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Longitudinal assessment of factors contributing to Mutans streptococci colonization in young children

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LONGITUDINAL ASSESSMENT OF FACTORS CONTRIBUTING TO MUTANS STREPTOCOCCI COLONIZATION IN YOUNG CHILDREN

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CERTIFICATE OF APPROVAL

MASTER'S THESIS

This is to certify that the Master's thesis of

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has been approved by the Examining Committee
for the thesis requirement for the Master of Science degree in
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To Aai-Baba, Mahesh, and Ketan

Karmanye Vadhikaraste Ma Phaleshu Kada Chana

(Perform your duty, but not with the expectation of
any rewards. Let not the fruits of action be your motive)

Vyas, Bhagavad-Gita

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CHAPTER 1

INTRODUCTION

Dental caries or tooth decay is a communicable oral disease, prevalent throughout the world with a potential to affect anyone, regardless of age. As per the National Health and Nutritional Examination Survey (NHANES) 1999-2002 report, 91% of adults, 42% of adolescents, and 41% of children between 2 to 11 years of age had caries experience [Beltrán-Aguilar et al., 2005]. Dental caries, especially when manifested as cavitation, is irreversible, and its prevalence is cumulative. The prevalence of untreated decay in young children has increased over the past decade [CDC website, 2011], which can be suggestive of further increase in caries prevalence in adult population in upcoming years. Hence, it is imperative to address the problem of dental caries in early childhood in order to prevent future increase in disease burden for the population. The Federal Interagency Workgroup (FIW) considered this while designing Healthy People 2020, and included 'reducing the proportion of children with dental caries experience in their primary dentition' as the first oral health objective [Healthy People 2020].

The mechanism of caries involves progressive demineralization of teeth by the acids secreted by micro-organisms in the oral cavity. Studies conducted in this field have shown a strong association between acid-producing micro-organisms and presence of caries in an individual. Gram positive Mutans Streptococci (MS) are among these organisms, which can not only produce strong acids, but also survive and proliferate in the highly acidic environment typical of dental caries. Among all the micro-organisms found in the oral cavity, MS are found in high proportions in individuals with active caries.

The communicable nature of dental caries is imparted to it by MS – one of the causative organisms. Studies have shown the initial MS infection occurring as early as the first few months of life. The source is usually found to be the mother, or the primary caretaker. MS are present in the dental plaque and saliva of infected individuals. Mode of transmission involves exchange of saliva through shared utensils, pre-tasted food, and kissing. A specific period of time in the infant's life, usually referred as the "window of infectivity", is associated with the maximum susceptibility of the child to acquire MS for the first time. This period roughly matches with the period of eruption of the deciduous first molar tooth in the child.

In addition to age of the child and maternal factors, other demographic, dietary, behavioral, and environmental factors also play a role in MS infection and subsequently caries incidence in children. In the past, many studies have analyzed the relationship between dental caries and risk factors, such as diet, including the content and frequency; demographic characteristics, including socio-economic status and race/ethnicity; behavioral characteristics including oral hygiene habits and feeding practices; and environmental factors including number of teeth and presence of plaque, etc.

However, in most of these studies dental caries was the primary outcome. Very few studies were based on MS colonization as the primary outcome. Also, a majority of the studies were cross-sectional analytical studies. Few studies had all variables considered simultaneously to evaluate their effects on each other. Others were limited by the sample size which limited the generalization of their findings. Moreover, sometimes, samples were not representative of the desired age-group or target population. Longitudinal studies had limitation of loss of data due to loss of subjects to follow up.

Studies enrolling volunteers encountered selection bias. Chapter 2, the Review of Literature, provides an overview of the published scientific literature in this field.

In light of the previous studies' limitations, this study involving 129 infants and toddlers between six months to twenty-four months of age was designed. The subjects were the enrollees of Supplemental Nutrition Program at the Women, Infant, and Children (WIC) center in Muscatine, Iowa. They were followed at five time points over a period of 18 months between June 2003 and July 2006. The salivary samples obtained from the children were used to predict the MS levels at three time points (baseline, nine-months, and eighteen-months). The data for the study were collected both clinically (at baseline, nine-months, and eighteen-months) and from a validated questionnaire (additionally, at four- and thirteen-months). Chapter 3, Methods, provides more details about the study design, hypotheses, and statistical framework.

The data are analyzed at baseline, nine-months, and eighteen-months – those time points with information from both clinical examinations and questionnaires. The dependent variable was salivary MS levels, and subjects were divided into three groups based on their colonization status. The independent variables were assessed using univariate, bivariate, and multivariate logistic regression models specific to each time point to check for association with MS colonization status of the subjects. The analysis confirmed association of caries with MS colonization, age, consumption of sugar sweetened beverages, and plaque. Details about the findings are listed in Chapter 4, Results.

Chapter 5, Discussion, talks about the strengths and limitations of this study. It also dwells upon possible implications of the results and areas for further research. Like other communicable diseases, dental caries, too, can be controlled and prevented if appropriate measures are taken. This study identifies the factors contributing to early acquisition of MS in infants. This may help in designing preventive strategies for such population subgroups.

CHAPTER 2

REVIEW OF LITERATURE

Overview

This chapter seeks to provide a comprehensive overview of the scientific literature in the field of dental caries in young children, and the role of oral bacteria in it. It starts with defining 'caries', its prevalence, the caries process, causative organisms, the infection and window of infectivity, and the associated etiologic risk factors. At the end of each section, the main findings of the studies discussed in that section are summarized. The chapter also provides a thorough assessment and discussion of the gaps in the literature. It concludes with an overall summary which will brief the reader about the findings and conclusion of the studies to further point out the gaps in the literature. The purpose of the study and the research questions are listed after the overall summary.

Dental Caries

The mouth and face are considered to be a mirror of general health and disease. Oral health plays a vital role in the well-being and integrity of an individual. Teeth are a prominent component of the oral cavity, and one of the most common diseases affecting teeth is dental caries, which is also known as tooth decay or more commonly as 'cavities' [Medline Plus Dental Cavities].

The contemporary word "caries" originates from the Latin word for rotten. Beginning in the Middle Ages a tooth worm was thought to be causing this 'rottenness'. Caries were then described as holes in the teeth and were not called "cavities" until the early twentieth century. This description, still used today, may not be completely incorrect, but is misleading, as non-cavitating white-spot lesions are now categorized under dental caries. In either case, the etymology and early beliefs are suggestive of acknowledgement of bio-chemical alteration in tooth structure resulting in caries. Over the years, research has demonstrated a role for bacteria and bacterial products (mainly acid) being responsible for both cavitating and non-cavitating dental caries [Loesche W., 1986].

Dental caries manifest as deciduous tooth caries, permanent tooth caries, root caries, and as cavitated or non-cavitated lesions. In all these forms, the basic pathogenesis of the disease involving bacteria, diet, tooth, and time remains the same. The interplay of these four factors leads to alteration in the mineralized or hard tissue and can subsequently result in caries. In other words, dental caries is a bacterial infection causing acid production through hydrolysis of the food debris accumulated on the tooth surface, ultimately leading to demineralization of the hard tissues (enamel, dentin, and cementum) and destruction of the organic matter of the tooth [Selwitz et al., 2007].

Presence of a tooth in the mouth is necessary but not sufficient condition for the manifestation of dental caries. Infection with the bacteria, the causative agents for dental caries, is more important and decisive. The disease of dental caries is non-discriminatory with potential to affect any individual regardless of age, gender, race, or ethnicity. Severe symptoms include irreversible destruction the tooth structure, with the infection extending up to the periapex, periodontal ligaments, bone, and sometimes the adjacent soft tissue. Death has also been reported at rare instances. Loss of tooth structure to caries is irreversible. Caries is nearly ubiquitous and correspondingly, its treatment from a broader population perspective is expensive in terms of health, time, and money. Nearly all people are at risk of developing dental caries, and the risk starts with the eruption of deciduous teeth [Selwitz et al., (2007), Ripa (1984), Wan et al., (2001)].

Early Childhood Caries (ECC)

ECC has been known by many names such as baby bottle tooth decay, nursing caries, rampant caries, labial caries, and others. Such a plethora of names might be a result of the multi-factorial nature of the disease, its worldwide prevalence, and its strong association with diet [Ismail and Sohn, 1999].

According to the American Association of Pediatric Dentistry (AAPD), ECC is now defined as "the presence of one or more decayed (non-cavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces in any primary tooth in a child 71 months of age or younger" [AAPD Definition of ECC]. Also, any sign of smooth-surface caries in children younger than 3 years of age is considered to be indicative of severe early childhood caries (S-ECC). The AAPD elaborates on their definition, asserting that "From ages 3 through 5, 1 or more cavitated, missing (due to caries), or filled smooth surfaces in primary maxillary anterior teeth or a decayed, missing, or filled score of ≥4 (age 3), ≥5 (age 4), or ≥6 (age 5) surfaces constitutes S-ECC" [AAPD Definition of ECC]. However, this definition may be too broad, as it does not really distinguish between a real problem and minor decalcification.

As a more practical definition, many dental professionals define ECC as multiple cavitated lesions in deciduous dentition observed in children less than 5 years of age. These definitions provide an understanding of the nature of ECC and S-ECC, and also provide a basis upon which further exploration about caries status and etiology can be performed. In the sections that follow, the term ECC includes both cavitated and non-cavitated lesions.

Prevalence of ECC

Based on the National Health and Nutritional Examination Survey (NHANES) findings, the Surgeon General's Report of 2000 states that, "dental caries is the most prevalent chronic disease of childhood. It is five times more common than asthma, and seven times as common as hay fever" [Surgeon General's Report , 2000]. The data collected over the past few decades in the United States have shown that the overall prevalence of dental caries decreased from the early 1970's until the mid-1990 in the primary and mixed dentitions in the 2 to 11 year old age group. However, the most recent NHANES report (1999-2004) demonstrated a reversal in this trend. In this survey, caries prevalence, especially in the deciduous dentition, showed a small but significant increase among two to five year-olds [NIDCR website, 2012]. Studies from other countries have reported similar findings [Seow WK, 1998; Deavis et al., 2013].

Tomar and Reeves used the national- and state-specific data derived from annual surveys to examine the changes in oral health of US children after the release of the Healthy People 2010 objectives. They used the national dental data collected by NHANES in 1988-1994 and again in 1998-2004. The state data were derived from Basic Screening Surveys (BSS) developed and supported by the Association of State and Territorial Dental Directors (ASTDD) [Tomar and Reeves, 2009].

The prevalence of dental caries in children in the two to four year age range increased from the baseline of 18% in 1988-1994 to 24% in 1998-2004, in spite of other positive developments. For example, the proportion of communities with water fluoridation increased from 62% in 1992 to 69% in 2004. Utilization of the oral care system by children aged two to seventeen years increased from 48% in 1996 to 52% in 2004, and the proportion of low-income children and adolescents receiving preventive dental services in the past one year increased from 25% in 1996 to 31% in 2004. Thus, while these figures indicate progress towards achieving some of the Healthy People 2010 objectives, there remains a substantial gap between the measures and the desired effect [Tomar and Reeves, 2009].

The authors also noted the persistence of racial disparities observed in the young population, with Mexican and non-Hispanic Black children being more affected by dental caries than non-Hispanic whites. The highest prevalence of dental caries (68.5% in 1999-2004) was found in six to eight year old Mexican American children. Interestingly, higher prevalence was noted in children with family income levels marginally below the Federal Poverty Level (FPL) than in those at greater than 200% of the FPL across all age groups and in both time periods. According to the state-data, Connecticut had the lowest scores with 40.6% prevalence of caries among third graders, and Arkansas was the highest with 72.2% children from the same category being affected with caries [Tomar and Reeves, 2009].

The article concluded by highlighting the high proportion of untreated dental caries in young children, suggestive of the need for greater attention toward the oral health of children. The authors also claimed that dental caries is preventable, and therefore advocated that adequate help from child care specialists can contribute to solving this public health issue. Also, as is evident from the national statistics reviewed in the article, socioeconomic status and racial or ethnic origin can affect the prevalence of caries in the population [Tomar and Reeves, 2009].

Overall, findings from this and other relevant studies suggest the family's socio-economic status has a strong impact on the oral health of children. A targeted approach for the socio-economically underprivileged consistently exhibiting compromised oral health, generally referred to as 'high-risk population', is being practiced in many of the US government programs. The Women, Infants, and Children (WIC) program; Head Start program; Medicaid program; and Children Health Insurance Program (CHIP) are examples of federal and state governments' efforts in this direction. These programs are discussed mainly in relation with their economically underprivileged, young children enrollees in this chapter. Detailed descriptions of each of these programs are available in a later section of this chapter.

Etiology of ECC

As per the definition of caries, the role of microorganisms is an unquestioned requirement for caries progression. Mutans Streptococci (MS) is a group of microorganisms which include strains of *S. Mutans* and other organisms commonly found to be associated with caries in all age groups of the human population. Some of these are involved in caries initiation, manifested as white spot lesions; whereas others are associated with caries progression, manifested as cavitated lesions.

However, dental caries is a multifactorial disease and has a very wide spectrum of risk factors. Fermentable carbohydrates and tooth/host are the other two factors responsible for manifestation of caries. According to caries tetralogy, when all three of these (microorganisms, fermentable carbohydrates and tooth/host) interact over a period of time, caries result [Newbrun (1967), Ripa (1988)]. Other factors such as demographics, diet, plaque, and oral hygiene habits contribute directly or indirectly in the MS infection and progression of the disease. The following section provides a brief overview of the studies which have focused on the risk factors for dental caries in children.

MS have been referred as the causative organisms for dental caries since the 1960's and is considered as the primary etiological factor in dental caries. Many researchers all over the world have confirmed the presence of MS in saliva as an indicator of caries experience for that individual. One such early study confirming this association was by Berkowitz et al. (1984). The study was aimed at exploring the microflora of seven preschool children with extensive dental caries enrolled as study subjects. Plaque samples were collected from these children mainly from three intra-oral sites – sound enamel, white spot lesions, and carious lesions; and were cultured on selective agar media (MSB agar, Rogosa SL agar, and blood agar). The samples were cultured mainly for *S. mutans* and *lactobacilli* species [Berkowitz et al., 1984].

S. mutans were ubiquitously found in all the samples in very high proportions. Also, consistent with earlier studies, *lactobacilli* showed higher proportions in plaque samples collected from the areas of dental caries as compared to those from white spot lesions or smooth enamel surfaces. The finding supported the role of *S. mutans* in caries initiation and *lactobacilli* in caries progression. The authors referred to a few studies in the past, to point out the correlation between dental caries and with high *S. mutans* counts and high sucrose diet. The history of nighttime bottle-feeding with potentially cariogenic contents in the bottle was observed in all of their subjects; and led to a conclusion that microbial specificity promoted by dietary habits characterizes dental caries. They also proposed that only those infants who were colonized with *S. mutans* are at risk of developing dental caries [Berkowitz et al., 1984].

The role of other factors was assessed in a study conducted by Warren et al. (2008). The study included 212 six to twenty-four month-old children at baseline from the WIC program. The children participants of the WIC program from low-income families were enrolled and examined from time to time over a period of 18 months. The demographic information collected at baseline included data regarding family income, the child's racial/ethnic information, and the mother's education level and beverage consumption. The validated questionnaires for the children completed by the mother (or caregiver) included information regarding child's fluoride exposure, defined by the researchers as the use of fluoride toothpaste. To measure the beverage consumption and nighttime bottle-feeding practices of the child, the questionnaire included closed-ended (yes/no) questions about specific beverages, and open-ended questions (amounts) for servings per week, and average amount per serving. A summary variable combined all added-sugar beverages, and since added-sugar content of the beverage was the primary focus, 100% juices with no added sugar were excluded from the summary variable [Warren et al., 2008].

A single trained examiner assessed the children for caries and visible plaque at baseline, after nine months, and after eighteen months. The examinations were primarily visual examinations, using a halogen headlight, explorer, and mirror. The carious lesions were classified as either cavitated (d_{2-3}) or non-cavitated (d_1). A cavitated (d_{2-3}) lesion was defined as a clinically evident loss of enamel structure (frank caries) or softness at the base of the lesion when probed with controlled modest pressure using an explorer. A non-cavitated (d_1) lesion was defined as a distinct chalky white enamel area either on a smooth tooth surface or adjacent to a pit or fissure site. Saliva samples were collected with a sterile tongue blade in order to semi-quantitatively assess the MS levels by manually counting bacterial colonies. The MS levels of the subjects were grouped into categories as none, <10, 10–100, 100– 200 or as ‘too many to count’ [Warren et al., 2008].

Data were analyzed using SAS program. For these analyses, baseline data were used as the predictor for the $d_{2\text{-}3}f$ caries in the children eighteen months after baseline. Risk factors for caries development were assessed by logistic regression for incidence density ratio and odds ratios (OR). The independent variables used were sugared beverage consumption, nighttime bottle-feeding, plaque, demographics, and fluoride exposure. Incidence rates were evaluated based on caries prevalence and levels of MS at baseline and the eighteen month follow-up [Warren et al., 2008].

The findings of the study showed a strong association between caries and age ($p = 0.006$). Only 11% of those below twelve months of age at baseline developed $d_{2\text{-}3}f$ caries during the study, but 39% of those aged between 18 to 24 months at baseline developed frank caries. Children positive for MS who also consumed sugared beverages were four times (OR = 4.4; 95% CI: 1.4 – 13.9) as likely to develop caries as those who only consumed sugar or sugared beverages, and three times (OR = 3.0; 95% CI: 1.1 – 8.6) as likely as those who were only positive for MS. The article concluded that consumption of sugar-sweetened beverages in children up to 24 months of age can be used as a predictor of ECC in the population under study. The study also supported findings from other studies about the association between MS acquisition in early age and severity of ECC. Most importantly, the study demonstrated the strong role of microorganisms in the caries process [Warren et al., 2008].

Another study conducted by the same group of researchers aimed at assessing the factors associated with the presence of caries in the same group of subjects (children aged 6 to 24 months). They analyzed the cross-sectional data at baseline from the longitudinal study described earlier. Out of the 212 children at baseline, only 164 who had teeth present were included in this analysis. The mean age of study sample was 14 months, and 49% of the children were females. The majority (66%) of the children was Caucasian, over 80% of the children were from families with annual incomes less than $25,000, and the majority of the mothers had education less than high school. Caries (d_1, d_{2-3}, or filled lesions) were evident in 23 children at baseline [Warren et al., 2008].

Bivariate analyses found that caries were positively associated with each of the following variables: older age group, presence of MS in the child's saliva, lower annual family income level (<$25,000), presence of plaque, sugared beverage consumption, sippy cup use, and use of fluoridated toothpaste ($p < 0.05$). The odds of child developing caries are increased by 43% with each passing month. In multivariate analyses, the age of the child was used as a surrogate for exposure and all the odds ratios were calculated after adjusting for age. Presence of MS and plaque score were the variables that remained significant in the age-adjusted model at $p < 0.01$. As per the authors, both sugared beverage consumption and use of fluoridated toothpaste were not significant in the final model as both are functions of time and became non-significant after age adjustment in the final model. The positive relation between caries experience and brushing with fluoridated toothpaste at bivariate level was suggested to be reflective of difficulty in tooth brushing in young children [Warren et al., 2008].

The article concluded that, caries experience in young children can be attributed to the presence of MS and plaque score, along with the other age related factors. The findings of the study were supportive of findings from other studies. Although, the study was unique in inclusion of high risk population with very young children as study subjects, it had some limitations. The cross-sectional analysis did not have sufficient power due to small sample size. The young age of the subjects was also a limitation, as they did not have sufficient numbers of teeth and caries to be representative of children exhibiting caries as a problem. Subsequently, inclusion of non-cavitated (white-spot lesions) is neither comparable nor representative. The sample, predominated by Caucasians, was another limitation for generalizability [Warren et al., 2008].

While Warren et al. (2008) identified MS as the cause of ECC development, they also acknowledged other significant risk factors associated with ECC. The following section will discuss diet, behavior, and environment as risk factors for ECC. Etiologic factors responsible for MS acquisition in young children are discussed later after discussing the caries process and other oral microbial flora of oral cavity.

Diet

As teeth are primarily associated with food, diet plays a key role in dental caries. Since the study described in this document is focused on caries experience of very young children, the following section will mainly consider diet in terms of liquid intake of the subjects. However, there are a few studies referred to in this section which discuss children from other age groups (e.g. Thitasomakul et al., Marshall et al., Chankanka et al.). They have analyzed other patterns of dietary intake than that of the present study. Those studies are included in the review as their methods closely resemble the methods used in this study.

Food habits – Frequency and Timing of Food Intake

Thitasomakul et al. (2009) conducted a longitudinal study in Thailand to identify the risk factors for caries experience in children from the prenatal period up to the child's first birthday. The study sample consisted of 495 children examined at three time points – ages nine, twelve, and eighteen months (approximately). The population studied was mainly rural, with diet predominated by rice, spicy cooked meat, fish, and vegetables. The fluoride concentration of water was between 0.1 to 0.2 parts per million [Thitasomakul et al., 2009].

The children were examined in knee-to-knee position and each examined surface was categorized into one of the following categories: (1) unerupted tooth, (2) normal enamel surface, (3) initial caries or caries limited to enamel, (4) caries in dentin, and (5) caries involving pulp. Intra- and inter-examiner reliability (kappa values) between the five examiners ranged between 0.68 and 0.91. The caries incidence was described as (a) Caries increment – number of events in which a sound surface converted to a carious surface (Beck et al., 1997; Broadbent and Thomson, 2005), (b) Incidence Density – "Number of new caries affected surfaces per surface time at risk" (Thitasomakul et al., 2009), and (c) Incidence Density Ratio – "the ratio of incidence density among those exposed to the incidence density to those unexposed to the particular independent variable concerned" (Thitasomakul et al., 2009). Questionnaires were distributed to mothers at various time points to gather data about children's dietary habits and socio-demographic variables [Thitasomakul et al., 2009].

Data were analyzed using t-test, one-way ANOVA, and correlation coefficient. Multivariate analysis was performed with a negative binomial model. The results of both kind of analyses indicated significantly higher risk for caries in children who were breast-fed, had sweet-tasting food at 5 months, started snacking at 5 months, had soft drinks and sugary snacks, having mothers with >10 decayed teeth, lower income, mothers with only primary education, and those who did not receive calcium supplements during pregnancy ($p < 0.05$) [Thitasomakul et al., 2009].

The high frequency of common, on-demand breast-feeding was a peculiar characteristic of this cohort. The accumulation of milk on the upper incisors, those already compromised with less salivary flow as compared to the rest of the teeth, due to frequent breast-feeding especially at night results in higher caries experience in this group. The higher proportion of bottle-feeding than from breast-feeding, easy access to convenience stores, and the sticky nature of commercial snacks are some of the other ECC risk factors. The study also supported previous findings about the importance of tooth brushing, and other oral hygiene habits [Thitasomakul et al., 2009].

The study concluded that mother's oral hygiene status and calcium intake before the child's birth are important determinants for the child's caries experience; whereas, child-rearing practices, mainly the mode and frequency of feeding, after birth impact the oral health of the child [Thitasomakul et al., 2009].

Composition of Diet

A study conducted by Marshall et al. (2003) analyzed the dietary intake of 642 participants of the Iowa Fluoride Study (IFS). The study explored the consequences of contemporary beverage consumption patterns on dental caries in young children. Three day food and beverage records were obtained from children up to five years of age. The cohort was followed from birth of the child and diet diaries were collected at six weeks after birth, 3, 6, 9, and 12 months of age, after every 4 months through 3 years of age, and every 6 months thereafter. Visual clinical examinations were conducted to assess dental caries by one of two calibrated examiners [Marshall et al., 2003].

Results from statistical analyses of the data indicated that a high intake of soda pop was associated with increased caries experience. Along with the regular soda pop, regular powdered beverages and 100% juice were also found to be cariogenic, but the amount of milk consumption did not alter individuals' caries experience. The odds of caries experience increased with inadequate intake of vitamins (vitamin B complex and vitamin D) and other nutrients (copper). The study concluded by supporting the contemporary guidelines for children's food intake and highlighted that daily sugared beverage consumption increases the risk of caries experience in the children [Marshall et al., 2003].

Another study based on the IFS data was conducted by Chankanka et al. (2011), in order to assess the risk factors for caries for children in deciduous (age 5), mixed (age 9), and permanent (age 13) dentitions. The questionnaire and diet diaries collected periodic data on beverage exposure frequencies, tooth brushing habits, fluoride exposure, and socio-economic status. Surface-specific count of new cavitated and non-cavitated lesions reported by standardized examiners was the outcome variable. A total of 156 children who attended all three dental exams and had at least two completed questionnaires at ages 5, 9, and 13 were included in the study [Chankanka et al., 2011].

The beverage consumption was averaged over the period of diet diary recordings and categorized into high, middle, and low frequency for each of the time points (5, 9, and 13 years). Fluoride exposure through drinking water was calculated as weighted average amount of water consumption from all possible water sources [Chankanka et al., 2011].

The statistical tests used repeated measure modeling framework with Generalized Linear Models Mixed (GLMMs) based on negative binomial distribution. The repeated measure framework accounted for correlated outcomes of newly cavitated and non-cavitated caries from the same individual across three time points including primary, mixed, and permanent dentitions. Individual differences among subjects were accounted for by the GLMM modeling approach. Correlation analyses were performed between significant beverage variables and other caries related factors, such as, fluoride exposure and toothbrushing. In presence of significant correlations, only those variables with more significant association with caries were retained in the model. This procedure was repeated for all the three time points [Chankanka et al., 2011].

The negative binomial GLMM model resulted in 100 % juice being the only variable that was significantly associated with both fewer new non-cavitated caries ($p = 0.003$) and fewer new cavitated caries ($p < 0.0001$). Stage of dentition ($p< 0.0001$) and powdered beverage consumption ($p = 0.003$) were associated with new cavitated caries only. For non-cavitated lesions, the significant variables included higher 100% juice consumption (p =0.041), low tooth brushing frequency (p = 0.044), low SES (p = 0.02), mixed dentition, and a higher proportion of new cavitated lesions (p =0.004). These variables were regarded to be clinically as well as statistically significant. Since the above results are based on models which included exam (first, second, or third) as control (representative of time/age/dentition type) for each of the models, the findings are applicable for all three stages of the dentition [Chankanka et al., 2011].

The only significant protective factor for dental caries was 100% juice (with higher exposure, fewer new cavitated and non-cavitated lesions). No other beverage exposure was proven to be significant as either risk or protective factor. Lower SES and lower toothbrushing frequencies were found to be significantly associated with greater caries experience, and this was in accordance with other studies described in this chapter (Schwarz et al. 1998, Chan et al. 2002, Santos et al. 2002). The strengths of the study findings included the longitudinal nature of the data, inclusion of beverage consumption, and the use of GLMM statistical techniques [Chankanka et al., 2011].

- Infant Formula

A study conducted by Sheikh et al. (1996) explored the cariogenic potential of infant formula. The ability of a product to alter the pH was considered as the measure of cariogenicity for that particular product. The researchers analyzed eight different infant formulae available in the market (Mead Johnson Nutritionals and Ross Laboratories products). They belonged to four groups: soy formulas, formulas with iron, formulas with low iron, and protein formulas. The researchers used in vivo/vitro combination techniques and found that all the formulae under study significantly reduced intra-oral pH and thus, can be considered cariogenic [Sheikh et al. 1996].

Erickson et al. (1998) conducted a study to estimate the cariogenic potential of 26 infant formulae in a clinical trial using plaque pH of adult volunteers and laboratory procedures. The findings showed that most of the formulae are capable of reduction in pH to different extents, some can support bacterial growth, and a few could support dissolving enamel [Erickson et al. 1998].

- Sugar Intake

As described previously, Warren et al. (2008) showed that there is a strong association between caries and sugar-sweetened beverage consumption [Warren et al., 2008]. Cross-sectional analyses of the same group of subjects had shown significant association between sugar consumption and caries experience at the bivariate level. At the multivariate level, age was used as surrogate for exposure which made the sugar intake insignificant [Warren et al., 2008]. Marshall et al. (2003) had similar findings showing higher soda pop, powdered beverage, and total sugared beverage consumption among the subjects with caries [Marshall et al., 2003]. Law and Seow found significantly higher proportions of children exhibiting caries had sugar-containing snacks than children without caries ($p<0.05$) [Law and Seow, 2006].

Mode of Feeding

In their review article, Nainar and Mohummed (2004) used the literature on infant feeding practices to assess its impact on oral health of children and summarized the anticipatory guideline with regard to diet counseling. They quoted the American Academy of Pediatrics (AAP) on exclusive breast-feeding being protective up to six months of age. The article considered the increased prevalence of bottle-feeding and pacifiers in noticeable proportion of US infants, as risk factors for ECC. Multiple snacking habits; night-feeding practices; consumption of cariogenic diet, such as sweetened fluids and acidogenic infant formula; beverage such as fruit juices and soda pop consumption, were found to be associated with dental caries in childhood [Nainar et al., 2004]. The dietary counseling guidance at the end of the article included these points in addition to recommendations for the parents and professionals regarding regular follow up.

The Law and Seow study discussed in the next session also found significant differences in snacking frequencies of children with caries (daily 3 or more) as compared to children without caries (daily 1-2) ($p<0.025$) [Law and Seow, 2006]. This kind of association was supported by Warren et al. (2008), Thitasomakul et al. (2009), and Marshall et al. (2003).

Behavioral and Environmental Factors

Oral Hygiene Habits and Plaque

A prospective cohort study was conducted by Law and Seow in Australia (2006) in order to identify the factors associated with both the MS infection and caries experience in young children (<6 years of age). They followed a group of 63 children aged twenty-one to seventy-two months at baseline, for 24 months. All the enrolled children were divided in two groups at baseline as a) those who were infected with MS and, b) those who were not infected with MS. The subjects were recalled every 3 months to update their medical, social, and dental histories [Law and Seow, 2006].

For the eight children who developed caries during the study period, significant association was found between frequent consumption of sugared snacks ($p<0.05$) and lack of regular use of chlorhexidine for tooth brushing ($p<0.01$). Enamel hypoplasia ($p<0.05$) was more prevalent in the children with caries. Lack of oral hygiene was found to be a significant risk factor for both MS infection and caries experience [Law and Seow, 2006].

A cross-sectional study was conducted by Chan et al. (2002) involving 666 children. All the children were less than four years of age and attended one of the six randomly chosen Maternal and Child Health Centers (MCHC). The questionnaire answered by the parents or caregivers encompassed questions about dietary habits, oral hygiene habits, and attitude towards dental care. Two calibrated examiners (inter-examiner reliability Kappa=0.95) recorded clinically visible cavitated lesions (using WHO criteria for caries), and plaque deposition on maxillary incisors of these children. The data were cross-sectional and analyzed using unpaired t-tests, Chi-square, and Fisher's exact tests [Chan et al., 2002].

