ALLERGY-ASSOCIATED SYMPTOMS ARE LINKED TO EARLY CHILDHOOD CARIES

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DECLARATION

I hereby declare that this thesis is my original work and it has been written by me in its entirety and I have duly acknowledged all the sources of information which have been used in the thesis.

This thesis has also not been submitted for any degree in any university previously.

Signature ..... December 10, 2014

Dr. Bindu Karunakaran

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ABBREVIATIONS

ECC	Early Childhood Caries
SECC	Severe early childhood caries
AAPD	American Academy of Pediatric Dentistry
CAT	Caries risk assessment tool
WHO	World Health Organization
NIDCR	National Institute of Dental and Craniofacial Research
ICDAS	International Caries Detection and Assessment System
DMFT	Decayed Missing Filled Teeth Index for permanent dentition
DMFS	Decayed Missing Filled Surface Index for permanent dentition
dmft	decayed missing filled teeth index for deciduous dentition
dmfs	decayed missing filled surface index for deciduous dentition
MS	Mutans streptococci
LB	Lactobacilli
DMH	Deciduous molar hypomineralisation
HASECC	Hypoplasia associated severe early childhood caries
EHP	Enamel hypoplasia
OHRQOL	Oral health - related quality of life
ISAAC	International study of asthma and allergies in childhood
NSCH	National survey of children's health
AHR	Airways hyperresponsiveness
COPSAC	Copenhagen Prospective Study on Asthma in Childhood
SIgA	Secretory immunoglobulin A
OR	Odds ratio
RR	Relative risk

OE	Oral examination
SM	Streptococcus mutans
KKH	KK Women's and Children's Hospital
NUH	National University Hospital
GUSTO	Growing Up in Singapore Towards healthy Outcomes
NUSIRB	National University of Singapore Institutional Review Board
PI	Plaque index
HI	Household Income
IV	Input variables
PC	Potential confounders
OV	Outcome variable

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SUMMARY

ALLERGY-ASSOCIATED SYMPTOMS ARE LINKED TO EARLY CHILDHOOD CARIES

OBJECTIVE: To evaluate if allergy-associated symptoms are linked to early childhood caries (ECC) among Singapore toddlers.

METHODS: In "Growing Up in Singapore Towards healthy Outcomes" (GUSTO) - oral health cohort study, 543 children aged 24 months (M) were examined from the main study and ECC was scored using modified ICDAS criteria. Maternal and infant factors related to allergy-associated symptoms and demography were determined at first visit and infant ages - 3 weeks, 3M, 6M, 9M, 12M, 15M and 18M using questionnaires. Multivariate analysis model (Poisson regression) was built to analyze the relationship between ECC and allergy-associated symptoms - eczema, rhinitis and wheezing with nebulizer controlling the confounding factors.

RESULTS: The prevalence rate of caries was 17.7%. The chance to have one more caries lesion are high among preschoolers who ever-had eczema [RR(95%CI)-5.97(2.87-12.43], wheezing with prescribed nebulizer [6.35(1.01-40.08)], breastfeeding at night-time [3.97(1.21-13.02)], plaque score [3.56(2.29-5.53)], frequent snacks at night-time [3.42(1.69-6.92)] and whose mother's with non-tertiary educational level [2.2(1.21-4.06)].

CONCLUSION: Oral health care professionals willing to prevent caries among toddlers should consider the child's allergy-associated symptoms like eczema and wheezing with prescribed nebulizer together with plaque, diet habits at night-time and maternal education.

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CHAPTER I

INTRODUCTION and

LITERATURE REVIEW

1. INTRODUCTION AND LITERATURE REVIEW

1.1. Overview

Children commonly have significant allergy problems, they are often undiagnosed as the child may not complain or the allergy is not recognised. There is an increasing trend of allergies in children (Bousquet et al., 2001). As the child grows, allergy may change and children may "grow out" of an allergy or develop new allergies. For instance, babies are more troubled by eczema but as they grow, the problems of rhinitis and asthma become more prevalent and eczema may subside (Health, 2007). Environmental exposure to dust, pollen, pets and diet may play a critical part in these changes.

If atopic eczema and allergic rhinitis left untreated, it may lead to increased risk of asthma (Bousquet et al., 2001; Health, 2007; Scadding, 2008). Allergic diseases involves both the genetic and environmental factors (Soh et al., 2012). A child experiencing recurrent colds, runny nose, sneezing, glue ears and eczema should be evaluated for allergies, as successful management of allergies will lead to a better quality of life with less frequent accompanying medical problems (Mark D. Scarupa, 2005).

Global report shows that all over the world, hundreds of millions of people suffer from avoidable chronic respiratory disease and allergy, more than 500 million of these people are from low and middle income countries, chronic respiratory diseases account for four million deaths annually (Bousquet, 2007). International study of asthma and allergies in childhood have shown that Singapore is one of the countries where the highest prevalence of asthma

is seen (Wang et al., 2004). During childhood, asthma is the most common chronic medical condition or disease characterized by obstruction of airflow due to increased airway hyper responsiveness and it is a major public health problem in adulthood (Alavaikko et al., 2011; Thomas et al., 2010). Globally, the needs of oral health has been increasing (Marcenes et al., 2013), dental caries of permanent teeth (38.1%) has shown to be one of the cause for years lived with disability with all age groups and ranked number 1 among the global prevalence of the 50 most common sequelae (Vos et al., 2012). Both chronic diseases, asthma (Bousquet, 2007; Rees and John, 1995) and tooth ache (Gift et al., 1992) have been the reason for more children to be absent in preschool/school and miss significant part of their education and has been estimated that both direct and indirect cost in treating asthma among Singapore population alone could be US$ 33.93 million annually (Chew et al., 1999a).

In 1989, D. P. Strachan proposed the concept of hygiene hypothesis (Arbes Jr and Matsui, 2011). Due to its widespread awareness, it has caused researchers to look into the early exposure of oral microbes on the development of allergic disease. It was indicated that early colonization of microorganism occurs in infants and evidence for early immune response to those exposed bacteria is shown by the presence of serum antibodies to those bacteria (Arbes Jr and Matsui, 2011). The hygiene hypothesis suggests, the prevalence of asthma and other allergic disease is increased as the oral infection are decreased (Arbes Jr and Matsui, 2011; Tanaka et al., 2008).

Though numerous studies have been conducted on early childhood caries among allergic population, there is no study to investigate the association between early childhood caries and allergy-associated symptoms like asthma, eczema and rhinitis among local population. This study serves as a tool to monitor oral health among young children over time, providing an insight into potential factors causing development of caries in primary dentition.

1.2. Early Childhood Caries

1.2.1. Preface

Dental caries can be defined as the localized destruction of the tissues of the tooth by bacterial action. It is a diet-related chronic infectious disease with a multi-factorial etiology, multiple cariogenic bacteria in the biofilm on tooth surfaces ferment the carbohydrates and produce acid leading to demineralization of hard tissues and destruction of tooth structure (Marsh and Martin, 2009). Dental caries has become a major public health problem among preschool children (Mannaa et al., 2013; Tang et al., 2013). United States report has shown that dental caries is the single most common chronic childhood disease and it is five times more common than asthma and seven times more common than hay fever (RockvilleMD, 2000). It is one of the most common preventable chronic childhood diseases, people of all age groups throughout their lifetime are susceptible to caries. World oral health report shows that dental caries is affecting about 5 million people of all age group (Health-Report, 2003).

1.2.2. Terminology

Dental caries in preschool children has been recognized as a unique entity with distinguishing clinical characteristics, it was initially identified among infants and toddlers associated with nursing habit as a distinctive pattern of carious lesion affecting their primary maxillary incisors (Fass, 1962). In 1912, Harries coined the term “COMFORTER CARIES” based upon his observations of children who had otitis media and used “comforters” or “pacifiers” (Harries, 1912). According to the international literature, to describe this condition the term "*NURSING BOTTLE MOUTH*" was first adopted by Fass [Fass, 1962]. Since then terminology has been progressively refined to include the terms: *Baby bottle tooth decay, Nursing bottle syndrome and Nursing caries* (Arkin, 1986; Ripa, 1988). In the year 1978, term "Nursing Bottle Caries" was released by American Academy of Pedodontics with American Academy of Pediatrics to address the severe form of caries associated with bottle usage (AAPD, 2011). Though in many cases, improper exercise of feeding practices play a major role but it is not the sole and most important factor in the caries development. However, nursing bottle caries focuses that inappropriate nursing practice is the main or exclusive etiology. Recognizing that the distinctive clinical presentation of caries was not consistently associated with poor feeding practices, to better reflect the multi-factorial nature and etiology of this clinical syndrome and to avoid such inconsistencies "Early Childhood Caries" (ECC) term was suggested to replace other terms like nursing bottle caries in 1994 by the Centers for Disease Control and Prevention (Ismail and Sohn, 1999; Reisine and Douglass, 1998). Thereby, American Academy of Pediatric Dentistry (AAPD)

adopted the term *"Early Childhood Caries"* (AAPD, 2011). In 2002, based on a set of physical, environmental and general health factors, AAPD has developed the caries risk assessment tool (CAT). One of the components of CAT is children with chronic medical conditions requiring long term medication being at a risk of dental caries as a side effect (AAPD, 2009).

1.2.3. Definition

The word caries is a Latin word meaning "DRY ROT' (Pandit I K, 2007). It is a process of slow disintegration that may affect any of the biological hard tissues as a result of bacterial action (caries, 2010). WHO defines caries as localized post eruptive, pathological process of external origin involving softening of the hard tooth tissue and proceeding to the formation of a cavity (WHO, 1962). ECC is a virulent form of dental caries that can destroy the primary dentition of toddlers and preschool children (Berkowitz, 2003). Dental Caries also known as tooth decay or cavity is the infectious microbiologic disease of the calcified tissues of the teeth characterized by demineralization of the inorganic portion and destruction of the organic substance of tooth (Shulman et al., 2001). ECC definition was proposed at a workshop convened by the National Institute of Dental and Craniofacial Research (NIDCR) and published in peer-reviewed journals between 1996-1998 (Drury et al., 1999; Ismail and Sohn, 1999).

AAPD defines caries as biofilm (plaque) induced acid demineralization of enamel or dentin, mediated by saliva as well. *Early childhood caries* is defined as the presence of one or more decayed (non-cavitated or cavitated lesions), missing or filled tooth surfaces (due to caries) in any primary tooth in 6 yr old

child or younger. *Severe early childhood caries* (SECC) represents of one or more cavitated, missing or filled smooth surface (due to caries) in primary maxillary anterior teeth or DMF score of ≥ 4 (age 3), ≥ 5 (age 4), ≥ 6 (age 5) in children from 3 - 5 yr of age, also if any sign of smooth surface caries is seen among children younger than 3 yrs (AAPD, 2008; Evans et al., 2013).

As ECC does not accurately describe several aspects of disease like risk factors, characteristics of condition, rampant nature, inability to define the age of children affected and its prevention, many authors have raised objection to the name ECC which is currently being used in a vital way and suggest that ECC is not an ideal term due to their weaknesses (Vadiakas, 2008).

1.2.4. Classification

In the last 30 over years, many researchers have attempted to classify ECC based on different criteria.

- Johnsen and colleagues have classified ECC into three main patterns: this system has been tested (Greenwell et al., 1990; Johnsen et al., 1984). (A) Lesion associated with developmental defects [Pit & fissure defects and hypoplasia]. (B) Smooth surface lesions [Facio-lingual lesions, approximal molar lesions, combination of both facio-lingual and approximal lesions]. (C) Rampant caries - when 14 out 20 primary teeth having carious lesion including at least one mandibular incisor.
- Caries Analysis system defines four patterns of caries in primary teeth. This system has been validated. [Fissure pattern, maxillary anterior pattern,

posterior proximal pattern, posterior buccal/lingual pattern] (Douglass et al., 1994).

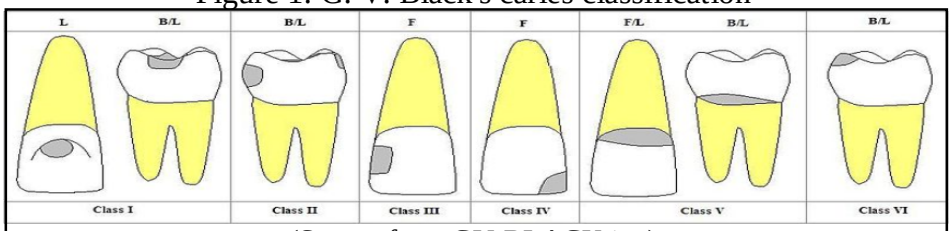
♦ Another classification system was proposed by Veerkamp and Weerheijm to account for the stage of dentition and severity of dental caries. Their four stages were referred to initial, damaged, deep lesions and traumatic. This system assumes that according to successive stages starting from 10 Months to 48 Months the caries follows and mention that at each stage, different group of teeth are involved and caries can range from opaque enamel demineralization to cavitation involving enamel and dentin. This system has not yet been validated (Ismail and Sohn, 1999; Veerkamp and Weerheijm, 1995).

♦ G. V. Black's classification of caries lesions: Greene Vardiman Black classified caries into five types depending upon the site of tooth, later Simon has added class 6 in this classification (GVBlack, 1917)

Table 1: G. V. Black's caries classification

Class I	Caries affecting pits and fissures on occlusal third of molars and premolars, occlusal two thirds of molars and premolars, and Lingual part of anterior teeth.
Class II	Caries affecting proximal surfaces of molars and premolars.
Class III	Caries affecting proximal surfaces of central incisors, lateral incisors, and cuspids.
Class IV	Caries affecting proximal including incisal edges of anterior teeth.
Class V	Caries affecting gingival 1/3 of facial or lingual surfaces of anterior or posterior teeth.
Class VI	Caries affecting cusp tips of molars, premolars, and cuspids.

Figure 1: G. V. Black's caries classification



(Source from GV-BLACK.jpg)

♦ Graham J (1997) proposed a simple digital system for classification of caries, which can be more compatible with use of computers for housekeeping as well allow the dentist to define the complexity and extend of a cavity. At the same time, encourages a conservative approach to preserve the natural tooth surface in the presence of adhesive restorative materials lesions (Graham J, 1997).

Table 2: Classification of caries lesions

SIZE / SITE	No Cavity (0)	Minimal (1)	Moderate (2)	Enlarged (3)	Extensive (4)
Pit/fissure (1)	1.0	1.1	1.2	1.3	1.4
Contact area (2)	2.0	2.1	2.2	2.3	2.4
Cervical (3)	3.0	3.1	3.2	3.3	3.4

(Modified from Graham J (1997))

♦ International Caries Detection and Assessment System (ICDAS) caries codes: ICDAS presents a new paradigm for the measurement of dental caries that was developed based upon the insights gained from a systematic review of the literature on clinical caries detection system (Ismail et al., 2007). For classification of caries, this system has been recently practicing.

♦ World Health Organization (WHO): The below table shows the WHO recommended coding for the dentition status (WorldHealthOrganization).

Table 3: WHO code for dentition status

Code			Condition/status
Primary teeth	Permanent teeth		
Crown	Crown	Root	
A	0	0	Sound
B	1	1	Caries
C	2	2	Filled, with caries
D	3	3	Filled, no caries
E	4	-	Missing due to caries
-	5	-	Missing for any other reason
F	6	-	Fissure sealant
G	7	7	Fixed dental prosthesis abutment, special crown or veneer/implant
-	8	8	Unerupted teeth (crown)/unexposed root
-	9	9	Not recorded

♦ Decayed Missing Filled Index (DMF Index) for permanent dentition: Introduced in 1938 by Henry Klein, Carrole E Palmer and Knutson JW. Later in 1986, WHO modified the DMF Index, they suggested to include all the third molars, temporary restorations and carious cavities are to be considered as 'D', decayed. 'def' index was developed by Gruebbel AO in the year 1944 for deciduous dentition, the basic principle and rules are the same as that of DMF index (Rao, 2012).

Comparison of the visual examination of WHO and ICDAS criteria used in clinical evaluation is shown in Figure 2.

Figure 2: Criteria used in clinical evaluation

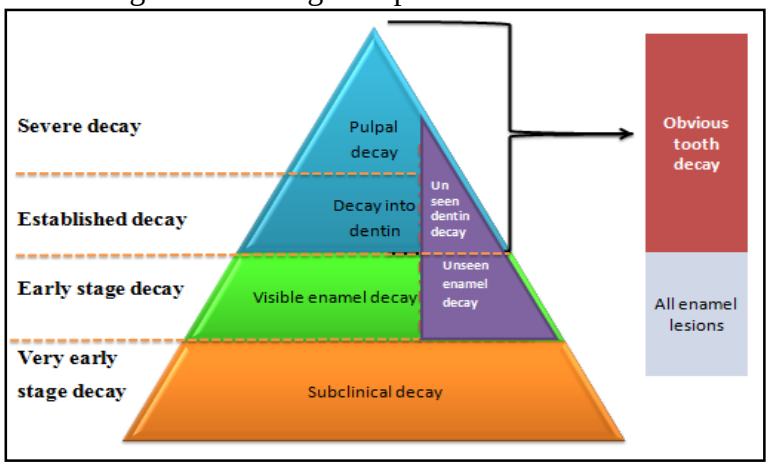
ICDAS criteria	WHO criteria	
Code 0	D0	Sound tooth surface
Code 1	D1	Non-cavitary enamel lesion
Code 2		
Code 3	D2	Cavitary enamel lesion
Code 4	D3	Cavitary dentinal lesion
Code 5		
Code 6		Cavitary lesion with pulp complication

{Modified from Simona Stoleriu (2012)}

The below Figure 3 describes the iceberg metaphor of caries which identifies the different stages of caries scored at various diagnostic thresholds. Initially

the caries are subclinical level like white spot lesion that is difficult to diagnose and extends in such a way it is visible, most of the enamel lesions are visible though there are unseen enamel decay. As the decay is progressive, the enamel lesions reach the dentin and pulpal layer where most of the decays are obviously seen and need treatment. It also explains the four stages of decay - very early, early, established and severe decay (Selwitz et al., 2007).

Figure 3: Iceberg metaphor for dental caries



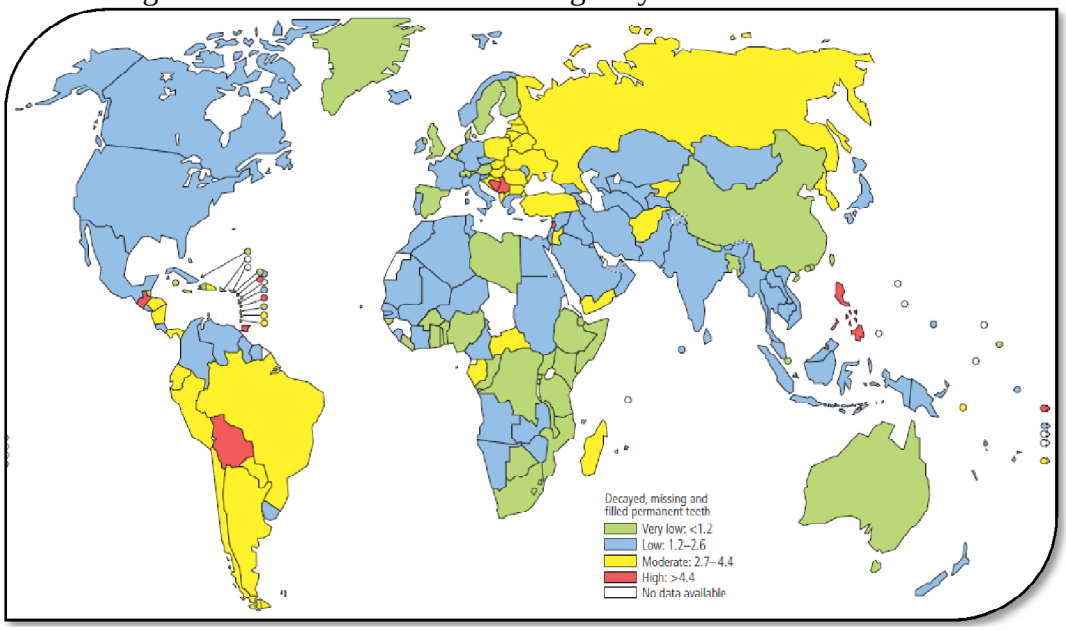
Modified from (Selwitz et al., 2007)

1.2.5. Epidemiology

Dental caries is one of the most common chronic diseases affecting millions of people globally, In most industrialized countries it affects 60-90% of school aged children and also vast majority of adults thereby it still remains a major health problem (Petersen et al., 2005). Regardless of the remarkable reduction in caries among western world, dental caries still remains a major problem among pre-school populations as it affects significant percentage of pre-schoolers in both developing and developed countries (Vadiakas, 2008). ECC is seen more among families with less education as well who have limited access to dental care facilities (Gomez, 2013). Comparisons of global frequency and distribution of caries are complicated by their diagnostic criteria

that differs from study to study (Selwitz et al., 2007). At present, the severity and distribution of caries varies in different parts of world, it also varies within the same country or region. Figure 4 describes the worldwide data of DMFT index among 12-year-old children (Petersen et al., 2005).

Figure 4: Dental caries levels among 12-year-olds worldwide

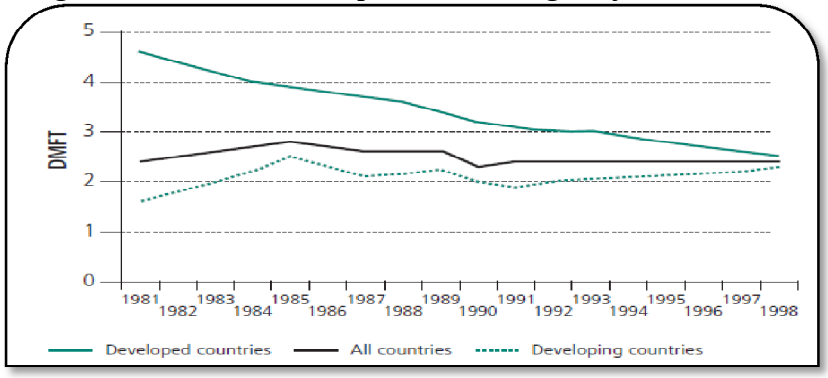


(Source from Petersen et al. (2005).

The time trends and changing levels in dental caries experience of 12-year-old children of all countries and also both the developing and developed countries are illustrated in the below Figure 5. Until recent years, the levels of dental caries were low in most developing countries. Recently, the prevalence rates and experience of dental caries are tending to increase which is largely due to inadequate exposure to fluorides and increase in consumption of sugars (Petersen et al., 2005). In contrast, over the past 20 years in most industrialized countries a decline in caries has been observed which could be the result of public health measures, effective use of fluorides along with change in living conditions, lifestyles and improved self-care practices.

However, it must be emphasized that dental caries as a disease of children has not been eradicated, but only controlled to a certain degree.

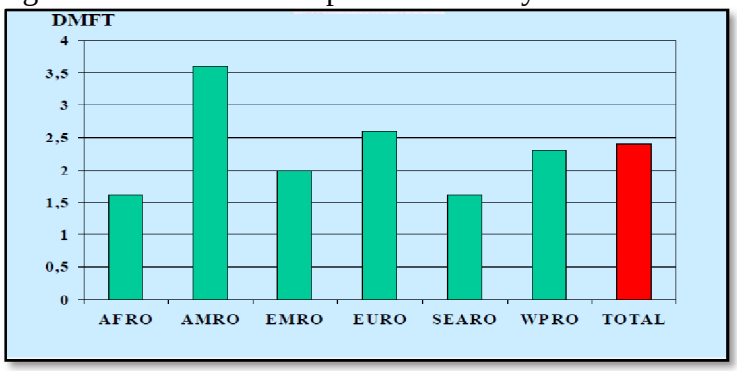
Figure 5: Dental caries experience among 12-yr old children



(Source from Petersen et al. (2005).

In most industrialized countries, dental caries is still a major oral health problem, affecting 60 - 90% of schoolchildren. Figure 6 highlights the dental caries experience among 12-year-old children in the six WHO regions (AFRO = African Region; AMRO = Region of the Americas; EMRO = Eastern Mediterranean Region; EURO = European Region; SEARO = South-East Asia Region; WPRO = Western Pacific Region) in the year 2000, based on the DMFT Index. Currently, the disease level is low in Africa but relatively high in America. However, it is expected that the incidence of dental caries will increase in many developing countries, due to increased consumption of sugars and inadequate exposure to fluorides (Health-Report, 2003).

Figure 6: Dental caries experience of 12-year old children.



(Source from Health-Report (2003).

The prevalence rate of ECC in England (3yr old), Finland (3yr old), United States (18-36M old), Indonesia (<5yr old), Western China (2yr old), Hong Kong (5yr old) and Taiwan (<6yr old) were reported to be 4%, 6%, 20.2%, 48%, 20.2%, 31.5% (Milnes, 1996) and 56% respectively (Tsai et al., 2006). Table 4 shows the experience of caries among 5 yr olds, reported by European national surveys (Vadiakas, 2008). The prevalence of ECC among children aged 1-2 years has shown to be 32.19% with mean dmft of 2.01 (Kumarihamy et al., 2011).

Table 4: Dental caries among 5-yr old reported in European national surveys

Country	Prevalence (%)	Mean dmft
England & Wales [Pitts et al., 2005]	39.6	1.55
Scotland [Pitts et al., 2005]	55.4	2.76
Norway [Haugejiorden et al., 2002]	38.9	1.50
Denmark [Poulsen et al., 2002]	29.0	1.00
Greece [Oulis et al., 2005]	42.8	1.77

(Source from Vadiakas (2008)

In Singapore, 40% of pre-schoolers have ECC (49% for 5-6 yrs, 37% for 4-5 yrs and 26% for 3-4 yrs) (Gao et al., 2009) and in a 1 year follow up study the incidence rate of 44 % have been observed among pre-schoolers, with higher caries rate among boys, low economic status and Malay community. Herewith, they conclude caries is a severe oral health problem among preschoolers in Singapore (Gao et al., 2010a). Data / news published in 2012 by Sing health indicate that four in ten children aged 3-6 yrs have caries lesions and by 5 yrs of age, one in five children would have suffered from dental caries (Kiat, 2012). A recent study conducted among Singapore children aged 36.3±6.9 months indicates that caries active lesions was present

among 48.4 % of children and high plaque scores was also evident, though the country has 100% civilized with water fluoridation (Hong et al., 2013).

At present the severity and distribution of caries varies in different parts of world and within the same country or region. The prevalence and severity of ECC in children across many countries are shown below (Mohebbi, 2008).

Table 5: Prevalence and Severity of ECC among Europe, North and South America.

Author	Year of report	Country	Age group	% with dmft > 0	dmft
Davies et al.	2001	England	36-49 mos	32	1.4
Hinds & Gregory	1995		18-30 mos	4	
			30-42 mos	14	
WHO [a]	2005	Sweden	3 yr	5	
Grindefjord et al.	1995		24-36 mos	11	
Hallonsten et al.	1995		18 mos	2	
Schroder et al.	1994		12-23 mos	1	
Wendt et al.	1991		12-14 mos	1	
			23-26 mos	8	
Nordblad et al.	2004	Finland	3 yr	16	0.3
WHO [a]	1997		3 yr	0	
Alaluusua & Malmivirta	1994		19 mos	8	
			36 mos	14	
Paunio et al.	1993		3 yr	6	
WHO [a]	1994	Italy	3 yr	1	0.3
Szatko et al.	2004	Poland	3 yr	56	2.9
Lopez Del Valle et al.	1998	Island	13-18 mos	11	
			19-24 mos	21	
			25-36 mos	27	
			37-48 mos	50	
Douglass et al.	2001	US	13-24 mos	4-13	
			25-36 mos	17-25	
			34-36 mos	25	
Tang et al.	1997		1 yr	6	
			2 yr	22	
			3 yr	35	
O' Sullivan et al.	1994	Native American	0-3 yrs	44-90	
Peressini et al.	2004	Canada, Manitoulin	3 yr	67	3.5
Rosenblatt & Zarzar	2002	Brazil	12-18 mos	9	0.4
			19-24 mos	28	1.5
			25-36 mos	46	2.0

World Health Organization (WHO). Available at http://www.whocollab.od.mah.se/euro/(2007a)

Source from Mohebbi (2008).

Table 6: Prevalence and severity of ECC among Asia and Africa

Author	Year of report	Country	Age group	% with dmft > 0	dmft
King et al.	2003	China	0-4 yr	18	
Du et al.	2000		3 yr	36	
Douglass et al.	1995		3 yr	67	3.5
Mayanagi et al.	1995	Japan	2 yr	43	1.8
			3 yr	72	4.0
Tsubouchi et al.	1994		12-23 mos	13	
Fujiwara et al.	1991		1-1.5 yr	2	0.1
			1.5-2 yr	5	0.5
			2-2.5 yr	26	3.2
			2.5-3 yr	37	6.3
Tsai et al.	2006	Taiwan	1 yr	0	0
			2 yr	5	0.1
			3 yr	66	2.6
Vachirarojpisan et al.	2004	Thailand	11-14 mos	11	0.3
			15-19 mos	41	1.3
Carino et al.	2003	Philippines	3 yr	85	7.4
Jose & King	2003	India	8-48 mos	44	1.8
Rajab & Hamdan	2002	Jordan	1 yr	8	0.2
			2 yr	21	1.2
			3 yr	22	1.7
Hattab et al.	1999		12-23 mos	13	
			24-35 mos	21	
			36-47 mos	34	
Al-Hosani & Rugg-Gunn	1998	United Arab Emirates	2 yr	36-47	1.7-3.2
Al-Malik et al.	2001	Saudi Arabia	3 yr	61	3.6
Masiga & Holt	1993	Nairobi	3 yr	38	1.4
Kiwanuka et al.	2004	Uganda	3 yr	45	1.7
Roberts et al.	1993	South Africa	12-48 mos	37	

Source from Mohebbi (2008).

1.2.6. Etiology

In general, dental caries resembles other diseases that are produced by bacteria but actually, it differs from them. In this the relative organisms live outside the body (in the mouth) that they need not invade tissues but passively grow into cavities produced by their own products as well they cause damage by acids produced by themselves as a metabolic product and not by their exotoxins, endotoxins or allergic phenomenon (Robinson, 1952). Dental caries

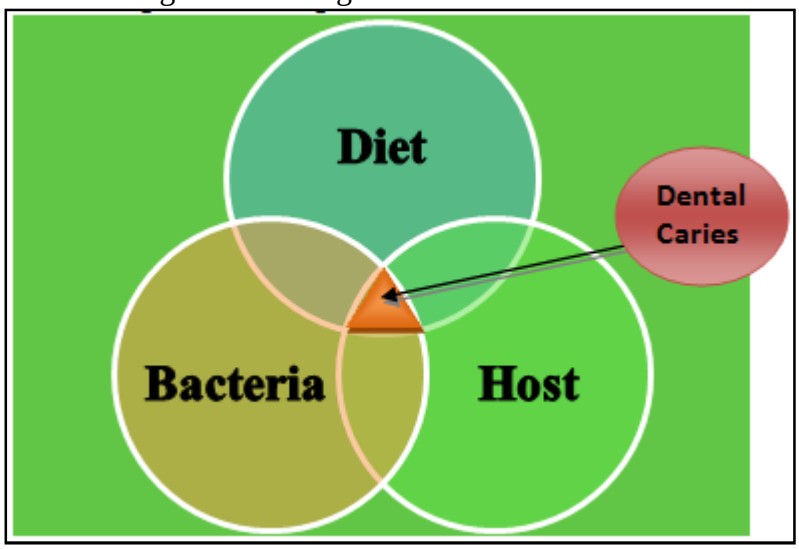
is a common chronic infectious transmissible disease resulting from tooth-adherent specific bacteria, primarily *mutans streptococci (MS)* that metabolize sugars to produce acid which over time demineralizes tooth structure (Loesche, 1986).

Initially many theories were proposed to understand the etiology of dental caries like Worm theory (Black, 1972; Gerabek, 1999), Humour theory (Stelmack and Stalikas, 1991), Chemical theory (Featherstone et al., 1979; Parmly., 1819; Van Dijk et al., 1979), later in 1835 Robertson proposed the chemical theory (Guttorm Toverud, 1952), Parasitic or septic theory by Erdl 1843 (Guttorm Toverud, 1952; Searle, 1884). The recent theories are Acidogenic theory proposed by W.D.Miller in year 1980 (Ring, 2002), *Proteolysis theory* by Gottlieb in 1944 (Gottlieb, 1944), Proteolysis - chelation theory by Schatz and Martin in 1955 (Schatz et al., 1972). Acidogenic theory or Miller's Chemico-parasitic theory says that dental decay is a chemico-parasitic process consisting of two stages. Firstly, the decalcification of enamel results in total destruction and decalcification of dentin and followed by dissolution of enamel which signifies its total destruction. According to Millers' theory along with carbohydrates, micro-organism and tooth structure, saliva (acidic pH) also plays a major role (Miller, 1883).

