual elements but also the combination of them and to consider seasoning and meteorological factors and classical contaminant as Pb, Hg, Cd show low values. The measures in hair demonstrated that the procedure is viable for applying to future massive campaigns and for example levels of some elements are higher that our reference values in sample in population in Spain. Therefore human biomonitoring of metals would be consider of high interest in future programs. In a Third Step the obtained data will be revised, provisional conclusion will be presented in Leon to authorities and representation of the communities

We intend to have a final report of our data by March 2013 and an Action Plan proposing global actions for improving water quality actions and controls and human biomonitoring by May 2013.

(*) Acknowledgements:
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CLINICAL CHARACTERISTICS AND 3 YEAR FOLLOW-UP OF PATIENTS WITH CHRONIC KIDNEY DISEASE WHO LIVE IN SANTA CLARA SUGARCANE COOPERATIVE DEPARTMENT OF LA PAZ, EL SALVADOR

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INTRODUCTION

The Santa Clara's sugarcane cooperative is located 44 kilometers from San Salvador, at an altitude of 121 meters above sea level. This cooperative was formed as a result of agricultural reform. Cotton was cultivated on those lands from 1967 to 1986 and then was replaced by sugarcane plantations. The cooperative has 396 members and our Nephrology Department was contacted in the year 2006. In order to do a research on Chronic kidney Disease (CKD) because of the increasing number of deaths among their workers.

In a previously research conducted by PAHO and the hospital network of El Salvador in 2003, the department of La Paz had the highest incidence of ESRD in the country (25.3 per 100,000 populations), the etiology was unknown in 66.8% of cases and the male / female ratio was 18.1/7.0 per 100,000 populations, aged 40-60 years.

OBJECTIVES

To identify CKD prevalence among sugarcane workers of Santa Clara's cooperative, their epidemiological and clinical characteristics after a 3 year monitoring.

MATERIAL AND METHODS

The study was a retrospective and descriptive.

The local physician performed a screening of 396 workers and then she referred to us those with creatinine ³ 1.2 mg /dl or abnormal renal ultrasound.

The patients were evaluated by Nephrologists that performed a complete medical history; laboratory tests (blood count, creatinine, urea nitrogen, uric acid, electrolytes, lipid profile, glucose, urinalysis, and creatinine and protein clearance in a 24 hours urine collection).

Transportation of the patients to the hospital was provided by the cooperative trough a Social Worker.

RESULTS

Of the group of 396 workers, we found that 58 (14.6%) had CKD, their ages were in the range of 40 to 70 years(X55 SD 12 y); most of them had lived in that area for X35years (SD 2.11); the family group consisted of at least X4.58 members (SD 1.72), the mean number of children was X4.3 (SD 2.21). The average daily fluid intake was X3.24 L (SD 0.47 L); 84.5% drank water from wells without any treatment; 10.3% drank potable water and 5.2% both. All of them had contact with pesticides.

NSAID was consumed by 59% occasionally, 67% drank alcohol regularly, and 20.6% had a family history of CKD. The etiology of the disease was identified in 9 patients: 5 with high blood pressure, 3 with DM, and 1 with kidney stones; in the rest of them, 84.5% , the etiology was unknown; 24.1% consulted because of arthralgias in the rest of the group the diagnosis of CKD was incidental.

The average weight was 67Kg (SD 10.2); average height was 1.59 m (SD 0.07).

We categorized the blood pressure using JNC-7's classification: (57%) were normotensive, 10 (17%) had pre-hypertension, 8 (13%) stage 1, and 8 (13%) stage 2 hypertension.

According to the classification of Chronic Kidney Disease, we found 7 workers (12%) in stage 1; 13 (22%) in stage 2; 20 (34%) in stage 3; 14 (24%) in stage 4 and 4 (8%) in stage 5 .

Regarding laboratory tests, normocytic normochromic anemia was found in 86% , glucose levels > 110 mg/dl in 3 patients (5%); hyperuricemia (uric acid levels >7 mg/dl) in 40 patients (69%); cholesterol> 200 mg/dl, 17 (29%), triglycerides >150mg/dl 18 (31%), 93 % had non nephrotic proteinuria and only 7% didn't have proteinuria. There were no significant findings in the urinary sediment.

The main treatment was enalapril in 50% of the cases, allopurinol in 49% , and hypolipidemic drugs in (12%).

There was a significant drop out during follow up; in the first control at Hospital Rosales, only 58 patients came, and by the third month only 27 pa-

tients showed up (47%). By the end of the first year only 29 remained (50%), after 2 years there were 21 patients (36%), and after 3 years only 13 patients (22%) continued their follow up; this group had a rapid progression of their CKD

In the study period, 6 patients died 5 of uremia and only one accepted dialysis therapy. Another one died of stroke.

CONCLUSIONS

The results of this research show the high prevalence of CKD of unknown etiology in sugarcane workers of Santa Clara cooperative. The most important findings were anemia, hyperuricemia and non nephrotic proteinuria; 57% of them had no hypertension.

The withdrawal from follow up was striking, despite that they had the viability to get to the hospital. This population needs more information about CKD in order to recognize the importance of medical follow up.

More research is needed to determine the etiology of CKD in this population.

AN INTERDISCIPLINARY DISCUSSION OF RENAL DISEASE IN NATIVE NORTH AMERICANS· WHAT IS THE ROLE FOR GENOMICS?

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The burden of CKD and rapid and cloaked transition to ESRD in American Indian/Alaska Native (AI/AN) populations is higher than national aggregate. Our team's goal is to bring together a multidisciplinary team to analyze the roots of renal disease disparities among AI/AN groups and find pathways to health benefit. We will explore the interplay of environmental factors, social determinants, and genetic variation as components of these disparities with an eye toward reducing health disparities in this context and perhaps more broadly.

Component examples include the degree and pace of dietary transition, pesticide/herbicide exposure in occupational settings, diet, and genetic susceptibility and resilience. Other topics of interest include fetal origins of health and disease, allostatic load and overload, and pharmacogenomics. We also will assess the pre-diabetic contributors in this population such as IgA nephropathy & fibrosis from sclerosis.

This represents the information our group has gathered to date and emphasizes genetic components as a first step.

EQUATIONS TO ESTIMATE GLOMERULAR FILTRATION RATE

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BACKGROUND:

Clinical assessment of kidney function is part of routine medical practice for adults and is essential for assessing overall health, interpreting signs and symptoms, as well as detecting, evaluating, and monitoring acute and chronic kidney disease. The Glomerular Filtration Rate (GFR) is considered the best overall index of kidney function in health and disease. The GFR cannot be measured easily in clinical practice; instead, it is estimated from equations by using serum creatinine level, age, race, sex and body size.

1. MDRD STUDY EQUATION

Estimated GFR = 175X standardized $S_{cr}^{-1.154}$X $age^{-0.203}$X 1.212 {if black} X 0.742 {if female }. In which GFR is expressed as mL/min per 1.73 m^2 of body surface area and S_{cr} {serum creatinine} is expressed en mg/dl.

2-COCKCROFT-GAULT:

Creatinine clearance (Ccr mL/ minute).

Ccr= {(140-Age) x weight (kg)} /{ S_{cr} (mg/dl) x 72} x 0.85 in women

3-CKD-EPI EQUATION (WHITE OR OTHERS)

Sex	Serum creatinine (mg/dl)	
Female >	0.7	GFR= $144x\,(Scr/0.7)^{-1.209}\,x\,(0.993)^{Age}$
Male >	0.9	GFR= $141x\,(Scr/0.9)^{-1.209}x\,(0.933)^{Age}$

The equations are inadequate in the following situations:

a. Too high or too low body weight, more than 35 kg/m2 or lower than 19 kg/m2

b. Important disturbances of the muscle mass

c. Acute Renal Failure

d. Pregnancy

e. Serious liver disease

f. Edema

CHRONIC KIDNEY DISEASE OF UNKNOWN ETIOLOGY (CKDu) IN THE NORTH CENTRAL PROVINCE IN SRI LANKA

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BACKGROUND:

During 2000, a rising number of CKD patients were observed in the North Central Province (NCP) of Sri Lanka. A retrospective study of hospital records reported a type of CKD unassociated with the conventional risk factors of diabetes, hypertension and an aging population (Athuraliya 2011). In 2009, the Sri Lankan Ministry of Health officially confirmed a high hospital rate of CKDu in the North Central Province (WHO 2008). In 2012, the World Health Organization announced that 15% of Sri Lankans in the North Central Province have CKDu. This high prevalence rate creates a massive need of hemodialysis and kidney transplantation in Sri Lanka. Affordability and access to renal replacement therapy is limited in affected communities and creates a substantial public health burden (WHO 2012).

In recent years, epidemiologic and histological investigations have made significant progress identifying CKDu as a tubule-interstitial disease. Past and on-going research projects have investigated the role of cadmium, arsenic, fluoride and lead in the cause of CKDu. A few investigators believe that agrochemicals and pesticides may be contaminating the soil and water sources, adding nephrotoxic elements to the environment. However, the current data cannot point to a single or multi-factorial cause.

RESEARCH NARRATIVE:

The World Health Organization, Ministry of Health and in-country clinical researchers have stream-lined their resources to create a comprehensive research effort to investigate CKDu in Sri Lanka. Unpublished studies completed by this research effort include a population prevalence study, a case-control study of arsenic, cadmium, and lead in water, rice and soil samples, a case-control study of arsenic and cadmium in hair and nail samples, and a case-control study of cadmium, lead and arsenic in urine samples. The WHO should publish this data soon.

The National Institutes of Health's Kidney Disease Section is collaborating with several local universities in Sri Lanka to conduct a case-control study investigating the biological mechanisms in early-stage CKDu subjects. In their protocol, they will be collecting serum, blood, urine and kidney tissue of early-stage CKDu subjects for genetic and metabolic analysis. The goal of this study is to generate hypothesis-based follow up studies.

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INTERVENTIONS AND TREATMENT OPTIONS

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The Global Risk 2010 Report prepared by the World Economic Forum shows the correlation between the probability and economic severity of different risks, including earthquakes, flooding, food and oil prices, collapse of prices of production, financial crises, crime and corruption, international terrorism, infectious diseases and non-communicable diseases (NCDs). Surprisingly, NCDs are located in the right superior quadrant, meaning high probability and high costs.

What place does chronic kidney disease (CKD) have in the NCD's scenario? On September 16, 2011, the UN General Assembly approved a resolution on the prevention and control of NCDs, timidly including the recognition that kidney, bucodental and eye diseases are an important burden for the health sectors of many countries, and that these diseases share risk factors and could benefit from the common responses to NCDs. However, the nephrology community views CKD as important as other well-recognized NCDs including diabetes, cardiovascular disease, hypertension, and obesity. In addition, CKD may impose extreme financial burdens to health systems, given the high costs of renal replacement treatment (RRT) (dialysis and transplantation), which are the only options for patients with end stage renal disease (ESRD). Statistics like the following below show that this view is justified.

In Mexico, CKD occupied the 5th place for hospital discharges in 2007; nevertheless, the four causes that preceded CKD were not NCDs. In fact, CKD is the first cause of hospital discharge of all chronic diseases (NCDs) (Sistema de Información en Salud, 2009). At the turn of the millennium, a yearly increase of 11% was estimated between 2000 and 2010 in number of ERSD patients on dialysis (hemodialysis or peritoneal dialysis), from a baseline of 25 thousand in 2000 to 70 thousand in 2010 (Correa-Rotter et al., Plan Nacional de Salud, 2001). Current conservative projections of a 10% annual increase go from approximately 68 thousand patients in 2010 to over 180 thousand in 2020. The prevalence of patients with renal replacement therapy (RRT) in Mexico increased from 129 per million population (pmp) in 1992 to 478 pmp in 2005, and reached approximately 630 pmp in 2010 (Correa-Rotter et al, 2008). Extrapolating US and Canadian data of number of patients on RRT to Mexico, for example, would imply that there should be some 151 thousand CKD cases on RRT and some 34 thousand annual incident cases in an estimated population of 105 million (Correa-Rotter, unpublished data).

Between 1996 and 2008, the number of nephrologists per million population in Central America and Mexico has only slightly increased according to data from the Education Committee of the Latin American Society of Nephrology (SLANH) presented at the Latin American Congress of Nephrology, Cartagena Colombia, 2012 (Gonzalez et al, 2012). This indicator, in itself low for most Latin American nations, is in Mesoamerican countries well below the mean for Latin America and more than tenfold below Uruguay, the country with the highest number of nephrologists pmp (Table 1).

Table 1. Number of nephrologists per million population in Mesoamerica as compared to Latin America.

COUNTRY	NUMBER OF NEPHROLOGISTS	
	1996	2008
Costa Rica	4.4	4.8
El Salvador	2.4	5.3
Guatemala	1.7	2.2
Honduras	1.3	1.7
Mexico	2.1	5.5
Nicaragua	2.6	2.9
Panama	6.0	7.9
Uruguay	36.3	52.7
Mean Latin America	8.3	13.8

The United States Renal Data System (USRDS 2010) report depicts the highly varying incidence rates of CKD reported in 2009 for different countries worldwide, from around 20 pmp in Bangladesh to close to 400 in the US and the state of Jalisco in Mexico and around 600 pmp in the state of Morelos in Mexico. It is clear however that these incidence rates do not represent true incidences as in many countries there are severe deficiencies in both health care as well as data collection systems. It merely gives a picture of what is reported without having a standardized system of data collection. In a study among 3560 subjects of a 40 thousand population of a midsize Mexican city, the prevalence of kidney disease was around 8.5 per 100 inhabitants; of relevance, and not

unexpected, was the fact that the majority was CKD of unknown etiology (CKDu). Diabetes and hypertension explained around 30% of the cases (Amato et al, 2005).

The establishment of reliable registries of CKD and its treatment is of utmost importance to aid prevention, and only very few Latin American nations and none in Mesoamerica have such a register in place. CKD must be prevented as early as possible, in fact before kidney damage becomes apparent. Primary CKD prevention focuses on the reduction and control of risk factors. Secondary prevention focuses on early detection of CKD, through National programs, and early interventions carried out by general practitioners and other health workers to delay progression. Early treatment is often highly efficient and may significantly prolong life expectancy as compared with the natural course of the disease. In addition, it guarantees the appropriate follow up and timely referral to a nephrologist when it should be performed. Tertiary prevention aims to increasing access to and quality of dialysis services and to the creation or expansion of kidney transplant programs. It is noteworthy that in Mesoamerican countries the prevalence of cases of RRT is not equal to the prevalence of cases of ESRD, which may be much higher, i.e. a situation that implies an insufficient coverage of patients affected by the disease. It is therefore clear that there is a strong need to know how many patients are affected by CKD and in potential need of RRT, how many are actually on dialysis, and how many have received a kidney transplant.

Risk factors for CKD that are susceptible to interventions of life style changes and pharmacological interventions are multiple and include among others, reduction of overweight, appropriate management of hypertension, dyslipidemia and glycemic control in diabetics, and early treatment in the presence of proteinuria. Biomarkers for diagnosis and follow-up of traditional CKD are serum creatinine to estimate glomerular filtration rate (MDRD, CKD-EPI) and proteinuria-albuminuria. Although risk factors of chronic kidney disease of unknown origin (CKDu) are not known, there are nonetheless interventions that can and should be carried out today. Specifically actions related to changes in lifestyle and working practices will reduce risks of CKDu and improve quality of life.