Chan et al. (2002) found caries to be significantly associated with the age of the child, plaque deposition, late weaning age, eating candies, infrequent use of toothpaste, low education level of the parents, and low household income ($p < 0.05$). The findings reflect the low awareness of parents or caregivers for the child's oral condition. The article demonstrated the need to improve parental education and awareness, and the authors recommended providers such as pediatricians be engaged in this activity, as they are more likely to come in contact with the new mothers. The role of parents in providing accurate and appropriate information to the professionals, especially regarding night-time bottle-feeding, use of non-dairy products in the bottle, and child's tooth brushing habits with specific toothpaste is very pivotal as well. [Chan et al., 2002]

Nobre et al. (2002), in a cross-sectional study, analyzed the plaque samples of 60 preschool Brazilian children in order to understand the relationship between sugar exposure, plaque composition, and caries patterns. The children belonged to three groups – caries free, those with pit and fissure caries, and those with nursing caries. They were from the same daycare center and were subjected to toothbrushing with fluoridated toothpaste twice a day at the center. Also, all the children received the same meals during the day at the same time. Two dental plaque samples were collected from each child for the purpose of biochemical and microbiological analyses. Detailed dietary history was obtained from the parents using the diet chart of three consecutive days [Nobre Santos et al., 2002].

The three groups differed significantly ($p<0.05$) in daily sugar exposure frequency, whether total, solid, or liquid. The caries free children had the lowest sugar exposure, and their plaque samples comprised of the highest proportions of fluoride, calcium, and inorganic phosphates. Similarly, children with nursing caries were those with the highest sugar exposure. Their plaque sample contained the highest amount of insoluble polysaccharides and MS, and the least proportions of fluoride, calcium, and inorganic phosphorus. The study confirmed the relationship between microbiological and biochemical composition of dental plaque and caries patterns. The authors concluded that the difference in caries patterns could be attributed to differences in sugar exposure, which can also contribute to differential plaque composition [Nobre Santos et al., 2002].

Thus, not only the mere presence of plaque, but also its composition can predict caries in children. Toothbrushing and parental awareness about oral hygiene can help in mechanical removal of the plaque. The type and contents of the toothpaste can alter the plaque composition, and fluoridated toothpastes for young children are commonly available today. An individual can be exposed to fluoride through water, salt, and other dietary sources. The following section discusses effects of fluoride, both topical and systemic, on caries progression.

Fluoride Exposure

Nobre et al. (2002) found that the concentration of fluoride (F) was the highest in the plaque of caries free children, as compared to children with caries. This was in spite of both groups of children having similar exposure to fluoride through diet and toothbrushing habits [Nobre Santos et al., 2002]. Schwarz et al. (1998) found that daily toothbrushing with fluoridated toothpaste (1000ppm) resulted in slowing down caries progression in 251 Chinese preschool children over a period of three years [Schwarz et al., 1998].

Twetman (2008) conducted a systematic review of the scientific literature published in English between 1998 and 2007 to assess the effectiveness of methods for prevention of ECC. The inclusion criteria included study designs that were prospective controlled trials with non-invasive prevention approaches, and focused on children younger than three years of age. Out of the 22 articles shortlisted, six studies were focused on fluoride as the intervention. Three of the studies were carried out in low socio-economic / immigrant areas, and found that fluoridated dentifrices [Davies et al.

2001], NaF tablets [Wehnall et al. 2005], and professional fluoride varnish applications [Weintraub et al. 2006], were significantly effective against caries in the primary dentition. The Weintraub et al. (2006) study was considered to be of better quality than the other two, as it was a two-year randomized controlled trial with two parallel arms – counseling only and counseling + fluoride varnish twice/year[Twetman, 2008].

The fourth article reviewed was a cross-sectional study in Israel [Sgan-Cohen et al. 2001], based on a community intervention project examining 2.5 year old enrollees who received free toothbrushes and fluoridated toothpastes. The findings did not show any significant reduction in ECC after use of regular tooth-brushing with fluoridated tooth paste in the subjects. The fifth study [Lin and Tsai, 2000] assessed effectiveness of fluoride in a group of Taiwanese children with special needs. It demonstrated a beneficial effect of daily fluoride, in the form of tablets and liquids, in caries prevention for cleft lip/palate children. The last study in the group [Aaltonen et al. 2000] assessed the use of 0.25 mg sodium fluoride loaded in "fall-asleep-pacifiers." Despite having a positive outcome, the results were not directly applicable because of the noncompliance with the pacifier/dummy in the target population.

Overall, the evidence was not enough to confirm role of F as a preventive measure for ECC [Twetman, 2008]. Milgrom et al. (2009) considered the findings from similar studies, systematic reviews, and overall cost-effectiveness of the fluoride exposure by the means of fluoridated toothpaste; and recommended the use of "smear" of fluoridated toothpaste for high-risk children younger than two years [Milgrom et al., 2009].

Thus far it is evident that dental caries is a very common disease and is a major public health concern due to its high prevalence in children. Several studies have demonstrated that caries in children have MS as the causative organisms. Other factors like lower SES, lower parental education levels, sugar-rich diets, and behavioral factors can be considered as potential risk factors for caries. To improve the health of economically disadvantaged children, the federal and state governments have designed programs that may also improve oral health, and are common settings for studies of low-SES children. The following section talks about such programs and policies in the United States.

Government Programs and Policies & High Risk Populations

Governmental Programs and Policies

The US government has designed programs targeting specific population subgroups in an attempt to improve the general health status and promote healthier lifestyles. Programs such as Head Start, Women Infants and Children (WIC), Medicaid, and a few others deal with poverty among children. They are state, federal, or jointly funded, and serve the disadvantaged populations through a variety of approaches such as nutrition, education, and provision of healthcare, including dental care. These programs often serve as a setting for academic studies.

The WIC, Head Start, Medicaid, and CHIP programs are described below, followed by a brief review of additional studies in the high risk populations.

The Women Infants and Children (WIC) program

The "Special Supplemental Nutrition Program for Women, Infants and Children" (WIC) is a federally funded program targeting the low-income population. It is designed to meet the nutritional needs of pregnant women, postpartum breast-feeding and non-breast-feeding women, infants, and children up to five years of age. Dietary supplements, immunization, nutritional education, and counseling are offered to the beneficiaries at the WIC clinics, in addition to periodic screenings and referrals. The referral system is a coordinated approach with medical and dental healthcare professionals either housed in the WIC clinic itself or in the other health, welfare and social service. It is a nationwide program with 90 state agencies and up to 1900 local agencies. WIC services encompass hospitals, mobile clinics, community centers, schools, migrant health centers, and camps [WIC at Glance, FNS website].

The Head Start Program

Head Start is a group of federal programs for young children designed to enhance their cognitive, social, and emotional development. These programs aim to promote the readiness of low-SES preschoolers for school. Apart from the education and cognitive development services, they also provide comprehensive health and nutritional services, including dental screenings, as determined by the family need assessment. Starting in 1965, the Head Start program has served nearly 30 million children. Currently, over a million children and their families are served each year at Head Start centers in all 50 states, the District of Columbia, Puerto Rico, and the U.S. territories [Head Start website].

Medicaid and CHIP

Both the Medicaid and the Children's Health Insurance Program (CHIP) vary from state to state; and are jointly funded by the federal and state governments. More than the half of all low-income children in the U.S are covered by Medicaid and CHIP [Medicaid website].

Medicaid is the primary federal government healthcare program for low income children, pregnant women, individuals with disabilities, and also low-income-non-disabled adults. The minimum requirements set by the federal government are flexible, and can be expanded by states through use of state funds. Most states cover children with family income above the minimum of 100% of the FPL through the Medicaid program. Generally, children in families with incomes up to $44,700/year (for a family of four in 2011) are likely to be eligible for either the Medicaid or the CHIP coverage in all states. The benefits of Medicaid for children include early periodic screening, diagnosis, and coverage for the comprehensive treatment of health issues, including dental diseases [Medicaid website].

The CHIP program was designed to serve uninsured children up to 19 years of age, who are from the families with modest income, but still higher than the requisite for Medicaid benefits. Similar to the Medicaid, most of the states have increased the income eligibility criteria for the CHIP up to or above 200% FPL. Many (24 states) offer coverage to children from families at the income level 250% of FPL and some up to 300% of FPL ($67,050 for a family of four in 2011). [CHIP Eligibility Standards – medicaid.gov]. The state of Iowa offers Medicaid expansion and separate CHIP program under the name of Healthy and Well Kids in Iowa (*hawk-i*) coverage for infants and children up to 300% of the FPL [Iowa CHIP fact sheet].

The populations targeted by these programs are usually referred as 'high-risk populations.' The following section attempts to explore the how specific characteristics of these population subgroups make them high risk populations.

High Risk Populations

As discussed earlier, SES of an individual can generally be used as a predictor for his/her health status. It is often found to be correlated with the individual's demographic characteristics as well. Families with low SES and specific demographic groups are thus targeted by the governmental programs in an attempt to improve their health and nutrition. To study the impact of these socio-demographic factors, a study was conducted by Psoter et al. (2006) evaluating race/ethnicity, household income, and other socio-economic factors, as predictors of ECC [Psoter et al., 2006].

The study was conducted in Arizona during 1994-95. A total of 5,171 children aged five to fifty-nine months were visually examined for caries by one of the five examiners. Cohorts of pre-school children were recruited from the governmental and private healthcare agencies. The recruitment occurred in 32 centers distributed in both urban and non-urban areas of Arizona. Every center had a minimum of 25 children in each year of age from one through four enrolled into the study. The data about each child's dental caries were collected by visual clinical examination, and the demographic and other information were collected by a self-administered questionnaire answered by the caregivers [Psoter et al., 2006].

Data analysis was carried out by cluster analyses for contralateral paired tooth surfaces, and was interpreted using dendograms. Unconditional multivariate logistic regressions were modeled with caries as a dichotomous dependent variable and race/ethnicity, education level, and income level as independent explanatory variables [Psoter et al., 2006].

Household income and race/ethnicity were found to be statistically significant risk factors for caries. Children from the lowest income category had mean dmft scores three times higher than the children from the highest income category. Annual family income >$30,000 was a protective factor for caries [Adjusted OR = 0.37]. The OR for race/ethnicity ranged from 1.4 to 3.6 for all non-white children. The study led to the conclusion that the pattern of caries development can be predicted from socio-demographic indicators of the individual [Psoter et al., 2006].

Similar findings were observed in other studies emphasizing the important role of SES in caries prevalence and progression in the U.S. and other nations [e.g. NAHNES Reports, Freire et al. (1996), Tang et al. (1997), Warren et. al. (2008), Tomar and Reeves (2009)].

In summary, the established positive association between caries and low SES suggests that low SES populations are at high risk of caries. Recent U.S. national statistics have confirmed this, as evidenced by an increase in caries prevalence in children from low SES backgrounds over the past few decades. However, as discussed earlier, in addition to MS and family SES, other factors also play a role in etiology-pathogenesis of the MS infection. In the light of increasing prevalence of caries in the most vulnerable age group, it is imperative to scientifically explore these factors. The subsequent sections will discuss the caries process, MS as cariogenic bacteria, and factors responsible for MS infection in young children.

The Caries Process

Loesche (1986) discussed the ecological plaque hypothesis in reference with the specific and non-specific plaque hypotheses. The specific hypothesis is about the role of certain bacteria in caries manifestation; whereas, the non-specific hypothesis focuses on plaque more than its bacterial composition. The specific hypothesis is based on the quality and microbial composition of the plaque, and non-specific hypothesis is based on the quantity and location of plaque in caries process.

The ecological plaque hypothesis combines both of these and states that at neutral pH, both cariogenic and non-cariogenic micro-organisms exist in balance and in non-threatening numbers. With excess intake of sucrose, the balance is disturbed and the oral environment becomes acidic. Reduced salivary flow or inefficient mechanical plaque removal can also contribute to the acidic environment. Only acidogenic (those which can produce acid by fermentation of sucrose), and aciduric microorganisms (those which can survive in the low pH environment), proliferate under such conditions, leading to a further drop in the intraoral pH. The low pH has detrimental effect on the mineral component of enamel resulting in demineralization, destruction of the organic matrix, and finally cavitation [Loesche W, 1986]. These properties are discussed in greater detail by Featherstone (2004) and Law et al. (2007).

Featherstone (2004) described the dynamic process of dental caries and also presented a brief overview of the established theories. The acidogenic bacteria like MS produce organic acids by fermentation of carbohydrates. The commonly found acids are lactic acid, formic acid, acetic acid, and propionioc acid. Their diffusion into the tooth enamel leads to dissolution of mineral crystals (carbonated hydroxyapatite). As the dissolved minerals diffuse out of the tooth, cavitation may occur. This is the process of de-mineralization.

De-mineralization is counteracted by the process of re-mineralization. Re-mineralization involves diffusion of calcium, phosphate, and fluoride ions back into the enamel. This is particularly observed in non-cavitated lesions, when the diffusing ions form a new veneer on the existing crystal remnants. This new layer of re-mineralized enamel is more resistant to acid attack as compared to the original hydroxyapatite crystal structure. Both the processes are cyclic, and can repeat multiple times during a day. It is their balance which determines the progression of the lesion into cavitation, reversal, or maintenance of the existing situation [Featherstone, 2004].

According to Featherstone, under the normal conditions there is a balance between de-mineralization and re-mineralization of the enamel. Pathologic factors such as acidogenic bacteria, fermentable carbohydrates, and reduced salivary function cause de-mineralization. Protective factors such as saliva, antimicrobials, and fluoride are responsible for re-mineralization. Predominance of pathological factors leads to cavitation. Prevention or reversal of initial caries can be achieved by enhancing the protective factors [Featherstone, 2004].

Law et al. (2007) reviewed understanding of the role of different factors involved with MS colonization in children. Interactions among bacterial virulence factors, host related factors, and environmental factors, result in *S. mutans* colonization in children.

The complexity of the process increases with the eruption of deciduous dentition as evident from more than 80% of twenty-four-month olds being infected with MS as compared to only 30% of the three-month olds. The stark difference in the prevalence of MS positive infants is observed in the period around tooth eruption. The post-eruptive flora, if predominated with *S. mutans*, can be considered as a marker for the individual to be at high risk of developing caries [Law et al., 2007].

Law et al. (2007) emphasized that the virulence of *S. mutans* is related to their property of adherence to the host surfaces by means of salivary pellicle. This is vital in colonization of the organism, as it is initially independent of sucrose. The initial adherence is achieved through MS bacterial adhesins like, antigen I/II (*S. mutans*) and Spa A (*S. Sobrinus*) interacting with salivary proteins from the pellicle. Later, glucan binding proteins from enzymatic secretions of MS promote binding of the organism to the tooth surface. This is the sucrose dependent part of adherence process. In addition to producing glucan binding proteins, the enzymes glucosyltransferase and fructosyltransferase from MS result in thickening of the layer of plaque promoting increased sugar diffusion and consequently more acid production [Law et al., 2007].

Acidogenecity and aciduarance are the properties of MS that give these organisms an ecologic advantage over all the other oral micro-organisms in an acidic environment. MS generate acids by anaerobic metabolism of carbohydrates available in the surrounding (acidogenecity) environment. The characteristic of aciduarance can be attributed to ATPase driven proton pumps present in the cell walls of MS. These pumps can actively remove H^+ from the cytoplasm of MS. This maintains the acidic environment ($pH<5.5$) despite of depletion of the dietary carbohydrates. The low pH environment is particularly harmful during the periods of low salivary secretion such as sleep and fosters de-mineralization of the tooth structure leading to cavitation [Law et al., 2007].

Hence, as evident from the studies discussed so far, characteristics of MS make them the predominant cariogenic bacteria. Similar to other strains of bacteria, MS also have many types and subtypes. The following section explores these bacteria in greater details.

MS as Cariogenic Bacteria

By definition, "the MS are those streptococci which are found in plaque and which ferment mannitol and sorbitol, produce extracellular glucans from sucrose, and with the exception of *Streptococcus ferus*, are cariogenic in animal models" [Loesche W, 1986]. The group of MS includes *Streptococcus mutans* (*S. mutans*), *S. sobrinus, S. cricetus, S. rattus*, and *S. ferus*. Amongst them *S. mutans* and *S. sobrinus* are pathogenic and are considered as the main cause of dental caries in humans [Loesche WJ, Barron's Medical Microbiology, edition 4, 1996]. The other members of the family include *S. cricetus* and *S. rattus* both of which were isolated from laboratory animals. The role of *S. macacae,* another member of the family, is yet unexplored. All these species of streptococci are collectively known as Mutans Streptococci and are commonly abbreviated as MS.

S. mutans is the most common pathogen associated with dental caries in humans. They are gram positive, anaerobic cocci, members of family *Viridans Streptococci*, and are one of the 25 species of oral streptococci. The *Viridans Streptococci* are typically commensal streptococci, as many of the members accompany humans from birth until death. They typically inhabit skin, nose, oral cavity, lungs, intestines, and urinary tract. The overall pathogenicity of the family *Viridans Streptococci* is considered to be low. However, as discussed earlier, certain species and their strains like *S. mutans* and *S. sobrinus*, are pathogenic, especially in the oral cavity.

The c, d, e, f, and g serotypes of *S. mutans* exhibit various degrees of pathogenicity based on the characteristics such as acidogenicity and aciduranc. Alaluusua et al. (1995) studied a group of 12 one and half to three year old Finnish children in order to examine the differences in serotypes and ribotypes of MS acquired by the children with ECC (n=6) and caries-free children (n=6). Pooled samples of dental plaque from carious as well as non-carious surfaces of teeth were collected from cases and controls. Maternal salivary samples were also collected [Alaluusua et al., 1996].

Data analyses and bacterial cultures indicated predominance of only the c serotype of *S. mutans*. The other serotypes of *S. mutans* found occasionally were e and g. Maternal salivary samples were rich in multiple serotypes of MS as well as many other organisms as compared to their children from either of the categories. Also, children with caries had a greater number of both serotypes and ribotypes, along with other species of bacteria, mainly *S. sobrinus*, as compared to caries-free children. Nine out of 25 maternal ribotypes were found in the children with ECC, whereas some of the ribotypes were typical for children only. This confirmed the mothers as the primary source of infection for their children, but did not rule out other sources of infection including horizontal transmission [Alaluusua et al., 1996].

Another important finding was about the heavy and diverse colonization observed in caries-prone children. The caries-free children were colonized with only one, or occasionally two, ribotypes. As noted by the authors, the difference was a consequence of frequency and mode of consumption of fermentable carbohydrates, mainly sucrose. The authors concluded that, the diversification in MS colonies in caries active children may be due to the favorable circumstances promoting simultaneous colonization by strains with differing virulence further increasing the risk for caries [Alaluusua et al., 1996].

Bacterial testing and identification

The process of bacterial identification can be done in many ways. The common method in the dental setting is the identification using morphologic and colony characteristics. Salivary or plaque samples obtained from individuals are processed and cultured on selective agar media. It not only provides evidence of a suspected oral bacterial infection, but also gives an estimate of the severity of the infection. This method has been the traditional approach for bacterial identification and has been used by many researchers in the past including, Warren et al. (2008), Nair et al. (2010), Weber-Gasparoni et al. (2012), Law and Seow (2006), and others. The other methods of bacterial identification include differential staining and microscopic examination, serological methods, flow cytometry, and protein and nucleic acid analyses. Recently, DNA testing methods such as Polymerase Chain Reaction (PCR) [Li and Caufield (1998), and others], genetic probes [Papapanou et al. (1997) and others], and DNA microarrays [Ajdić and Pham (2007) and others] have become more widely used for oral bacterial identification.

Morphological identification is carried out by quantitative [e.g. Caufield et al. (1993)] or semi-quantitative methods [e.g. Warren et al. (2008)]. In the quantitative method, the sample (either plaque or saliva) is obtained from the subject, converted into an aliquot with defined concentration of the sample, and then used for bacterial culture by streak plate, pour plate, or spiral plate method. In the quantitative method, the bacterial colonies obtained from the incubated agar plates are counted and the result is given in terms of number of colony forming units (CFU)/ml of saliva of the concerned organism for that individual. In the semi-quantitative approach, the samples are directly impressed into the agar plates and the number of CFUs are estimated and grouped into categories 0, I, II, III with 0, 1-10, 11-99, and >100 CFU respectively. The semi-quantitative method provides an estimate of bacterial concentration and disease severity [Finegold & Martin, Schuster], and studies have shown that this method is more cost- and time-effective, while still providing all the information necessary for diagnosis [Buchnan et al. 1985].

Some of the other studies in this field include Edelstein and Tinanoff (1989) reporting 57% sensitivity and 95% predictive value negative of the semi-quantitative test for *S. mutans* in young children. Linossier et al. (2002) also concluded that the semi-quantitative method is accurate enough to be used for population studies, based on the findings from the comparison between semi-quantitative and quantitative method.

Sample Collection and Culture Medium

However, the utility of the microbial tests depends upon the selective agar medium used for culture. Wan et al. (2000) conducted a study to compare five selective media for *S. mutans* namely, mitis salivarius with bacitracin (MSB), MSB with kenamycin (MSKB), glucose-sucrose-tellurite-bacitracin (GSTB), trypticase soy with sucrose and bacitracin (TYS20B) and tryptone-yeast extract-cysteine with sucrose and bacitracin (TYCSB). A standard strain of *S. mutans* was cultured on each of these using sterile pipettes and spreaders. After anaerobic incubation at 37C for 72 hours, the colonies were counted using a colony counter. Mean CFU and percentage recoveries were measured for each of the culture media. The results suggested that TYCSB was the most appropriate culture media for *S. mutans* and MSKB showed lower recovery rates and sensitivity, as compared to all the other culture media under study [Wan et al., 2002].

Kimmel and Tinanoff (1991) have compared the MSKB media with the MSB media. They found significantly more growth of non-MS organisms on MSB as compared to MSKB. They also observed 13% less recovery rates for MS on MSKB medium [Kimmel and Tinanoff, 1991]. Tanabe et al. (2006) found 92% specificity and 60% sensitivity for MSKB medium for the growth of MS obtained from salivary samples of 50 subjects aged 10 to 36 months. The findings were statistically significant [OR=17.3 (3.3-89.0) and p = 0.001].

Loesche et al. (1974) compared the paraffin-stimulated saliva samples with that of plaque samples either obtained from occlusal and proximal surfaces or from carious/non-carious fissures. They observed weak association between the MS and decay when paraffin–stimulated saliva samples were used. They attributed this to the contribution of flora from tongue and the mucosa typically infiltrating the saliva samples, diluting the actual concentration of cariogenic bacteria. The authors concluded that plaque samples give close-to-accurate estimation of the MS, and subject's plaque level can be considered as a practical way to gauge individual's MS colonization status and a good predictor for caries. The study is discussed again later in the chapter [Loesche et al., 1975].

Kohler and Bratthall (1979) devised a method as an alternative to traditional quantitative method. The traditional aliquot method had limitations such as difficulty in obtaining stimulated saliva or ideal plaque sample, especially in very young children. The traditional method is also technique sensitive and follows a complex methodology. It needs more time and material at both sampling site and laboratory. In their method, Kohler and Bratthall suggested using 1.8mm wide wooden spatula contaminated with saliva to develop bacterial culture. Approximately 3 cm of sterile spatula is introduced in the subject's mouth, and is gently pressed against the subject's tongue alternatively on each side for approximately ten times. Excess saliva is wiped against the lips of the subject and then each side of the spatula is pressed on the Replicate Organism Detection And Counting (RODAC) plate with elevated level of selective agar [Kohler and Bratthall, 1979].

The tongue blade method by Kohler and Bratthall would give an estimate of the microbial count differentiating high-risk and low-risk individuals, and so, can be considered equivalent to the semi-quantitative method discussed earlier. They compared the bacterial cultures obtained using the tongue blade method with the traditional method using paraffin stimulated saliva samples. Salivary counts as low as 10,000 *S. mutans* per ml of saliva were detected by the tongue blade method. The authors recommended this method for epidemiological studies mainly geared towards identifying high risk children [Kohler and Bratthall, 1979].

Another comparison study was conducted by Weinberger and Wright (1990). They compared the tongue blade/RODAC plate method with each Cariescreen dip slide method and modified Cariescreen dip slide method. The use of tongue blade to gather the saliva sample was one of the modifications in modified Cariescreen method. The authors found 86% agreement between the modified Cariescreen and the tongue blade method [Weinberger and Wright, 1990]. This is suggestive of the reliability of semi-quantitative tongue blade/RODAC plate method, and also the efficiency of tongue blade as a means to collect salivary samples.

Finally, while comparing the RODAC plate counts obtained from both the spiral plate method and tongue blade method, Olson et al. (2004) found significant correlation between the two methods for both grouped (Kendall's tau-b = 0.42) and ungrouped (Spearman's rank correlation r = 0.79) counts. Both the results were statistically significant ($p<0.0001$). Higher correlation was particularly observed in children with higher MS counts. Spiral plate counts gave higher estimates than the RODAC plate method in subjects with low MS levels [Olson et al., 2004]. It can be concluded that the RODAC plate counts can be a convenient method for semi-quantitative assessment of bacterial counts in high-risk individuals.

Many studies discussed in this chapter have used the tongue blade method for salivary MS determination. Some of them are Grindfjord et al. (1991), Mohan et al. (1997), Wan et al. (2003), Law and Seow, (2006), Warren et al. (2008), and Weber-Gasparoni et al. (2012). Some have used plaque samples (Alaluusua et al. 1996). Others have used quantitative method with stimulated saliva samples such as Caufield et al. (1993), Li et al. (1994), Loesche et al. (1975). In the present study, the bacterial counts were assessed semi-quantitatively using the tongue blade and MSKB/RODAC plate method.

MS Acquisition and Window of Infectivity in Infants

Caufield et al. (1993) conducted a study to investigate the time period during which children are most susceptible to MS acquisition and the factors affecting this process. Forty-six mother-infant pairs were selected from a group of third trimester pregnant women, who were screened positive for high levels of MS in saliva ($\geq 2.5 \times 10^4$ colony forming units (CFU) /ml of saliva). They were followed until the child's fifth birthday. Water fluoridation levels were the same for all the participants, as all of them lived in the same community. The mothers were given comprehensive dental treatment post-partum for a period of six months. Afterwards, they were randomly divided into either a treatment group, which included prophylaxis and iodine-sodium-fluoride topical application, or a placebo group. This was done after the infants' first tooth eruption, but before the infants became positive for salivary MS (mean age 8.5 months). Samples were obtained from the unstimulated whole saliva of mothers, while cotton tip applicators were used for the infants [Caufield et al., 1993].

The sample collection was carried out every three months for both the mothers and their children. They were cultured using the standard protocols on to MSB agar. A second sample was repeated two weeks after the first positive sample. The age for MS acquisition for an infant was defined after two consecutive positive cultures for MS from plaque, oral swab, or saliva. Information about primary and secondary caretakers was obtained through interview with a single-blinded health-care professional. Statistical analysis was carried out using the logarithm of the bacterial counts with both parametric and non-parametric tests [Caufield et al., 1993].

Out of the forty-six infants in the study, thirty-eight acquired MS at a median age of 26 months. The remaining eight children were not infected during the entire study period of fifty-six months. Child's race, gender, age at the first tooth eruption; and maternal factors such as age, DMFT/DMFS indices, past dental history, as well as maternal MS levels, did not differ significantly ($p>0.05$) between the MS-colonized and MS-absent groups. The variable for preventive intervention in the mothers only had a short term effect, and, thus, was excluded from the analyses. The only significant difference between the MS-colonized ($n=12$) and MS-absent ($n = 8$) children was related to the time of infection. The MS-colonized children were significantly younger ($p = 0.0001$) at the initial MS acquisition and correspondingly had lesser time lapse ($p=0.0001$) between the time from emergence of the first tooth to the detection of MS infection as compared to MS-absent children [Caufield et al., 1993].

Nine children out of the 38 who were MS-colonized developed active caries by the age of three years. When they were compared with the eight MS-free, caries-free children, the only statistically significant difference was that the mother was the primary care-taker of the caries-active children, especially during the first two years of the child's age ($p=0.03$). They did not differ significantly on birth weight, gestational age, or antibiotic episodes [Caufield et al., 1993].

The initial acquisition of MS occurred in infants during a well-delineated time period termed as "the window of infectivity" by the authors and was a major finding of this study. As per the observations from the study, this "window period" spans over the duration of seventeen months from seven months up to twenty-four months of age. A sharp rise was observed in the graph for cumulative probability of MS acquisition by age (in months) between 16 to 24 months. This period of time roughly coincides with primary molar eruption and corresponding increase in tooth (non-desquamating) surface area in the child's mouth. The authors postulated that, the newly emerged teeth provide "virgin" habitat for MS colonization and so heighten infants' susceptibility to MS infection during that period. The article concluded by emphasizing the importance of this window period in a child's life and suggested possible preventive measures targeting this period [Caufield et al., 1993].

Many other studies have confirmed MS acquisition in infants during a similar period in infant life. One of them was reported by Tedjosasongko and Katsuyuki, who followed a group of 39 two to twenty-five month-old Japanese children for a period of thirty months. At baseline, none of these children were infected with MS. Considering the infectious nature of MS acquisition, they used the set-up of a day care nursery for their research. Out of 39 children, 10 were siblings. Plaque samples from all regions of the teeth of the children were collected once every month for 30 months, and a single collection was made for parents and caregivers in order to isolate and culture the bacteria. The first detection of MS in dental plaque was identified as initial acquisition. A DNA fingerprinting method was used to identify the source of infection [Tedjosasongko and Katsuyuki, 2002].

A total of 21 children acquired MS during the study period with mean age for acquisition being 24 months, with cumulative probability of initial acquisition increasing rapidly between twelve to twenty-five months. It matched with the eruption of deciduous molars in children, as the cumulative probability graph for prevalence of infection against the number of teeth observed a sharp rise after the number of teeth reached ten [Tedjosasongko and Katsuyuki, 2002]. The only contrasting finding of this study with that of Caufield et al., was that, the most common path of transmission was horizontal (58.4%) followed by maternal transmission (33.3%). Caufield et al. (1993) had inferred that children in their study had acquired the infection "almost exclusively" from their mothers. Tedjosasongko and Katsuyuki, concluded that the initial acquisition of infants is affected by child's social environment in addition to his/her oral condition [Tedjosasongko and Katsuyuki, 2002].

Etiology of MS Infection

Griendefjord et al. (1991) studied a group of 1095 one-year old Swedish children to assess MS colonization. The study was cross-sectional and the bacterial samples were obtained using the tongue blade method. The tests indicated that 6% of the subjects were infected with MS and colonization was significantly associated with non-Swedish background and maternal behavior patterns, such as dietary habits with high sugar consumption. The authors predicted a high risk of caries in the primary dentition of children who were colonized because of these factors [Griendefjord et al. 1991].

In a study to determine the prevalence of MS colonization and the associated factors, Wan et al. (2001) performed a cross-sectional observational study on a group of 172 (60 pre-term, 112 full-term) mother-infant pairs. The mean age of infants at the time of examination was 26.7+/- 3.8 weeks. Saliva samples were collected from the infants using cotton swabs swabbed along their alveolar ridges. Samples were repeated every three months until two years of age of the child. Only after two consecutive positive samples an individual was considered positive for MS. The mothers' teeth were also swabbed with sterile cotton tips [Wan et al., 2001].

