Turun yliopisto
University of Turku

# RELATIONSHIP BETWEEN ORAL MICROBIOTA, CARIES AND DAILY HABITS, WITH SPECIAL REFERENCE TO XYLITOL

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TURUN YLIOPISTON JULKAISUJA – ANNALES UNIVERSITATIS TURKUENSIS
Sarja - ser. D osa - tom. 1127 | Medica - Odontologica | Turku 2014

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ISBN 978-951-29-5822-1 (PRINT)
ISBN 978-951-29-5823-8 (PDF)
ISSN 0355-9483
Painosalama Oy - Turku, Finland 2014

# ABSTRACT

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Annales Universitatis Turkuensis, Sarja - Ser. D, Medica-Odontologica. Painosalama Oy, Turku, Finland 2014.

Caries is a plaque-associated multifactorial chronic disease. Oral hygiene habits, sugar, and oral micobiota interactions are important for caries to occur. Xylitol has been shown to reduce caries mainly due to its effects on mutans streptococci (MS). The purpose of this study was to evaluate the relationship of daily oral health habits and bacterial level on the caries occurrence and to study the effect of xylitol on the composition of oral microflora.

A total of 192, 10-12 years old, male school children were screened for salivary MS. Healthy subjects with high MS counts participated in two parallel double-blinded, randomised, controlled trials. In the first 5-week trial, subjects were assigned into xylitol (n=35) and sorbitol gum (n=38) groups. At baseline, children were examined using International Caries Detection and Assessment System (ICDAS) criteria and interviewed for oral health habits. In the second 4-week trial, subjects were assigned into xylitol (n=25) and saccharine mouthrinse (n=25) groups. Saliva samples were collected at the end of both interventions. The samples were analysed for changes in MS counts and changes in the composition of the oral microbiota was assessed by the Human Oral Microbe Identification Microarray (HOMIM). Relationships between daily habits, bacterial levels and caries were evaluated.

Daily use of sweets and soft drinks were the habits significantly associated with caries severity measured by ICDAS Caries Index (CI), while toothbrushing was the only habit associated with the low caries severity. *Abiotrophia defectiva* and *Actinomyces meyeri/ Actinomyces odontolyticus* were significantly higher in caries-affected children while *Shuttleworthia satelles* was significantly higher in caries-free children. Xylitol showed significant reduction in salivary levels of MS in both trials. No significant effects on other members of the microbiota were found when evaluated by HOMIM.

In conclusion, other members of oral microbiota than MS may be associated with caries occurrence or absence. The use of xylitol had significant effect on MS with no effects on the other members of the salivary microbiota.

**Key words:** Caries, Saliva, Mutans Streptococci, HOMIM, ICDAS CI, Oral Microbiota

# TIIVISTELMÄ

**Mohamed ElSalhy**

## SUUN MIKROBISTON, KARIEKSEN, PÄIVITTÄISTEN SUUN TERVEYSTOTTUMUSTEN JA KSYLITOLINKÄYTÖN KESKINÄISET SUHTEET.

Hammaslääketieteen laitos/Sosiaalihammaslääketiede, Lääketieteellinen tiedekunta, Turun yliopisto ja Hammaslääketieteellinen tiedekunta, Kuwaitin yliopisto, Kuwait.

Annales Universitatis Turkuensis, Sarja - Ser. D, Medica-Odontologica. Painosalama Oy, Turku, 2014.

Hampaiden reikiintyminen on plakkiperäinen, monen tekijän aiheuttama krooninen sairaus. Suun hygieniatottumusten, sokerin ja suun mikrobien yhteisvaikutus ovat avainasemassa karieksen kehittymiselle. Ksylitolin on osoitettu vähentävän kariesta vaikuttamalla suussa oleviin mutans streptokokki (MS) -bakteereihin. Tämän tutkimuksen tarkoituksena oli selvittää suun terveystottumusten ja suussa kasvavien eri bakteerien yhteyttä karieksen esiintymiseen ja ksylitolin vaikutusta suun mikrobeihin.

Yhteensä 192, 10-12-vuotiasta perustervettä poikaa seulottiin mittaamalla syljen MS-määrä. Poikia, joilla oli korkea MS-määrä (n = 123), pyydettiin osallistumaan satunnaistettuun kaksois-sokkoutettuun tutkimukseen. Ensimmäisessä tutkimuksessa, 35 poikaa käytti ksylitoli- ja 38 poikaa sorbitolipurukumia viiden viikon ajan. Karies rekisteröitiin alussa International Caries Detection and Assessment System (ICDAS) -menetelmällä ja suun terveystottumukset kartoitettiin haastattelemalla. Toisessa tutkimuksessa, 25 poikaa käytti ksylitolia ja 25 sakkariinia sisältävää suuhuuhdetta neljä viikkoa. Molempien tutkimusten päätyttyä otettiin sylkinäytteet. Näytteistä tutkittiin muutokset MS bakteerien määrässä. Muutokset suun mikrobiston muiden bakteerien esiintyvyydessä määritettiin Human Oral Microbe Identification Microarray (HOMIM) –menetelmällä. Lisäksi tutkittiin päivittäisten suun terveystottumusten, bakteerien määrän ja karieksen välisiä yhteyksiä.

Virvoitusjuomien ja makeisten päivittäinen käyttö olivat tilastollisesti merkitsevästi yhteydessä runsaaseen hampaiden reikiintymiseen ja karieksen vakavuusateeseen (mitattuna ICDAS karies indeksilla, CI). Hampaiden harjaus puolestaan oli yhteydessä matalaan karieskokemukseen. Bakteereista *Abiotrophia defectiva* ja *Actinomyces meyeri/A. odontolyticus löytyiv*ät syljestä merkittävästi useammin pojilla, joilla oli reikiä hampaissa, ja *Shuttleworthia satelles* niillä lapsilla, joilla ei ollut kariesta. Ksylitolin käyttö alensi merkittävästi syljen MS-määrää molemmissa interventioissa. Kun muiden mikrobien esiintyvyys suusssa määritettiin HOMIM-menetelmällä, ei ksylitolin käyttö muuttanut niiden määrää.

Tutkimuksen perusteella muutkin suun mikrobiston bakteerit kuin MS ovat yhteydessä karieksen esiintyvyyteen. Ksylitolin vaikutus mutans streptokokki –bakteereihin vahvistettiin, mutta merkittävää yhteyttä suun muuhun mikrobistoon ei löytynyt.