Improvements of working conditions, in particular in those workers exposed to extreme or harsh working conditions such as agricultural or mining workers, may include reduction of heat exposures and improvement of hydration practices with adequate replacement of water and salt intake, interventions that should be implemented on a large scale basis. In addition, the use of nonsteroidal anti-inflammatory drugs (NSAIDs), common practice in Mesoamerica among these overexerted workers, must be avoided in general and even more in the presence of CKDu. Finally, all highly toxic exposures that anyway are hazards to health and that could be potentially responsible for kidney or other organ injury should of course be avoided.

Early detection of CKD brings important benefits as health risks deriving from CKD are high, the diagnostic tests are simple and low cost, early treatment may reduce progression and complications, treatment of underlying causes is feasible, and life expectancy may be improved. Early detection of CKDu brings about the same benefits, with the exception of treatment of underlying causes since they are unknown.

A number of challenges are intrinsic to CKDu in Mesoamerica, the biggest being that the etiology must be understood to establish specific therapeutic and preventive measures. The scarce human resources in health care must be increased, especially the number of nephrologists but also other health personnel. Sufficient financial resources for diagnosis, prevention, and treatment of CKD must become available. Actions to improve medical education are necessary through inclusion of nephrology in medicine curricula, and training of general practitioners and family doctors in early detection, preventive measures and appropriate referral of the renal patient. An important aid may be guidelines as the Latin American guidelines for clinical practices and prevention of CKD (Guias latinoamericanas para diagnóstico y tratamiento de la enfermedad renal crónica estadios 1 a 5, SLANH/FMR 2012). Guidelines to deal with CKDu are, of course, not available since we do not know the pathophysiology. Finally, it is very clear that we badly need research to define causality of CKDu in Mesoamerica, and from there we can move faster to create better preventive measures and specific potentially therapeutic options.

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THE HEALTH SYSTEM AND CKD IN EL SALVADOR

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SUMMARY

- ESRD in El Salvador is a serious health problem, especially in the coastal areas, overwhelming the health services.
- Patients have no possibility for transplants and very few can be treated with hemodialysis. Peritoneal dialysis is the first option, although not all patients qualify for the CAPD program due to the poverty conditions in which they live.
- The highest risk population is young (20-60 years).
- There is a clear predominance of males.
- There is no identifiable etiology for about 70% of cases, i.e. there is no known previous chronic pathology in these cases.
- There seems to be higher prevalence of cases in pesticide-exposed populations.
- A limitation of the studies is their cross-sectional design and the possibility of bias.
- Complementary studies are needed in order to determine the etiology of ESRD in El Salvador. We need to develop a true program with enough funding in order to prevent, early detect and treat CKD, in its different stages all around the country.

The health system of El Salvador is fragmented and oriented to healing actions. A significant part of the population does not have proper access to health services. El Salvador faces a double challenge, with an epidemiological profile of pathologies characteristic of an underdeveloped country, together with a first world profile. There is a high morbidity and mortality due to violence, trauma and chronic diseases. Among the chronic diseases, an alarming rise in the number of patients with End Stage Renal Disease (ESRD) has been observed in the internal medicine and nephrology wards, nationwide.

El Salvador reported to the Latin-American Society of Nephrology and Hypertension (SLANH) the following statistics over a time period of 16 years:

YEAR	NUMBER OF REGISTERED ESDR PATIENTS
1995	346
2002	1142
2003	1588
2006	2598
2007	3381
2008	3342
2009	3315
2010	3653

El Salvador's health system and End Stage Renal Disease

The large number of patients with ESRD is overwhelming the nephrology services and has exceeded by far the capacity for dialysis and transplants. The Hospital Nacional Rosales is a 450-bed hospital, where the most common admission diagnosis is renal failure (300-400 new patients per year). Outpatient Hemodialysis and Continuous Ambulatory Peritoneal Dialysis (CAPD) are offered but due to the high incidence of ESRD, most new patients can only be offered intermittent weekly peritoneal dialysis with a rigid peritoneal dialysis catheter [1]. There is no formal kidney transplant program, and the first two transplants were performed were performed last year.

There are only 32 nephrologists in the country and many of them are only working part time in the public health system. There are around 80 hemodialysis machines in the public health system in the entire country. The Salvadorian Institute of Social Security has more resources, but only covers around 18% of the population. It has had a kidney transplant program since 1985, and performs around 16 transplants per year, and very few patients can be admitted for hemodialysis.

Chronic kidney disease (CKD) research programs

Responding to the increasing crisis and frustrations from not being able to solve patients' needs, with great effort, a number of isolated studies were conducted over the last decade by nephrologists, individually or with some support of organizations such as the Association of Nephrology and Hypertension of El Salvador, PAHO and SALTRA [2-5]. These studies characterized the demographic and clinical characteristics of ESDR and CKD patients and showed clearly an epidemic of CKD of unknown etiology. More recently, the government initiated efforts towards the prevention and early detection of CKD in the lower Lempa river area [6]. So far, no collaboration has been established with the Association of Nephrology and Hypertension of El Salvador, which is desirable for efficient use of resources towards improvement of health services for all CKD patients and carrying out needed research.

All studies show that the pattern regarding the etiology of CKD in El Salvador is different from most countries in the world. A study conducted in 2003, in Hospital Nacional Rosales, the largest public reference hospital for CKD in the country, showed that around 70% of the ESRD patients did not have previous diagnosis related to CKD, 87% were male, the mean age was 50 years, over 63% were farmers (many sugarcane workers), and the majority of ESRD cases lived in coastal areas [4]. Below a comparison between an unpublished national report for ESRD, based on 2006 data from the Hospital Nacional Rosales, and 2010 data of the US Renal Data System, with regard to underlying cause, showing the very high relative frequency of ESRD of unknown etiology and, hence, the lower proportions of ESRD linked to traditional causes of diabetes and hypertension.

ETIOLOGY ESRD	US (%)	EL SALVADOR (%)
Diabetic nephropathy	44.2	14.9
Hypertension	28.1	7.4
Chronic glomerulonephritis	6.4	2.9
(Poly)cystic kidney disease	2.2	2.0
Urologic disease (US) / Renal lithiasis (El Salvador)	1.4	1.5
Other known cause	12.8	..
NSAID consumption	..	4.5
Unknown etiology	3.4	66.8
Missing cause	1.6	..

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COSTA RICA· HEALTH SYSTEM RESPONSE TO CHRONIC KIDNEY DISEASE

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Chronic kidney disease (CKD) is defined as a structural or functional damage of the kidney, regardless of the cause that originated it, for a period of 3 months or more. It is classified in five stages according to the degree of glomerular filtration. Chronic kidney insufficiency is defined as the permanent loss of kidney function and corresponds to stage CKD 3 or higher, with less than 60 ml glomerular filtration / minute, for a period of 3 months or more.

According to WHO, CKD has reached figures considered as epidemic, with a growth rate of 5 to 8% a year in developed countries. Although few data are available, it is believed that by 2030, 70% of people with CKD will reside in developing countries, which do not have the economic resources to address it.

With respect to causality, chronic diseases (diabetes, hypertension) can be mentioned as traditional causes as well as other risk factors such as obesity, dyslipidemia, poor nutrition, smoking, and sedentary lifestyle, all of them with a growing trend in developing countries. This is combined with the aging of our population.

On the other hand, there is the non-traditional causality related to environmental, toxic and occupational factors, particularly agricultural practices. It has been mentioned that there is a double burden of causal factors and CKD progression factors, with the most affected populations living in conditions of poverty, exclusion and vulnerability.

To achieve not just a socioeconomic impact, but also to improve healthcare services with an impact on the quality of life, the Council of Ministers of Health in Central America and Dominican Republic, in their 35th Regular Meeting, resolved to:

- Consider CKD as a priority public health problem among the group of chronic non-communicable diseases (CNCDs) because of its high human, social and economic costs and because it is impeding human development of our societies.
- Advocate that into the group of CNCD risk factors to be monitored also those risk factors will be included that are considered non-traditional and that are not mentioned in the political statements about CNCDs but that are important to take into account, such as occupational hazards and environmental pollution.
- In addition, increase the efforts at all levels to monitor and deal with the CKD originating from known risk factors.
- Urge Member countries to make a joint effort to conduct multi-centric investigations that will identify the magnitude, frequency, distribution, social determinants, and traditional and non-traditional risk factors of CKD in the region.
- Promote cooperation agreements between the countries for the development of policies and strategies for the prevention and control of CKD.

We are detecting the CKD cases in a late stage; they are detected passively in the health services in stages 3 - 4, or in stage 5, where the condition is irreversible and the patient requires peritoneal dialysis, hemodialysis or renal transplantation. This implies an overload of the health services with a high cost of care and a negative social impact on low-income families, where often the affected person is the head of household.

During the period 2006-2011, 28,540 hospitalizations due to CKD were registered in the hospitals of the Ministry of Health of El Salvador, with a case-fatality rate of 12.4% . During 2009-2011, in this same country 66,947 peritoneal dialysis and 70,684 hemodialysis were performed.

The analysis of the hospital discharges in Costa Rica during the quinquenium between 2005 and 2010 showed that the hospitalization rate of patients with CKD is higher in the province of Guanacaste as compared to other provinces, starting at young ages, with 112.9 cases per 100 000 inhabitants between age 20 to 29, far higher than in the province of Cartago, which occupies the second place with 43.8 cases per 100 000 population, for this same age group. The rates in the Guanacaste province are higher also in older age groups and only gets similar to the rest of the provinces for the population of 70 years and older. This trend here described for the Guanacaste province occurs among males.

The foregoing has led to that in this province the provision of health services, specifically the services network of the Hospital Enrique Baltodano in Liberia, has implemented the program Unity of Peritoneal Dialysis which allows a greater social inclusion of patients.

It is necessary to inquire more about the causality of the excess morbidity in this province to identify the factors associated with it.

A case-control study is about to be implemented by the CCSS, in the counties where the hospital discharge rates are highest. There are also plans to carry out a prevalence study and a study about the health costs.

The knowledge generated by these studies will allow the implementation of strategies towards a reduction of morbidity and mortality, as well as to address the social and economic situation of the affected population.

HOW CAN RESEARCH RESULTS BE APPLIED TO POLICIES THAT CONTRIBUTE TO SOCIAL AND ENVIRONMENTAL IMPROVEMENTS?

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"...the stubbornness of social inequality, which despite enormous progress in material well-being is still very much with us today, despite the fact that we now know it to be strongly associated with inequality in the length of life (Mackenbach & Dreier, 2012)."

Evidence-Informed Policy-Making

When discussing research to inform policy making, there are at least two questions that should be addressed:

- Are we developing research that is relevant, appropriate, and sufficient to respond to the most important public health problems of the population we are intending to protect?
- Are we using the results of research in the formulation of policies, programs, and laws to protect public health?

In the world of public health, the issue of bridging the know-do gap has been extensively addressed, but still, simple, cheap and effective interventions are sometimes not used or simply unavailable, while unnecessary, and sometimes expensive interventions are still applied not only in rich countries, but also in resources poor countries. A WHO World Report for Better Health (2004) has a whole chapter on the need to link research to action, and the Ministerial Summit on Health Research (2004) indicated in its final statement that there is a need to develop and use "research for better health and strengthening health systems" (Pablos-Mendez et al., 2005). A Resolution of the World Health Assembly (58th WHA, 2005)[1] urges Member States "to establish or strengthen mechanisms to transfer knowledge in support of evidence-based public health and health care delivery systems and evidence-based health related policies."

Evidence-informed health policy-making is an approach to policy decisions that aims to ensure that decision making is well-informed by the best available research evidence. Evidence-informed policy-making enables the use of research evidence adapted to specific conditions, where contextual constrains and modifiers are taken into consideration. Additionally, it protects against misuse of research evidence, including researchers and advocates of particular policy options. As decisions are always a matter of judgment - based on facts and circumstances or political power -, introducing a platform to do that in a systematic way will increase the transparency of the decision making process, and the accountability of the interventions proposed. That is the reason a structured dialogue with different stakeholders should take place in the process of examining the research evidence to prioritize the interventions proposed based, for example, on evidence assessed through systematic reviews or on the best research evidence available (Oxman et al, 2009). Following the WHO EVIPNET[2] (Evidence-Informed Policy Network) methodology, it is the process of adapting and adopting the research results, and translating it in a policy brief that should be written in accessible language and respond to specific questions in a particular context.

As an example, let us assume that the Ministry of Health of a particular country wants to introduce screening for Chronic Kidney Disease (CKD) in public health services. Some of the questions that could be asked are:

(a) What is the best screening strategy for early detection of CKD in that particular country? Target specific population groups defined by: Age? Hypertension? Diabetes? Occupation? Certain geographical areas? Both genders? Ethnic groups? Other groups?

(b) Which tests should be used for the screening?

The answers to these questions would only inform what is the best way to introduce a particular screening strategy in public health services, but say nothing about the idea of introducing screening as a strategy to prevent new cases of CKD or end stage renal disease. The latter would require other questions such as:

(a) What are the available policy options to prevent CKD in the country?

1. Resolution of the World Health Assembly, 58th WHA (2005). Available at: http://www.paho.org/english/gov/ce/ce136-25-e.pdf

2. Evidence Informed Policy Network (EVIPNET) available at: http://search.bvsalud.org/evipnet/index.php?output=html&site=evipnet&col=global&lang=en&filter_chain=tag:% 22Tools% 22

(b) Or more specifically: Is screening a good strategy for early detection of CKD in the general population?

(c) Or: Is early detection through screening strategies cost-effective to prevent End Stage Renal Disease (ESRD)?

A study in Norway, for instance, reviewed data of screening for CKD in the population of 20 years and older in a certain area and concluded that the most effective strategy to identify CKD in the general population was screening people with diabetes, hypertension or age > 55 years. Screening all persons from age 20 would detect one case in 20.6 cases screened, and the proposed strategy would detect one in every 8.7 cases screened (Hallan et al, 2006).

› *System thinking for decision making*

There are many tools that can help policy decision making by analyzing the context in which the health effects occur (see EVIPNET). Noteworthy is also the DPSEEA framework, a method of system thinking that was developed on behalf of the World Health Organization (Kjelström & Corvalán, 1995; Corvalán et al, 1996), initially as a basis for developing environmental health indicators. The framework presents health impacts as originating in driving forces **(D)**, which lead to pressures on the environment **(P)** in the form of production, consumption, waste generation, among others, and their consequent releases into the environment. These contribute to changes in the state of the environment **(S)** - for example, contamination of water sources by pesticides in agricultural fields, or by mercury in mining and industrial areas. Exposures **(E1)** occur when humans come into contact with these hazards, leading to potential health effects **(E2)**. Policy and other actions **(A)** are taken to control adverse health effects.