Maternal factors like oral hygiene, caries experience, and periodontal health were also measured according to standard criteria used by WHO. Mothers were interviewed using a validated questionnaire to gather information about demographics, medical conditions, and feeding habits. Interviews and dental examinations were conducted by a single examiner (intra-examiner reliability was over 95%). The selected cohort of the mother-infant pairs was representative of the population on the SES levels of the families. [Wan et al., 2001].

While assessing the mode of feeding (breast- versus bottle-feeding), breast-feeding was found to have a statistically significant association with increased susceptibility to MS acquisition. Both on-demand feeding [OR = 26.1 (6.5-103.5)] and night feeding [OR = 11.4 (2.8-46.8)] were significantly associated with MS acquisition ($p < 0.001$ for both) [Wan et al., 2001].

The amount of sugar exposure in the routine diet of the children was another significant finding. Exposure to sugar from solids (meals or snacks) had OR as high as 125.9 (CI: 2.6-620.9) (in pre-term infants having sugar from meals) for colonization by MS. This finding was statistically significant (p=<0.001). Younger age for starting solids was also identified as a risk factor with OR = -0.7 (0.6-0.8) ($p < 0.001$). Although the use of a pacifier was not significantly associated with MS acquisition, a high percentage of non-infected preterm children were observed to use pacifiers regularly sterilized with Milton's solutions ($p < 0.001$) [Wan et al., 2001].

The article provided an overview of possible factors associated with MS infection. They were both child and maternal factors. The child factors included breast-feeding, especially night-time feeding; frequent bottle-feeding; higher sugar exposure, mainly through solids; poor oral hygiene habits, such as lack of regular gum cleaning; and other habits like sucking adult's fingers and food pre-tasting practice. The maternal factors were presence of plaque, high salivary MS levels, poor periodontal health, unhealthy dietary habits, and maternal demographics. All of them were significantly ($p<0.05$) associated with high salivary MS levels of the child in bivariate analyses. Sugar from fluids in diet, food pretested by others, and maternal plaque and calculus levels were the only factors that were non-significant in either pre-term or full-term infants [Wan et al., 2001].

The article also provided a framework for assessing acquisition of MS in infants. The authors discussed follow-up areas such as controlled clinical trials with larger sample size, and a focus on maternal transmission. However, there was no mention of conducting multivariate analyses to consider all these factors in a single model. This remains as a limitation of this study as collinearity amongst the variables remains unexplored.

Weber-Gasparoni et al. (2012) conducted a similar study aiming to assess relationships between the presence of MS and other factors in very young children (twelve to forty-nine months old). Healthy WIC enrollees who participated in a psycho-educational study (n = 411) were considered for this purpose. The sample population was composed of whites (57%) and non-whites including African-Americans, Hispanic/Latino, and others (43%). Household income was less than $5,000 per annum (abbreviated as 'p.a.' henceforth) for 25% of the mothers, with more than 80% of the study population being below $25,000 p.a.. The children were examined in knee-to-knee position and the mothers were asked to respond to a questionnaire about demographics, snacking and oral hygiene habits. The samples were collected using tongue blade method and a semi-quantitative method was used to assess MS levels [Weber-Gasparoni et al., 2012].

The logistic regression models suggested that the presence of MS in very young children was positively associated with the presence of cavitated lesions, higher numbers of teeth, higher proportion of teeth with plaque, non-white race, and lower level of maternal education ($p<0.05$). Child's sugared beverage consumption was found to be associated with presence of MS, whereas children without MS more commonly exhibited milk consumption ($p \leq 0.001$). Also, the proportion of teeth with plaque was significantly related to percentage of added sugar beverage (such as sports drinks, soda-pop, sugared powder drinks) consumption and percentage of all sugared beverages which was a combination of natural and added sugared beverages [Weber-Gasparoni et al., 2012].

The study found significant use of fluoridated toothpaste in children who were positive for MS. This was an unexpected result, as usually children using fluoridated toothpaste have better oral hygiene and less susceptibility to caries than those who do not use it. The authors speculated the use of toothpaste was related to age and number of teeth, allowing for a greater time period and surface area for MS colonization, respectively. However, this association was not significant after adjusting for the covariates in multivariable modeling. The dietary variables were significant only at the bivariate level, and became insignificant at the multivariate level [Weber-Gasparoni et al., 2012].

Although, this study was focused on cross-sectional data mainly collected by means of questionnaire and clinical examinations of the children, it encompassed a high risk population with a fairly representative sample. The study also provided a comprehensive overview of demographic, dietary, and behavioral factors involved in predicting MS colonization in young children. The statistically significant findings of the study thus can be generalized to other high-risk populations [Weber-Gasparoni et al., 2012].

A study by Tanner et al. (2011) was aimed at analyzing microbiota of infants with severe ECC pre- and post-treatment. They enrolled 53 children with S-ECC and 32 caries-free children between two to six years of age. The parents and caregivers were surveyed for demographic, dietary, and hygiene habits. Children with S-ECC were subjected to a comprehensive restorative and extraction treatment. Clinical measurements and plaque samplings were performed at baseline, 6 months, and 12 months after the treatment. If new caries on unrestored surfaces were detected during either of the follow up visits, that visit was counted as the last visit for that child [Tanner et al., 2011].

The microbial analysis was carried out using PCR technique and microarray. The t test was used to detect mean differences in age, diet, plaque, and gingival and bleeding indices between the caries-free and S-ECC children. Chi-square statistics were used to detect differences in proportions between disease categories and demographic characteristics. Multivariate linear regression model analysis was carried out using the partial-least squares method to detect correlations between matrices of descriptor and response variables [Tanner et al.., 2011].

Cariogenic food intake, drinking frequencies, and plaque and gingival indices were significantly lower in caries-free children as compared to the S-ECC groups ($p<0.05$). Specific PCR analyses demonstrated strong associations between S-ECC and presence of *S. mutans*, *S. sobrinus,* and *Bifidobacteriaceae*. Post-treatment microarray reports of S-ECC childrens who did not develop new lesions after the treatment, showed significant reduction in *S. mutans* post-treatment. The multivariate model clustered the S-ECC children into C1 subgroup (34 children) having more carious surfaces before treatment and lesser new lesions after the treatment and C2 subgroup (19 children) who had multiple recurrent carious lesions post-treatment. The two variants of S-ECC groups had different characteristics on the parameters of oral microbiota, dietary, and hygiene habits. The C2 (recurrent caries) subgroup showed minimal change in the microbiota post-treatment as compared to C1 subgroup [Tanner et al., 2011].

Along with the confirmation of association between MS, diet and ECC, a main finding of the study was that of the association between the bacterium *Slackia exigua* and S-ECC. The authors suggested possible role of *S. exigua* as a risk factor for ECC. *S. exigua* was found consistently with *Actinobactria* and other aciduric/acidogenic bacteria typically associated with periodontitis. The authors suggested that the presence of these species may reflect an ecological niche of deep dentinal caries as often occurs with S-ECC [Tanner et al., 2011].

Overall, the study highlighted the diversity within ECC based on higher counts of cariogenic bacteria in subsets of children. This is consistent with its varied clinical appearance. The use of microarray and other advanced technical tools were the strengths of the study, whereas the weaknesses of the study included the follow-up of only 12 months and the power of the statistical analyses. The authors speculated about the generalizability of the findings and suggested that the groups of children enrolled for the study were representative of the population with aggressive caries. The study tried to explain the differences in response to therapy and recommended further research in this field. It can be concluded that ECC is a complex infection with different oral microorganisms and diet simultaneously playing roles in the clinical course of ECC [Tanner et al., 2011].

Caries Causing Bacteria in Infants

The oral cavity is inhabited by numerous micro-organisms. According to Rotimi and Duerden (1981), a normal fetus is sterile until shortly before birth. The fetus is exposed to micro-organisms of skin, nose, mouth, and conjunctiva, during birth from the vaginal canal in a normal vaginal delivery. These organisms are usually commensals – non-pathogenic. However, in case of a weak host response, they potentiate to become opportunistic pathogens. Rotimi and Duerden collected samples at 1, 2, 3, and 6 days after birth of 23 newborn normal infants. They found *Viridans Streptococci* and *Streptococcus salivarius* were the most common organisms found in mouth swabs 8 hours after the birth. Out of 233 total oral isolates, almost 31% were positive for *S. viridans* and 25% were positive for *S. salivarius*. Other organisms that showed transient presence during the study period were *H. influenza*, *Enterobacteria*, *Neisseria*, and *Enterococci* species [Rotimi and Duerden, 1981].

The article concluded that the normal commensal flora of the oral cavity is comparatively more complex than skin and fecal flora. The authors noted that though originally sterile, the mouth of a newborn is rapidly colonized by aerobic organisms, facultative species, and commensals within the first few days of infant life. However, the appearance of strict anaerobes occurs only after the eruption of the deciduous dentition. It can be concluded that the neonatal oral microflora consists of mainly aerobic organisms and facultative bacteria which inhabit the cheek and tongue mucosa, such as, *S. viridans* and *S. salivarius* [Rotimi and Duerden, 1981].

A study was conducted by Marchant et al. (2001) to compare the microflora of sound enamel surfaces in caries-free children and aciduric microorganisms from carious lesions in caries-prone children (three to five years of age). The sample consisted of 52 teeth extracted due to caries collected from 14 caries-prone children and plaque samples collected from another 14 caries-free children. The organisms were cultured onto a range of specific, non-specific, and aciduric culture media. Strains of the microorganisms were genotyped using Polymerase Chain Reaction (PCR) [Marchant et al., 2001].

The results showed predominance of MS in the oral flora of 94% of the teeth from caries-prone children, whereas, it appeared in only 27% of the samples from caries-free children ($p<0.01$). MS was successfully isolated 34% of the time at pH=5.2 (aciduric culture), where it remained undetected in samples from caries-free children using the same culture media ($p<0.01$). The authors found other strains of Streptococci such as *S. oralis*, *S. sanguinis*, *S. gordiniiin* in the caries free plaque samples. Carious plaque samples were rich in *Actinomyces israelii*, *Candida albicans*, *Lactobacillus spp.*, and *Veillonella spp.* in addition to the MS. The aciduric flora included *S. oralis*, *S. mutans* and *A. israelii* [Marchant et al., 2001].

Marchant et al. concluded by stating that it is the physiologic characteristics of the infecting flora, and not its composition, which determines caries initiation and progression. As described in an earlier section, Tanner et al. (2011) found *S. exigua* along with MS to be associated with ECC. These studies provide insight into the etiology of the caries process and suggest the importance of dietary and other intra-oral environmental factors as targets for preventive measures for ECC.

Mutans Streptococci (MS)

In the latter half of 19th century Miller et al. confirmed microbial acid production as the etiology of dental decay. However, they could not associate any single species with the concerned activity of acid production [Loesche WJ, 1986]. Sucrose restriction in the diet of Japanese and European populations during World War II was followed by a notable reduction in caries prevalence in these populations. This helped establish the role of sucrose in caries progression. It is MS that has the unique ability to use the excess sucrose and convert it as part of its pathogenicity. Chemical analyses have shown the unique property of MS to form adhesive colonies in the presence of sucrose medium on any surface, including a tooth, a wire, or culture vessel. The colonization was not observed with non-MS bacteria or non-sucrose media [Loesche WJ, 1986].

Scientists have found that the initial metabolism of sucrose leads to the formation of glucans and fructans, partially diffusing into the oral environment and lowering the pH. A part of these metabolites remain associated with the cariogenic microbial cell. Tracing this link leads to the MS using these polymers and their homopolymers to form adhesive colonies sticking to surfaces both *in vitro* and *in vivo*. This is the characteristic of MS that makes it cariogenic [Loesche WJ, 1986].

A systematic review of 313 published English-language articles by Tanzer et al. (2001) analyzed the role of bacteria in dental caries. The studies included randomized-blinded-interventional, longitudinal, case-control, and cross-sectional studies, all weighted as per the quality of evidence. Results indicated the central role of MS in caries initiation on both smooth surfaces and pit-and-fissure surfaces. Other findings included the contribution of *lactobacilli* in caries progression and vertical spread of MS. The review also discussed some of the challenges or limitations of the studies included, such as scarcity of studies on salivary or plaque sampling and the role of dietary carbohydrates in influencing the oral flora [Tanzer et al., 2001]. This paper is discussed again later in the chapter.

The Loesche et al. (1975) study discussed earlier in relation to the reliability of plaque sampling, also found significant differences in *S. sanguinis* levels of caries-free and caries affected children. The levels of *S. sanguinis* in plaque samples from carious fissures were lower than those in plaque samples from caries-free fissures. The authors concluded that, the presence of other oral micro-organisms, such as, *S. sanguinis,* can influence MS infection and caries progression. These specific organisms will be discussed in the subsequent sections.

Streptococcus sanguinis (S. Sanguinis)

Carlson et al. (1970) conducted a longitudinal study on 27 newborns in Sweden in order to compare oral streptococcal flora in the early period of life. Oral swabs were obtained from these children in the neo-natal period, as well as periodically up to the fourteenth month of infant life. The initially sterile samples became positive with *S. sanguis* in the period matching with that of tooth eruption (5-10 months). After assessing the salivary samples from the family members of the children, the authors found that infants acquired *S. sanguis* earlier and more easily than they would acquire *S. mutans*. This could be due the common habitat of teeth, but also because different factors govern the colonization of *S. mutans* and *S sanguis*. The authors recommended more research in this field using more advanced techniques [Carlson et al., 1970].

Based on the application of advanced molecular and chemotaxonomic approaches, in taxonomy of oral streptococci was updated between 1990 and 2000. *S. saguine* became *S. sanguinis* and its characteristics were redefined along with the entire *Viridans* group. Caufield et al. (2000) conducted another study to investigate infants' acquisition of *S. sanguinis*. This study was a re-analyses of the samples collected in a longitudinal study conducted by the same group of investigators and is described earlier in this chapter. The change in the taxonomical definitions and characteristics of the entire *Viridans* group of *Streptococci* necessitated the comparison and confirmation of the bacterial samples for the older cohort and acted as the impetus for the re-analyses [Caufield et al., (2000)].

The study followed two separate cohorts. The first was a group of 46 mother infant pairs (1984-89) called "the natural history cohort" described earlier for the main study. The other cohort, derived from the same population, was composed of 38 infants between six to thirty-six months of age and was referred to as "the taxonomy cohort".

Selection criteria and sample collection procedures were similar for both the groups. A total of 291 samples were obtained from the second group [Caufield et al., 2000].

Data were analyzed by both parametric and non-parametric statistical tests using the time of initial colonization as a dependent variable. The findings confirmed that the time of initial colonization by *S. sanguinis* and infant's age at first tooth eruption were significantly correlated. (p=0.0001). Another important finding of the study was that late MS colonization was correlated with early *S. sanguinis* colonization. (p=0.03, Cox regression analysis). Observations from the natural history group showed that the eight infants who remained free of MS infection during the study period had a higher proportion of *S. sanguinis* in their salivary samples (5.7 X 10^5 CFU/ml). This was notably higher than the thirty seven infected with MS who had an average 4.6 X 10^5 CFU/ml. (p=0.03 Wilcoxon rank sum test) Comparison of *S. sanguinis* levels at pre- and post- MS acquisition in both the cohorts revealed significantly (p=0.05) higher *S. sanguinis* levels (4.5 X 10^5 CFU/ml) pre-acquisition than post-acquisition (1.9 X 10^5 CFU/ml) [Caufield et al., 2000].

The authors acknowledged limitations of the study, such as generalizability of the sample, accuracy of methods, and concluded that MS and *S. sanguinis* are competitors of each other in colonizing infants' mouths. In other words, one group of organisms has negative effect on colonization by the other. The authors speculated that this may have significance in developing preventive strategies and also suggested that *S. sanguinis* could possibly be used as a caries vaccine in the future [Caufield et al., 2000].

Although there have been many studies exploring preventive strategies at a microscopic level, no confirmed associations have been established. Thus, exploration of more established aspects of the caries process, such as dietary and behavioral factors, which can be controlled by every individual, is needed.

Diet

A study was conducted by Palmer et al. (2010) in Boston to examine the association between bacterial colonization and diet in children with S-ECC. They recruited 72 children with S-ECC and 39 caries-free children between the ages of two to six years. Both the cohorts were followed for 12 months with monitoring scheduled every three months. A questionnaire about the child's diet, in the form of a twenty-four hour diet recall, was answered by the parents. Plaque samples were collected from subject's molars using toothpicks. Microbial analyses for *S. mutans*, *S. sobrinus*, and Bifidobacterium species were carried out using Polymerase Chain Reaction (PCR) [Palmer et al., 2010].

The groups were comparable in child's age, gender, race, family income, parent/guardian's education, and fluoride exposure. The mean consumption frequency (including bedtime snacking) ($p=0.003$) and amounts (0.002) of food and beverages were significantly higher for children with S-ECC than caries-free children. In addition to being significantly associated with the caries status, *S. mutans* were also associated with developing new lesions, food frequency, and estimated cariogenecity of the food ($p<0.05$). Mean putative food cariogenecity was also positively correlated with the presence of *S mutans* ($p=0.03$). The study concluded that, putative food frequency, cariogenecity, and cariogenic bacteria should be targeted for treatment or prevention purposes [Palmer et al., 2010].

Grinefjord et al. (1991), as discussed earlier, found a significant association between early MS colonization and dietary habits, especially sugar-containing beverages at night and total sugar containing beverages, in one-year old Swedish children. The authors discussed the impact of maternally induced behavior patterns, such as dietary habits, on a child's susceptibility to MS infection [Grinefjord et al. 1991].

Food habits - Frequency of Food Intake

Wennerholm et al. (1995) carried out a clinical trial with 20 subjects heavily colonized by MS, who were also regular sugar consumers and harbored *S. mutans* and *S. sobrinus* in saliva. The test group subjects (n=12) refrained from sugar consumption between meals and total sugar intake in main meals for 6 weeks. The control group was not subjected to any dietary restrictions. Salivary samples collected at baseline, 3, 6, and 12 weeks showed that the bacterial levels, including those of MS, were reduced during the six week sugar restriction period. In the six weeks post-restriction follow-up visit, the levels in the test group had increased again, but were still lower as compared to baseline ($p<0.01$). The control group did not show significant differences in the bacterial levels at any point of time. The authors also noted significant differences in the buccal counts as compared to interdental counts post-intervention. They concluded that the dietary sugar restriction affects salivary counts of both *S. mutans* and *S. sobrinus* to similar extent and more effect is noted on the buccal than on the interdental plaque levels [Wannerholm et al., 1995]. Similar results were noted by Grindefjord et al. (1991), and Tanzer et al. (2001).

- Night Time Bottle-Feeding

A study conducted by Plonka et al. (2012) explored the colonization patterns for MS and *Lactobacilli* in 957 pre-dentate Australian children. The recruited mother-child dyads were followed for seven months from the birth of the child. The second visit was conducted at the age of seven months, and 283 children who did not have any teeth erupted at that time, were included in the study. The prevalence of MS positive children increased from 9% at baseline to 11% at seven months. The prevalence of *Lactobacilli* infection increased significantly from 24% to 47% ($p<0.0001$) over the same period. Predictors for the presence of MS were maternal MS counts, presence of MS at baseline, and nighttime bottle-feeding. Presence of *Lactobacilli* was associated with maternal *Lactobacilli* counts, and that was the only predictor for *Lactobacilli* infection at seven months [Plonka et al., 2012]. Findings from Grindefjord et al. (1991) also support that nighttime feeding predisposes the child to early MS infection.

- Frequent Snacking

Thitasomakul et al. (2009) analyzed 495 Thai children (discussed earlier) and concluded that frequent snacking in the form of on-demand breast-feeding resulted in higher caries susceptibility [Thitasomakul et al., 2009]. The results from this and other studies suggest the need for further investigation of the relationship between frequent snacking and MS levels. Carlson (1989) reviewed data from the Vipeholm study (an early study on the effects of candy chewing on caries) after the identification of MS as cariogenic agent. He found that MS levels did not differ significantly among the groups with differing sugar consumption. He concluded that differences in the diet are sufficient to create differences in caries experience, but not sufficient enough to create differences in oral bacterial levels [Carlson, 1989].

As reviewed so far, many studies have found relationships between early MS colonization and dietary habits, especially high frequency of sugar-rich foods [Alauusua et al. (1995), Karn et al (1998), Tanzer et al. (1991), Wan et al. (2003), Weber-Gasparoni et al. (2012)] . Others have found no association between frequency of food or beverage consumption and MS colonization [Carlson (1989), Gudino et al., (2007), Kowash et al. (2002)]. However, as discussed in the systematic review by Tanzer et al. (2001), more studies are needed to investigate the effect of dietary carbohydrates on the MS levels in children. The following section will review studies that have assessed diet composition and salivary MS levels.

Composition of Diet

- Solid/Liquid/Semisolid

Beighton et al. (1999) found that both consumption of sugar-rich food in high frequencies, especially solid food, resulted in increased MS levels in a group of 20 dental students. The study assessed the effects of lemonade and biscuit consumption, which did not have significant impact on MS levels, as compared to toffee and sugar lumps consumption, which increased the MS levels significantly. The study is discussed in subsequent sections in greater detail [Beighton et al., 1999].

- Infant Formula

A study conducted by Sheikh et al. (1996) demonstrated that salivary pH was decreased by consumption of eight different infant formulae included in the study. The authors concluded that infant formulae are cariogenic in higher quantities and frequencies [Sheikh et al., 1996]. These results suggest that infant formulae should be under consideration for contributing to MS infection as well.

The finding by Sheikh et al. (1996) was supported by another study by Erickson et al. (1998), where the alteration in pH was analyzed in healthy adult volunteers using 26 infant formulae. The study concluded that most of the infant formulae resulted in a significant decrease in plaque pH and also supported significant bacterial growth. Dissolution of enamel mineral of incubated extracted teeth with certain infant formula was observed in the study [Erickson et al., 1998].

Holgerson et al. (2013) conducted a study to assess the difference among the oral microbial profiles of 207 infants who were exclusively breast-fed (70.5%), partially breast-fed (18.4%), and exclusively formula fed (11%). After analyzing the swab samples with Human Oral Microbe Identification Microarray (HOMIM) and by PCR technique, the authors found that the oral microbial profile of breast-fed infants differed significantly from those that were formula-fed. Growth of cariogenic MS were inhibited by multiple commensal organisms like *L vaginalis*, and *L gasseri* in the prior group as compared to the latter. The authors concluded that the possible reason for these differences would be species suppression by *Lactobacilli* indigenous to breast milk leading to inhibited growth of MS in these infants [Holgerson et al., 2013].

- Sugar intake

There has been established connection between sugar intake and caries experience as discussed earlier in this chapter (Warren et al., Marshall et al., Thitasomakul et al., Chankanka et al., and others). The direct relationship between sugar intake and MS infection has not been not completely explored and is more often discussed through correlation with caries. This section focuses on studies which have considered MS levels as primary outcome and compared sugar intake of the enrolled subjects.

Cross-sectional studies of Swedish children (Grindefjord et al., 1991), Australian-Caucasian children (Wan et al. 2001), and Iowa-WIC children (Weber-Gasparoni et al., 2012) have shown the association between high sugar consumption and early MS colonization. They are discussed earlier in this chapter. Mohan et al. (1998) conducted a study to investigate the association between MS levels in children and bottle usage. They collected information on the contents of the bottle and categorized the children (n=118, aged 6 to 24 months old) into three groups no bottle, milk, and sweetened beverages. After adjusting for age, number of teeth, and bottle usage, sweetened beverages were associated with a statistically significant, four-fold increase in the odds of MS colonization relative to milk consumers [Mohan et al., 1998]. The study will be discussed later in greater detail.

Beighton et al. (1999) studied a group of 20 dental students exhibiting high levels of salivary MS (>10^4 CFU/ml of saliva). The effects of oral hygiene habits and dietary changes on the oral microbial count were analyzed at two time periods in the same group of subjects. Initially, the subjects refrained from toothbrushing for 24 hours and plaque samples were collected on the next day. Another time they were asked to resume oral hygiene practices but were subjected to dietary supplementation for five days with lemonade, biscuits, chocolate and caramel toffees, and sugar lumps.. Plaque samples were collected at the end of 5 day regime [Beighton et al., 1999].

The authors did not find significant differences in microbial composition as an effect of oral hygiene habits. The decreased proportion of MS in subjects after good oral hygiene practices were attributed to the use of fluoridated toothpaste. Significant differences were noted with total bacterial counts as well as MS counts, especially after consumption of sugar lumps. Lemonade and biscuit supplements were not associated with significant alteration in oral microflora. The authors concluded that high frequency consumption of certain sugar-containing food items was associated with increased plaque accumulation and correspondingly increased susceptibility to MS [Beighton et al., 1999].

Tanzer et al. (2001) conducted a systematic review of 313 published articles on bacteria and caries. The found that the frequent sucrose consumption leads to ecological emergence of MS and *lactobacilli* subsequently leading to caries. They also included two studies on patients with no sucrose consumption – patients with genetic diseases such as hereditary fructose intolerance and intestinal sucrose deficiency. The results indicated a great impact of sugars on colonization of the bacteria as well as on the development of carious lesions [Tanzer et al., 2001].

Law and Seow (2006), as discussed earlier, found significant association ($p<0.01$) between intake of sugar snacks and MS colonization in their prospective cohort study involving 63 young children [Law and Seow, 2006].

Mode of Feeding

The mode of feeding has an effect on MS acquisition in infants. A study conducted by Thakur et al. (2011) aimed to determine the correlation between mode of delivery and other factors associated with MS colonization in infants. Sixty mother-infant pairs were enrolled from a defined geographic area in Southern India for the study. Thirty of the children were born through caesarean section (C-section) and the other 30 were born through vaginal delivery. Depending on tooth eruption period, salivary or plaque samples were obtained from the infants. Salivary samples were mainly collected from the dorsum of the tongue and the alveolar ridge. The final study included data collected from 57 mother-child pairs followed for 1 year with sample collection repeated every 3 months. [Thakur et al., 2011]

Results demonstrated a significant positive association between the duration of bottle-feeding and MS acquisition. Statistically significant correlation ($\chi 2 = 12.1$; $p= 0.007$) was observed in 13 of the 21 prolonged bottle fed infants who were positive for MS. The results also did not support any possible association between birth history and MS acquisition ($p>0.05$) as there was no difference between the groups. The study results suggested a narrow range of risk factors for MS acquisition in infants by its negative findings regarding birth history and emphasized the effect of feeding practices [Thakur et al., 2011].

Thitasomakul et al. (2009) explored the relationship between caries experience and mode of feeding and found significant associations between caries experience and on-demand – breast-feeding in 495 Thai children [Thitasomakul et al., 2009]. The study has been discussed elsewhere, and the findings suggested a potential correlation between

MS levels and mode of feeding. The following section discusses a few studies which were targeted at analyzing the relationship between breast- or bottle-feeding or sippy cup use and MS levels.

- Breast-Feeding

The American Academy of Pediatric Dentistry (AAPD) policy supports American Academy of Pediatrics' (AAP) statement regarding the benefits of breast-feeding. Breast-feeding does not eliminate the risk of caries development in breast-fed infants, but breast-feeding and the consumption of human milk have positive effects on dento-facial growth and oral health. According to AAPD, risk of ECC for the breast-fed child is related to "the extended and repetitive feeding times with prolonged exposure of teeth to fermentable carbohydrate without appropriate oral hygiene measures." [AAPD, 2000-2001]

In their article, Hanson et al. (1998) described the immunological role of breast-feeding. Components of mature human milk are secretory IgG (SIgA), lactoferrin protein, large oligosaccharide fractions which act as receptor analogues for microbes, and nucleotide fractions which form the building blocks for DNA in the developing lymphoid system. Breast-feeding has also been shown to have long term protection against infections by enhancing antibody responses to vaccines [Hanson et al., 1998], by stimulating infants' immune system by secreting anti-idiotypic antibodies and lymphocytes in the milk [Hanh-Zoric et al., 1993], and by stimulating the increase in the size of thymus [Hanson et al., 2001].

Li et al. (1994) studied a group of 486 three to four year old Chinese children. Almost 48% of the children (n = 234) were diagnosed with enamel hypoplasia. Amongst the MS positive children, the majority (49.6%) exhibited high levels of salivary MS (>100 CFU per strip). The authors also recorded birth history, dietary habits, including length of breast-feeding, as well as body height and weight. They did not find significant association between differences in MS concentrations and length of breast-feeding in the cases and the controls. The authors observed a significant correlation between enamel hypoplasia and high MS counts, and so concluded that the surface irregularities on the enamel are contributing factors for higher MS infection [Li et al. 1994].

- Bottle-Feeding

A study conducted in Connecticut by Mohan et al. (1998), was focused on the factors associated with MS acquisition in infants. The study was a cross-sectional study and enrolled 122 children in the six to twenty-four months age group from a low SES population attending WIC clinics in Hartford. Salivary samples were collected using sterile wooden tongue blades. Clinical examinations were carried out by a single examiner. Data about bottle content / usage were obtained using open-ended questions answered by the parents. The children were classified in three groups according to bottle content – milk / formula, sweetened beverages, and no bottle. Four out of 122 children used only water in bottles. They were eliminated from the sample and data analysis was carried out for the remaining 118 children [Mohan et al., 1998].

One half of the total children were positive with MS with 20% of them being under fourteen months of age. MS colonization was significantly associated with age, number of teeth, and bottle content. Prevalence odds ratio was found to be 4.0 (95% CI = 1.2-12.6) for consumption of sweetened beverage as bottle contents when adjusted for age and number of teeth. Children aged eighteen to twenty-one months were more susceptible to MS infection than other age groups and susceptibility to infection increased as the number of teeth increased. The authors concluded that sweetened beverage consumption from the bottle increased the risk of MS colonization four times as much as when milk was used as the bottle contents [Mohan et al., 1998].

- Use of Pacifiers / sippy Cups

No study was found in the literature which analyzed the effect of the use of pacifiers / sippy cups on the MS levels of a child. Almost all of the search results were studies about possible role of pacifiers / sippy cups in prevention of ECC by means of probiotics or lozenges [Caglar et al. (2008), Suhonen et al. (1994), Taipale et al. (2007, 2012)]. Because of this shortcoming in the literature, the present study will analyze the effect of sippy cups, and whether their use has any potential to affect the MS in children.

Oral Hygiene Habits

Many studies have suggested that oral hygiene habits such as toothbrushing, use of fluoridated toothpastes, rinsing after every meal, and not sharing utensils, have the potential to reduce plaque accumulation and decrease caries experience. Some of them have been discussed earlier in the chapter [Chan et al. (2002), Santos et al. (2002), Law and Seow (2006)]. Tenovuo et al. (1992) conducted a study on 151 one year old children and their mothers. The subjects were divided into three groups based on the maternal salivary MS levels. Children form the test group, where mothers received chlorhexidine treatment, experienced MS infection and caries at significantly older age than did children in the control group [Tenovuo et al. (1992)]. These findings are suggestive of the impact of oral hygiene habits on plaque as well as on transmission of MS. However, as discussed in the successive sections, evidence of the relationship between oral hygiene practices and bacterial levels are varied. Effects of different oral hygiene practices are discussed separately below.