Few more theories have been proposed like Sucrose chelation (Phosphorylating) theory by Lura in 1967 (Lura, 1961), Autoimmunity theory by Burch and Jackson in 1970 (Jackson, 1968; Jackson and Burch, 1970) and Sulfatase theory by Pincus et al in 1950 (Srivastava, 2011) however they have

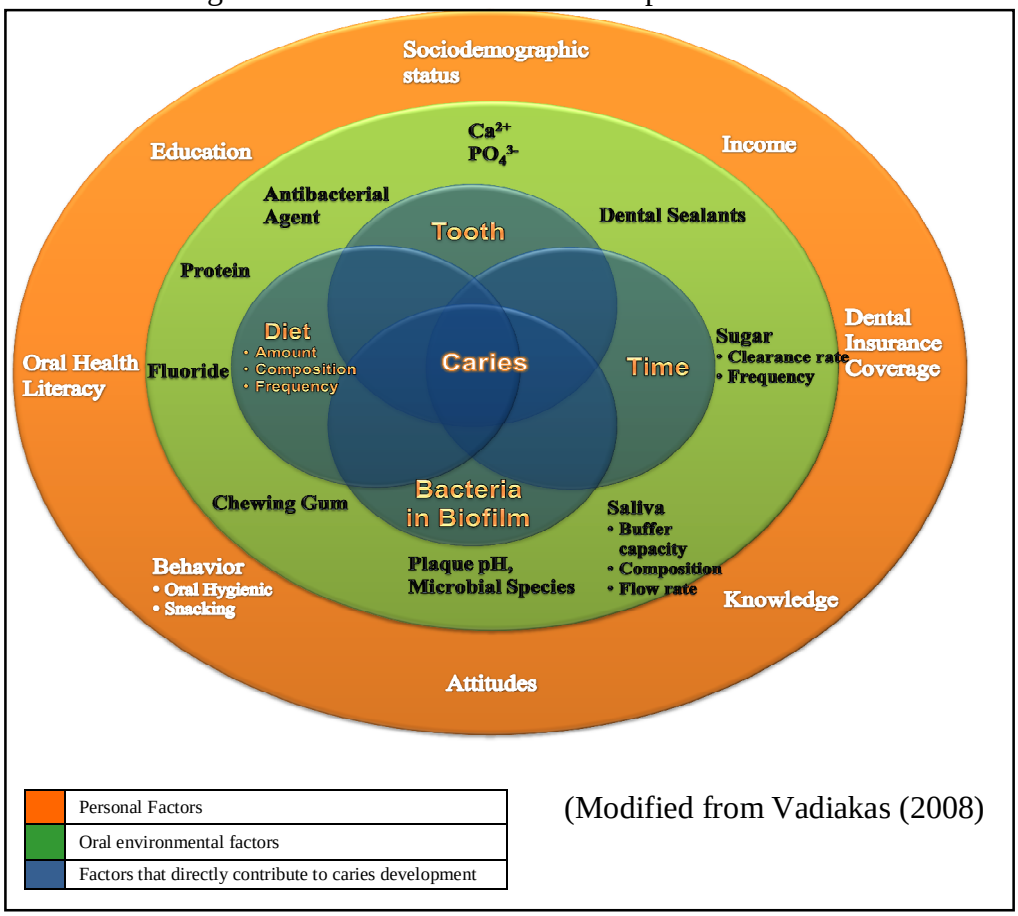
been discarded as the researchers failed to confirm these theories. In 1960's heat has been proposed as cause for dental decay (Taylor, 1967). During the 20th century, many researchers and dentists recognized that dental caries is a product of interplay of many factors. Dr. Keyes in 1954 redefined dental caries (Keyes, 1954) and explained the role of local cariogenic bacteria in plaque, fermentable carbohydrates, constitutional factors related to those species / strains and the tooth structure. Thereby the research work / effort of Dr. Keyes and Fitzgerald proved that caries is an infectious process of teeth (Keyes, 1960; Keyes P and RJ, 1961) thereby leading to definition of *Dental Caries* as a "MULTIFACTORIAL DISEASE". This proposed concept has been valid till today though we understand more about the biological determinants and interactions among different factors (Amid I. Ismail, 2001), thereby caries results from an ecological imbalance between the tooth minerals and oral microbial biofilms in the physiological equilibrium (Fejerskov, 2004) and various etiological factors must be simultaneously present to initiate as well progress the disease. The most important factors are cariogenic microorganisms, fermentable carbohydrates (substrate), susceptible tooth surface or host (Vadiakas, 2008). In the biological process of caries development especially in young children factors like newly established bacterial flora, low resistance of the newly erupted teeth, immaturity of the host defense system along with extremes in the dietary substrate component are responsible in accelerating this process (Vadiakas, 2008). Figure 7 shows the Keyes triad, it explains the result of interaction between oral microbes (acidogenic bacteria colonizing the tooth surface), diet (primarily sucrose) and host factors, including saliva buffering capacity (Keyes, 1962).

Figure 7: Etiological triad of dental caries



(Modified from Keyes (1962)

Figure 8: Factors involved in development of caries

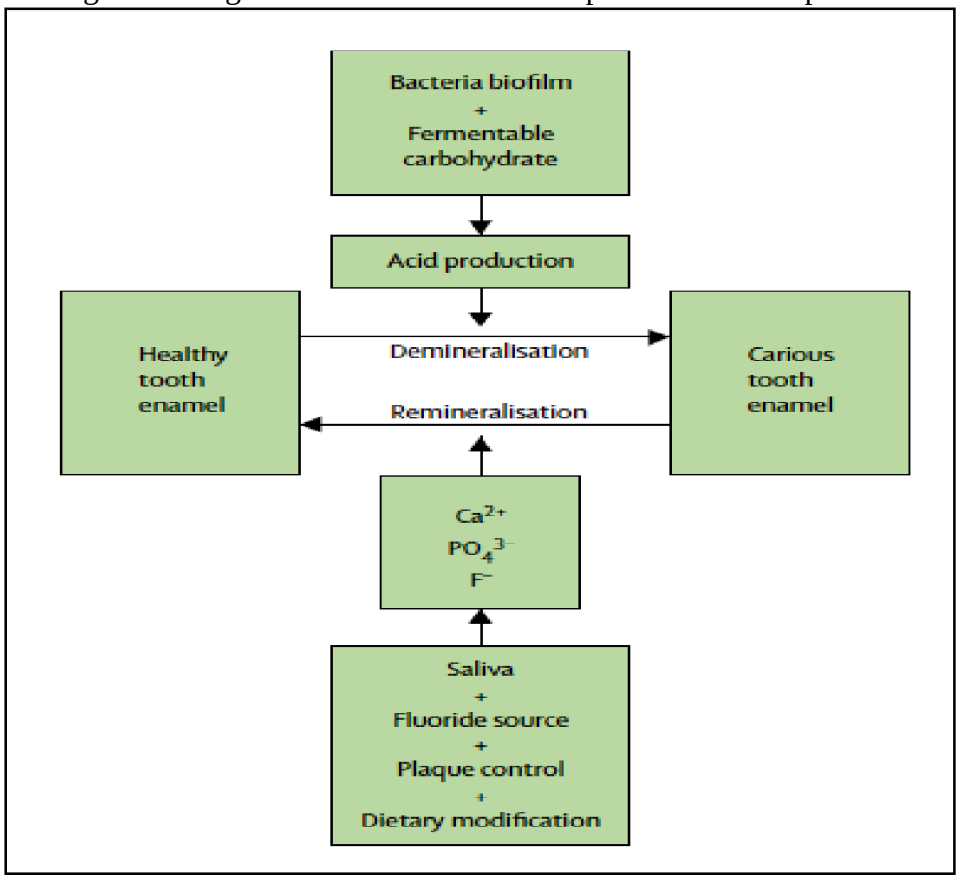


(Modified from Vadiakas (2008)

Figure 8 demonstrate the newly proposed concept of factors responsible for development of caries lesion. They indicate that four factors - tooth, diet, biofilm and time directly contribute to the development of caries. Oral

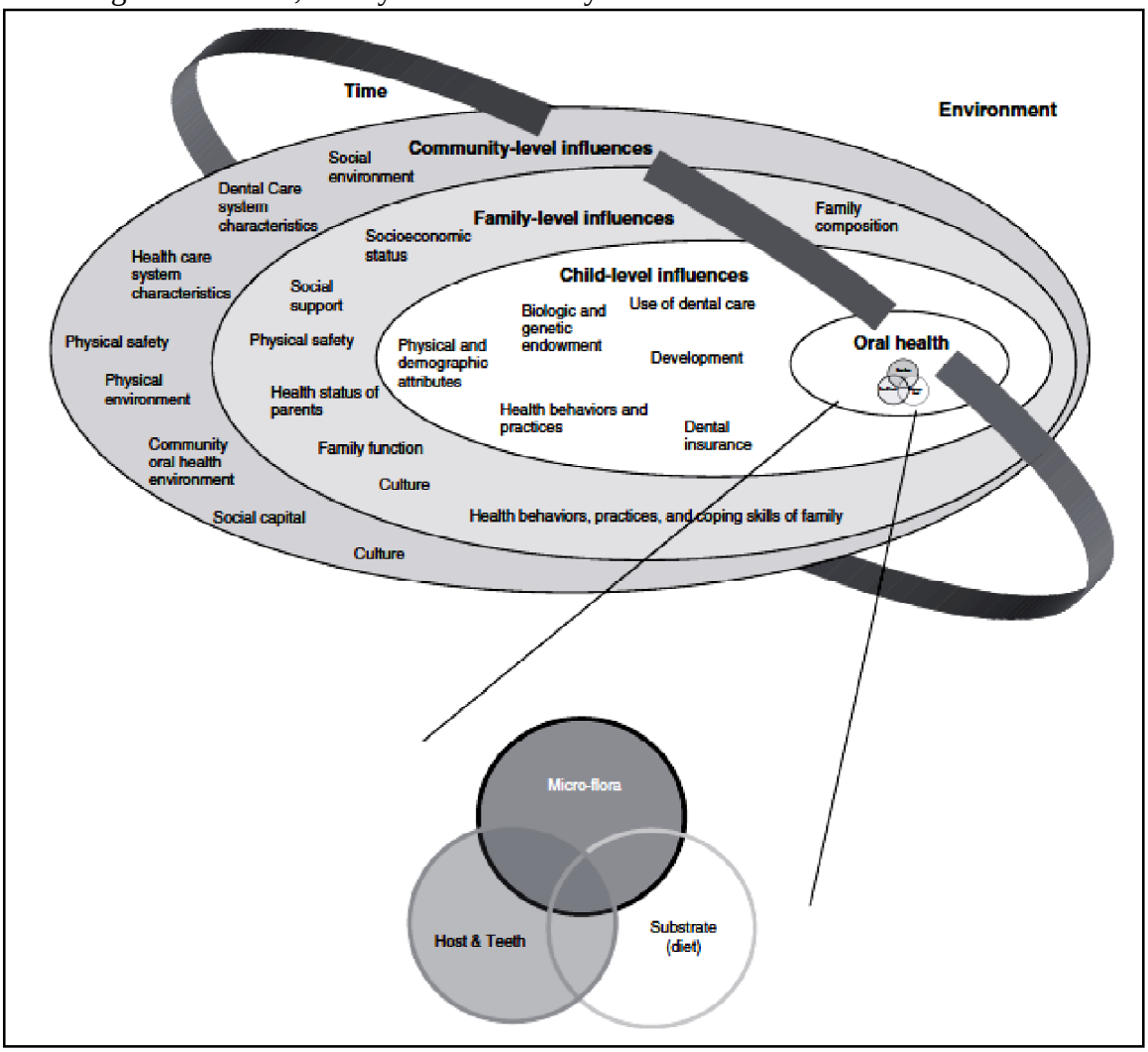
environmental factors like saliva, fluoride, frequency of consumption of sugars and some personal factors like education, income, cost, attitude, oral hygiene are considered as the essential factors in development of caries (Selwitz et al., 2007). The healthy teeth after its eruption in oral cavity are subjected to various changes like demineralization and remineralisation by acids and minerals respectively. The below Figure 9 explains the regular flux of demineralization and remineralization process in development of caries (Kidd EAM, 1988; Selwitz et al., 2007). 22 oral health domains across three levels - the community, family and child level including time (Figure 10) have been identified that has effect on oral health outcomes of children (Bramlett et al., 2010).

Figure 9: Regular flux of mineralization process in caries process



Source from (Selwitz et al., 2007)

Figure 10: Child, family and community influences on oral health



(Source from Bramlett et al. (2010)

1.2.6.1. Microorganisms

Many studies have shown the presence of *S. mutans* in both saliva and plaque (Karjalainen et al., 2004; Lindquist and Emilson, 2004; Okada et al., 2010; Roeters et al., 1995). *S. mutans* and *Lactobacilli (LB)* have shown to be strongly associated with early caries lesions and cavitated caries lesions respectively (Parisotto et al., 2010). The initial acquisition of *MS* is seen during the median age of 26 months (19-21 months) during the first window of infectivity period (Caufield et al., 1993; Law et al., 2007; Lindquist and

Emilson, 2004). Microorganism for development of caries can be transmitted either vertically (Berkowitz, 2006; Caufield et al., 1993; Emanuelsson et al., 1998; Law et al., 2007; Lindquist and Emilson, 2004; Ng and Chase, 2013; Shulman et al., 2001; Straetemans et al., 1998; Vadiakas, 2008) or horizontally (Berkowitz, 2006; Emanuelsson et al., 1998; Kohler et al., 2003; Vadiakas, 2008).

Most recent studies using sophisticated genetic methods that involve the sequencing of amplified 16rRNA genes have identifies numerous taxi of bacteria from sound and carious tooth sites. Some of these types have not been identified previously, at present their role is to be investigated (Crielaard et al., 2011) and suggest apart from *S. mutans* and *LB* other bacteria like *S. salivarius, S. wiggsiae* & *V. atypica* are also involved in caries process like developing white spot lesions (Torlakovic et al., 2012). *S. mutans, S. sobrinus* and *Bifidobacteria* have shown to be significantly associated with SECC as well development of new lesions (Palmer et al., 2010). Becker et al., 2002 suggest *Actinomyces* species and specifically *A. gerencseriae* were associated with caries initiation, while Van Houte et al., 1996 indicates *Bifidobacterium* species was associated with deep lesions. A recent article conveys that *C. albicans* genotypic subgroup A is the dominant strain and important component of dental biofilm children associated with SECC (Yang et al., 2012). Pyrosequencing analysis based on 16S rRNA gene V1-V3 hypervariable regions have shown that three genera, *Streptococcus, Granulicatella,* and *Actinomyces* were increased significantly in children with SECC (Jiang et al., 2013). In infants low threshold of bacteria (*MS* and *LB*) are sufficient for onset and progression of ECC. As well, in infants and toddlers

significant association between caries and bacterial levels are noticed (Ramos-Gomez et al., 2003). The microbiology of the oral cavity is itself an ecological system of great complexity, with interactions creating properties beyond what can be assumed from the sum of individual microbial characteristics (Eriksen and Dimitrov, 2003).

1.2.6.2. Teeth

Tooth is a host factor. Infants do not harbor organisms until sometime after teeth emerge into oral cavity as microorganisms require hard surface for colonization (Caufield et al., 1993). Window of Infectivity is the critical time for the initial acquisition of *S.mutans* in children between age group of 19-31months (Lindquist and Emilson, 2004). Several windows of infectivity may exist, if colonization of *S.mutans* occurs along with tooth eruption of primary incisors, primary molars and permanent first molars (Law et al., 2007). Second window of Infectivity has been postulated during the eruption of first permanent molars or permanent teeth (Carlen et al., 1996; Caufield et al., 1993; Lindquist and Emilson, 2004). It is the period where the risk of acquisition of *S.mutans* will be apparently high (Alves et al., 2009). Caufield et al., 1993 shows that children who are negative for *MS* at younger age may become positive at later age, due to increase in tooth surface for colonization of bacteria.

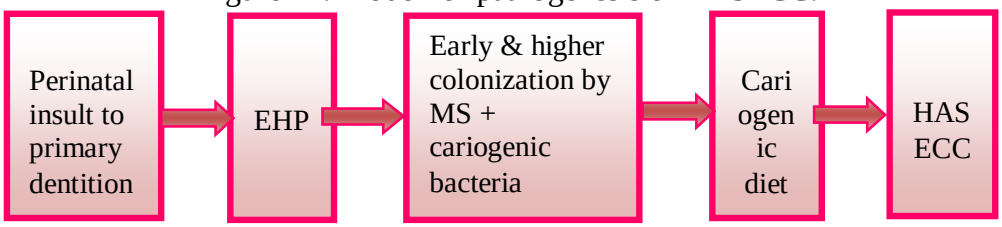
Developmental defects of teeth like enamel hypoplasia (EHP) has shown to have strong association with dental caries in primary dentition (Caufield et al., 2012). In 1992, EHP was identified by Federation Dentaire Internationale Commission on Oral Health and it is defined as a quantitative disturbance of

mineralized tissue formation during tooth development (Caufield et al., 2012). The children with enamel hypoplasia are more likely to predispose for initiation and progression of caries and in a community they are predictor of high caries susceptibility (Li et al., 1996). Enamel hypomineralisation is a qualitative defect of the enamel due to disturbance during initial calcification or maturation (Weerheijm et al., 2003). Disturbance during late matrix formation and early maturation results in hypoplasia and hypomineralization respectively (Fearne et al., 1994). Clinically, hypomineralisation can be seen as opacity i.e., abnormality in the translucency of the enamel (Weerheijm et al., 2001). Many studies have shown that EHP has been associated with SECC and designated as Hypoplasia associated severe early childhood caries (HASECC) (Caufield et al., 2012). Compared to normal enamel, a higher carbon content and lower calcium and phosphorus concentrations in the affected enamel can be seen (Jalevik et al., 2001). Studies have shown that newly erupted teeth due to their low resistance are prone to caries (Vadiakas, 2008). In primary dentition, hypomineralisation are seen in molars and incisors, deciduous molar hypomineralisation (DMH) are defined as idiopathic hypomineralisation of 1 - 4 second primary molars (Elfrink et al., 2008).

Figure 11 shows the model of sequence of events which leads to HASECC, indicating that the perinatal teeth are adversely affected by various insults to embryonic cells responsible for formation of enamel and dentin. These insults are compromised mainly of covariates possibly associated with dietary deficiencies, poverty, low birth weight, pre or post natal infections, these impacts collectively results clinically as EHP, when these teeth are exposed to

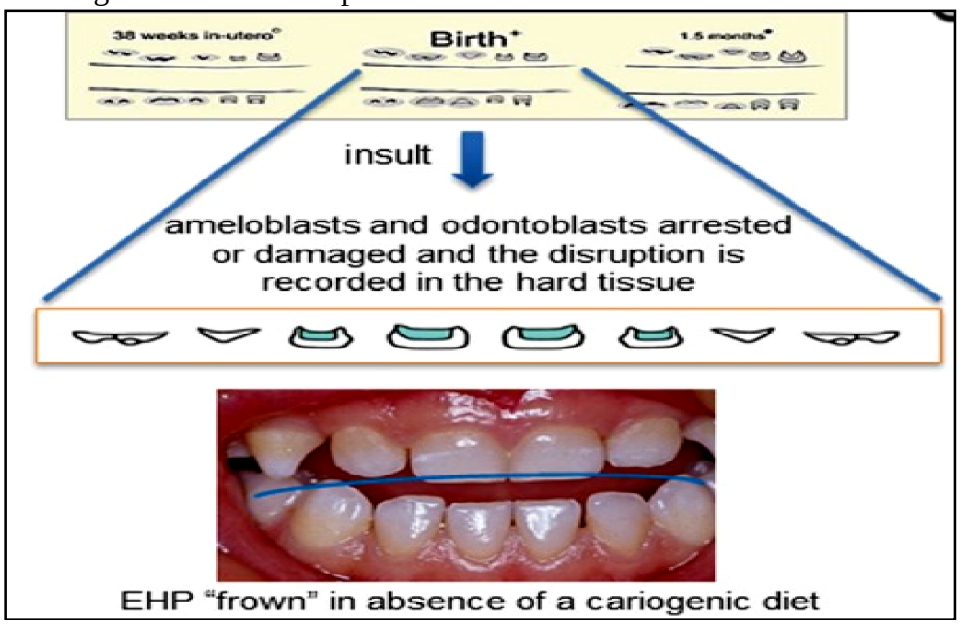
early colonization with cariogenic diet promoting HASECC (Caufield et al., 2012).

Figure 11: Model for pathogenesis of HASECC.



(Modified from Caufield et al. (2012)

Figure 12: Clinical representation of EHP occurred around birth



(Source from Caufield et al. (2012)

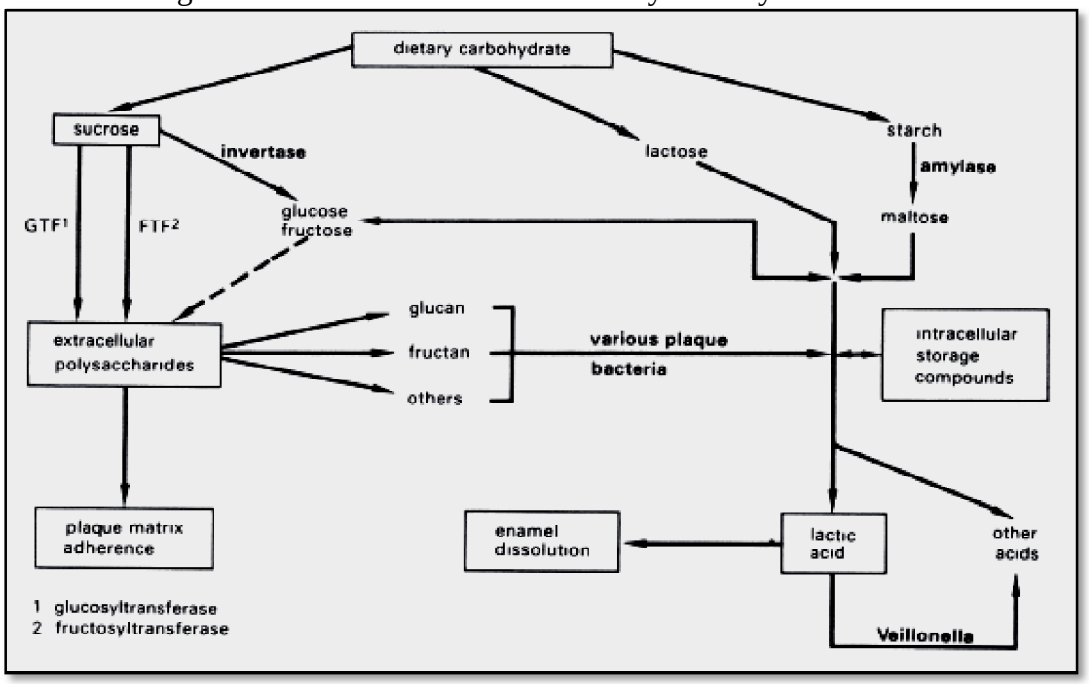
Significantly, higher levels of *MS* are shown among children with enamel hypoplasia. Severe enamel hypoplasia are associated with higher levels of *MS* (Li et al., 1994) which is mainly due to retentive surface defects associated with EHP (Caufield et al., 2012). Figure 12 shows that EHP occurred at birth, with the downward curve or "frown" coincidental to the stage of individual tooth development at time of insult. Collectively, this suggests that children with HASECC are prone to early colonization by *MS*, due to retentive surface defects associated with EHP (Caufield et al., 2012).

1.2.6.3. Diet

Dietary factors contribute to the severity of caries (Ramos-Gomez et al., 2003). The inter-relationship of carbohydrates in causing dental caries is complex (Shaw, 1983). All fermentable carbohydrates are capable of producing acids in which monosaccharide's and disaccharides produce the most (Sheiham, 1983). Significant evidence suggests, for initiation of caries development dietary sugars are required, both sugar-sweetened beverages and added sugars in food have been implicated in the development of caries and showed that they play an important role in SECC (Evans et al., 2013). Sugar has been the principal cause for caries; brown sugars are cariogenic as white. The main sugars implemented in caries, in decreasing order of cariogenicity, are sucrose, glucose and fructose (Sheiham, 1983).

Due to the relationship between dietary sugars, low pH, and dental caries, more attention has been paid to the metabolism of carbohydrates. Figure 13 shows the simplified diagram of metabolic fate of dietary carbohydrates. Sucrose can be down into glucose and fructose molecules by extracellular bacterial 'invertase', which can be directly taken up by bacteria. It can also be utilized extracellularly by glycosyltransferases that produces both soluble and insoluble glucans. They are important in plaque formation and consolidation of bacterial attachment to the teeth. Starches, which contain mixtures of amylase and amylopectin, can be broken into their constituent sugars by amylases of salivary and bacterial origin. Some *Streptococci (S. gordon ii, S. mitis)* are able to bind salivary amylase, which might provide additional metabolic capability (Marsh and Martin, 2009).

Figure 13: The metabolic fate of dietary carbohydrates

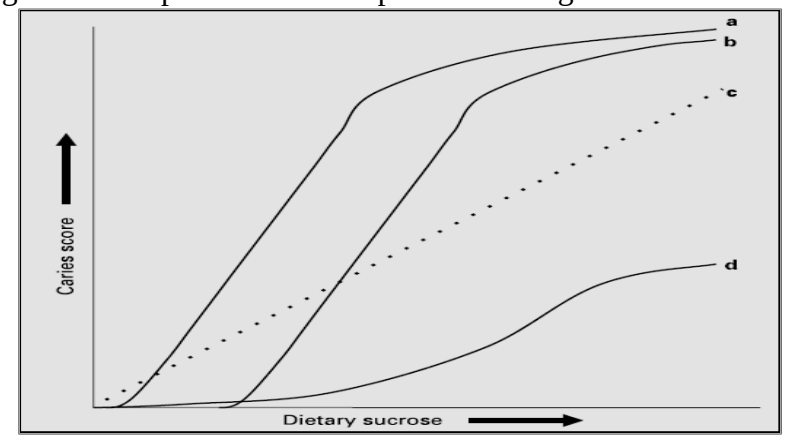


(Source from Marsh and Martin (2009)

The classic evidence supporting the role of sugars in dental caries can be seen from Vipeholm Study (Gustafsson et al., 1954), Turku Sugar Study, World War II Food Rationing, Hopewood House Study (Harris, 1967), Tristan da Cunha, Hereditary Fructose Intolerance, Experimental Caries in Man, and Stephan plaque pH response as reviewed by Zero (2004).

Sucrose are more cariogenic because they can be rapidly converted into acids by *S. mutans* and also to extracellular polysaccharides like glucan which enhances the adherence of bacteria to teeth (Sheiham, 1983) herewith they are considered as the arch criminal of dental caries (Newbrun, 1969). Increase in dosage, frequency (Parisotto et al., 2010) and sticky form of sugars exposure (Gustafsson et al., 1954) plays an important role in development of ECC.

Figure 14: Proposed relationships between sugar intake and caries



(Source from **Newbrun (1982)**

In 1982, Newbrun. E proposed the concept of 'S' shaped curve to better explain the relationship between sucrose and dental caries as well hypothesized that in pre- fluoride and post-fluoride era, the 'S' shape curve can be moved to right (Fig. 14a & 14b respectively) (Newbrun, 1982). Based on the analysis of sugar consumption in 90 countries, Woodward and Walker (1994) have showed that the relationship is linear (Fig. 14c) and among individuals with regular exposure of fluoride and good oral hygiene the author proposes that higher levels of sugar consumption may be tolerated (Fig. 14d). Studies of the dynamics of sucrose metabolism by investigations of experimental caries in animals, cariogenic organisms and clinical observations of the inter-relationship of dietary sucrose intake and caries experience all provide compelling facts that the proportion of sucrose in a food is one important determinant of its cariogenicity (Newbrun, 1982).

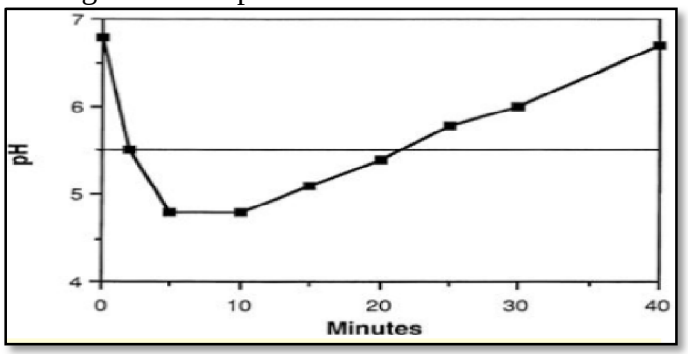
1.2.6.4. Saliva

Human saliva is an important oral environmental factor in the role of caries. It is not only lubricating the oral mucosal surface along with oral

functions like speaking, eating and swallowing but also protects teeth and oral tissue (Lenander-Lumikari and Loimaranta, 2000). The flow and composition of saliva is an important factor in maintaining the pH of saliva. Buffering capacity of both stimulated and un stimulated saliva involves three major buffer systems: the bicarbonate, the phosphate and protein buffer systems (Lenander-Lumikari and Loimaranta, 2000).

Reduced flow of saliva directly affects its pH resulting in a critical pH of 5.5 (Stephan, 1944). Figure 15 demonstrate the Stephan curve immediately after sucrose rinse and the importance of saliva in development of caries(Stephan and Miller, 1943).

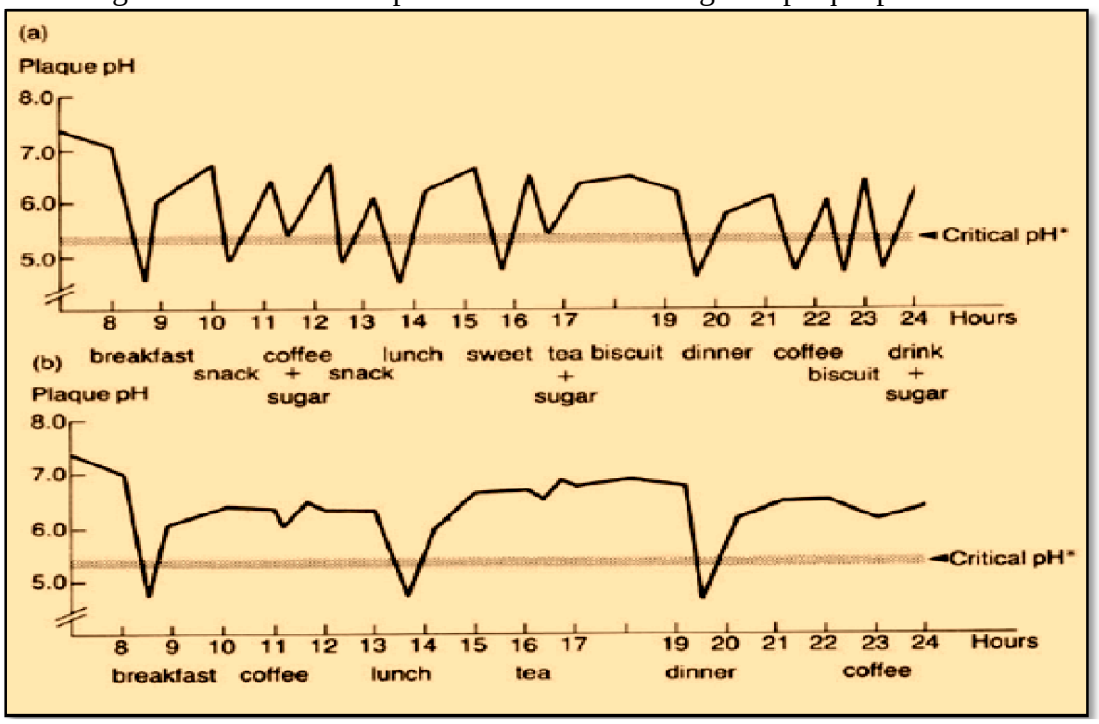
Figure 15: Stephan Curve after sucrose rinse



(Source from (Stephan and Miller, 1943)

Stephan curve demonstrates that time is also an important factor in remineralization of demineralized tooth surface. Remineralization of tooth surface does not occur due to continuous or repeated intake of carbohydrates, demonstrating that frequency of sucrose exposure, time is more important than quantity of sugar intake in caries formation. Figure 16 demonstrates the changes in plaque pH in an individual who (a) has frequent intakes of fermentable carbohydrate during the day (b) limits their carbohydrate intake to main meals only (Marsh and Martin, 2009).

Figure 16: Schematic representation of the changes in plaque pH



(Source from Marsh and Martin (2009)

Hypo salivation or Xerostomia, an uncomfortable condition may occur with the use of medications, as a complication of connective tissue and autoimmune diseases, with radiation therapy to head and neck, or with a number of other conditions like diabetes, renal dialysis. Among xerostomic patients, dental caries is one of the major complication which needs attention by a dentist (Guggenheimer, 2003).

1.2.7. Consequences

Globally, dental caries is still a major public health problem as poor oral health has a profound effect on general health and quality of life (Petersen et al., 2005). Among the global prevalence of the most common sequelae, dental caries of permanent teeth (35.29% of population) has shown to be the first common sequelae followed by tension-type headache (20.77%), migraine

(14.7%), fungal skin diseases (14.3%) and caries of primary teeth (9.02 %) is in 10th place compared to asthma (4.85%), chronic obstructive pulmonary disease (4.77%), osteoarthritis (3.64%), eczema (3.33%), and diabetes mellitus (3.3%) which are more commonly seen among community (Vos et al., 2012).

Though ECC is a preventable disease its prevalence has been increasing (Ng and Chase, 2013). Their impact on individuals and communities because of pain, impairment of function and reduced quality of life, is considerable. Its consequences are mainly higher risk of new carious lesions in both primary and permanent dentition (al-Shalan et al., 1997; O'Sullivan and Tinanoff, 1996), hospitalizations and emergency room visits (al-Shalan et al., 1997; Ladrillo et al., 2006), high treatment cost (Berkowitz, 2003; Kanellis et al., 2000), risk for delayed physical growth and development (Acs et al., 1992; Ayhan et al., 1996), loss of school days and increased days with restricted activity (Gift et al., 1992; Reisine, 1985), diminished ability to learn (Blumenshine et al., 2008), as well along with school performance. Sleeping problems are also encountered by the children affected by caries (Smith and Riedford, 2013), malnutrition and overall weight / nutrition of child may be affected (Duijster et al., 2013). Normal growth & development as well social adaptation of preschoolers are affected (Vadiakas, 2008), malocclusion, poor speech pronunciation (Ramos-Gomez et al., 2003), reduced oral health - related quality of life (OHRQOL) are seen (Filstrup et al., 2003).

Preschoolers with SECC has shown to have disturbed nutritional status like iron deficiency anemia which may be caused by aversions to eating because of tooth ache (Tang et al., 2013), as well they are physically underdeveloped (Acs et al., 1999). Its serious consequences include pain, infection, chewing

difficulty, malnutrition, gastrointestinal disorders, and low self-esteem (Ramos-Gomez et al., 2003) and another article has shown that due to difficulties in chewing the patient went on only fruits resulting in thiamine deficiency - Beriberi (Probert, 1989).

A study mentions that the growth, body weight and quality of life may be associated by three possible mechanisms: firstly, untreated caries and their infection may cause discomfort and reduce intake of foods due to pain. Secondly, disturbed sleep may affect the gluco-corticosteroid production and growth and finally the growth may be affected due to chronic infection or inflammation associated with pulpitis affecting the metabolic pathways where cytokines affect erythropoiesis e.g. Interleukin 1 can induce inhibition of erythropoiesis in bone marrow leading to suppression of hemoglobin (Sheiham, 2006). Even after complete oral rehabilitation of children with ECC under general anesthesia, ECC may recur after 6 months (Berkowitz et al., 1997) or 19 - 24 months of treatment (Amin et al., 2010).