The policies and actions may be targeted at different points in the causal chain: later interventions aimed at reducing exposures or mitigating the health impacts are in general very specific, while early policy interventions aimed at preventive measures are broader and in general offer a wider range of other environmental and social benefits. Targeting intervention on the effects tend to be easier and more attractive for policy makers because the action can be applied directly at specific population groups and health outcomes. The sustainability of the applied policies though, will depend on the capacity to refrain new people entering at risk, and not only by improving diagnosis and treatment. The best way to avoid waste of limited resources is to be conscious of the process, and make informed decisions on where and when to take action.

Several examples of the use of the DPSSEEA framework for MeN are presented in table 1 (the problems and actions in the table are only presented as an exercise, and should not be used for any other purposes).

Table 1 - Examples of the use of the DPSEEA framework

CAUSAL CHAIN	ACTIONS
Driving forces - agriculture-based economies; political agendas; global economic crises; dependent economies - "biofuel" – the green economy; health systems (national policies, financing, legal framework etc.); growing of new markets (China, for instance); agricultural subsidy elsewhere; alcoholic beverages production	Implement: International Conventions (e.g. POPs); trade agreements; legislation on pesticides; FAO Save and Grow program; among others.
Pressures - export based production; intensive pesticides use; obsolete pesticides use and storage; intensive large scale and small scale mining; low health coverage; low compliance with regulations on workers' health; frailty of workforce and popular organizations; job insecurity; food insecurity; production-based payment of the workforce; low salaries (to compete with subsidized production elsewhere); perinatal, infant and/or life-course nutritional status; frailty of national governments; sovereignty challenged by international agreements; low investment in health	Commitment of the Ministry of Health to intersectoral work; PLAGSALUD; social protection (social security); national work plans on chemical safety; among others.
State - overuse of pesticides on the less regulated agricultural production (products for internal or regional market); overworking hours; unregulated workforce; seasonality of the workforce; use of high energetic, low-protein food; stunting; low high "allostatic load" (Mcewen, 1998); NEFROLEMPA (Orantes et al, 2009); low diagnostic capacity; low response capacity; increased awareness of CKD by the health workforce; low laboratory capacity; fragile health information systems (under-registration, incomplete forms); among others	Resolutions of the WHA; Pan American Health Conference and sub-regional agencies; capacity building; information systems; PLAGSALUD; SALTRA; legislation on worker's health; among others

Exposure – re-use of containers of pesticides; low implementation of individual safety equipment; contamination of diverse media (water, soil, air, food); increased individual vulnerability to environmental risks; low access to care; low resolution of health care systems; dehydration at work	National Committees on CKD in Central America; adoption of the Non-communicable Diseases (NCDs) strategy by Member States; decision of COMISCA to include NCDs in the political agenda; Public health risk assessment; work education; strengthen health systems; Water and Sanitation projects; enforcement of legislation; workers' health programs; SALTRA; among others
Effect – intoxications (acute and chronic); "heat stress"; NCDs – CKD – ESRD, premature death; limited availability of health indicators, and limited possibility of analysis of health indicators	Early diagnosis and treatment; Improvement of health surveillance systems

Acronyms:
POPs – Persistent Organic Pollutants
FAO – Food and Agriculture Organization
WHA – World Health Assembly of WHO Member States
PLAGSALUD – It is an acronym in Spanish for the project "Strengthening of Management Capacity for Pesticide-related llnesses"
SALTRA – University-based Program on Work, Environment and Health in Central America, founded in 2003
COMISCA: *Consejo de Ministros de Salud de Centroamérica y República Dominicana*

About the MeN know-do gap

To inform prevention strategies for CKD in Mesoamerica, the following considerations regarding knowledge gaps and research needs are in place:

- What is the variation of the incidence and prevalence rates of CKD and CKDu between countries, and within each country according to socioeconomic position and occupation/ activity? And what is the variation over time?
- Genetic variations are generally larger within than between populations whereas environmental exposures are usually larger between populations and this is important if studies of causality are looking at environmental exposures. More and better designed studies are needed to produce the necessary evidence, as the traditional designs (cohort and case-control studies) may be unsuccessful if there are not sufficient differences in exposure within the study population.
- Most chronic diseases do not have a well-established cause.
- We know more about the characteristics of the individuals that are more susceptible to them, but little about the determinants of the incidence rate.
- What is the best approach for prevention of CKDu?

- How much do we need to know to work on prevention strategies?

- How is etiologic research related to prevention policies in Mesoamerica?

A set of ideas to bridge the know-do gap for MeN

- Prioritize research that creates knowledge relevant for policy decision making;
- Frame research based on the social determinants of health as well as other determinants in the chain of causes;
- Establish systems that could include peers, donors and stakeholders dialogue in the research agenda setting;
- Frame research using designs that could answer appropriately the legitimate fears, beliefs, disbeliefs and specific questions of the population affected by MeN;
- Whenever possible, summarize evidence by performing systematic reviews in topics of interest for policy making;
- Share results of research and of conferences and expert meetings with policy decision-makers, and inform implications of the findings, including limitations of the studies and of the research evidence;
- Include the topics of options to health care, disease management in poor resources settings, and primary prevention in current research needs (explore the idea of how to build health systems that are "more high touch, less high tech", and the principles of "universal health coverage" and "health in all policies");
- Publish results in peer review journals, but always communicate to national and regional health officials and authorities about the research findings in the official language of the country;

- Contribute to respond to the main questions prompted by the ministries of health;
- Based on research evidence, what are the best options available for policy interventions in Mesoamerica, and how should they be ranked?
- What are the steps to build a list of best policy options for MeN in each of the countries? For example, to study causal relationship, to improve survival, to establish cost-effective treatments and ESRD care, others?
- What are the best options to study causality of MeN? For instance, what are the actions that could only be done by, or in collaboration with the ministries of health?

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CONCLUDING REMARKS

The international, interdisciplinary expert group attending the I[st] International Research Workshop on MeN concluded the following:

GEOGRAPHICAL OCCURRENCE OF CKDu:

MeN is well-identified in certain parts of Nicaragua, El Salvador and Costa Rica, but not universally throughout these countries, and has not yet been documented in the rest of Mesoamerica. CKDu is probably a hitherto unrecognized global problem although it is not clear if the CKDu epidemics observed in other parts of the world, specifically in Sri Lanka and India, are the same disease or are caused by the same factors as in Mesoamerica.

LEADING HYPOTHESIS OF MEN:

Probably, there is more than one causal factor for MeN, but important evidence exists to help us narrow hypotheses and focus future research and intervention efforts. Currently, the strongest hypothesis for MeN is heat stress and dehydration, i.e. heavy work in a hot climate with inadequate rehydration practices, associated with repeated episodes of dehydration. These episodes are believed to lead to subclinical acute kidney injuries that develop into chronic damage over time. The heat stress hypothesis is supported by results of epidemiologic, occupational hygiene, experimental and biopsy studies presented at the workshop by different research groups. In Nicaragua, El Salvador and Costa Rica, clearly the most affected populations are sugarcane cutters who perform hard physical labor while exposed to extreme ambient heat, and there is some additional evidence for workers in other hot occupations. Experimental data presented at the workshop show induction of tubular kidney damage in mice exposed to repeated dehydration through the activation of the fructokinase-enzyme pathway in the kidney. Fructose intake in combination with dehydration is an interesting new aspect that needs further experimental elucidation. Biopsy findings presented at the workshop show glomerular and tubular lesions that are in concordance with the dehydration hypothesis.

ALTERNATIVE HYPOTHESES AND CO-FACTORS:

The workshop participants considered most well-known nephrotoxic agents to be unlikely single causes of MeN, but many merit further attention as potential co-factors through interactions or by influencing the progression of CKDu, in particular excess use of non-steroidal anti-inflammatory drugs (NSAIDs). Other priority hypotheses include leptospirosis, inorganic arsenic, and other nephrotoxic medications (also traditional herbal medications). Based on existing evidence, pesticides and urinary tract infections are considered unlikely causes, but important to address because of community concerns and because they are important for general public health. Lead, mercury, cadmium, uranium, aristolochic acid and hard water are considered unlikely causes and of low research priority for MeN, although hard water is a community concern and as such should be addressed. Genetic susceptibility and childhood exposures that increase susceptibility may be important but have so far not been addressed. Most importantly, social determinants, including factors of work organization, migration patterns, and macro socioeconomic drivers strongly influence disease occurrence and must be integrated in study designs.

RESEARCH PRIORITIES:

Agreements on case definitions for clinical purposes and epidemiologic research, along with the development and validation of biomarkers of early and late disease were seen to be of utmost importance for maximizing the potential of future research. More biopsy studies, under well controlled circumstances, are needed. Correct measurements of the above mentioned priority risk factors as well as personal risk factors need carefully planned procedures. Different study designs to achieve complementary objectives were elaborated. Prevalence studies will continue to be important to characterize the magnitude of MeN and to identify other populations at risk. Case - control studies can contribute to determining which hypotheses merit further exploration through cohort studies, the preferred design for etiologic research. The feasibility for retrospective cohort studies, which are more rapid and cheaper than prospective cohort studies, should be assessed but was deemed to be low in the region. Prospective cohort studies in occupational groups or contrasting communities should be pursued despite their high costs.

INTERVENTIONS IN THE FACE OF UNCERTAIN ETIOLOGY:

Even though the etiology of MeN is uncertain, important interventions can be implemented that can be expected either to reduce the risk of developing CKDu or slow the progression of the disease. In workplace settings, it is important to reduce heat

stress and to conduct controlled trials to improve hydration with adequate electrolyte replacement and safe drinking water, free of the nephrotoxic metals and pesticides.

RESEARCH COLLABORATION:

Interdisciplinary and multicenter research collaboration is undoubtedly desirable to address MeN, as well as the CKDu epidemics elsewhere in the world. The workshop concluded with the establishment of a Research Consortium to develop collaboration that builds upon the ongoing work in the region and the progress made at this meeting. The consortium model consists of a network of affiliated interdisciplinary scientific researchers working together to increase understanding and public awareness of the disease.

A Temporary Consortium Board was formed[1]. Activities of the Consortium will include: information sharing, compiling and disseminating research, facilitating collaboration in fund seeking, and serving as a bridge for translation of research results to policy makers. We hope that the extraordinary efforts made by the participants in this workshop continue through the Consortium, maximizing the potential of research initiatives to solve the devastating public health issue of MeN.

1. The Temporary Board is currently in the process of drafting the organization structure with vision, mission, tasks, and bylaws for the Board, and is preparing the election of the permanent Consortium Board to be coordinated by SALTRA.

ACKNOWLEDGEMENTS

This workshop was possible thanks to the dedication of many individuals, including those who participated. We extend our most profound thanks to the people who helped make the workshop and this report possible:

Andrés Esteban Sánchez Barboza
Douglas Barraza Ruíz
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Laura Ortiz Cubero
María Zúñiga Mena
Marianela Rojas Garbanzo
Marta Castillo Sevilla
Mauricio Salas Salas
Pablo Cruz Rodríguez
Raquel Campos Calvo
Sara González Bonilla
Karen Herrera Benavides

FUNDING CONSIDERATIONS

The workshop was organized by SALTRA, a program funded by the European Union. SALTRA participants received travel funds from SALTRA (European Union) and the Universidad Nacional in Costa Rica (as visiting professors with support from IRET and the Facultad de Ciencias de Tierra y Mar). All other participants either paid their own way or obtained external funding to attend. We know that many participants went to great lengths to be able to attend, and that this is a reflection of their willingness to work towards find the cause of MeN.

PROGRAM

The program will consist of **sessions on key topics** related to chronic kidney disease (CKD) of unknown origin in Mesoamerica and elsewhere.

Each topic will be introduced **with a plenary presentation**, summarizing what we already know, and what we need to know to move forward.

Round table discussions will allow all participants to bring forward additional data, knowledge, hypotheses, obstacles and opinions. Results of the discussions will be presented in **plenary sessions**. There will also be **workings groups**, where participants will bring their area of expertise and concern to the design of future studies. The last session, in the afternoon of November 30, will summarize the main results from the workshop.

All workshop material will be distributed digitally. We will be distributing essential documents and conference presentations via Dropbox daily during the workshop. All participants are highly encouraged to bring a laptop.

There will be 1-4 page summaries for each of the topics prepared by the main speaker of the sessions. These summaries, with revisions and extensions after the workshop discussions will form the basis for a final workshop report to be published.
NOTE: All presenters are kindly asked to give their ppt-files to Mauricio at the secretary´s desk outside the main room the morning of the presentation.

THEME NOV 28: WHAT DO WE KNOW?

8.00-9.00 REGISTRATION
9.00-10.00 INAUGURATION
10.00-10.15 COFFEE BREAK
10.15-10.30 ANNOUNCEMENTS AND EXPLICATIONS OF THE DAY'S ACTIVITIES

10.30-11.45 EPIDEMIOLOGY OF CKD OF UNKNOWN ORIGIN

CHAIR
Catharina Wesseling,
Universidad Nacional, Costa Rica

- The epidemiology of CKD of unknown origin in Central and Latin America (30 min)
Daniel Brooks, Boston University, USA; Oriana Ramírez, Boston University and Autonomous University of Madrid, USA/Spain
- The epidemiology of spatially clustered CKD elsewhere (30 min)
Agnes Soares, PAHO-Washington; Kristina Jakobsson, Lund University, Sweden, and input from Sri Lanka
- Time for discussion (15 min)

11.45-12.15 PATHOLOGY OF CKD

CHAIR
Catharina Wesseling,
Universidad Nacional, Costa Rica

- Pathology and pathophysiology of CKD (including a general introduction). The specific characteristics of CKD of unknown origin in Mesoamerica
Annika Östman-Wernerson, Karolinska Institutet, Sweden

12.15-13.30 LUNCH

13.30-14.15 BIOMARKERS OF EFFECT

CHAIR:
Annika Östman-Wernerson,
Karolinska Institutet, Sweden

- Biomarkers for early diagnosis of renal disease, suitable for use in epidemiological studies, and for elucidation of pathophysiology
Ricardo Correa-Rotter, National Medical Science and Nutrition Institute Salvador Zubiran, Mexico; Manuel Cerdas, Social Security, Costa Rica; Carl Gustaf Elinder, Karolinska Institutet, Sweden

14.15 -16.00 ENVIRONMENTAL AND LIFESTYLE-RELATED RISK FACTORS

CHAIR
Richard Johnson,
University of Colorado Denver, USA

- Renal effects from heavy metals (20 min)
Carl Gustaf Elinder, Karolinska Institutet, Sweden
- Special discussion on arsenic (10 min)
Rebecca Laws, Boston University, USA
- Drug nephrotoxicity: current state of knowledge (10 min)
James Kaufman, New York Harbor Healthcare System and Boston University, USA; Carl Gustaf Elinder, Karolinska Institutet, Sweden
- Smoking and alcohol intake and nephrotoxicity: current state of knowledge. Use in Mesoamerica, in different socioeconomic groups (10 min)
Carl Gustaf Elinder, Karolinska Institutet, Sweden

- Pesticides: current state of knowledge (10 min)
Michael McClean, Boston University, USA
- Other possible etiologies (15 min)
Infectious/vector-borne diseases
Alejandro Riefkohl, Juan José Amador, Oriana Ramírez, Boston University, USA
- Plant and mould toxins
Kristina Jakobsson, Lund University, Sweden
- Hardness of water: data from Sri Lanka (10 min)
Channa Jayasumana, Rajarata, University of Sri Lanka
- Panel discussion – Can these risk factors explain the MeN epidemic? (20 min)
Chair: Richard Johnson,
University of Colorado Denver, USA

16.00 – 16.15 COFFEE BREAK

16.15 -18.00 HEAVY WORK IN HOT CLIMATE

CHAIR
Ricardo Correa-Rotter,
National Medical Science and Nutrition Institute Salvador Zubiran, Mexico

- Physiological changes during work in a hot climate and methods of quantification: Current state of knowledge (20 min)
Rebekah Lucas, Umeå University, Sweden
- Empirical data from Costa Rica (20 min)
Jennifer Crowe, Universidad Nacional, Costa Rica
- The dehydration and heat stress hypothesis (20 min)
Daniel Brooks, Boston University; James Kaufman, New York Harbor Healthcare System and Boston University, USA
- Fructose related kidney injury: Experimental studies; fructose intake in Mesoamerica (30 min)
Richard Johnson, Gaby Sánchez-Lozada, University of Colorado Denver, USA
- Time for discussion (15 min)

18.00-18.30 REFLECTIONS ON THE DAY

- Panelists: David Wegman, University of Massachusetts Lowell (chair); Aurora Aragon, UNAN-León, Nicaragua; Ricardo Leiva, Hospital Nacional Rosales, El Salvador

THEME NOV 29 AM (CONTINUED): WHAT DO WE KNOW?