Toothbrushing Habits

In their article, Law and Seow (2006) demonstrated that commencement of tooth brushing after 12 months of age is significantly associated with early MS infection in young children [Law and Seow, 2006]. Beighton et al. (1999) did not find a significant impact of oral hygiene habits, especially toothpaste, on MS counts of the study subjects. However, they noted a non-significant decrease in MS levels post-toothbrushing with fluoridated toothpaste. The authors attributed this to the use of antimicrobial toothpaste containing fluoride, zinc citrate and triclosan. Commencement of tooth brushing after 12 months of age and lack of parental assistance while tooth brushing were found to be significant risk factors in developing MS infection [Beighton et al., 1999].

Mattos-Granner et al. (2001) followed children aged 12 to 30 months for one year in order to assess the effect of their sucrose-rich diet on MS levels. All of their subjects were attending school nurseries where they received sucrose-rich meals four times a day for five days a week. Children older than two years of age also received daily toothbrushing with fluoridated toothpaste by the health agents at the nurseries. Most of the children (70-86%) were MS positive at baseline and the groups did not differ significantly on other determinants of MS colonization such as number of teeth, age, and dietary habits. Significantly higher proportions of those aged 25-30 months showed a decrease in MS levels at the end of 1 year [OR = 3.2 (1.07-9.57)]. However, findings from the younger age group (12-24 months) indicated an increase in the proportion of infected children [OR = 1.0]. The authors attributed this difference to the increased exposure to fluoride through fluoridated toothpaste in the older age group, even though they were more heavily colonized at baseline. The findings suggest that regardless of early MS colonization and high sucrose intake, the MS levels may remain relatively stable after two years of age [Mattos-Granner et al., 2001].

Seow et al. (2003) studied the effect of toothbrushing in 107 children who were an average 20 months old. They randomly selected these children from community child health clinics in Australia and collected swab samples from their teeth and mucosa. The presence of MS was determined using a commercial microbiological kit and the mothers were instructed about the soft-scrub tooth brushing method and also educated about oral health. The children were followed after four weeks, and re-evaluation of MS levels was performed. Twenty-nine percent of the children who were positive for MS at baseline were converted to negative status at the follow-up period. The authors attributed this to the toothbrushing practice during the four week period. The significant differences in the toothbrushing and snacking habits between MS positive and MS negative children at both the time points were also credited to the health education of the mothers [Seow et al., 2003].

Plaque

Results from studies assessing effects of plaque on caries progression [Santos et al. (2002), Chan et al. (2002), Law and Seow (2006),] are suggestive of correlation between MS levels and plaque.

As discussed earlier, Beighton et al. (1999) concluded that high frequency consumption of certain sugar-containing food items was associated with increased plaque accumulation and correspondingly increased MS counts [Beighton et al., 1999].

D. Demographics

As discussed earlier under the demographics section on caries, age, SES, race and ethnicity are important determinants of an individual's caries experience [Warren et al. (2008), Tomar and Reeves (2009), Psoter (2006), Chan et al. (2002), NHANES reports]. The established relationship between MS and caries suggests that there may be a possible role of these variables in MS infection as well.

Law et al. (2007) concluded that complex interactions among virulence factors, environmental factors and host-related factors including demographics determines the age at which initial colonization by MS occurs [Law et al., 2007]. Studies such as Weber-Gasparoni et al. (2012), Wan et al. (2001) have looked into MS levels of children and analyzed the role of demographic factors. They were discussed earlier in this chapter. As appropriate, a few of the demographic factors are reviewed below.

Demographic factors

Age

Warren et al. (2008) and Tomar and Reeves (2009) found that age is a risk factor in caries experience of an individual. Caufield et al. (1993) defined the 'window of infectivity' period of infant life being the most susceptible time span for MS infection. Tedjosasongko and Katsuyuki (2002) confirmed this period using a DNA fingerprinting method. Chan et al. (2002) found significant association between caries experience and age and age related functions such as weaning period and the consumption of candies in 666 Hong Kong preschool children. All of these studies suggest that MS infection becomes more likely as children get older and have more teeth.

Weber-Gasparoni et al. (2012) found significant association between MS levels and some age related functions such as toothbrushing, number of teeth and breast-feeding practices. After adjusting for age, these factors did not remain significant in the final model [Weber-Gasparoni et al., 2012]. Karn et al. (1998) studied a group of 149 WIC enrolled children from eight months to fifteen months of age, in order to explore the relationship between age and MS colonization. All of their subjects were using a bottle and had four or more teeth. Salivary samples were collected using a tongue blade and were cultured on MSKB agar medium. Thirty-five percent of the subjects were identified to be MS positive. The findings indicated that infants (1 year or younger) were also susceptible to MS colonization based on their dietary, behavioral and demographic determinants [Karn et al., 1998]. Mattos-Granner et al. (2001) found decreased or stable MS levels in children aged two years and older. They considered the role of increased fluoride exposure in the older children as one of the contributing factors to having lower MS levels in older children. [Mattos-Granner et al, 2001].

Family SES

Psoter et al. (2006), Tomar and Reeves (2009), Warren et al. (2009), Chan et al. (2002) and many other studies have found positive relationships between caries experience and low SES. Freire et al. (1996), Tang et al. (1997), and Psoter et al. (2006) suggested preventive measures and health education should be directed towards areas of social deprivation. These study findings are suggestive of higher susceptibility of low SES children to MS infection.

The SES of the family directly impacts the quality of nutrition of the child and this, in turn, affects the child's susceptibility to infections common in childhood. In a study done by Turell et al. (2002) to explore the relationship between household income and diet on the basis of nutrient intake, a probability sample of 1003 urban households in Austria, was surveyed. Face-to-face interviews were used for data collection. An individual's Socio-Economic Position (SEP) was determined based on education, occupation and household income. Diet and nutrient intake was determined by examining the grocery items including vegetables, fruits, and meat. [Turell et al., 2002]

The results indicated that the choice for food purchase and SEP of an individual were strongly associated. Individuals with low SEP purchased lesser proportions of grocery foods that were fiber-rich and low in fat, salt and sugar than those who were at an advantageous SEP. Similarly, individuals with lower levels of SEP, education, and those employed in manual occupations purchased fewer types of vegetables and fruits as compared with individuals from higher SEP. The article concluded that to improve health of the population, health promotion efforts need to be directed to narrow the differences in food purchasing based on SEP [Turell et al., 2002]. These results are also suggestive of higher susceptibility to MS infection in low SEP individuals due to poor choices of food and sugar-rich, unhealthy diets.

Parental Education and Health Related Behavior

Kowash et al. (2002) found that parental health education and putative health related behavior of the parents/caregivers does not affect the levels of MS in children. Both Weber-Gasparoni et al. (2012) and Wan et al. (2001), found that low levels of maternal education usually results in higher MS levels of the child in their cross-sectional analyses.

Floride Exposure

Kowash et al. (2002) compared MS levels of five groups of 228 children based on maternal dental health education and oral hygiene habits including toothbrushing with fluoridated toothpaste. The study found no significant differences among the groups at the end of three years. [Kowash et al, 2002].

Santos et al. (2002) studied a group of 60 Brazilian children in order to compare their plaque composition based on their caries status. The participants of the groups were children from the same daycare center and were subjected to toothbrushing with fluoridated toothpaste twice a day, every day, under supervision. The authors found significantly higher proportion of fluoride and lower proportions of MS in plaques of caries free children as compared to those suffering from nursing caries [Santos et al., 2002].

Weber-Gasparoni et al. (2012) analyzed a group of 411 WIC enrolled children and compared their MS levels in relation to various demographic and behavioral factors. They found that children exhibiting high levels of MS also had a history of tooth brushing with fluoridated toothpastes. The authors considered it as a function of age and concluded that MS infection was associated with age of the child and older children with a greater number of teeth also used fluoridated toothpaste, resulting in the correlation observed in this study [Weber-Gasparoni et al., 2012].

Race, Ethnicity and Gender

In a review article by Helderman et al. (1996), a meta-analysis of fourteen African studies about MS infection in Africa in comparison with European and North American studies was carried out. The review attributed differences in the cariogenicity of the diets as the reason for differences in caries experience in children of the three continents and not the prevailing mutans streptococci species. The authors concluded that, MS infections are ubiquitous in children ages seven years and older in Africa, Europe, and North America. The low daily sucrose intake and consumption frequency in African populations were the reasons for the low caries experience in the African populations. Thus, the authors concluded that the diet, rather than the race or ethnicity, should be considered as risk factor for MS acquisition and caries [Helderman et al., 1996].

Summary

The literature review has documented that MS is the causative organism for early childhood caries. Many studies conducted have shown strong association between diet, plaque, socio-economic status, and caries experience. Very few studies have assessed the role of these factors in establishing MS infection in infants. As MS has been recognized as a causative organism for dental caries, this knowledge plays a crucial role in understanding the etiology and progression of the disease. This also plays a very important role in deciding the course of the treatment and planning preventive strategies.

As per the literature, dietary, behavioral, and socio-demographic factors play key roles in the MS infection initially and the progress of ECC later on. Several studies have reviewed caries as an outcome measure, and exploration about factors related to ECC has been quite extensive. However, there are very few studies published which have analyzed MS levels in children as the primary outcome measure. Thus, a longitudinal study assessing possible risk factors in MS acquisition is needed.

The present study was aimed at analyzing factors associated with the presence of MS in infants and toddlers. The study utilized longitudinal data on beverage consumption for subjects who could be differentiated into groups based on their salivary MS levels. Besides the detailed dietary data, the study also collected behavioral and demographic data of the subjects which allows for consideration of a broad array of factors involved with MS colonization. To date, most of the published literature has focused on only a limited number of factors with regards to MS infection in infants. Thus, the present study will contribute to the literature by longitudinally assessing a wide range of factors that may potentially affect MS colonization in young children.

CHAPTER 3

MATERIALS AND METHODS

Introduction

The purpose of this secondary analysis was to identify the factors associated with mutans streptococci (MS) colonization in infants and toddlers participating in a longitudinal study who were aged six- to twenty-four months at baseline. The data for the study were collected by researchers from the University of Iowa during June 2003 - 2006 from 212 children enrolled in the Special Supplemental and Nutrition Program for Women, Children and Infants (WIC) program in Iowa.

Research Question

The primary research question was:

"What factors are associated with the presence of MS in infants and toddlers?"

General Research Hypothesis

The overall general hypothesis was that demographic, behavioral, and environmental factors affect MS colonization in low income children enrolled in the WIC program aged six- to twenty-four months at baseline.

Study Population and Inclusion Criteria

Study Population

The sample was composed of participants in the WIC program from two rural Iowa counties, based at the WIC center in Muscatine, Iowa. The population consisted of children mostly from low socioeconomic status and included a large Hispanic minority. The children and their mothers or primary caregivers (in some cases) were recruited by the study coordinator at the WIC sites in Iowa during June 2003 to December 2004.

Inclusion Criteria

The only inclusion criteria were the age of the infant (6 to 24 months) and the mother's and child's intentions of staying in the area for approximately18 months.

All the participating children were healthy and normally developed, except one who had special health care needs. A total of 268 children's parents were approached to enroll in the study and 212 children ranging from six- to twenty-four months of age participated at baseline. All the children received a clinical examination either at the time of recruitment or in the next few months, and then returned for examinations 9 and 18 months later. As will be described later, the study also obtained data via questionnaires completed by parents at baseline and 4.5, 9, 13.5, and 18 months after baseline.

IRB Approval

Informed consent was obtained from all the participants at the time of enrollment using protocols approved by the University of Iowa Institutional Review Board, Human Subjects Committee.

A separate application was made to University of Iowa Institutional Review Board for secondary analysis of the data on July 03, 2012. The chair of the UI-IRB determined that this secondary analysis using de-identified data was exempt from IRB regulation as it no longer constituted human subject research.

Data Collection

Non-clinical Information

The information about demographics and behavioral patterns was collected using questionnaires. They were completed by the mothers or primary caregiver. These questionnaires were adopted from questionnaires developed, validated and used for the Iowa Fluoride Study (Marshall et. al., 2008). The information collection was done at five time points during the study – at baseline and at 4-5, 9, 13-14, and 18 months after baseline. Questionnaires were administered at the time of examination at baseline, 9 months, and 18 months. At the two other time points (4-5 months and 13-14 months) the questionnaires were sent to the participants by mail. The study participants were asked to return these completed questionnaires by return mail using a pre-addressed, stamped envelope.

The questionnaires included questions regarding demographic characteristics of the child and family which were recorded at baseline only. These questions mainly sought information about the child's age, sex, mother's age, race/ethnicity, mother's occupation, total annual household income, mother's marital status, and mother's highest level of education. In addition, separate questionnaires were used to collect data on the diet of the child and mother. These data were collected at five time points using the same survey instrument each time. The questions included yes/no questions, multiple choice questions, and questions where mothers provided estimates of beverage serving amounts and number of servings. The following table lists the variables used in the questionnaire along with the levels or answer choices for each variable. The table includes only those questions which were in the questionnaire for children. The mothers were given separate questionnaire.

Table 3.1. Dietary Variables and Their Description

Dietary Variables	**Levels**
Breast-feeding habits	Dichotomous (yes/no)
Bottle-feeding habits	open cup / sippy cup / cup with straw mechanism, night feeding
Product container	juice box, pop can or bottle
Infant formula	amount / serving and servings / week
Cow's milk	amount / serving and servings / week
100% juice	amount / serving and servings / week
Water	amount / serving and servings / week
Flavored water	amount / serving and servings / week
Sugared beverages	amount / serving and servings / week
Sugar free beverages	amount / serving and servings / week
Regular pop	amount / serving and servings / week
Diet pop	amount / serving and servings / week
Sports drinks	amount / serving and servings / week
Other sugared beverages	amount / serving and servings / week
Juice drinks	amount / serving and servings / week
Type of milk	Whole, 2% milk, 1% or skim milk, chocolate milk, other flavored milk

Table 3.2. Behavioral Variables and Their Description

Behavioral Variables	**Levels**
Toothbrushing	dichotomous (yes/no)
Use of fluoridated toothpaste	dichotomous (yes/no)
Main water source for the child	bottled water or public water supply
City for the public water supply	Open question
Brand name for the bottled water	Open question

A copy of the actual, detailed questionnaire is included in the appendix.

Clinical Examination

The clinical examinations conducted at baseline, 9 months and 18 months after enrollment included dental examinations of each child and salivary sample collection from both the children and their mothers/caregivers. These are described in detail below:

Dental examinations were primarily visual examinations, based on published criteria, using a halogen headlight, explorer and mirror. The knee-to-knee position was used and exams were done in a dedicated room at the Muscatine WIC clinic site. Dental caries were assessed using d1 (non-cavitated) d2-3 (cavitated) f (filled) criteria (Warren et. al. 2002).

Maxillary and mandibular arches of the children were also assessed for the presence of plaque. The observations were recorded separately as plaque – present or absent on the upper and lower incisors and molars. Data were also collected for the presence or absence of teeth.

Examinations were carried out by a single examiner (Dr. Karin Weber-Gasparoni). The teeth were dried with gauze, and were primarily visual with suspected cavitation confirmed with a shepherd's hook explorer, which was also used to remove debris.

Bacterial counts for MS were obtained via salivary sample collected from the mother and the child and were quantified using the method of Edelstein and Tinanoff [Edelstein B., Tinanoff N., 1989]. Using this method, a sterile tongue blade was gently pressed against the dorsum of the subject's tongue one side at a time in an alternating fashion for a total of ten times. Each side of the blade was then imprinted on a raised agar plate containing selective agar for MS. These plates were then incubated at 37˚C in 5%

CO_2 for 48 hours at the University of Iowa laboratories. The numbers of MS colonies were counted and categorized into groups as

i) None

ii) 10 or less

iii) 11 to 100

iv) 101 to 200

v) Too many to count

The clinical examinations including salivary sample collection were repeated at three time intervals – at baseline, 9 months and at 18 months.

Data Management

All data were entered and verified using SPSS Data entry 3.0 (SPSS Data Entry II, Chicago, SPSS, Inc 2001). These data were de-identified prior to analyses and then imported into SAS for analysis. Descriptive statistics were subsequently conducted to identify outliers using SPSS (SPSS v. 19, Armonk, New York: IBM SPSS Statistics, 2013) and SAS (v9.3, SAS Institute Inc, Cary, NC, USA).

Variables

For the present analyses, the subjects were divided into three groups based on salivary MS levels:

Group 1: Children with no MS at any of the three study time points (n=58)

Group 2: Children positive for MS at baseline and at subsequent measurements (n=35)

Group 3: Children who acquired MS during the 18-month study period (n=36) [i.e. subjects who had no MS at baseline, but MS were detected at the 18 month follow-up].

Primary dependent variable

The primary dependent variable was presence of MS in the salivary sample of the children as grouped above.

Independent variables

Independent variables obtained via questionnaires used for data analysis were categorized into three main domains as follows.

Demographic Domain

1. Age of the child: Measured in months from the date of birth until the last complete month at baseline.
2. Sex: Male or female
3. Age of the mother: Measured in years from the date of birth until the last complete year at baseline.
4. Race/Ethnicity: Dichotomized as non-Hispanic and Hispanic
5. Family income level: Annual household income <$25,000, or $25,000 or more.
6. Parental education: Highest level of education of the mother. It was dichotomized as: Less than high school and High school or more.

   The high school or more category included:

   a. High school diploma or GED
   b. Some college including associate degree

c. Baccalaureate college degree or graduate degree.

Dietary Domain

7. Diet of the child:

A. Diet Composition (beverage only)

For each of the following beverages variables indicating whether any was consumed at baseline and subsequent 3 intervals, as well as total amount consumed [amount (in ounces) /serving X servings/week]

i. Infant formula – yes/no and ounces/week

ii. Cow's milk – yes/no and ounces/week

iii. Chocolate milk – yes/no

iv. 100% juice – yes/no and ounces/week

v. Juice drinks – yes/no and ounces/week

vi. Water – yes/no and ounces/week

vii. Flavored water – yes/no and ounces/week

viii. Sugared beverages made from powder – yes/no and ounces/week

ix. Sugar-free beverages – yes/no and ounces/week

x. Regular pop – yes/no and ounces/week

xi. Diet pop– yes/no and ounces/week

xii. Sports drink– yes/no and ounces/week

xiii. Other sugared beverages – yes/no and ounces/week

xiv. Other sugar-free beverages – yes/no and ounces/week

xv. Any sugared beverage intake – chocolate milk /100% juice/ juice drinks / flavored water / sugared beverages made from powder / regular pop / sports drinks / other sugared beverages, – yes/no and combined sugared beverage intake for all these beverages in total ounces/week

B. Diet Behavior: Related to beverage intake.

i. Breast-fed at the time of interview – yes/no
ii. Bottle-fed at the time of interview – yes/no
iii. Use of open cup at the time of interview – yes/no
iv. Use of sippy-cup at the time of interview – yes/no
v. Nighttime feeding practices at the time of interview – yes/no
vi. Child falls asleep with a bottle at the time of interview – yes/no
vii. Bottle-feeding at the middle of the night at the time of interview – yes/no
viii. Snack at bedtime at the time of interview – yes/no
ix. Snack in the middle of the night at the time of interview – yes/no

Clinical Domain

8. Proportion of mothers positive with MS
9. Number of teeth: teeth were considered present if erupted to any degree and the number was summed; dichotomized for regression models depending upon the mean number of teeth and the level of significance.

10. Plaque: Presence or absence of visible plaque was recorded by tooth type for incisors and molars of both maxillary and mandibular arches. The proportion of children with any tooth with visible plaque was used for assessment.
    i. Mean number of maxillary incisors (maxillary molar, mandibular incisors, and mandibular molars at eighteen months' time point only) with visible plaque on them at the time of examination
    ii. Proportion of children with plaque present of any of the maxillary incisors
    iii. Proportion of children with plaque present of any of the mandibular incisor
    iv. Proportion of children with plaque present of any of the maxillary molars
    v. Proportion of children with plaque present of any of the mandibular molars
    vi. Proportion of children with plaque present of any of the teeth
11. Child's caries experience:
    i. Any caries experience (d1d2-3fs score >1)
    ii. Presence of d1 lesions - yes/no variable (only at 18 month time point).
12. Oral hygiene habit assessed as habit of toothbrushing
    - Yes, daily
    - Yes, once in a while
    - No

    Assessed only at descriptive level, due to missing values and procedural errors
13. Use of fluoridated toothpaste - yes/no variable.
14. Exposure to fluoridated water: yes/no variable

    Yes = City public water (from the city of Muscatine)

    No = Bottled water / well water / private water source

<u>Individual alternative hypotheses within each domain:</u>

Demographic Domain

1. Mean age of the children in group 1 (no MS at any point of time) is less than that of group 2 (MS positive at baseline and at subsequent measures) at baseline, and group 3 (acquired MS during the study period) at baseline, nine months, and eighteen months.
2. Group 1 has lower proportions of children, who are girls, have mothers younger than 26 years of age, with Hispanic ethnicity, who belong to low SES (family income <$25,000/year), and have mothers with education less than high school, than those from group 2 at baseline, and group 3 at baseline, nine months, and eighteen months.

Dietary Domain

A. Diet composition:

3. Children from group 1 have lower mean sugared beverage intake as compared to children from group 2 at baseline, and group 3 at baseline, nine months, and eighteen months.
   a. Children from group 1 have lower consumption of individual as well as combined sugared beverages; such as, chocolate milk, 100% juice, juice drinks, flavored water, sugared beverages made from powder, regular pop, sports drinks, and other sugared beverages; as compared to from group 2 at baseline, and group 3 at baseline, nine months, and eighteen months.
   b. Children from group 1 have higher intake of liquids considered to be protective (infant formula, cow's milk, water) and sugar-free beverages [sugar-free soda

pop (e.g. diet pop), sugar-free beverages made from powder (e.g. Crystal Light) and other sugar free beverages (e.g. ice tea, coffee)] against MS infection and caries as compared to group 2 at baseline, and group 3 at baseline, nine months, and eighteen months.

B. Diet Behavior:

4. A higher proportion of children in group 1 were breast-fed compared to group 2 at baseline, and group 3 at baseline, nine months, and eighteen months.
5. Lower proportions of children from group 1 were bottle-fed, used open cup, and closed (sippy) cup, had nighttime feeding practices, were bottle-fed at bedtime and in the middle of night, and had snacks at bedtime and in the middle of night as compared to group 2 at baseline, and group 3 at baseline, nine months, and eighteen months.

Clinical Domain

6. Lower proportions of children from group 1 had mothers infected with MS as compared to group 2 at baseline, and group 3 at baseline, nine months, and eighteen months.
7. Children in group 1 have lower mean number of teeth as compared to group 2 at baseline, and group 3 at baseline, nine months, and eighteen months.
8. Children from group 1 have higher proportion of teeth (maxillary incisors, mandibular incisors, maxillary molars, mandibular molars, and any of these in

combinations) with visible plaque as compared to group 2 at baseline, and group 3 at baseline, nine months, and eighteen months.

9. Lower proportions of children from group 1 have any caries experience as compared to from group 2 at baseline, and group 3 at baseline, nine months, and eighteen months.
    i. Lower proportions of children from group 1 have d1 lesions than group 3 children at eighteen months' time points.
10. Higher proportions of children from group 1 were exposed to fluoride through fluoridated toothpaste and fluoridated water as compared to group 2 at baseline, and group 3 at baseline, nine months, and eighteen months.

Data Analyses

The data were separated into three sets corresponding to each of the time point (baseline, 9 months, and 18 months). Missing values in each of the set were replaced with 0 for clinical variables, such as, number of teeth, number of teeth covered with plaque, and number of d1d2-3fs lesions. For other variables, missing values were considered missing and no replacement was done. For the primary analyses, descriptive statistics were generated for each of the three time-points for all the variables. The variables were categorized into four domains – demographics, dietary, behavioral, and environmental. Each subsection contains analyses of the relationships between group membership and variables under each of these domains. The categorization of variables under these domains is arbitrary and an attempt to match with the questionnaire used for data collection.

After completing descriptive analyses for each time point, the subjects were divided into groups and bivariate analyses were performed on each variable to test its association with group membership. These analyses included Cochran-Mantel-Haenszel test for categorical data, and ANOVA and Kruskal Wallis Test for continuous data for comparisons involving three groups. Chi-square, Fisher exact test, and Wilcoxon Rank-Sum test were used appropriately for the data to be compared across two groups. Pairwise comparisons such as groups 1 vs. 2, 2 vs. 3, and 1 vs. 3, were done at bivariate level for all the variables. Variables that showed significantly in bivariate analyses were included for the multivariate analyses using logistic regression.

Multivariate analyses were conducted for each of the four domains (demographics, dietary – composition & behavior, and clinical). There were a few variables that were significant ($p<0.05$) at bivariate level from each of the four domains. These were selected to enter into the domain specific model. Each domain specific model was formed by subjecting the chosen variables to forward selection procedure with entry 0.1 level of significance set. . The variables that remained in the domain-specific models are summarized in a table for each time point. These variables entered the next stage modelling titled ‘preliminary model’. The preliminary model also was subjected to forward selection procedure with entry level significance set as 0.1. Preliminary model for each time point along with the model statistics, its variables and their ORs are summarized in a table for each time point.

The final model consisted of significant variables selected by the forward selection procedure with similar entry level of significance (alpha=0.1). The final model was checked for multicollinearity using the variance inflation factor (VIF) and its performance was confirmed using c-statistics, Hosmer-Lemeshow Goodness of Fit test, and Akaike Information Criteria (AIC).

Analyses for data points at 9 months and 18 months were done to compare Group 1 vs. Group 3. Group 2 children, those infected with MS at baseline, were not accounted for further analyses. Bivariate analyses for pairwise comparisons for Group1 and Group 3 were carried out. The significant variables were used to construct a model for that particular time point in similar fashion as discussed above.

All tests utilized a 0.05 level of significance, except for the forward selection procedure for logistic regression models which set the 0.1 level of significance for entry into the model. Only those variables from the final model with p-value of 0.05 or less were considered to have significant association with the group membership. SAS for windows (v9.3, SAS Institute Inc., Cary, NC, USA) was used for the data analyses.

CHAPTER 4

RESULTS

This chapter discusses results from the secondary analysis of the data from a longitudinal study of caries in children enrolled in Women, Infant, and Children (WIC) Supplemental Nutrition Program in Muscatine, Iowa. The procedures for the analyses were discussed in Methods section. However, as the chapter progresses, the statistical method used for that specific analysis will be described in greater detail.

The chapter is divided into four sections matching with the time of clinical examinations conducted during the study i.e. baseline, 9 months and 18 months, with the last section presenting the longitudinal results. Each of the first three sections will discuss the frequency distribution for categorical variables and overall distribution with mean and standard deviation for continuous variables at that specific time point. The pairwise group comparisons for all the variables at each time point are then described in the corresponding sections followed by multivariate analyses and results. Baseline pairwise comparisons include three pairs of comparison, whereas successive time points include only one pairwise comparison between Groups 1 and 3. As group 2 consists of subjects who were positive for Mutans Streptococci (MS) at the baseline itself; Group 2 was excluded from analyses at successive time points that were aimed at analyzing relationship between factors responsible for MS infection. Also, a majority of the subjects from group 2 were lost to follow-up, whereas the number of subjects for group 1 and 3 remained unchanged for all the time points discussed in the study.

Baseline

This section is further divided into four subsections:

Overall comparison of all the groups

Pairwise comparison of groups 1 and 3

Pairwise comparison of groups 1 and 2

Pairwise comparison of groups 2 and 3

As mentioned earlier, each subsection contains analyses of the relationships between group membership and variables under each of the following domains: demographics, dietary – composition & behavior, and clinical. The categorization of variables under these domains is arbitrary and an attempt to match with the questionnaire used for data collection. However, each section begins with descriptive statistics about the entire cohort regardless of the group membership.

Baseline – Univariate Analyses

Demographic Variables

Age: The study included 129 subjects at baseline. The minimum age of the children was 6 months and the maximum was 24 months. The subjects' age was normally distributed (based on Shapiro-Wilk test of normality) over the span from 6 to 24 months with mean age of 12.9 months and standard deviation of 5.5 months.

The sample was composed of 45% female children and 55% male children. Twenty-four of 129 subjects (18.6%) answered "Hispanic" for their race/ethnicity. The sample included Black Americans (3.1%), Caucasians (77.5%), Asian (0.8%), and others (3.9%).

Socioeconomic Status and Maternal Education: There were 60.5% (n=78) of the subjects who had annual household incomes of $20,000 or less, while 39.5% (n = 51) of the subjects had annual household income of $20,001 or more. Most of the mothers were high school graduates or had earned a GED (61.9%), while 21.4% had less than a high school education. Very few had two year (8.7%) or four year college degrees (4.7%). Even fewer had graduate degrees (3.1%). The maternal education variable was later dichotomized into 'Less than High School' and 'High School or More'. Thus, a total of 78.6% (n = 99) of the subjects had mothers with education high school or more. This variable had 3 missing values.

Maternal Age: The children were categorized as those with mother aged 26 years or younger and those with mothers aged 27 years or more. Almost three quarters (75.4%) of the children belonged to the first category and one quarter (25.6%) belonged to the latter.

Table 4.1. Frequency and Descriptive Statistics for Baseline Demographic Variables for the Entire Sample (N=129)

Variable	**Frequency**	**Valid Percentage**
Sex		
Male	71	55.04
Female	58	44.96
Mother's Age Group		
26 or younger	96	74.42
27 or older	33	25.58
Race		
Black American	4	3.10
Caucasian	100	77.52
Hispanic	24	18.60
Asian	1	0.78
Other	5	3.88
Ethnicity		
Hispanic	24	18.60
Non-Hispanic	71	81.40
Income Category		
$20,000 or less	78	60.47
$20,001 or more	51	39.53
Mother's highest level of education		
Less than High School	27	21.43
High School Diploma or GED	78	61.90
Two Year College Degree	11	8.73
Four Year College Degree	6	4.76
Graduate Degree	4	3.17
Mother's education (dichotomous)		
Less than High School	27	21.43
High School or More	99	78.57

Dietary Variables

- Diet composition

The mother was asked if the child consumed any specific type of beverage (dichotomous - yes or no) and then was asked about the amount and frequency of consumption of that particular beverage for which the answer was yes. Accordingly, the dietary variables were both dichotomous (yes/no) and continuous (amount per serving x number of servings/week = amount per week).

As mentioned earlier in Chapter 3, a new variable (apart from those in the questionnaire) was created for any milk (whole, 2%, 1%, skimmed, and infant formula) consumption. It consisted of any kind of milk consumption by the participating children (yes/no variable). However, there was no equivalent for the total amount of any type of milk consumed by these children. Another variable ‘any sugared beverage consumption’ represented consumption of any of the sugar-rich beverages, such as, regular pop (e.g., Pepsi, Coke), sugared beverages made from powder (e.g., Kool-Aid), sports drinks (e.g., Gatorade, Powerade), 100% juice, juice drinks, chocolate milk, flavored water, and other sugared beverages (e.g. lemonade, sweetened tea).

The following table (Table 4.2) presents frequency distributions (as percentages) for each of the beverages listed in the questionnaire, along with the average amount of that beverage consumed by the children. Based on the analyses of beverage variables at baseline, infant formula, cow’s milk, 100% juice, water, and milk were most commonly consumed (>50% answered affirmatively). Water was the most popular drink among the subjects (86.1% subjects drinking 50.5 ounces/week) and 100% juice was the next common drink with more than 80% of the children drinking 54.8 ounces of juice/week.