**Avainsanat:** karies, sylki, mutans streptokokit, HOMIM, ICDAS CI, suun mikrobisto

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# ABBREVIATIONS

| | |
|---|---|
| CFU | Colony Forming Unit |
| CI | Confidence Interval |
| $D_{1-3}S$ | Decayed Surfaces with ICDAS codes 1-3 (enamel caries) |
| $D_{4-6}S$ | Decayed Surfaces with ICDAS codes 4-6 (dentine caries) |
| DT/dt | Decayed permanent Teeth/decayed primary teeth |
| DMFS | Decayed, Missing and/or Filled Surfaces in permanent teeth |
| dmfs | Decayed, missing and/or filled surfaces in primary teeth |
| DMFT | Decayed, Missing and/or Filled permanent Teeth |
| dmft | Decayed, missing and/or filled primary teeth |
| DNA | Deoxyribonucleic Acid |
| HBSC | Health Behaviour in School-aged Children |
| HOMIM | Human Oral Microbe Identification Microarray |
| ICDAS | International Caries Detection and Assessment System |
| MS | Mutans Streptococci |
| MSB | Mitis Salivarius Bacitracin |
| PCR | Polymerase Chain Reaction |
| SOHP | School Oral Health Program |
| SD | Standard Deviation |
| TE buffer | Tris-EDTA buffer |
| TSB | Tryptic Soy Broth |
| WHO | World Health Organization |

# LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the original publications listed below which are referred to in the text by the Roman numerals I–IV.

**I.** ElSalhy M, Honkala S, Söderling E, Varghese A, Honkala E (2013). Relationship between daily habits, *Streptococcus mutans*, and caries among schoolboys. *J Dent* 41:1000-1006.

**II.** ElSalhy M, Söderling E, Honkala E, Fontana M, Flannagan S, Kokaras A, Paster BJ, Varghese A, Honkala S. Salivary Microbiota and Caries Occurrence in Mutans Streptococci-positive School Children. (Submitted)

**III.** Söderling E, ElSalhy M, Honkala E, Fontana M, Flannagan S, Eckert G, Kokaras A, Paster BJ, Tolvanen M, Honkala S (2014). Effects of Short-term Xylitol Gum Chewing on the Oral Microbiome. *Clin Oral Invest* 10.1007/s00784-014-1229-y

**IV.** ElSalhy M, Sayed Zahid I, Honkala E (2012). Effects of Xylitol Mouthrinse on *Streptococcus mutans*. *J Dent* 40*:*1151-1154.

In addition, some unpublished data is presented. The original publications are reproduced with the permission of the copyright holders.

# 1. INTRODUCTION

Dental caries is considered the most prevalent chronic disease in children. It is defined as dissolution of tooth structure by acids produced by bacteria (Selwitz et al., 2007; Fejerskov and Kidd, 2008). Dental caries is the outcome of an overtime, complex interaction between acid-producing bacteria, fermentable carbohydrates, and host-related factors including tooth and saliva. It starts with a shift in microbial composition within a complex biofilm. This microbial shift moves the balanced demineralisation-remineralisation process toward demineralisation and loss of tooth substance. Factors that affect caries include consumption of dietary sugars, salivary flow, exposure to fluoride and preventive behaviours (Selwitz et al., 2007). Complexity of the disease makes it challenging to prevent. The complete knowledge relating to ways to change the etiological factors of the disease is needed. For prevalence and severity rates of caries to be reduced, a multidimensional approach to disease treatment and prevention must be applied.

The role of bacteria in caries formation is essential. Species that have been associated with caries include mutans streptococci (MS), *Veillonella*, *Actinomyces*, *Lactobacillus*, and *Bifidobacterium*. The common characteristic of these bacteria is their ability to produce acids and survive acidic conditions (Aas et al., 2008). The acid production of these bacteria is enhanced by sugar exposure which enhances demineralisation. Habits like brushing and flossing disrupt plaque formation and can deliver fluoride from the toothpaste to the tooth which reduces the effects of demineralisation and encourages remineralisation. Diet, hygiene, and bacteria are all important factors to study in relation to caries formation (Takahashi and Nyvad, 2011).

Xylitol has been shown to prevent caries as it is non-acidogenic in dental plaque, and habitual xylitol consumption reduces the amount of plaque and MS counts (Söderling, 2009; Milgrom et al., 2012). Its effects on MS are well-known, however little is known about its effects on other members of oral microflora. In addition, it is not known if the effect of xylitol on MS is specific or if it affects the total composition of oral microflora.

# 2. REVIEW OF LITERATURE

## 2.1 Microbiology of Caries

In the 1880s, W.D. Miller proposed that oral bacteria are the causative agents in dental caries development. Loesche (1975) introduced the specific plaque hypothesis and described specific microorganisms which contribute to caries formation. Later, nonspecific plaque hypothesis was proposed and suggested that caries is the outcome of the overall activity of the total plaque microbiota (Theilade, 1986). As these two hypotheses could not explain the presence of caries associated bacteria on sound teeth, the ecological plaque hypothesis was proposed. The ecological plaque hypothesis stated that caries is the result of a shift in the balance of the plaque microflora as a result of a change in local environmental conditions (Marsh, 1994; 2004). Takahashi and Nyvad (2008, 2011) expanded the caries ecological hypothesis and described the dental plaque as a microbial ecosystem in which bacteria other than MS are essential for maintaining dynamic stability of the biofilm on the tooth surface, whereas MS dominate during caries process.

In the oral cavity, over 700 different oral microbes have been identified (Paster et al., 2001; Socransky and Haffajee, 2005). Most of these microbes are acquired by transmission via saliva from the mother, father, or siblings during the first 18-24 months of life (Berkowitz et al., 1981; Köhler and Andreen, 1994; Könönen, 2000; Berkowitz, 2003; Köhler et al., 2003). Mutans streptococci, *Streptococcus mutans* and *Streptococcus sobrinus*, are the most studied bacteria associated with caries. MS may colonise the oral cavity at any age during childhood if the favourable environment existed and their levels may increase with age (Köhler et al. 1983; Söderling et al. 2000; Köhler and Andreen, 2012).