08.15 – 8.30 ANNOUNCEMENTS AND DESCRIPTIONS OF THE DAY'S ACTIVITIES

08.30-8.50 SUSCEPTIBILITY

CHAIR
Daniel Brooks,
Boston University, USA

- Genetic susceptibility to kidney disease, and possible interactions with risk factors
David Friedman, Harvard University; Daniel Brooks, Boston University, USA

08.50-9.50 POLICIES AND SOCIO-ECONOMIC DRIVERS

CHAIR
Daniel Brooks,
Boston University, USA

- Experiences from La Isla Foundation (30 min)
Ilana Weiss and Y-Vonne Hutchinson, La Isla Foundation, Nicaragua
- PAHO and the global health agenda (30 min)
Agnes Soares, PAHO-Washington

9.50-11.00 COFFEE BREAK AND POSTER EXHIBITION

THEME NOV 29, AM: WHAT DO WE NEED TO KNOW AND UNDERSTAND?

11.00-12.30 ROTATING ROUND TABLE DISCUSSIONS TO IDENTIFY RESEARCH GAPS

Groups consisting of 6-8 participants visit each table for 15 min (thus each group discusses all themes). At each table there is a facilitator and a rapporteur. After all rounds, the facilitator and rapporteur select 4-6 main messages from the discussions, and write them on ONE ppt-slide. These summaries of the seven Round Table themes are presented in plenum

Table A. Prevalence and incidence of CKD in general populations and occupational groups
Table B. Biomarkers for early detection of CKD, and how/if biomarkers can be used for elucidations of pathophysiology
Table C. Diagnostic practices for early and late detection of CKD in different regions
Table D. Exposure levels of nephrotoxins and

risk factors for CKD in humans and the environment with validity aspects
Table E. Social and working conditions in affected populations (incl migrant workers)
Table F. Hard work, heat, dehydration, and access to water and other liquids
Table G. Beliefs, disbeliefs, political and legal acceptance of a CKD epidemic in Mesoamerica and elsewhere

12.30-13.45 LUNCH

13.45-14.30 SUMMARY OF TABLE DISCUSSIONS

CHAIR
Christer Hogstedt,
Karolinska Institutet, Sweden
The rapporteurs present 1 ppt slide each.
Do the participants agree that these are the most urgent questions?

THEME NOV 29, PM: TOP PRIORITY RESEARCH TO ADDRESS CKD OF UNKNOWN ORIGIN

14.30 - and as long as needed WORKING GROUPS

Questions to be addressed by all groups:
1. Interdisciplinary frameworks and tools to integrate knowledge on health, ecologic, economic, technologic, socio-cultural and behavioral phenomena and their interactions
2. Participatory research, qualitative research, ethics, access to study populations
3. Funding and priorities
4. Multilevel communication strategy
The working groups will have co-chairs and a rapporteur. These are asked to summarize the recommendations from the working group on a flip chart, which will be posted by 8:00 am on Friday for participants.
Themes:
– Cross-sectional studies with attention to target groups (occupational and other) and exposure measures (short and long term)
– Case-Control studies with attention to diagnostic specificity and effective assessment of historical and current exposure
– Cohort studies - prospective and retrospective
– Intervention studies at the workplace and in the population/health care system
– Experimental studies; in humans and animals to elucidate pathophysiologic mechanisms and to inform interventions. Attention to susceptibility
– Eco-health and spatial epidemiology

17:30–18:00 REFLECTIONS ON THE DAY

- **Panelists:** Ricardo Correa-Rotter, National Medical Science and Nutrition Institute Salvador Zubiran, Mexico (chair); Donna Mergler, University of Quebec in Montreal (UQAM), Canada; Agnes Soares, PAHO-Washington; Catharina Wesseling, Universidad Nacional, Costa Rica

THEME NOV 30, AM: TOP PRIORITY RESEARCH AND POLICY INITIATIVES TO ADDRESS CKD OF UNKNOWN ORIGIN (CONTINUED)

08.15 – 8.30 ANNOUNCEMENTS AND DESCRIPTION OF THE DAY'S ACTIVITIES

08.30-09.30 WORKING GROUP PROGRESS REPORTS

CHAIRS
Jennifer Crowe,
Universidad Nacional, Costa Rica;
Kristina Jakobsson,
Lund University, Sweden

09.30-11.30 SECOND ROUND OF WORKING GROUPS (incl coffee)

The same co-chairs and rapporteurs, but group members may join another group.

THEME NOV 30: MOVING FORWARD

11.30-12.30 REPORTS OF SECOND ROUND WORKING GROUP AND DISCUSSIONS ON CONCRETE PROPOSALS FOR EACH THEME

CHAIRS
Jennifer Crowe,
Universidad Nacional, Costa Rica;
Kristina Jakobsson,
Lund University, Sweden

The proposals should suggest as far as possible study populations and design, methods and tools for measurements of exposure and effect, national and international collaborators, and funding potential/options.

12.30 – 13.30 LUNCH

13.30-14.00 CONTINUED REPORTING FROM WORKING GROUPS

14.00-14.45 MOVING FORWARD – SETTING PRIORITIES FOR A POLICY AGENDA

CHAIR

David Wegman,
University of Massachusetts Lowell, USA

- **Interventions and regulations, and clinical treatment options (20 min)**
Ricardo Correa-Rotter, National Medical Science and Nutrition Institute Salvador Zubiran, Mexico
- **Response of health care systems (10 min)**
Ricardo Leiva, Hospital Nacional Rosales, El Salvador
- **How can research results and policies be applied to contribute to social and environmental improvements? (15 min)**
Agnes Soares, PAHO-Washington

14.45-15.45 PANEL AND PLENARY DISCUSSION

- **Moderators:** Catharina Wesseling, Universidad Nacional, Costa Rica; Christer Hogstedt, Karolinska Institute, Sweden
- **Panelists:** Aurora Aragón, UNAN-León, Nicaragua; Daniel Brooks, Boston University, USA; Ricardo Correa-Rotter, UNAM, Mexico; Carl Gustaf Elinder, Karolinska Institute, Sweden; Ilana Weiss, Isla Foundation, Nicaragua

15.45-16.00 WORKSHOP ENDING

A

Acosta, Hildaura

Panama - Universidad de Panamá/CIIMET/SALTRA-Panama
Toxicology, pharmacology

Amador, Juan José

Nicaragua/USA - Boston University School of Public Health (BUSPH) - *Public health*

Aragón, Aurora

Nicaragua - UNAN-León/CISTA/SALTRA-Nicaragua - *Occupational health, CKD research*

B

Bonilla,Cinthya

Honduras - Universidad de Honduras/Medical Faculty SALTRA-Honduras - *Microbiology/Clinical chemistry*

Brooks, Daniel

USA - Boston University School of Public Health (BUSPH)
Public Health, epidemiology, CKD research

C

Carmenate, Lino

Honduras - Universidad de Honduras/Medical Faculty/ SALTRA-Honduras
Medicine, occupational health

Castillo, Luisa Eugenia

Costa Rica - Universidad Nacional, IRET

Cerdas, Manuel

Costa Rica - Caja Costarricense de Seguro Social (CCSS), Hospital México - *Clinical nephrology*

Correa Rotter, Ricardo

Mexico - National Medical Science and Nutrition Institute Salvador Zubirán National Institute of Medical Science and Nutrition, Mexico City - *Clinical nephrology, CKD research*

Crowe, Jennifer

Costa Rica - Universidad Nacional/IRET/SALTRA
Heat stress, field studies with sugarcane workers

Cruz de Trujillo, Zulma

El Salvador - Ministry of Health, Hospital Rosales; Society of Nephrology - *Nephrology*

D

de Voogt, Pim

The Netherlands - University of Amsterdam
Environmental chemistry, water contaminants

E

Elinder, Carl-Gustaf

Sweden - Stockholm County Council and Karolinska Institute, Department of Evidence Based Medicine - *Nephrology, heavy metals*

F

Friedman, David J.

USA - Harvard Medical School
Genetics

G

García Trabanino, Ramón Antonio

El Salvador - National Institute of Health
Nephrology

González, Marvin

Nicaragua - UNAN-León/CISTA/SALTRA-Nicaragua
Occupational health, Epidemiology, CKD research

Guzmán, Carolina

Guatemala - USAC/CIAT/SALTRA-Guatemala - *Occupational health/Toxicology*

H

Hogstedt, Christer

Sweden - Karolinska Institute, IMM - *Epidemiology, CKD research*

Hutchinson, Y-Vonne

Nicaragua - La Isla Foundation - *Human rights, law*

J

Jakobsson, Kristina

Sweden - Department of Occupational and Environmental Medicine, Lund University - *Environmental medicine*

Jayasumana, Channa

Sri Lanka - Rajarata University - *Toxicology*

Jensen, Olaf

Denmark/Panama - Centre for Maritime Health and Society, University of Southern Denmark - *Occupational medicine*

Johnson, Richard

USA - University of Colorado, Denver - *Nephrology, pathogenesis*

K

Kaufman, James

USA - Boston University School of Medicine, VA New York Harbor Healthcare System - *Clinical nephrology*

L

Laws, Rebecca

USA - Boston University School of Public Health, in the Department of Environmental Health
Field studies, environmental epidemiology, exposure assessment

Leiva, Ricardo

El Salvador - Minsitry of Health, Hospital Rosales
Nephrology

Lucas, Rebekah

Sweden - Umeå International School of Public Health, Umeå University; Hothaps Program
Exercise physiology

Lundberg, Ingvar

Sweden - Occupational and Environmental Medicine, Uppsala University
Occupational medicine, CKD research

M

McClean, Michael

USA - Boston University - *Biological markers in environmental and occupational health*

Mergler, Donna

Canada - University of Quebec at Montreal/CINBIOSE
Ecosystem health approach, environmental health, neurotoxicology

O

Orantes, Carlos Manuel

El Salvador - Instituto Nacional de Salud; Ministerio de Salud - *Medicine, Research*

Östman-Wernerson, Annika

Sweden - Karolinska Institute - *Pathology*

P

Peraza, Sandra

El Salvador - Universidad de El Salvador/SALTRA-El Salvador
Chemistry, pharmacy, occupational health, CKD research

Porras, Rafael

Costa Rica - Ministerio de Salud
Public health policy

R

Raines, Nate

USA - Mount Siani School of Medicine
Epidemiology and medicine

Ramírez, Oriana

España/USA - Universidad Autónoma de Madrid/Boston University - *Public health, CKD research*

Riefkohl, Alejandro

Mexico - Boston University - *Internal medicine (Infectious Diseases/Leptospirosis)*

Robles, Andrés

Costa Rica - Instituto Tecnológico de Costa Rica/EISLHA/SALTRA-Costa Rica - *Industrial hygiene*

Ruepert, Clemens

Costa Rica - Universidad Nacional/IRET/LAREP - *Environmental analytical chemistry, occupational health*

Ruiz, Roberto Antonio

Spain - Universidad Miguel Hernández de Elche, Alicante - *Toxicology*

S

Sánchez Lozada, Laura Gabriela

Mexico - Laboratorio de Fisiopatología Renal, INC Ignacio Chávez - *Nephrology, animal studies, hydration*

Soares da Silva, Agnes

PAHO Headquarters - Sustainable Development and Environmental Health - PAHO HQ, Washington DC, USA - *Pan American Health Organization (PAHO/WHO) - Policies, prevention, public health, epidemiology*

T

Trejos, Maria Ethel

Costa Rica - Ministerio de Salud - Epidemiology, *public health policy*

Turcios Ruiz, Reina

USA/Guatemala - CDC-Atlanta - *Public health*

V

Vanegas, Ramón

Nicaragua - CISTA/UNAN-León - *Nephrology*

Vilanova, Eugenio

Spain - Universidad Miguel Hernández de Elche, Alicante - *Toxicology*

Vinda, Pedro

Panama - Caja Seguro Social de Panamá; Universidad de Las Américas; SALTRA-Panama - *Occupational medicine*

W

Wegman, David

USA - University of Massachussetts Lowell - *Occupational epidemiology*

Weiss, Ilana

Nicaragua - La Isla Foundation - *Policies, prevention*

Wesseling, Catharina (Ineke)

Costa Rica - Universidad Nacional/IRET/SALTRA - *Occupational and environmental epidemiology, CKD research*

Williams, Desmond

USA - CDC-Atlanta - *Public Health, kidney disease*

Wong, Roy

Costa Rica - Caja Costarricense de Seguro Social (CCSS) - *Field studies (Case-control in Costa Rica)*

Y

Yracheta, Joe

USA - University of Washington - *Genetics*

ADDENDUM ON PESTICIDES AS A POSSIBLE CAUSAL FACTOR OF MeN

In the workshop, pesticides were not considered a likely cause based on available evidence to date, but it was acknowledged that pesticides are an important factor to address because of community concerns. In addition, some researchers insisted in that the pesticide etiology should be investigated much further. An important post-workshop report was submitted by Sri Lanka colleagues to sustain their case. The Organizing Committee beliefs that this information must be integrated with the rest of the considerations of the report, and that it is wise not to dismiss the pesticide issue prematurely.

Arsenic, lead and cadmium in technical organic pesticide compositions and in fertilizers available in the Sri Lankan market

Jayasumana MACS, Paranagama PA, Amarasinghe MD, Fonseka SI.
University of Kalanyia, Kelaniya, Sri Lanka

A review of the toxicological evidence for nephrotoxicity for the active components of a wide range of pesticides has been given in this report (McClean, this volume p.77), and it has been concluded that exposure to pesticides is not among the main hypothetical risk factors for MeN (Concluding remarks p. 227)

However, the technical formulation of a pesticide is not only composed of the active ingredient, i.e. the specific compound designed either to kill or debilitate the pest. In general, the active ingredient forms a certain percentage of the pesticide mixture and the rest comprises the inert ingredients that improve their storage, handling, application, effectiveness, or safety. Additionally, there may be chemical compounds present as impurities.