Only a small proportion of infants consumed flavored water, sugar-free beverages, sugar-free beverages made from powder, or diet pop. Almost 43% of the subjects consumed at least one of the sugared beverages with average amount of 60.5 ounces/week. The variable milk consumption included breast milk, cow’s milk, whole milk, 2%, and 1% milk and infant formula. Fifty-nine percent of the subjects were drinking at least one of these.

Table 4.2. Frequency and Amount (in Ounces) Consumption for Beverage Consumption for the Entire Sample at Baseline (N=129)

Variables	**Proportion Reporting Use**		**Amount (ounces) Per Week**	
	Frequency	**Valid Percentage**	**Mean**	**Standard Deviation**
Infant formula	62	48.06	92.29	117.78
Cow's milk	74	57.81	72.14	100.87
Chocolate milk	9	6.98	--	--
Any milk (human milk, cow's milk (whole, 2%, 1%, or skimmed), or infant formula)	76	58.91	--	--
100% Juice	75	80.65	5.13	54.80
Juice drinks	27	21.09	8.81	30.77
Water	111	86.05	50.45	63.15
Flavored water	6	4.69	3.27	23.70
Sugared beverages made from powder	22	17.19	7.10	24.98
Sugar-free beverages made from powder	3	2.34	0.80	8.63
Regular pop	29	22.48	2.86	7.22
Diet pop	9	6.98	0.88	4.32
Sports drinks	16	12.50	3.30	13.48
Other sugared beverages	21	16.28	3.02	9.99
Other sugar-free beverages	8	6.25	1.02	6.31
Any sugared beverage intake	55	42.64	28.36	60.48

- Diet behavior:

The mothers were asked about certain behavioral characteristics of their children such as feeding practices and toothbrushing habits. From the behavioral variables, bottle-feeding (58%) and use of sippy cup (64.3%) were common among the subjects at baseline. Only 1.6% of the subjects had the habit of having snacks in the middle of the night and 9.3% had snacks at bedtime. Almost 42% subjects had night time bottle-feeding habit, and 20.2 % had the habit of bottle-feeding in the middle of the night. Forty-one percent of the children had none of the nigh time feeding practices. Almost 50% of the children subjects brushed or had their teeth brushed regularly, 28% brushed or had their teeth brushed once in a while, and 22.4% never brushed or had their teeth brushed at baseline.

Table 4.3 describes the frequency and percentage distribution of these variables. A majority of children were reported to have engaged in bottle-feeding, drinking from closed (Sippy) cup, and regular toothbrushing.

Table 4.3. Frequency and Descriptive Statistics for Variables on Dietary Behavior at Baseline for the Entire Sample (N=129)

Variables	**Frequency**	**Valid Percentage**
Breast-feeding	14	10.85
Bottle-feeding	75	58.14
Open cup	15	11.63
Closed cup (sippy cup)	83	64.34
Night-time feeding	76	58.91
Night time bottle-feeding	54	41.86
Bottle-feeding in the middle of the night	26	20.16
Snacks at bedtime	12	9.30
Snacks in the middle of the night	2	1.55

Clinical Variables

Maternal MS levels were considered in the clinical variables and up to 85% of the mothers were infected with MS at baseline. Use of fluoridated toothpaste was not reported by all the subjects (missing data = 20), but up to 40% of the remaining subjects reported regular use of the same. Similarly, 76 of the subjects did not report if they were drinking fluoridated water. Among the remaining 53 subjects, 77% were exposed to fluoridated water through the city or public water source.

Among the tooth and plaque related variables, only 17 % of the maxillary molars, 31% of the maxillary incisors, 11.6% of the mandibular molars, and 7% of the mandibular incisors were covered with visible plaque. Twelve percent of the children had history of caries (any d1, d2, or filled surfaces). The mean number of teeth present in these subjects was 7. The mean number of d1d2-3f surfaces was 0.7 and mean number of teeth covered with visible plaque was 1.4.

Table 4.4 describes the frequency and percentage distribution of clinical variables such as maternal MS levels (positive or negative); number of teeth, maxillary and mandibular incisors and molars with visible plaque; use of fluoridated toothpaste; main water source and any exposure to fluoridated water; and if there were any decayed, missing, or filled surfaces. Most of these variables were the dichotomized for further analyses. The table describes categorical distributions of the variables.

Table 4.4. Frequency and Descriptive Statistics for Clinical Factors at Baseline for the Entire Sample (N=129)

Variable	**Frequency**	**Valid Percentage**
Mother is infected with MS		
No	19	14.73
Yes	110	85.27
Maxillary molars with visible plaque		
0	107	82.95
1	11	8.53
2	11	8.53
Maxillary incisors with visible plaque		
0	89	68.99
1	13	10.08
2	13	10.08
3	3	2.33
4	11	8.53
Mandibular incisors with visible plaque		
0	120	93.02
1	1	0.78
2	2	1.55
3	1	0.78
4	5	3.88
Mandibular molars with visible plaque		
0	114	88.37
1	9	6.98
2	6	4.65
Children with visible plaque present on any teeth	49	37.98
Any decay (d1d2-3fs >0)		
No	114	88.37
Yes	15	11.63
Are your child’s teeth brushed?		
Yes (brushed regularly)	62	49.6
Yes, once in a while	35	28.0
No	28	22.4
Use of fluoridated toothpaste for the child		
No	66	60.55
Yes	43	39.45
Missing	20	
Main water source		
Well water or private water source	4	7.55
City or public water source	41	77.36
Bottled water	8	15.09
Missing	76	
Exposure to fluoridated water (City water)		
No	12	22.64
Yes	41	77.36
Missing	76	

Table 4.5 describes the mean and standard deviation of clinical variables – total number of teeth, number of decayed, missing, or filled tooth surfaces, and total number of teeth with visible plaque. These data indicate that on average more than one tooth had visible plaque, and that subjects had average 7 teeth present. Caries prevalence in the subject population was 11.6%, as 15 of the subjects had one or more decayed (at the d1 or higher level) or filled surfaces, with an overall mean of 0.7 surfaces affected.

Table 4.5. Descriptive Statistics for Clinical Factors at Baseline for the Entire Sample (N=129)

Variable	**Mean**	**Standard Deviation**
Number of teeth	7.28	6.09
d1d2-3fs	0.67	3.27
Plaque total	1.35	2.57

Baseline – Bivariate Analyses (Overall)

The following section consists of bivariate analyses of demographic, dietary, behavioral, and environmental variables by group membership at baseline. For this section all the three groups are considered.

Demographic Variables

Age: The variable age was not normally distributed between the three groups [Shapiro-Wilk Test ($p<0.0001$)]. The mean age for group 1 was 10.8 months (6 – 24 months), group 2 was 16.7 months (7 – 24 months), and group 3 was 12.6 months (6 – 23 months). The Kruskal-Wallis test was used to test if the mean or median ages differed significantly between the groups. The mean age of the subjects differed significantly with group 2 having the oldest children ($p < 0.0001$).

Among the other demographic variables, the proportion of females differed significantly between the groups and the maximum (60%) proportion of females was in group 2. Group 2 also had significantly higher proportion of Hispanic population (31.4%) than other two groups. Maternal age and education differed significantly between the groups with group 1 having more educated and younger mothers as compared to the other two groups. Annual household income did not differ significantly between the groups [Table 4.6]. Bivariate analyses were performed using Chi-square/Fisher's exact test and Kruskal-Wallis test (data were not normally distributed) respectively. The significant demographic variables were sex, age of the mother, race, and mother's education ($p<0.05$ in all instances). The findings are elaborated in Table 4.6.

Table 4.6. Bivariate Analysis of Demographic Factors by Group Membership at Baseline (N=129)

Variables	**Group1 n=58(%)**	**Group2 n=35(%)**	**Group3 n=36(%)**	**P-value**
Sex				**0.0229***
Female	27 (46.6)	21 (60.0)	10 (27.8)	
Male	31 (53.5)	14 (40.0)	26 (72.2)	
Age of the mother				**0.0046***
26 or younger	49 (84.5)	19 (54.3)	28 (77.8)	
27 or older	9 (15.5)	16 (45.7)	8 (22.2)	
Ethnicity				**0.0412****
Non-Hispanic	48 (82.8)	24 (68.6)	33 (91.7)	
Hispanic	10 (17.2)	11 (31.4)	3 (8.3)	
Income category				0.2876
$20000 or less	31 (53.5)	22 (62.9)	25 (69.4)	
$20001 or more	27 (46.5)	13 (37.1)	11 (30.6)	
Maternal education				**0.0289***
Less than high school	6 (10.7)	11 (32.4)	10 (27.8)	
High school or more	50 (89.3)	23 (67.7)	26 (72.2)	

Note: Statistically significant using * Cochran-Mantel-Haenszel Statistics
** Fisher's exact test

Dietary Variables

- Diet composition

The proportion of infants having infant formula was significantly higher in groups 1 and 3 as compared to group 2. The relationship was reversed when milk (any kind of milk), juice, juice drinks, sugared beverages made from powder, regular pop, sports drinks, other added sugar beverage consumption, and combined sugared beverage consumption were considered. In the latter case, group 2 showed greater consumption than Groups 1 and 3. [Table 4.7 and Table 4.8]

Table 4.7. Bivariate Analysis of Proportion Reporting Beverage Intake by Group Membership at Baseline (N=129)

Variables	**Group 1 (n =58)**	**Group 2 (n =35)**	**Group 3 (n=36)**	**P-value**
Infant formula	**63.79**	**20.00**	**50.00**	**0.0002***
Cow's milk	**40.35**	**91.43**	**52.78**	**<0.0001***
Chocolate milk	**1.72**	**20.00**	**2.78**	**0.0043**
Any milk (human milk, cow's milk (whole, 2%, 1%, or skimmed), or infant formula)	100.00	100.00	27.34	0.5504
100% Juice	**73.68**	**91.43**	**91.67**	**0.0252***
Juice drinks	**8.62**	**42.86**	**20.00**	**0.0005***
Water	81.03	85.71	94.44	0.1891
Flavored water	3.45	5.71	5.71	0.7630
Sugared beverages made from powder	**6.90**	**34.29**	**17.14**	**0.0032***
Sugar-free beverages	5.17	0.00	0.00	0.2507
Regular pop	**10.34**	**45.71**	**19.44**	**0.0003***
Diet pop	3.45	11.43	8.33	02628
Sports drinks	**5.26**	**25.71**	**11.11**	**0.0201****
Other sugared beverages	**5.17**	**34.29**	**16.67**	**0.0011***
Other sugar-free beverages	3.45	11.43	5.71	0.3288
Any sugared beverage intake	**25.86**	**71.43**	**41.67**	**<0.0001***

Note: Statistically significant using * Cochran-Mantel-Haenszel Statistics
** Fisher's exact test

Table 4.8. Bivariate Analysis of Mean Amount (in Ounces per Week) of Beverage Consumption by Group Membership at Baseline (N = 129)

Variables	**Group 1 (n =58)**	**Group 2 (n =35)**	**Group 3 (n=36)**	**P-value**
Infant formula	**75.99**	**45.39**	**66.36**	**0.0002***
Cow's milk	**55.48**	**86.64**	**59.29**	**0.0001***
100% Juice	**52.48**	**75.61**	**74.84**	**0.0025***
Juice drinks	**58.94**	**79.16**	**60.99**	**0.0004***
Water	58.43	70.67	70.07	0.1931
Flavored water	64.71	66.16	64.34	0.8066
Sugared beverages made from powder	**59.69**	**72.97**	**65.81**	**0.0301***
Sugar-free beverages made from powder	66.84	63.5	63.5	0.1549
Regular pop	**57.53**	**79.16**	**63.28**	**0.0005***
Diet pop	62.72	67.80	65.94	0.3285
Sports drinks	**60.78**	**72.29**	**64.72**	**0.0353***
Other sugared beverages	**58.62**	**75.84**	**64.73**	**0.0022***
Other sugar-free beverages	63.22	68.37	64.58	0.3007
Combined added sugar beverages	**53.88**	**84.81**	**63.65**	**<0.0001***

Note: * Statistically significant using Kruskal-Wallis test; the different letters as superscript indicate the group that is different from the others.

- Diet behavior

Among the behavioral factors, only the proportion of children with bottle-feeding practice, those using open cup, and those using fluoridated tooth-paste differed significantly between the three groups. Group 2 had lowest proportion of children with bottle-feeding practice and the highest proportion of infants using open cup and fluoridated toothpaste. These contradictory findings can be attributed to the higher age of children in group 2 as compared to the other two groups [Table 4.9].

Table 4.9. Bivariate Analysis of Proportion of Variables on Dietary Behavior by Group Membership at Baseline (N=129)

Variables	**Group 1 (n =58)**	**Group 2 (n =35)**	**Group 3 (n=36)**	**P-value**
Breast-feeding	10.34	14.29	8.33	0.7678
Bottle-feeding	**72.41**	**34.29**	**58.33**	**0.0015***
Open cup	**6.90**	**25.71**	**5.56**	**0.0095***
Closed cup (sippy cup)	58.62	80.00	58.33	0.0768
Sugared beverage intake at meals	**36.21**	**62.86**	**72.22**	**0.0013**
Sugared beverage intake between the meals	55.17	74.29	66.67	0.1612
Night-time feeding	56.90	62.86	58.33	0.8490
Snack at bedtime	6.90	17.14	5.56	0.1697
Snack in the middle of the night	0.00	5.71	0.00	0.0653
Children sleeping with a bottle	41.38	40.00	44.44	0.9259

Note: *Statistically significant using Cochran-Mantel-Haenszel Statistics;

Clinical Variables

Bivariate analyses for environmental factors found many significant variables ($p<0.05$ in each instance), including mean number of teeth, d1d2-3fs lesions, and maxillary and mandibular incisors and molars with visible plaque. The teeth covered with visible plaque were dichotomized as to whether the subject had any maxillary/mandibular incisor/molar with visible plaque versus no visible plaque at all. All the new dichotomized variables showed statistically significant as well. Caries experience was assessed as subjects with any decayed or filled teeth and those who had none. The variable indicating such proportion of subjects with any decay was significantly with the high in group 2 [Table 4.10].

Table 4.10. Bivariate Analysis of Proportion / Means of Environmental Factors by Group Membership at Baseline (N = 129)

Variable	**Group 1 (n =58)**	**Group 2 (n =35)**	**Group 3 (n=36)**	**P-value**
Proportion of mothers positive with MS	82.8	80.0	94.4	0.1591
Mean number of teeth	**4.8[a]**	**11.54[b]**	**7.13[c]**	**<0.0001^**
Mean number of maxillary incisors with visible plaque	**0.33[a]**	**1.54[b]**	**0.53[a]**	**<0.0001^**
Proportion of maxillary incisors with visible plaque	**8.62[a]**	**48.57[b]**	**8.33[a]**	**<0.0001***
Proportion of maxillary molars with visible plaque	**6.90**	**34.29**	**16.67**	**0.0032***
Proportion of mandibular incisors with visible plaque	**1.72**	**17.14**	**5.56**	**0.0124****
Proportion of mandibular molars with visible plaque	**1.72**	**31.43**	**8.33**	**<0.001****
Mean number of teeth with visible plaque	**0.48[a]**	**3.26[b]**	**0.89[a]**	**<0.0001^**
Proportion of children with visible plaque present on any tooth	**17.24**	**71.43**	**38.89**	**<0.001****
Any decay (d1d2-3fs >0)	**1.72[a]**	**34.29[b]**	**5.56[a]**	**<0.001****
Mean number of d1d2-3fs lesions	**0.00[a]**	**1.29[b]**	**0.00[a]**	**0.0123^**
Toothbrushing	**66.66**	**93.94**	**80.00**	**0.0031**
Fluoridated toothpaste	**32.00**	**61.29**	**28.57**	**0.0127**
Fluoridated water	63.64	86.67	87.50	0.1322

Note: Statistically significant using ^Kruskal-Wallis test * Cochran-Mantel-Haenszel Statistics **Fisher's exact test; the different letters as superscript indicate the group that is different from the others.

The subsequent sections consist of pairwise comparisons for demographic, dietary, behavioral, and environmental factors between three different pairs of groups as follows:

Pair 1 – group 1 and group 3

Pair 2 – group 1 and group 2

Pair 3 – group 2 and group 3

For the overall comparison across the three groups, multi-category logistic regression model was built using significant variables at bivariate level. Akaike

Information Criteria (AIC) of the model was 3487.8. The fit of the model was questionable and hence it was excluded from the analyses.

Baseline – Pairwise Comparison 1 (Groups 1 and 3)

Group 1 [A group that had no MS at any of the three study time points (n=58)] is compared with group 3 [A group that acquired MS during the 18-month study period (n=36)]. Group 2 was excluded from the analyses for this particular comparison.

Means of continuous variables and frequencies of categorical variables are compared between the two groups and the significant variables are included in the logistic regression model at the end of the section.

Demographic Variables

Age: The variable age was normally distributed [from the Shapiro-Wilk Test (p = 1.0000)]. The mean age for group 1 was 10.8 months (6 – 24 months) and for group 3 was 12.6 months (6 – 23 months). T-tests were used to test if the means differed significantly between the groups. The p-value of 0.1065 indicated a non-significant difference in mean age between the two groups.

Among the other demographic variables only mother's education level differed significantly (p = 0.0351) between groups 1 and 3, with group 3 having a higher proportion of mothers with less than high school education [OR = 0.31; 95% CI 0.10-0.95]. Children with educated mothers were 70% less likely to be in group 3 as compared to children with mothers educated less than high school. Table 4.11 describes the detailed results for pairwise comparisons of demographic variables. The variables were analyzed using Chi-Square test and Fisher's exact test.

Table 4.11. Pairwise Comparison of Frequencies (Proportion) of Demographic Factors for Group 1 and 3 at baseline (N = 94)

Variables	**Bivariate**		
	Group1 n=58(%)	**Group3 n=36(%)**	**P-value**
Sex			0.0701
Female	27 (46.6)	10 (27.8)	
Male	31 (53.5)	26 (72.2)	
Age of the mother			0.4116
26 or younger	49 (84.5)	28 (77.8)	
27 or older	9 (15.5)	8 (22.2)	
Race			0.3574
Non-Hispanic	48 (82.8)	33 (91.7)	
Hispanic	10 (17.2)	3 (8.3)	
Income category			0.1245
$20000 or less	31 (53.5)	25 (69.4)	
$20001 or more	27 (46.5)	11 (30.6)	
Maternal education			**0.0351***
Less than high school	6 (10.7)	10 (27.8)	
High school or more	50 (89.3)	26 (72.2)	

Note: * Statistically significant using Chi-Square test

Dietary Variables

- Diet composition

Among the dietary variables, the proportion of children drinking 100% juice differed significantly between groups 1 and 3 with more children from group 3 (~92%) drinking 100% juice. The children consuming 100% juice were 3 times as likely to develop infection as compared to non-juice consumers [OR = 3.8, 95%CI =1.03-14.36]. Similarly, the average amount of 100% juice consumed was significantly higher for group 3 (74.8 ounces/week) as compared to group 1 (54.8 ounces/week). None of the other dietary variables were significantly different. The categorical variables were analyzed using Chi-Square test and Fisher's exact test. The continuous variables were analyzed using Wilcoxon Rank Sum test. [Table 4.12 and 4.13]

Table 4.12. Pairwise Comparison of Proportion Reporting Beverages Intake in Groups 1 and 3 at Baseline (N = 94)

Variable	**Group 1 (n =58)**	**Group 3 (n=36)**	**P-value**
Infant formula	63.79	50.00	0.1870
Cow's milk	40.35	52.78	0.2408
Chocolate milk	1.72	2.78	1.0000
Any milk (human milk, cow's milk (whole, 2%, 1%, skimmed), or infant formula)	100.00	97.22	0.3830
100% Juice	**73.68**	**91.67**	**0.0325***
Juice drinks	8.62	20.00	0.1128
Water	81.03	94.44	0.0671
Flavored water	3.45	5.71	0.6301
Sugared beverages made from powder	6.90	17.14	0.1689
Sugar-free beverages made from powder	5.17	0.00	0.2835
Regular pop	10.34	19.44	0.2141
Diet pop	3.45	8.33	0.3676
Sports drinks	5.26	11.11	0.4238
Other sugared beverages	5.17	16.67	0.0812
Other sugar-free beverages	3.45	5.71	0.6301
Any sugared beverage intake	25.86	41.67	0.1101

Note: * Statistically significant using Chi-Square test

Table 4.13. Pairwise Comparison of the Mean Amount (in Ounces per Week) of Beverages Intake of Groups 1 and 3 at Baseline (N = 94)

Variables	**Group 1 (n =58)**	**Group 3 (n=36)**	**P-value**
Infant formula	75.99	66.36	0.1990
Cow's milk	55.48	59.29	0.5954
100% Juice	**52.48**	**74.84**	**0.0069***
Juice drinks	58.94	60.99	0.6531
Water	58.43	70.07	0.1314
Flavored water	64.71	64.34	0.8883
Sugared beverages made from powder	59.69	65.81	0.1565
Sugar-free beverages made from powder	66.84	63.5	0.1718
Regular pop	57.53	63.28	0.2281
Diet pop	62.72	65.94	0.3125
Sports drinks	60.78	64.72	0.2933
Other sugared beverages	58.62	64.73	0.1268
Other sugar-free beverages	63.22	64.58	0.6325
Combined added sugar beverages	53.88	63.65	0.1720

Note: * Statistically significant using Wilcoxon Rank-Sum test

- Diet behavior

The pairwise comparison for behavioral factors revealed no significant differences among groups 1 and 3 for any of the variables. The proportions and corresponding p-values for these variables are described in Table 4.14. The categorical variables were analyzed using Chi-Square test and Fisher's exact test.

Table 4.14. Pairwise Comparison of Proportion Reporting Use of Variables on Dietary Behavior for Group 1 and 3 at Baseline (N = 94)

Variable	**Group 1 (n =58) %**	**Group 3 (n=36) %**	**P-value**
Breast-feeding	10.34	8.33	1.0000
Bottle-feeding	72.41	58.33	0.1581
Open cup	6.90	5.56	1.0000
Closed cup (sippy cup)	58.62	58.33	0.9781
Night-time feeding	56.90	58.33	0.8911
Bottle-feeding in the middle of the night	18.97	22.22	0.7023
Night time bottle-feeding	41.38	44.44	0.7710
Snack at bedtime	6.90	5.56	1.0000
Snacks in the middle of the night	--	--	---

Clinical Variables

Table 4.15 presents pairwise comparisons for clinical variables. Only the mean number of teeth and mean number of teeth with visible plaque differed significantly ($p < 0.05$) between groups 1 and 3. The older group 3 had higher number of teeth (7.1) and mean number of teeth with visible plaque (0.89) than group 1 (4.8 and 0.5 respectively). The variable for number of teeth was continuous and not normally distributed. It was dichotomized into a new variable based on mean number of teeth (5) for the group 1 and 3 collectively. Group 3 had significantly more children with more than five teeth than children with five teeth or less ($p = 0.0116$) [Table 4.15]. The categorical variables were analyzed using Chi-Square test and Fisher's exact test. The continuous variables were analyzed using Wilcoxon Rank Sum test.

Table 4.15. Pairwise Comparison of Proportion Reporting Use of Clinical Variables for Group 1 and 3 at Baseline (N = 94)

Variable	**Group 1 (n =58)**	**Group 3 (n=36)**	**P-value**
Proportion of mothers positive with MS	82.76	94.44	0.1218
Mean number of teeth	**4.81**	**7.13**	**0.0106****
Children with more than 5 teeth	**34.48**	**61.11**	**0.0116***
Mean number of maxillary incisors with visible plaque	0.33	0.53	0.2476
Proportion of maxillary incisors with visible plaque	8.62	8.33	0.2939
Proportion of maxillary molars with visible plaque	6.90	16.67	0.0914
Proportion of mandibular incisors with visible plaque	1.72	5.56	0.2726
Proportion of mandibular molars with visible plaque	1.72	8.33	0.1358
Mean number of teeth with visible plaque	**0.48**	**0.89**	**0.0254****
Proportion of children with visible plaque present on any tooth	**17.24**	**38.89**	**0.0193***
Any decay (d1d2-3fs >0)	1.72[a]	5.56	0.5561
Mean number of d1d2-3fs lesions	0.00	0.00	0.2950
Toothbrushing	66.67	80.00	0.1072
Fluoridated toothpaste	32.00	28.57	0.7530
Fluoridated water	63.64	87.50	0.0991

Statistically significant using ** Wilcoxon Rank-Sum test *Chi-Square test

Baseline - Multivariate analyses

As discussed earlier in Chapter 3 – Methods, multivariate analyses were conducted in four domains – demographics, dietary – composition and behavior, and clinical. Among the demographic variables, maternal education was the only significant variable at a 0.05 level of significance. For the diet -composition domain, the dichotomous variable for juice consumption (yes/no) and total amount of juice consumed entered the model for diet. None of the dietary behavioral variables were significant in bivariate analyses, so no domain-specific modelling was done for behavioral variables.

Among the clinical variables, number of teeth erupted and number of teeth with visible plaque were significant and hence they were entered into the clinical model. Hosmer-Lemeshow Goodness of Fit test and its p-value were used to evaluate the fitness of each of the models. [Table 4.16] A summary of the four domain specific models is given in Table 4.16. These variables entered the preliminary model for baseline comparison of Group 1 and 3.

The preliminary model contained four variables. It included age based on the evidence from literature. The convergence criteria were satisfied and the tests of multicollinearity indicated no correlation between the independent variables. When subjected to forward selection procedure, only two variables remained in the model – mothers' education and more than five teeth erupted. These variables were used to build the final model. The detailed results are described in the Tables 4.17 and 4.18.

Table 4.16. Summary of Logistic Model Development for Domain Specific, Preliminary, and the Final Model at Baseline

Variables	**AIC**	**c-statistics**	**Variables Included in Model**	**Chi-Square**	**P-value**
Model with significant demographic variables					
1	125.156	0.693	Age, higher maternal education	4.7538	0.7835
Model with significant dietary variables					
2	126.142	0.677	Juice (yes/no), total juice consumption	4.6737	0.6997
Model with clinical variables					
1	127.115	0.633	More than five teeth erupted	--	--
Preliminary model with demographic, dietary, and clinical variables					
4	125.156	0.709	Child's age, Mother's education, Juice consumption, More than five teeth erupted	1.4311	0.9846
Final model					
2	125.156	0.695	Mother's education, More than five teeth erupted	0.3469	0.8408

Table 4.17. Preliminary Model for Comparison of Groups 1 and 3 at Baseline

Independent variables	**OR**	**95% CI**	**P-value**
Age	0.985	0.86 – 1.12	0.8207
Higher maternal education	0.311	0.09 – 1.02	0.0573
Juice	2.664	0.73 – 12.7	0.1648
More than five teeth erupted	2.830	0.79 – 10.4	0.1104

Note: AIC = 118.614; Hosmer-Lemeshow Goodness of Fit test p =0.9846, c-statistics = 0.709

Table 4.18. Final Model for Comparison of Groups 1 and 3 at Baseline

Independent variables	**OR**	**95% CI**	**P-value**
Higher maternal education	0.279	0.08 – 0.88	0.0340
More than five teeth erupted	3.549	1.46 – 9.04	0.0062

Note: AIC =125.156; Hosmer-Lemeshow test statistics =0.3469, p =0.8408; c-statistics 0.695

According to the final model, maternal education was a significant protective factor whereas number of teeth (>5) was a significant risk factor for MS infection ($p<0.05$). The OR of 0.279 indicated that the odds of getting infected were (1 – 0.279~) 72% less in children with mothers educated more than high school as compared to mothers with education less than high school. Children having more than five teeth erupted were 3.6 times as likely to develop MS infection as compared to children with less than five teeth.

Baseline – Pairwise Comparison 2 (Groups 1 and 2)

Group 1 [A group that had no MS at any of the three study time points (n=58)] is compared with group 2 [A group that had >0 MS at the baseline measurement and at subsequent measurements (n=35)]. Group 3 was excluded from the analyses for this particular comparison.

Demographic Variables

Age: The variable age was normally distributed [from the Shapiro-Wilk Test ($p = 0.0950$)]. The mean age for group 1 was 10.8 months (6 – 24 months), and for Group 2 was 16.7 months (7 – 24 months). Student's t-test was used to assess if the means differed significantly between the groups. As described below, the results indicated significant differences between the two groups at $p < 0.05$.

Among the other demographic variables, the age of the mother ($p = 0.0015$) and mother's education ($p = 0.0110$) were significantly different between groups. Group 1 had higher proportion of mothers aged 26 or younger (84.5%) and with higher education (89.3%) as compared to group 2. The detailed description of demographic variables can be found in Table 4.19.

Table 4.19. Pairwise Comparison of Proportion of Demographic Variables for Group 1 and Group 2 at Baseline (N = 93)

Variables	**Bivariate**		
	Group1 n=58(%)	**Group2 n=35(%)**	**P-value**
Sex			0.2087
Female	27 (46.6)	21 (60.0)	
Male	31 (53.5)	14 (40.0)	
Age of the mother			**0.0015***
26 or younger	49 (84.5)	19 (54.3)	
27 or older	9 (15.5)	16 (45.7)	
Ethnicity			0.1129
Non-Hispanic	48 (82.8)	24 (68.6)	
Hispanic	10 (17.2)	11 (31.4)	
Income category			0.3746
$20000 or less	31 (53.5)	22 (62.9)	
$20001 or more	27 (46.5)	13 (37.1)	
Maternal education			**0.0110***
Less than high school	6 (10.7)	11 (32.4)	
High school or more	50 (89.3)	23 (67.7)	

Note: * Statistically significant using Chi-Square test

Dietary Variables

Diet composition

Table 4.20 describes proportions of beverage consumption and their p-value based on Chi-Square or Fisher's exact test. Infant formula consumption, milk consumption, any milk (whole, 2%, 1%, skimmed, chocolate or other flavored) consumption, juice drink consumption, sugar beverage (made from powder e.g. Kool-Aid) consumption, regular pop consumption, sports drink consumption, other sugared beverage consumption, and any sugared beverage consumption ($p<0.05$ in all instances), were significantly different between the two groups. Higher proportions of children in group 2 consumed all of these beverages, except for infant formula, where children from group 1 were more likely to have had infant formula as compared to Group 2.

All these variables differed significantly ($p < 0.05$ in each instance) when compared for the amount of consumption in ounces per week. In addition, juice consumption (ounces/week) was also significantly different ($p = 0.0024$). Similar to the proportions, group 2 children consumed greater mean amounts of the beverages than group 1, except infant formula. Group 1 children had an average of 76 ounces of infant formula per week, whereas group 2 children had 45.4 ounces per week on average. The variable any milk (whole, 2%, 1%, skimmed, chocolate or other flavored) was significantly different with a greater proportion of children from group 2 drinking any milk. Table 4.21 describes these results in greater detail.