As caries is a biofilm (plaque) associated disease, its formation starts with pellicle formation. Dental pellicle is an acellular proteinaceous film composed of salivary glycoproteins, phosphoproteins, and lipids (Ericson, 1968; Kopec and Bowen, 1995). *Streptococcus sanguinis*, *Streptococcus oralis* and *Streptococcus mitis* are the main initial colonisers on tooth surfaces together with *Actinomyces* spp., *Haemophilus* spp., and *Neisseria* spp. (Nyvad and Kilian, 1990). Later, shift happens in the microbial plaque composition to become *Actinomyces* dominated (Syed and Loesche, 1978*)*. As the plaque matures, its composition changes depending on the site as well as the surrounding environment. *Streptococcus*, *Actinomyces* and *Veilonella* species are the major constitute of bacterial biofilm on fissures while streptococci, and *Actinomyces* and anaerobic gram

positive rods are the predominant species in the gingival cervical area of sound teeth (Fejerskov and Kidd, 2008). Of the streptococci, *S. mutans* colonises mainly fissures and grooves while *S. sobrinus* colonise mostly smooth surfaces (de Soet et al., 1991).

Cariogenic bacteria must be both acidogenic to produce acids and aciduric to survive within an acidic environment. Examples of these bacteria include MS, *Veillonella*, *Propionibacterium*, *Actinomyces*, *Atopobium*, *Lactobacillus*, and *Bifidobacterium* (Aas et al., 2008).

MS are the most extensively studied caries-associated bacteria (Loesche, 1975; Parisotto et al., 2010) and are considered an important predictors for caries (Tanzer et al., 2001; Tinanoff and Reisine, 2009; Colak et al., 2013). MS are highly acidogenic and aciduric and have the ability to promote bacterial adhesion to the tooth surface by production of water-insoluble glucan (Hamada and Slade, 1980). Early acquisition of MS is associated with higher caries experience (Alaluusua and Renkonen, 1983; Köhler et al., 1983). The prevention of early MS colonisation reduces caries occurrence many years later (Köhler and Andreen, 2010; Laitala et al., 2012). They are also considered valid markers for cariogenic flora (Fejerskov, 2004). On the other hand, many other bacteria participate in caries formation with MS. MS can be present on caries-free tooth surfaces and caries may develop occasionally in the absence of MS (Takahashi and Nyvad, 2008). Therefore, MS are very important contributors but not direct causative agents of caries (Beighton, 2005). In early childhood, however, MS colonisation is a reliable indicator of high caries risk (Thenisch et al., 2006; Meurman and Pienihäkkinen, 2010).

## 2.2 Caries Detection and Assessment

Detection and assessment of dental caries lesions has been always a challenging task in dentistry. There is a need for reliable and reproducible methods for caries evaluation which give reliable information when studying the factors that contribute to caries formation and/or its prevention. It is also important in oral health monitoring, assessing dental needs, prevention and intervention program design, and for policy making (Burt, 1997; Bonecker et al., 2002; Pitts, 2004).

In epidemiological surveys as well as clinical trials, caries detection generally was analysed as the total conversion from sound tooth surface to cavitated one. According to the World Health Organization (1997), the main reason behind caries detection in surveys performed at cavitation level is that examiners frequently cannot reliably detect the non-cavitated caries lesions. However, these non-cavitated lesions can give a clearer picture on caries initiation and progression and how different factors contribute in caries

formation. They are also more important as these can be arrested through preventive management. This lowers the need for more complicated interventions which make the approach more cost-effective (Pitts and Fyffe, 1988; Ismail et al., 1992; Pitts, 2004; Assaf et al., 2006). The purpose of introducing a measure which includes non-cavitated caries lesions is to improve sensitivity of caries epidemiology and clinical trials. This is important in populations with low prevalence of dental caries, in which lesions have a slow progression rate and are found mostly in the initial stages (Assaf et al., 2006). In addition, the presence of non-cavitated lesions can be a more sensitive measure to evaluate caries progression. The transition from sound tooth surface to non-cavitated lesion can be an alarm of increased caries risk compared to those that stay sound over time.

### 2.2.1 WHO Caries Assessment and DMF Index

Worldwide, dental caries has been recorded for years using variations of the decayed, missing and filled (DMF) index developed in the 1930s by Klein, Palmer and Knutson (Klein et al., 1938). The index is still in use 70 years after its first description, indicating how successful it has been and how difficult it is to develop and gain acceptance for any alternative. The DMF score, whether calculated by teeth (DMFT) or surfaces (DMFS) affected, can be collected for both permanent (DMF) and primary (dmf) teeth. The index has a particular meaning to dental epidemiologists, researchers, and oral health care development.

Originally, D in the DMF was for decayed teeth, M for teeth missing/extracted due to decay and F for previously filled teeth due to decay. The index can be used as a measure of caries in whole teeth (designated as DMFT) ranging from 0 to 32. It also can be used as a measure of caries in surfaces (DMFS). In the DMF system, the score D is given only when there is a cavitation (Burt, 1997).

The DMF method is understood internationally and facilitates the ready comparison of data sets. However, it has its limitations (Fejerskov and Kidd, 2008; Braga et al., 2009). The main limitation is that only teeth or surfaces with cavitated caries lesions extending into the dentine are included and enamel caries lesions are excluded. This makes diagnosis of caries lesions unreliable. Also, secondary caries to old restorations are not included. DMF lacks lesion activity and its value is not caries specific. Missing, untreated, or restored teeth are weighted equally. As preventive resin restorations (PRR) and cosmetic restorations are counted as restorations in the DMF index, it can overestimate caries experience. DMF index cannot be used in estimating treatment needs, and DMF index does not include fissure sealants.

### 2.2.2 International Caries Detection and Assessment System (ICDAS)

The International Caries Detection and Assessment System (ICDAS) was developed as a standardised system based on the best available evidence for detecting early and later stages of caries lesions. Its aim was the acquisition of better quality information which can be used in clinical research and practice as well as in epidemiological studies (Pitts, 2004). The system was designed to be practical and easy to use in epidemiological surveys. It was also intended to detect cavitated and non-cavitated caries lesions at different stages with acceptable reliability (Pitts, 2004; Ismail et al., 2007). The system detects and categorises early enamel caries lesions and the "obvious" dentine caries lesions according to the stage of their progression (Pitts, 2009a; b). The validity and reproducibility of ICDAS have been examined, and it seems evident that the WHO criteria of caries underestimates the presence of caries (Agustsdottir et al., 2010). In addition, caries data collected using ICDAS codes can be used to compare data generated by WHO caries criteria, but include non-cavitated lesions (Braga et al., 2009).