The presence of arsenic and other toxic elements as impurities, or as deliberately added compounds in the technical products to enhance toxicity, has recently been tested, with special emphasis on potentially nephrotoxic substances.

Sealed samples of pesticides (insecticides, weedicides and fungicides) were purchased from the major sales outlets in Districts of Anuradhapura, Trincomalee, and Kurunegala and from company show rooms in Colombo. These sales outlets are the major centres through which agrochemicals are distributed in the adjacent areas. Brand names of pesticides to be sampled were selected to include the same active ingredient (according to the labels on the containers), but distributed by different companies. Samples of the same brand too were purchased from sales outlets of the above Districts.

Analyses using atomic absorption spectroscopy (AAS) were carried out at the analytical chemistry laboratory in the University of Kelaniya and confirmed by repeating the tests using aliquots from the same pesticide samples at the Water Resources Board, Sri Lanka.

Twenty different active substances, from 31 different purchased brands were investigated. All 319 different samples were analyzed and Arsenic (As) was found in 29 out of the 31 brands, at levels ranging from 180 to 2586µg/kg. Taking glyphosate, a commonly used herbicide, as an example: Three batches from one specific company had no detectable As levels. Samples from a second company ranged between 233 and 1452µg/kg, with lowest levels found in samples from Colombo, where rice is not grown in large scale. Samples from a third company ranged between 785 and 1462µg/kg, also here with lowest levels in samples from Colombo and the highest in the rice growing areas.

Mercury was analyzed in 50 samples from 8 different brands of insecticides and herbicides. The levels observed ranged from 903 to 4552 ppb (total mercury, analyzed by AAS (Paranagama PA, personal communication).

A recent WHO report (2) also has information on As in 26 pesticide samples from the CKDu endemic area, ranging from 10 to 94930µg/kg, and in 8 samples from the non-endemic areas, ranging from 10 to 13150µg/kg. This latter study also reports levels of cadmium, ranging from 50 to 9340µg/kg in endemic and 50 to 2000µg/kg, in non-endemic areas. For lead, the corresponding results were 830 to 93,0810µg/kg, and 1010 to 56,390 µg/kg, respectively.

The arsenic content in fertilizers has also been investigated (3) and shown that As was not detected, or detected only at low levels in locally produced organic fertilizers from manure, while that was 2 to 4 mg/kg in the commercially available triple phosphate fertilizers (TSP) used in vegetable cultivation. Much higher levels were observed in TSP fertilizers used in rice production, from 25 to 38 mg/kg. The determinations were based on >15 specimens for each type of fertilizer.

CONCLUSION

It is evident that the nephrotoxicity of technical formulations of pesticides and industrial fertilizers

purchased in Sri Lanka, and most likely also in other developing countries, cannot be evaluated on basis of the evidence from toxicity studies of the active substances only.

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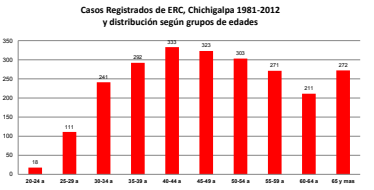
Servicios de Salud para Personas con Enfermedad Renal Crónica, Municipio de Chichigalpa, Nicaragua

Dr. Juan José Amador - Universidad de Boston
Lic. Damaris López - Universidad de Boston



Antecedentes

- Comportamiento epidémico de la Insuficiencia Renal Crónica en Occidente de Nicaragua. León y Chinandega son los dos departamentos más afectados.
- León tiene algunas alternativas de tratamiento para pacientes en Estadio 5 en cambio Chinandega no lo tiene. Los pacientes de Chichigalpa fallecen sin tratamiento apropiado.
- CAO/Banco Mundial elaboró en 2011 un diagnóstico situacional del Centro de Salud de Chichigalpa y una propuesta de mejora de la atención en salud .

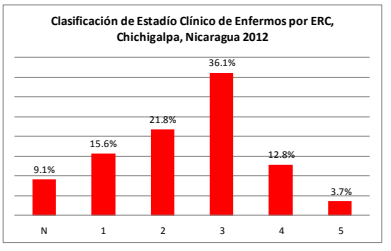
Contexto 2012

- 2012: Hospital Primario y Servicios Clínicos para la ERC/IRC
  - Febrero y Mayo 2012: Vice Ministro confirma el interés del MINSA para una respuesta de mayor nivel resolutivo para la enfermedad renal en Chichigalpa.
  - Junio 2012: Meg Taylor (CAO), Ute Sudmann (DEG) e ISA confirmaron el interés de contribuir financieramente para construir la nueva clínica de salud renal en el Hospital Primario de Chichigalpa..

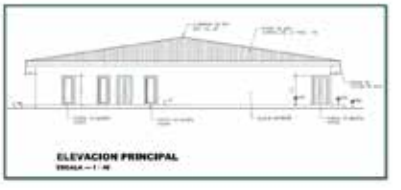
Proyecto 2012-2013

- El Gobierno de Nicaragua destinó US$ 3.25 millones del tesoro nacional para la construcción del hospital primario. ISA y Alcaldía donaron el terreno de 5 hectáreas. ISA y DEG donarán US$ 320,000 adicionales para creación de la nueva clínica renal.
- La clínica renal proporcionará atención integral incluyendo servicios de hemodiálisis y diálisis peritoneal para la sustitución de la función renal. Incluye un programa de mejora de la calidad de la atención y procesos educativos para pacientes, familia y trabajadores de la salud.
- Octubre 2012: Inicia la construcción del Hospital de Chichigalpa el que será finalizado en 12 meses.

Sitio para Hospital Primario y Clínica ERC



Diseño arquitectónico de la Clínica de Atención Renal del Hospital de Chichigalpa



Visita conjunta CAO/BM, DEG e ISA , Junio 2012, sitio de construcción del Hospital

Reunión de Asamblea de ASOCHIVIDA, Marzo 2012

Perspectivas

- Fortalecer la prevención de la ERC en base a nuevos conocimientos sobre las causas de la misma.
- Mejoramiento de la atención médica, dotación de medicamentos y exámenes para personas con ERC en cada una de las etapas clínicas. Capacitación a personal que brinda la atención en salud en base a normas y protocolos actualizados para niños y adultos.
- Coordinaciones efectivas con las redes de apoyo al acceso a la diálisis peritoneal y la hemodiálisis.
- Apoyar el proceso nacional en curso para una nueva ley y un programa de salud para la donación de órganos y trasplante renal.

Kidney Damage Markers in Nicaraguan Adolescents in a Region of an Epidemic of CKD of Unknown Etiology

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[1]Boston University School of Public Health, [2]Universidad Autónoma de Madrid, [3]VA Boston Healthcare System, [4]Yale University School of Medicine, [5]Tufts University School of Medicine

Background

An epidemic of CKD is occurring across Central America with over 20,000 deaths, mainly among younger men. Most studies have focused on occupational factors, but the large number of cases at a young age suggests that initial damage may begin in childhood.

Methods

We studied markers of kidney damage in 200 students (age 12-18) with no prior work history from 4 schools in Nicaragua (1 Jinotega, 2 Masaya, 3 North Chichigalpa, 4 South Chichigalpa). Schools were selected to represent a range of risk based on adult CKD mortality data. Urine was tested by dipstick and analyzed for ACR, NGAL, NAG, and IL-18.

Results

Dipstick proteinuria (3%) or glucosuria (1%) were rare, and only 8% had ACR >30 mg/g. The median IL-18 level (pg/ml) was higher than in healthy controls identified from other studies (45, IQR 21-115 vs. 15, IQR 7-28). The ratio of mean levels for NGAL, NAG, and IL-18 by sex and school are shown in the table.

Males had lower levels for all markers, particularly NGAL and IL-18. The results by school were consistent with their *a priori* risk: among males, the highest mean levels of NAG, NGAL, and IL-18 were at the highest risk school. Females at the two highest risk schools had elevated levels of NAG. Results were the same regardless of whether measurements were normalized for urine creatinine.

	Mean ratio (95% CI)		
	NAG	**NGAL**	**IL-18**
Sex			
F	Ref	Ref	Ref
M	0.78 (0.58-1.05)	0.25 (0.20-0.32)	0.29 (0.23-0.37)
School (Males)			
1 (lowest risk)	Ref	Ref	Ref
2	1.43 (0.70-2.92)	1.36 (0.87-2.13)	0.58 (0.35-0.96)
3	2.15 (1.13-4.07)	0.77 (0.52-1.16)	0.85 (0.54-1.33)
4 (highest risk)	3.25 (1.61-6.55)	1.53 (0.98-2.38)	1.62 (0.99-2.66)
School (Females)			
1 (lowest risk)	Ref	Ref	Ref
2	1.28 (0.86-1.90)	0.76 (0.45-1.27)	0.65 (0.40-1.05)
3	2.00 (1.36-2.94)	1.28 (0.77-2.13)	0.64 (0.40-1.04)
4 (highest risk)	2.27 (1.46-3.52)	1.40 (0.79-2.50)	0.57 (0.33-0.98)

Conclusions

The results suggest that tubular kidney damage may be present among children in an area of epidemic CKD. If confirmed, factors in addition to occupational exposure should be studied as possible causes of CKD.

Sugar-cane harvesting and history of kidney disease

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2 Federación Nacional de Trabajadores Agroindustriales, Campesinos e Indígenas Libres del Ecuador (FENACLE), Guayaquil Ecuador
3 Sindicato de Campo del Ingenio Valdez (SCIV), Milagro Ecuador
4 University of Massachusetts Lowell, Lowell MA, USA

INTRODUCTION:

Sugar-cane harvesting requires extremely strenuous physical labor in hot climates. In Milagro, Ecuador (altitude = 71 meters), cane cutting is relatively well-paid compared to others available in this agricultural region.

This project results from a **binational, union-academic collaboration** with the union (SCIV) representing 1200 workers who harvest sugar cane in Milagro, Ecuador, and the national agricultural workers' federation (FENACLE).

Mixed methods (qualitative/quantitative) have been used to characterize these jobs, with the hope of being able to develop ergonomics and safety measures that are feasible, effective, and acceptable to the workers.

METHODS:

- Group interviews at the union hall provided detailed information about work organization and job content (tasks by season, pay basis, etc.).
- Harvesting work was observed and videotaped by the lead investigator (RH).
- A standardized questionnaire was developed to cover medical and injury history, work history, physical and psychosocial job features, MSDs, and demographics.
- The questionnaire was pilot-tested and revised through one-on-one interviews with union leaders and members.
- At the end of the harvest season (2011), the survey was distributed at a general assembly of the union.

RESULTS:

Of about 400 assembly participants, nearly all completed the questionnaire.

They were primarily young and middle-aged men, with an average age of 45 years.

A majority of them reported their health as "very good" (16%), "good" (47%), or "regular" (28%).

Demographic and occupational characteristics of 386 male sugarcane workers, Ecuador, 2011

	Mean	S.D.	Range
Age (yr)	45	12	21 - 76
Height (cm)	165	8	124 - 190
BMI	24.5	3.9	14.9-37.9
Years work	13.8	8.6	1 - 46

Workload:

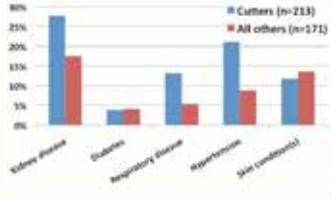
An exposure index was constructed by summing 13 physical workload variables (self-reported). Cutters had a somewhat higher workload index than other workers (37.3 vs 32.1, p< 0.0001).

Cutters also had a slightly lower BMI, although they were 4 years older, on average than other study participants..

Cutters' output (in tons), as reported on questionnaire

	Mean	Std. Dev.
Per day	8.5	4.3
Per week	47.9	17.4

Self-reported history of diagnoses



Kidney Disease:

Kidney disease diagnosed by MD was reported by 23% of participating workers, and much more frequently by cane cutters than by other workers.

Less than half of those with self-reported history of renal disease also reported diabetes or hypertension, two of the most common causes of kidney disease in working-age people.

Select Characteristics and CKD Risk Factors among Self-Reported Cases of Kidney Disease

	Kidney Disease	
	Yes (n=89)	No (n=297)
Age	47.7	43.5
BMI	24.3	24.5
Years Worked in Sector	17.3	12.6
CKD Risk Factors		
Diabetes (n=9)	10%	2%
Hypertension (n=60)	36%	9%

The Work Process

Harvest begins at end of the rainy season. The "zafra" in Ecuador lasts from about July to December.

Key jobs include planting, irrigation, application of fertilizer and herbicides, in addition to harvesting ("cutting") of the mature cane. Weeding is performed with a machete, in a manner similar to the harvesting procedure but somewhat less strenuous. Some jobs are seasonal and others are year-round. Some cutters perform other tasks during the rest of the year.

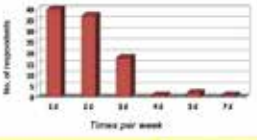
Primary job assignments of 386 male sugarcane workers, Ecuador, 2011

	(n)	Percentage of respondents
Cutting	213	55%
Irrigation	68	18%
Herbicides	15	4%
Planting	30	8%
Weeding	37	10%
Mixed	21	5%

All workers have a state-guaranteed minimum wage.

However, cutters are paid primarily on the basis of production. The complicated incentive system includes double wages for weekend work.

In a typical work week, how many days do you have to leave work early because of excessive fatigue?



Despite being paid for the amount that they harvest, interviewed cane cutters reported regularly being too fatigued to be able to work more than 6 hours per day, even when more work was offered.

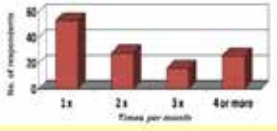
The fields are burned before harvest, to reduce weeds and to drive away snakes.

Often they are still hot – even smoking - when the cane cutters enter.

Dehydration and lack of potable water are common complaints.

Air-borne ash was a frequent exposure for 73% of study respondents.

In a typical month, how often do you have to enter a field that was recently burned or is still burning?



DISCUSSION:

Cane cutting is a relatively well-paid job compared to others in this region but involves high physical demands with significant climate stressors.

The combination of quantitative and qualitative findings indicated that kidney disease may be excessive in this population.

A follow-up investigation of kidney disease biomarkers and work practices is warranted in this work setting.