Table 4.20. Pairwise Comparison of Proportion Reporting Use of Beverages for Group 1 and Group 2 at Baseline (N = 93)

Variables	Group 1 (n =58)	Group 2 (n =35)	P-value
Infant formula	**63.79**	**20.00**	**<0.0001***
Cow's milk	**40.35**	**91.43**	**<0.0001****
Chocolate milk	**1.72**	**20.00**	**0.0038**
Any milk (human milk, cow's milk (whole, 2%, 1%, skimmed), or infant formula)	100.00	100.00	--
100% Juice	73.68	91.43	0.0565
Juice drinks	**8.62**	**42.86**	**<0.0001***
Water	81.03	85.71	0.5620
Flavored water	3.45	5.71	0.6301
Sugared beverages made from powder	**6.90**	**34.29**	**0.0013***
Sugar-free beverages	5.17	0.00	0.2934
Regular pop	**10.34**	**45.71**	**0.0001***
Diet pop	3.45	11.43	0.1935
Sports drinks	**5.26**	**25.71**	**0.0085****
Other sugared beverages	**5.17**	**34.29**	**<0.0003****
Other sugar-free beverages	3.45	11.43	0.1935
Any sugared beverage consumption	**25.86**	**71.43**	**<0.0001***

Note: Statistically significant using * Chi-Square test ** Fisher's exact test

Table 4.21. Pairwise Comparison of Mean Amount (in Ounces per week) of Beverages Consumption for Group 1 and Group 2 at Baseline (N = 93)

Variable (in ounces/week)	**Group 1 (n =58)**	**Group 2 (n =35)**	**P-value**
Infant formula	**75.99**	**45.39**	**<0.0001***
Cow's milk	**55.48**	**86.64**	**<0.0001***
Juice	**52.48**	**75.61**	**0.0024***
Juice drinks	**58.94**	**79.16**	**0.0003***
Water	58.43	70.67	0.1358
Flavored water	64.71	66.16	0.6117
Sugared beverages from powder	**59.69**	**72.97**	**0.0076***
Sugar free beverages	66.84	63.50	0.1779
Regular pop	**57.53**	**79.16**	**0.0001***
Diet pop	62.72	67.80	0.3125
Sports drinks	**60.78**	**72.29**	**0.0099***
Other sugared beverages	**58.62**	**75.84**	**0.0005***
Other sugar-free beverages	63.22	68.37	0.1338
Combined added sugar beverages	**53.88**	**84.81**	**<0.0001***

Note: *Statistically significant using Wilcoxon Rank Sum test

Diet behavior:

Among the behavioral variables, the proportion of infants with bottle-feeding practice (p = 0.0003), using open cup (p = 0.0152), using sippy cup (p = 0.0341), and those using fluoridated toothpaste (p = 0.0097) differed significantly between the two groups. Group 1 had significantly lower proportions of all these variables than group 2, except for the bottle-feeding practice. Group 1 had more infants (72.4%) with bottle-feeding practice than group 2 (32.3%). Table 4.22 describes these findings in greater detail.

Table 4.22. Pairwise Comparison of Percentage Proportion of Variables on Dietary Behavior for Group 1 and Group 2 at Baseline (N = 93)

Variable	**Group 1 (n =58)**	**Group 2 (n =35)**	**P-value**
Breast-feeding	10.34	14.29	0.7418
Bottle-feeding	**72.41**	**34.29**	**0.0003***
Open cup	**6.90**	**25.71**	**0.0152****
Closed cup (sippy cup)	**58.62**	**80.00**	**0.0341***
Night-time feeding	56.90	62.86	0.5710
Night time bottle-feeding	41.38	40.00	0.8957
Bottle-feeding in the middle of the night	18.97	20.00	0.9026
Snack at bedtime	6.90	17.14	0.1689
Snacks in the middle of the night	0.00	5.71	0.1391

Note: Statistically significant using *Chi-Square test ** Fisher's exact test

Clinical Variables

Table 4.23 describes clinical factors and almost all of them differed significantly (p< 0.05) between the two groups, except for presence of maternal MS. These included the mean number of teeth, mean number of maxillary incisors with visible plaque, proportion of maxillary and mandibular incisors and molars with visible plaque, mean number of teeth with visible plaque, mean number of d1d2-3fs lesions, and proportion of teeth with any decay.

Overall, the pairwise comparison between groups 1 and 2 showed many significant differences. Most of them can be explained as the function of age. Since children from group 2 were older, on average, than those from group 1, they had different dietary and behavioral habits, and also their environments differed, with higher mean numbers of teeth and associated factors in group 2 children as compared to group 1 children.

Table 4.23. Pairwise Comparison of Means and Proportions of Clinical Variables for Group 1 and Group 2 at Baseline (N = 93)

Variable	Group 1 (n =58)	Group 2 (n =35)	P-value
Proportion of mothers positive with MS	82.76	80.00	0.7388
Mean number of teeth	**4.81**	**11.54**	**<0.0001^^**
Mean number of maxillary incisors with visible plaque	**0.33**	**3.26**	**<0.0001^^**
Proportion of maxillary incisors with visible plaque	**8.62**	**48.57**	**<0.0001***
Proportion of maxillary molars with visible plaque	**6.90**	**34.29**	**0.0007***
Proportion of mandibular incisors with visible plaque	**1.72**	**17.14**	**0.0099****
Proportion of mandibular molars with visible plaque	**1.72**	**31.43**	**<0.0001****
Mean number of teeth with visible plaque	**0.48**	**3.26**	**<0.0001^^**
Proportion of children with visible plaque present on any tooth	**17.24**	**71.43**	**<0.0001****
Any decay (d1d2-3fs >0)	**1.72**[a]	**34.29**	**<0.0001****
Mean number of d1d2-3fs lesions	**0.02**	**2.31**	**<0.0001^^**
Fluoridated toothpaste	**32.00**	**61.29**	**0.0097***
Fluoridated water	63.64	86.67	0.1527

Note: Statistically significant using ^^ Wilcoxon Rank-Sum test
*Chi-Square test **Fisher's exact test

Baseline – Pairwise Comparison 3 (Groups 2 and 3)

Group 2 [A group that had >0 MS at the baseline measurement and at subsequent measurements (n=35)] was compared with group 3 [A group that acquired MS during the 18-month study period (n=36)]. Group 1 was excluded from the analyses for this particular comparison. Means of continuous variables and frequencies of categorical variables were compared for the two groups and the significant variables were included in the logistic regression model at the end of the analyses.

Demographic Variables:

<u>Age:</u> The variable age was not normally distributed [from the Shapiro-Wilk Test (p = 0.0149)]. The mean age for group 2 was 16.7 (range: 7 – 24) months and for group 3 was 12.6 (range: 6 – 23) months. The Wilcoxon Rank-Sum test was used to test if the mean or median age differed significantly between the groups. The results indicated significant difference between the two groups with p <0.05.

Table 4.24 describes other demographic factors for this comparison. The sex of the child (p = 0.0062), age of the mother, (p = 0.0364), and proportion with Hispanic ethnicity (p = 0.0145) were significantly different between the two groups. Group 2 had more female children (60%) than group 1 (28%). A greater proportion mothers were 27 or older in group 2 (45.7%) than in group 3 (22.2%). Group 2 also had more Hispanic children (17.2%) than Group 3 (8.3%).

Table 4.24. Pairwise Comparison of Proportion of Demographic Variables for Group 2 and Group 3 at Baseline (N = 71)

	Bivariate		
Variables	**Group2 n=35(%)**	**Group3 n=36(%)**	**P-value**
Sex			**0.0062***
Female	21 (60.0)	10 (27.8)	
Male	14 (40.0)	26 (72.2)	
Age of the mother			**0.0364***
26 or younger	19 (54.3)	28 (77.8)	
27 or older	16 (45.7)	8 (22.2)	
Ethnicity			**0.0145***
Non-Hispanic	24 (82.8)	33 (91.7)	
Hispanic	11 (17.2)	3 (8.3)	
Income category			0.5574
$20000 or less	22 (62.9)	25 (69.4)	
$20001 or more	13 (37.1)	11 (30.6)	
Maternal education			0.6763
Less than high school	11 (32.4)	10 (27.8)	
High school or more	23 (67.6)	26 (72.2)	

Note: * Statistically significant using Chi-Square test

Dietary Variables

Diet composition

Pairwise comparison of dietary variables showed significant differences among the groups for variables such as, infant formula (p = 0.0081), milk (p = 0.0003), any milk (human milk, cow's milk (whole, 2%, 1%, skimmed), or infant formula) (p = 0.0006), juice drinks (p = 0.0394), regular pop (p = 0.0181), and any sugared beverage consumption (p = 0.0115). A higher proportion of infants from group 2 consumed all of these beverages, except for infant formula. A higher proportion of group 3 children (50%) consumed infant formula than Group 2 (20%) children. The detailed findings are described in Table 4.25.

The corresponding variables were found to be significant when compared for mean amount of beverages consumed in ounces per week. Similar to earlier comparisons, group 2 showed higher mean amount of beverages consumed in ounces per week than group 3, except for infant formula. Group 2 infants consumed less (45.4 ounces per week) infant formula than group 3 (66.4 ounces per week). The variable any kind of milk consumption (whole, 2%, 1%, skimmed, chocolate or other flavored) was significant with group 2 children having a greater proportion of children consuming milk. The questionnaire item did not allow for the total amount of any type of milk consumed by these children to be determined. The findings are described in detail in Table 4.26.

Table 4.25. Pairwise Comparison of Proportion Reporting Consumption of Beverages for Group 2 and Group 3 at Baseline (N = 71)

Variable	**Group 2 (n =35)**	**Group 3 (n=36)**	**P-value**
Infant formula	**20.00**	**50.00**	**0.0081***
Cow's milk	**91.40**	**52.78**	**0.0003***
Any milk (human milk, cow's milk (whole, 2%, 1%, skimmed), or infant formula)	100.00	97.22	1.0000
100 % Juice	91.40	91.70	1.0000
Juice drinks	**42.90**	**20.00**	**0.0394***
Water	85.70	94.44	0.2603
Flavored water	5.71	5.71	1.0000
Sugared beverages from powder	34.30	17.14	0.1008
Sugar-free beverages	0.00	0.00	--
Regular pop	**45.70**	**19.44**	**0.0180***
Diet pop	11.40	8.33	0.7101
Sports drinks	25.70	11.11	0.1117
Other sugared beverages	34.29	16.67	0.0880
Other sugar-free beverages	11.43	5.71	0.6733
Any sugared beverage consumption	**71.43**	**41.67**	**0.0115***

Note: * Statistically significant using Chi-Square test

Table 4.26. Pairwise Comparison of Mean Amount (in Ounces per Week) for Beverage Consumption of Group 2 and Group 3 at Baseline (N = 71)

Variable in ounces/week	**Group 2 (n =35)**	**Group 3 (n=36)**	**P-value**
Infant formula	**45.39**	**66.36**	**0.0059***
Cow's milk	**86.64**	**59.29**	**0.0026***
100% Juice	75.61	74.84	0.9221
Juice drinks	**79.16**	**60.99**	**0.0108***
Water	70.67	70.07	0.9039
Flavored water	66.16	64.34	0.5766
Sugared beverages from powder	72.97	65.81	0.2852
Sugar-free beverages	63.50	63.50	1.0000
Regular pop	**79.16**	**63.28**	**0.0377***
Diet pop	67.80	65.94	0.7148
Sports drinks	72.29	64.72	0.2120
Other sugared beverages	75.84	64.73	0.1047
Other sugar-free beverages	68.37	64.58	0.3872
Combined added sugar beverages	**84.81**	**63.65**	**0.0231***

Note: ** Statistically significant using Wilcoxon Rank-Sum test

Diet behavior

Significant differences were observed between group 2 and 3 for variables such as: proportion of infants with bottle-feeding practice (p = 0.0422), use of open cup (p = 0.0189), sippy cup (p = 0.0484), and fluoridated toothpaste (p = 0.0118). Group 2 had significantly higher proportions than group 3 for all these variables, except for the bottle-feeding practice. Group 3 had more infants (58.3%) with bottle-feeding practice than Group 2 (34.3%). Table 4.27 describes these findings in greater detail.

Table 4.27. Pairwise Comparison of Proportion Reporting Use of Variables on Dietary Behavior for Group 2 and Group 3 at Baseline (N = 71)

Variable	**Group 2 (n =35)**	**Group 3 (n=36)**	**P-value**
Breast-feeding	14.29	8.33	0.4782
Bottle-feeding	**34.29**	**58.33**	**0.0422***
Open cup	**25.71**	**5.56**	**0.0189***
Closed cup (sippy cup)	**80.00**	**58.33**	**0.0484***
Night-time feeding	62.86	58.33	0.6969
Night time bottle-feeding	40.00	44.44	0.7047
Bottle-feeding in the middle of the night	20.00	22.22	0.8186
Snack at bedtime	17.14	5.56	0.9750
Snacks in the middle of the night	5.71	0.00	0.2394

Note: * Statistically significant using Chi-Square test

Clinical Variables

Table 4.28 describes comparison of clinical factors between groups 2 and 3. The significant variables were: mean number of teeth, mean number of maxillary incisors with visible plaque, proportion of maxillary incisors with visible plaque, proportion of mandibular molars with visible plaque, mean number of teeth with visible plaque, mean number of d1d2-3fs lesions, and proportion of infants with any decay (all p <0.05). All the significant variables had higher values for group 2 than group 3.

Table 4.28. Pairwise Comparison of Means and Percentage Proportions of Clinical Variables for Group 2 and Group 3 at Baseline (N = 71)

Variable	**Group 2 (n =35)**	**Group 3 (n=36)**	**P-value**
Proportion of mothers positive with MS	80.00	94.44	0.0847
Mean number of teeth	**11.54**	**7.13**	**0.0023****
Mean number of maxillary incisors with visible plaque	**1.54**	**0.53**	**0.0023****
Proportion of maxillary incisors with visible plaque	**48.57**	**8.33**	**0.0002***
Proportion of maxillary molars with visible plaque	34.29	16.67	0.0880
Proportion of mandibular incisors with visible plaque	17.14	5.56	0.1514
Proportion of mandibular molars with visible plaque	**31.43**	**8.33**	**0.0145***
Mean number of teeth with visible plaque	**3.26**	**0.89**	**0.0027****
Any decay (d1d2-3fs >0)	**34.29**	**5.56**	**0.0024***
Mean number of d1d2-3fs lesions	**1.29**	**0.00**	**0.0035****
Fluoridated toothpaste	**61.29**	**28.57**	**0.0118***
Fluoridated water	86.67	87.50	1.000

Note: Statistically significant using **Wilcoxon Rank-Sum test *Chi-Square test

Nine Months

Nine Months' – Univariate Analyses

The following section consists of descriptive statistics followed by bivariate analyses of demographic, dietary, behavioral, and environmental variables by group membership at the nine month time point. Since group 2 consisted of children who were positive for MS at baseline and remained positive throughout the study, we did not include them for these analyses. Only groups 1 and 3 are considered for analyses at this time point. Both groups had retained all the subjects enrolled at baseline for the nine month time point (group 1 n = 58 and group 3 n = 36).

Demographic Variables

Age: The study consisted of 94 subjects at the nine month time point. The minimum age of the children in the sample was 14 months and the maximum was of 38 months. The age variable was not normally distributed (based on Tests of Normality) over the span from 14 to 38 months with mean age of 21.1 months and standard deviation of 5.3 months.

The other demographic characteristics of the sample changed from that of the baseline findings mainly because of the deletion of group 2 individuals. This cohort consisted of more males (61%), non- Hispanics (86%), educated (83%) and younger (82%) mothers as compared to the baseline cohort. Detailed findings are described in the Table 4.29.

Table 4.29. Frequency and Descriptive Statistics for the Nine Month Demographic Variables for the Entire Sample (N=94)

Variable	Frequency	Valid Percentage
Sex		
Male	57	60.64
Female	37	39.36
Age of the mother		
26 or younger	77	81.91
27 or older	17	18.09
Ethnicity		
Hispanic	13	13.83
Non-Hispanic	81	86.17
Income Category		
$20,000 or less	56	59.57
$20,001 or more	38	40.43
Maternal education		
Less than high school	16	17.39
High school or more	76	82.61

Dietary Variables

- Diet composition

Among the dietary variables, the observed changes from baseline were pertinent to age changes from baseline. Negligible proportions of the individuals from either of the groups consumed infant formula. Cow's milk, 100% juice, and water were the most popular beverages. Flavored water (9%), sugar free beverages made from powder (6%), and other sugar free beverages (6%) were the least popular at nine-months. The combined sugared beverage consumption variable was formed if the subjects answered yes to any one of chocolate milk, 100% juice, juice drinks, sugared beverages made from powder, regular pop, sports drinks, and other sugared beverages. 62% of the subjects had consumption of one or more of these. The average amount consumed was approximately 45 ounces/week. The detailed findings are described in the Table 4.30.

Table 4.30: Frequency and Descriptive Statistics for Beverage Consumption at Nine Month Time Point for the Entire Sample (N=94)

Variable	**Proportion Reporting Use**		**Amount (ounces) Per Week**	
	Frequency	**Valid Percentage**	**Mean**	**Standard Deviation**
Infant formula	1	1.06	0.89	8.66
Cow's milk	88	93.62	126.39	82.37
Chocolate milk	14	12.61	--	--
Any milk (human milk, cow's milk (whole, 2%, 1%, skimmed), or infant formula)	89	94.98	--	--
100% juice	86	91.49	71.31	59.22
Juice drinks	35	37.23	16.68	45.56
Water	83	88.30	86.77	86.43
Flavored water	8	8.51	3.10	14.06
Sugared beverages made from powder	25	26.60	11.44	27.36
Sugar-free beverages made from powder	6	6.38	1.11	6.16
Regular pop	26	27.66	5.80	18.47
Diet pop	11	11.70	1.46	5.26
Sports drinks	15	15.96	3.51	13.05
Other sugared beverages	15	15.96	3.44	12.46
Other sugar-free beverages	6	6.38	0.94	5.99
Any sugared beverage consumption	57	61.96	44.99	85.72

- Diet behavior

Table 4.31 describes the frequency distribution of behavioral variables at nine month time points. A minority of the group still practiced bottle-feeding, night time bottle-feeding, and bottle-feeding at the middle of the night and none had human milk. A majority were using sippy cup, and a considerable portion practiced night time feeding and use of open cups. For the toothbrushing behavior, a majority (70%) of the group brushed regularly, some (27%) occasionally, whereas a negligible proportion (<3%) did not brush at all. A considerable number of subjects did not answer the question about toothbrushing.

Table 4.31. Frequency and Descriptive Statistics for Variables on Dietary Behavior at the Nine Month Time Point for the Entire Sample (N=94)

Variable (those with Yes)	**Frequency**	**Valid Percentage**
Breast-feeding	0	0.00
Bottle-feeding	11	12.09
Open cup	36	39.56
Closed cup (sippy cup)	76	80.85
Night-time feeding	38	41.76
Bottle-feeding in the middle of the night	6	6.59
Snack at bedtime	26	27.66
Snack in the middle of the night	0	0.00
Children sleeping with a bottle	9	9.57

Clinical Variables

Table 4.32 describes percentage distribution of clinical variables at the nine month time point. The change in the distribution of variables as compared to the baseline can be attributed to deletion of Group 2 from the analyses and increased age of the subjects. At nine months, the cohort was composed of more mothers who were positive for MS, a higher proportion of maxillary and mandibular teeth with visible plaque. Also, there was a reduction in the proportion of subjects who did not use fluoridated toothpaste, and an increase in those who used fluoridated toothpaste occasionally and those who used it regularly. This variable was later dichotomized as those who used fluoridated toothpaste (occasionally or regularly) and those who did not use it. A higher proportion of subjects were exposed to fluoridated drinking water through municipal water supplies (74%) than non-fluoridated water.

Table 4.32. Frequency and Descriptive Statistics for Clinical Variables at the Nine Month Time Point for the Entire Sample (N=94)

Variable	Frequency	Valid Percentage
Mother is infected with MS		
No	8	8.51
Yes	86	91.49
Maxillary molars with visible plaque		
0	55	58.51
1	13	13.83
2	24	25.53
3	2	2.13
Maxillary incisors with visible plaque		
0	55	58.51
1	6	6.38
2	15	15.96
3	8	8.51
4	10	10.64
Mandibular incisors with visible plaque		
0	74	78.72
1	7	7.45
2	8	8.51
3	1	1.06
4	4	4.26
Mandibular molars with visible plaque		
0	61	64.89
1	16	17.02
2	14	14.89
3	1	1.06
4	2	2.13
Children with visible plaque present on any teeth		
Any decay (d1d2-3fs >0)		
No	74	78.72
Yes	20	21.28
Are your child's teeth brushed?		
Yes	57	70.37
Yes, once in a while	22	27.16
No	2	2.47
Missing	13	
Use of fluoridated toothpaste for the child		
No	26	31.71
Yes	56	68.29
Missing	12	
Main water source		
Well water or private water source	13	15.85
City or public water source	61	74.39
Bottled water	9	9.89
Missing	11	

Table 4.32. Continued

Exposure to fluoridated water (City water)		
No	21	25.61
Yes	61	74.39
Missing	12	

Table 4.33 describes continuous clinical variables at nine months' time point. The findings are reflective of age related changes. The subjects had 13.6 as the mean number of teeth, and about 1/10 of a surfaces was either decayed or filled per person on average, with approximately 3 teeth having visible plaque.

Table 4.33. Descriptive Statistics for Clinical Variables at the Nine Month Time Point for the Entire Sample (N=94)

Variable	**Mean**	**Standard Deviation**
Number of teeth	13.64	4.74
d1d2-3fs	0.09	0.48
Plaque total	2.81	3.17

Nine Month – Pairwise Comparison (Group 1 and 3)

Group 1 [A group that had no MS at any of the three study time points (n=58)] was compared with Group 3 [A group that acquired MS during the 18-month study period (n=36)] on a number of independent variables. Group 2 was excluded from the analyses for this particular comparison.

Means of continuous variables and frequency distributions of categorical variables were compared for the two groups and the significant variables were included in the logistic regression model at the end of the analyses.

Demographic Variables

Age: The variable age was not normally distributed [from the Shapiro-Wilk Test ($p < 0.05$)]. The mean age for group 1 was 20.5 (range: 14 – 34) months and for group 3 was 22.2 (range: 15 – 38) months. A non-parametric Wilcoxon rank sum test was used to test if the means differed significantly between the groups. The test results indicated non-significant difference between the two groups ($p>0.05$).

The rest of demographic characteristics remained the same as the baseline comparisons between group 1 and 3. Similar to the baseline, only maternal education was a significant variable among all the demographic variables (refer to table 4.11 for details on individual variables).

Dietary Variables

- Diet composition

Table 4.34 and 4.35 describe comparison of dietary variables between groups 1 and 3 at the nine month time point. The proportion of children having chocolate milk, juice drinks, and sports drinks were significantly ($p<0.05$) higher in group 3 as compared to group 1. This was reflected in continuous variables for the amount of consumption (ounces/week) of the same beverages. Children from group 3 consumed higher amounts of juice drinks ($p\sim0.05$) and sports drinks ($p<0.05$) at the nine month time point than did group 1 children, and this was statistically significant.

The children drinking chocolate milk were 6.8 times as likely to develop infection as compared to non-drinkers [OR = 6.8, 95%CI =1.31-34.67]. The children drinking juice drinks were 2.4 times as likely to develop infection as compared to non-drinkers [OR =

2.4, 95%CI =1.02-5.72]. The children drinking sports drinks were 4.3 times as likely to develop infection as compared to non-drinkers [OR = 4.3, 95%CI =1.31-13.80]. The detailed results are describes in Table 4.34 and 4.35.

Table 4.34. Pairwise Comparison of Proportion Reporting Use of Beverages for Group 1 and Group 3 at the Nine Month Time Point (N=94)

Variable	**Group 1 (n =57)**	**Group 3 (n=35)**	**P-value**
Infant formula	1.72	0.00	1.0000
Cow's Milk	6.90	5.56	1.0000
Chocolate milk	**3.57**	**20.00**	**0.0247****
Any milk (human milk, cow's milk (whole, 2%, 1%, skimmed), or infant formula)	93.10	97.22	0.6459
100% Juice	93.10	88.89	0.4770
Juice drinks	**29.31**	**50.00**	**0.0511***
Water	87.93	88.89	1.0000
Flavored water	10.34	5.56	0.7061
Sugared beverages from powder	22.41	33.33	0.2441
Sugar-free beverages	8.62	2.78	0.4011
Regular pop	22.41	36.11	0.1490
Diet pop	8.62	16.67	0.3242
Sports drinks	**8.62**	**27.78**	**0.0137***
Other sugared beverages	15.52	16.67	0.8824
Other sugar-free beverages	6.90	5.56	1.0000
Any sugared beverage consumption	40.35	34.29	0.5607

Note: Statistically significant using *Chi-Square test ** Fisher's exact test

Table 4.35. Pairwise Comparison of the Mean Amount (in Ounces per Week) for of Beverage Intake for Group 1 and 3 at Nine Month Time Point (N=94)

Variable in ounces/week	**Group 1 (n =58)**	**Group 3 (n=36)**	**P-value**
Infant formula	1.45	0.00	0.4437
Milk	130.22	120.22	0.6398
100% Juice	62.67	85.22	0.0582
Juice drinks	**10.29**	**26.97**	**0.0525***
Water	84.10	91.11	0.7933
Flavored water	4.28	1.19	0.4079
Sugared beverages from powder	7.97	17.20	0.2524
Sugar-free beverages	0.83	1.56	0.2870
Regular pop	2.89	10.63	0.1519
Diet pop	1.35	1.64	0.2650
Sports drinks	**0.95**	**7.69**	**0.0114***
Other sugared beverages	2.91	4.28	0.9313
Other sugar-free beverages	1.17	0.56	0.8257
Combined added sugar beverages	29.77	70.50	0.0733

Note: * Statistically significant using Wilcoxon Rank-Sum test

- Diet behavior

Table 4.36 compares behavioral variables between groups 1 and 3 at the nine month time point. Among all of the variables, only use of open cup differed significantly ($p<0.05$) with group 3 having 54% of its children using open cup compared to 30% in group 1. The variable for night time bottle-feeding practices was marginally significant ($p =0.0554$). It was considered as significant and for the domain specific model based on the literature review and findings from analyses of other time-points for this dataset. [Children having snack at midnight ($p = 0.0552$) is not considered though as it was significant in further analyses.]

The children with no night feeding practices were 57% less likely to develop infection as compared to those with any night feeding practices [OR = 0.4, 95%CI = 0.18-1.03]. The children using open cup were 2.7 times as likely to develop infection as compared to non-open-cup-users [OR = 2.7, 95%CI =1.14-6.54]. Use of sippy cup (80.9) and toothbrushing (70.4%) were commonly practiced in this cohort. None of the children were having snacks in the middle of the night (0%) Exposure to fluorides through fluoridated toothpaste (68%) and fluoridated water (74%) was common at this time point. Detailed results are described in Table 4.36.

Table 4.36. Pairwise Comparison of Proportion Reporting Use of Variables for Dietary Behavior for Group 1 and Group 3 at the Nine Month Time Point (N=94)

Variable (Yes/No)	**Group 1 (n = 58) %**	**Group 3 (n= 36) %**	**P-value**
Breast-feeding	--	--	--
Bottle-feeding	10.71	14.29	0.7437
Open cup	**30.36**	**54.29**	**0.0231***
Closed cup (sippy cup)	83.93	82.86	0.8934
Night-time feeding	33.93	54.29	0.0554
Night time bottle-feeding	7.14	14.29	0.2976
Bottle-feeding in the middle of the night	5.35	8.57	0.6721
Snack at bedtime	20.69	38.89	0.0552
Snacks in the middle of the night	5.36	8.57	0.6721

Note: * Statistically significant using Chi-Square test

Clinical Variables

Table 4.37 compares clinical variables between groups 1 and 3. Group 3 children had significantly ($p<0.05$) more teeth erupted, more mandibular molars with visible plaque, higher mean number of d1d2-3fs lesions, and more children with any decay (d1d2-3fs lesions) as compared to group 1. Based on mean number of teeth erupted at this time point, the children were divided into two categories – those with less than 13 teeth, and those with 13 teeth or more. The dichotomized version of this variable was significant ($p<0.05$) as well.

The children with more than 13 teeth were 3.2 times as likely to develop infection as compared to children with less than 13 teeth [OR = 3.2, 95%CI = 1.28-8.05]. The children having mandibular molars with visible plaque were 2.9 times as likely to develop infection as compared to children with none of the mandibular molars with visible plaque [OR = 2.9, 95%CI = 1.19-6.90]. The children with previous caries experience were 7.8 times as likely to develop infection as compared to children with no previous caries experience [OR = 7.8, 95%CI = 1.28-8.05]. For a child in the study, on average, almost three of 14 erupted teeth were with visible plaque and one of the erupted teeth exhibited caries. Detailed results are described in Table 4.37.

Table 4.37. Pairwise Comparison of Proportion and Means of Clinical Variables for Group 1 and Group 3 at the Nine Month Time Point (N = 94)

Variable	**Group 1 (n =58)**	**Group 3 (n=36)**	**P-value**
Proportion of mothers positive with MS	91.38	91.67	1.0000
Mean number of teeth	**12.78**	**15.03**	**0.0117****
Children with more than 13 teeth	**17.37**	**74.29**	**0.0112***
Mean number of maxillary incisors with visible plaque	0.95	1.25	0.1802
Proportion of maxillary incisors with visible plaque	34.48	52.78	0.0801
Proportion of maxillary molars with visible plaque	36.21	50.00	0.1870
Proportion of mandibular incisors with visible plaque	22.41	19.44	0.7324
Proportion of mandibular molars with visible plaque	**25.86**	**50.00**	**0.0172***
Mean number of teeth with visible plaque	2.40	3.47	0.0644
Proportion of children with visible plaque present on any tooth			
Any decay (d1d2-3fs >0)	**8.77**	**42.86**	**<0.0001***
Mean number of d1d2-3fs lesions	**0.00**	**0.22**	**0.0271****
Toothbrushing	96.00	100.00	0.7341
Fluoridated toothpaste	62.00	78.13	0.1258
Fluoridated water	74.00	75.00	0.9194

Note: Statistically significant using **Wilcoxon Rank-Sum test *Chi-Square test

Nine Months' – Multivariable Analyses

As discussed earlier, the multivariate analyses at the nine month time point was also conducted in four domains – demographics, dietary - composition and behavior, and clinical. Mother's education and child's age (demographic domain), chocolate milk and sports drink (both dichotomous, dietary composition domain), use of open-cup for feeding and no habit of night feeding practices (dietary behavior domain), and child's caries experience (as d1d2-3fs >0) (clinical domain) are the variables that remained in their respective models after the forward selection procedure applied to variables significant at bivariate level. Hosmer-Lemeshow Goodness of Fit test and its p-value were used to assess fitness of each of the models [Table 4.38]. These variables entered the preliminary model for comparison of Group 1 and 3 at nine months' time point.

The preliminary model consisted of all seven variables selected by forward selection procedure from each of the domains. It included age variable based on the evidence from literature. The preliminary model had AIC of 119.4, Hosmer-Lemeshow test statistics as 3.8 (p-value = 0.8060), and c-statistics as 0.841. The convergence criteria were satisfied, and tests of multicollinearity indicated no correlation between the independent variables. The fit of the model was good and the relationship given by the model was significant. Sports drinks [OR =5.5 (1.32-26.43); p = 0.0240] and presence of any (d1d2-3fs) decay [OR = 9.7 (2.59-43.58); (p = 0.0014)] were the only significant relations given by the model. It was subjected to forward selection procedure to get the final model [Table 4.39].