Based on visual inspection, ICDAS records caries on a six ordinal scales (Pitts, 2004). In addition to caries, it also records the type of restoration combined with caries. Every surface is coded with two digit codes. The first is the preventive or restorative treatment code while the second code is the caries code (Table 1). By measuring non-cavitated and cavitated caries lesions, as well as, sealants and recurrent caries lesions, ICDAS overcomes the deficiency in the WHO examination criteria. Currently, ICDAS is the internationally recommended system for dental health surveys (EGOHID, 2008). Although, ICDAS can be considered an integral examination system, it lacks an index that gives an overall caries status of the subjects.

**Table 1.** Coding system for ICDAS (Ismail et al., 2007).

| Restoration and Sealant Codes | Caries Codes |
|---|---|
| 0 = Not sealed or restored | 0 = Sound tooth surface |
| 1 = Sealant (partial) | 1 = First visual change in enamel |
| 2 = Sealant (full) | 2 = Distinct visual change in enamel |
| 3 = Tooth coloured restoration | 3 = Enamel break down. No dentin visible |
| 4 = Amalgam restoration | 4 = Dentinal shadow (not cavitated into dentin) |
| 5 = Stainless steel crown | 5 = Distinct cavity with visible dentin |
| 6 = Porcelain, gold, PMF crown or veneer | 6 = Extensive distinct cavity with visible dentin |
| 7 = Lost or broken restoration | **Missing Teeth** |
| 8 = Temporary restoration | 97 = Extracted due to caries |
| | 98 = Missing for other reason |
| | 99 = Un-erupted |
| | P = Implant |

## 2.3 Diet, Oral Hygiene Habits and Caries

### 2.3.1 Diet and Caries

The relationship between dental caries and diet was documented 60 years ago when Gustafsson et al. (1954) demonstrated an increased caries incidence in subjects with a high sugar diet during and between meals in the famous "Vipeholm Dental Caries Study". Dental caries has been always defined as diet-associated bacterial infectious disease (van Houte, 1994). Diets rich in sugars affect the composition of the oral microflora toward caries-promoting type (Fejerskov and Kidd, 2008). The frequent exposure to sugar has been established as a caries causing factor for decades (Fejerskov and Kidd, 2008).

Frequent exposure to sugar plays an important role in the microbiota of the dental plaque. It causes a change in the plaque environment to be acidogenic. This may cause a shift in the balanced demineralisation/remineralisation processes toward total mineral loss and caries initiation. Enamel demineralisation begins when pH level falls below the critical value of 5.5 (Stephan and Miller, 1943; Touger-Decker and van Loveren, 2003). This damages the tooth surface creating white spot lesions on the tooth surface which eventually progress to frank dental cavitation (Touger-Decker and van Loveren, 2003; Fontana and Zero, 2006).

Studies that evaluated caries prevalence rates among populations with consumption of low quantities of sugars compared to populations with high levels of sugar consumption, have found that high intake of sugars, especially sucrose, is a primary factor in development and progression of caries (Tinanoff and Palmer, 2000; Moynihan and Kelly, 2014). Sucrose is considered the most cariogenic sugar because of its ability to form extracellular glucans, which enables firm bacterial adhesion to teeth and limits diffusion of buffers into the plaque (Tinanoff and Palmer, 2000). These water-insoluble glucans enhance accumulation of MS on the surface of teeth resulting in enhanced acid production and further decreasing pH level, and thus promoting demineralisation (Zero et al., 1986; Tinanoff and Palmer, 2000; Zero, 2004). Frequent and prolonged consumption of sugars in the diet is a diet-related behaviour that have been highly correlated with caries (Berkowitz, 2003; Fisher-Owens et al., 2007; Tinanoff and Reisine, 2009; Kawashita et al., 2011).

On the other hand, the relationship between sugar consumption and caries experience seems not to be consistent when there is high fluoride exposure (Burt and Pai, 2001). It was concluded that the consumption of sweets and other sugary products does not seem to be a strong factor for the occurrence of caries (Sundin, 1990). However, combination of poor oral hygiene and sweets consumption has been shown to be particularly harmful especially without fluoride protection (Zero, 2004). Studies have

also shown that the high consumption of caries-risk products and drinks during the early years of life is associated with caries at kindergarten age (Wendt and Birkhed, 1995; Grindefjord et al., 1996).

Type of carbohydrate contained in the food, the stickiness of the food, and the presence of remineralisation factors (e.g. $Ca^{2+}$) are all factors that determine food cariogenicity (Lingström et al., 2003; Mobley, 2003; Sanders, 2004; Fontana and Zero, 2006). The ability of food or beverage to cause a decline in salivary pH reflects the acidogenic potential. Thus, foods and beverages that are considered highly acidogenic are also considered to be highly cariogenic (Lingström et al., 1994; Palmer, 2001). Sweetened drinks have been shown to be the main source of added sugar in children's daily diet and are potential risk factor for dental caries in children (Guthrie and Morton, 2000; Marshall, 2003; Marshall et al., 2003; 2005).

### 2.3.2 Oral Hygiene Habits and Caries

In addition to the influence of diet-related factors on oral pH equilibrium, a variety of oral self-care habits are important as well. Twice-a-day toothbrushing, flossing, fluoride exposure and chewing gum are all daily habits that may affect caries occurrence. Proper oral self-care helps to minimise accumulation of dental plaque and aid in the promotion of optimal oral pH balance.

Toothbrushing after intake of foods and beverages encourages rapid clearance of sugars and carbohydrates from the oral cavity, thereby minimising exposure to the demineralisation processes. Daily toothbrushing with fluoride toothpaste is believed to be the primary reason for the caries decline over the last few decays (Nyvad, 2004). The frequency of toothbrushing was significantly associated with caries occurrence (Wendt et al., 1994; Julihn et al., 2006). Establishing oral hygiene habits at an early age and maintained during pre-school age, appears to be crucial for good oral health (Grytten et al., 1988).

It is established that topical application of fluoride using different vehicles prevents caries. Fluoride toothpaste is the most widely used mode of fluoride delivery globally (Fejerskov and Kidd, 2008) and its efficiency is supported by strong scientific evidence (Marinho et al., 2003; Twetman et al., 2003; Mannaa et al., 2014).

The evidence of effectiveness of flossing on caries prevention is not very strong (Hujoel et al., 2006; Sambunjak et al., 2011). Under supervision flossing by adolescents on school days didn't show benefit on caries prevention (Granath et al., 1979). However, professional flossing performed in the 5 years-old children reduced caries risk by 40% (Wright et al., 1979). The benefit of professional flossing was assumed to be effective among children with poor oral hygiene with minimal fluoride exposure (Wright et al., 1979; Hujoel et al., 2006).