Acknowledgement:
This project was supported financially by the Post-Graduate Directorate, Ecuatorian Technological University (Universidad Tecnológica Equinoccial), Quito, Ecuador

Investigating occupational factors and biomarkers of kidney function among Nicaraguan workers

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Oriana Ramirez Rubio, Jose Marcell Sánchez Rodríguez, Michael McClean
Boston University School of Public Health, Department of Environmental Health, Boston, MA

Abstract

Introduction: In Nicaragua, an excess prevalence of chronic kidney disease (CKD) with unknown etiology has been described in young, male agricultural workers. Though the majority of recorded cases are sugarcane workers, it is unknown whether other industries are affected. Our goals were to characterize the type of kidney damage, evaluate occupational factors, and investigate the role of metals exposure.
Methods: Our study population included 284 sugarcane workers, 51 miners, 60 construction workers, and 53 port workers. Blood and urine samples were collected during two rounds of sampling, at the beginning and end of the harvest season (zafra) for sugarcane workers and only during the second round for other workers. We analyzed biological samples for metals and biomarkers of kidney injury and CKD. We used linear regression models to investigate predictors of kidney injury and CKD.
Results: Estimated glomerular filtration rate (eGFR) was significantly different by sugarcane job and decreased by 6.4 mL/min/1.73 m2 in cane cutters as compared to factory workers (p=0.006). Similarly, NGAL was significantly different by job and increased most among cane cutters, by 19.2 mg/g creatinine, compared to factory workers (p=0.04). More workers than expected in other industries had eGFR <60 mL/min/1.73 m2, indicating CKD. Generally, urine albumin was low in all workers. Heavy metals were not associated with markers of kidney function, with the exception of arsenic; workers with the highest arsenic exposures had significantly lower eGFR (p=0.01).
Conclusions: In sugarcane workers, biomarkers of kidney injury and CKD were highest among field workers and lowest among factory workers, supporting the hypothesis that workers with the greatest heat exposure are at greater risk of developing disease. These data provide evidence of CKD among workers in other industries and indicate a tubulointerstitial disease. Finally, there is some evidence that high exposure to arsenic is associated with biomarkers of CKD.

Objectives

- Evaluate characteristics of the disease to determine whether kidney damage is tubulointerstitial or glomerular.
- Evaluate biomarkers of kidney injury and CKD among sugarcane workers by investigating changes during the zafra and differences by job.
- Using job category as a surrogate exposure variable, identify which jobs have the highest risk of kidney injury, representing an important step in determining the causal agent(s).
- Determine whether there is evidence of kidney injury or CKD among workers in other industries.
- Analyze heavy metals in biological samples to characterize metals exposure and explore relationships with markers of kidney damage.


Seed cutting


Agrichemical application

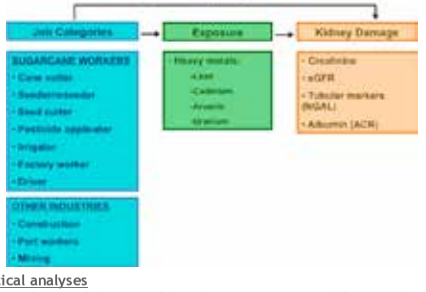

Cane cutting

Methods

Study population and design

- Study population includes:
  - 284 sugarcane workers representing 7 different job tasks; blood and urine samples collected twice, at pre- and late-zafra
  - 51 miners, 60 construction workers, and 53 port workers; blood and urine samples collected once
- Biological samples analyzed for markers of kidney injury (ACR and NGAL), markers of CKD (serum creatinine and eGFR), and heavy metals

Figure 1. Study design overview.

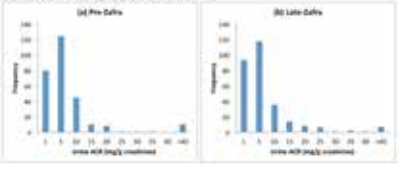


Statistical analyses

- Biomarkers with lognormal distributions were natural log-transformed.
- Linear regression models used to evaluate predictors of biomarkers of metals exposure, kidney injury, and CKD.

Results & Discussion

Figure 2. Distribution of urinary ACR (mg/g creatinine) at (a) pre-zafra and (b) late-zafra among sugarcane workers



- Very few workers had ACR ≥30 mg/g creatinine (Figure 2).
- ACR was not associated with reduced eGFR (p=0.9).
- Indicates that kidney damage is tubular in nature, not glomerular.

Table 1. Multivariate analysis of eGFR (mL/min/1.73 m²) by sugarcane job

ISA Job	Pre-zafra β	Pre-zafra p-value	Late-zafra β	Late-zafra p-value	Change during zafra β	Change during zafra p-value
Cane cutter	**-5.0**	**0.03**	**-11.4**	**<0.0001**	**-6.4**	**0.004**
Seed cutters	-4.3	0.2	**-12.0**	**0.002**	**-7.0**	**0.03**
Irrigators	1.2	0.6	-4.1	0.1	**-5.3**	**0.03**
Drivers	-2.4	0.3	-0.1	0.9	2.3	0.4
Seeders	2.4	0.5	-2.4	0.6	-4.4	0.2
Pesticide applicators	2.5	0.3	-1.1	0.7	-3.6	0.2
Factory workers	reference		reference		reference	

Analyses adjusted for age and sex.

- Decrease in eGFR during the zafra highest for seed cutters, cane cutters, and irrigators, as compared to factory workers (Table 1).

Results & Discussion

Table 2. Multivariate analysis of ln(NGAL) and change in NGAL (µg/g creatinine) by sugarcane job

ISA Job	Pre-zafra β	Pre-zafra e^β	Pre-zafra p-value	Late-zafra β	Late-zafra e^β	Late-zafra p-value	Change during zafra β	Change during zafra p-value
Cane cutter	0.1	1.1	0.6	**1.1**	**3.0**	**<0.0001**	**19.2**	**0.04**
Seed cutters	**0.6**	**1.8**	**0.04**	0.5	1.6	0.1	-2.8	0.8
Irrigators	0.05	1.1	0.8	**0.8**	**2.2**	**0.0007**	4.4	0.6
Drivers	-0.08	0.9	0.7	0.01	1.0	0.9	-2.3	0.8
Seeders	**0.8**	**2.3**	**0.005**	0.6	1.8	0.08	-0.6	0.9
Pesticide applicators	0.02	1.0	0.9	-0.01	1.0	0.9	-0.8	0.9
Factory workers	reference			reference			reference	

Adjusted for age and sex.

- Increase in NGAL during the zafra highest for cane cutters (Table 2).
- The highest tertile of late-zafra NGAL associated with an eGFR 7.3 mL/min/1.73 m2 lower than the lowest tertile (p=0.0005).

Table 3. Summary statistics for late-zafra eGFR (mL/min/1.73 m²) among workers in other industries

Industry	n	Mean	Minimum
Miners	51	103	39
Construction	60	107	15
Port workers	53	101	26

- 3 miners (6%), 3 construction workers (5%), and 4 port workers (8%), had eGFR <60, much higher than expected if compared to a similarly aged population of US men (Table 3).

Table 4. Summary statistics for exposure to arsenic

	n	Arsenic (µg/L) Pre-zafra GM	Pre-zafra Range	Late-zafra GM	Late-zafra Range
ISA Job					
Cane cutter	20	17	2.0 - 110	15	4.3 - 74
Irrigator	20	13	1.3 - 71	11	2.2 - 60
Seeder	20	12	2.7 - 52	13	2.2 - 48
Factory workers	19	8	2.5 - 21	13	1.6 - 45
Miners	20	NA		26	6.3 - 180
Exposure Guideline		100		100	
GM in US		8.4		8.4	

NA=Not available

- Concentrations of arsenic higher than expected and exceeded WHO's Exposure Guideline for 3 workers (Table 4).
- On average, eGFR among workers with high arsenic was 9.0 mL/min/1.73 m2 lower than workers with low arsenic (p=0.01).
- Lead, cadmium, and uranium not associated with markers of kidney function and generally consistent with levels in the US.

Conclusions

- Disease process appears to be tubular rather than glomerular.
- Measures of kidney damage not uniform among all sugarcane workers and were higher at late-zafra than pre-zafra; changes largest in field workers and smallest in factory workers, suggesting an occupational component to the disease possibly due to heat stress.
- Prevalence of eGFR <60 mL/min/1.73 m2 was higher than expected in sugarcane workers and in other industries.
- Arsenic was higher than expected and associated with lower eGFR.

Authors are supported through a contract with the Office of the Compliance Advisor/Ombudsman, the independent accountability mechanism for social and environmental concerns of IFC/MIGA, World Bank Group, Contract #7154314.

End Stage Renal Disease in El Salvador
Evidence of non-traditional risk factors

Ricardo Leiva, MD. Zulma Trujillo, MD. Silvia Leiva, MD. Luis Trujillo, MD
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Introduction

Hospital Nacional Rosales in San Salvador is the main safety net hospital for the uninsured in El Salvador. Outpatient hemodialysis and peritoneal dialysis are offered but due to the high incidence of end stage renal disease (ESRD), most new patients can only be offered intermittent peritoneal dialysis with a rigid peritoneal dialysis catheter (weekly treatment). Hospital Nacional Rosales is a 450 bed hospital and the most common admission diagnosis is renal failure with 300-400 new patients per year. Our previous work revealed that 67% of the incident patients with ESRD did not have traditional risk factors for chronic kidney disease such as diabetes or hypertension, and that the sub-group without traditional risk factors tended to be younger and predominately male (87%). Furthermore, this subgroup tended to be farm workers reporting contact with pesticides. (1,2)

Hospital Nacional Rosales is a referral hospital and to better understand the geographic distribution of the incident patients with ESRD we designed a study to estimate the incident rates of ESRD per department (the country is divided into 14 departments). (3)

Methodology

El Salvador does not maintain a precise ESRD registry. In cooperation with the Pan American Health Organization, we developed an epidemiological survey in which ESRD patients (Hemodialysis, Peritoneal Dialysis or Kidney Transplant) were interviewed. The probable causes of ESRD and demographic information was collected.

Results

The prevalence of ESRD was 12.5 cases per 100,000 habitants, and the most affected ages where between 20 and 70 years. The highest prevalence of ESRD was in the Department of La Paz with a prevalence rate of 25.3 cases per 100,000 habitants and with a male predominance of three times greater than expected. 67 % of the cases did not have traditional risk factors for chronic kidney disease. Potential risk factors were identified and included exposure to chemicals, mainly pesticides or fertilizers and drinking form wells.

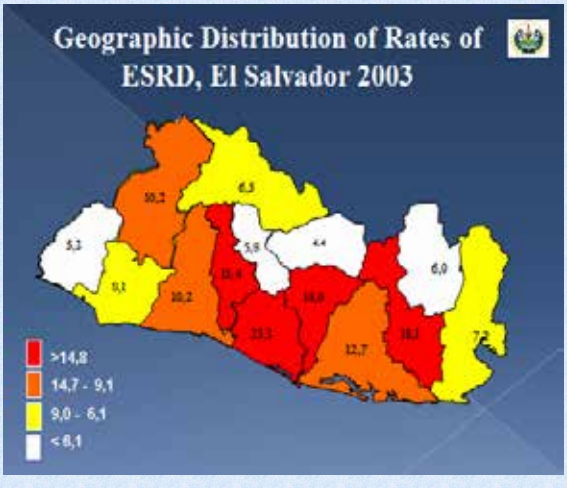
Conclusions

Similar to many countries, the rate of ESRD is rising and the treatment costs are prohibitive in El Salvador. Initial epidemiologic research suggests that non-traditional risk factors for chronic kidney disease could be responsible for some portion of the rampant rise in ESRD rates in El Salvador. There is an urgent need for further research into the possible causative role of pesticides, heavy metals, contaminated water, dehydration or other non-prescribed medications or herbs.

Figure 1

Figure 2

Figure 3



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Ministerio de Salud

INSTITUTO NACIONAL DE SALUD- MINISTERIO DE SALUD

ENFERMEDAD RENAL CRONICA Y FACTORES DE RIESGO ASOCIADOS EN EL BAJO LEMPA, EL SALVADOR. ESTUDIO NEFROLEMPA 2009 -2010

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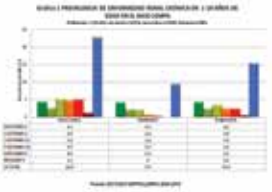
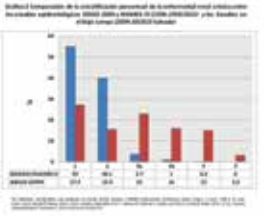
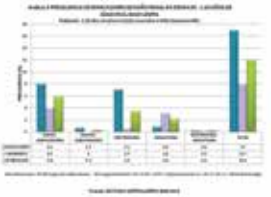
INTRODUCION Y OBJETIVOS: En El Salvador la Enfermedad Renal Crónica (ERC) constituye la primera causa de muerte hospitalaria. El proyecto Nefrolempa tiene como objetivo determinar la prevalencia de la ERC, marcadores de daño renovascular en orina, y factores de riesgo asociados.



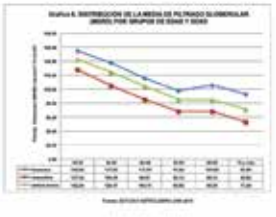
METODOS: Nefrolempa es un estudio epidemiologico-clinico transversal y analitico realizado durante los años 2009-2010, basado en la pesquisa activa de la ERC y los factores de riesgo asociados en las comunidades del Bajo Lempa, Usulután, Jiquilisco, El Salvador. Se contó con la participación activa de la comunidad, organizaciones sociales, instituciones de educación superior y de salud. Se seleccionaron y capacitaron: médicos, enfermeras, laboratoristas clínicos, estudiantes de medicina, de tecnología de la salud y promotores de salud. Previo consentimiento informado de los participantes, y luego de un estudio piloto, para valorar la metodología, se recopilaron: determinantes sociales, datos epidemiológicos y clínicos a través de la aplicación de una historia clínica familiar y personal elaborados al efecto. Se realizaron visitas casa por casa y consultas médicas: anamnesis, mediciones físicas, urianálisis de marcadores de daño renovascular a través de tiras reactivas; determinaciones en sangre de Creatinina y estimación del Filtrado Glomerular (FG) por la fórmula MDRD, Glucosa, Colesterol Total, LDLc, HDLc, Triglicéridos, con personal técnicamente capacitado y certificado. **[figure 1]**



RESULTADOS: Se estudiaron 554 familias y 1215 personas ≥ 18 años, de ambos sexos. La prevalencia de ERC fue de 15.4%; sexo masculino: 22.8% y en el sexo femenino: 9.5%. La Prevalencia de IRC fue de 8,8%; sexo masculino: 15.9% y sexo femenino: 3.2 %: La prevalencia por estadios fue: estadio 1: 4,2%; estadio 2: 2,4%; estadio 3a: 3.5%; estadio 3b: 2.5%; estadio 4: 2.3%; estadio 5: 0.5%. La prevalencia de marcadores de daño renal fue de: 12.1%; sexo masculino (17%) y sexo femenino (8.1%). Prevalencia por marcadores : microalbuminuria (A2) 5.9 %; Macroalbuminuria (A3): 0.3%; Proteinuria: (3.5%); Hematuria: 2.2%; Proteinuria-hematuria: 0.2% Se demostró una elevada prevalencia de factores de riesgo tradicionales: DM: 8.9 % HTA: 19.2 %; historia familiar de ERC: 21.2 %; Dislipidemia: 49.9%; Sobrepeso: 29.5%; Obesidad: 22.1%; Síndrome Metabólico: 15.2% y elevada prevalencia de exposición a nefrotóxicos: consumo de AINES: 80.9 %; consumo de plantas medicinales: 62.3% [tabla 1]; contacto con agroquímicos: 52.1%. [tabla 2]



CONCLUSIONES: Se demostró una elevada prevalencia de factores de riesgo de ERC en la población estudiada. Predominó la ERC de causa desconocida (ERCcd) no asociada a DM a HTA ni a proteinuria > 1 g/L. Se realizaron análisis univariado y multivariado mediante regresión logística múltiple (RLM) con un IC 95%. Los factores de riesgo asociados mediante RLM, fueron la edad (OR 4.630), el sexo masculino (OR 1.874), la ocupación agricultor (OR 1.761), hipertensión arterial (OR 1.750) la hipertrigliceridemia (OR 1.733) y la historia familiar de ERC (OR 1.712). Se evidenció la presencia de una doble carga de factores de riesgo tradicionales: vasculares ateroscleróticos y no tradicionales: tipo de ocupación laboral y factores tóxicos ambientales, que pueden actuar de forma sinérgica en el daño renal. Los resultados de este estudio y de otros realizados por la comunidad nefrológica salvadoreña, evidencian la presencia de una nueva entidad nosológica, aun no caracterizada a profundidad,: ¨nefropatía de las comunidades agrícolas salvadoreñas¨. La similitud de reportes de ERC en otros países centroamericanos y en el sur de México pudiera plantear la hipótesis de estar ante una ¨Nefropatía Regional Centroamericana o Mesoamericana. Para corroborar estas hipótesis se requieren de ulteriores estudios epidemiológicos, clínicos y toxicológicos. Finalmente, la información obtenida ha sido útil para planificar recursos necesarios para la atención médica de la población afectada y constituyó la base para el diseño de una Unidad de Salud Renal en el Bajo Lempa, integrada por un equipo multidisciplinario para las acciones de intervención preventivas y curativas en los pacientes detectados, implementada por el Ministerio de Salud de El Salvador y que permite dar continuidad al trabajo en el resto de las comunidades de la región estudiadas. Así como la extensión de nuevas pesquisas y acciones en salud a otras comunidades rurales salvadoreñas.