The final model consisted of six variables – age, mothers' education, sports drinks, no night feeding practices, caries experience (d1d2-3fs >0) and use of open cup.

Again, the age variable was included based on the evidence from literature. Convergence criteria were satisfied and the tests of multicollinearity indicated no correlation between the independent variables. The model statistics indicate reasonable fit of the model [AIC = 119.4, Hosmer-Lemeshow test statistics=10.1 with p=0.1819, and c-statistics = 0.830].

Model statistics indicated a reasonable fit for both the models and the association depicted by the model was significant. The detailed results are described in the Table 4.39 and 4.40.

Table 4.38. Summary of Logistic Model Development for Domain Specific, Preliminary, and the Final Model at Nine Months' Time Point

Variables	**AIC**	**c-statistics**	**Variables Included in Model**	**Chi-Square**	**P-value**
Model for demographic domain					
2	122.285	0.686	Higher maternal education, Age	9.6826	0.2880
Model for dietary composition domain					
2	121.334	0.669	Chocolate milk, Sports drinks (both yes/no),	0.2164	0.6418
Model for dietary behavior domain					
2	123.263	0.650	No night feeding practices, use of open cup	8.9508	0.0114
Model for clinical domain					
1	124.227	0.670	Any decayed or filled tooth	--	--
Preliminary model with demographic, dietary – composition and behavior, and clinical variables					
7	119.408	0.841	Age, higher maternal education, Chocolate milk, Sports drinks, No night feeding practices, Use of open cup, Any decayed or filled tooth	3.7685	0.8060
Final model					
6	121.293	0.808	Age, higher maternal education, Sports drinks, No night feeding practices, Any decayed or filled tooth, Use of open cup	29.614	<0.001

Table 4.39. Preliminary Model for Comparison of Groups 1 and 3 at Nine Months' Time Point

Independent variables	**OR**	**95% CI**	**P-value**
Age	0.989	0.88 – 1.11	0.8481
Higher maternal education	0.312	0.07 – 1.36	0.1212
Chocolate milk	3.397	0.47 – 34.12	0.2469
Sports drinks	5.446	1.32 – 26.43	0.0240
No night feeding practices	0.420	0.13 – 1.33	0.1450
Use of open cup	2.666	0.86 – 8.57	0.0907
Presence of any decay	9.661	2.59 – 43.58	0.0014

Note: AIC = 119.408; Hosmer-Lemeshow test statistics 3.7685, p = 0.8060; c-statistics = 0.841

Table 4.40. Final Model for Comparison of Groups 1 and 3 at Nine Months' Time Point

Independent variables	**OR**	**95% CI**	**P-value**
Age	0.971	0.87 – 1.09	0.6053
Higher maternal education	0.242	0.06 – 0.98	0.0495
Sports drinks	5.555	1.39 – 26.26	0.0198
No night feeding practices	0.349	0.11 – 1.04	0.0653
Presence of any decay	9.358	2.55 – 40.90	0.0014
Open cup	2.365	0.78 – 7.37	0.1287

Note: AIC = 119.408; Hosmer-Lemeshow test statistics 10.1189, p=0.1819; c-statistics = 0.830

According to the final model, maternal education [OR =0.2; 95%CI: 0.06-0.98]; p = 0.0496] was a marginally significant protective factor. Sports drinks [OR = 5.6; 95%CI: 1.39-26.26; p = 0.0198] and presence of any decay [OR =9.4 ; 95%CI: 2.55-40.90; p = 0.0014] were risk factors for MS infection. The OR of 0.249 indicated that the odds of getting infected were (1 – 0.249 ~) 74% less in children with mothers educated more than high school as compared to mothers with education less than high school. Children having sports drinks were 5.6 times as likely to develop MS infection as compared to children not having sports drinks. Children with previous caries experience were 9.4 times as likely to develop MS infection as compared to children with no history of caries.

Eighteen Months

Eighteen Months –Univariate Analyses

The following section consists of descriptive statistics and bivariate analyses of demographic, dietary, behavioral, and environmental variables by Group membership at the eighteen month time point. Since group 2 children were positive for MS at baseline and remained positive throughout the study, we did not consider that group in these analyses. Only groups 1 and 3 are considered in the following section.

Demographic Variables

Age: The analyses included 94 subjects at the eighteen month time point. The minimum age of the children in the sample was 23 months and the maximum was 47 months. The mean age was 30.7 months with standard deviation of 5.4 months. The age variable was not normally distributed (based on the Shapiro-Will test of Normality $p < 0.0001$).

The other demographic variables are same as that of nine month time point. In order to avoid redundancy, the rest of the demographic variables are not described here. Those interested can refer to Table 4.29 for details.

Dietary Variables

- Diet composition

Among the dietary variables, negligible proportions of the individuals from either of the groups consumed infant formula. The most and least popular beverages were similar to that of baseline and nine months. Cow's milk, 100% juice, and water were still the most popular beverages; while flavored water, sugar free beverages made from powder, and other sugar free beverages were still some of the least popular beverages. Sixty-nine percent of the subjects had consumption of one or more of any of the sugared beverages (chocolate milk, flavored water, regular pop, sports drinks, 100% juice, juice drinks, sugared beverages made from powder, and other sugared beverages). Almost 68% of the subjects consumed at least one of the sugared beverages with average amount of 59 ounces/week, which was also higher than that of nine months' consumption. Sugar-free beverages and flavored water were the least popular drinks (<10% children drinking < 5 ounces/week in each group) again at this time point. The detailed findings are described in the Table 4.41.

Table 4.41. Frequency and Descriptive Statistics for Beverage Consumption at the Eighteen Month Time Point for the Entire Sample (N=94)

Variable	**Proportion Reporting Use**		**Amount (ounces) Per Week**	
	Frequency	**Valid Percentage**	**Mean**	**Standard Deviation**
Infant formula	1	1.06	0.65	7.40
Cow's milk	88	93.62	112.23	86.11
Chocolate milk	11	12.22	---	---
100% juice	87	96.67	63.84	60.52
Juice drinks	44	89.36	16.06	41.86
Water	84	88.30	73.91	81.46
Flavored water	16	17.02	2.66	12.42
Sugared beverages made from powder	24	25.53	10.93	26.33
Sugar free beverages made from powder	4	4.26	1.46	9.03
Regular pop	41	47.13	5.46	16.39
Diet pop	10	10.64	1.51	6.65
Sports drinks	13	13.83	3.11	12.22
Other sugared beverages	14	14.89	3.87	13.15
Other sugar free beverages	7	7.45	3.21	5.81
Any sugared beverage consumption	65	69.15	58.66	20.00
Any milk (human milk, cow's milk (whole, 2%, 1%, skim), or infant formula)	89	94.68	--	--

- Diet behavior

Table 4.42 describes the frequency distribution of behavioral variables at the eighteen month time point. None of the children engaged in either breast-feeding or bottle-feeding anymore. A small minority had feeding practices during the night. Sippy cups were still popular though comparatively lower proportion of children used them compared to nine months. The popularity of open cups and regular toothbrushing increased consistently over the previous time points. For the toothbrushing behavior, a majority (76%) of the group brushed regularly, some (23%) occasionally, whereas a negligible proportion (~1%) did not brush at all. A considerable number of subjects (13) did not answer this question about toothbrushing.

Table 4.42. Frequency and Descriptive Statistics for Variables on Dietary Behavior at the Eighteen Month Time Point for the Entire Sample (N=94)

Variable (those with Yes)	**Frequency**	**Valid Percentage**
Breast-feeding	0	00.00
Bottle-feeding	0	00.00
Open cup	48	51.06
Closed cup (Sippy cup)	68	72.34
Night time feeding	36	38.30
Night time bottle-feeding	4	4.44
Bottle-feeding in the middle of the night	2	2.22
Snacks at bedtime	22	24.44
Snacks in the middle of the night	6	6.67

Clinical Variables

Table 4.43 describes the percentage distribution of clinical variables at the eighteen months' time point. The change in the distribution of variables as compared to the baseline can be attributed to deletion of Group 2 from the analyses and increased aged of the subjects. At eighteen months, the cohort had more mothers who were positive for MS, a higher proportion of maxillary and mandibular teeth with visible plaque. Also, there was an increase in the proportion of subjects who used fluoridated toothpaste. Most of these variables were the dichotomized for further analyses as described earlier. The table describes categorical distributions.

Table 4.43. Frequency and Descriptive Statistics for Clinical Variables at the Eighteen Month Time Point for the Entire Sample (N=94)

Variable	Frequency	Valid Percentage
Mother is infected with MS		
No	7	7.45
Yes	87	92.55
Maxillary molars with visible plaque		
0	18	19.15
1	16	17.02
2	35	37.23
3	11	11.70
4	14	14.89
Maxillary incisors with visible plaque		
0	28	29.79
1	14	14.89
2	18	19.15
3	5	5.32
4	29	30.85
Mandibular incisors with visible plaque		
0	48	51.06
1	14	14.89
2	16	17.02
3	3	3.19
4	13	13.83
Mandibular molars with visible plaque		
0	24	25.53
1	20	21.28
2	25	26.60
3	13	13.83
4	12	12.77
Children with visible plaque present on any teeth	80	85.11
Any decay (d1d2-3fs >0)		
No	29	30.85
Yes	65	69.15
Are your child’s teeth brushed?		
Yes	71	75.53
Yes, once in a while	22	23.40
No	1	1.06
Missing	13	
Use of fluoridated toothpaste for the child		
No	11	11.70
Yes	83	88.30

Table 4.43 Continued

Exposure to fluoridated water (City water)		
No	10	26.32
Yes	28	73.68
Missing	56	

Table 4.44 describes continuous clinical variables at eighteen months' time point. The findings are reflective of age related changes. The subjects had 18.18 as the mean number of teeth present, and more than 3 surfaces as d1d2-3f surfaces per person on average. Most of the surfaces recorded for caries experience had initial levels of cavitation. The subjects also had heavy plaque depositions with more than six teeth per person laden with visible plaque. All these variables showed an increase in comparison with both nine months and baseline recordings.

Table 4.44. Descriptive Statistics for Clinical Variables at the Eighteen Month Time Point for the Entire Sample (N=94)

Variable	**Mean**	**Standard Deviation**
Number of teeth	18.18	2.22
d1d2-3fs	3.38	5.31
d1 surfaces	2.88	3.76
d2 surfaces	0.50	6.20
Plaque total	6.60	4.29

Eighteen Months – Pairwise Comparison (Groups 1 and 3)

As before, for these analyses group 1 [A group that had no MS at any of the three study time points (n=58)] was compared with group 3 [A group that acquired MS during the 18-month study period (n=36)]. Group 2 was excluded from the analyses for this comparison.

Means of continuous variables and frequencies of categorical variables were compared for the two groups and the significant variables were included in the logistic regression model at the end of the analyses.

Demographic Variables

Age: The variable age was not normally distributed [from the Shapiro-Wilk Test (<0.0001)]. The minimum age of the children in the sample was 23 months and the maximum was of 47 months. The mean age of group 1 was 29.5 months and group 3 was 31.2 months. Student's t-test was used to test if the means differed significantly between the groups. The test results indicated non-significant differences between the two groups (p >0.05).

The rest of the demographic characteristics remained the same as in the baseline comparisons between Group 1 and Group 3. Similar to baseline and nine months, only maternal education was significantly different among all the other categorical demographic variables (refer to table 4.11 for details on individual variables).

Dietary Variables

- Diet composition

Table 4.45 and 4.46 describe the comparison of dietary variables between groups 1 and 3 at the eighteen month time point. The proportion of children having cow's milk, any kind of milk (whole, 2%, 1%, skimmed, or other flavored), were significantly (p<0.05) lower in group 3 as compared to group 1. Regular pop were significantly (p<0.05) more popular in group 3 as compared to group 1. Significant difference existed among the groups for regular pop intake (in ounces/week) as well. Group 3 children had higher intake of regular pop than group 1 ($p<0.05$).

Table 4.45. Pairwise Comparison of Proportion Reporting Use of Beverages for Group 1 and Group 3 at the Eighteen Month Time Point (N=94)

Variable	**Group 1 (n =58)**	**Group 3 (n=36)**	**P-value**
Infant formula	0.00	2.78	0.3830
Cow's Milk	98.28	96.88	0.6661
Chocolate milk	12.07	12.50	1.0000
Any milk (human milk, cow's milk (whole, 2%, 1%, skimmed), or infant formula)	**100.00**	**86.11**	**0.0069***
100% Juice	98.28	93.75	0.2871
Juice drinks	46.55	47.22	0.9495
Water	93.10	83.33	0.1742
Flavored water	15.52	19.44	0.6224
Sugared beverages from powder	20.69	33.33	0.1717
Sugar-free beverages	3.45	5.56	0.6357
Regular pop	**38.18**	**62.50**	**0.0284****
Diet pop	6.90	16.67	0.1742
Sports drinks	10.34	19.44	0.2141
Other sugared beverages	13.79	16.67	0.7036
Other sugar-free beverages	6.90	8.33	1.000
Any sugared beverage consumption	24.14	22.22	0.8311

Note: Statistically significant using *Fisher's exact test ** Chi-square test

Table 4.46. Pairwise Comparison of Mean Amount (in Ounces/week) for Beverage Consumption for Group 1 and Group 3 at the Eighteen Month Time Point (N=94)

Variable	**Group 1 (n =58)**	**Group 3 (n=36)**	**P-value**
Infant formula	0.00	4.67	0.2122
Milk	135.90	106.44	0.0539
100% Juice	72.98	68.00	0.3531
Juice drinks	20.35	23.50	0.5141
Water	86.90	76.72	0.2687
Flavored water	3.24	4.11	0.6994
Sugared beverages from powder	5.64	19.43	0.1977
Sugar free beverages	0.36	2.72	0.6012
Regular pop	**4.91**	**11.31**	**0.0424***
Diet pop	1.45	5.00	0.1288
Sports drinks	3.48	15.44	0.1862
Other sugared beverages	4.52	9.33	0.7072
Other sugar-free beverages	3.00	3.56	0.8176
Combined added sugar beverages	42.53	84.94	0.1760

Note: * Statistically significant using Wilcoxon Rank-Sum test

- Diet behavior

Table 4.47 compares behavioral variables between group 1 and 3 at the eighteen month time point. None of these variables were significant. The use of sippy cup differed marginally (p = 0.0552) between the groups. Proportion of children with night time feeding practices was considered as it was significant at nine months' time point. Group 3 had a higher proportion of children with nighttime feeding practices [OR = 2.1 (95% CI: 0.87 – 5.21)] as compared to group 1. Whereas, group 1 had a higher proportion of children using sippy cup [OR = 0.4 (95% CI: 0.16 – 1.03)] than group 3.

Table 4.47. Pairwise Comparison of Proportion Reporting Use of Variables on Dietary Behavior for Group 1 and Group 3 at the Eighteen Month Time Point (N=94)

Variable	**Group 1 (n =58) %**	**Group 3 (n=36) %**	**P-value**
Breast-feeding	0.00	0.00	---
Bottle-feeding	0.00	0.00	---
Open cup	51.72	50.00	0.8709
Closed cup (sippy cup)	79.31	61.11	0.0552
Night-time feeding	29.31	46.88	0.0956
Night time bottle-feeding	29.31	46.88	0.0956
Snack at bedtime	20.69	31.25	0.2645
Snack in the middle of the night	5.17	9.38	0.6620
Children sleeping with a bottle	3.45	6.25	0.6136

Clinical Variables

Table 4.48 compares clinical variables between groups 1 and 3 at the eighteen month time point. Group 3 children had significantly ($p<0.05$) higher numbers of teeth, and maxillary and mandibular molars with visible plaque than group 1. Group 3 also had higher mean number of teeth with visible plaque, d1d2-3fs lesions, as well as individual surfaces with d1 lesions and d2 lesions as compared to group 1.

Table 4.48. Pairwise Comparison of Proportion and Means of Clinical Variables for Group 1 and Group 3 at the Eighteen Month Time Point (N = 94)

Variable	**Group 1 (n =58)**	**Group 3 (n=36)**	**P-value**
Proportion of mothers positive with MS	89.66	97.22	0.2445
Mean number of teeth	**17.59**	**19.09**	**0.0017[c]**
Children with more than 18 teeth	**41.51**	**74.29**	**0.0025***
Mean number of maxillary incisors with visible plaque	1.74	2.22	0.1333
Mean number of maxillary molars with visible plaque	**1.50**	**2.44**	**0.0004[c]**
Mean number of mandibular incisors with visible plaque	0.96	1.42	0.2104
Mean number of mandibular molars with visible plaque	**1.39**	**2.11**	**0.0110[c]**
Proportion of maxillary incisors with visible plaque	63.79	80.56	0.0841
Proportion of maxillary molars with visible plaque	**71.14**	**91.67**	**0.0358***
Proportion of mandibular incisors with visible plaque	46.55	52.78	0.5572
Proportion of mandibular molars with visible plaque	**67.24**	**86.11**	**0.0414***
Mean number of teeth with visible plaque	**5.60**	**8.19**	**0.0023[c]**
Proportion of children with visible plaque present on any tooth	79.31	94.44	0.0715
Any decay (d1d2-3fs >0)	62.07	80.56	0.0592
Mean number of d1d2-3fs lesions	**1.83**	**5.89**	**0.0009[c]**
Mean number of d1 surfaces	**1.81**	**4.61**	**0.0048[c]**
Mean number of d2 surfaces	**0.02**	**1.28**	**0.0001[c]**
Fluoridated toothpaste	89.66	86.11	0.7435
Fluoridated water	70.69	77.78	0.4496

Note: Statistically significant using * Chi-Square test [c] Wilcoxon Rank-Sum test

Eighteen Months – Multivariable Analyses

As discussed earlier the multivariate analyses at eighteen months' time point was also conducted in four domains – demographics, dietary – composition and behavior, and clinical. For the demographic domain, mother's education and child's age were included in the model. Maternal education was significant at bivariate level, and hence entered the domain specific model. Age of the child, although not significant statistically at the bivariate level, was included in the domain specific model based on evidence from the literature and findings from earlier time points.

For the diet domain, among the three significant dichotomous and continuous dietary variables, regular pop consumption, for both categorical (yes/no) and continuous (ounces/week) versions were significant at bivariate level. The proportion of children consuming any kind of milk (whole, 2%, 1%, skimmed, or other flavored) was also significant at bivariate level and was eligible to be subjected to forward selection procedure along with the regular pop variables. Only the amount of pop consumed per week remained in the domain specific model.

The variable for any kind of milk intake had a heavily skewed distribution with everybody in Group 1 drinking some kind of milk, whereas five children from group 3 not drinking any kind of milk. Hence, group 1 had 100% in one of the cells for independent variable (no variation), and the other cell had 0 in it. This being quasi-complete separation of the data points, the fit of the model was questionable. It is not possible to do the computations for the “any milk” variable and thus, the variable for any kind of milk intake was taken out of the models, in spite of being significant at the bivariate level.

None of the dietary behavioral variables were significant at bivariate level. However, based on the literature and findings from analyses of earlier time points, variables for night feeding practices, use of open cup, and use of sippy cup were subjected to forward selection procedure for domain specific model. Only night feeding practices remained in the behavior model. Among the clinical variables, only child’s caries experience (as decayed or filled teeth) remained in the model after subjecting the ten variables, significant at bivariate level, to forward selection procedure.

The Hosmer-Lemeshow Goodness of Fit Chi-square test and associated p-value for each model indicate the significance of association between the group membership and the variables chosen for that particular domain. AIC and c-statistics can also be used to compare different models and evaluate the model fit. The detailed results are described in the Table 4.49.

Table 4.49. Summary of Logistic Model Development for Domain Specific, Preliminary, and the Final Model at Eighteen Months' Time Point

# of Variables entering	**AIC**	**c-statistics**	**Variables Chosen by Forward Selection Procedure**	**Chi-Square**	**P-value**
Model with significant demographic variables					
2	118.227	0.693	Mother's education, Age	5.9405	0.6539
Model with significant dietary composition variables					
3	116.454	0.649	Total soda pop consumption (ounces/week)	2.6143	0.4550
Model with dietary behavioral variables					
3	119.147	0.588	Night time feeding practices	2.6143	0.0141
Model with clinical variables					
10	127.115	0.716	Number of d1 surfaces, Number of d1d2-3 and filled surfaces	5.1716	0.5220
Preliminary model with demographic, dietary- composition & behavior, and clinical variables					
6	118.227	0.834	Age, Higher maternal education, Number of d1 surfaces, Number of d1d2-3 and filled surfaces	37.1626	<0.001
Final model					
3	125.156	0.760	Mother's education, Number of d1 surfaces, Number of d1d2-3 and filled surfaces	2.3375	0.8862

The preliminary model consisted of all six variables. Similar to nine months' time point, child's age was considered in the preliminary model at this time point as well. The other variables included in the model were maternal education, total soda pop consumption (ounces/week), no night feeding practices, number of d1 surfaces, and number of d1d2-3f surfaces. Among these only maternal education was marginally significant (p = 0.0510). When subjected to forward selection procedure, age, total soda pop consumption, and no night feeding practices were eliminated from the model (level of significance for entry into the model was set as 0.1).

The preliminary model was subjected to forward selection process to give the final model. The p-value for entry into the model was selected to be 0.1. The preliminary model had AIC of 118.2. The convergence criteria were satisfied and the tests of multicollinearity indicated no correlation between the independent variables. When subjected to forward selection procedure, only three variables remained in the model – mothers' education (yes/no), number of d_1 surfaces, and number of d1, d2-3, and filled surfaces. These variables entered the final model, which had AIC of 125.2 and Hosmer-Lemeshow Goodness of Fit test Chi square statistics as 6.4968 (p=0.5918). The detailed results are described in the Tables 4.50 and 4.51

Table 4.50. Preliminary Model for Comparison of Groups 1 and 3 at the Eighteen Month Time Point

Independent variables	**OR**	**95% CI**	**P-value**
Age	0.946	0.81 – 1.06	0.3226
Higher maternal education	0.253	0.06 – 0.99	0.0510
Total soda-pop consumption	0.974	0.91 – 1.04	0.4399
No night feeding practices	0.625	0.19 – 2.11	0.4388
Number of d1 surfaces	4.535	1.27 – 82.1	0.1239
Number of d1d2-3 and filled surfaces	0.189	0.01 – 0.66	0.0901

Note: AIC = 118.227; c-statistics = 0.821; Hosmer-Lemeshow Goodness of Fit test Chi-square= 6.81; p =0.5569

The final model comprised of higher maternal education, d1surfaces, and d1d2-3f surfaces. The model statistics indicated reasonable fit of the overall model. However, none of the individual variables were significant (p>0.05). Higher maternal education and d1d2-3f surfaces were found to be protective factors, whereas presence of d1 surface was a risk factor. Tests for multicollinearity indicated very high correlation (variance inflation factor >5) between d1surfaces and d1d2-3f surfaces. However, eliminating any one of the two (d1s or d1d2-3fs) made the model even less stable and parameters non-significant.

Table 4.51. Final Model for Comparison of Groups 1 and 3 at the Eighteen Month Time Point

Independent variables	**OR**	**95% CI**	**P-value**
Higher maternal education	0.322	0.09 – 1.09	0.0701
Number of d1 surfaces	5.161	1.29 – 94.36	0.1038
Number of d1d2-3 and filled surfaces	0.155	0.01 – 0.61	0.0645

Note: AIC = 125.156; c-statistics = 0.760; Hosmer-Lemeshow Goodness of Fit test Chi-square = 2.375; p =0. 8862

According to the final model, maternal education was a protective factor, and this relationship was not statistically significant (p>0.05). The OR of 0.322 indicated that the odds of getting infected were (1 – 0.322 ~) 68% less in children with mothers educated more than high school as compared to mothers with education less than high school. Similarly, d1d2-3fs was found to be a protective factor. Presence of additional d1d2-3f surface would reduce the chance of being infected by (1 – 0.155 ~) 84.5%. However, this relationship was statistically not significant.

Number of d1 surfaces was a risk factor. Children with more d1 surfaces were 5.2 times as likely to develop MS infection as compared to children with less d1 surfaces. Even this relationship was statistically not significant. The relationships given by the model are suggestive of high prevalence of untreated caries in the subject population in the post-study period. The model also reflects effectiveness of caries treatment in controlling the MS infection. Children with advanced but treated carious lesions (d1d2-3fs) were better protected from MS as compared to children with untreated white spot lesions.

Hence there have been some important findings and their possible implications. The next section, Chapter 5 – Discussion, elaborates more on these.

CHAPTER 5

DISCUSSION

This chapter consists of interpretation of the findings described in Chapter 4, more details on the model selection and other statistical procedures, and concludes with discussion of strengths and limitations of this study. The findings are discussed in the same order as that of the analyses presented in Chapter 4. At the end of each time point, a summary about the important findings is given.

Baseline

For baseline analyses of this study, the mean age of the subjects was 12.9 months with the youngest being 6 months old and the oldest being 24 months. A majority of the mothers were younger (less than 26 years of age) and educated. The population was more or less homogenous for annual family income. Socio-demographic characteristics of the cohort were comparable to studies by Warren et al. (2008), Wan et al. (2003), Mattos-Graner (2001), Karn et al. (1998), and Mohan et al. (1997).

Among these studies, Warren et al. (2008) was a longitudinal study assessing caries as the outcome, and Karn et al. (1998) and Mohan et al. (1997) were cross sectional studies assessing factors responsible for salivary MS levels. Wan et al. (2003) was a longitudinal study following children from birth until 24 months of age, and hence could not comment much on the crucial period of 23-35 months when second deciduous molars erupt. Mattos-Garner et al. (2001) had followed 12-30 month olds for one year studying the salivary MS levels. However, the study did not have any subjects in predentate period, had two points of examination separated by 12 months, had limited spectrum of liquid intake under consideration, and did not assess use of beverage containers except the baby bottle.

For the present study, at baseline, the children were young and most of the variable distributions can be considered as characteristics for this age group. A typical 12-13 month old child would typically have no more than four incisors erupted per arch. Accordingly, the mean number of erupted teeth was seven (ADA, 2005). The diet does not vary drastically among the children from a very young age group. Hence, there were fewer other beverage options reported by the parents of these children, as compared to later time points. Less frequent usage of other sugared and sugar-free beverages can be explained by this.

The developing motor coordination in younger children likely makes parents hesitant regarding the use of open cup and fluoridated toothpaste. Sippy cup use was therefore common (64.3%) at baseline. Contradictory advices and prevalent controversies in relation to fluoride exposure in children less than two year of age could be reflected in the missing answers for use of fluoridated toothpastes (20 out of 129). Lower education and family income can be contributing to lack of knowledge and awareness about water fluoride levels.

Baseline Comparisons

Among the demographic variables, mean age of the subjects and the proportion of females differed significantly between the groups. Group 2 (MS positive at baseline) had the oldest (16.74 months old) children and the highest proportion of females. Many dietary variables including infant formula, milk (cow's milk), and sugared beverages were significantly different among groups in both frequency and ounces/week analysis. Infant formula was popular in group 1 (MS negative at all three time points) children, whereas rest of the beverages and drinks were more common in the two other groups (2 and 3) than group 1 [Table 4.6 - 4.8].

This difference in beverage consumption pattern can be attributed to the younger children in group 1 as compared to older children in the other two groups. The younger children had comparatively smaller intake capacity and less variation in the diet. Thus, group 1 children were more homogenous in consumption of infant formula than the other two groups. The effect of this difference in beverage consumption pattern was evident in the status of MS infection as well as caries experience of children. Group 2, the infected children, had more consumption of sugared beverages as compared to the other two. Group 1 children, the non-infected ones, were more inclined towards infant formula. Weber-Gasparoni et al. (2012), Wan et al. (2003), Mottos-Garner et al. (2001) reported similar findings.

Among the behavioral variables, group1 children (the youngest) reported bottle-feeding at significantly higher proportion than groups 2 and 3. A higher proportion of group 2 children (the oldest) used the open cup as a container. Group 2 children also had a higher proportion using fluoridated toothpaste. These may have contributed to difference in their MS status. Bottle-feeding has been associated with MS infection [Mohan et al., (1997), Karn et al. (1998)]. However, the relationship between use of a particular container (baby bottle, sippy cup, open cup) can be correlated with the age of the child as well.

The correlation with the age variable can be explained by the underlying age differences among the groups. The older children from group 2 likely developed better motor coordination skills. They had better compliance regarding the use of toothpaste and toothbrush. Also, group 2 children had more teeth (mean number of teeth Group 2 = 11.5). Parents of group 2 children would thus be more inclined to use fluoridated toothpaste and open cup as compared to parents of group 1 and group 3 children. Similar findings and discussion were observed in the study by Weber-Gasparoni et al. (2012).

Number of teeth [Caufield et al. (1993), Tedjosasongko et al. (2002)] and previous caries experience [Kishi et al. (2009), Choi et al. (2009)] have often been associated with MS infection in children. Significant differences among the groups were observed in these two tooth related variables in this study as well. Mean number of erupted teeth was 11.5 for group 2 (4.8 for group1 and 7.1 for group 3). Proportion of children with any caries experience was the highest (34.3%) in group 2 (1.7% in group 1 and 5.6% in group 3). However, with group 2 being the oldest group of children both of these findings can be attributed to chronological difference in development.

Group 2 children (MS positive at baseline) also had a higher proportion of teeth with visible plaque, and higher mean number of d1d2-3fs lesions than the other two groups. The association between MS and plaque, higher caries indices, and hygiene habits was established by different studies [Loesche (1986), Beighton et al. (1999), Weber-Gasparoni et al. (2012)]. Both tooth-related variables including number of teeth, and dietary-behavioral practices affecting certain tooth related variables are functions of age. Hence, the significant differences between these tooth related variables can be explained by difference in the age among the groups.

Baseline – Logistic Regression Findings

As mentioned in Chapter III and IV, only group 1 and 3 were used for regression and follow-up analyses. The age of the children did not differ significantly between group 1 (10.8 months) and group 3 (12.6 months). Still, it was considered in the preliminary model owing to the evidence for its role in MS colonization [Weber-Gasparoni et al. (2012), Caufield et al. (1993)]. The other variables included in the model were maternal education, juice consumption, and presence of more than five teeth (dichotomized version of the variable for mean number of teeth). The final model consisted of only two variables – maternal education and more than five erupted teeth. The model statistics (AIC, Hosmer-Lemeshow test, and c-statistics) indicated a good fit for both the models. Hence the association depicted by the model was significant.

The odds ratio for both the variables indicated that higher maternal education was a protective factor. Higher maternal education can be considered as an indicator for higher family income and higher SES. Also, inverse relationship between education and health and health education-health behavior has been established in children (Psoter et al., 2006, Nunes et al. 2012). Weber-Gasparoni et al. (2012) had found that mother's education less than high school was a risk factor for MS infection in children (OR = 2.75 p<0.001). In the present study, more than five erupted teeth in a child's mouth was a risk factor for developing MS infection in children at this time point. This is similar to findings from Cufield et al. (1993), Tedjosasongko et al. (2002), and Mohan et al. (1998). Although, age was not significant, the effect of age is evident through number of teeth erupted. Older children had more teeth and hence more susceptible to develop infection.