## 2.4 Xylitol, Caries and Oral Microflora

### 2.4.1 Xylitol

Xylitol is a naturally occurring five carbon polyalcohol or polyol (pentitol). Most fruits and plants contain xylitol. It is also present in micro-organisms and in animal tissues. Plums, strawberries, raspberries, cauliflower, and endives are the richest nature sources of xylitol (Washüttl et al., 1973). In human metabolism, 5-15g of xylitol is formed (Hollmann, 1964). The absorption rate of xylitol in the gut is quite slow, and it is metabolised in the liver. Un-adapted adults can consume 30-60g oral xylitol per day safely while after adaptation, up to 400g per day can be tolerated (Mäkinen and Scheinin, 1976a).

### 2.4.2 Xylitol, Caries and MS

Xylitol was introduced in dentistry at the beginning of 1970s through the Turku Sugar Studies (Scheinin and Mäkinen, 1976). Total of 85% reduction in dental caries was observed in the xylitol group when used as the main dietary sugar with consumption of xylitol of 50-67g/day (Scheinin et al., 1974). While chewing 6.7g xylitol gum was associated with 82% reduction in caries compared to the sucrose gum (Scheinin et al., 1975). After the Turku Sugar Studies, many xylitol trials have been conducted in different places in the world (Mäkinen, 2011). The clinical studies have shown that the intake of xylitol has to be at least 5-7g/day in divided doses, preferably in 3-5 separate episodes, to have a caries-preventive effect (Campus et al., 2013, Alanen et. al., 2000; Mäkinen, 2011). Studies with lower doses showed less effectiveness (Isokangas et al., 1988; Mäkinen, 2000; Machiulskiene et al., 2001; Oscarson et al., 2006).

MS have been suggested to be the target organism of xylitol judged by both *in vitro* and *in vivo* studies (Rogers et al., 1991; Bradshaw and Marsh, 1994; Söderling, 2009). Reduction in plaque/saliva MS levels was observed after habitual use of xylitol. The effects were seen from two-week studies as well as in studies lasting for months (Loesche et al., 1984; Söderling et al., 1997; Campus et al., 2009). Six-month habitual xylitol consumption demonstrated decrease of MS counts in dental plaque (Mäkinen et al., 2005; Milgrom et al., 2006; Haresaku et al., 2007), and in both unstimulated (Milgrom et al., 2006) and stimulated saliva (Haresaku et al., 2007).

Xylitol consumption by mothers decreased the mother-child transmission of MS (Söderling et al., 2000; Thorild et al., 2003; Nakai et al., 2010). Decreasing the early MS transmission with xylitol has reduced caries occurrence of the children (Thorild et al., 2006; Olak et al., 2012; Laitala et al., 2013).

Consumption of xylitol chewing gum has reduced both salivary and plaque MS levels but not for other streptococci or lactobacilli (Loesche et al., 1984; Söderling et al., 2011). In vitro inhibition of bacteria growth by xylitol has been observed in *Escherichia coli*, *Lactobacillus casei*, *Streptococcus pneumoniae*, and several *Actinomyces* species (Vadeboncoeur et al., 1983; Birkhed et al., 1985; Tapiainen et al., 2001). Apart from these studies very little is known about the effects of xylitol on the oral microbiota.

### 2.4.3 Mechanisms of Action of Xylitol

The caries-preventive effects of xylitol are attributed to its non-fermentability, and ability to decrease plaque and MS (Söderling, 2009; Mäkinen, 2011). In addition to these mechanisms, several other actions have been suggested (Mäkinen, 2011). Xylitol is believed to contribute in remineralisation process by forming complexes with $Ca^{2+}$ (Mäkinen and Söderling, 1984; Mäkinen, 2010). It decreases the growth of most *S. mutans* strains, and affects bacterial ultrastructure, cell envelope, acid production, and the formation of extracellular insoluble dextrans (Tuompo et al., 1983; Lee et al., 2009). Xylitol decreases the cariogenicity of plaque by decreasing carbohydrate-associated metabolism and increasing nitrogen metabolism (Mäkinen and Scheinin, 1976b). Use of xylitol causes increase in plaque and saliva levels of ammonia and amino acids resulting in partial neutralization of acids formed by acidogenic bacteria (Mäkinen and Scheinin, 1976b; Mäkinen, 1985). It also causes a reduction in the level of lipopolysaccharides resulting in lowered adhesivity of bacterial cells on tooth surfaces and to each other (Rølla et al., 1980; Tuompo et al., 1983). In addition, some strains of MS transport xylitol with the formation of intracellular xylulose and/or xylitol 5-phosphate which interfere with the intracellular bacterial metabolism and consumes energy. Part of the C5-phosphates is expelled back into the medium (‘futile xylitol cycle’), providing no energetic advantage (Söderling and Pihlanto-Leppälä, 1989; Pihlanto-Leppälä et al., 1990; Kakuta et al., 2003). In addition, xylitol has been shown to inhibit otopathogens and prevent acute otitis media (Uhari et al., 1998; Uhari et al., 2000; Azarpazhooh et al., 2011).

## 2.5 Bacterial Detection

As caries is a bacteria-associated disease, detection of the causative bacteria has been an important target for many years. The most of the known caries associated bacteria have been detected through cultivation. Introduction of molecular based techniques into dentistry enabled a high diversity of flora with unidentified phylotypes to be found (Kroes et al., 1999). More than 600 predominant oral bacterial species have been identified by the molecular techniques, 35% of which, have not yet been cultivated (Paster and Dewhirst, 2009; Dewhirst et al., 2010; Nyvad et al., 2013). 16S rRNA gene has been

the most widely used in molecular detection of oral bacteria (Wade, 2011; Nyvad et al., 2013). Techniques mostly used are denaturing gradient gel electrophoresis, PCR-based methods, 16S rRNA gene microarrays, checkerboard hybridization, and sequencing.

### 2.5.1 Human Oral Microbe Identification Microarray (HOMIM)

HOMIM is a taxonomic microarray that has been developed by the Forsyth Institute (Preza et al., 2009). It can detect about 300 of the most prevalent oral bacterial species. It has been used in the identification of subgingival bacteria and in root caries (Preza et al., 2009; Olson et al., 2011). The major disadvantage is that the technique uses bacteria specific probes. This restricts the detection to only micro-organisms that are targeted by the probes. Microarrays are not open-ended techniques, and although they are easy to perform, they are quite expensive (Nyvad et al., 2013).