Dental diseases, mainly caused by imbalances in the resident microflora, are highly prevalent and extremely costly to treat (Marsh and Martin, 2009). The traditional treatment of caries is expensive as well a significant economic burden. As in many low income countries, the prevalence of caries is about 80% but over 90% of affected people are left untreated. If dental caries among children are treated, the costs would exceed the total health care budget of children. The oral health care investment is low, funds are primarily allocated for pain relief and emergency oral care. In both developing and developed countries, the treatment of dental caries is an expensive scheme for governments. However in some industrialized countries it costs about 5 - 10%

of total health expenditure (Kathmandu, 2002). Dental Caries is the fourth most expensive disease to treat thereby the burden has been tackled through establishment of advanced oral health care systems that primarily offer restorative services to patients. Countries like Scandinavia and United Kingdom provides oral health care, particularly to children and disadvantaged population groups by public health services (Petersen et al., 2005).

Despite great achievements in oral health of populations globally, issues still remain in many societies all over the world especially among under privileged groups in under developed and developing countries. Historically, dental caries have been considered as the most important global oral health burdens. Presently, the severity and distribution of oral diseases vary among different parts of the world and within the same country or region. An extensive number of epidemiological surveys has shown the significant role of socio-behavioral and environmental factors (WHO, 2014). In extreme cases, ECC and its treatment may lead to serious disability and mortality, ECC related mortality, secondary to infection and treatment is underreported owing to inadequate surveillance, lack of ECC registry, issues of confidentiality, terms of legal settlement and missing or incorrect diagnosis (Casamassimo et al., 2009).

1.2.8. Caries Management

Dental caries is a transmissible infectious disease, preventive strategies which are important in improving the oral health can be enhanced by understanding the acquisition of cariogenic microbes (AAPD, 2011), roles of diet, eating behaviors, demographics and environmental factors (Roberts, 2008).

Dental caries is a dynamic process, progression and reversal depends upon various factors that alter the balance between demineralization and remineralization visualized as *"Caries balance"*. It is determined upon the sum of pathologic and protective factors (Featherstone, 2006; Fontana and Wolff, 2011). The *Featherstone caries balance concept* states that the balance of pathologic factors can be altered in favor of protective factors to slow down the caries lesion. Among individuals with active caries lesions, if the balance towards protective factors are altered in comparison to pathologic factors caries continue forming new or recurrent lesion (Featherstone, 2006).

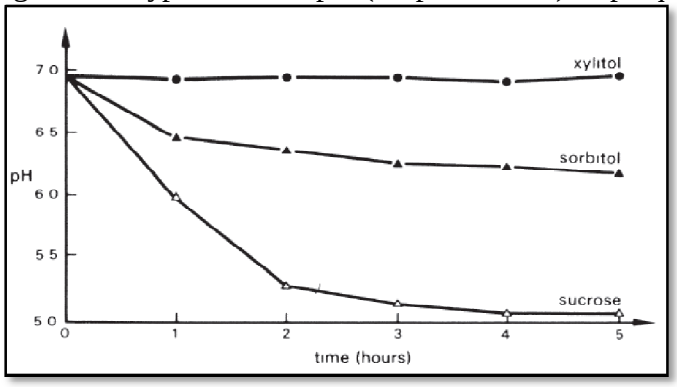
Management of chronic disease differs from a traditional approach (Ng and Chase, 2013). To address the causes of ECC, risk based prevention and management have to be done which requires family engagement in day to day behavior modifications (Ng et al., 2012). Risk factors have to be improved by modifying certain social and behavioral factors that promote caries e.g., diet, tooth brushing habits, fluoride intake, altering the saliva sharing activities and feeding behavior (Smith and Riedford, 2013).

Caries cannot be addressed by restoring the cavity alone. Along with treatment preventive tools and management should be considerate in reducing the risk of caries (Ng and Chase, 2013). Primary prevention of ECC should begin in the prenatal period, the health of both mother and child needs to be addressed (Milgrom, 1998). By providing specific dietary guidance to parents in regards to sweetened beverages (Evans et al., 2013) and eliminating saliva-sharing activities i.e. sharing utensils, orally cleansing a pacifier (Berkowitz, 2006) the prevalence of ECC may decrease.

Cottrell (2013) shows that the large window of risk would begin if oral hygiene is neglected along with fluoride supply and reduced protection from saliva and also indicates that it may be an impossible task to reduce window of risk to such an extent that window is not completely filled (Cottrell, 2013).

American Academy of Pediatrics has recommended that children 1-6 years of age should consume no more than 4-6 ounces of fruit juice per day, from a cup i.e. not a bottle or covered cup and as part of a meal or snack (Pediatrics, 2001). Evidence increasingly suggests that preventive interventions within the first year of life are critical (Lee et al., 2006). This may be best implemented with the help of medical providers who, in many cases, are being trained to provide oral screenings, apply preventive measures, counsel caregivers and refer infants and toddlers for dental care (Douglass et al., 2009). Polyol of glucose (sorbitol), mannose (mannitol) and pentose alcohol (xylitol) have been proposed as non-cariogenic natural sweeteners which are useful for partial substitution of sucrose in foods thereby sugar alcohols, sucrose substitutes or polyols are considered as suitable means of preventing or controlling dental caries (Jori, 1984). Figure 17 shows that sucrose reduces the pH (Stephan curve) of plaque drastically when suspended in buffer, incubated in the presence of 0.75% (w/v) xylitol, sorbitol and sucrose.

Figure 17: Typical fall in pH (Stephan curve) of plaque



Source from Marsh and Martin (2009)

The policy statement of AAPD encourages professional to take measures that may reduce the parents and siblings *MS* levels to decrease its transmission to each other, implement oral hygiene measure not later than 6 months of first tooth eruption, high frequency consumption of liquids and solid foods containing sugar or sugar-containing beverages (e.g. juices, soft drinks, sweetened tea, milk with sugar) in a baby bottle or no-spill training cup should be avoided. Infants should not be put to sleep with a bottle filled with milk or liquids containing sugar.

Once the first primary tooth begins to erupt, Ad libitum breast-feeding should be avoided and dietary carbohydrates should be introduced. Parents should be encouraged to have infants drink from a cup as they approach their first year birthday. Infants should be weaned from the bottle between 12 to 18 months of age (AAPD, 2011). In general, the community needs to be encouraged to work with medical providers to ensure all infants and toddlers have access to dental screenings, counseling, and preventive procedures. Ismail (1998) shows that development in community like educating society, water fluoridation as well professional and home care is important in prevention of caries (Figure 18).

Figure 18: Strategies for prevention of ECC

(Modified from Ismail (1998)

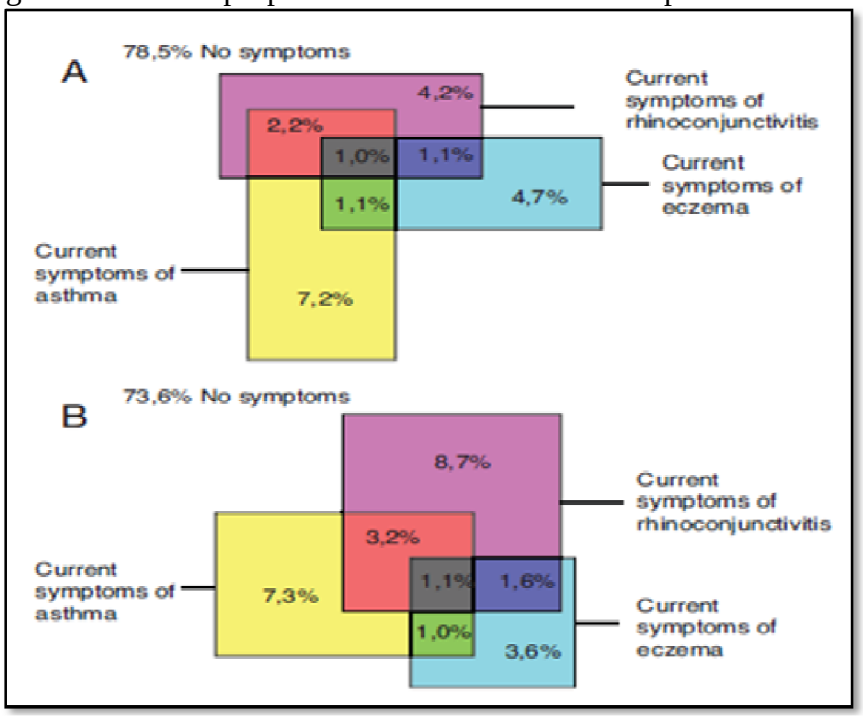
1.3. Allergic disease

1.3.1. Overview

Allergy-associated symptoms like asthma, eczema and rhino-conjunctivitis in children have become a major public health problem worldwide (Beasley, 1998). Globally the prevalence of asthma, eczema and rhinoconjunctivitis has been increasing which can be understood from the recent International study of asthma and allergies in childhood (ISAAC) phase three.

The worldwide recent data of prevalence among 6 - 7 yr age group was 11.7%, 8.5% and 7.9% in asthma, rhinoconjunctivitis and eczema respectively. In the age group of 13-14 years, it was 14.1%, 14.6% and 7.3% respectively. Among the Asia pacific group (Singapore, Malaysia, India, Philippines, Myanmar, Pakistan, Japan, Iran, Iraq, Bangladesh, Saudi Arabia, Sri Lanka, Thailand etc.) the intermediate prevalence values was found for asthma, rhinoconjunctivitis and eczema in the younger age group (Mallol et al., 2013). Figure 19 shows the overall proportions of children with current symptoms of asthma, rhinoconjunctivitis or eczema or combinations of symptoms (16-A: 6-7 yr age group and 16-B: 13-14 yr age group for ISAAC phase three study (Mallol et al., 2013).

Figure 19: Overall proportion of children for ISAAC phase three study



(Source from Mallol et al. (2013)

Cross-sectional data was compared between two ISAAC surveys conducted in 1994 and 2001- which was carried out among Singapore school children aged 6-7 yrs and 12-15 yrs of age to study the prevalence of asthma, rhinitis and eczema, their results showed the symptoms of eczema was significantly increased in both age group, symptoms of rhinoconjunctivitis did not show significant change in any age group and the symptoms of asthma was decreased in 6-7 yrs age group and also 6.4% reduction in 12 month prevalence of wheeze and among 12-15 yr old there was slight increase of 2% in 12 month prevalence of wheeze and they showed that 27.4% of 12-15 yr old have been medically diagnosed with asthma and also indicate that the prevalence of cumulative asthma has been increased from 5.5% in 1967 to 13.7% in 1987 and 20.0% in 1994 (Wang et al., 2004).

Goh *et al.*, 1996 contends that allergic diseases are common in Singapore school children and their prevalence can be compared to other western

countries (Goh et al., 1996). The prevalence and the severity are influenced by demographic and socio-economic status. It has been observed that Malays and Indians have more severe asthma related symptoms compared to Chinese. Higher prevalence of rhinitis and asthma is shown among males and with higher socio-economic status (Goh et al., 1996). A recent article indicates that one in every five school children suffer from eczema and the prevalence has been increasing (How et al., 2013). Another article have shown that 20.8% of school children are affected by acute dermatitis in Singapore (Tay et al., 2002). Furthermore a comparative study in Singapore shows lower prevalence of asthma, rhinitis and eczema among children residing in northern region of Singapore when compared to other regions (Chew et al., 1999b).

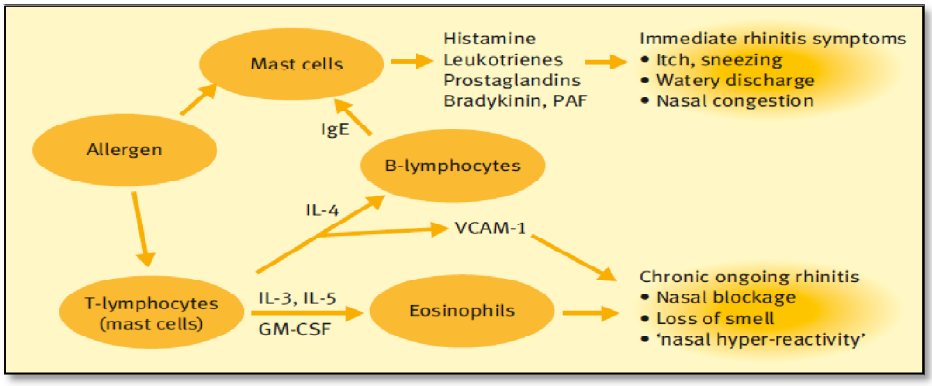
Few studies hypothesize that the prevalence of allergic disease is increased due to increase in hygiene practice, better environmental conditions, reduced exposure to bacteria and small family size indicating that general hygiene hypothesis is still valid (e.g., pets in house) (Brooks et al., 2013; Figueiredo et al., 2013). Another study suggest that allergic diseases in children can also be reduced by cross infection from siblings or maternal exposure to infections, as it may stimulate the immunological memory which protects against allergic respiratory disease (Hersoug, 2006).

1.3.2. Mechanism of allergic diseases

An exposure of allergen causes stimulation of T cells or mast cells, which results in release of histamine and interleukins, that may activate B cells and eosinophils, release of these interleukins may cause loss of smell. Figure

20 schematically shows the basic mechanism of allergic reactions (Scadding, 2008).

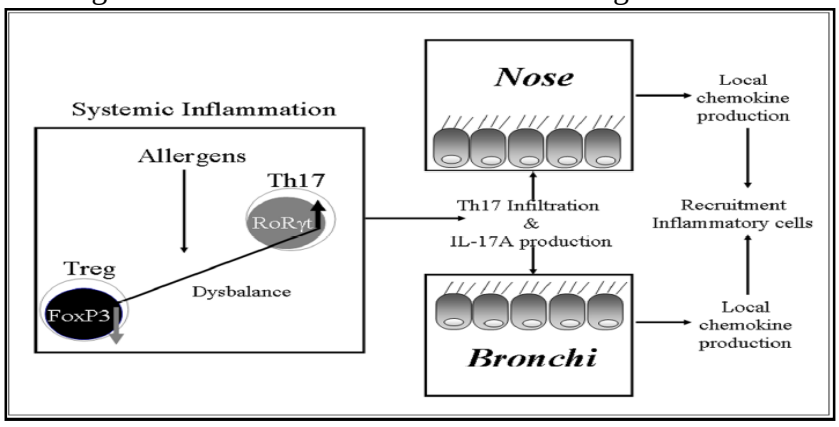
Figure 20: Shows basic mechanism of allergic reactions



(Source from Scadding (2008)

A recent study shows that IL17A and Th17 cells play an important role in the development and progression of allergic diseases, Figure 21 explains the role of Th17 and Treg balance among asthma and rhinitis children (Albano et al., 2013).

Figure 21: Shows the role of Th17 and Treg imbalance



(Source from Albano et al. (2013)

However, the exact mechanism of hygiene hypothesis is not very well known, the immunological explanation was put forward for supporting hygiene hypothesis, postulating that early exposure of both microbial and parasitic infections lead to an increase in anti-inflammatory cytokines IL-10, that may reduce both TH1 and TH2 responses. It may also shift the balance of maturing

immune system from pro-allergic TH2 responses to TH1 responses thereby reducing auto-immune and allergic diseases (Figueiredo et al., 2013).

1.3.3. Eczema

Eczema also known as atopic dermatitis is the chronic inflammatory itchy skin condition. In most of the cases, this atopic dermatitis is seen in early childhood generally involving face, extensor surfaces of limbs, sometimes seen over trunk. Atopic eczema often involve genetic factor, due to this the child's eczema may be elicited when exposed to allergens/irritant factors. Few children may end up with allergic rhinitis and asthma as they grow. In preschoolers, the food allergy may be occasionally associated with atopic eczema (Health, 2007; How et al., 2013).

In Singapore among 16 yr old, they have observed that the onset of disease occurs before 10 yrs of age (49.5%). In comparison to Indians (16%) and other races (14%), eczema is more common among Chinese (21.6%) and Malays (19.8%) (Tay et al., 2002). Emollients, mild to potent corticosteroids, topical calcineurin inhibitors, phototherapy and systemic therapy are used for treatment for eczema depending upon the severity/progression of disease (Health, 2007).

The Ryukyus child health study was carried out on 21,792 children aged between 6 to 15 years in Japan to know more about the association between allergic disease like atopic eczema and dental caries, prevalence of eczema was 6.8% and the findings of this study did not provide a strong relationship (Tanaka et al., 2008). Based on reports from Food and Drug Administration

(FDA), a recent ongoing study on 2,192 eczema subjects has been created by eHealthMe, their recent report shows that 0.09% have dental caries, this report have been updated regularly. As of now they do not show any statistical reports (FDA-reports). The dental examination of Hyper-IgE syndrome (HIES - characterized by the clinical triad of high serum levels of IgE >2000 IU/ml, eczema and recurrent skin and lung infections) patients have shown that they are severely prone to caries besides retention of deciduous teeth and delayed eruption of permanent teeth (Esposito et al., 2012; Olczak-Kowalczyk et al., 2013).

A large national survey of children's health (NSCH) who examined the association of atopic dermatitis with atopic and non-atopic co-morbid conditions (poorer oral health) have shown that eczema was associated with a high prevalence of multiple dental problems (tooth ache, broken teeth, bleeding gums, tooth decay - $P \leq 0.015$) within the past 6 months (Silverberg and Simpson, 2013). Hence, we would like to study more about the association of caries among eczema children and to signify the importance of dentist role among them.

1.3.4. Rhinitis

Rhinitis is the inflammation of nasal mucosal lining, allergic rhinitis is mediated through IgE and it leads to cardinal symptoms of sneezing, nasal itching, rhinorrhea and congestion by involving degranulation of mast cells, mediator release and entering of inflammatory cells (Scadding, 2008).

Rhinitis can be caused due to

- Allergy (intermittent/persistent, seasonal/perennial/mixed),
- Infection (acute/chronic, viral/bacterial)
- Others (PCD, cystic fibrosis, immunoglobulin deficiency, GORD, autonomic, hormonal, NARES, drug-induced) (Scadding, 2008)

Generally, in children allergic rhinitis is the recurrent chronic respiratory disease. Its prevalence varies from 1.3% - 52% which is recorded in various countries. Their symptoms are obstruction, pruritus, sneezing and rhinorrhea which can indirectly cause increased oral infection due to long-term mouth breathing (Vázquez-Nava et al., 2008). ISAAC study (1977) has observed the range of prevalence of rhinitis with rhinoconjunctivitis from 0.8% to 14.9% in 6 - 7yr old and 1.4% to 39.7% in 13 - 14 yr old across countries (Strachan et al., 1997).

Management of rhinitis includes education, allergen and irritant avoidance, nasal douching, topical corticosteroids, systemic glucocorticosteroids, antihistamines, antileukotrienes, anticholinergics (Ipratropium bromide nasal spray), decongestants, chromones, allergen immunotherapy, other therapies like anti-IgE & surgery (Wallace et al., 2008).

Not many studies have shown the prevalence of caries among allergic rhinitis children. Tanaka et al. (2008) concludes that there was no significant association between caries and allergic rhinoconjunctivitis but caries was inversely related to rhinoconjunctivitis children only among those parents who had allergic history. Vázquez-Nava et al. (2008) has shown that children with

allergic rhinitis and pacifier use have double the risk of developing caries than rhinitis alone. A recent study published in this year shows that patients with allergic rhinitis had an increase in salivary *MS* level although there was no significant difference in DMFT/dmft index, salivary flow, buffering capacity, *LB* levels and sugary food consumption (Wongkamhaeng et al., 2014 May).

1.3.5. Asthma

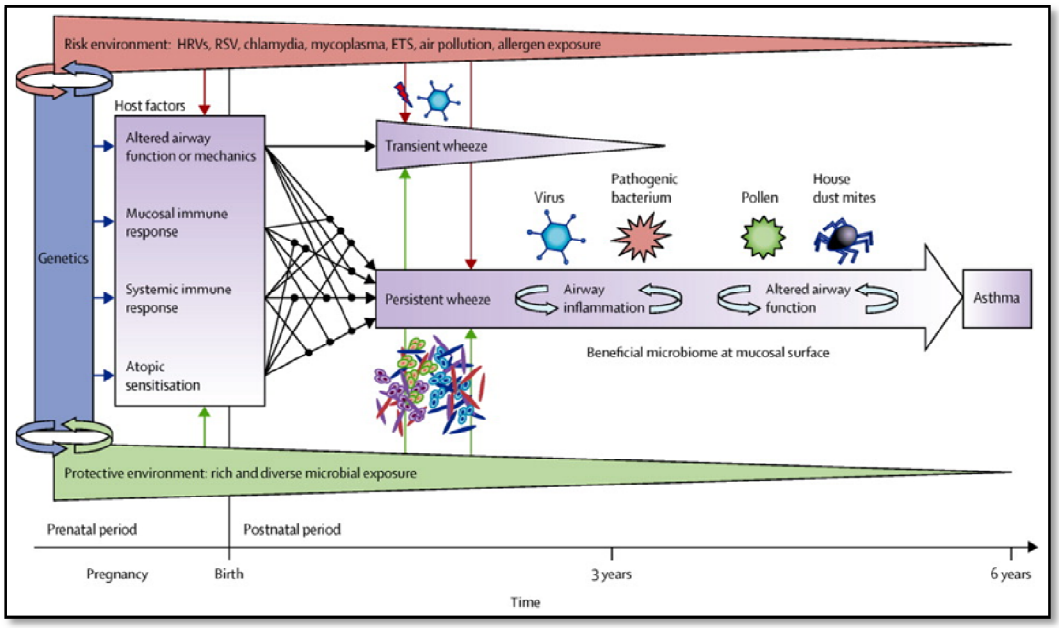
In childhood, Asthma is the most common chronic disease. It is a chronic inflammatory disorder of the airways, characterized by episodic and reversible symptoms of airflow obstruction (Stensson et al., 2008; Stensson et al., 2010) and chronic inflammation of the airway leads to symptoms like dyspnea, wheezing, chest tightness and coughing (Alavaikko et al., 2011).

1.3.5.1. Risk Factors

Genetic, viral respiratory infections, bacterial colonisation, hypo-pharyngeal infections, allergic sensitisation, environmental factors like smoking, all predisposes towards risk factors for asthma (Bisgaard et al., 2007; de Nijs et al., 2013). Some studies indicated that use of antibiotics by mothers increases the risk of asthma in children (Stensballe et al., 2013). In the development of asthma both allergic and non-allergic rhinitis plays a major role with an odds ratio of 3 (Scadding, 2008). In the pathogenesis and susceptibility to atopy and asthma, many genes like *ORMDL3, ADAM33, DPP10, PHF11, GPRA, TIM-1, PDE4D* and *OPN3* have been included (Agrawal and Shao, 2010). Figure 22 explains the contribution of viruses and bacteria in the development of asthma. Depending on genetic background and

environmental exposures four essential host factors altered airway function or mechanics, mucosal immune response, systemic immune response, atopic sensitisation are formed both pre and postnatally. Although viruses and bacteria primarily interact with the mucosa, there is also interaction with the systemic immune response, local changes of which will be further boosted by atopic sensitisation, allergen exposure and continuous infection. Transient wheeze is triggered by virus infections on the basis of altered airway function but will be outgrown by children. Development of asthma might be determined by one or a combination of the four primary host factors and prenatal and postnatal environmental exposures, which might contribute to or protect from the development of asthma. (HRVs =human rhinoviruses, RSV=respiratory syncytial virus, ETS=environmental tobacco smoke).

Figure 22: Contributions of viruses and bacteria to the development of asthma



(Source from Fuchs and von Mutius (2013)

Thereby indicating that early exposure of bacteria and virus may cause transient or persistent wheeze, altered airway function and airway inflammation resulting to asthma.

1.3.5.2. Bacterial Infection in Respiratory track

Recent studies have shown that apart from virus respiratory infections, bacterial infection with certain pathogens like *Chlamydia, Haemophilus influenza* and *Streptococcus pneumonia* in early life may promote permanent deleterious changes in immunity, lung structure and function (damage to the alveoli) that predisposes to or increase the severity of chronic respiratory disease in later life. Sensitivity of airways to non-specific stimuli in adjunct with inflammatory response results in bronchial smooth muscle contraction known as airways hyperresponsiveness (AHR). Eosinophils, Th2 lymphocytes and innate lymphoid cells drive the inflammatory responses that emphasize asthma, these cells release inflammatory mediators and cytokines and damage the bronchial mucosa and airway epithelium resulting in structural changes and mucus hyper secretion (Starkey et al., 2013).

Immunologically they show that early bacterial infection cause increase in mucus secretion, IL-13 and AHR thereby increasing the severity of allergic airway disease in later life. Increase in mucus secretion along with AHR results in increase airway obstruction leading to hospitalization and sometimes may even result in death due to asphyxiation (Starkey et al., 2013).

A review article stated that early life bacterial colonization may reduce immunity in children, which may induce neutrophilic inflammation of airways (characteristic of non-atopic infection induced asthma) (Korppi, 2010). Compared to typical bacteria (*S. pneumonia, H. influenza, M. catarrhalis*) atypical bacteria *M. Pneumonia, C. Pneumonia* and also *C. trachomatis* may

induce wheezing and acute exacerbations in asthma (Korppi, 2010) and involves in pathogenesis of asthma (Sutherland and Martin, 2007).

Table 7: Pre/post natal exposure of bacteria & antibiotics as risk factor for asthma

Microbe/Antibiotics	Colonization/Exposure to Antibiotics	Outcomes
Streptococcus pneumoniae, non-typeable *Haemophilus influenzae, Moraxella catarrhalis*	Colonization at <4 weeks of age	Increased asthma risk at 5 years of age
Staphylococcus aureus	Maternal colonization during pregnancy	Increased asthma risk at 5 years of age
Antibiotic therapy	Maternal use during pregnancy	Increased asthma risk at 5 years of age
Antibiotic therapy	Children at age <1 month	Wheezing at age <12 months
Antibiotic therapy	Children at age <3 months	Wheezing at age 1.5 years
Antibiotic therapy	Children at <12 months of age	Increased asthma risk at 6–7 years of age

(Source from Korppi (2010)

Korppi 2010 have also mentioned that Acute episodes of *C. pneumoniae* infection could be a primary infection or re-activation of chronic infection may be stimulated by acute viral infections, this infection may cause occult chronic infection and airway inflammation thereby involving in pathogenesis of asthma. In treatment resistant asthmatics, *Chlamydophila* bacteria were cultured in a 3rd of bronchoalveolar lavage samples. Role of atypical bacteria in exacerbation of asthma, chronic stable asthma & onset of new asthma in children is shown in table 8.

Table 8: Role of atypical bacteria in asthma

Microbe	Asthma Exacerbation	Stable Asthma	Incipient Asthma
Mycoplasma pneumoniae	Yes	No	No
Chlamydophila pneumoniae	Yes	Yes	Yes?
Chlamydia trachomatis	Yes?	Yes?	No?

(Source from Korppi (2010)

In a cohort study (COPSAC), the neonates (born to asthmatic mothers) were followed till 5 yrs of age (Bisgaard et al., 2007), hypo-pharyngeal samples were collected and cultured for *S. pneumonia, H. influenza, M. catarrhalis* and

S. aureus at 1 month and 1 yr of age. Blood samples were taken at 6M, 18M and 4 yr to measure total and specific IgE, eosinophil count. Doctors in the research unit diagnosed wheezing /asthma.

Table 9: Shows the percentage of bacteria's present in Neonates (1month)

At 1M in asymptomatic neonates	
S. Pneumonia	9%
H. influenza -	9%
M. catarrhalis	8%
S. aureus	61%
S. pyogens	< 1%
S. pneumonia, H. influenza, M. Catarrhalis	21 % (with more than 1 species)

(Modified from Bisgaard et al. (2007)

Results indicate that colonization of these bacteria either alone or in combination *(S. pneumonia, H. influenza, M. catarrhalis)* at 1M in asymptomatic neonates increased the risk of wheeze by a factor of 2- 4 and showed increase in eosinophil count & total IgE and ultimately increased reversibility of airway resistance. Thereby it is concluded that the neonates colonized with above bacteria are at increased risk for development of asthma and recurrent wheeze at 5 yrs or early in life (Bisgaard et al., 2007).

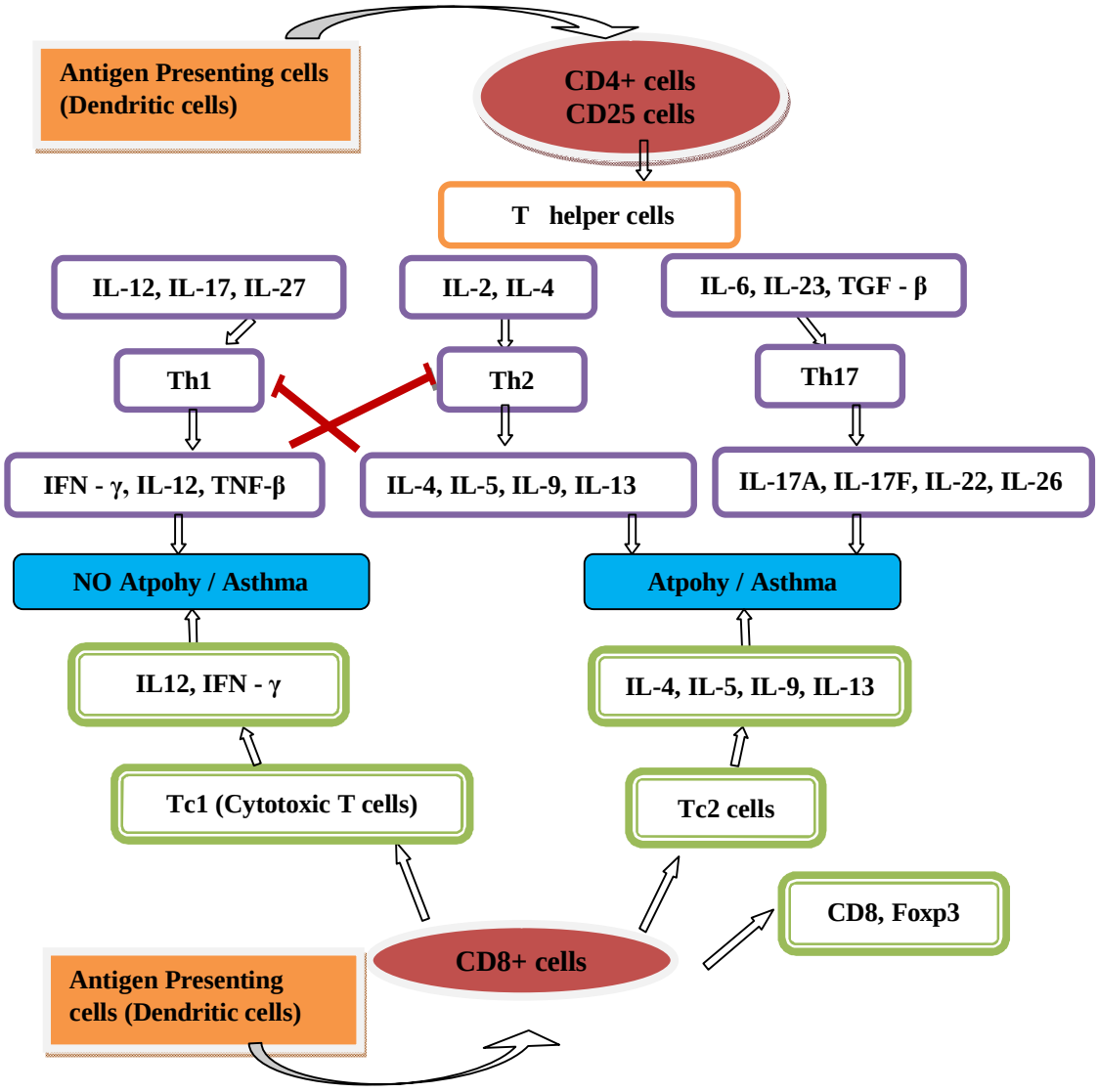
The recent data of the same cohort study (COPSAC) showed that bacterial colonization induces significant stimulation of the immune profile of airways (Folsgaard et al., 2013). After analysis they conclude that airway colonization with *M. catarrhalis* and *H. influenza* is associated with inflammatory immune response of airway mucosa which may result in chronic inflammation (Folsgaard et al., 2013). A recent study have showed that *Chlamydophila* or *Chlamydia* species were detected by PCR in 68% of fluid samples which were collected by broncho-alveolar lavage (43% for *C. pneumoniae*, 42% for *C. trachomatis* and 26% for both) and 46% of culture were positive for *C. trachomatis*, In conclusion they indicate that among Rx resistant asthma *C.*

trachomatis and *C. pneumoniae* occur more frequently in comparison to earlier belief (Webley et al., 2009). Peter wark et al., 2013 study have shown that those with both virus and bacterial infection had lower FEV_1, longer length of stay and likely to be readmitted again (Wark et al., 2013).

1.3.5.3. Role of CD4/CD8 cells in asthma

CD4, CD8 and CD25 cells play an important role in the development of asthma, this is shown in the below Figure 23 (Agrawal and Shao, 2010; Zhu and Paul, 2008)

Figure 23: Role of CD4/CD8 cells (OR) TH1/TH2/TH17 response

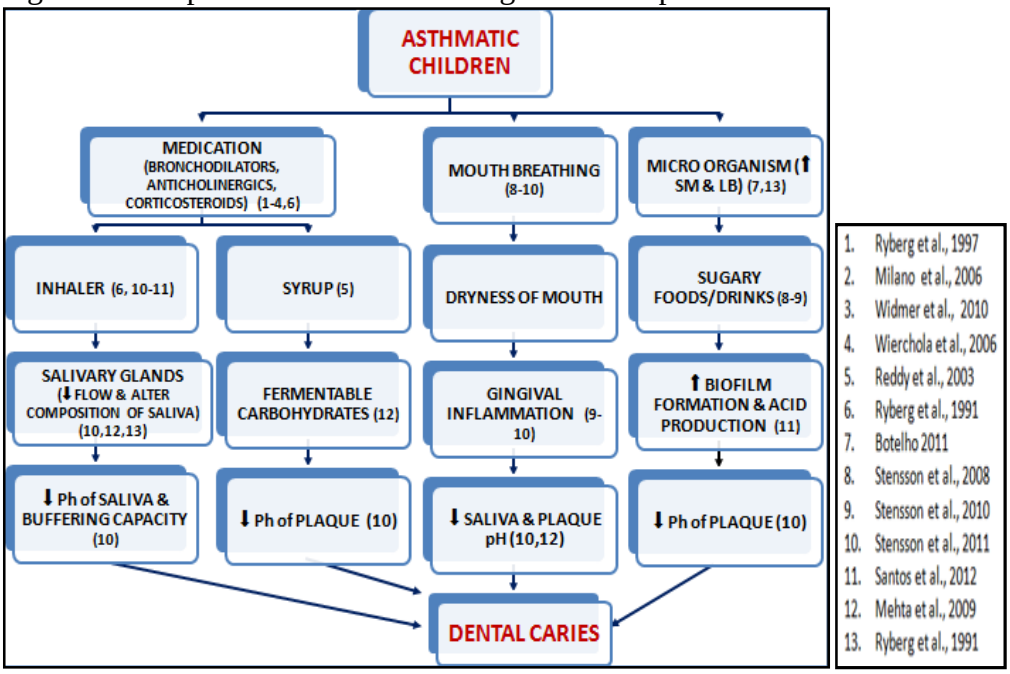


1.3.5.4. Effects of asthma on oral cavity

As the prevalence of asthma has been increasing, the side effects associated with its medication could result in significant global oral health issue (Widmer, 2010). Studies have shown the differences in gender, females are more asthmatics in comparison to males (Anjomshoaa et al., 2009).