The number of erupted teeth hence can be considered as a surrogate or proxy variable for child's age in this model.

None of the dietary or behavioral variables were significant at this stage in the final model. This is again reflective of the age-effect. At very young ages, most of the dietary and behavioral practices are similar for children belonging to the same population group [Table 4.16 – 4.18]. This was different from Wan et al. (2003), as all the children enrolled in their study belonged to same age group, and still exhibited significant differences in dietary and behavioral practices depending on status of MS infection. One of the reasons for this difference of findings can be due to the diversity in socio-demographic background of the subjects in Wan et al. study. As mentioned earlier, the population for this study was more or less homogenous in terms of socio-demographic characters except age, likely due to all subjects being enrolled in the WIC program.

Nine Months

The mean age of children at this time point was 21.14 (14 – 38) months, and both the groups under comparison were homogenous for the age variable ($p>0.05$). Group 3 was near the higher end of the age-range (mean age = 22.17 months) and group 1 was near the lower end of the range (mean age = 20.51 months). This is comparable to the Seow et al. (2003) study involving 107 children selected from a community child health clinic. The study analyzed short-term effect of tooth brushing with fluoridated toothpaste. The analyses were focused on oral hygiene habits and did not explore diet and diet related behavior in detail. The present study has a more comprehensive assessment of dietary, environmental, and tooth related factors in addition to behavioral practices.

At the nine months' time point chocolate milk, juice drinks, sports drinks, use of open cup, presence of more than 13 teeth, a higher proportion of mandibular molars with visible plaque, and history of any decay were risk factors at the bivariate level. Higher maternal education and no night feeding practices at this time point were protective factors for MS infection. Distribution of remaining non-significant variables was similar to age related dietary and behavioral changes observed in other studies as well.

Marshall et al. (2005) had found significant associations between sugared beverages (including flavored milk, juice drinks, and sports drinks) and caries experience at the age of two years. Weber-Gasparoni et al. (2012) had found significant associations between added sugar beverages and presence of MS in a similar age group in their cross-sectional analysis. Other studies have supported the association between sugared beverages and MS infection at different age groups [Thakur et al. (2012), Mattos-Graner et al. (2001), Mohan et al. (1997)]. Significant association between MS infection and number of teeth [Caufield et al., 1993], presence of plaque [Marsh, 1994], and history of caries [Tanner et al., 2011] have been established before.

Weber-Gaparoni et al. (2012) noted lower maternal education as a risk factor [OR = 2.78 (1.52-5.06)] in their cross-sectional study of young children (mean age 27 months), and Wan et al. (2003) had observed nighttime feeding practices as a risk factor at 21 months of age [OR = 7.3 (2.7-20.1)]. Use of open cup was not assessed in any of these studies. Wan et al. (2001) examined use of pacifiers in their 112 predentate infants, and found no association between MS colonization and use of pacifiers. The Seow et al. (2003) study was comparable for age but not in terms of other variables. At baseline, they found significant association between MS and daily tooth brushing, use of toothpaste and fluoride supplements, frequent snacking, and solid food intake. The study concluded that oral health education of the mothers can effectively reduce MS levels on all these indicators/risk factors.

Nine months – Pairwise Comparison Regression Findings

Child's age was considered in both the preliminary and final models owing to the strong evidence for the role of age in MS colonization [Weber-Gasparoni et al. (2012), Caufield et al. (1993)]. The presence of some of the unexpected variables in the model was also thought to be function of age. Both the preliminary and final models were composed of more than five variables and hence the stability of the models was not very good. The six other variables included in the preliminary model were maternal education, chocolate milk, sports drinks, no night feeding practices, use of open cup, and presence of any decay. Among these only chocolate milk was excluded for the final model.

Based on these models, the relationship of both sports drinks and caries history at nine months' time point was significant with MS infection. The final model also showed maternal education as marginally significant protective factor for MS infection. This is different from the baseline regression model when maternal education was a significant protective factor, and number of teeth was a significant risk factor. The change in key factors from baseline is indicative of effect of age on child's potential to develop infection. At the nine months' time point, the children were almost 21 months old (mean age). There were more diet and beverage options available to them. Sugar content of these dietary products is an important facilitator for MS colonization [Marshall et al. 2005, Law et al., 2007]. Maternal education was still significant as beverage options also depend on maternal education and corresponding SES of the family [Weber-Gasparoni et al., 2012].

As discussed earlier, the older children from group 3 had more teeth, more hard surfaces available for plaque deposition and MS colonization [Marsh, 1994], and also higher chances of caries experience [Mohan et al., 1997] in comparison with younger children from group 1 based on baseline analyses [similar to findings from Warren et al., (2008) with respect to caries]. Baseline analyses in this study found number of teeth as significant risk factor for MS infection, whereas nine months' analyses detected another tooth related variable – caries experience – as a risk factor for MS infection. This is additional evidence for temporal change in risk factors for MS infection.

The variables for age, no night feeding practices, and use of open cup were present in the model but were non-significant ($p>0.05$). Selective significance of the variables, in the context of nine months' analyses only, can be explained with the difference in the mean age for the two groups. Both group 3 (22.2 months old) and group 1 (20.5 months old) belonged to a comparable age group in terms of behavior and developmental milestones. Past twelve months of age children have developed better circadian rhythm and start sleeping undisturbed at night for more than 10 hours [Anders et al., (1992)]. The practice of night time feeding is not as common at this stage as compared to infants [Mindell et al., (2006)]. Similarly, developed motor coordination in children of approximately two years age [CDC, 2014], makes the parents more likely to allow use of an open cup as a container for their children. Both of these characteristics are typical for children between one and two years. As both groups 1 and 3 belong to the same age range, these variables became non-significant after adjusting for age.

However, groups 1 and 3 differ in terms of tooth eruption. At the mean age of 22 months for group 3, mandibular second molars may have started erupting (age of eruption for deciduous mandibular second molars is 23 – 31 months) (Tooth Eruption: The Primary Teeth; 2005). Whereas, for the mean 20 month old group 1 children, they likely have teeth only up to the first deciduous molars (Tooth Eruption: The Primary Teeth; 2005). The newly erupting molars are usually associated with systemic as well as local inflammation [Ramos-Jeorge et al., 2011]. This may result in reduced use and toothbrushing in the area adjacent to the erupting tooth, in this case mandibular molars. Bivariate analyses of clinical variables indicate significantly higher proportion of mandibular molars with visible plaque in group 3 children as compared to group 1 children.

Group 3 children had more teeth (15.03) than group 1 (12.78) and more chances of having a history of caries (pit and fissure caries and smooth surface caries both). By providing the habitable environment for the MS, the increased surface area available after second molar eruption makes the older children more susceptible to infection. Findings from Caufield et al. (1993) indicated a steep rise in the cumulative probability of MS infection between 20 to 36 months of age corresponding to the erupting second molars. According to caries etiology, MS infection is communicable and progressive (Ripa, 1988), which supports this finding of increased susceptibility to MS infection for children with more teeth and caries history.

Considering the group of subjects was primarily from low SES background, the quality of nutrition may be low. In this age group (1-2 years), children are susceptible to systemic diseases [Cohen S, 1999] and infections including malnutrition and diarrhea

[Feldens et al., 2010], along with caries. The economic conditions can make the parents opt for sports drinks for their content of electrolytes and calories (report from Committee on Nutrition and the Council on Sports Medicine and Fitness) as a replacement of expensive rehydration beverages and fruit juices. This might be one of the explanations for higher consumption of sports drinks in this cohort leading to the significant correlation between sports drinks and MS infection.

Although the variable for sports drinks consumption was considered in earlier studies [Marshall et al., 2003, 2005, Weber Gasparoni et al., 2012], it was not found to be significant, or was a part of bigger group (such as added sugar beverages). Influence of maternal education is evident in the model and also can be used to explain the relation between SES [Weber-Gasparoni et al., 2012], sports drink consumption and MS infection. Thus, this study contributes in understanding the effect of individual variables, like sports drinks and maternal education, on MS infection in the presence of other socio-demographic factors.

<u>Eighteen Months</u>

The mean age of the cohort was 30.68 months, and again both the groups under comparison were homogenous for this variable ($p>0.05$). The cohort is comparable with Kishi et al. (2009) where 30 months old children were enrolled and followed prospectively for 2 years to assess the effect of fluoride intervention. Kishi et al. also evaluated caries status of the children and maternal *S. mutans* and *S. sobrinus* levels. They found maternal MS levels detected by PCR technique can be used as predictor for child's caries experience and maternal transmission of MS. In addition to being a cross-sectional study, their study focused on maternal and clinical factors, such as maternal MS levels and caries in children, and did not assess any of the socio-demographic and dietary variables.

For the present study at eighteen months' time point, maternal education, any kind of milk (breast milk, cow's milk, or infant formula) consumption, regular pop intake, number of teeth, number of teeth with visible plaque, and caries related experience were significant variables at bivariate level. Different studies have found evidence supporting association between each of these variables and either MS or caries. That is, studies with similar findings about maternal education [Weber-Gaparoni et al. (2012)], number of teeth [Caufield et al. (1993)], and plaque [Kishi et al. (2009), Loesche WJ (1986), Marsh (1994)] have been discussed earlier. Palmer et al. (2010) found association between elevated levels of mean putative cariogenicity of food such as regular soda (as a cariogenic food) and higher MS levels in two to six years olds. They found evidence for protective action of milk against caries, but did not analyze the association between milk and MS levels. Marshall et al. (2005) and Warren et al. (2008) had similar findings with respect to caries. Again, none of these studies longitudinally assessed effects of all the factors in combination.

The age of the subjects did not differ significantly between groups 1 and 3 at eighteen months. None of the behavioral variables were significant at p = 0.05 for eighteen months' time point. This is likely due to the effect of age. At eighteen months' time point (mean age of the sample 23 months), a months' difference in age may imply major differences in intra-oral findings, such as presence or absence of a mandibular molar. However, behavioral practices do not vary that greatly. For example, a parent of a

29.5 month old child (group 1 mean age) is equally likely to use open cup to feed his/her child as a parent of a 31 month old (group 3 mean age) [open cup use for group 1= 52% and for group 3 = 50%]. These findings were consistent with the findings from baseline and nine months' time point and support the conclusion about the effect of age in MS infection at follow-up time points as well. Other studies have confirmed effect of age/time in different ways [Ripa (1988), Caufield et al. (1993), Karn et al. (1998), Weber-Gasparoni (2012)].

Eighteen months – Pairwise Comparison Regression Findings

The final model comprised of higher maternal education, d1surfaces, and d1d2-3f surfaces. Higher maternal education and d1d2-3f surfaces were protective factors, whereas, the presence of d1surface was a risk factor. None of the relationships given by the model were significant. As mentioned in Chapter IV, d1 surfaces indicated presence of untreated early carious lesions, and would more likely place the child at a risk of developing MS infection. Presence of advanced, but treated caries (f = filled component) is represented by d1d2-3fs variable. The relationship given by the model suggests that treatment reduces the risk of MS infection. As discussed earlier in this section, maternal education is not only a potential indicator of SES of the family, but also health related behavior of the mother. This variable has been consistently associated with MS infection of the children at all three time points.

Considering the mean age of the cohort, the children had most of their teeth erupted including the primary mandibular first molars. As discussed earlier for nine months' time point, the availability of more hard surfaces including pit and fissure surfaces of molars increases susceptibility to MS colonization [Caufield et al. (1993), Marsh (1994)]. This is increased drastically after second molar eruption seen at eighteen months' time point, which is most likely had happened with all the children from in this cohort [mean age = 30.68 months (23–47)]. Hence, at this time point all the teeth related and clinical factors take precedence over external dietary and behavioral factors.

Thus, the positive correlation between age, number of teeth, and caries experience; and communicable and progressive nature of MS infection is well portrayed by this model. Studies discussed earlier have similar findings to this. For example, Caufield et al. (1993) indicated a steep rise in the cumulative probability of MS infection between 20 to 36 months of age corresponding to the erupting molars.

Comparison across the three time points

Although age was the only variable differing in three time points among all the demographic variables, age related changes were observed in some of the variables from other domains. Many children had acquired the habit of regular toothbrushing (76 %) as compared to baseline (62%) and nine months (70%). More parents had started using fluoridated toothpaste (baseline 39.5%, nine months 68.3% and eighteen months 88.3%). Mean number of teeth increased from 5.7 at baseline and 13.6 at nine months to 18.2 at eighteen months. Simultaneously, there was increase in both the number of teeth with visible plaque (baseline 0.6, nine months 2.8, and eighteen months 6.6) and the mean d1d2-3fs (baseline 0.1, nine months 0.7, and eighteen months 3.4) of the subjects.

Increases were observed in ounces/week of beverage consumption such as water, milk, and sugared beverages. Infant formula consumption had declined, whereas breast milk was no longer consumed at the eighteen months' time point. Behavioral variables like bottle feeding and night time feeding practices decreased over the study period whereas use of open cup increased (baseline 11.63%, nine months 39.56%, eighteen months 51.0%). Use of a sippy cup increased at nine months' time points but decreased at eighteen months (baseline 64.34%, nine months 80.85%, eighteen months 72.34%). This can be attributed to stages of motor development in the children.

Regarding regression analyses of the three time points, maternal education was consistently significant for its negative association with MS infection. This is contradictory to Habibian et al. (2002) who found no association between maternal education and MS infection in their longitudinal study enrolling newborns followed up to 18 months. However, many studies with either caries as an outcome (Warren et al. 2008, and others) or MS as outcome (Wan et al. 2003, and others) had noticed similar association with either maternal education or SES.

Clinical variables related to teeth were consistently present. However, they seem to have changed over time from presence of teeth to advanced stages of caries. The number of teeth erupted ($p=0.0109$), the presence of decay ($p=0.0003$), and the number of d1 ($p = 0.1038$) and d1d2-3f ($p = 0.0645$) surfaces reached the final models for the baseline, 9-month and 18-month time points, respectively. This is another example of temporal pattern in aging and MS infection. Similar findings were observed with other cross-sectional studies or studies with caries as an outcome.

Among the few other longitudinal studies conducted in the field, Mattos-Granner et al. (2001) also found number of erupted teeth to be significant at baseline [9-19 erupted teeth: OR = 6.7 (2-22) and for 20 erupted teeth: OR = 15 (1.58-142.07)] only. They did not conduct multiple regression analyses due to statistical limitations. Habibian et al.'s (2002) study subjects exhibited no caries, and the only tooth related variable significant in their final model was age at the time when children started toothbrushing. The study by Wan et al. (2003) did not collect any data on clinical or tooth related variables and also had no multivariable regression models to assess relationship between different variables at the same time.

Sports drink intake, use of open cup, and no night feeding practices were the only other variables significant at nine months' time point. These can be functions of age as described earlier during the discussion for nine months' regression analyses. This also represents age-related changes in the dietary and behavioral practices exhibiting different effect at different stages of development.

To summarize, maternal education and number of teeth/age were the most consistently significant risk factors over the three time points considered in this study. There were some dietary (such as sugared beverages) and clinical factors (such as plaque related variables) that became significantly associated with MS infection at different time points. Sometimes they entered the regression models corresponding to that time point as well, but did not remain in the final regression models consistently for all the three time points.

Strengths

The study captured the children in the age of deciduous tooth eruption, especially molar teeth eruption, and as such was strength of the study. Incisors are the first teeth to erupt in deciduous dentition. However, due to smaller surface area, and comparatively more smooth surfaces than pits and fissures, incisors are not the ideal sites for MS to adhere and colonize [Caufield et al. (1993)]. Caufield et al. (1993) had noted a sharp rise in the cumulative probability of MS infection with eruption of molars. Tedjosasongko and Kozai (2002), had noted that the mean age for MS acquisition is 24 months (rage 8 – 52 months). Many other studies have found supportive evidence for this phenomenon [Catalanotto et al. (1975), Loesche (1986), Dasnayake et al. (1995), Wang et al. (2005)]. Thus, to identify factors associated with MS colonization, it was important to observe children from 13 to 33 months, around the time of eruption of primary molars.

This study enrolled subjects when they were as young as 6 months old with average 7 teeth erupted at baseline. At the time of completion of the study, the youngest subject was 23 months old, and the average number of teeth erupted was 18. Hence, most of the children were followed when their primary molars were erupting. Comprehensive information about dietary, behavioral, and environmental factors was obtained from the enrolled children and their parents during the study period. Because of the tighter age-range of the enrolled children, it was easier to magnify and analyze effect of age on dietary, behavioral and environmental variables.

At a very young age, the diet of an individual predominantly consists of liquids. The beverage questionnaire used for the study considers all the possible liquids children less than 2 years may consume. As the same questionnaire was used for all follow-up time points as well, pattern of change in the liquid intake could be analyzed. The same holds true for behavioral and environmental factors. The questionnaire for behavioral practices was comprehensive for children in the very young age group. As it was used for all the follow-up time points, temporal change in behavioral practices could be analyzed.

This was one of the strength of the study design, but limited scope of this thesis does not include longitudinal assessment.

Clinical examinations were conducted by single calibrated examiner (inter-examiner reliability with the gold standard examiner 96% and kappa = 0.84 for d2 or filled lesions and 96.3% agreement and kappa = 0.48 for d1 lesions). Hence, the reliability and validity of the data for environmental variables was standardized. All of these factors can be considered as study strengths.

Limitations

One limitation of the study is that measurement of salivary MS was carried out using semi-quantitative method. This may not be an accurate measure for assessment of the status of colonization. Although it is recommended for epidemiologic studies [Kohler and Bratthall (1979), Buchnan et al. (1985)] and has been used by many studies conducted in this field of research [Warren et al. (2008), Weber et al. (2012)], it has limitations. Edelstein and Tinanoff (1989) reported that semi-quantitative method of microbial analysis has only 93% sensitivity with 30% false positive results. Linossier et al. (2002) concluded that semi-quantitative method is accurate enough for the use in population studies, but did not recommend for cohort studies.

Use of tongue blade-RODAC plates and use of MSB medium also does not assure accurate reproduction of salivary MS levels. These are again approximate means commonly used in epidemiological studies [Wan et al., (2002), Weinberger and Wright, (1990), Olson et al., (2004)]. As discussed earlier, use of other testing methods such as PCR, genetic probes, immunoassays, and DNA microarrays are more accurate and commonly observed for bacterial identification in recent times [Uhl et al. (2002)]. The arbitrary categorization of subjects into groups based on salivary MS levels assessed by semi-quantitative tongue blade-RODAC plate method is one of the major limitations of the study.

Moreover, the subjects were divided into three groups based on MS levels only at the beginning of the study and at the end of the study. In spite of the availability of the microbial data for the nine months' time point, the MS levels at that time point were not considered. This was mainly due to limited sample size. Group 3 (those who were MS negative at baseline and turned MS positive at eighteen months) comprised of those who acquired MS infection at some point between baseline and nine months, and those who acquired MS infection at some point between nine months and eighteen months. Dividing subjects from group 3 (n = 36) into these two such groups would have limited most of the statistical procedures and decreased the power of the study. A larger sample size and follow-up on a shorter period (e.g. monthly or quarterly follow-up) may have been a better way to assess the outcome of MS infection.

Limited sample size may have been a major reason for some of the non-significant or unusual findings as well. Regression models constructed for each of the time points had better fit with maximum four variables. A bigger sample size could have allowed more variables in the multivariate analyses to explain the relationship between MS colonization and demographic, dietary, behavioral, and environmental factors. For example, at nine months' time point, the final model had six variables, two (use of open cup and age of the child) of which were non-significant. When tested, the two were neither correlated nor had any interaction. The presence of open cup in the final model was a function of age of the child more than a factor responsible for MS infection. Accordingly, after adjusting for age, it became insignificant. However, better statistical explanation with higher power could have been available, if the sample size was large enough.

Inclusion of chocolate milk in the beverage questionnaire was one of the strengths of the study. However, the questionnaire did not confirm details on the quantity of chocolate milk consumed. Another shortcoming of the questionnaire was a question about most frequently used container (infant bottle, open cup, sippy cup, and cup with non-spilling-straw mechanism). This question was asked for the container, but not the content of the container when it was being used. A child using infant bottle or sippy cup containing water would likely not be at as much risk as a child using either of the containers with chocolate milk in them. Similarly, an older child using open cup containing water was likely to be comparatively safer than a younger child using sippy cup containing sugared beverages.

The questionnaire did not assess the general health status of the subjects. The children enrolled in the study were from low SES and most of their parents' were educated up to high school diploma or GED. These children were thus at risk for compromised health status common in low SES children [Vallejo-Torres et al. (2014), Emerson et al. (2006), Chi et al. (2014)]. Also, the measures taken to prevent or recover from the ill-health (e.g. night-time snacking, sports drinks) might be those inclined towards inexpensive approaches. It is plausible that some of the night-time snacking habits and use of sports drinks or energy drinks are manifestations of recurrent ill-health of children, especially those from group 3.

The lack of assessment of maternal and family related factors; lack of data on other oral microorganisms, lack of history of dental office visits or antibiotic usage, and lack of information on recent systemic infections were some other limitations of the study. Information on enamel and dentin anomalies, structural defects, and conditions, such as dental fluorosis, also were not collected during the clinical examinations. Among all these, data on maternal factors were collected, but not analyzed due to limited scope of this thesis.

Overall, the analyses had some significant and some non-significant findings. A larger sample size, more follow-up covering shorter time-periods, and improved tools of data collection were attributes that could be improved if the study were to be repeated. Despite of these limitations, the study provided a longitudinal assessment of factors associated with MS infection in children.

Future Direction

The study had certain limitations as listed above. To analyze the role of different factors affecting MS infection in children, more detailed and comprehensive research is needed. The study enrolled children as early as 6 months of age, who had some teeth erupted. However, evidence has shown that MS colonization can occur in the presence of any non-desquamating surface [Caufield et al., (1993)]. Wan et al. (2001) found that many (30%) infants in their prospective cohort study were infected with *S. mutans* since predentate period. Thus, enrollment of all infants in the pre-eruptive stage would be more appropriate for future studies.

Also, the youngest child was 23 months old at the completion of study. Hence, not all the subjects had their complete set of deciduous dentition erupted while in the study. It is possible that the result might have differed if all the subjects had all of their teeth, especially deciduous second molars, erupted during the study period. Future studies could be designed for longer time periods starting from the pre-eruptive period, and continuing up to completion of eruption of the entire deciduous dentition.

The questionnaire could also be improved to overcome a limitation of this study. The beverage and behavioral questionnaire could thus include questions about quantities of certain beverages and drinks (e.g. chocolate milk), content of the most used containers, and information regarding quantity and nature (e.g. sticky vs fibrous) of solid food intake. That would portray a better dietary profile of the subjects and provide a better understanding of dietary factors contributing to MS infection.

Questions about general health of the child, health literacy/health behavior of the parents, and regularity of dental office visits could also be included in the demographic questionnaire. Presence of enamel hypoplasia, fluorosis, and other tooth structure anomalies could be assessed during the clinical examination in future studies. If studied in a way like this study, the additional information would help in better understanding the effect of dietary and demographic characters on certain clinical and microscopic conditions.

CHAPTER 6

CONCLUSION

The study provided cross-sectional analyses of longitudinal data at three time points. MS infection is a multifactorial condition and the importance of each factor varies with time. At a very young age with a minimal number of teeth erupted, the role of external and environmental factors is more important than that of personal or dietary factors. At baseline, maternal education and number of teeth were the most significant factors, the prior being protective and the latter being a risk factor. In the next follow-up at nine months' time point, maternal education still played a role, but dietary factors started to show significance. The role of night feeding practices, sports drink consumption, and history of caries experience at this time point also influenced MS infection. At eighteen months' time point clinical factors played the key role with history of caries being the other significant variable in addition to maternal education.

Although the age of the child was not significant in any of these analyses, the role of time was found to be crucial. Time, as measured by the age of the child, influenced dietary practices. Clinical variables were time-dependent as well, as older children had more teeth and were at a higher risk of MS infection. Maternal education was a significant variable throughout all the three time points, and can be considered as reflective of SES and health related behavior of the family. Although, the later were not assessed and/or measured in this study, the association between these socio-demographic and behavioral factors has been established in the literature.

Although the study had its own limitations, it was successful in assessing the impact of time on MS infection in children. The study also found that maternal education is an important factor, which can be used to characterize population for targeted approach in caries prevention. To conclude, MS infection in children is a function of age/time and attempts to control it can be directed towards close monitoring of high-risk population factors.

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APPENDIX

Baseline Beverage Questionnaire

Please complete the following table by circling **YES** or **NO** if your child consumed the beverage **during the past week**. For beverages that your child drank, write the **number of servings** that your child drank and the **amount drank per serving** during the past week.

Beverage	**YES NO**	**Number of Servings per week**	**Amount per Serving**
Example: Water	YES NO	|___|___|	|___|___| oz
1. Infant Formula	YES NO	|___|___|	|___|___| oz
2. Human Milk	YES NO		
3. Cows' Milk	YES NO	|___|___|	|___|___| oz
4. 100% Juice	YES NO	|___|___|	|___|___| oz
5. Juice Drinks	YES NO	|___|___|	|___|___| oz
6. Water	YES NO	|___|___|	|___|___| oz
7. Flavored Water	YES NO	|___|___|	|___|___| oz
8. Sugared beverages made from powder (e.g., Kool-Aide®)	YES NO	|___|___|	|___|___| oz
9. Sugar-free beverages made from powder (e.g., Crystal Light®)	YES NO	|___|___|	|___|___| oz
10. Regular Pop (e.g., Pepsi,® Coke®)	YES NO	|___|___|	|___|___| oz
11. Diet Pop	YES NO	|___|___|	|___|___| oz
12. Sports Drinks (e.g., Gatorade, Powerade)	YES NO	|___|___|	|___|___| oz
13. Other sugared beverages (e.g., lemonade, sweetened tea)	YES NO	|___|___|	|___|___| oz
14. Other sugar-free beverages (e.g., ice tea, coffee)	YES NO	|___|___|	|___|___| oz

Please circle the most appropriate response to the following questions.

1. If your child drinks cows' milk, what type of cows' milk does your child usually drink?
    i. Whole milk
    ii. 2% milk
    iii. 1% or skim milk
    iv. Chocolate
    v. Other flavored milk (e.g., strawberry, vanilla)
    vi. Doesn't drink milk

2. What type of container does your child usually use for beverages?
    i. Infant bottle
    ii. Open cup
    iii. Closed cup (sippy cup)
    iv. Cup with nonspilling, straw mechanism
    v. Water bottle
    vi. Product container (e.g., juice box, pop can or bottle)

3. What beverages does your child consume at meals?
    i. Infant formula, human milk or cows' milk
    ii. Juice or juice drinks
    iii. Water
    iv. Regular pop or other sugared beverages
    v. Diet pop or other sugar-free beverages
    vi. Other:

4. What beverages does your child consume between meals?
    i. Infant formula, human milk or cows' milk
    ii. Juice or juice drinks
    iii. Water
    iv. Regular pop or other sugared beverages
    v. Diet pop or other sugar-free beverages
    vi. Other:

5. Which statement best describes your child's nighttime feedings?
    i. My child falls asleep with a bottle.
    ii. My child has bottle in the middle of the night.
    iii. My child has a snack at bedtime.
    iv. My child has a snack in the middle of the night.
    v. None of the above.

Thank you for your participation!

Baseline Demographic Questionnaire

INSTRUCTIONS: For each question, please write your response in the space provided or check the box next to the most appropriate response. All information will be kept confidential. Thank you for your participation in this study! Today's date ___________________________________

1. Mother's name ____
(last/first/middle)

2. Mother's date of birth ____
(month/day/year)

3. Permanent Address ____

4. What is the mother's occupation ____

5. Your child's date of birth ____
(month/day/year)

6. What category best describes the total annual household income? Include salaries, alimony, child support, government assistance or any other income you receive such as food stamps.
   - ❑ $0 – 5,000 ❑ $5,001 – 10,000 ❑ $10,001 – 15,000
   - ❑ $15,001 – 20,000 ❑ $20,001 – 25,000 ❑ $25,001 – 30,000
   - ❑ $35,000 - 40,000 ❑ More than $40,000

   If it would be easier for you, you can give either a weekly or monthly amount:
   $ ___________ per week OR $ ___________ per month

7. What is your (the child's mother's) marital status?
   - ❑ Married ❑ Separated ❑ Divorced
   - ❑ Single, never married ❑ Live with significant other ❑ Widowed

8. What is your (the child's mother's) highest level of education completed?
   - ❑ Less than High School ❑ High School Diploma or GED ❑ Two Year College Degree
   - ❑ Four Year College Degree ❑ Graduate Degree

9. Would you consider your child (please, check only **one**):
   - ❑ Caucasian or white ❑ American Indian/Alaska Native ❑ Hispanic
   - ❑ Asian ❑ Black or African American ❑ More than one race
   - ❑ Pacific Islander ❑ Other, please specify _________________

10. How long have you lived in the Muscatine area? ______ years ______months

11. Are you likely to remain in the Muscatine area for the next two years? ___ yes ___ no

12. Breastfeeding:
- ❑ My child is currently being breastfed
- ❑ My child was completely weaned from breastfeeding at _________ (age in months)
- ❑ My child has never been breastfed

13. Bottle feeding:
- ❑ My child is currently being bottle fed
- ❑ My child was completely weaned from bottle feeding at _______________ (age in months)
- ❑ My child has never been bottle fed

If you answered in question # 13 that your child has <u>never</u> been bottle-fed, please go straight to question # 16 and do <u>not</u> answer questions #s 14 & 15.

14. What do/did you usually put inside your child's bottle? (please, check <u>all</u> that apply):

❑ Formula ❑ Cow's milk ❑ Breast milk
❑ Water ❑ Juice ❑ Other, please specify ___________________________

15. Nighttime bottlefeeding (please, check <u>all</u> that apply):
- ❑ I am currently bottlefeeding my child to sleep
- ❑ I am currently bottlefeeding my child in the middle of the night
- ❑ I have never bottlefed my child to sleep
- ❑ I have never bottlefed my child in the middle of the night
- ❑ My child stopped being bottlefed to sleep at ________________ (age in months)
- ❑ My child stopped being bottlefed in the middle of the night at __________ (age in months)

16. Does your child use a sippy-cup?

❑ Yes ❑ No

If you answered "no" for question # 16, please go straight to the next section and do <u>not</u> answer question # 17.

17. What do you usually put inside the sippy-cup?

❑ Cow's milk ❑ Water ❑ Juice
❑ Other, please specify __________________________________

Fluoride Exposure Questionnaire

1. Are your child's teeth brushed?
   ❑ Yes, daily ❑ Yes, once in a while ❑ No

2. Are you using fluoridated toothpaste to brush your child's teeth?
   ❑ I am not using toothpaste

   ❑ Yes, once in a while Name of Toothpaste (brand):
   ❑ Yes, daily

3. What is the main water source from which your child is drinking?
   ❑ Well water or private water source
   ❑ City public water City:

   ❑ Bottled water Brand name(s):