# 3. AIMS OF THE STUDY

The aim of the study was to evaluate the relationship between daily habits, oral microbiota and caries and to investigate the effects of xylitol on oral microbiota.

The specific objectives were:

1. To evaluate the relationship between daily habits, MS and caries using a measure of caries severity calculated from ICDAS scores (I). The hypothesis was that caries severity is correlated with unhealthy daily habits and high levels of MS.

2. To identify organisms in the salivary microbiota which are associated with caries presence or absence in MS-positive subjects (II). The hypothesis was that other bacteria species may be associated with caries occurrence in addition to MS.

3. To study the effect of using xylitol chewing gum on the composition of the oral microbiota. The hypothesis was that chewing xylitol gum would not affect the composition of the oral microbiota (III).

4. To evaluate the use of xylitol as a mouthrinse on salivary levels of MS. The hypothesis was that the xylitol mouthrinse would reduce salivary MS levels (IV).

# 4. MATERIALS AND METHODS

## 4.1 Subjects and Study Designs

All studies were conducted in Kuwait. Kuwait is a small country where all the Kuwaiti population are considered to represent one socioeconomic class. Caries rates as well as oral health habits are very similar between different regions in Kuwait (Al-Mutawa et al., 2006; Honkala et al., 2006).

The target population of the study was school children in late mixed dentition (age 10-12 years old). All studies were conducted during the 2011-2012 academic year in Jabria area, Kuwait. Jabria was chosen because of its proximity to the university area for accessibility for close monitoring of the intervention studies and handling of the samples. Two intermediate schools and one youth centre present in Jabria were included in the sampling frame. Jabria intermediate school for boys was selected over the girl's school for easier access for male investigators due to cultural limitations.

Studies I and II were observational cross-sectional studies performed in 11-12 years old school children. Studies III and IV were randomized, double-blind, placebo-controlled, intervention studies. Study III was conducted in 11-12 years old school children while study IV was performed in 10-12 years old children at Al-Ghadeer Islamic Youth Centre. Both studies III and IV consisted of two parallel groups. The clinical trial profile for studies III and IV is presented in Figure 1.

In study I, children at Jabria Intermediate School for boys were screened for mutans streptococci (MS), clinically examined and interviewed for daily habits. Subjects with high MS counts ($>10^5$CFU/mL) either in saliva or in plaque, healthy and willing to participate in a 5 weeks intervention participated in study III.

In study III, the children were randomly allocated to xylitol and sorbitol groups, using classrooms as clusters. This was done mainly to prevent exchange or mixing of the gums by children and/or teachers. Group of Study III subjects were randomly selected for HOMIM analysis (II).

In study IV, children at Al-Ghadeer Islamic Youth Centre participated. The inclusion criteria were healthy, willing to participate and having high salivary MS levels. Children were randomly allocated to xylitol and sorbitol groups using the random number table.

In studies III and IV, the required sample size was based on earlier studies showing the effects of xylitol on MS counts (Söderling et al., 1989; 1997). Probability power of 0.8 and type I error of 0.05 were used. Since the study is short, the dropout percentage of less than 5% was expected.

## 4.2 Ethical Aspects (I-IV)

The Joint Committee for Protection of Human Subject in Research of Kuwait University and Ministry of Health approved the clinical studies (project number DD02/10). Written informed consent was obtained from parents/guardians of every child. All data were treated confidentially. The studies were conducted in accordance with the Helsinki Declaration. Products of xylitol, sorbitol, and saccharine used are considered safe for dental health because they cannot be used by dental plaque microorganisms. Sugar alcohols are considered safe. The studies were registered at ClinicalTrials.gov with identifier ID of NCT0528969.

## 4.3 Test Chewing Gums and Mouthrinses (III, IV)

In study III, xylitol and sorbitol chewing gums were used. Xylitol gum (1.5 g/pellet) contained 65 % xylitol w/w while sorbitol gum contained sorbitol 63 % with 2 % maltitol and Acesulfame K. Maltitol was used for the texture and Acesulfame K was used for sweetness. Both gums had the same texture, flavour, colour and sweetness. They were packaged in letter coded identical plastic containers with a humidity-removing bag. The non-commercial gums were manufactured and donated to the study by Karl Fazer Ab, Vantaa, Finland. The gums were administered to children by teachers allocated to each group of students. Two pieces of gum, three times a day (6 g xylitol/day in the xylitol group; 6 g sorbitol/day in the sorbitol group) were used. The timing during the day was after breakfast and during lunch breaks at school and just before leaving the school in the afternoon. A bag of 12 pieces of gums was given a day before the weekend for the weekend use.

In study IV, xylitol and saccharine mouthrinses were used. The xylitol and the saccharine mouthrinses had concentration of 20% and 16% w/w. Both had similar sweetness and colour. Mouthrinses were prepared and filled in letter coded plastic bottles. Subjects were advised to use the mouthrinse five times a day.