Many studies have concluded that asthmatic children has shown increase in decay, missing and filled (DMF) score in both primary and permanent dentition (Alavaikko et al., 2011; Anjomshoaa et al., 2009; Ryberg et al., 1991), decrease in flow & alter in the composition of saliva (protein, amylase, hexosamine, Lysozyme, secretory IgA, peroxidase) (Ryberg et al., 1991; Sag et al., 2007), reduced pH of saliva, altered buffering capacity of saliva, mouth breathing (Stensson et al., 2010), increase in *S. mutans* and *LB* bacteria (Botelho et al., 2011; Ryberg et al., 1987; 1991), increase in biofilm formation (Botelho et al., 2011)(Ersin et al., 2006; Sag et al., 2007) and gingival inflammation (Stensson et al., 2010).

Figure 24: Proposed flow chart showing relationship between asthma & caries.



Asthmatic children may be more prone to caries due to side effects of medication, which alters the pH of saliva and plaque. Asthmatics are more prone to breathe in mouth it may cause inflammation of gingiva and due to dryness of mouth they may have reduced pH of saliva and plaque. Studies have shown that *SM* and *LB* levels among asthmatics are higher and they tend to consume more sugary foods conclusively may result in caries formation.

1.3.5.4.1. Effect of Asthmatic drugs on saliva

1.3.5.4.1.1. Asthmatic drugs

Asthmatic drugs consist of bronchodilators (β-adrenergic receptor agonist, muscarinic acetylcholine receptor antagonist/anti-cholinergic drugs and xanthines like theophylline) and corticosteroids. Many studies have shown that inhaled corticosteroids are used in the treatment of chronic asthmatic patients to provide relief and open airway due to anti-inflammatory effects. These drugs are mainly used to control/reduce airway inflammation and broncho constriction (Cazzola et al., 2012; Papi et al., 2009; Thomas et al., 2010) but these drugs have effect on the salivary glands by reducing the flow of saliva (hypo salivation) and altering the saliva composition (Scully, 2003).

The asthmatic drugs may contain fermentable carbohydrates like lactose and sugar which has a cariogenic effect. The chronic use of these drugs may cause demineralisation of teeth (Maguire et al., 1996; Mehta et al., 2009; Steinbacher and Glick, 2001). O'Sullivan and Curzon have mentioned that all inhalant powders may have pH < 5.5 which may cause dental erosion (O'Sullivan and Curzon, 1998). Some studies showed effects on dental caries are contributed by certain inhalation techniques and medication. The criteria

and score for inhalation use technique were: 1) removing the cap of canister and shake the inhaler; 2) slow deep expiration; 3) placing the mouthpiece into mouth; 4) slow deep inspiration; 5) pressing the canister to release drug simultaneously with inspiration; 6) holding the breath at the end of inspiration for 10 second; 7) washing the mouth (Boskabady et al., 2012).

O'Sullivan and Curzon (1998) have shown the pH of asthmatic drugs which are commonly used either in powder or aerosol form (Table 10) and indicates the residual detrimental effects on hard tissues of mouth.

Table 10: pH of various asthmatic drugs

Generic name of Drug	Proprietary name	Dose (µg)	pH*
Beclomethasone diproprionate	Becotide	200 (P)	4.76
		200 (A)	7.76
Fluticasone	Flixotide	100 (P)	4.76
		125 (A)	7.73
Budesonide	Pulmicort	400 (P)	6.47
		200 (A)	8.34
Salbutamol	Ventolin	200 (P)	5.94
		100 (A)	9.30
Salmeterol	Serevent	50 (P)	5.49
		25 (A)	7.24
Terbutaline sulphate	Bricanyl	500 (P)	4.31
		250 (A)	7.03
Ipratropium bromide	Atrovent	20 (A)	7.88
Sodium cromoglycate	Intal	20 (P)	5.54
	Cromogen	05 (A)	7.34

(Modified from O'Sullivan and Curzon (1998)

1.3.5.4.1.2. Effects of asthmatic drugs on Salivary glands

There are three major paired salivary glands - parotid, submandibular and sublingual glands (Grist, 1990). Many minor salivary glands are located on the labial, buccal and palatal mucosa of oral cavity and they open into oral mucosa through short excretory ducts, their excretion is seromucinous containing more amounts of IgA (Geerling et al., 2008) and some minor glands are also present in pharynx and larynx (Grist, 1990).

These salivary glands are generally innervated either directly or indirectly by parasympathetic and sympathetic arms of autonomic nervous system. Parasympathetic innervation is carried out by cranial nerves; Sympathetic innervation takes place directly through pre-ganglionic nerves in thoracic segments T1-T3 which synapse in superior cervical ganglion.(Frederick S. Rosen, 2001; Walker, 1990a; b). The inferior salivatory nucleus (via Glossopharyngeal (IX) nerve through otic ganglion) innervates the parotid and von Ebner's (lingual) salivary glands**.** The superior salivatory nucleus (via Facial (VII) nerve through Submandibular ganglion) controls sublingual and submandibular salivary glands**.** The axons from the salivatory neurons (preganglionic) synapse in a peripheral ganglion gives rise to postganglionic fibers that synapse on the salivary gland acinar cells (Bradley and Kim, 2007).

Guggenheimer (2003) shows that anticholinergics, antihistamines and analgesic drugs causes xerostomia which may result in dental caries as their side effects shown in table 11.

Table 11: Drugs associated with Xerostomia

Category	Generic Name
Anticholinergic Agents	Atropine, benztropine, oxybutynin, scopolamine, trihexyphenidyl
Analgesic Agents	Codeine, meperidine, methadone, ibuprofen, naprozen, piroxicam
Antihistamines	Brompheniramine, chlorpheniramine, diphenhydramine, loratadine, meclizine

Both the parasympathetic and sympathetic stimuli results in increase salivary gland secretions (Bavikatte et al., 2012). Due to nervous stimulation of salivary glands the secretion of secretory immunoglobulin A (SIgA) which is derived from B-lymphocytes in the vicinity of salivary glands is increased, once derived they are transported into saliva through cell membranes by

polymeric immunoglobulin receptor. When the flow of saliva is reduced the SIgA is also affected. Thereby SIgA, alpha-amylase is related to the flow of saliva (Salimetrics, 2011).

1.3.5.4.1.1. Mechanism of action of asthmatic drugs on salivary glands

The drugs readily reaches the saliva/salivary glands by passive diffusion, through the lipo-protein membranes of the secretory cells in salivary glands (Salimetrics, 2011). The Anticholinergic drugs acts by blocking the Ach, neurotransmitter for parasympathetic pathway thereby inhibiting the muscarinic (M3Rs) stimulation of salivary glands through the cAMP-protein kinase A pathway and DAG-protein kinase C pathway resulting in reduced flow of saliva (Ipratropium bromide, Tiotropium bromide - available only in inhaler form). β2 adrenergic agonist drugs binds to the β receptors present on the salivary glands there by inhibiting their action (Kelly, 2007; Scully, 2003). In asthmatics with long term use of medication it has been observed that the secretory rate was decreased by 26% and 36% of whole and parotid saliva (Ryberg et al., 1987).

1.3.5.4.2. Mouth Breathing

Mouth breathing is the abnormal breathing pattern where the individual inhales and exhales through mouth due to airway obstruction. Asthma is one of the reasons for mouth breathing, thereby among young asthmatics the prevalence of mouth breathing was high (26%) comparative to non-asthmatics (Stensson et al., 2008; Stensson et al., 2010). In mouth breathers the facial growth pattern gets changed and the relationship of dental

tissues gets altered, the overjet is significantly increased, the inter molar width is decreased resulting in narrow maxillary arch (Bresolin et al., 1983; Mattar et al., 2004) and incompetent lip seal (Peltomäki, 2007) may cause dry mouth and gingival inflammation among mouth breathers (Jacobson, 1973).

Studies have proposed that in asthmatics, due to airway inflammation or any exposure of pollen or allergens, exhaled nitric oxide (ENO) level increases and during mouth breathing habit this ENO passes through the oral cavity, if it comes in contact with water or saliva in mouth, it may change to a strong acid (nitric acid), which may directly affect the calcified structures of teeth resulting in demineralization and dental caries or erosion. (Maupome et al., 2010; Szefler et al., 2008; Vahlkvist et al., 2006).

1.4. Allergic disease and Dental Caries: A Divergent Clinical Evidence

Both allergy and caries are common chronic conditions with multifactorial causes. Various clinical appearances, challenges in their diagnosis and different types of pharmacotherapy used in their treatments may cause difficulties in the efforts to study them. As the prevalence of asthma is increasing its necessary to know how this disease affects the other areas of health care especially oral health. Many studies show that allergic diseases may be associated with dental caries but some studies show that asthmatic patients with medication are linked to dental diseases, Furthermore few studies have shown that relation between asthma and caries is not statistically significant. (Bjerkeborn et al., 1987; Meldrum et al., 2001; Shulman et al., 2001).

Table 12: Summary of studies on negative association between allergic disease and dental caries based only on caries

1st Author, Geographic Location	Study Design, Sample size (Age in year)	Objective	Allergic disease measure	Caries measure	Outcome / Result
Stensson et al. (2008) Jőnkőping, Sweden	Case - control study. **A**: 66 (3yr) C : 62 (3yr) **A**: 61 (3yr) C : 55 (6yr)	Oral health in two groups (3 yr old & 6 yr old) of asthmatic children	Pediatrician diagnosed asthma as well duration of medication exposure	Calibrated examiner - dfs (initial and manifest caries), plaque index, 2 posterior radiographs in 6 yr old group, Saliva sampling (SM and LB culture) & oral hygiene.	**3 yr old A** :- Mean dfs* - 1.4 (3.2) [⬆ sugary drinks, gingivitis & mouth breathing*] **6 yr old A** :- NS
Bjerkeborn et al. (1987) Huddinge, Sweden	Case - control study. N # 116 (5-18 yr) **A** - 61, C - 55	Children with Asthma using medication.	Diagnosed - extrinsic asthma without other systemic disease	DF(S)scores, Salivary flow rate, buffer capacity, lactobacilli, Bitewing radiographs, gingival condition	**A**-MEAN DFS - 7.8 : dfs - 3.8, **C**- DFS - 6.9 : dfs - 4.1. DFS & dfs - **NS,** Salivary secretion rate (5-10yr)*****
Shulman et al. (2001) USA	Cross-sectional study, N # 6,938: **A** - 1129, C-5809 (4-16yr)	Caries in asthmatic children & adults	Physician diagnosed asthma & medication	DMFS, deft	**A:** ⬇ caries than control
Tanaka et al. (2008) Japan	Cross sectional survey study, N # 21,792 aged 6-15 yr	Allergic disease like asthma symptoms, eczema, rhino conjunctivitis among children and caries activity.	By Questionnaire	Data on dental caries were obtained from school records	No association b/w caries and allergic disease. But inversely associated with prevalence of rhinoconjunctivitis children among +ve parental allergic history.

Meldrum et al. (2001) New Zealand	Cohort study (longitudinal DMHDS study) N # 781 [(**A** - 140, C - 206) @ 15 & 18 yrs	Determine association of caries in long term asthmatics	Wheeze or medication determined asthmatics (WDA/MDA)	DFS scores at 15 and 18 yrs (3yr increment)	Mean DFS of WDA + MDA - 2.61 (4.89), among no asthma is 2.13 (3.68) - **NS**
Vázquez et al. (2011) Mexico	Cross-sectional in cohort study N # 1160 (4-5 yr)	Relationship of asthma & caries in primary teeth	By questionnaire	dmft, dmfs	No association OR = 1.17 (0.81 -1.69) p=0.220
Lindemeyer et al. (2011) New York	Case control study **A -** 86, C - 86 matched with age, sex, socioeconomic status (≤ 71M)	Severity of bronchial asthma is a risk factor for ECC	Physician diagnosed asthma	dmft + plaque index	**A -** less caries + less plaque

Table 13: Summary of studies on negative association between allergic disease and dental caries based on caries + microorganism measurement

1st Author, Geographic Location	Study Design Sample size (Age in year)	Objective	Allergic disease measure	Caries measure	Outcome / Result
Wongkamhaeng et al. (2014 May) Thailand	Case - control study N # 80 (6 - 13 yrs)	caries in allergic rhinitis (AR) patients	AR + persistent / intermittent AR/allergic conjunctivitis control group matched for age and SES	DMFT / dmft +, salivary levels of *MS* and LB + oral hygiene and dietary habits.	AR - ↓ DMFT / dmft, salivary flow rate, buffer capacity, LB levels, and sugary food consumption*. ↑ *MS* levels*

Vázquez-Nava et al. (2008) Mexico	Cross-sectional study based on cohort of 1,160 children aged 4 - 5 yrs	Effect of AR, breast /bottle fed, SES, oral status pacifier on caries	Parents confirmed AR + skin prick test	dmft / deft , digit sucking	AR alone had no effect but AR + pacifier use had more than doubled the risk of developing caries.
Ryberg et al. (1987) Umea, Sweden	Case control study **A** - 24 (10-20yr) C - matched with age, sex, social background	Effect of β_2-adrenergic drugs on salivary gland and teeth.	Chronic asthmatic with medication, as well with no other disorders	Examiner was blinded: - DFS (initial and manifest caries based on 2 bitewing radiograph), whole & parotid salivary rate (SM + LB culture).	**A** - ⬇ salivary (Whole & Parotid) secretion flow*, ⬆ SM and LB count*, higher DFS score but NS **(P=0.07)**
Alaki et al. (2013) Saudi Arabia	Case control study (Randomly selected) **A** - 30, C -30 (5 - 13 yr)	Effect of asthma and its medication on dental caries + saliva in children	Physician diagnosed asthma (mild moderate and severe)	CPI, dmf index, saliva flow, buffering capacity, *MS*, *LB* + sugar intake	A - ⬆ sugar intake, Severe asthma - ⬇ flow*, β2 agonist inhaler with CS has ⬆ LB**, medication ≥ 3times/day - ⬆ *MS* + *LB*, DMFT or dmft scores - NS
Hyyppä and Paunio (1979) Finland	Case control study **A** - 30, C -30 (10 - 12 yr)	Oral health and salivary factors among asthmatics	Long term extrinsic asthma using steroid and β adrenergic drugs -inhaler	DMFS (OE + OPG) and saliva pH, flow, buffering capacity and LB	A - ***LB*,*** No change in salivary flow, pH and buffering capacity.

To summarize, the above studies have shown that allergic diseases like eczema, rhinoconjunctivitis and asthma are not significantly associated with dental caries, though there is increase in caries score among asthmatics but they are not statistically significant in comparison to their control group. Authors suggest the negative association could be due to the potential confounders like fluoride, but due to increase in caries activity among asthmatics they conclude that preventive measures has to be taken to reduce the micro-organisms level or to decrease the active caries lesions.

Table 14: Summary of studies on positive association between allergic disease and dental caries based only on caries measurement

1st Author, Geographic Location	Study Design Sample size (Age in year)	Objective	Asthma measure	Caries measure	Outcome / Result
Arnrup et al. (1993) Sweden	Retrospective - Cross sectional study 269 & Mean age 7.7±5.1	Caries among children under medications with IDDM, asthma and epilepsy.	Doctor diagnosed asthma with long term medication	Clinical and radiographic diagnosis - df(s) and gingivitis.	**A** - more df(s) - 3.5±4.3 in primary dentition*****
Ersin et al. (2006) Turkey	Case control study **A**: 106, C : 100 (6-19 yr)	Children with Asthma using medication in two groups 6-10 & 11-19 yr.	Doctor diagnosed asthma with long term medication	Clinically - DMF(S), dmf(s), plaque index, salivary samples - pH, buffering capacity, S.m level.	**A** (6-10 yr) had more caries: mean dfs±SD***** -9.2 ± 7.6 and low salivary flow
Kankaala et al. (1998) Finland	Cohort -Case-control study **A**: 51, C : 102 (> 3yr born in 1980's)	To analyze the time of 1st filling in asthmatic receiving CS	Children receiving daily asthma medication (Inhaled CS)	Time of placement of first restoration	**A** - received more restorations earlier shown only in graph
Wierchola et al. (2006) Poland (North)	Case control study **A** - 326 (3-15yr), C - matched with age, sex, social background	Association of asthma and caries at developmental age	Chronic asthmatic seeking care at referral hospital - medication β_2, CS	DMFT and dmft score measured according to their age groups	DMFT*: **A** - 6.76,C-5.06 at age 13 yr group

Stensson et al. (2010) Jönkőping, Sweden	Cohort - Case control study N # 114, **A** - 64 & C - 50 at 3 yr and 6 yr	Oral health in preschool children on follow up	Doctor diagnosed asthma with duration of medication & usage of inhaled CS	defs (initial and manifest caries based on clinic examination & 2 bitewing radiograph @ 6yr),plaque index, saliva sampling	**A :** ↑ caries* from 29% to 61%, ↑ in mouth breathing**, ↑ in sugary drinks**
Stensson et al. (2011a) Jönkőping, Sweden	Case-control study **A** - 20, C - 20 (12-16 yr)	Caries & associated factors in chronic asthmatic adolescents.	Diagnosed with asthma along with duration of disease & medication	DF (initial and manifest caries based on clinic examination & 4 bitewing radiograph gingival bleeding & plaque pH + index,	**A:** DF* (initial and manifest caries) - 4.9±5.5. C: 1.4±2.3
Stensson et al. (2011b) Jönkőping, Sweden	Case - control study **A** - 20, C - 20 (18 - 24 yr) C - matched with age, sex.	Oral health in chronic asthmatic adults	Long term asthmatic with Rx (Inhaled β_2, CS > 2yrs).	D (initial + manifest) based on clinic examination & 4 bitewing radiograph),GCF, erosion, salivary analysis, gingival index & plaque pH, index	Caries prevalence ↑ *
Reddy et al. (2003) Mangalore, India	Cross-sectional study **A** - 205 (3-18 yrs)	Caries status in bronchial asthma	Doctor diagnosed asthma with medication (Inhaler, syrup, tablet, combination)	DMFT, deft	Caries prevalence ↑ with syrup form -90.47%**, deft*= 5.02 ±4.05, mixed dentition -3.54 ±2.89, DMFT= 4.83±3.66*.
Boskabady et al. (2012) Mashhad, Iran	Case -control study **A** - 40, C - 40 (20 - 30 yr) C - matched with age, sex, socioeconomic status.	Caries in chronic asthmatic adults or its severity using inhaler / dose/ technique	Physician diagnosed asthma & medication (Medical records), PFT	DMFT index	**A:** ↑ Caries**** in young adults
Anjomshoaa et al. (2009) USA	Cross sectional survey study	Relationship b/w oral health	Self-reported medical history	Data extracted from registry record of	Caries is associated with asthma (DMFT*, DMFS****) ↑

	N # 318 (17-84 yr)	& systemic disease (Asthma, Epilepsy, CVD and diabetes)		Pittsburgh school of dental medicine.	females.
Santos et al. (2012) Brazil	Cross sectional design of case control study A - 40, C - 40 (10 - 18yr) C - matched for age and sex	Association of caries, plaque & salivary flow in asthmatic using inhaled CS	Use of inhaled CS for 3M & inhaled β_2 agonists for 1/wk	DMFT, DMFS, plaque index, salivary flow, pH & leukocyte in saliva	**A**: ↑caries lesion (DMFS)**** and plaque**** and high salivary leucocytes*
Mehta et al. (2009) Mangalore, India	Case control study, **A** - 80, C - 80 (11 - 25 yr) C- matched for age, sex & SES	Dental caries status in young asthmatics	Asthmatic patients receiving Rx (β_2, CS / inhaled device)	DMFT and DMFS index	Significant difference b/w mean of 2 groups DMFT & DMFS****
Esther (1998) England	Case control study. **A** - 100, C - 100 (4-16 yr) C- matched for age, sex & SES.	Prevalence of caries among asthmatic school children	Doctor diagnosed asthma with using inhaler	DMFT, DMFS, dmft, dmfs index	**A**: ↑caries lesion @ 11-16yr DMFT* - 2..48±3.05, DMFS* - 3.39±4.48
Shashikiran et al. (2007), India.	Case control study **A** - 105, C - 106 (6 - 14 yr)	Caries & periodontal status in asthmatic medicated children	Children taking Salbutamol inhaler, tablet, beclamethasone inhaler	DMFT, DMFS, dft, dfs, periodontal index (CPITN)	**A:** DMFT, DMFS was ↑ in Salbutamol inhalers and tablets (Mean ± SD- 1.40 ± 1.19 & 2.00 ± 1.66 respectively**) poor periodontal status.
Samec et al. (2013) Slovenia, USA	Case control study **A** - 220, C - 220 (2 - 17 yr)	To know the influence of anti-asthmatics & other factors on caries	Chronic bronchial asthmatics under Rx in university of children hospital	DMFS and dmfs (ICDAS II)	**A :** ↑ DMFS* & dmfs*

Wogelius et al. (2004) North Jutland, Denmark	Cohort study N # 4,920 (5-7yr)	Caries risk in medicated asthmatics	Asthmatics receiving both inhaled CS and inhaled β2-agonists	Caries in primary canine, molars and in any permanent teeth	Caries increased in newly erupted permanent teeth :RR - 1.45 (95% C.I.: 0.99-2.11)
Kargul et al. (1998) Turkey	Case study (Randomly selected) **A** - 30 (6-14 yr)	Effect of inhaled β2-agonist + CS on saliva + plaque pH	**A** - using inhaler for 1 yr	**A** - 4 to 10 DMFS index. Saliva + plaque pH using inhaler + water + sugar free chewing gum or any combination	**A** with medication (β2 agonist + CS) reduced saliva and plaque pH
(Silverberg and Simpson, 2013) US	National survey of Children's health. Eczema children - 91,642 (0-17yr)	Impact of atopic eczema on atopic / non-atopic co-morbidities	Self / parent reported medical history (Eczema - mild, moderate and severe)	Eczema - Higher prevalence of dental problems* (toothache, broken teeth, bleeding gums and tooth decay).	Atopic dermatitis was associated with impaired dental hygiene including bleeding gums and tooth ache.

Table 15: Summary of studies on positive association between allergic disease and dental caries based on caries + microorganism measurement

1st Author, Geographic Location	Study Design Sample size (Age in year)	Objective	Asthma measure	Caries measure	Outcome / Result
Mazzoleni et al. (2008) Italy	Case-control study **A**: 30, C : 30 (6-12 yr)	Caries among asthmatics on short acting β_2 agonists	Doctor diagnosed asthma with long term medication	DMFT/dmft scores, plaque index, salivary samples - buffering capacity , LB and S.m level	**A :** ↑ DMFT* - 1.2±1.8 & ↓ buffering capacity*, ↑ cariogenic bacteria*

Ryberg et al. (1991) Umea, Sweden	Case control study **A** - 21(14-24yr) C - matched with age, sex, social background	Effect of β_2-adrenergic drugs on salivary gland and teeth. (FOLLOW UP OF PREVIOUS 1987 study)	Chronic asthmatic with medication (β_2), as well with no other disorders	DFS - initial and manifest caries based on OE & bitewing radiograph, whole and parotid salivary rate, saliva sampling (SM and LB culture), Conc. of stimulated parotid saliva.	**A** - ⬇ whole & parotid salivary secretion flow by 20% and 35%, ⬆ LB count***,** higher DFS score (17.6) - initial****** and manifest***** & ⬆ new caries lesion*****
Botelho et al. (2011) Londrina, Brazil	Cross sectional case control study **A** - 80, C - 80 (3 - 15yr) C - matched for age, sex.	Caries in asthmatics due to micro-organism, oral hygiene & caries	Asthmatics under Rx	DMFT, dmft, saliva samples (*MS* & *LB* level), biofilm index	**A :** ⬆ Caries****** in permanent teeth DMFT - 2.11±0.36, biofilm index***** - 1.47±0.06, ⬆ MS***** - 70.40±8.95 CFU/ spatula
Khalilzadeh et al. (2007) Tehran - Iran	Case control study **A** - 45, C - 46 (5 - 15 yr)	Prevalence of caries in asthmatics	Asthmatic patients receiving Rx (β_2-agonists, CS)	DMFT, Saliva sampling - *lactobacilli* & Streptococcus mutans	**A** : Mean DMFT :- 4.30 ± 2.81, ⬆ SM*

A - Asthmatic group, C - Control group, ⬆ - Increase, ⬇ - Decrease, IDDM - Insulin dependent diabetes mellitus, DF(S) - decayed filled surface in permanent dentition, df(s) - decayed filled surface in deciduous dentition, defs - decayed extracted filled surface in primary dentition, deft - decayed extracted filled tooth in primary dentition, PFT - Pulmonary function tests, OR - Odds ratio, RR - Relative risk, OE - Oral examination, OPG - Orthopantomogram, CPI - Community periodontal index, *SM - Streptococcus mutans, LB - Lactobacilli, MS - Mutans Streptococci,* CS - Corticosteroids, NS - Not Significant, * - $p < 0.05$, ** - $p<0.001$.

In summary, the above studies have shown that allergy-associated symptoms mainly asthma is associated with dental caries in both primary and permanent dentition. Though they do not explain whether the disease itself is directly related to caries, they indicate that medication or pharmacotherapy received for asthma treatment may have deleterious effect on oral health. Many authors suggest that they have observed increase in cariogenic microorganism especially *S.mutans* and *LB* among asthmatics which may be the cause for caries and some more authors have indicated that due to medication (especially the inhaler form without proper spacer) the salivary glands may be affected resulting in reduced flow as well altering the composition of saliva resulting in low pH and buffering capacity of saliva.

As well medication in the form of syrup may have more effect as the syrup based medication may contain fermentable carbohydrates which is again more harmful to teeth. Some authors have measured the plaque and mentioned that plaque index is high among asthmatics with or without treatment and also observed decrease in plaque pH. Thereby use of asthmatic medication in the form of inhaler or syrup may have their side effects on salivary glands affecting their pH resulting in more caries lesions among children suffering from asthma.

The potential mechanisms for how allergy-associated symptoms may predispose toward poorer dental hygiene include the following: (a) decreased attention toward flossing and brushing their tooth as parents or children may be pre-occupied with the symptoms and management of their allergy-

associated symptoms (b) long term use of corticosteroid for eczema, rhinitis and asthma either orally, intra-nasal or inhaler form (c) oral antihistamine associated xerostomia (d) potentially increased oral infections secondary to impaired innate or cellular immunity (Silverberg and Simpson, 2013). Future studies are necessary to elucidate these points.

1.5. Knowledge Gap

- Many researchers have explored the effect of allergic disease on dental caries in children aged above 3years but its effect among children below 3 years have not been explored.
- Many studies have been carried out in relation to asthma and its effect on hard / soft tissue of oral cavity but there are not many studies to know the relation between eczema and rhinitis towards caries.
- Although some articles provide pharmacological evidence for the association of both chronic conditions (allergy and caries) but their exact mechanism of action or bio-physiological relationship is not clearly explained.

1.6. Objectives

My objective is to evaluate whether allergy-associated symptoms are linked to dental caries among preschoolers.

The specific aim of this study is to assess the impact of eczema, rhinitis and asthma individually as well their cumulative effect on dental caries among preschoolers aged 2 years and also to appraise if allergy-associated symptoms

with medication has an effect on primary teeth amongst 24 month old preschoolers.

1.7. Hypothesis

1.7.1. Primary hypothesis

Allergy-associated symptoms like eczema, rhinitis and asthma causes increase in early childhood caries among toddlers.

CHAPTER II
MATERIALS and METHODS

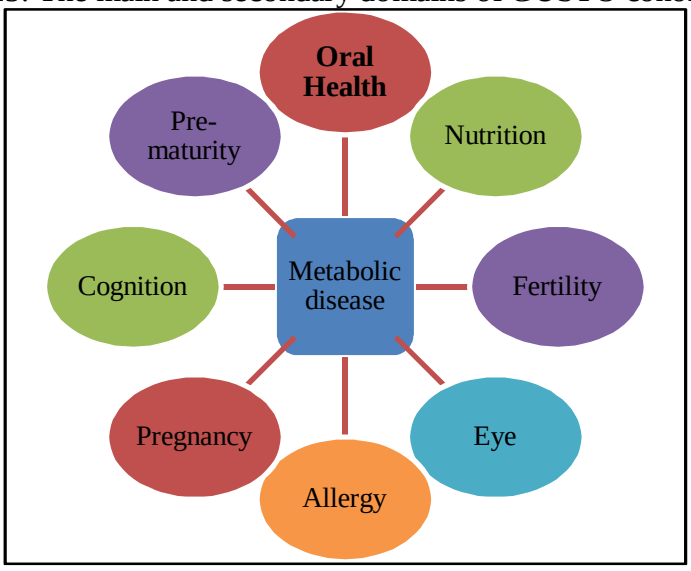
CHAPTER II MATERIALS AND METHODS

2.1. INTRODUCTION TO GUSTO STUDY

Growing Up in Singapore Towards healthy Outcomes (GUSTO) is Singapore's on-going largest birth cohort study. Pregnant women aged ≥ 18 yrs attending their first trimester antenatal dating ultrasound scan at Singapore's two major public maternity units namely KK Women's and Children's Hospital (KKH) and National University Hospital (NUH) between June 2009 and September 2010 were recruited. Participants approached were Singapore citizens or permanent residents who were of Chinese, Malay or Indian ethnicity with homogeneous parental ethnic background and who had intention of eventually delivering in KKH or NUH site and living in Singapore for the next 5 years. Mothers who agreed to donate birth tissues including placenta, cord and cord blood at time of delivery were included and women receiving chemotherapy, psychotropic drugs or who had type I diabetes mellitus were excluded (Soh et al., 2013).

The main aim of GUSTO is to evaluate the role of developmental factors in the early pathways to metabolic compromise namely obesity and type 2 diabetes mellitus. With the intention of being a holistic cohort study, the GUSTO research team is further subdivided into several secondary domains with entity aims to maximise the research opportunities that have arisen shown in Figure 25 (Soh et al., 2012). Oral health is one of the domains and the primary concern of this present study. Parents were asked questions related to tooth eruption status, dental caries, oral hygiene habits, their knowledge towards it and oral examination was conducted at 24 month old.

Figure 25: The main and secondary domains of GUSTO cohort study.



2.2. RECRUITMENT OF SUBJECTS: TARGET POPULATION

Recruitment for the study was completed in September 2010 with 1,163 pregnant women recruited into the main GUSTO cohort (current drop-out rate = 12.1%).

2.2.1. Subjects inclusion and exclusion Criteria.

All pregnant mothers (and their offspring) enrolled in the main GUSTO study were eligible for recruitment into the oral domain. To qualify for GUSTO, mothers had to be Singapore citizens or permanent residents aged 18 years and above who are Chinese, Malay or Indian with homogenous parental ethnic background and have the intention to eventually deliver in the 2 major public maternity units (NUH and KKH) and reside in Singapore for the next 5 years. In addition, only healthy women who also agreed to donate birth tissues (i.e., cord blood, cord and placenta) at delivery were included. Mothers on chemotherapy, psychotropic drugs or with type 1 diabetes mellitus were excluded (Soh et al., 2012).

2.3. ETHICAL CONSIDERATIONS

2.3.1. Ethical Approval

Before the beginning of this study, the protocol and supporting documents have been reviewed and approved by the National University of Singapore Institutional Review Board (NUSIRB) [NUH site - National Healthcare group Domain Specific Review Board (NHG DSRB) Ref: 2009/00021; KKH site - CIRB Ref: 2009/280/D], oral health study at 24 and 36 month time points - 2012/00436). Any change in the document was again sent to NUSIRB for review and approval.

Table 16: DSRB approved documents related to oral health used in this study.

Document	Appendix Number
24 month oral questionnaire (English, Chinese and Malay version)	Appendix I
Participation Information sheet for Parents/Guardians and consent form (English version - NUH and KKH sites)	Appendix II
Oral Examination Record (English version)	Appendix III
Biofilm Sample form (English version)	Appendix IV

2.3.2. Parents Informed consent

2.3.2.1. Consent (1): for participation in the study

Parents were clearly explained about their voluntary participation for this study using the participation information sheet (PIS) written in English, Chinese, Tamil and Malay by calibrated examiners. Complete details of the study were provided in PIS including project title, principal investigator, and significance of this study, procedures followed, their responsibility, risks and benefits of participation, confidentiality of study and contact details of researchers (Appendix II).

The parents were reminded to read the PIS carefully and sign on the Consent form if they are willing to participate. Only the legal parents were approached to endorse, guardians were not considered to sign. From all participants in this study, informed written consent was obtained.

2.3.2.2. Consent (2): for oral examination

In order to further enhance the study, oral plaque samples were collected along with oral examination [Silness - Loe plaque Index and International Caries Detection and Assessment System (ICDAS) score]. Any lesions on mucosa tongue or lips were also noted.