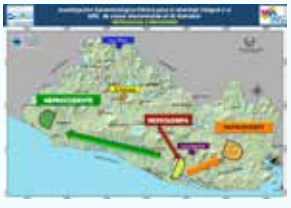
Salud Renal

Instituto Nacional de Salud

Unidad de investigaciones renales

Estudios epidemiológicos

Se han realizado investigaciones epidemiológicas de base poblacional. Durante 2 años se han estudiado 11 comunidades, 1306 familias y 5018 personas de todas las edades. Realizados en comunidades rurales de los municipios de Jiquilisco (Bajo Lempa), San Miguel y Guayapa Abajo respectivamente, las cuales reportaron prevalencias elevadas de 16 a 20% y disminución de las funcion renal desde edades tempranas en menores de 20 años.



Patrón epidemiológico

Se ha confirmado un patrón epidemiológico muy particular en las poblaciones agrícolas, caracterizado por afectación en ambos sexos, adultos y adolescentes, predominante en el sexo masculino (relación hombre-mujer de 2:1 entre las edades de 20 a 59 años de edad) En cuanto a la causa, en la mayoría de los pacientes diagnosticados no están asociadas a la diabetes mellitus ni la hipertensión arterial, ni a otra enfermedad renal primaria, es decir, su causa es desconocida.

Resultados

Se ha confirmado una coexistencia elevada de factores de riesgo que plantean la presencia de una doble carga de factores causales y de progresión.

Los **tradicionales**: diabetes mellitus, hipertensión arterial, obesidad, dislipidemia; y los **no tradicionales**: riesgos de toxicidad laboral y medioambiental, que pudieran actuar de forma sinérgica en el daño renal.

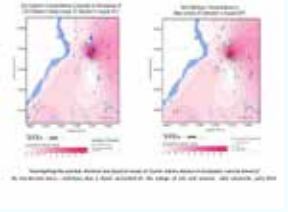
Toxicidad ambiental y ocupacional

La investigación sobre el contenido de pesticidas y metales pesados en aguas superficiales y subterráneos, suelos y sedimentos han comenzado en el Bajo Lempa, con la colaboración de investigadores de la Universidad de Ohio, el Ministerio de Salud, Ministerio de Medio Ambiente, y ANDA.

Las muestras de suelo se han recogido teniendo en cuenta las áreas eran las personas que tienen enfermedad renal han estado trabajando.

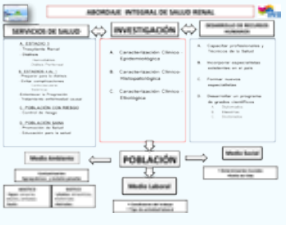
Metales pesados

Resultados de esta investigación muestran la presencia de metales pesados en los suelos, los niveles de cadmio son los más altos en la zona donde el mayor número de trabajadores enfermos han estado trabajando. Estos resultados sugieren una posible conexión entre la presencia de cadmio (un metal pesado, altamente tóxico que ataca el riñón) y la presencia de la enfermedad.



Nuevo paradigma

Se ha implementado un nuevo paradigma de abordaje de la salud renal, a traves de la investigación clínico-epidemiológica para el desarrollo de acciones integradas e intersectoriales en la promoción y prevención de la enfermedad renal crónica, los factores de riesgos asociados a nivel comunitario a traves de LA RIISS. Las Intervenciones benefician a personas, familias y comunidades de las regiones de salud intervenidas, contemplados en un programa integral para la prevención y atención de la enfermedad renal crónica en proceso de implementación a nivel nacional como parte de las acciones enmarcadas dentro del proceso de la Reforma de Salud.



Sri Lankan Agricultural Nephropathy (SAN)

Sri Lanka - an Island

- Pearl in the Indian ocean
- Area - 65,610 km^2
- Population - 21 million

SAN

- First reported in 1994
- Total number of patients (up to July 2012)-**22321**
- Total number of deaths (up to July 2012)-**14256**

Risk Factors,

- Drinking well/hard water
- Being a male
- Being a farmer
- Exposure to agrochemicals
- Monthly income less than Rs.15,000/=
- Exposure to profound repeated daily dehydration?

Already exclude,

- Fluoride
- Aluminium Fluoride
- Fungal toxins (aflatoxins, ochratoxins and fumonisins)
- Cynobacterial toxins

as etiological factors.

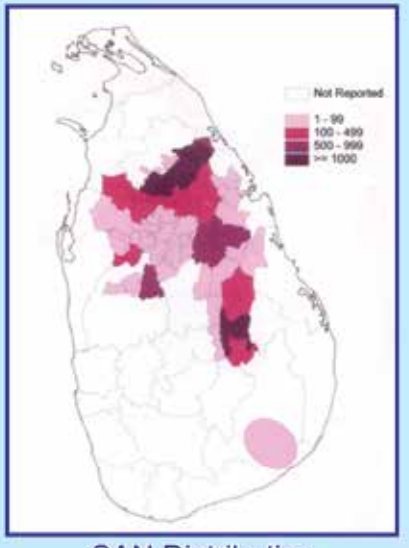
SAN and High ground water hardness –Possible link

- A statistically significant positive correlation (P <0.008)) was revealed between occurrence of SAN and hard water consumption.
- 96% of the SAN patients have consumed hard or very hard water at least for 5 years before the diagnosis of the disease.
- We have already published data on presence of Arsenic(As), Cadmium (Cd) and Uranium (U) in agrochemicals used in Sri Lanka that have a possible link to SAN.
- It was found that most of the pesticides and chemical fertilizers containing phosphate were contaminated with As,Cd,U and our findings revealed that As and Cd content in soil gradually decreases with depth, particularly in the agricultural areas implying that it is not present naturally in soils nevertheless has been introduced from the surface, most probably due to anthropogenic activities.
- Hardness may act through several mechanisms to carry these toxic substances to victim's body and enhance the toxicity. Although it is believed that hardness alone does not cause any damage to human health it may become hazardous in the presence of arsenic, cadmium and other pollutants.

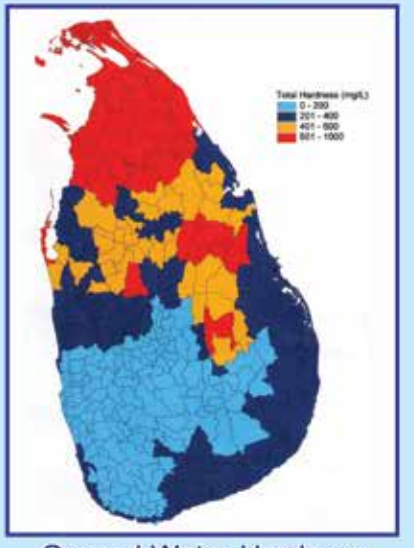
Histopathological features of SAN

- Histopathological observations of 34 renal tissues obtained at Padavi Sripura Hospital from December 2010 to July 2012 were scored according to Banff 97 Working Classification of Renal Allograft pathology.
- Tubular Interstitial nephritis with or without nonspecific interstitial mononuclear cell infiltration was the dominant histopathological observation.
- Glomerular sclerosis and glomerular collapse were also common.
- Study concludes that tubulointerstitial damage which is commonly seen in toxic nephropathies is the major pathological lesion in SAN.
- The disease process appears to mainly affect the proximal tubules and the interstitium giving rise to characteristic, recognizable histopathological and clinical features.
- Presences of α-1 and β-2 microglobulin in patient's urine samples are compatible with the histopathological observation.

Climate Zones



SAN Distribution



Ground Water Hardness

Soil Map


Objetivos
CHISTATA
ERC
Infecciones Tracto Urinario (ITUs)
Metodología
Entrevistas semi-estructuradas
Chinandega
León
Muestreo
Enfermedad Renal Crónica de causas desconocidas en Nicaragua:
análisis cualitativo de entrevistas semi-estructuradas con médicos y farmacéuticos locales
Ramírez-Rubio O, Brooks D, Amador JJ, Kaufman J, Weiner D, Scammell M
Universidad Autónoma de Madrid, Universidad de Boston
Conclusiones
Chistata
¿ITUs?
ERC
Resultados
2 ¿Causas de ERC? ¿Sol fuerte, trabajo duro y agua?
3 "Chistata" e ITUs
TRATAMIENTO
5 Las percepciones de los médicos confirmaron la permisividad de los farmacéuticos frente a la automedicación de los pacientes
4 ERC, ITUs y chistata
TRATAMIENTO

Possible Association Between *Leptospira* Infection and Chronic Kidney Disease of Unknown Origin in Central America

A. Riefkohl, O. Ramírez, J. J. Amador, M. McClean, R. Laws, J. Kaufman, D. Weiner, M. Sánchez, D. Brooks

Boston University School of Public Health; Boston, MA

Introduction

- Chronic kidney disease of unknown origin (CKDu) in Central America is a major public health problem
- It is characterized by chronic kidney disease of non-glomerular origin that disproportionately affects young male agricultural workers[1-3]
- Leptospirosis, a bacterial zoonosis with epidemiologic characteristics similar to CKDu is among the causal hypothesis[4-6]
- It causes renal injury, and although it is not known to cause chronic kidney disease in humans, biologic plausibility exists[7]
- A link between leptospirosis and chronic kidney disease represents an evidence-gap due to lack of research and limited data on long-term outcomes in existing studies[8,9]
- It is important to explore whether recurring mild or asymptomatic leptospirosis can lead to multiple episodes of subclinical acute interstitial nephritis, resulting in progressive kidney fibrosis and ultimately chronic kidney disease (figure 1)
- Boston University with collaboration from the Centers for Disease Control and Prevention (CDC) is conducting a study in Nicaragua among workers in sugarcane and other industries to determine if leptospirosis is associated with urine markers of kidney injury

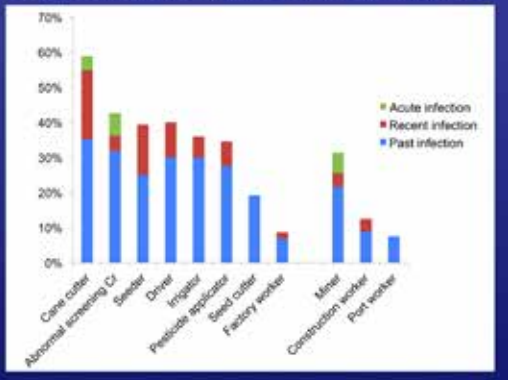
Figure 1. Suggested mechanism by which leptospirosis can lead to CKDu

Study Design

- Study population includes:
  - 282 sugarcane workers representing 7 different job tasks within the industry
  - 47 individuals that had abnormal serum creatinine (Cr) during initial screening for hire
  - 160 workers from non-sugarcane industries including mining, construction and port workers, who have never worked in sugarcane
- Exposure to *Leptospira* was determined by using the microscopic agglutination test, ELISA and urine PCR
- Serum Cr and an estimated glomerular filtration rate were used to determine kidney function
- Urinary markers of kidney injury include urine albumin, neutrophil gelatinase-associated lipocalin, N-acetyl-beta-D-glucosaminidase, and interleukin-18

Preliminary Results

Figure 2. Leptospirosis among workers in sugarcane and other industries



Conclusions

- Past exposure to *Leptospira* is high, particularly among cane cutters (35.3%)
- The high percentage of recent infections among workers in the sugarcane industry suggests that many exposures occurred in a non-occupational setting or in the previous harvest season
- Acute infection is common among cane cutters (4%), subjects with an abnormal screening Cr (6.4%), and miners (6%)
- These acute infections may represent mild or subclinical disease that would have otherwise gone unrecognized
- Urine PCR was negative in all subjects, arguing against chronic colonization of renal tubules by leptospires

Future Work

- Correlation between *Leptospira* exposure and urine markers of kidney injury is still not available, and will be important to determine if leptospirosis is associated with subclinical kidney injury

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CLINICAL CHARACTERISTICS AND 3 YEAR FOLLOW-UP OF PATIENTS WITH CHRONIC KIDNEY DISEASE WHO LIVE IN SANTA CLARA SUGARCANE COOPERATIVE. DEPARTMENT OF LA PAZ, EL SALVADOR.

Dr. Luis Antonio Trujillo, Dra. Zulma Cruz de Trujillo, Dr. Ricardo Alberto Leiva Merino, Dra. Silvia Lazo de Leiva* * Dra. Viviana Cruz **

*Department of Nephrology, Hospital Nacional Rosales. **Santa Clara cooperative clinic.

INTRODUCTION: [illegible]

OBJECTIVES: To identify CKD among sugarcane workers of Santa Clara's cooperative, their epidemiological and clinical characteristics after the 3 year monitoring.