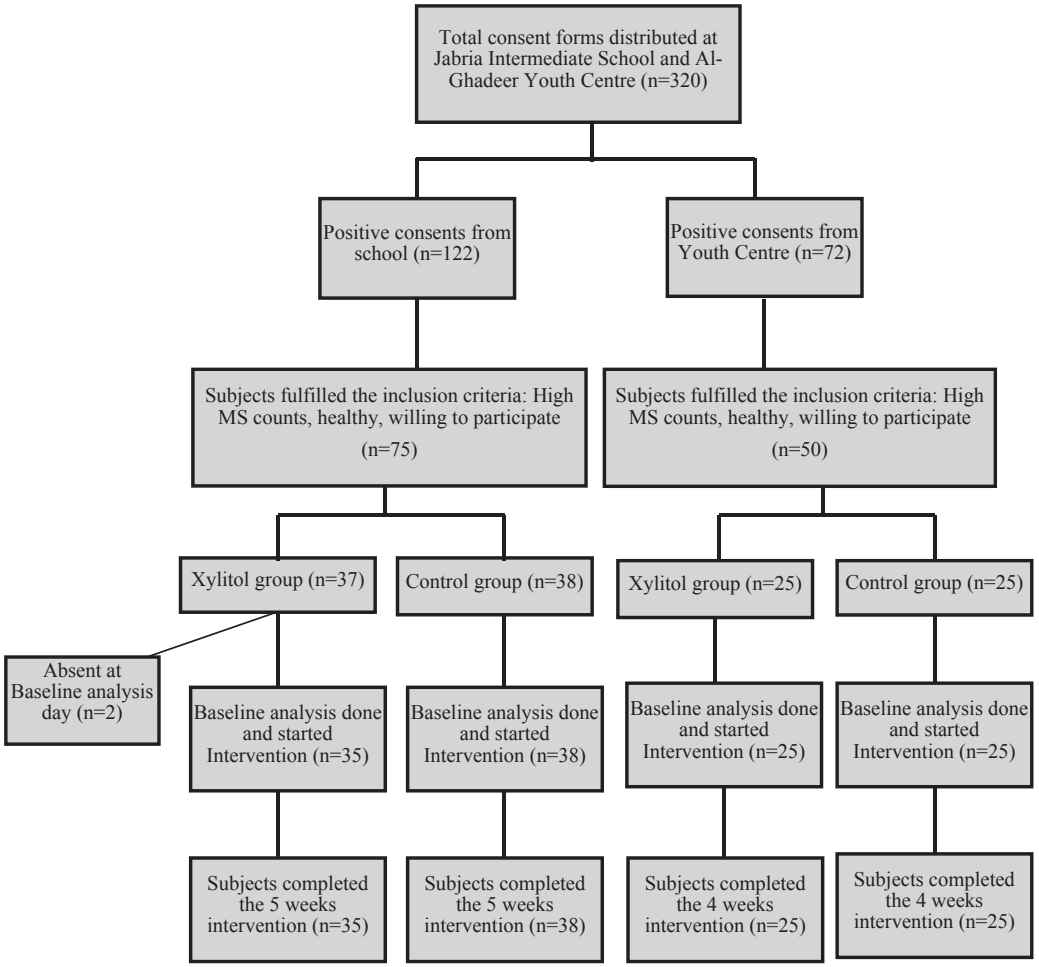



**Figure 1.** Clinical trial profile.

## 4.4 Analytical Methods

### 4.4.1 Oral Microbiological Sample Collections and Analyses

In study I, stimulated saliva and plaque samples were collected from the children. Plaque samples from interproximal spaces between primary second molar/first premolar and permanent first molar of all quadrants using sterile microbrushes were collected. Stimulated saliva was collected in 15 ml sterile Tubes (Corning®, Tewksbury, MA, USA). Children's MS levels at plaque and saliva were determined using the Dentocult® SM Strip mutans test (Orion Diagnostica, Espoo, Finland) (I).

In study II and III, unstimulated and stimulated saliva was collected in 15 ml sterile Tubes (Corning®, Tewksbury, MA, USA). One hundred microliters of the unstimulated

and stimulated saliva were added into 900 µl Tryptic Soy Broth with 10 % glycerol (TSB; Scharlau Chemie S.A., Barcelona, Spain) and stored at -70 °C before microbial analyses were performed at the Institute of Dentistry, University of Turku, Finland. Also another 1 mL sample of the stimulated saliva was pipetted onto 10 µl TE-buffer (Sigma-Aldrich, St. Louis, MO, USA) and stored at -70°C for the HOMIM analyses. Saliva was collected twice, before and after intervention (III). The tubes were stored at –70˚C and transported on dry ice to the Institute of Dentistry, Turku, and to USA for HOMIM analysis on dry ice. Samples were stored there at –70˚C before microbiological analysis.

In Study IV, stimulated saliva was collected before and after intervention as described above. MS level in saliva was determined by CRT bacteria (Ivoclar Vivadent®, Amherst, NY, USA).

#### *4.4.1.1 Determination of MS in Saliva (I, III, IV)*

##### *4.4.1.1.1 MS Plate Culturing (III)*

The plate-culturing of MS was performed as follows: After 10-fold serial dilutions, the samples were plated on Mitis salivarius agars (Difco, Detroit, MI, USA) containing sucrose and bacitracin (MSB; Gold et al., 1973). The MS grown on the MSB agar were incubated for 3 days in a 7% $CO_2$ atmosphere at 37˚C. MS were identified on the basis of colony morphology and counted using stereomicroscope as described previously (Söderling et al., 2011). *S. mutans* was identified as based on "rough" colony morphology on the MSB plate with positive fermentation with sorbitol, mannitol, raffinose, and melibiose, and negative dextran agglutination. *S. sobrinus* was identified based on "smooth" colonies on the MSB plate, positive fermentation with mannitol but negative with raffinose, and melibiose, and positive dextran agglutination (Söderling et al., 2000).

##### *4.4.1.1.2 Chair-side Assay of Mutans Streptococci (I, IV)*

Two types of chair-side MS assays were used in the present study Dentocult® SM Strip mutans test (Orion Diagnostica, Espoo, Finland) and CRT bacteria (Ivoclar Vivadent®, Amherst, NY, USA).

The strips of the Dentocult® SM Strip mutans test (Orion Diagnostica) were incubated in their vials at 35– 37˚C for 48– 72 hours. Dried strips were read by three dentists (Eino Honkala, Sisko Honkala and Mohamed ElSalhy) with the naked eye according to the manufacturer's classification chart. The strip was considered positive if typical round and spherical MS colonies were detected. Codes of 0.0, 1.0, 2.0, and 3.0 were used as an indication of the bacterial count as follow 0 = $<10^4$, 1.0 = $10^4$–$10^5$, 2.0 =

$10^5$–$10^6$, and 3.0 = >$10^6$ CFU of MS/ml of saliva. The mode of the readings of all readers was used. If there was no mode, the mean of all readings was used.

For CRT bacteria, vials were incubated in at 37˚C for 48 hours and the strips were read by two dentists (Mohamed ElSalhy and Ismail Sayed Zahid). Mean of both readings was used. Different codes were used as follows: 1.0 = <$10^4$, 2.0 = $10^4$–$10^5$, 3.0 = $10^5$–$10^6$, and 4.0 = >$10^6$ CFU of MS/ml of saliva.

Both commercially available chair-side tests measure MS counts, the combination of *S. mutans* and *S. sobrinus*. The tests are employing the same culture medium as the plate culturing of MS described above. The Strip mutans test has been used in several clinical studies in children and adults and it has been validated by Karjalainen et al. (2004).