2.4. DATA COLLECTION

2.4.1. Preparations

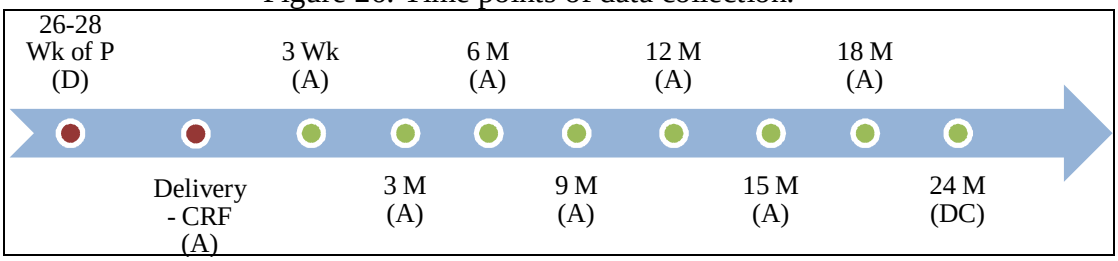
Before the start of data collection, a qualified team was formed and examiners were trained using photographic slides with clinical examples. The criteria and procedures were thoroughly discussed and all the examiners (trained nurses / home visitors) were calibrated. Appointments were given for all the participants at the clinical site with each mother / child coded confidentially. All the facilities required were arranged at both the clinical sites.

2.4.2. Questionnaire Survey

Using questionnaires, maternal and infant data related to demographic background (D) and allergy-associated symptoms (A) was collected at different time points by trained nurses and home visitors (Refer to table # 20

for the list of all variables). Questionnaires related to oral health and data related to caries (outcome variable of interest in this study) was collected at 24M, shown in Figure 26.

Figure 26. Time points of data collection.



- Before delivery, - After delivery, Wk - Weeks, P - Pregnancy, CRF - Case report form, M - Months.

2.4.3. Clinical visit

2.4.3.1. Maternal and Infant data: Questionnaire

Detailed 45 to 60 minutes interview were conducted at the clinical site as well at mother's home. Medical history of mother and child, sociodemographic profile and questionnaires related to allergy-associated symptoms were collected from mother. In cases where mother cannot answer questionnaires, father or grandparents (child caregiver) answered them.

2.4.3.2. Infant data: Oral Examination

After taking consent from parents, oral examination of the subjects were conducted in a knee-to-knee position using torch light, sterilized mouth mirror and a blunt probe / explorer. Mucosa, tongue and lips were also examined for lesions. The light intensity used was the same for all subjects.

All the children were refrained from food, drinks and tooth brushing for at least 1 hour before the oral examination and plaque collection.

(a) Caries status examination: The caries status of each tooth surface was evaluated with examination procedures and diagnostic criteria recommended by ICDAS. ICDAS protocol was followed for dental examination except drying the surfaces for 5 seconds due to time constraints and cooperation of toddlers.

Table 17: Classification of the restoration, sealant / missing status in ICDAS

Code	Description
0	Sound Surface is not restored or sealed.
1	Sealant, partial A sealant that does not cover all pits and fissures on the tooth surface.
2	Sealant, full A sealant that covers all pits and fissures on a tooth surface.
3	Tooth colored restoration The tooth has a colored (resin or glass-ionomer cement) restoration.
4	Amalgam restoration
5	Stainless steel crown
6	Porcelain or gold or PFM crown or veneer
7	Lost or broken restoration
8	Temporary restoration
9	Tooth does not exist or other special cases, Used in as the following: 96 = Tooth surface cannot be examined: surface excluded 97 = Tooth missing because of caries (tooth surfaces will be coded 97) 98 = Tooth missing for reasons other than caries (all tooth surfaces will be coded 98) 99 = Un-erupted (tooth surfaces coded 99)

Modified from (Ismail et al., 2007)

Table 18: ICDAS Caries Codes

Code	Description
0	Sound
1	First visual change in enamel (seen only after 5-second air drying or restricted to within the confines of a pit or fissure)
2	Distinct visual change in enamel
3	Localized enamel breakdown (without clinical visual signs of dentinal involvement)
4	Underlying dark shadow from dentin (no cavity)
5	Distinct cavity with visible dentin
6	Extensive distinct cavity with visible dentin

Modified from (Ismail et al., 2007)

The ICDAS protocol is as follows, ask patient to remove any removable appliances, clean the tooth surface, remove excess/frothy saliva, visual examination of the surface wet, dry the surface for 5 seconds and finally visual examination of the dry surface (ICDAS). As dental examination was done at NUH/KKH clinical site, air was not available for drying the tooth for 5 seconds; instead, gauze was used to dry and to remove the excess plaque from the tooth surface. Using sterilized mouth mirror, tooth status was evaluated by the examiner, which was based on visual examination, if doubt arised it was confirmed by tactile inspection using and blunt probe/explorer. To avoid damage to the sound enamel surfaces probe was used with cautious. After plaque collection, the debris obscuring the visual inspection was removed using sterile gauze as the teeth was either cleaned / dried before the assessment. A recording chart was used to register the status of all the tooth surfaces (Appendix III).

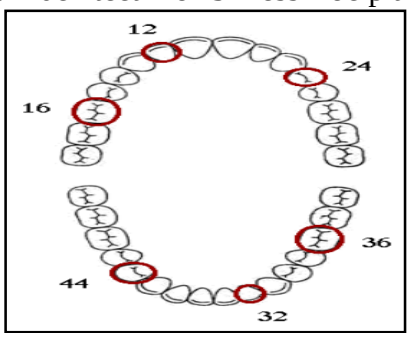
If any part of the tooth or its surface was not clearly visible for examination, it was recorded as "tooth erupting". Caries was scored as present if there was presence of first or distinct visual change in enamel, breakdown of enamel, underlying dentinal shadow, distinct or extensive distinct cavity with visible dentin. A tooth was recorded as filled when partial or full sealants or tooth colored restorations was present; as extracted only when normal exfoliation would not be a sufficient explanation for the absence of the tooth at that age.

(b) Oral hygiene status examination

The oral hygiene status was evaluated using the modified Silness-Löe plaque index (PI) (Silness and Loe, 1964). For PI, six teeth (55, 52, 64, 75, 72 and 84)

were selected to closely simulate the original index teeth (16, 12, 24, 36, 32 and 44). When the designated teeth were missing or un-erupted, an alternative tooth in the same quadrant was taken as substitute.

Figure 27: Index teeth for Silness-Löe plaque index



Source from Silness and Loe (1964)

The amount of plaque on four surfaces (buccal/labial, lingual/palatal, mesial and distal) of the 6 teeth was scored. To represent the oral hygiene status of that tooth the average score of the 4 surfaces was calculated, while to represent the oral hygiene status of that individual the average score of the entire 6 index tooth was calculated.

Table 19: Scoring criteria for Silness-Löe Index

Scores	Criteria
0	No plaque
1	A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen in situ only after application of disclosing solution or by using the probe on the tooth surface.
2	Moderate accumulation of soft deposit s within the gingival pocket, or the tooth and gingival margin, which can be seen with the naked eye.
3	Abundant of soft matter within the gingival pocket and or on the tooth and gingival margin.

2.4.4. Information for parents after data collection.

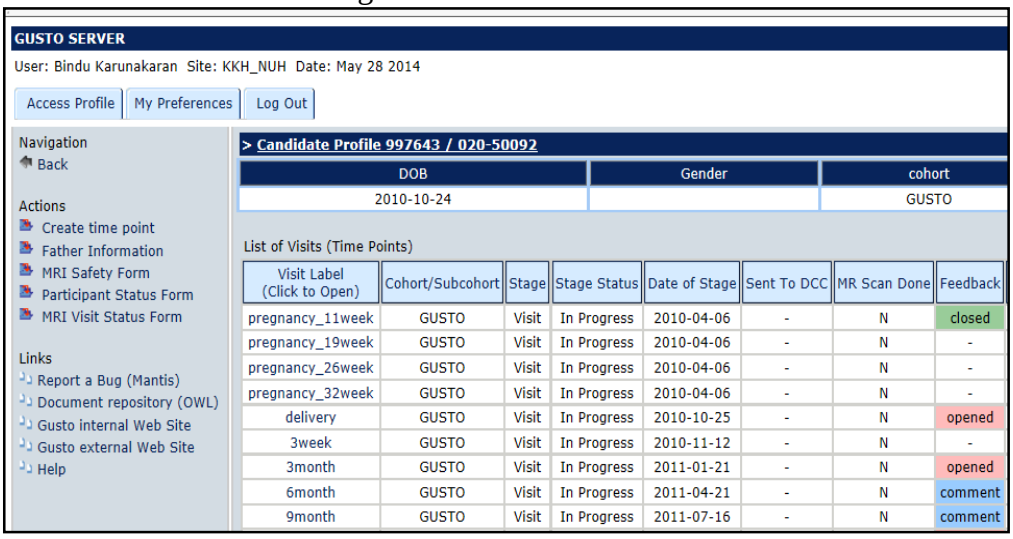
After completing the oral examination and sample collection, parents were informed about their child oral health status and instructions were also

given about tooth brushing and dietary habits. In cases where treatment is required, parents were encouraged to take the child to a dentist. In regards to general health of child, child health diary was also given to the parents at 36 months so they can monitor child progress in growth.

2.5. DATA ENTRY AND VERIFICATION

A separate database named LORIS was created and maintained by the data team. The first round of data entry (recorded in the hard copy) was done by the examiners themselves and answers to questionnaires were also directly entered by the staff during interview session at the clinical site in LORIS online database. Later, second and third round of entry was done by GUSTO administrators to ensure internal validity of data. Only domain members and data coordinators were given access to this database. Data manager gave individual login id and password to all in charge staff and necessary actions were taken to ensure confidentiality of data. Cross verification of the data was done by the domain leaders at regular intervals before proceeding to data analysis.

Figure 28: LORIS database



2.6. DATA ANALYSIS

Oral health domain is under the main GUSTO birth cohort study, Early childhood caries data was collected for 543 (43.9%) cases at month 24 using ICDAS scoring criteria due to the time constraints. To ensure that the sample size is big enough and to avoid type I and II error in this study, power analysis calculation was done. The sample size of 543 will provide a precision of ± 4% on the 95% confidence interval on the prevalence of caries obtained and a 90% power with a 2 sided 5% to show at least a predictor risk of 2.

2.6.1. Dealing with missing data

For both descriptive and inferential analysis, all the missing data were not replaced nor removed, thereby the sample size was slightly different for each tested variables.

2.6.2. List of all variables used in this study.

The below table shows the list of all independent and potential confounding variables used in this study. The dependent variable, dental caries (dmft and dmfs) was considered as count and dichotonomous variable. During analysis, all the below variables shown in table 20 were taken into consideration.

Table 20: Variables used in this study.

Independent Variables	Confounding Variables
1. Child had sneezing, blocked or running nose for ≥ 2 wks (Yes/no) 2. Skin prick test (Positive / negative) 3. Child diagnosed with eczema (Yes/no). 4. Child had wheezing	1. Mother suffer from asthma (Yes/no) 2. Mother suffer from eczema (Yes/no) 3. Mother had sneezing, blocked/ running nose (Yes/no) 4. Child use any oral medicine (Yes/no)

(Yes/no). 5. Child used nebulizer (>12<24M, <12M, never)	5. Ever used topical steroids (>12<24M, <12M, never) 6. Child - oral health (dietary habits) a. Child brushing habits- (>once/ day, once a day, never) b. Frequent snacks at night time (Frequently or almost every night, occasionally, never) c. Frequent snacks / drinks between meals during day time (> once/day, once a day, never) 7. Gender (Male/Female) 8. Ethnicity (Chinese/Malay/Indian) 9. Child's Medical health - suffer from any disease other than allergic condition (Yes/No) 10. Household Income (≥ 4,000, <4,000) 11. Mother educational level (Tertiary / non-tertiary) 12. Mode of delivery (C-section/vaginal) 13. Breast feeding during sleep (>12<24M, <12M, never) 14. Bottle feeding habit during sleep (>12<24M, <12M, never) 15. Plaque Index (continuous)

2.6.3. Selection of Statistical Model

Before we proceeded with the model selection, frequency of all variables and univariate analysis was done to verify if the variables can be selected into the multivariate model. Based on these results from univariate analysis, coding of all the independent variables was done and variables was taken into the multivariate analysis.

We also encountered issues with the main independent variables - eczema, asthma and rhinitis as they were correlated to each other. Thereby we

considered the analysis in two different models like if the child had ever-had or suffered from allergy-associated symptoms at all time points, ensuring the rest of the variables are same.

The outcome variable was collected as a value based on a count from a set of distinct whole values and as the outcome variable value cannot take the value of a fraction between one value and the next closest value, therefore we considered the outcome variable as a count variable rather than continuous variable. Thereby, we did not use the generalized linear model.

As well in this study, the dependent variable was a single variable and as generalized estimating equation (GEE) method is an inferential procedure concerning the marginal mean of a multivariate outcome through regression models (Paik and Lee, May 2010) we did not consider the GEE, which is also used to estimate the parameters of a generalized linear model with a possible unknown correlation between outcomes.

Outcome variable was considered as categorical and count variable - number of teeth decayed (dmft) and number of caries lesion or surface decayed (dmfs) to analyse the data using logistic and poisson regression models respectively. Univariate and multivariate logistic regression analysis and poisson regression analysis with negative binomial distribution were performed to understand more about the important variables and collinearity.

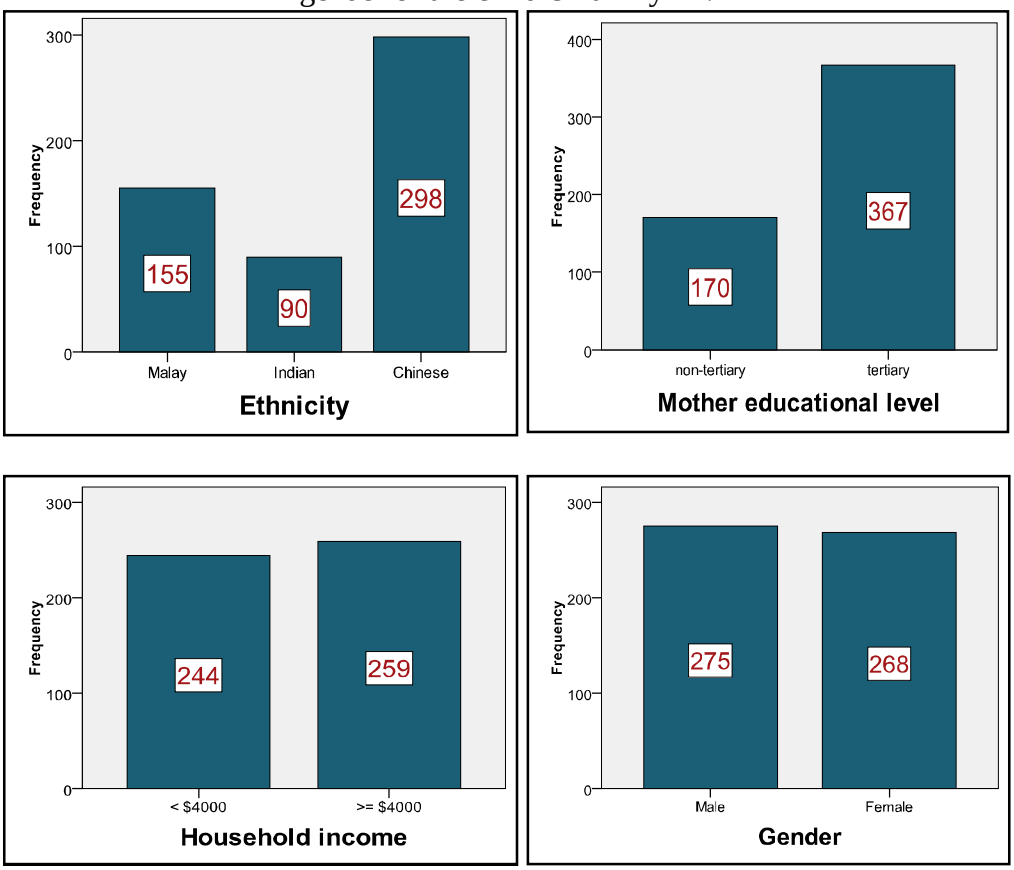
CHAPTER III
RESULTS

CHAPTER III RESULTS

3.1. Demographic and socioeconomic profile of GUSTO cohort study

In the main GUSTO study, 1237 pregnant women were recruited consisting of different ethnical background, household income and educational background. Under oral health domain, sample size was 543 children, of which 54.9% were Chinese followed by Malay (28.5%), Indian (16.6%). Figure 29 shows the distribution of GUSTO population according to ethnicity, mother's educational level, gender and household income (HI).

Figure 29: Distribution of subjects by ethnicity, maternal educational level, gender of the child & family HI.

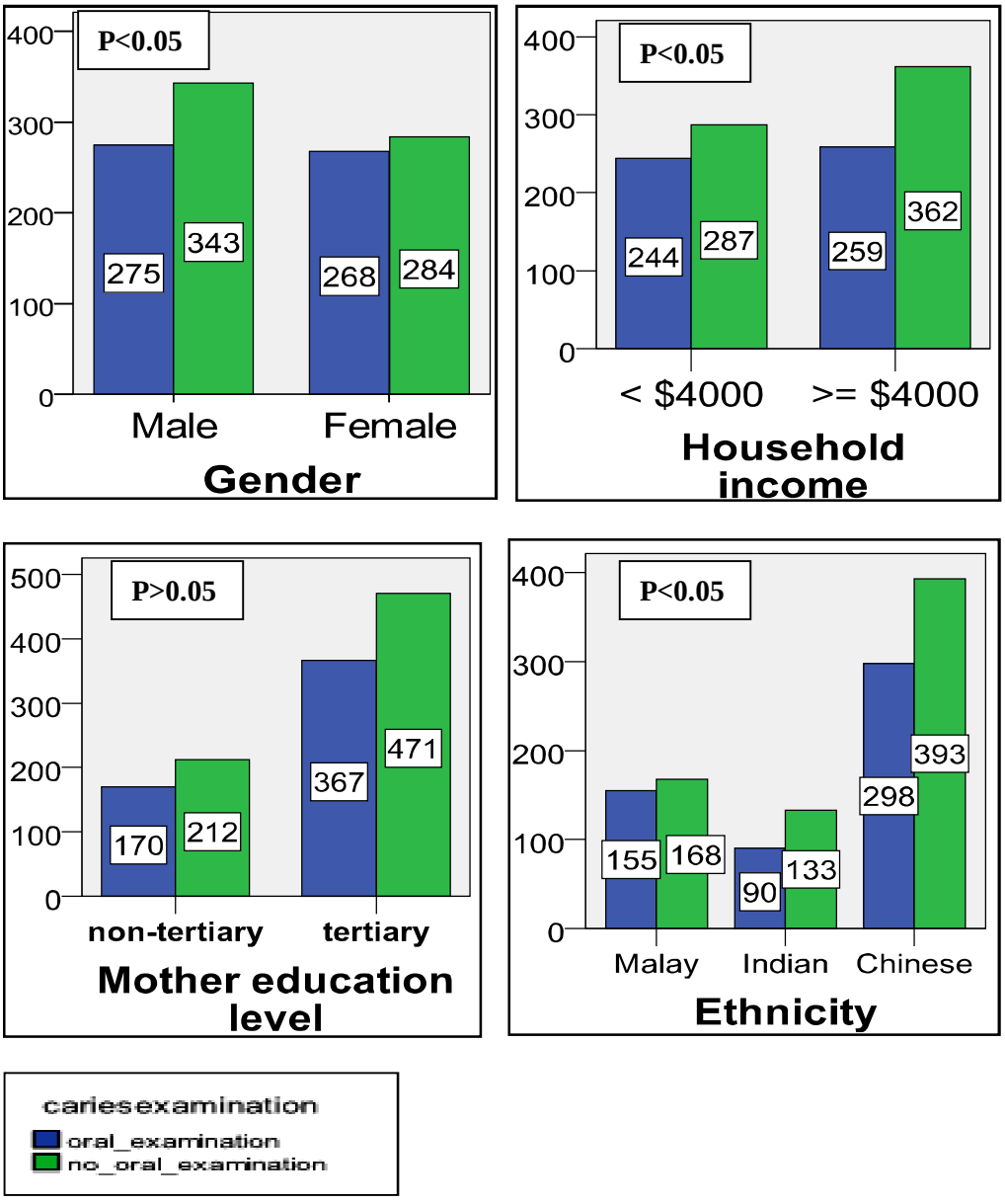


The study sample under oral health domain was representative of the national population, in regards to distribution by ethnicity, mother's educational level and household income. Majority of the parents were Chinese, household

income were ≥ S$ 4,000 and greater part of mother's had received tertiary level of education.

In comparison to the main GUSTO study sample size, oral health domain sample size was less. Figure 30 demonstrates the difference in socioeconomic maternal factors and demographic factors between children participated and not participated under oral health study.

Figure 30: Distribution of difference in children participated and not participated in oral health study.



3.2. Inferential data from multi-variate regression

3.2.1. Association of Allergic diseases on Early Childhood Caries

3.2.1.1. Distribution of outcome variable - ECC according to the various variables included in the study.

The descriptive (frequency) of number of teeth and surfaces affected by dental caries; number of teeth and surfaces erupted in oral cavity at 24M time point is shown in table 21-24.

Figure 31: Frequency of number of dmft score

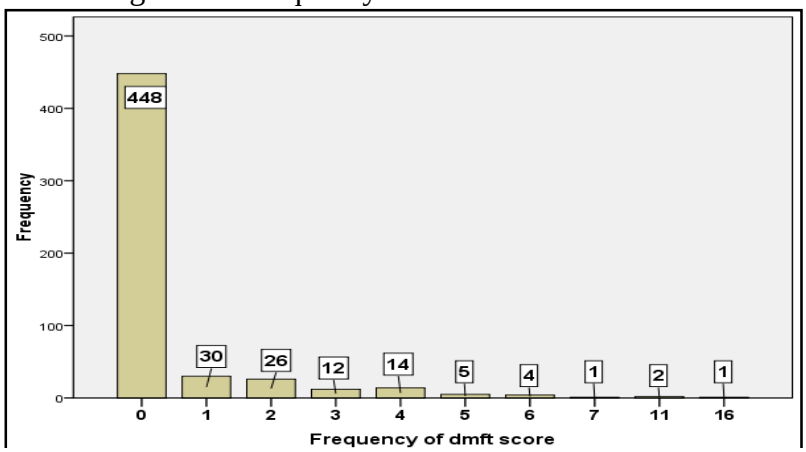


Figure 32: Frequency of number of teeth erupted

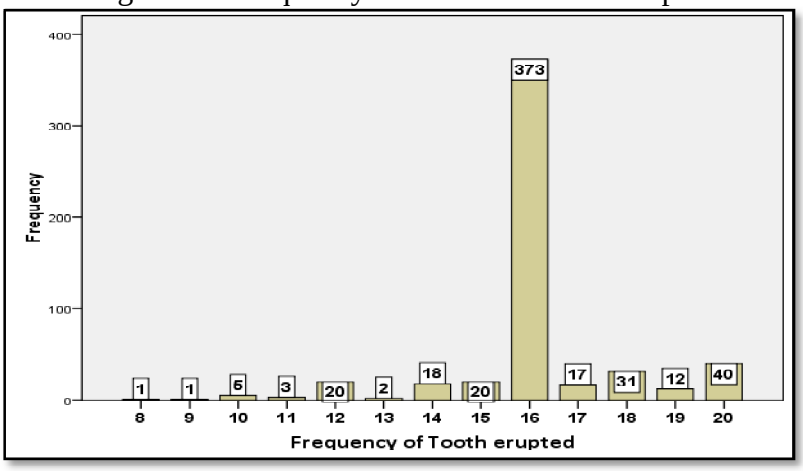


Figure 33: Frequency of number of surfaces decayed

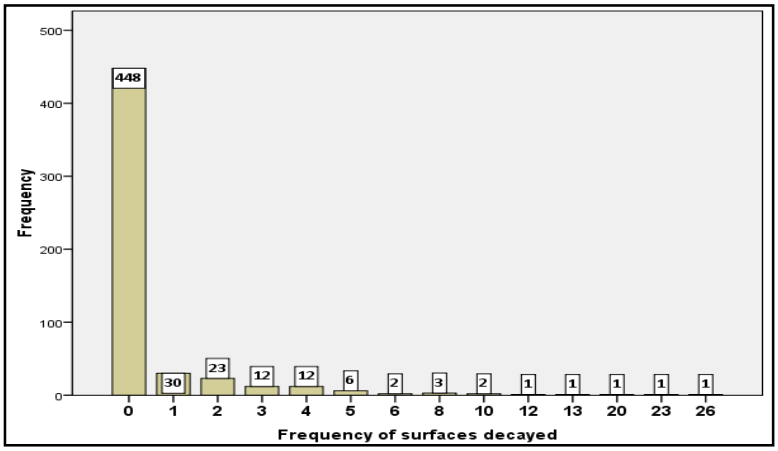


Figure 34: Frequency of number of surfaces erupted

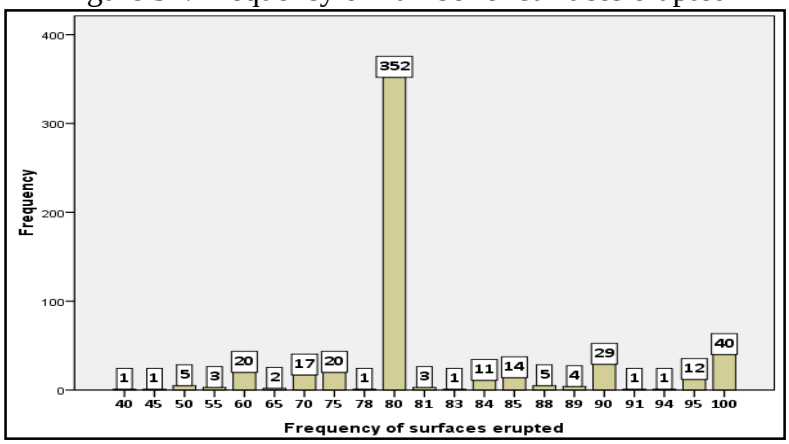


Table 21 shows the frequencies of ECC according to demographic variables.

Table 21: Frequency of ECC in relation to confounder variables

Demographic variable		ECC - N (%)		Total sample size (N)
		No	Yes	
Ethnicity	Chinese	243 (54.4%)	55 (57.3%)	543
	Indian	80 (17.9%)	10 (10.4%)	
	Malay	124 (27.7%)	31 (32.3%)	
Mother Educational level	Tertiary	309 (69.8%)	58 (61.7%)	537
	Non-Tertiary	134 (30.2%)	36 (38.3%)	
HI	< $ 4,000	207 (50.5%)	52 (55.9%)	503
	≥ $ 4,000	203 (49.5%)	41 (44.1%)	
Gender of Child	Male	220 (49.2%)	55 (57.3%)	543
	Female	227 (50.8%)	41 (42.7%)	
Mode of Delivery	C-section	134 (30%)	25 (26%)	543
	Vaginal delivery	313 (70%)	71 (74%)	

3.2.1.2. Preliminary Analysis - First Approach

In the preliminary round of analysis, the caries data was considered as counts and binary variable, to explore the most significant variables according to three types of allergy-associated symptoms - asthma, eczema, rhinitis and as well as cumulative, all the independent variables listed in table 26 were taken into consideration.

Table 22: List of all variables in the preliminary round of analysis

Outcome variables for all below hypothesis - Caries as Count and Categorical variable	
Hypothesis 1a: There is a significant association between caries and asthma	
Input variables(IV):	Child had wheezing, if yes, how old, child diagnosed asthma.
Potential confounders (PC):	Mother suffer from asthma, how many attacks of wheezing, has child diagnosed bronchitis, child used nebulizer, Mouth breathing.
Hypothesis 1b: There is a significant positive link between caries and rhinitis	
IV:	Child had sneezing, blocked/running nose for 2 wks, If yes, hw old, Child diagnosed with rhinitis
PC:	Mother had sneezing, blocked/running nose, child diagnosed bronchitis, mouth breathing.
Hypothesis 1c: There is a significant positive link between caries and eczema	
IV:	Child diagnosed with eczema
PC:	Mother suffers from eczema, child had itchy rash, did you use oral medicine to treat, ever used topical steroids).
Hypothesis 1d: There is a significant association between early childhood caries and allergic disease (Cumulative and at time points)	
IV:	Child had sneezing, blocked/running nose for 2 wks, child diagnosed with rhinitis, eczema, asthma, child had wheezing.
PC:	Mother suffer from asthma, eczema, sneezing, blocked/ running nose, child had itchy rash, use oral medicine to treat, ever used topical steroids. Child diagnosed bronchitis, mouth breathing, child used nebulizer, skin prick test.
Common confounders for all hypothesis	Gender, ethnicity, child's health, HI, mother educational level, mode of delivery, frequent snacks/drinks between meals, tooth brushing habits, breast or bottle feeding during sleep at night, have frequent snacks and sleep without brushing.

In this study, very few people (50/1237) had answered mouth breathing variable, it was found that none of them had mouth breathing habit. Though it is an important factor, we removed it from the final model due to low sample size, data shown in table 23.

Table 23: Frequency of Mouth breathing habit

Mouth breathing habit	Frequency	Percent
Missing	1185	95.8
No	50	4.0
Not answered	1	0.1
Not applicable	1	0.1
Total	1237	100 %

Hypothesis 1a, 1b and 1c was initially proposed and tested to find if there is any association between them individually. After analysing the preliminary results, assessment was done and hypothesis 1d - research question of this thesis (primary hypothesis), was taken into consideration to know the effects of allergy-associated symptoms like wheezing, itchy rash and running nose more than or equal to 2 weeks of cumulative effect and at various time-points on ECC among Singapore toddlers.

3.2.1.3. Final model and analysis.

To test the hypothesis 4, final model was constructed after preliminary round of analysis. In this final model, all the allergy-associated symptoms - asthma, eczema and rhinitis were considered at all time points and also as ever-had till 18M. The outcome variable (OV) was taken as dmfs; list of all the variables is shown in table-24.

At last, in this final model (OV - dmfs) for ever-had till 18M - 308 (24.9%) cases and for allergic history taken at all time points until 18M - 271 (21.9%) cases were included for poisson regression with incidence model, reduced sample size could be due to subjects missing, not answered or not applicable at different time points. Results from this final model showed the prevalence of caries among Singapore toddlers at 24M was 17.7%. The results of outcome

variable (OV) - dmft (Poisson regression with incidence model) and main independent variables of ever-had history and collected at various time points models are shown in appendix V. Table 24 shows the frequency distribution of all the variables in this final analysis.

Table 24: Frequencies of all variables used in Final Model (Ever-had history)

List of all Variable		No. of cases included - 308
Ever-had eczema	yes	50 (16.2%)
	no	258 (83.8%)
Ever-had Wheezing with Nebulizer	yes	35 (11.4%)
	no	273 (88.6%)
Ever-had Rhinitis	yes	87 (28.2%)
	no	221 (71.8%)
Skin prick test	positive	41 (13.3%)
	negative	267 (86.7%)
Frequent snacks / drinks given btw meals during day time at 24M	> once a day	114 (37%)
	once a day	138 (44.8%)
	none	56 (18.2%)
Frequent snacks / drinks given at night time - 24M	frequently or almost every night	81 (26.3%)
	occasionally	76 (24.7%)
	never	151 (49%)
Tooth brushing habits	> once a day	137 (44.5%)
	once a day	124 (40.3%)
	none	47 (15.3%)
Topical steroid at 18M	>12 months	56 (18.2%)
	≤12 months	20 (6.5%)
	never	232 (75.3%)
Medical condition	≤12months	104 (33.8%)
	never	204 (66.2%)
Dry mouth	yes	11 (3.6%)
	never	297 996.4%)
Oral medicine	yes	7 (2.3%)
	never	301 (97.7%)
Ethnicity	Malay	87 (28.2%)
	Indian	43 (14%)
	Chinese	178 (57.8%)
Mother education level	non-tertiary	93 (30.2%)
	tertiary	215 (69.8%)
Household Income	< $4000	142 (46.1%)
	≥ $4000	166 (53.9%)
Gender	Male	154 (50%)
	Female	154 (50%)

Mode of delivery - Vaginal*	c-section	90 (29.2%)
	vaginal delivery	218 (70.8%)
Breast fed at night	>12 months	37 (12%)
	≤ 12 months	16 (5.2%)
	never	255 (82.8%)
Bottle fed at night	>12 months	170 (55.2%)
	≤ 12 months	27 (8.8%)
	never	111 (36%)
Mother suffer from asthma	yes	41 (13.3%)
	no	267 (86.7%)
Mother suffer from eczema	yes	36 (11.7%)
	no	272 (88.3%)
Mother suffer from rhinitis	yes	90 (29.2%)
	no	218 (70.8%)

Using the final model with all the main and confounding variables, poisson regression analysis with incidence model was done to achieve maximum accuracy in the results. Table 25 shows the results of poisson regression with incidence model, OV - dmfs and independent variables - ever-had allergic history until 18M, sample size - 308.

Table 25: Results from Poisson regression of ever-had model - dmfs.

Independent variables		P value	RR	CI lower	CI upper
Ever-had eczema - No*	**Yes**	**<0.001**	**5.97**	**2.87**	**12.43**
Ever-had Rhinitis - No*	Yes	0.971	1.01	0.48	2.14
Skin prick test 18M - No*	Positive	0.982	1.01	0.40	2.54
Ever-had Wheezing+ Nebulizer - No*	Yes	0.932	0.95	0.27	3.35
Frequent snacks / drinks given btw meals during day time No*	> Once/day	0.767	0.87	0.35	2.19
	Once a day	0.367	1.45	0.64	3.27
Frequent snacks / drinks given at night time - No*	**>Once/day**	**0.003**	**2.45**	**1.37**	**4.40**
	Once a day	0.132	1.71	0.85	3.43
Tooth brushing habits	> Once/day	0.584	1.28	0.53	3.07
	Once a day	0.983	0.99	0.43	2.26
Topical steroid - No*	**>12 <18 M**	**0.011**	**0.24**	**0.08**	**0.72**
	≤ 12M	0.177	1.76	0.77	3.98
Medical condition - No*	Yes	0.951	1.02	0.57	1.81
Dry mouth - No*	Yes	0.267	1.66	0.68	4.07

Oral medicine - No*	Yes	0.221	0.46	0.13	1.60
Ethnicity - Chinese*	Malay	0.781	0.90	0.42	1.90
	Indian	0.941	0.95	0.23	3.82
Mother education - tertiary*	**Non - tertiary**	**<0.001**	**3.19**	**1.75**	**5.84**
HI ≥ $4,000*	< $ 4,000	0.531	1.27	0.60	2.67
Gender - Female*	Male	0.234	0.72	0.42	1.23
Mode of delivery - Vaginal*	C-section	0.887	0.95	0.48	1.88
Breast fed at night - No*	**>12 <18 M**	**0.006**	**4.45**	**1.53**	**12.96**
	≤ 12M	**<0.001**	**4.87**	**2.08**	**11.42**
Bottle fed at night - No*	>12 <18 M	0.158	1.59	0.83	3.04
	≤ 12M	0.49	1.49	0.48	4.68
Plaque Index		**<0.001**	**2.84**	**1.91**	**4.22**
Mother asthma - No*	Yes	0.886	0.90	0.22	3.64
Mother eczema - No*	**Yes**	**0.01**	**0.07**	**0.01**	**0.55**
Mother running nose - No*	Yes	0.858	0.94	0.47	1.86

* - Reference group

Table 26 shows the results of poisson regression with incidence model, OV - dmfs and independent variables - allergic history taken at all time points until 18M, Sample size - 271.