MATERIAL AND METHODS: The study was a retrospective and descriptive. [illegible]

RESULTS: [illegible]

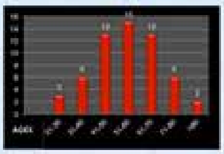
GEOGRAPHICAL LOCATION IN SAN LUIS TALPA, TIME OF RESIDENCE, AND FAMILY NUMBER

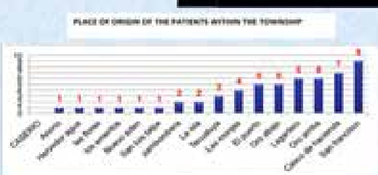
TIME OF RESIDENCE

NUMBER OF FAMILY MEMBERS

NUMBER OF CHILDREN

AGE OF SANTA CLARA'S WORKERS





[illegible]

ETIOLOGY OF CKD

UNKNOWN ETIOLOGY OF CKD

[illegible]

PERSONAL HISTORY

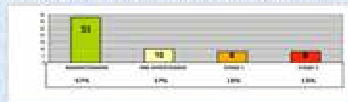
[illegible]

PHYSICAL EXAMINATION RESULTS

[illegible]

[illegible]

BLOOD PRESSURE VALUES USING THE JNC-7 CLASSIFICATION



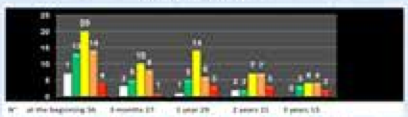
STAGES OF CHRONIC KIDNEY DISEASE TO INCOME

[illegible]

LABORATORY TESTS

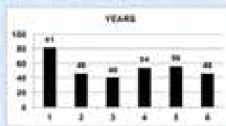
[illegible]

FOLOW UP IN 3 YEARS



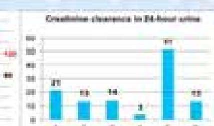
[illegible]

YEARS



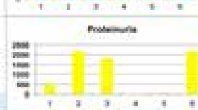
Blood Pressure

Creatinine clearance in 24-hour urine



Uric Acid

Proteinuria



CONCLUSIONS: [illegible]

REFERENCES:

[illegible]

DEVELOPMENT OF A HEALTH INTEGRAL PLAN OF WATER RESOURCES IN TWO DEPARTMENTS OF THE DRY REGION DRY OF NICARAGUA: PROTECTION AGAINST ENVIRONMENTAL POLLUTANTS.

IB-UMH (Elche España):
Eugenio Vilanova (Coordinador), Roberto A Ruiz (NI), Miguel A Sogorb, Carmen Estevan, Jorge Estevez

CISTAS-UNAN (Leon Nicaragua):
Edmundo Torres (Coordinador), Edipcia Roque, Aurora Aragón, Luis E Blanco, Teresa Rodriguez, Ervin Esquivel, Juan Centeno

CONFIRMED WITH A REALISTIC OBSERVATION THAT CHRONIC KIDNEY DISEASE IS A SERIOUS PUBLIC HEALTH PROBLEM AND WITH GREAT SOCIAL AWARENESS

Consequently: The project focuses its effort in chronic kidney disease and its relationship with the quality of the water

Objetivos

1. Develop a strategic plan for the acquisition and use of quantitative and qualitative data on water resources in two departments of the dry area of Nicaragua.
2. **Create and share knowledge about the quality of water resources in that zone..**
3. Develop a comprehensive plan for health protection against environmental pollutants.

Three stages:

1. Exploration, information and planning.
2. Training, exchange of knowledge, laboratory work, pilot sampling and chemical analysis.
3. Presentation of the final report of the comprehensive plan (long term project).

First stage (Nicaragua)

- Contact of the research teams,
- Familiarization with the problem.
- Assessment of existing epidemiological information



Visit to the communities, field work.

- Realistic in situ observation shows "local" geographical factor as decisive.
- Occupational factors are contradictory the social perception of causal agents varies according to the place and it is attributed to local occupational and economical activities.
- Why also animals died in communities with renal incidence?
- Why there are cases of children without working activity?
- There are associations to occupational factors but **what is the actual primary cause?** What they eat?, what they drink?, what they breathe? **Searching environmental "local" factors!**.

El Porvenir



Los Zanjones


San Jacinto

Villa 19

4 esquinas de Amayo-Los Palacios



Second stage (Spain)

Visit of the Nicaraguan team at the Institute of bioengineering: presentation of the issue to the members of the unit of Toxicology and chemical safety.
Analysis of water and hair samples in the ICP-MS Spain
Microbiology and analysis of pesticides (Nicaragua, work going on)
(There are no photos but a lot





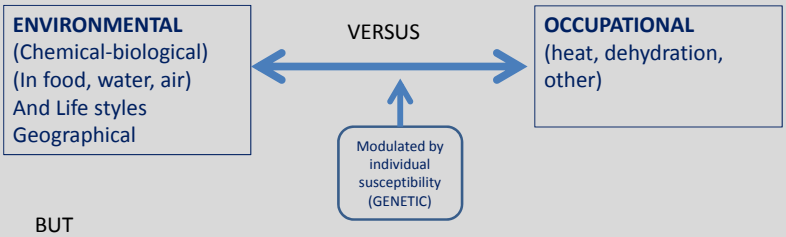




Third stage.

- Design and presentation of an Action Plan for long-term project.
- Workshop in Leon (6 December).
- Presentation of an Report-Project (February 2013)

Chronic kidney disease (CKD)
Association with some factors:



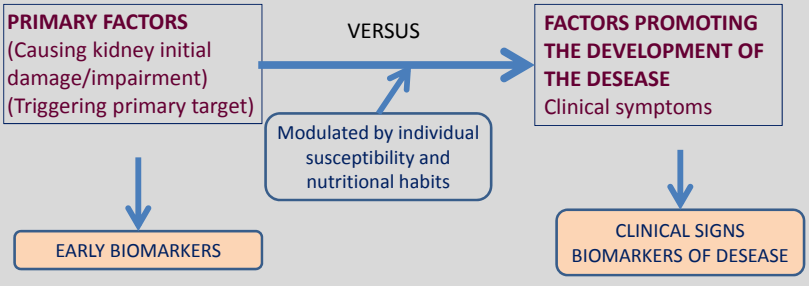
BUT

Statistical association is not necessarily a CAUSE → EFFECT relationship

Which are the PRIMARY FACTORS

Eugenio Vilanova
University Miguel Hernandez, Elche-Alicante Spain)
Coordinator of Collaborative project (AECID):
CISTAS UNAN Leon Nicaragua and
UMH, Elche Spain

Chronic kidney disease (CKD)
Association with some factors:



Statistical association is not necessarily a CAUSE → EFFECT relationship

Which are PRIMARY initiators and which are the PROMOTORS

Eugenio Vilanova
University Miguel Hernandez, Elche-Alicante Spain)
Coordinator of Collaborative project (AECID):
CISTAS UNAN Leon Nicaragua and
UMH, Elche Spain

An Interdisciplinary Discussion of Renal Disease in Native North Americans: What is the Role for Genomics?

Joe Yracheta B.S. Lorelei Walker B.S., Kate West M.S., Nirupama Shridhar MPH, Wylie Burke PhD, & Ken Thummel PhD
Center for Genomics & Healthcare Equality (CGHE), Department of Bioethics & Humanities, University of Washington - Seattle

UNIVERSITY OF WASHINGTON
CENTER FOR GENOMICS & HEALTHCARE EQUALITY

Introduction

The burden of CKD and transition to ESRD in American Indian/Alaska Native (AI/AN) populations is higher than the national aggregate. Our team's goal is to bring together a multidisciplinary team to analyze the roots of renal disease disparities among AI/AN groups and find pathways to health benefit.

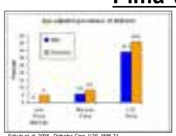
We will explore the interplay of environmental factors, social determinants, and genetic variation as components of these disparities, with an eye toward strategies that could reduce health disparities in this context and perhaps more broadly.

Components to be considered include social factors such as the degree and pace of dietary transition, pesticide/ herbicide exposure in occupational settings, and sequelae of poverty; genetic contributors to kidney disease and conditions associated with kidney disease, including diabetes; and epigenetic mechanisms. Our discussion aims to identify and evaluate competing explanations for CKD disease burden, in order to identify a research agenda aimed at defining and assessing interventions.

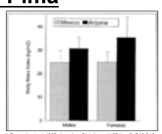
Social Determinants

Original unmodified figure from Norris KC, Agodoa LY: Unraveling the racial disparities associated with kidney disease. *Kidney Int* 68: 914–924, 2005

Pima vs Pima



Schulz et al. 2006, Diabetes Care, V.29, 1866-71



Ravussin et. al. 1997, Jour. Am. Diet Assc., V.97, Iss. 7, 542-546

- Social determinants are the greatest determining factors for poor health outcomes. This is evidenced by the highest rates of diabetic nephropathy among the world's poor, regardless of ethnicity (genetics).
- Environment: In a study of Yaqui girls, mammary tissue was decreased due to possible pesticide exposure (Field Yaqui vs. Mountain Yaqui).
- Stress: One example regarding border differences is the highly visible income gap on the U.S. side of the border.
- Diet: A recent study showed that Southwest Indigenous groups ate vegetable diets high in saponins (Solanaceae), fructo-oligosaccharides (roots), and phytates (legumes). All bind minerals such as iron, calcium and phosphate, and affect glycemic load. In this instance of diet, social determinants and genetics may act demonstratively in the areas of access and stress. Recently many Indigenous communities have recognized this intersection and self advocated via Food Sovereignty movements.

Genomic Background/Template Genes

Due to the purported restricted genome introduced into the Americas with effective population size of 70 individuals (range:50 – 300) from central and north east Asia, some highly frequent gene changes must be considered. Here are six:

EDAR chr.2q13 (*ectodysplasin A receptor)*, rs3827760,*MAF 0.70-1.0 and 0.40-0.55 in mixed Mexican and Muskogee.* Nicknamed the "Thick Hair" gene it is associated with increased secretion of lipid from epithelial cells, increased endothelial repair and increased NF-kB signaling.

ABCC11/MRP8 chr.16q12, *rs17822931, MAF 0.17-0.8 in a checkerboard pattern across the continent.* Nicknamed the "Dry Earwax" gene is associated with trafficking of amphipathic molecules across membrane, including pro-inflammatory & immunomodulatory mediators and drug elimination in the kidney. Variant undergoes rapid proteosomal degradation.

CD40 20q12-13.2, *rs11086998, MAF 0.20-0.45 higher in Central and South America.* Associated with hyper-immune response and autoimmunity. May show involvement with T1D. CD40 Ligand is on X chromosome and it has not yet been screened for variants in AI populations.

ABCA1 chr.9q31,(HDL variant), *rs9282541, MAF 0-0.31, x=0.12 increases clinally from North to South.* Carriers cannot efflux HDL. Varying association level with T2D and obesity depending on Amerind group. Strongest association in Mexicans. Animal models demonstrate β-cell damage. Not found anywhere else in the world to date.

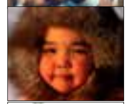
TMPRSS6 22q13.1(Iron Deficiency Variants), *rs855791 and rs4820268, both have a MAF: 0.24-1.0 and increase clinally from North to South.* Iron overload is associated with T2D. HbA1c glycosylation affected by iron dyscrasias. People carrying both variants were protected from T2D as opposed to those carrying either alone.

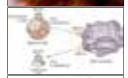
Umbelliferae:Yampa (Perideridia spp.)

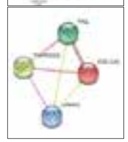
Solanaceae: Groundcherry (Physalis spp.)

Legumes: Tepary bean (Phaeolis spp.)

Saponin Roots &Stems: Yucca (Yucca spp.)



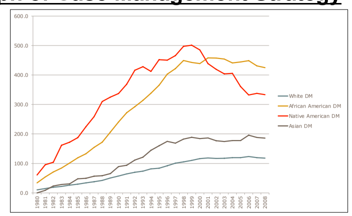




Triumph of Case Management Strategy

Incident Rates of ESRD Due to Diabetes 1980-2008

Per million population by age, gender, race, ethnicity.
IHS National Diabetes Program, Dr. Andrew Narva, NIDDK, NIH



- The U.S. Indian Health Service provides health care to Native Americans at about half the public capitated expenditure of the U.S. Civilian population ($2158 vs. $5291)
- Due to this budgetary constraint, they embarked upon a model with many similarities to the Chronic Care Model for a high risk population.
- This model emphasizes the holistic engagement of patients and healthcare teams through comprehensive Case Management via methods including but not limited too:
  - Wellness care of increased screening and monitoring (eGFR, UACR, Proteinuria, Lipids)
  - Cultural education for health providers about the community they service
  - Community education about diabetes, renal disease, lifestyle changes and IHS services
  - Health care teams that reflexively adapt to "system" changes and who make patients feel like active partners.

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Putative Gene/ Renal Disease Associations

Studies with American Indian/ Alaska Native Populations

PVT1 chr.8q24.21, *rs2720709, MAF 0.58, rs2648875, MAF 0.60.* Involved in regulation of cell division, highly expressed in kidney, associated with T1D & T2D.

ELMO1 7p14.2, *rs1345365, MAF 0.15-0.67.* Much higher frequency in African populations and much lower in Europeans. Increased expression of this gene and dedicator of cytokinesis 1 may promote glioma cell invasion. Associated with diabetic nephropathy.

WFS1 chr.4p16.1, *rs10010131, MAF unreported,* Associated with high eGFR, increased albuminuria, UACR, T2D, obesity and Wolfram's syndrome.

VDR 12q-12q-14, *rs544410, GG frequency 0.88-1.0,* Associated with Ca+ hyperabsorption, hypercalciuria, and renal leak in Canadian aboriginal children (6-13 yo).

ABCG2 4q22.1, *rs2231142, MAF 0.35-0.67.* Associated with increased uric acid, gout and reduced renal function.

SLC22A12 11q13.1, *rs505802 MAF 0.46-0.9, rs476037, MAF 0.16-0.5.* Associated with high serum uric acid because of lack of transport across renal tubules.

Studies with Mexican Populations:

ENPP1 6q22-23, *rs6918013 MAF unreported, rs1044498 MAF 0.188-0.22 (12.9 AI).* Associated with high fasting glucose, HDL and T2D. Associations sometimes found in Asians. Association can be abolished with modified diet.

CTGF 6q23.2, *rs9402373, MAF unreported, rs12526196, MAF 0.2.* Associated with increased rates of fibrosis in liver.

SGK1/SLC2A12 6q23 Implicated in Genome scan of Mexican Americans with high serum uric acid. The 6q22-23 region also is highly associated with IgA Nephropathy that is highly prevalent in some AI/AN groups.

Genes of Interest

PON 1 & 2 - AI/AN populations have shown poor metabolism.

MVP & Angiotensin – AI/AN populations have a higher frequency of mitral valve prolapse with beneficial effects. Studies with Asian populations have shown this to be associated with compensatory angiotensinogen variants.

***Structural proteins*:**

P-Selectin – Changes in P-selectin have been associated with increased hemorrhagic stroke in Asians and Mexicans.

Caveolin1 – Renal tubular Cavelolin1 has shown to undergo reactive changes after ischemic/reperfusion injury.

Filamins A&B - Renal tubular Filamin has shown to undergo reactive changes after ischemic/reperfusion injury prior to the main biomarker, Actin.

PTH target genes:

***FGF23 *SLC34A1 *IGF2 Receptor *Osteopontin**

All of these genes may be implicated in the renal phenomena of hypercalcemia/ hyperphosphatemia in Canadian aboriginals.


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