Table 26: Results from Poisson regression of allergic history taken at all time points until 18M model - dmfs.

Independent variables		P value	RR	CI lower	CI upper
Eczema 3M - No*	Yes	0.917	0.909	0.151	5.461
Eczema at 6M - No*	Yes	0.903	1.117	0.187	6.676
Eczema at 12M - No*	**Yes**	**0.003**	**6.976**	**1.922**	**25.321**
Eczema at 15M - No*	Yes	0.074	0.168	0.024	1.187
Eczema at 18M - No*	**Yes**	**0.001**	**6.4**	**2.096**	**19.541**
Rhinitis at 3 wk - No*	Yes	0.444	0.369	0.029	4.743
Rhinitis at 3M - No*	Yes	0.657	0.538	0.035	8.341
Rhinitis at 6M - No*	Yes	0.62	0.479	0.026	8.759
Rhinitis 9M - No*	Yes	0.096	0.252	0.05	1.278
Rhinitis 12M - No*	Yes	0.265	2.086	0.572	7.608
Rhinitis 15M - No*	Yes	0.481	1.86	0.331	10.468
Rhinitis with skin prick test 18M - No*	Positive	.	1.19E-10	0	0

Wheezing + Nebulizer at 6M - No*	Yes	0	3.35E-11	4.94E-12	2.27E-10
Wheezing + Nebulizer at 9M - No*	Yes	0	1.86E-11	1.69E-12	2.04E-10
Wheezing + Nebulizer at 12M - No*	Yes	0.383	1.907	0.447	8.144
Wheezing + Nebulizer at 15M - No*	Yes	0	1.65E-10	2.75E-11	9.93E-10
Wheezing + Nebulizer at 18M - No*	**Yes**	**0.049**	**6.355**	**1.008**	**40.088**
Frequent snacks / drinks given btw meals during day time - M24 - No*	> Once/day	0.835	0.913	0.386	2.157
	Once a day	0.81	0.912	0.43	1.934
Frequent snacks / drinks given at night time - M24 - No*	**> Once/day**	**0.001**	**3.425**	**1.695**	**6.922**
	Once a day	**0.028**	**2.4**	**1.098**	**5.246**
Tooth brushing habits	> Once/day	0.202	2.009	0.688	5.872
	Once a day	0.98	1.011	0.42	2.435
Topical steroid at 18M - No*	**>12 <18 M**	**0.023**	**0.246**	**0.073**	**0.826**
	≤ 12M	**0.007**	**3.314**	**1.389**	**7.905**
Medical condition - No*	Yes	0.713	0.863	0.395	1.888
Dry mouth - No*	Yes	0.079	2.425	0.904	6.507
Oral medicine - No*	Yes	0.198	0.124	0.005	2.978
Ethnicity - Chinese*	Malay	0.932	1.037	0.45	2.392
	Indian	0.998	0.998	0.223	4.463
Mother education - tertiary*	**Non - tertiary**	**0.009**	**2.226**	**1.217**	**4.069**
HI ≥ $4,000*	< $ 4,000	0.892	1.064	0.437	2.589
Gender - Female*	Male	0.907	0.962	0.501	1.849
Mode of delivery - Vaginal*	C-section	0.915	1.044	0.473	2.303
Breast fed at night - No*	**>12 <18 M**	**0.023**	**3.972**	**1.211**	**13.026**
	≤ 12M	**0.001**	**9.204**	**2.493**	**33.986**
Bottle fed at night - No*	>12 <18 M	0.25	1.629	0.709	3.744
	≤ 12M	0.463	1.531	0.491	4.772
Plaque index		**<0.001**	**3.564**	**2.295**	**5.535**
Mother asthma - No*	Yes	0.43	1.8	0.418	7.757
Mother eczema - No*	**Yes**	**0.034**	**0.141**	**0.023**	**0.866**
Mother running nose - No*	**Yes**	**0.053**	**0.453**	**0.203**	**1.011**

* - Reference group

In both these models, children who ever-had eczema till 18M and children who had diagnosed with eczema during 9M - 12M and 15M - 18M seemed to have increased risk of having one more caries lesion ($P<0.05$). Children who had suffered from wheezing and doctor prescribed nebulizer during 15M -

18M also have shown to be at risk for having one more caries lesion compared to children who did not suffer from wheeze and used nebulizer (P<0.05). Consistently, significant higher dental caries risk was observed among children who were breastfed at night till 18M, having frequent snacks or drinks given at night time either once or more than once a day and who have used topical steroid till 1 year of age, as well among children whose mother have non - tertiary educational level (P<0.05). Chances of having one more caries lesion increased with plaque score, statistically significant (P<0.001). Logistic regression was done to verify if the model shows similar results, Table 27 shows the results of outcome variable - dental caries (yes/no) with all the independent variables including the maternal medical history.

Table 27: Results from logistic regression, OV - dental caries (Yes/No).

Independent variables		P value	OR	CI lower	CI upper
Eczema 3M - No*	Yes	0.666	0.495	0.02	12.041
Eczema at 6M - No*	Yes	0.678	0.663	0.096	4.601
Eczema at 12M - No*	Yes	0.233	3.35	0.46	24.399
Eczema at 15M - No*	Yes	0.64	0.581	0.06	5.643
Eczema at 18M - No*	**Yes**	**0.067**	**4.153**	**0.907**	**19.013**
Rhinitis at 3 wk - No*	Yes	0.984	1.023	0.107	9.743
Rhinitis at 3M - No*	Yes	0.699	0.67	0.089	5.077
Rhinitis at 6M - No*	Yes	0.569	1.67	0.286	9.751
Rhinitis 9M - No*	Yes	0.209	0.207	0.018	2.418
Rhinitis 12M - No*	Yes	0.349	0.247	0.013	4.605
Rhinitis 15M - No*	Yes	0.571	1.72	0.263	11.232
Rhinitis with skin prick test 18M - No*	Positive	1	0	0	.
Wheezing + Nebulizer at 6M - No*	Yes	0.999	0	0	.
Wheezing + Nebulizer at 9M - No*	Yes	1	0	0	.
Wheezing + Nebulizer at 12M - No*	Yes	0.641	2.167	0.084	56.076
Wheezing + Nebulizer at 15M - No*	Yes	0.999	0	0	.
Wheezing + Nebulizer at 18M - No*	Yes	0.442	3.538	0.141	88.667
Frequent snacks / drinks	> Once/day	0.489	0.68	0.228	2.027

given btw meals during day time - M24 - No*	Once a day	0.314	0.59	0.211	1.648
Frequent snacks / drinks given at night time - M24 - No*	> Once/day	0.77	1.152	0.446	2.979
	Once a day	0.103	2.155	0.856	5.424
Tooth brushing habits	> Once/day	0.862	1.112	0.337	3.67
	Once a day	0.585	1.385	0.431	4.454
Topical steroid at 18M - No*	>12 <18 M	0.156	0.391	0.107	1.43
	≤ 12M	**0.033**	**5.262**	**1.144**	**24.212**
Medical condition - No*	Yes	0.909	0.952	0.41	2.209
Dry mouth - No*	Yes	0.83	1.229	0.187	8.072
Oral medicine - No*	Yes	0.585	1.98	0.171	22.92
Ethnicity - Chinese*	Malay	0.559	0.74	0.27	2.03
	Indian	0.922	0.949	0.328	2.747
Mother education - tertiary*	Non - tertiary	0.095	2.074	0.881	4.88
HI ≥ $4,000*	< $ 4,000	0.13	0.52	0.223	1.214
Gender - Female*	Male	0.578	1.241	0.579	2.662
Mode of delivery - Vaginal*	C-section	0.364	1.472	0.638	3.393
Breast fed at night - No*	>12 <18 M	0.054	3.188	0.979	10.379
	≤ 12M	0.218	2.916	0.531	16.006
Bottle fed at night - No*	>12 <18 M	0.901	1.059	0.431	2.599
	≤ 12M	0.577	1.496	0.363	6.158
Plaque Index		**0.018**	**2.358**	**1.161**	**4.792**
Mother asthma - No*	Yes	0.807	1.183	0.308	4.538
Mother eczema - No*	**Yes**	**0.023**	**0.139**	**0.025**	**0.763**
Mother running nose - No*	Yes	0.204	0.537	0.206	1.401

In this analysis, results have shown that main allergy-associated symptoms like running nose > 2weeks, wheezing with nebulizer and eczema till 18M did not increase the caries lesion, however trend towards significance was observed with eczema at 18M. Children using topical steroid until 12M and children with higher plaque score may tend to have increase in caries lesion ($P < 0.05$). Child with maternal eczema history has shown to have decrease in caries lesion ($P<0.05$). Logistic regression analysis was done for outcome variable dental caries - yes / no (<2 and ≥2, <3 and ≥3) to know if there is any association with severity of caries, results are shown in appendix VI.

CHAPTER IV

DISCUSSION

CHAPTER IV: Discussion

Singapore is a city-state and island country in Southeast Asia with a diverse ethnic resident population of approximately 5.3 million population (Statistics, 2014). The majority (74.2%) of the resident population are Chinese ethnic group followed by Malays 13.3%, Indians 9.2% and others (3.3%) (MOH, 2014). The multiethnic nature of this study was a great advantage where we could assess the impact of ethnicity on early childhood caries with various potential confounders. Furthermore, it is also a great platform to study the window period of exposure of allergic diseases on ECC. This study is the largest prospective cohort study in Singapore with the following main findings; ECC is a severe oral health problem affecting 17.7% of toddlers. The knowledge and attitude towards oral health needs to be encouraged especially in breast feeding habits, knowledge about fluoride, skills about oral hygiene and dental care services utilization.

4.1. Oral Examination

4.1.1. Examination methods

Oral examination was conducted at 24M clinical visit and performed by a trained and calibrated dental professionals using mouth mirror, ball ended probe and tweezer. Status of teeth was diagnosed using mouth mirror; ball ended WHO probe was used to confirm the diagnosis in doubt cases by tactile sensation. Throughout the procedure, sharp probe was not used to ensure the tooth structure is not disturbed or destructed. As examination was not conducted at the dental clinical site, sterile gauze was used instead of suction to control the saliva.

4.1.1.1. Diagnostic criteria

In this study, ICDAS scores with epidemiological criteria were used. The main advantage in using ICDAS was, it can detect both cavitated and non-cavitated lesions, in other words it enables the detection of all stages of caries lesion (ICDAS) and also includes the initial white spot lesions in the classification. Based on the staging of caries activity, we can clearly differentiate whether it is initial, moderate and severe stage of decay and the treatment planning can be done accordingly.

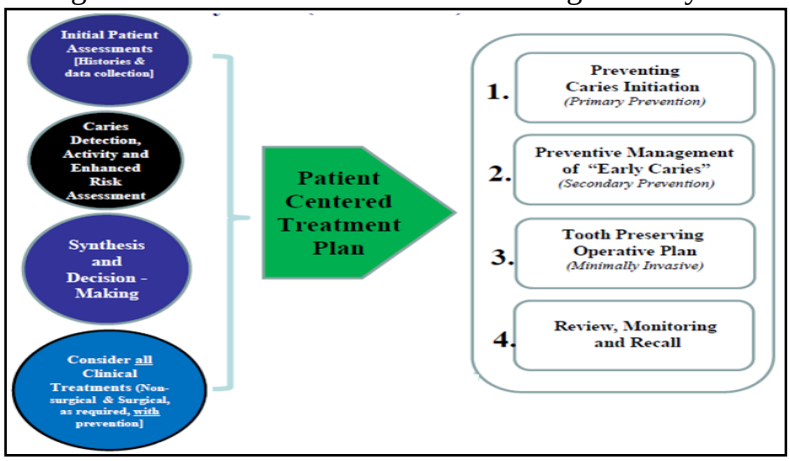
4.1.1.2. Caries Incidence

Dental caries is the most common chronic childhood disease (Oral Health in America: A Report of the Surgeon General—Executive Summary.), prevalent infectious disease in the world (Milano et al., 2006) and one of the leading cause for years lived with disability (Vos et al., 2012). Despite great improvements in the oral health in several countries, global problems still persist. The burden of oral disease is particularly high for the poor and disadvantaged population groups in both developing and developed countries. Globally, dental caries is still a major public health problem as poor oral health has a profound effect on general health and quality of life (Petersen et al., 2005).

Local data has shown that, in 2009 - 40% of children have suffered from caries (Gao et al., 2009), another recent study in Singapore has also shown that 48.4% of children aged 18 - 48M had active caries lesion (Hong et al., 2013). Similar to these studies, our study has also shown increase in caries among Singapore toddler group with a prevalence rate of 17.7%. Likewise, previous

study which evaluated the prevalence of dental caries among Singaporean children indicates that dental caries was a serious problem in this country (Cynthia M Pine et al., 2004). ICDAS's International caries classification and management system (ICCMS) caries management cycle (Figure 32) may be used in the management of caries (ICDAS).

Figure 35: ICDAS's ICCMS caries management cycle.



(Source from (ICDAS)

4.2. Questionnaire Survey

4.2.1. Interview-administered approaches

To ensure the competence of staff, periodic training was done by the research co-ordinators before they finally go ahead to the main questionnaire survey. The questionnaires was asked by the trained and calibrated home visitors and nurses to the mothers / guardian at different time points namely first clinical visit, 3 week, 3M, 6M, 9M, 12M, 15M, 18M and 24M.

4.2.1.2. Child's Allergic diseases

Allergy-associated symptoms - asthma, eczema and rhinitis have become a major public health problem (Beasley, 1998). All these symptoms were

considered in a single model to know their association along with confounders on ECC. Though the examiners explained very clearly to mothers to ensure the questionnaires were accurately answered by them, but still how accurate the answers is a question, thereby to reduce false positive and false negative results, we combined "wheezing" and "nebulizer" variable, if children had wheezing and used nebulizer it was considered as positive towards asthma. As skin prick test was done at 18M, we combined "skin prick test" with "child having running nose ≥ 2weeks" at 18M to consider being positive for rhinitis, thereby the results obtained from this collected data will be more reliable.

4.2.1.2.1. Asthma with nebulizer

Our results shows that children who ever-had wheezing with prescribed nebulizer did not have statistically significant association with caries. But children with wheezing and using prescribed nebulizer during 15 to 18M time point period has shown that the chance of having one more caries lesion is higher compared to the children who do not suffer from asthma and using nebulizer ($P < 0.05$). Our findings are similar to many other studies. Though these studies were not done on toddler group, they show that asthmatic children with medication are more prone to have higher caries lesion compared to non-asthmatics (Arnrup et al., 1993; Ersin et al., 2006; Reddy et al., 2003; Stensson et al., 2010). Children with asthma and using nebulizer may be more prone to caries due to the immunological response of these children from disease (Hyyppä and Paunio, 1979) or could be due to medication - nebulizer, the anti-asthmatic drugs used in these nebulizer have shown that they have low pH and the residual drugs in the oral cavity may

have effect on salivary glands by reducing its flow and altering composition of saliva (Bjerkeborn et al., 1987; Scully, 2003). Ryberg et.al (1991) have shown in asthmatics, the flow of whole parotid saliva is reduced by 20%. Furthermore, asthmatic drugs contain fermentable carbohydrates like lactose monohydrate which has cariogenic effect (Mehta et al., 2009).

Bronchodilators and corticosteroids are mainly used to control and reduce airway inflammation and broncho-constriction (Cazzola et al., 2012; Papi et al., 2009; Thomas et al., 2010). These drugs reach the salivary glands by passive diffusion passing through the lipoprotein membrane of glands. Anticholinergic drugs acts by blocking the acetylcholine resulting in reduced flow of saliva. β2 adrenergic agonist drugs binds to the β receptors present on the salivary glands thereby inhibiting their action (Kelly, 2007; Scully, 2003). O'Sullivan and Curzon have mentioned that all inhalant powders may have pH < 5.5 which may cause dental erosion (O'Sullivan and Curzon, 1998). In particular, along with inhaled medication, dental caries are also contributed by improper inhalation technique (Boskabady et al., 2012). Long-term use of these drugs may cause demineralisation of teeth (Maguire et al., 1996; Mehta et al., 2009) resulting in dental caries. Increased IgE in blood (Bisgaard et al., 2007), leukocyte (Santos et al., 2012) and secretory IgA in saliva (Ryberg et al., 1991) may provide link to the severity and length of disease in association with caries, as duration of asthma has statistically shown significant association with DMF-T/S scores (Mehta et al., 2009). Asthmatics have shown to be mouth breathers compared to non-asthmatics (Stensson et. al., 2010), higher MS and lactobacilli bacteria (Ryberg et. al., 1991; Botelho et al., 2011)

and they have frequent snacks or drinks to overcome the dry mouth (Stensson et. al., 2010). In conclusion, our present findings show that children with wheezing and using prescribed nebulizer may have more chance to have one more caries lesion compared to children who do not wheeze and use nebulizer. This study do not support the hygiene hypothesis that dental caries was protective against allergic diseases.

4.2.1.2.2. Eczema

Among children who ever-had eczema till 18M, chances of having one more caries lesion is nearly 6 times higher compared to children who did not suffer from eczema (P<0.001, RR-5.97, 2.87-12.43). Consistently, this variable have shown to be associated with caries. Children with eczema during 9-12M and 15-18M period (Table: 26) have shown they are more prone to have dental caries at 24M and chances of having one more caries lesion is higher compared to children who do not have eczema at 9-12M and 15-18M. However in contrary, the findings from Ryukyus child health study did not provide a strong relationship between eczema and caries (Tanaka et al., 2008). Though there are not many studies done in relation to eczema and caries, studies have been done to examine the prevalence of caries among Hyper-IgE syndrome (HIES) where eczema is one of the triad of disease and shown that children are more prone to caries lesion (Esposito et al., 2012; Olczak-Kowalczyk et al., 2013). Currently, HIES is known as a multi-systemic disorder with both immunologic and non-immunologic features. It is a rare disease with no difference for race and gender and characterized by a triad of signs and symptoms, high serum levels of IgE (2000 IU/ml), eczema and recurrent staphylococcal skin and lung infections. Osteopenia, minimal trauma

fractures, scoliosis, central nervous system abnormalities, arterial aneurysms, characteristic facial appearance, and dental anomalies, such as retention of primary dentition and alterations of oral mucosa and gingiva are common features. They conclude that it is more important to check the patients oral health regularly by a dentist or orthodontist and motivate them to maintain better oral hygiene (Esposito et al., 2012).

The results of this study indicate that the increased caries lesion among children diagnosed with eczema may be more likely linked with the changed immunological response of these children. Hahn et al. (2000) have shown that multiple inflammatory cytokine mRNAs - interferon, IL-4 and IL-10 were detected in human dental pulp, higher prevalence of interferon was obtained in *S. mutans* predominant shallow caries, suggesting a type-1 cytokine mechanism in early pulpitis where *S. mutans* predominates. Possible explanation may be the immunological changes among children with caries and eczema. Thereby further studies are required to explore the association of caries and eczema among children.

4.2.1.2.3. Rhinitis

In regards to rhinitis, we considered the children are positive to rhinitis if they have running nose ≥ 2 weeks from the day of previous interview to the day of interview - data collection. In this present study, children who ever-had running nose ≥ 2 weeks not shown to have statistically significant association with caries, even at any time-point, which is similar to other studies published in 2008 and 2014 (Tanaka et al., 2008; Vázquez-Nava et al., 2008;

Wongkamhaeng et al., 2014 May). Though Ryukyus study showed no significant association between child rhinitis and caries, it showed an inversely significant association with the prevalence of allergic rhinoconjunctivitis only among children with parental allergic history. Vázquez-Nava et al., 2008 has shown that children with allergic rhinitis and using pacifier had doubled the risk of developing caries but allergic rhinitis alone didnot have any effect. Wongkamhaeng et al., 2014 also shows that allergic rhinitis patients aged 6 - 13 years had reduced dmft / DMFT together with increased sugary food consumption.

Our study results have shown that children with running nose ≥ 2weeks during 12 to 15M may have increase in development of caries, however it is not statistically significant (RR-1.86, 0.33-10.47). We combined both the running nose ≥ 2 weeks with skin prick test to make the data more reliable but after combining, there was only one child with positive result and the rest 271 children were negative, thereby results was not reported. More studies should be done to explore the association of rhinitis on caries and general health of children.

4.3. Demographic Background

4.3.1. Maternal Factors

In our study, we considered mother's educational level, medical history - ever suffered / diagnosed from asthma, rhinitis and eczema, HI to verify their link with child's dental caries status.

4.3.1.1. Maternal Educational Level

In this study model, mother's educational level has shown to play an important role in child's caries status. About 69.8% of mothers received tertiary level of education. Our results shows children of mothers with non-tertiary level of education may have an increase in caries activity as compared to children of mothers with tertiary level of education and it's statistically significant (P<0.001, RR-3.19, 1.75-8.84). Mothers with high educational level may be more cautious about the child's general and oral health that could be due to their knowledge and attitude (Gao et al., 2010b). Studies have shown that attitude towards their oral health has an effect on susceptibility to caries and also shows that people whose attitudes are more towards health conscious may have reduced caries incidence could be due to their sensible eating habits and using fluoridated dentifrice (Reich et al., 1999), possibly could be due to their awareness of the importance of oral hygiene.

4.3.1.2. Household Income

Similarly, we evaluated whether HI has any effect on dental caries activity. However, in this study model HI did not show any statistically significant association, indicating that family HI does not play an important role in children caries activity compared to educational level - knowledge and attitude towards their health. Irrespective of HI, educational programs towards oral hygiene have to be encouraged; there by community will be benefited in maintaining the oral and general health. Our study results are similar to other studies indicating that monthly family income is not related to dmft and they

point out that mothers employment plays an important role and strongly related to ECC (Kumarihamy et al., 2011).

4.3.1.3. Maternal Medical history

In this exploratory study, mother's medical history was examined to explore their association with caries. Results show that the children with ever-had allergy-associated symptoms and whose mother suffer from eczema, asthma and running nose ≥ 2weeks may have reduced caries activity but statistically not significant. In the model, children with allergy-associated symptoms at time point variable; trend towards significance have been observed among children with maternal history of eczema and running nose and may have reduced caries. This shows an inversely significant association of caries among children with maternal allergic rhinitis and eczema history, which may probably be explained by mothers' attitude towards oral hygiene. Mothers suffering from eczema or running nose compared to mothers who do not suffer may be more cautious in health of children. To know more about the effect of maternal medical history on their child's oral health, we further proceeded with split data analysis; results are shown in appendix VII. In this analysis child ever-had eczema has been consistently shown to be associated with dental caries, Thereby our study results indicate that the changes in immunological response either to the disease or its treatment may have an effect on child's oral health. We encourage the children of this population group need to be followed until the eruption of permanent teeth to know their window effect. Further studies are also required to find their immunological association with oral bacteria.

4.3.2. Child factors

4.3.2.1. Gender

Our present study findings have shown that, gender of the child did not show statistically significant association with dental caries. Contrast to our study, other studies among Singapore children have also shown that males are more prone to have rampant caries lesion, the mean number of filled teeth for boys (0.16) was significantly higher than that for girls (0.07) (p=0.007) (Gao et al., 2009). Prakasha Shrutha et al., 2013 also show that boys (57%) had more caries than girls (43%) did. Further studies are required to compare the incidence of dental caries between genders.

4.3.2.2. Ethnicity

Our study finding shows that, in allergy-associated symptoms at all time point model, Malay children may be prone to have one more lesion of caries compared to Chinese, however not statistically significant. This is in accordance with other studies that surveyed the racial difference in caries susceptibility (Gao et al., 2009; Prakasha Shrutha et al., 2013). Gao et al., shows that the percentage of children with untreated teeth was significantly higher among Malay children (43.4%) than that of Chinese children (37.8%) (P=0.04). Along with, the mean number of decayed teeth was also significantly higher for Malays (1.72) compared to Chinese (1.33) and Indians (1.15) (p=0.017 and p=0.024, respectively). A significantly higher mean defs was found in Malays (4.13) than in Indians (2.79) (p=0.050). The proportion

of children with rampant caries was significantly higher in Malays (24.4%) than in Chinese (15.5%) and Indians (10.6%) (P <0.001).

The possible reason for the racial difference may be due to difference in parent's education - dental awareness or knowledge as well the supervision of child's oral hygiene measures, cultural characteristics, hurdles to dental care services, detrimental behaviors - bottlefeeding and breastfeeding during nighttime sleep. Diet pattern - frequent intake of sweets or sugary foods / drinks in between meals may play a major role in the increase of caries among Malay children and finally could also be due to expensive dental treatment and oral health education / intervention for this population group is not effective (Gao et al., 2009).

4.3.2.3. Mode of delivery

In relation to general health of children who are C - section delivered than vaginally born children, it have been demonstrated that they have significantly higher rates of cumulative infectious diseases, lower respiratory tract infections, and cough. Mode of delivery appears to have some lasting effect on child health even after 3 to 6 years of birth, in particular respiratory health. Further research is essential to elucidate the causative effect of mode of delivery on child health whether they can have any immunological effect on children who are born vaginally (Merenstein et al., 2011).

In our study, we considered mode of delivery to find out if it has any association with ECC. Currently, studies have been focussed to verify if the microbiota of breastfed infants differed based on vaginal or C - section

delivery (Holgerson et al., 2013). Interestingly, infants who were delivered through caesarean (C) - section has shown less incidence of dental caries compared to those who were delivered by normal vaginal delivery, though not statistically significant. In contrast Peretz and Kafka (1997) shows that babies born from C-section or Pregnancy complications were significantly higher in the ECC. This may be confirmed in future work when more specific details of delivery and labour are taken into consideration to understand the potential mechanisms leading to the decreased risk of developing caries lesions during early childhood.

Other studies in relation to C - section on general health also shows that infants delivered by C - section had significantly higher rates of cumulative infectious diseases, lower respiratory tract infections and cough (Merenstein et al., 2011). Holgerson et al. (2013) have shown that microbiota of breastfed infants differed on vaginal delivery or C - section. As the mode of delivery plays a role in the early acquisition of bacteria, more studies should be done to explore the effect of normal and C - section delivery on the general and oral health of children.

4.3.2.4. Feeding habits at nighttime

The breastfed infants either exclusively or partially has shown that increase in species of *LB* compared to formula - bottle fed infants (Holgerson et al., 2013) thus we explored to find if there is a link in breastfed or bottlefed on caries among Singapore toddler group.

4.3.2.4.1. Breast feeding at night

The children who are breastfed at nighttime until 18M are more prone to have caries lesion (P=0.006, RR - 4.45, 1.53 - 12.96) compared to children who did not breastfed at night during sleep. A positive association has been revealed between caries and breastfeeding practice. Our study results are similar to other study conducted among Singaporeans, which mentions that children who are breastfed for more than 10 months were significantly more likely to have severe dental decay (Hong et al., 2013), the duration of breast feeding increased the prevalence of caries (Gao et al., 2010a). Moreover Prakasha Shrutha et al. (2013) also found that caries prevalence was high among those who were breastfed for longer duration especially during night time ($P < 0.05$).

The underlying reason for the increased caries risk among children breastfed at night during sleep could be explained due to high lactose content and low buffering capacity compared to bovine milk (Thomson et al., 1996). In contrast other studies have shown that breastfeeding will reduce the ECC but these studies did not verify only breastfeeding at night during sleep, they compared to the children who were bottlefed and other oral hygiene habits on ECC (Alaluusua et al., 1990; Caplan et al., 2008; Nunes et al., 2012; Weerheijm et al., 1998). As prolonged breastfeeding practice at night may provide a continuous source of fermentable carbohydrates resulting in demineralisation of teeth and caries (Thomson et al., 1996). This may be due to the milk pooling between the upper lip & deciduous maxillary central incisor when children are fed at night and during sleeping there is reduced salivary flow (Dawes, 2008).

Interestingly, similar to this present study Li et al. (2000) have shown that children who were breastfed for > 9M were likely to harbor *MS* strains which are common to their mothers and experienced more dental caries at 36M. They also suggest that prolonged breastfeeding may associate with the fidelity of transmission of *MS* among 2 to 3 year old children and may contribute to a inclined caries rate, suggesting that the duration of breast feeding rather than the history, may play a significant role in caries activity (Li et al., 2000). With this professional guidance and interventions on feeding practice delivered by health professionals must be an important factor for Singaporeans.

4.3.2.4.2. Bottlefeeding at night

Though not statistically significant, as expected children with bottlefeeding at nighttime during sleep until 18M may have one more caries lesion compared to children who did not use bottle at night, thereby parents or the caretakers have to be encouraged about the tooth brushing habits with fluoride content before going to sleep for their children. Studies in relation to feeding practice and caries have shown that prevalence of caries are high among children who are bottlefed for longer duration and with those falling sleep with bottle (Mohebbi et al., 2008; Prakasha Shrutha et al., 2013). This indicates that bottlefeeding at nighttime especially during sleep has some unfavorable effect. Perhaps could be due to the addition of sugar in bottle milk (Mohebbi et al., 2008). In addition, this finding also suggested that determining the role of feeding practices on ECC could help in the development of appropriate oral health promotion strategies.

4.3.2.5. Medical Conditions

Child suffering from any other medical disease was taken into consideration to explore if they have any confounding effect on allergic diseases with caries. The results show that other medical condition apart from allergic disease also increase the caries activity but not statistically significant. Studies indicate that pediatric medicines used for general medical conditions or diseases are more cariogenic and has erosive potentials due to the presence of high sugar concentration (Neves et al., 2008). Use of fluoridated dentifrice during consumption of antihistamine syrup may assist in diminishing their erosive effect (C.C.Costa, 2006). Bigeard (2000) suggests to use sugar free medicines by pressurizing the manufacturers to produce both sugar and sugar-free liquid medicines.

4.3.2.6. Topical Steroid

We considered steroids as one of the possible confounder on independent variable, as intake of steroids have shown increase in caries due to its effect on salivary flow and composition along with reduced plaque pH (Kargul et al., 1998). Wogelius et al. (2004) have shown that asthmatics (aged 5 - 7 years) receiving inhaled corticosteroids and bronchodilators have increased caries in newly erupted permanent teeth. In this study it has shown that use of topical steroids by toddlers below 12M increases the chance of having one more caries lesion whereas at 12M to 18M it declines the caries activity among toddlers ($P < 0.05$) possibly the use of topical steroids may not have any action on salivary glands, plaque pH and cariogenic dietary patterns. Mothers of children using topical steroids may be aware of oral hygiene

practice resulting in reduced caries lesion suggesting that children need to be followed up to explore their association in this study till 36M and 50M. Wogelius et al. (2004) Wogelius et al. (2004) Wogelius et al. (2004) Wogelius et al. (2004) Wogelius et al. (2004) Wogelius et al. (2004) Wogelius et al. (2004)

4.3.2.7. Oral medicine

Children using oral medicine for other than allergic disease until 18M have not shown to have statistically significant association with caries possibly could be due to less usage of oral medicines (2.3%) in this model or mothers would encourage children to rinse their mouth after consuming oral liquid medicines. Tables 28 and 29 describes the pediatric liquid medicines with sugar sweeteners and the organic acid content, pH, titratable acidity and viscosity values of the evaluated medicines (Neves et al., 2010).

Table 28. Liquid pediatric medicines with sugared sweeteners

Brand names	*Label content (sweeteners)*	*Sucrose*	*Glucose*	*Fructose*	*Total sugars*	*Sorbitol*
Bricanyl broncodilatador®	Sugar	16.78 ± 0.15	11.93 ± 0.46	14.66 ± 0.46	43.37	ND
Bricanyl expectorante®	Sugar, sodium saccharin	11.36 ± 0.20	4.64 ± 0.08	5.09 ± 0.06	21.09	ND
Claritin D®	Sorbitol, sugar	57.00 ± 0.8	ND†	ND	57.00	5.39 ± 0.06
Claritin®	Sucrose	ND	40.19 ± 0.53	46.71 ± 0.40	86.90	ND
Desalex®	Sorbitol, sucrose	62.60 ± 1.80	ND	ND	62.60	12.51 ± 0.12
Dimetapp elixir®	Sorbitol, fructose syrup, sodium saccharin	ND	ND	43.72 ± 0.30	75.93	32.21 ± 0.39
Mucolitc®	Sucrose	63.08 ± 2.03	ND	ND	63.08	ND
Muricalm®	Sucrose	34.69 ± 0.48	22.05 ± 0.03	26.30 ± 0.03	83.04	ND
Polaramine expectorante®	Sorbitol, sucrose	57.05 ± 1.05	ND	ND	57.05	8.45 ± 0.07
Polaramine®	Sorbitol, sucrose	56.84 ± 0.68	ND	ND	56.84	17.35 ± 0.26
Vibral®	Sucrose, sodium cyclamate	85.99 ± 2.40	ND	ND	85.99	ND
Vick Mel®	Sugar, aspartame, acesulfame K	ND	23.18 ± 0.55	32.89 ± 0.88	56.07	ND
Vick®	Sugar, sodium saccharin	49.55±0.20	ND	ND	49.55	ND

* Concentrations are expressed in g% (g/100g) (median ± standard deviation).
† ND = not detected.

(Source from Neves et al. (2010)

Table 29. Organic acid content and pH of medicines

Brand names	*Acid content according to labels**	*pH*	*Vol NaOH (mL)†*	*Viscosity‡*
Aeroflux®	Citric acid	4.2	16.63 ± 0.28	30.2
Aerolin®	Citric acid	3.6	7.37 ± 0.10	10.9
Berotec®	Citric acid	3.5	3.08 ± 0.09	10.7
Bisolvon®	Tartaric acid	4.1	2.18 ± 0.05	36.6
Bricanyl broncodilatador®	Citric acid	3.8	4.92 ± 0.18	4.3
Bricanyl expectorante®	Citric acid	3.9	4.85 ± 0.29	2.8
Brondilat®	Citric acid	4.9	0.28 ± 0.00	4.9
Claritin D®	Citric acid	3.7	2.39 ± 0.10	40.9
Claritin®	Citric acid	2.8	14.59 ± 0.32	19.7
Desalex®	Citric acid	5.7	0.59 ± 0.05	13.7
Dimetapp elixir®	Citric acid	2.7	11.96 ± 0.05	13.3
Fluimucil®	---	6.4	0.83 ± 0.03	4.7
Mucolitic®	---	5.4	0.26 ± 0.02	6.1
Mucosolvan®	Benzoid acid, tartaric acid	2.6	3.65 ± 0.02	18.3
Muricalm®	Acetic acid	3.8	1.20 ± 0.02	6.7
Polaramine expectorante®	---	5.6	0.36 ± 0.01	7.9
Polaramine®	---	6.0	0.54 ± 0.05	9.7
Silomat Plus®	Chloridric acid	3.3	2.09 ± 0.02	21.3
Silomat®	Chloridric acid	3.3	1.05 ± 0.02	13.9
Vibral®	Citric acid	5.0	0.65 ± 0.00	14.2
Vick Mel®	Citric acid	4.7	7.36 ± 0.12	146.6
Vick®	Citric acid	4.9	4.17 ± 0.14	412.3
Zyrtec®	---	5.0	1.40 ± 0.09	5.1

* (---) not mentioned in labels.
† Volume of NaOH solution required to achieve neutral pH or indicator change.
‡ Viscosity values are presented at shear rate of $20s^{-1}$ (unit of viscosity: cP – centi-Poise = millipascal second, mPa·s).

(Source from Neves et al. (2010)

4.3.2.8. Dry mouth

In this study, children with dry mouth at 18M were observed to be 3.6%. We considered this variable in this model to explore if they have any confounding effect on dental caries. As children suffer from rhinitis and asthma, they may experience dry mouth situation. Possibly, could be due to blocked / running nose or difficulty in breathing which may make them to breathe in mouth resulting in dry mouth. It is one of the factors for reduced salivary pH. The anti-histamine medications taken to get relieve from allergic diseases may cause xerostomic effect which is their common side effect (pharmacology weekly newsletter, 2009). This present model showed that children with dry mouth might experience of having one or more caries lesion compared to children who do not have dry mouth. However, it is not statistically significant.

Patients with dry mouth or xerostomia are at higher risk for developing dental caries because of a loss of saliva. A loss of saliva may increase the acidity of the mouth, which may affect many factors such as proliferation of acid-producing bacteria, inability to buffer the acid produced by bacteria or from ingested foods, loss of minerals from tooth surfaces and inability to replenish the lost minerals, and loss of lubrication that contribute to the development of caries. In a dry mouth patients, natural remineralization and protection may not occur because of the lack of salivary calcium and phosphate ions (Su et al., 2011). Studies show that dry mouth resulting from dehydration of mouth breathing causes increase in gingivitis. Thereby, more studies are required to explore the effect of dry mouth on ECC.

4.3.2.9. Plaque Index

The presence of bacterial plaque is an important clinical indicator of caries risk, a significant difference with increase in caries prevalence has shown between those children with and without visible plaque (Mattos-Graner et al., 1998). In this study it has been observed that ECC risk inclined with the increase in plaque index score ($P<0.001$, RR-2.84, 1.91-4.22). Botelho et. al., 2011 and Santos et al., 2012 have also shown that biofilm index increases the risk of caries among asthmatic children under medication. Similar to this present study, plaque has been demonstrated to be a strong predictor of dental caries for 3 year old children therefore plaque should be considered as a key element in health education (Mattila et al., 1998).

4.3.2.10. Oral habits

Child consuming frequent snacks / drinks between meals during day and at nighttime: Child's dietary factors play a major role in development of caries. Our study has shown that having frequent snacks or drinks at night time more than once a day may increase the chance of having one more caries lesion (P=0.003, RR-2.45, 1.37-4.40). Evans et al. (2013) have shown that daily consumption of both sugar sweetened beverages and added sugars in food have been implicated in the development of caries in addition to their less resistance to newly established bacterial flora, immaturity of the host defense system and low resistance of the newly erupted teeth along with extremes in the dietary substrate component.

Tooth brushing habits: Though children in their first 2 yrs of life are not developmentally ready to independently brush their teeth, their personal hygiene habits are crucial factor. Thus, parents regular brushing of their children teeth may be a reflection of their child's brushing habits (Mattila et al., 2000). In our study we did not find statistical significant association of tooth brushing habits with caries possibly could be due to 44.5% of children brush their teeth more than once a day and 40.3% of children brush their at least once a day by 24M.

4.4. Limitations

It was quite hard to accurately quantify the running nose for more than 2 weeks and wheezing of toddlers at each time point. Therefore, these

variables were combined with skin prick test and using nebulizer to make the data more reliable. Questionnaire about mouth breathing variable was asked only at 18M time-point, if this questionnaire was asked at 24M may be more mothers would have answered and we would have used this variable in this present study. Different data analysis approaches were incorporated to increase the accuracy and consistency. In the logistic regression all the maternal and child variables till 18 M were incorporated, but the results were not significant with multicollinearity. In the next approach, Poisson regression with incidence model provided better results with narrower 95% CI. Based on the results from poisson regression, the major trends were identified, the data analysis confirmed significant results.

4.5. Conclusion and future studies

4.5.1. Conclusion

The results have showed a significant increase in caries scores among asthmatics as compared to the control group. Research on the relationship between caries and systemic diseases has provided evidence that caries may be associated with eczema, asthma with their medications. ECC, an oral health problem, affects considerable proportion of toddlers in Singapore. Hence, there is a need to educate this group of patients about their increased risk of caries and the importance of proper plaque control. There is a need to create awareness among dental practitioners regarding the increased caries risk among eczema and asthmatic patients using medications. In conclusion, this study demonstrated that despite being an affluent country, caries remains high

in preschool children in Singapore. Our present findings do not support the hygiene hypothesis that dental caries was protective against allergic diseases. Overall relationship between dental caries and the prevalence of asthma, eczema, or their medications has been shown.

4.5.2. Future studies

Further studies and development need to consider, based on the current findings.

a) The subjects need to follow at 3yrs and 5 yrs to explore the link between the prevalence and incidence of caries of primary teeth and allergic diseases.

b) We recommend the participants need to be followed at 11 years (time where most permanent teeth are erupted) to evaluate the incidence of caries in permanent teeth and also to know more about the second window of infectivity which has been postulated during the eruption of first permanent molars or permanent teeth (Carlen et al., 1996; Caufield et al., 1993; Lindquist and Emilson, 2004).

c) Oral microbiome analysis: The biofilm samples collected during the 24M can predict the most common organism causing the ECC among Singapore toddlers.

d) Follow up of GUSTO children to examine permanent teeth status may provide the link for first and second window of infectivity.

PUBLICATIONS

Conference publications

- **Bindu Karunakaran**, Stephen Hsu, Lynette Shek, Lee Bee Wah, Hugo van Bever, Teoh Oon Hoe, Anne Goh Eng Neo, Saw Seang Mei, Keith Godfrey, Peter D Gluckman, Kenneth Kwek, Chong Yap Seng. Effect of Chronic Rhinitis on Early Childhood Caries among Singapore Toddlers. YLLSoM 4th Annual Graduate Scientific Congress, Singapore, **Poster** presentation - March 2014.

- **Bindu Karunakaran**, Stephen Hsu, Lynette Shek, Lee Bee Wah, Hugo van Bever, Teoh Oon Hoe, Anne Goh Eng Neo, Saw Seang Mei, Keith Godfrey, Peter D Gluckman, Kenneth Kwek, Chong Yap Seng. Effect of Allergic diseases on Early Childhood Caries among Singapore Toddlers. International Association for Dental Research - Southeast Asian Division Regional Meeting, Malaysia, **Poster** presentation - August 2014.

Manuscript in preparation

- **Manuscripts:** Evaluation of Allergic diseases on early childhood caries among Singapore Toddlers. Bindu Karunakaran, Stephen Hsu, Lynette Shek, Lee Bee Wah, Hugo van Bever, Teoh Oon Hoe, Anne Goh Eng Neo, Saw Seang Mei, Keith Godfrey, Peter D Gluckman, Kenneth Kwek, Chong Yap Seng. Manuscript for Journal of Dental Research.

- **Manuscripts:** SYSTEMATIC REVIEW ARTICLE - Dental Caries Risk among Asthmatics of Allergic diseases on early childhood caries among Singapore Toddlers. Bindu Karunakaran, Stephen Hsu. Manuscript for ----- Journal.

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APPENDIX

Appendix I: Oral Health Questionnaire - English, Chinese & Malay version.

GUSTO
GROWING UP IN SINGAPORE TOWARDS HEALTHY OUTCOMES

MONTH 24 ORAL HEALTH QUESTIONNAIRE

Study ID: _____ - _____ **Date:** _____
Interviewer: _____ **Interview Starting Time:** _____

1. **How many times a day do you brush your teeth?**
   (1) None (2) Once/day (3) Twice/day (4) 3 times/day (5) More than 3 times/day

2. **Did you take fluoride tablets during pregnancy and/or lactation?**
   (1) Yes (2) No
   If YES, what are the dose and frequency? _____

3. **Do you receive dental checkups regularly (on a yearly basis)?**
   (1) Yes (2) No
   If YES, how often? _____ times per year

4. **If you do not receive dental check-up every year, what is the main reason?**
   (1) No money (2) No time (3) Difficult transportation (4) Fear of drills, injection and dentists
   (5) My teeth do not bother me (6) Others, please specify _____

5. **At what age do you think is appropriate to start dental check-ups for your child?** ___ year(s) old

6. **Do you think baby teeth are important?**
   (1) Yes (2) No

7. **What do you think is the main reason for tooth decay? (Choose one)**
   (1) Tooth worms (2) Heatiness (3) Ineffective tooth-brushing (4) Sugar (5) Bacteria (6) Sugar and bacteria

8. **Does your child have a finger-sucking habit?**
   (1) Never (2) Seldom (a few times a month) (3) Occasionally (a few times a week) (4) Frequently (a few times a day)

9. **Has your baby ever used a pacifier?**
   (1) Yes (2) No
   If YES, please specify the month(s) he/she used it.

First Year												Second Year											
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24

   If your child has stopped using the pacifier, did he/she (1) stop using it voluntarily or (2) was it taken away from him or her?

10. **Do you share feeding/drinking utensils with your child?**

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MONTH 24 ORAL HEALTH QUESTIONNAIRE

(1) Never (2) Seldom (a few times a month) (3) Occasionally (a few times a week) (4) Frequently (a few times a day)

11. **How frequent do you give sweet snacks or drinks to your child between meals during a day?**
(1) None (2) Once (3) Two to three times (4) Four to five times (5) More than five times

12. **How often does your child take sweet snacks/drinks and sleep without further brushing his/her teeth?**
(1) Never (2) Occasionally (3) Frequently (4) Almost every night

13. **Do you agree with the statement "I have the ability to withhold frequent sugar snacks from my child between meals even when he/she is crying for it"?**
(1) Strongly agree (2) Agree (3) Neutral (4) Disagree (5) Totally disagree

14. **How many times a day do your child's teeth get brushed?**
(1) None (2) Once/day (3) Twice/day (4) 3 times/day (5) More than 3 times/day

15. **Do you agree with the statement "I can do a good job brushing my child's teeth each day thoroughly even when I am very busy"?**
(1) Strongly agree (2) Agree (3) Neutral (4) Disagree (5) Totally disagree

16. **Is fluoride-containing toothpaste used for brushing your child's teeth?**
(1) Yes (2) No (3) Don't know

Interview Ending Time: _____

第 24 个月口腔健康问卷

研究 ID: _____ - _____ **日期:** _____

访问者: _____ **采访开始时间:** _____

1. **您在一天之内刷了多少次牙?**
(1)没有 (2)一天 1 次 (3)一天 2 次 (4)一天 3 次 (5)一天 3 次以上

2. **您是否有在怀孕或哺乳期间使用氟片?**
(1)有 (2)没有
若有, 是什么剂量和频率? _____

3. **您是否有定期接受牙齿检查 (按照每年)?**
(1)有 (2)没有
若有, 频率是? 每年 _____次

4. **若您没有每年接受牙齿检查, 主要的原因是什么?**
(1)没钱 (2)没时间 (3)交通不方便 (4)害怕钻孔机, 注射和牙医
(5)我的牙齿没有干扰我 (6)其他, 请注明 _____

5. **您认为在什么年龄开始适合让您的孩子接受牙齿检查?** ___岁

6. **您认为宝宝的乳牙重要吗?**

(1)重要　(2)不重要

7. **您认为蛀牙的主要原因是什么?(任选其一)**

(1) 蛀虫 (2) 热气 (3) 刷牙不彻底 (4) 糖 (5)细菌 (6) 糖和细菌

8. **您的孩子是否有吸手指的习惯?**

(1)从来没有　(2)很少 （一个月几次）　(3)偶尔（ 一周几次）　(4)经常（一天几次）

9. **您的宝宝是否有使用过奶嘴?**

(1)有　(2)没有

若有,请注明他/她使用的月份.

第一年												第二年											
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24

若您的孩子已经停止使用奶嘴,他/她是 (1) 自愿停止使用或是 (2) 从他/她身边取走?

10. **您是否和您的孩子共用喂食或喝水的用具?**

(1)从来没有　(2)很少 （一个月几次）　(3)偶尔（ 一周几次）　(4)经常（一天几次）

11. **在一天两餐之间，您会给您的孩子甜点零食或饮料多少次?**

(1)没有　(2)1 次　(3)2-3 次　(4)4-5 次　(5)5 次以上

12. **您的孩子会在吃甜点零食或喝饮料之后没有刷牙的情况下入睡么?**

(1)从来没有　(2)偶尔　(3)经常　(4)几乎每晚

13. **您同意这项陈述 "我有能力拒绝给予我的孩子在两餐之间频繁的甜点零食即使他/她吵着要"?**

(1)非常同意　(2) 同意　(3)中立　(4)不同意　(5)完全不同意

14. **您的孩子每天会刷多少次牙?**

(1) 完全不刷　(2)一天 1 次　(3)一天 2 次　(4)一天 3 次　(5)一天 3 次以上

15. **您同意这项陈述 "即使在我很忙的时候,我也可以把孩子的牙齿彻底地刷干净"?**

(1)非常同意　(2) 同意　(3)中立　(4)不同意　(5)完全不同意

16. **您是否使用含氟牙膏来刷孩子的牙齿?**

(1)是　(2)不是　(3)不知道

采访结束时间: _____

GUSTO
GROWING UP IN SINGAPORE TOWARDS HEALTHY OUTCOMES

SOAL SELIDIK KESIHATAN MULUT BULAN KE-24

ID Kajian: _____-_____ **Tarikh:** _____

Penemuduga: _____ **Masa Temuduga Bermula:** _____

1. **Berapa kalikah anda memberus gigi setiap hari?**
   (1) Tiada (2) 1 kali sehari (3) 2 kali sehari (4) 3 kali sehari (5) Lebih daripada 3 kali sehari

2. **Adakah anda mengambil pil-pil fluorida semasa mengandung atau penyusuan?**
   (1) Ya (2) Tidak
   Jika YA, apakah dos dan kekerapannya? _____

3. **Adakah anda menerima pemeriksaan gigi secara tetap (pada setiap tahun)?**
   (1) Ya (2) Tidak
   Jika YA, berapa kerap? _____ kali setiap tahun

4. **Jika anda tidak menerima pemeriksaan gigi setiap tahun, apakah sebab utamanya?**
   (1) Tiada wang (2) Tiada masa (3) Kesukaran pengangkutan (4) Takut gerudian, suntikan dan doctor gigi
   (5) Gigi saya tidak mengganggu (6) Lain-lain, sila nyatakan _____

5. **Pada umur tahun berapakah anda fikir adalah sesuai bagi mulakan pemeriksaan gigi untuk anak anda?**
   ___ tahun

6. **Adakah anda berfikir gigi susu bayi adalah penting?**
   (1) Ya (2) Tidak

7. **Yang manakah yang anda fikir adalah sebab utama bagi kerosakan gigi? (Pilih salah satu)**
   (1) Ulat gigi (2) Kepanasan (3) Berusan gigi yang tidak berkesan (4) Gula (5) Bakteria (6) Gula dan bakteria

8. **Adakah anak anda mempunyai tabiat menghisap jari?**
   (1) Tidak pernah (2) Jarang (beberapa kali setiap bulan) (3) Kadang-kadang (beberapa kali setiap minggu)
   (4) Selalu (beberapa kali setiap hari)

9. **Adakah bayi anda pernah menggunakan puting?**
   (1) Ya (2) Tidak
   Jika YA, sila nyatakan bulan yang dia menggunakannya.

Tahun Pertama												Tahun Kedua											
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24

   Jika anak anda telah berhenti menggunakan putting, adakah dia (1) berhenti menggunakannya secara sukarela atau (2) telah diambil jauh daripada dia?

10. **Adakah anda berkongsi peralatan makan/minum dengan anak anda?**
    (1) Tidak pernah (2) Jarang (beberapa kali setiap bulan) (3) Kadang-kadang (beberapa kali setiap minggu)
    (4) Selalu (beberapa kali setiap hari)

11. **Berapa kerapkah anda memberi snek/ makanan ringan atau minuman yang manis kepada anak anda antara waktu makan dalam sehari?**
    (1) Tiada (2) Sekali (3) 2-3 kali (4) 4-5 kali (5) Lebih daripada 5 kali

12. **Berapa kerapkah anak anda mengambil snek/ makanan ringan atau minuman yang manis dan tidur tanpa memberus giginya?**
    (1) Tidak pernah (2) Kadang-kadang (3) Selalu (4) Hampir setiap malam

13. **Adakah anda bersetuju dengan kenyataan "Saya menpunyai kemampuan untuk manahan snek gula yang kerap deripada anak saya antara waktu makan walaupun dia menangis untuknya"?**
    (1) Sangat setuju (2) Setuju (3) Neutral (4) Tidak setuju (5) Sangat tidak setuju sekali

14. **Berapa kalikah dalam sehari gigi anak anda diberus?**
    (1) Tiada (2) 1 kali sehari (3) 2 kali sehari (4) 3 kali sehari (5) Lebih daripada 3 kali sehari

15. **Adakah anda bersetuju dengan kenyataan "Saya boleh melakukan kerja yang baik bagi memberus gigi anak saya setiap hari dengan teliti walaupun saya sangat sibuk"?**
16. (1) Sangat setuju (2) Setuju (3) Neutral (4) Tidak setuju (5) Sangat tidak setuju sekali

17. **Adakah ubat gigi mengandungi fluorida yang digunakan untuk memberus gigi anak anda?**
    (1) Ya (2) Tidak (3) Tidak tahu

Masa Temuduga Berakhir: _____

Appendix II: Participation Information Sheet - NUH and KKH site

PARTICIPANT INFORMATION SHEET
GUSTO - ORAL HEALTH ADDENDUM

Study Information
Protocol Title:
GUSTO – Growing Up in Singapore Towards Healthy Outcomes
Substudy – Building Oral Microbiome to Identify Novel Biomarkers/Modulators for Early Childhood Caries and Oral-Systemic Link

Principal Investigator & Contact Details:
Associate Professor Hsu Chin-Ying, Stephen
Department of Preventive Dentistry, Faculty of Dentistry
National University of Singapore
11 Lower Kent Ridge Road
Singapore 119083
Office: +65 6772-6832

Purpose of the Research Study
We invite you and your child to participate in this study on early childhood caries and its impact on both oral and general health. You are invited to take part in this study as you have previously consented to be part of the medical research study – GUSTO (Growing Up in Singapore Towards Healthy Outcomes) or its substudy – Substudy in Women Conceived Through Assisted Reproductive Technology. During the GUSTO 24-month clinic visit, a maximum of 300 children will be recruited for this study ending in June 2013. A follow-up session will be conducted at the GUSTO 36-month clinic visit.

Dental caries is the most common chronic childhood condition, of which eighty percent of the disease is concentrated in twenty-five percent of the pediatric population. This study will help identify the maternal and infant factors related to oral conditions including tooth eruption timing and dental caries, as well as to develop and validate a cost-effective model for caries risk assessment and prediction model appropriate for infants and toddlers.

What Procedures will be Followed in this Study
You and your child will be undergoing the same procedures during your 24- and 36-month clinic visit.

1. ***Questionnaire survey:***
   - You will be interviewed by a dental professional for a short set of questions related to oral health.
2. ***Dental check-up (child):***
   - A simple dental check-up (knee-to-knee position) will be conducted using a mouth mirror and when necessary, a blunt dental explorer will be used to confirm the decay.
3. ***Dental plaque and tongue sample collection (child):***
   - The sample collection of both sites will be carefully conducted using sterile toothpicks, which are safe to use in children.
   - A sterile toothpick will be used to gently scrape dental plaque from one upper front tooth within ten seconds.
   - A sterile toothpick will be used to gently scrape over the tongue dorsum within ten seconds.
   - The samples will be transported to the laboratory for microbiological analysis.

Your Responsibilities in This Study
If you agree to participate in this study, you should follow the advice given to you by the study team. You will be required to visit the clinic twice, first during the 24-month clinic visit and second during the 36-month clinic visit. You can choose to end your participation in the study at any time, without any consequences to your dental care or your participation in the main GUSTO study.

What is not Standard Care or Experimental in This Study
The two procedures involved in bacteria sample collection are not yet routinely practiced in a normal clinical setting.

Possible Risks and Side Effects
The risks in taking part of this study are essentially the same as have a routine dental check-up, of which it will be performed by a dental professional from NUS. Disposable materials and sterilized instruments will be used. Your child might experience minor discomfort when having the toothpick in his/her mouth. However, it will carefully come into gentle contact with the dental plaque located on the tooth and with the first two-thirds of the tongue dorsum. All procedures do not have side-effects.

Possible Benefits from Participating in the Study
Not only you will be informed of your child's oral health status, but your participation in this study will also allow us to understand further the etiology of dental caries in baby teeth and to develop an adequate and appropriate caries risk and prediction model for the young generation.

Alternatives to Participation
If you choose not to take part in this study, you may receive standard dental check-up and care at any institution/clinic in Singapore.

Costs & Payments if Participating in the Study
All expenses derived from the procedures will be fully covered by the study.

Voluntary Participation
Your participation in this study is voluntary. You may stop participating in this study at any time. Your decision not to take part in this study or to stop your participation will not affect your dental care or any benefits to which you are entitled. If you decide to stop taking part in this study, you should tell the Principal Investigator.

Your Doctor or the Investigator of this study may stop your participation at any time if they decide that it is in your best interest. They may also do this if you do not follow instructions required to complete the study adequately.

In the event of any new information becoming available that may be relevant to your willingness to continue in this study, you or your legal representative will be informed in a timely manner by the Principal Investigator or his/her representative.

Compensation for Injury
The institution does not make any provisions to compensate subjects for research-related injury.

However, if you follow the directions of the doctors in charge of this study and your child is physically injured due to the substance or procedure given under the plan for this study, the National University Health System (NUHS) will pay the medical expenses for the treatment of that injury. Payment for management of the normally expected consequences of your child's treatment will not be provided.

By signing this consent form, you will not waive any of your child's and your legal rights or release the parties involved in this study from liability for negligence.

Confidentiality of Study

To protect your child's confidentiality, the samples will be coded. All identifiable information (e.g. names, NRIC numbers) will be kept separate from the specimens. The link between your identifiable information and the code number will be kept confidential by the Principal Investigator or a trusted third party. Information collected for this study will be kept confidential.

Regulatory Agencies, NHG Domain Specific Review Board, SingHealth Centralized Institutional Review Board, and Ministry of Health will be granted direct access to your original medical records to check study procedures and data, without making any of your information public. Data collected and entered into the Case Report Forms are the property of the NUHS and KKH. By signing the Informed Consent Form attached, you or your legally acceptable representative is authorizing such access to your study and medical records.

In the event of any publication or presentation regarding this study, your child's and your identity will remain confidential.

Who To Contact if You Have Questions

If you have questions about this research study, you may contact the Principal Investigator, Associate Professor Stephen Hsu Chin-Ying at 6772-6832 or the main research coordinator of this study, ***Dr. Carolina Un Lam at 9721-8427***.

In case of any injuries during the course of this study, you may contact the Principal Investigator, Associate Professor Stephen Hsu Chin-Ying, at 6772-6832.

This study has been reviewed by the NHG Domain Specific Review Board (the central ethics committee) for ethics approval. If you want an independent opinion of your rights as a research subject, you may contact the NHG Domain Specific Review Board Secretariat at 6471-3266.

If you have any complaints about this research study, you may contact the Principal Investigator or the NHG Domain Specific Review Board Secretariat.

CONSENT BY RESEARCH SUBJECT

Details of Research Study

Protocol Title:

GUSTO – Growing Up in Singapore Towards Healthy Outcomes
Substudy – Building Oral Microbiome to Identify Novel Biomarkers/Modulators for Early Childhood Caries and Oral-Systemic Link

Principal Investigator:

Associate Professor Hsu Chin-Ying, Stephen
Department of Preventive Dentistry, Faculty of Dentistry
National University of Singapore
11 Lower Kent Ridge Road
Singapore 119083
Office: +65 67726832

Part I – to be filled by participant

I, _____ (Name of Mother), (NRIC/Passport No._____), mother of _____ (Name of Child), (NRIC/Passport No._____), hereby consent to participate in the research study as described and on the terms set out in the Patient Information Sheet.

The nature of my participation in the proposed research study has been explained to me in _____ (Language/Dialect) by Dr/Mr/Ms _____ (Name of Healthcare Worker).

I have fully discussed and understood the purpose and procedures of this study. I have been given the Participant Information Sheet and the opportunity to ask questions about this study and have received satisfactory answers and information.

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reasons and without my medical care being affected.

Please choose one answer. *Please delete where applicable.*

☐ Yes, I agree to allow my child's data/samples and my data to be used for future research.

☐ No, I do not agree to allow my child's data/samples and my data to be used for future research.

In any event of publication, I understand that this information will not bear my child's name or other identifiers and that due care will be taken to preserve the confidentiality of this information.

_____ _____
[Signature/Thumbprint (Right / Left) of Participant] (Date of Signing)

Part II – to be filled by witness, where applicable

An impartial witness should be present during the entire informed consent discussion if a subject or the subject's legally acceptable representative is unable to read. After the written informed consent form and any written information to be provided to subjects, is read and explained to the subject or the subject's legally acceptable representative, and after the subject or the subject's legally representative has orally consented to the subject's participation in the study and, if capable of doing so, has signed and personally dated the consent form, the witness should sign and personally date the consent form.

Witnessed by: _____ _____
(Name of Witness) (Designation of Witness)

_____ _____
(Signature of Witness) (Date of Signing)

Part III – Investigator's Statement

I, the undersigned, certify to the best of my knowledge that the patient/patient's legally acceptable representative signing this informed consent form had the study fully explained and clearly understands the nature, risks and benefits of his/her / his ward's / her ward's participation in the study.

_____ _____ _____
Name of Investigator Signature Date

Study Information
Protocol Title:
GUSTO – Growing Up in Singapore Towards Healthy Outcomes
Substudy – Early Childhood Caries and Oral-Systemic Health Links among Infants and Toddlers

Principal Investigator & Contact Details:
Associate Professor Hsu Chin-Ying, Stephen
Department of Preventive Dentistry, Faculty of Dentistry
National University of Singapore
11 Lower Kent Ridge Road
Singapore 119083
Office: +65 6772-6832

Purpose of the Research Study
We invite you and your child to participate in this study on early childhood caries and its impact on both oral and general health. You are invited to take part in this study as you have previously consented to be part of the medical research study – GUSTO (Growing Up in Singapore Towards Healthy Outcomes) or its substudy – Substudy in Women Conceived Through Assisted Reproductive Technology.

Dental caries is the most common chronic childhood condition, of which eighty percent of the disease is concentrated in twenty-five percent of the pediatric population. This study will help identify the maternal and infant factors related to oral conditions including tooth eruption timing and dental caries, as well as to develop and validate a cost-effective model for caries risk assessment and prediction model appropriate for infants and toddlers.

What Procedures will be Followed in this Study

1. ***Questionnaire survey:***
   - You will be interviewed by a dental professional for a short set of questions related to oral health.
2. ***Dental check-up:***
   - Your child will have a simple routine dental check-up using a mouth mirror. A blunt dental explorer will be used to confirm the decay when necessary.
3. ***Dental plaque and tongue biofilm sample collection:***
   - A sterile toothpick will be used to gently scrape dental plaque from one upper front tooth within ten seconds.
   - A sterile toothpick will be used to gently scrape over the tongue dorsum within ten seconds.
   - The samples will be transported to the laboratory for microbiological analysis.

Your Responsibilities in This Study
If you agree to participate in this study, you should follow the advice given to you by the study team. You can choose to end your participation in the study at any time, without any consequences to your dental care or your participation in the main GUSTO study.

What is not Standard Care or Experimental in This Study
The two procedures involved in bacteria sample collection are not yet routinely practiced in a normal clinical setting.

Possible Risks and Side Effects
The risks in taking part of this study are essentially the same as have a routine dental check-up, of which it will be performed by a dental professional from NUS. Disposable materials and sterilized instruments will be used. The sterile toothpick will come into gentle contact with the dental plaque located on the tooth and with the first two-thirds of the tongue dorsum. All procedures do not have side-effects.

Possible Benefits from Participating in the Study
Not only you will be informed of your child's oral health status, but your participation in this study will also allow us to understand further the etiology of dental caries in baby teeth and to develop an adequate and appropriate caries risk and prediction model for the young generation.

Alternatives to Participation
If you choose not to take part in this study, you may receive standard dental check-up and care at any institution/clinic in Singapore.

Costs & Payments if Participating in the Study
All expenses derived from the procedures will be fully covered by the study.

Voluntary Participation
Your participation in this study is voluntary. You may stop participating in this study at any time. Your decision not to take part in this study or to stop your participation will not affect your dental care or any benefits to which you are entitled. If you decide to stop taking part in this study, you should tell the Principal Investigator.

Your Doctor or the Investigator of this study may stop your participation at any time if they decide that it is in your best interest. They may also do this if you do not follow instructions required to complete the study adequately.

In the event of any new information becoming available that may be relevant to your willingness to continue in this study, you or your legal representative will be informed in a timely manner by the Principal Investigator or his/her representative.

Compensation for Injury
The institution does not make any provisions to compensate subjects for research-related injury. However, if you follow the directions of the doctors in charge of this study and you are physically injured due to the substance or procedure given under the plan for this study, the National University Health System (NUHS) will pay the medical expenses for the treatment of that injury. Payment for management of the normally expected consequences of your treatment will not be provided.

By signing this consent form, you will not waive any of your legal rights or release the parties involved in this study from liability for negligence.

Confidentiality of Study
To protect your confidentiality, your samples will be coded. All identifiable information (e.g. names, NRIC numbers) will be kept separate from the specimens. The link between your identifiable information and the code number will be kept confidential by the Principal Investigator or a trusted third party. Information collected for this study will be kept confidential.

Regulatory Agencies, SingHealth Centralized Institutional Review Board, and Ministry of Health will be granted direct access to your original medical records to check study procedures and data, without making any of your information public. Data collected and entered into the Case Report Forms are the property of the NUHS and KKH. By signing the Informed Consent Form attached, you or your legally acceptable representative is authorizing such access to your study and medical records.

In the event of any publication or presentation regarding this study, your identity will remain confidential.

Who To Contact if You Have Questions
If you have questions about this research study, you may contact the Principal Investigator, Associate Professor Stephen Hsu Chin-Ying at 6772-6832 or ***Dr. Carolina Un Lam at 9721-8427***.

In case of any injuries during the course of this study, you may contact the Principal Investigator, Associate Professor Stephen Hsu Chin-Ying, at 6772-6832.

This study has been reviewed by the NHG Domain Specific Review Board for ethic approval, as well as SingHealth Centralized Institutional Review Board (the ethics committee). If you want an independent opinion of your rights as a research subject, you may contact the SingHealth Centralized Institutional Review Board Secretariat at 6323-7515.

If you have any complaints about this research study, you may contact the Principal Investigator or the SingHealth Centralized Institutional Review Board.

PARTICIPANT INFORMATION SHEET
GUSTO - ORAL HEALTH ADDEDNDUM

CONSENT BY RESEARCH SUBJECT
Details of Research Study
Protocol Title: GUSTO – Growing Up in Singapore Towards Healthy Outcomes Substudy – Early Childhood Caries and Oral-Systemic Health Links among Infants and Toddlers
Principal Investigator: Associate Professor Hsu Chin-Ying, Stephen Department of Preventive Dentistry, Faculty of Dentistry National University of Singapore 11 Lower Kent Ridge Road Singapore 119083 Office: +65 67726832

Part I – to be filled by participant

I, _____(NRIC/Passport No._____)
(Name of mother)

hereby consent to participate in the research study as described and on the terms set out in the Patient Information Sheet. The nature of my participation in the proposed research study has been explained to me in

_____ by Dr/Mr/Ms _____
(Language / Dialect) (Name of healthcare worker)

I have fully discussed and understood the purpose and procedures of this study. I have been given the Participant Information Sheet and the opportunity to ask questions about this study and have received satisfactory answers and information.

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reasons and without my medical care being affected.

I also give permission for information in my dental records to be used for research. In any event of publication, I understand that this information will not bear my name or other identifiers and that due care will be taken to preserve the confidentiality of this information.

_____ _____
[Signature/Thumbprint (Right / Left) of participant] (Date of signing)

Part II – to be filled by witness, where applicable

An impartial witness should be present during the entire informed consent discussion if a subject or the subject's legally acceptable representative is unable to read. After the written informed consent form and any written information to be provided to subjects, is read and explained to the subject or the subject's legally acceptable representative, and after the subject or the subject's legally representative has orally consented to the subject's participation in the study and, if capable of doing so, has signed and personally dated the consent form, the witness should sign and personally date the consent form.

Witnessed by: _____ _____
(Name of witness) (Designation of witness)

_____ _____
(Signature of witness) (Date of signing)

Part III – Investigator's Statement

I, the undersigned, certify to the best of my knowledge that the patient/patient's legally acceptable representative signing this informed consent form had the study fully explained and clearly understands the nature, risks and benefits of his/her / his ward's / her ward's participation in the study.

_____ _____ _____
Name of Investigator Signature Date

Appendix III: Oral Examination Record

GUSTO
GROWING UP IN SINGAPORE TOWARDS HEALTHY OUTCOMES

MONTH 24 CHILD'S ORAL EXAMINATION RECORD

Study ID: _____ - _____ **Examiner:** _____ **Date:** _____

Sample Collection

Collection Time: _____ Incisor: 51 or 61 Tongue

Examination Starting Time: _____ Examination Ending Time: _____

Silness-Löe Plaque Index

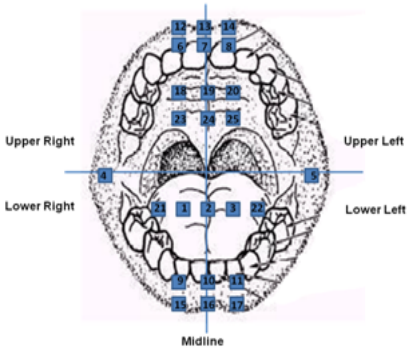
Teeth	55				52				64				75				72				84			
Surfaces	M	B	D	L	M	B	D	L	M	B	D	L	M	B	D	L	M	B	D	L	M	B	D	L
Score																								
PI																								

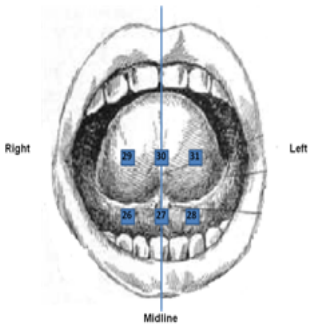
Dental Examination

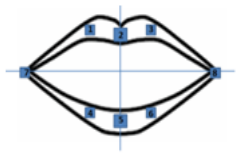
Teeth / Surfaces	55	54	53	52	51	61	62	63	64	65
Occlusal										
Mesial										
Buccal										
Distal										
Lingual										

Teeth / Surfaces	85	84	83	82	81	71	72	73	74	75
Occlusal										
Mesial										
Buccal										
Distal										
Lingual										

Lesions on Mucosa, Tongue and/or Lips ___ yes / ___ no







Version 1.2 dated 12-16-11
Page 1 of 1

Appendix IV: Biofilm (Specimen) Processing Form

GUSTO
GROWING UP IN SINGAPORE TOWARDS HEALTHY OUTCOMES

SPECIMEN PROCESSING FORM

24TH MONTH VISIT (ORAL HEALTH)

SUBJECT ID: _____

DATE OF VISIT: _____

Biofilm Sample Collection

To be filled-in by Examiner	To be filled-in by Laboratory Staff	To be filled-in by Laboratory Staff
Collected Sample NUH ☐ KKH ☐	Sample for Storage Date received: Time received:	Sample for Further Analysis Date released: Time released:
Biofilm sample (2 tubes) Tooth 51 ☐ OR Tooth 61 ☐ Tongue ☐	Biofilm sample (2 tubes) ☐	Biofilm sample (2 tubes) ☐
Collected by:	Received by:	Released by:
Collection time:		
Comment:	Comment:	Comment:

Appendix V: Results from Poisson regression, OV - dmft

Results from Poisson regression - Outcome variable is dmft (count variable), Input main variable - Ever-had till 18M

Independent variables		dmft - included mothers medical history			
		P value	RR	CI lower	CI upper
Ever-had eczema - No*	yes	<0.001	4.842	2.472	9.483
Ever-had Rhinitis - No*	Yes	0.915	0.963	0.480	1.931
Skin prick test 18M - No*	Positive	0.439	1.377	0.612	3.100
Ever-had Wheezing+ Nebulizer - No*	Yes	0.690	0.801	0.268	2.393
Frequent snacks / drinks given btw meals during day time - M24 - No*	> Once/day	0.809	0.902	0.391	2.079
	Once a day	0.639	1.178	0.594	2.339
Frequent snacks / drinks given at night time - M24 - No*	> Once/day	0.057	1.828	0.982	3.402
	Once a day	0.097	1.683	0.910	3.113
Tooth brushing habits	> Once/day	0.601	1.260	0.529	3.000
	Once a day	0.993	0.997	0.462	2.150
Topical steroid at 18M - No*	>12 <18 M	0.009	0.253	0.090	0.710
	≤ 12M	0.671	1.190	0.534	2.653
Medical condition - No*	Yes	0.485	1.197	0.722	1.986
Dry mouth - No*	Yes	0.051	2.342	0.998	5.495
Oral medicine - No*	Yes	0.337	0.546	0.158	1.882
Ethnicity - Chinese*	Malay	0.348	0.717	0.358	1.435
	Indian	0.855	0.893	0.265	3.007
Mother education - tertiary*	Non-tertiary	<0.001	2.717	1.552	4.758
HI ≥ $4,000*	< $ 4,000	0.732	0.885	0.439	1.784
Gender - Female*	Male	0.495	0.844	0.518	1.375
Mode of delivery - Vaginal*	C-section	0.938	0.976	0.532	1.792
Breast fed at night - No*	>12 <18 M	0.009	3.672	1.374	9.815
	≤ 12M	0.001	3.963	1.812	8.664
Bottle fed at night - No*	>12 <18 M	0.215	1.467	0.801	2.687
	≤ 12M	0.448	1.512	0.519	4.401
Plaque Index		<0.001	2.774	1.956	3.933
Mother asthma - No*	Yes	0.467	0.626	0.177	2.211
Mother eczema - No*	Yes	0.024	0.133	0.023	0.769
Mother running nose - No*	Yes	0.215	0.649	0.328	1.285

* - Reference group

Results from Poisson regression - Outcome variable is dmft (count variable), Input main variable - taken at all time points

Independent Variables		dmft-included mothers medical history			
		P value	RR	CI lower	CI upper
Eczema 3M - No*	Yes	0.587	0.652	0.139	3.054
Eczema at 6M - No*	Yes	0.58	1.503	0.356	6.352
Eczema at 12M - No*	Yes	0.051	3.45	0.994	11.968
Eczema at 15M - No*	Yes	0.23	0.324	0.052	2.036
Eczema at 18M - No*	Yes	0.005	5.667	1.703	18.85
Rhinitis at 3 wk - No*	Yes	0.555	0.488	0.045	5.298
Rhinitis at 3M - No*	Yes	0.585	0.593	0.091	3.864
Rhinitis at 6M - No*	Yes	0.966	0.953	0.101	8.994
Rhinitis 9M - No*	Yes	0.121	0.211	0.029	1.511
Rhinitis 12M - No*	Yes	0.816	1.211	0.241	6.082
Rhinitis 15M - No*	Yes	0.715	1.298	0.32	5.257
Rhinitis with skin prick test 18M - No*	Positive	-	8.99E-11	0	0
Wheezing + Nebulizer at 6M - No*	Yes	-	4.45E-11	6.86E-12	2.88E-10
Wheezing + Nebulizer at 9M - No*	Yes	-	2.42E-11	2.15E-12	2.72E-10
Wheezing + Nebulizer at 12M - No*	Yes	0.876	1.131	0.241	5.32
Wheezing + Nebulizer at 15M - No*	Yes	-	3.01E-11	3.60E-12	2.52E-10
Wheezing + Nebulizer at 18M - No*	Yes	0.004	9.168	2.055	40.902
Frequent snacks / drinks given btw meals during day time - M24 - No*	> Once/day	0.753	0.884	0.412	1.898
	Once a day	0.409	0.767	0.409	1.439
Frequent snacks / drinks given at night time - M24 - No*	> Once/day	0.013	2.439	1.202	4.948
	Once a day	0.043	2.036	1.023	4.053
Tooth brushing habits	> Once/day	0.327	1.691	0.592	4.833
	Once a day	0.962	1.022	0.423	2.465
Topical steroid at 18M - No*	>12 <18 M	0.026	0.284	0.093	0.861
	≤ 12M	0.016	2.959	1.222	7.166
Medical condition - No*	Yes	0.768	0.898	0.439	1.836
Dry mouth - No*	Yes	0.062	2.342	0.959	5.718
Oral medicine - No*	Yes	0.341	0.231	0.011	4.696
Ethnicity - Chinese*	Malay	0.593	0.796	0.345	1.836
	Indian	0.927	0.943	0.269	3.309
Mother education - tertiary*	Non - tertiary	0.007	2.243	1.241	4.053

HI ≥ $4,000*	< $ 4,000	0.581	0.796	0.354	1.79
Gender - Female*	Male	0.963	1.014	0.566	1.815
Mode of delivery - Vaginal*	C-section	0.712	1.144	0.561	2.332
Breast fed at night - No*	>12 <18 M	0.018	3.835	1.264	11.636
	≤ 12M	<0.001	7.543	2.491	22.837
Bottle fed at night - No*	>12 <18 M	0.212	1.601	0.765	3.35
	≤ 12M	0.325	1.701	0.591	4.893
Plaque Index		<0.001	2.917	1.891	4.501
Mother asthma - No*	Yes	0.972	1.026	0.25	4.217
Mother eczema - No*	Yes	0.037	0.161	0.029	0.897
Mother running nose - No*	Yes	0.037	0.479	0.24	0.956

* - Reference group

Appendix VI: Results from Logistic regression based on severity of caries

Logistic Regression – child had caries (<2 and ≥ 2) - yes/no

Independent variables		dmft-included mothers medical history			
		P value	OR	CI lower	CI upper
Eczema 3M - No*	Yes	0.782	0.562	0.009	33.344
Eczema at 6M - No*	Yes	0.665	1.603	0.189	13.557
Eczema at 12M - No*	Yes	0.51	2.643	0.147	47.465
Eczema at 15M - No*	Yes	0.262	0.151	0.006	4.118
Eczema at 18M - No*	Yes	0.006	18.078	2.291	142.634
Rhinitis at 3 wk - No*	Yes	0.998	0	0	.
Rhinitis at 3M - No*	Yes	0.656	1.788	0.138	23.101
Rhinitis at 6M - No*	Yes	0.635	0.533	0.04	7.16
Rhinitis 9M - No*	Yes	0.083	0.021	0	1.654
Rhinitis 12M - No*	Yes	0.895	1.214	0.068	21.506
Rhinitis 15M - No*	Yes	0.589	2.01	0.16	25.25
Rhinitis with skin prick test 18M - No*	Positive	1	269.137	0	.
Wheezing + Nebulizer at 6M - No*	Yes	0.999	0	0	.
Wheezing + Nebulizer at 9M - No*	Yes	1	0	0	.
Wheezing + Nebulizer at 12M - No*	Yes	0.884	1.368	0.02	91.393
Wheezing + Nebulizer at 15M - No*	Yes	0.998	0	0	.
Wheezing + Nebulizer at 18M - No*	Yes	0.118	22.883	0.45	1162.963
Frequent snacks / drinks given btw meals during day time - M24 - No*	> Once/day	0.831	1.172	0.274	5.009
	Once a day	0.63	0.702	0.166	2.962
Frequent snacks / drinks given at night time - M24 - No*	> Once/day	0.557	1.424	0.438	4.635
	Once a day	0.294	1.919	0.568	6.485
Tooth brushing habits	> Once/day	0.848	1.161	0.252	5.363
	Once a day	0.861	0.872	0.188	4.042
Topical steroid at 18M - No*	>12 <18 M	0.042	0.13	0.018	0.925
	≤ 12M	0.064	6.834	0.892	52.351
Medical condition - No*	Yes	0.942	0.959	0.313	2.938
Dry mouth - No*	Yes	0.67	0.571	0.043	7.52
Oral medicine - No*	Yes	0.999	0	0	.
Ethnicity - Chinese*	Malay	0.477	1.546	0.466	5.133
	Indian	0.313	0.406	0.07	2.337
Mother education - tertiary*	Non - tertiary	0.048	3.046	1.01	9.181
HI ≥ $4,000*	< $ 4,000	0.584	0.733	0.242	2.226
Gender - Female*	Male	0.684	0.817	0.308	2.164
Mode of delivery - Vaginal*	C-section	0.642	1.297	0.433	3.889

Breast fed at night - No*	>12 <18 M	0.016	6.64	1.425	30.942
	≤ 12M	0.018	9.856	1.486	65.385
Bottle fed at night - No*	>12 <18 M	0.977	0.983	0.299	3.224
	≤ 12M	0.848	1.208	0.175	8.344
Plaque Index		<0.001	5.406	2.125	13.754
Mother asthma - No*	Yes	0.457	1.883	0.356	9.958
Mother eczema - No*	Yes	0.107	0.147	0.014	1.517
Mother running nose - No*	Yes	0.141	0.386	0.109	1.371

* - Reference group

Logistic Regression – child had caries (<3 and ≥ 3) - yes/no

Independent variables		dmft-included mothers medical history			
		P value	OR	CI lower	CI upper
Eczema 3M - No*	Yes	0.817	.549	.003	87.57
Eczema at 6M - No*	Yes	0.851	.758	.042	13.58
Eczema at 12M - No*	Yes	0.360	5.486	.143	210.3
Eczema at 15M - No*	Yes	0.658	.394	.006	24.23
Eczema at 18M - No*	Yes	0.097	13.23	.629	278.5
Rhinitis at 3 wk - No*	Yes	0.998	.000	.000	.
Rhinitis at 3M - No*	Yes	0.960	.903	.018	46.26
Rhinitis at 6M - No*	Yes	0.864	.719	.017	31.06
Rhinitis 9M - No*	Yes	0.278	.040	.000	13.41
Rhinitis 12M - No*	Yes	0.873	1.368	.029	64.23
Rhinitis 15M - No*	Yes	0.999	.000	.000	.
Rhinitis with skin prick test 18M - No*	Positive	1.000	4089.38	.000	.
Wheezing + Nebulizer at 6M - No*	Yes	0.998	.000	.000	.
Wheezing + Nebulizer at 9M - No*	Yes	1.000	.000	.000	.
Wheezing + Nebulizer at 12M - No*	Yes	0.877	0.680	0.005	89.82
Wheezing + Nebulizer at 15M - No*	Yes	0.998	0.000	0.000	.
Wheezing + Nebulizer at 18M - No*	Yes	0.041	190.391	1.226	29567.1
Frequent snacks / drinks given btw meals during day time - M24 - No*	> Once/day	0.347	2.773	0.331	23.26
	Once a day	0.816	.781	0.098	6.248
Frequent snacks / drinks given at night time - M24 - No*	> Once/day	0.138	3.360	0.679	16.64
	Once a day	0.896	1.139	0.162	8.031
Tooth brushing habits	> Once/day	0.235	3.917	0.412	37.24
	Once a day	0.764	.711	0.076	6.618
Topical steroid at 18M - No*	>12 <18 M	0.177	0.092	0.003	2.946

	≤ 12M	0.025	15.19	1.402	164.6
Medical condition - No*	Yes	0.882	1.124	0.240	5.255
Dry mouth - No*	Yes	0.773	1.579	0.070	35.46
Oral medicine - No*	Yes	0.999	.000	0.000	.
Ethnicity - Chinese*	Malay	0.782	1.253	0.254	6.177
	Indian	0.691	1.490	0.209	10.62
Mother education - tertiary*	Non - tertiary	0.075	3.986	0.869	18.28
HI ≥ $4,000*	< $ 4,000	0.806	0.825	0.179	3.801
Gender - Female*	Male	0.706	0.771	0.199	2.978
Mode of delivery - Vaginal*	C-section	0.873	1.132	0.249	5.147
Breast fed at night - No*	>12 <18 M	0.011	16.92	1.925	148.7
	≤ 12M	0.005	47.10	3.297	672.9
Bottle fed at night - No*	>12 <18 M	0.401	2.086	0.375	11.59
	≤ 12M	0.990	0.978	0.035	27.27
Plaque Index		0.001	6.712	2.076	21.70
Mother asthma - No*	Yes	0.806	0.68	0.03	13.67
Mother eczema - No*	Yes	0.998	.000	.000	.
Mother running nose - No*	Yes	0.288	.356	.053	2.39

* - Reference group

APPENDIX VII: Results from Poisson & Logistic regression - Split data analysis

Poisson regression - Outcome Variable - dmfs

Independent variables		Mother allergy history = no				Mother allergy history = yes			
		Sig.	RR	Lower	Upper	Sig.	RR	Lower	Upper
Ever-had eczema - No*	yes	.005	4.800	1.608	14.329	<0.001	11.527	2.926	45.406
Ever-had Wheezing+ Nebulizer - No*	Yes	.888	1.089	.331	3.588	.639	.610	.077	4.822
Ever-had Rhinitis - No*	Positive	.479	.733	.309	1.735	.754	.765	.143	4.081
Skin prick test 18M - No*	Yes	.917	1.061	.347	3.248	.315	2.154	.482	9.627
Frequent snacks / drinks given btw meals during day time - M24 - No*	> Once/day	.214	2.325	.615	8.782	.038	.010	.000	.766
	Once a day	.221	1.876	.685	5.138	.998	1.004	.036	27.840
Frequent snacks / drinks given at night time - M24 - No*	> Once/day	.041	2.305	1.034	5.138	.104	14.069	.579	341.857
	Once a day	.032	2.387	1.076	5.296	.083	10.261	.736	143.023
Tooth brushing habits	> Once/day	.705	1.227	.425	3.547	.629	1.987	.122	32.249
	Once a day	.348	.631	.241	1.651	.734	1.828	.056	59.513
Topical steroid at 18M - No*	>12 <18 M	.031	.102	.013	.809	.847	1.302	.090	18.918
	≤ 12M	.418	.605	.179	2.042	.225	12.706	.209	770.669
Medical condition - No*	Yes	.110	1.682	.890	3.180	.121	3.378	.725	15.732
Dry mouth - No*	Yes	.004	5.214	1.682	16.161		1.732E-13	0.000	0.000
Oral medicine - No*	Yes	.552	.596	.109	3.271	.732	.424	.003	56.953
Ethnicity - Chinese*	Malay	.644	1.256	.477	3.308	.388	2.160	.376	12.422
	Indian	.306	1.960	.541	7.110	.227	.268	.032	2.275
Mother education - tertiary*	Non-tertiary	.003	3.027	1.474	6.215	.029	5.214	1.185	22.949
HI ≥ $4,000*	< $ 4,000	.385	.585	.175	1.958	.383	1.896	.450	7.986
Gender - Female*	Male	.205	.646	.329	1.270	.570	1.381	.454	4.200
Mode of delivery - Vaginal*	C-section	.432	1.428	.587	3.471	.866	.860	.149	4.971
Breast fed at night - No*	>12 <18 M	.092	3.072	.832	11.342	.055	52.051	.919	2947.960
	≤ 12M	.010	4.626	1.439	14.869	.017	47.532	2.002	1128.458
Bottle fed at night - No*	>12 <18 M	.813	1.107	.476	2.574	.087	12.740	.694	233.878
	≤ 12M	.508	1.553	.422	5.725	0.000	3.092E-	4.268E-	2.240E-

							13	16	10
Plaque Index		<0.001	5.042	3.336	7.620	.545	.505	.055	4.617

Poisson regression - Outcome Variable - dmft

Independent variables		Mother allergy history = yes				Mother allergy history = no
		Sig.	RR	Lower	Upper	
Ever-had eczema - No*	yes	.006	7.944	1.828	34.519	No results
Ever-had Wheezing+ Nebulizer - No*	Yes	.728	.773	.181	3.296	
Ever-had Rhinitis - No*	Positive	.661	.753	.213	2.669	
Skin prick test 18M - No*	Yes	.176	2.241	.697	7.208	
Frequent snacks / drinks given btw meals during day time - M24 - No*	> Once/day	.118	.036	.001	2.306	
	Once a day	.856	1.274	.093	17.429	
Frequent snacks / drinks given at night time - M24 - No*	> Once/day	.299	5.411	.224	130.561	
	Once a day	.055	7.077	.961	52.118	
Tooth brushing habits	> Once/day	.905	1.164	.096	14.065	
	Once a day	.794	1.470	.081	26.649	
Topical steroid at 18M - No*	>12 <18 M	.915	1.114	.154	8.062	
	≤ 12M	.434	4.365	.108	175.845	
Medical condition - No*	Yes	.082	2.618	.884	7.757	
Dry mouth - No*	Yes		2.524E-13	0.000	0.000	
Oral medicine - No*	Yes	.510	.260	.005	14.305	
Ethnicity - Chinese*	Malay	.902	1.123	.178	7.074	
	Indian	.134	.279	.052	1.484	
Mother education - tertiary*	Non-tertiary	.014	4.616	1.367	15.584	
HI ≥ $4,000*	< $ 4,000	.571	1.492	.373	5.965	
Gender - Female*	Male	.456	1.580	.475	5.259	
Mode of delivery - Vaginal*	C-section	.665	.596	.057	6.192	

Breast fed at night - No*	>12 <18 M	.036	25.398	1.231	523.856	
	≤ 12M	.111	12.193	.564	263.418	
Bottle fed at night - No*	>12 <18 M	.207	4.697	.425	51.929	
	≤ 12M	<0.001	1.281E-13	5.921E-16	2.771E-11	
Plaque Index		.673	.694	.128	3.773	

Poisson regression - Outcome Variable - dmfs

Independent variables		Mother allergy history = no				Mother allergy history = yes
		Sig.	RR	Lower	Upper	No results
Eczema 3M - No*	Yes	<0.001	6640.692	1170.077	37688.794	
Eczema at 6M - No*	Yes	.083	7.061	.777	64.167	
Eczema at 12M - No*	Yes	.271	.330	.046	2.374	
Eczema at 15M - No*	Yes	.437	.332	.021	5.371	
Eczema at 18M - No*	Yes	.038	7.953	1.118	56.557	
Rhinitis at 3 wk - No*	Yes	.072	.153	.020	1.186	
Rhinitis at 3M - No*	Yes	.995	1.006	.170	5.936	
Rhinitis at 6M - No*	Yes	.025	4.676	1.218	17.954	
Rhinitis 9M - No*	Yes	<0.001	2.799E-10	6.697E-12	1.170E-08	
Rhinitis 12M - No*	Yes	<0.001	1.998E-08	2.661E-09	1.500E-07	
Rhinitis 15M - No*	Yes	.061	4.747	.933	24.136	
Rhinitis with skin prick test 18M - No*	Positive	<0.001	1.439E-08	9.805E-11	2.112E-06	
Wheezing + Nebulizer at 3M - No*	Yes		1			
Wheezing + Nebulizer at 6M - No*	Yes	<0.001	4.923E-09	9.137E-10	2.652E-08	
Wheezing + Nebulizer at 9M - No*	Yes	<0.001	8.488E-10	4.392E-11	1.640E-08	
Wheezing + Nebulizer at 12M - No*	Yes	.061	3.870	.940	15.937	
Wheezing + Nebulizer at 15M - No*	Yes	<0.001	3.308E-08	1.386E-09	7.897E-07	
Wheezing + Nebulizer at 18M - No*	Yes		.008	0.000	0.000	
Frequent snacks / drinks given btw meals during day time - M24 - No*	> Once/day	.064	3.788	.925	15.520	
	Once a day	.161	2.266	.722	7.114	
Frequent snacks / drinks given at night time - M24 - No*	> Once/day	.089	2.504	.869	7.213	
	Once a day	.127	2.135	.807	5.648	

Tooth brushing habits	> Once/day	.835	1.161	.285	4.737
	Once a day	.489	.710	.270	1.871
Topical steroid at 18M - No*	>12 <18 M	.142	.294	.058	1.504
	≤ 12M	.055	3.444	.975	12.161
Medical condition - No*	Yes	.467	.667	.224	1.986
Dry mouth - No*	Yes	<0.001	12.855	3.966	41.662
Oral medicine - No*	Yes	.286	.107	.002	6.534
Ethnicity - Chinese*	Malay	.347	1.518	.636	3.622
	Indian	.093	2.745	.845	8.922
Mother education - tertiary*	Non - tertiary	.013	2.827	1.246	6.418
HI ≥ $4,000*	< $ 4,000	.326	.556	.173	1.794
Gender - Female*	Male	.915	1.041	.495	2.192
Mode of delivery - Vaginal*	C-section	.224	1.837	.689	4.899
Breast fed at night - No*	>12 <18 M	.536	1.556	.384	6.310
	≤ 12M	.026	5.366	1.226	23.480
Bottle fed at night - No*	>12 <18 M	.982	1.010	.419	2.436
	≤ 12M	.623	1.408	.359	5.518
Plaque Index		<0.001	5.453	3.348	8.883

Poisson regression - Outcome Variable - dmft

Independent variables		Mother allergy history = no				Mother allergy history = yes
		Sig.	RR	Lower	Upper	No results
Eczema 3M - No*	Yes	<0.001	1270.357	258.182	6250.645	
Eczema at 6M - No*	Yes	.046	8.172	1.040	64.204	
Eczema at 12M - No*	Yes	.204	.299	.046	1.927	
Eczema at 15M - No*	Yes	.394	.320	.023	4.412	
Eczema at 18M - No*	Yes	.041	6.969	1.087	44.681	
Rhinitis at 3 wk - No*	Yes	.056	.149	.021	1.046	
Rhinitis at 3M - No*	Yes	.906	1.101	.220	5.504	
Rhinitis at 6M - No*	Yes	.020	4.312	1.253	14.840	
Rhinitis 9M - No*	Yes	<0.001	5.458E-11	1.461E-12	2.039E-09	
Rhinitis 12M - No*	Yes	<0.001	3.485E-09	4.868E-10	2.495E-08	
Rhinitis 15M - No*	Yes	.288	2.277	.499	10.389	

Rhinitis with skin prick test 18M - No*	Positive	<0.001	2.466E-09	2.328E-11	2.612E-07	
Wheezing + Nebulizer at 3M - No*	Yes		1			
Wheezing + Nebulizer at 6M - No*	Yes	<0.001	1.031E-09	2.169E-10	4.901E-09	
Wheezing + Nebulizer at 9M - No*	Yes	<0.001	3.962E-10	2.024E-11	7.753E-09	
Wheezing + Nebulizer at 12M - No*	Yes	.386	1.760	.490	6.319	
Wheezing + Nebulizer at 15M - No*	Yes	<0.001	2.469E-09	1.245E-10	4.897E-08	
Wheezing + Nebulizer at 18M - No*	Yes		.036	0.000	0.000	
Frequent snacks / drinks given btw meals during day time - M24 - No*	> Once/day	.046	3.435	1.021	11.557	
	Once a day	.201	1.929	.705	5.279	
Frequent snacks / drinks given at night time - M24 - No*	> Once/day	.266	1.820	.633	5.233	
	Once a day	.188	1.847	.741	4.603	
Tooth brushing habits	> Once/day	.884	.907	.243	3.382	
	Once a day	.266	.594	.237	1.487	
Topical steroid at 18M - No*	>12 <18 M	.169	.329	.068	1.604	
	≤ 12M	.020	3.352	1.207	9.306	
Medical condition - No*	Yes	.552	.732	.263	2.044	
Dry mouth - No*	Yes	<0.001	10.734	3.279	35.140	
Oral medicine - No*	Yes	.309	.145	.003	6.007	
Ethnicity - Chinese*	Malay	.502	1.333	.576	3.083	
	Indian	.194	2.150	.677	6.823	
Mother education - tertiary*	Non - tertiary	.008	2.922	1.321	6.466	
HI ≥ $4,000*	< $ 4,000	.122	.463	.175	1.228	
Gender - Female*	Male	.853	1.068	.531	2.148	
Mode of delivery - Vaginal*	C-section	.116	1.986	.845	4.668	
Breast fed at night - No*	>12 <18 M	.339	1.859	.522	6.622	
	≤ 12M	.024	4.359	1.214	15.649	
Bottle fed at night - No*	>12 <18 M	.900	1.052	.478	2.315	
	≤ 12M	.533	1.470	.438	4.929	
Plaque Index		<0.001	4.094	2.531	6.621	

Logistic Regression - Outcome Variable - dental caries (yes/no)

Independent variables		Mother allergy history = no				Mother allergy history = yes			
		Sig.	OR	Lower	Upper	Sig.	OR	Lower	Upper
Ever-had eczema - No*	Yes	.670	1.323	.365	4.791	.050	33.223	.996	1108.226
Ever-had Wheezing+ Nebulizer - No*	Yes	.519	.558	.095	3.284	.277	.140	.004	4.862
Ever-had Rhinitis - No*	Positive	.550	.714	.237	2.155	.372	.301	.022	4.202
Skin prick test 18M - No*	Yes	.339	1.842	.526	6.449	.775	1.471	.105	20.684
Frequent snacks / drinks given btw meals during day time - M24 - No*	> Once/day	.165	2.674	.668	10.710	.113	.059	.002	1.957
	Once a day	.958	1.036	.274	3.915	.306	4.875	.234	101.505
Frequent snacks / drinks given at night time - M24 - No*	> Once/day	.911	.941	.320	2.768	.369	4.038	.193	84.609
	Once a day	.279	1.802	.621	5.231	.030	20.844	1.344	323.335
Tooth brushing habits	> Once/day	.651	1.401	.326	6.019	.793	.648	.025	16.581
	Once a day	.863	1.128	.285	4.469	.357	4.094	.204	82.301
Topical steroid at 18M - No*	>12 <18 M	.426	.574	.146	2.253	.854	1.233	.132	11.525
	≤ 12M	.898	.908	.208	3.958	.539	3.673	.058	233.028
Medical condition - No*	Yes	.099	2.225	.861	5.749	.089	8.551	.721	101.395
Dry mouth - No*	Yes	.071	7.296	.841	63.290	.999	.000	0.000	
Oral medicine - No*	Yes	.750	1.527	.113	20.640	.517	.149	.000	47.107
Ethnicity - Chinese*	Malay	.705	.796	.244	2.594	.835	.754	.053	10.689
	Indian	.829	.865	.233	3.209	.295	.154	.005	5.124
Mother education - tertiary*	Non-tertiary	.023	3.175	1.175	8.582	.056	21.439	.919	500.176
HI ≥ $4,000*	< $ 4,000	.077	.408	.151	1.103	.496	2.364	.199	28.134
Gender - Female*	Male	.653	1.215	.519	2.848	.033	14.847	1.242	177.491
Mode of delivery - Vaginal*	C-section	.012	3.386	1.303	8.799	.100	.064	.002	1.687
Breast fed at night - No*	>12 <18 M	.808	1.196	.283	5.053	.009	130.757	3.441	4969.353
	≤ 12M	.845	1.221	.165	9.016	.321	8.584	.123	598.621
Bottle fed at night - No*	>12 <18 M	.377	.624	.219	1.776	.151	6.516	.505	84.056
	≤ 12M	.666	1.446	.270	7.736	.998	.000	0.000	
Plaque Index		.001	5.176	2.028	13.20	.383	.401	.051	3.127

Logistic Regression - Outcome Variable - dental caries (yes/no)

Independent variables		Mother allergy history = no				Mother allergy history = yes			
		Sig.	OR	Lower	Upper	Sig.	OR	Lower	Upper
Eczema 3M - No*	Yes	1.000		0.000		1.000	.000	0.000	
Eczema at 6M - No*	Yes	.033	45.569	1.357	1530.322	.999	.000	0.000	
Eczema at 12M - No*	Yes	.513	.173	.001	33.412	.999		0.000	
Eczema at 15M - No*	Yes	.345	.063	.000	19.344	.998	.000	0.000	
Eczema at 18M - No*	Yes	.184	4.877	.472	50.417	.998		0.000	
Rhinitis at 3 wk - No*	Yes	.376	.173	.004	8.392	.999	.000	0.000	
Rhinitis at 3M - No*	Yes	.822	1.461	.053	39.980	.999		0.000	
Rhinitis at 6M - No*	Yes	.406	3.267	.200	53.413	1.000	.000	0.000	
Rhinitis 9M - No*	Yes	.999	.000	0.000		1.000	.000	0.000	
Rhinitis 12M - No*	Yes	.999	.000	0.000		1.000	.000	0.000	
Rhinitis 15M - No*	Yes	.999	49922761 2.305	0.000		1.000	.000	0.000	
Rhinitis with skin prick test 18M - No*	Positive	1.000	.000	0.000					
Wheezing + Nebulizer at 3M - No*	Yes								
Wheezing + Nebulizer at 6M - No*	Yes	.999	.000	0.000		1.000	.000	0.000	
Wheezing + Nebulizer at 9M - No*	Yes	1.000	.000	0.000					
Wheezing + Nebulizer at 12M - No*	Yes	1.000	######## ####	0.000		1.000	.000	0.000	
Wheezing + Nebulizer at 15M - No*	Yes	.999	.000	0.000		1.000	17.050	0.000	
Wheezing + Nebulizer at 18M - No*	Yes	1.000	.084	0.000		1.000	########	0.000	
Frequent snacks / drinks given btw meals during day time - M24 - No*	> Once/day	.083	4.412	.822	23.684	.998	.000	0.000	
	Once a day	.831	1.203	.221	6.536	.999	########	0.000	
Frequent snacks / drinks given at night time - M24 - No*	> Once/day	.485	.606	.148	2.471	.999	########	0.000	
	Once a day	.451	1.686	.434	6.549	.999	########	0.000	
Tooth brushing habits	> Once/day	.183	.303	.052	1.757	1.000	.000	0.000	
	Once a day	.302	.422	.082	2.173	1.000	1.323	0.000	
Topical steroid at 18M - No*	>12 <18 M	.745	.738	.118	4.601	1.000	.000	0.000	
	≤ 12M	.045	13.762	1.064	177.983	.999	.000	0.000	
Medical condition - No*	Yes	.585	1.413	.408	4.894	.999	41878.10	0.000	

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Dry mouth - No*	Yes	.038	18.730	1.182	296.718	.999	.000	0.000	
Oral medicine - No*	Yes	.507	.287	.007	11.485	.999	########	0.000	
Ethnicity - Chinese*	Malay	.948	1.052	.231	4.800	.999	########	0.000	
	Indian	.601	1.545	.302	7.888	1.000	1.494	0.000	
Mother education - tertiary*	Non - tertiary	.044	3.952	1.038	15.047	1.000	########	0.000	
HI ≥ $4,000*	< $ 4,000	.032	.259	.076	.888	.999	########	0.000	
Gender - Female*	Male	.212	2.024	.669	6.125	1.000	2.649	0.000	
Mode of delivery - Vaginal*	C-section	.001	9.878	2.519	38.738	.999	.000	0.000	
Breast fed at night - No*	>12 <18 M	.877	.860	.127	5.815	1.000	.923	0.000	
	≤ 12M	.768	1.511	.097	23.596	.999	.000	0.000	
Bottle fed at night - No*	>12 <18 M	.275	.468	.120	1.827	.997	.000	0.000	
	≤ 12M	.665	1.561	.208	11.745	.999	.000	0.000	
Plaque Index		.001	6.951	2.198	21.983	.999	.000	0.000	