#### *4.4.1.2 DNA Isolation and Purification (II, III)*

Bacterial DNA was isolated and purified using the Ready-Lyse™ Lysozyme Solution and MasterPure Gram Positive DNA Purification kits (Epicentre Biotechnologies, Madison, WI, USA). Briefly, cells in 1ml saliva were pelleted by centrifugation and the supernatant was discarded. The pellet was re-suspended in 150 µl TE Buffer. One microliter of Ready-Lyse Lysozyme was added and incubated at 37°C overnight. After incubation, 150 µl of 2 X T & C Lysis Solution was added to each sample and pipetted up and down when adding. One microliter Proteinase K was added, mixed, and incubated at 65°C for 30 minutes and vortex mixed every 5 minutes. Samples were allowed to cool to 37°C then placed on ice for 3-5 min before DNA precipitation.

MPC protein precipitation reagent (175 µl) was added to the lysed sample and vortex mixed vigorously for 10 seconds. The mixture was then centrifuged for 10 minutes at greater than 10,000 x g to pellet the debris then placed immediately on ice after centrifugation. The supernatant was then transferred to a clean micro-centrifuge tube and the pellet was discarded. Five hundred microliter of isopropanol was added the recovered supernatant and inverted 30-40 times before placing it on ice for 10 minutes. DNA was pelleted. Isopropanol was poured off and the remaining isopropanol was pipetted. The pellet was washed with 500 µl 75% ethanol twice. Residual ethanol was removed and the DNA was re-suspended in 25 µl of TE Buffer. DNA was stored at -70$^o$C before HOMIM analysis (http://bioinformatics.forsyth.org/ homim/).

#### *4.4.1.3 HOMIM (II, III)*

In HOMIM, the concentration levels of approximately 300 oral taxa were determined by microarray hybridization using a fluorescent readout reverse-capture method (Colombo et al., 2009). Fluorescently labelled sample microbial DNA was captured

by 16S rRNA-based probes attached to glass slides. Briefly, labelled nucleotide Cy3-dCTP was incorporated during DNA amplification during a second nested PCR. DNA hybridization to the array was performed at 55°C overnight. The arrays were then washed at room temperature, spun dry and stored in a dark container until scanned. The scanning was done using an Axon 4000B microarray scanner. The fluorescent intensity for each probe was normalised and scaled as previously reported (Colombo et al., 2009). Double background signals were considered negative and assigned a score of 0. Positive hybridization signals were categorised into 5 levels, with 1 indicating a signal that was just detectable and 5 indicating a maximum signal intensity (Fine et al., 2013).

### 4.4.2 Caries Measurements (I-III)

Caries was evaluated at multiple levels. Indices for caries status, severity and experience were used. ICDAS Caries Index (ICDAS CI) was calculated by counting all ICDAS caries scores (1-6) of all surfaces divided by a total number of carious teeth was used as a measure of caries severity. Total number of carious teeth and total number of enamel and dentine carious surfaces was used as measure of caries status. DMFT/dmft and DMFS/dmfs were used as a measure of caries experience.

## 4.5 Clinical Examination

In studies I-III, the clinical examinations were conducted at the school clinic with a mobile dental chair, artificial spotlight, and mobile dental unit using a mouth mirror and air/water syringe. The ICDAS criteria (Ismail et al., 2007) were used in the clinical examinations, and it was conducted by one examiner (EH). The examiner had training and experience in the use of ICDAS from earlier studies with high consistency (kappa > 0.9). Radiographs were not included in the examinations. The caries status of each tooth surface was classified on an ordinal scale (from 0 to 6 depending on the severity of the lesion) and marked on an ICDAS recording sheet (Appendix 1). Restorations and missing teeth were also recorded according to ICDAS codes (Table 1). Students were asked to brush their teeth before the clinical examination.

## 4.6 Daily Habits Interviews (I)

Daily oral health habits interviews were conducted on all subjects. A standardized interview form was used (Appendix 2), where the questions had been validated in the Health Behaviour in School-aged Children (HBSC) survey (Currie et al., 2009) and also used earlier in a Kuwaiti study with the same age group (Honkala et al., 2006).

Subjects were asked about their frequency of brushing (type of toothpaste), flossing, use of mouthrinse (frequency and type), consumption of sweets, soft drinks (frequency and type) and chewing gum (frequency and type). They were also asked about their possible use of any antibiotics during the last two weeks.

## 4.7 Outcome Measures

The primary and secondary outcome measures are presented in Table 3. In all studies, the pre-specified outcome measures were defined prior to starting the clinical trial.

**Table 2.** The primary and secondary outcome measures of the study.

| Study | Primary Outcome | Secondary Outcome |
|---|---|---|
| I | Caries occurrence measured by ICDAS | Daily habits and salivary MS levels |
| II | Bacterial levels in saliva | Bacterial levels in caries-affected and caries free-children |
| III | MS level in stimulated saliva | MS level in unstimulated saliva |
| IV | MS level in stimulated saliva | |

## 4.8 Statistical Methods

Statistical analyses were done using SPSS 17.0-21.0 softwares (SPSS Inc., Chicago, Ill., USA) and SAS version 9.3. The data normality was tested by Shapiro–Wilk test. Mean and Standard Deviation (SD) were used to describe normally distributed data while median, 25th percentile and 75th percentile were used to describe data that did not follow normal distribution. P value of less than 0.05 was considered significant.

In study I, correlations between all caries indices were analysed by the non-parametric Spearman correlation coefficient (ρ). Relationships between caries, salivary and plaque MS, and daily habits were analysed by Kruskal–Wallis and Mann-Whitney U tests for non-parametric comparisons.

In study II, levels of bacteria were analysed by comparing caries-affected and caries-free subjects. Mann-Whitney analysis was used to determine statistical significance of the differences in bacterial levels between the study groups. Correlations between the levels of bacteria and caries indices were analysed by the Spearman correlation coefficient (ρ).

In Study III, paired sample T-test was used to study differences before and after intervention while independent samples t-test was used to compare the groups.

Spearman correlation coefficient (ρ) was used to calculate the correlations between the MS in stimulated vs. unstimulated saliva. Changes in HOMIM bacterial levels before and after intervention were tested within each group using Wilcoxon signed rank tests.

In study IV, changes in the distribution of MS levels between groups were evaluated by Chi-square test.

# 5. RESULTS

## 5.1 Subjects' Caries Status Measured by ICDAS (I)

### 5.1.1 ICDAS Caries Scores

The mean percentages of ICDAS codes in primary second molars are shown in table 3 and the mean percentages of ICDAS codes in permanent teeth according to tooth surfaces are summarized in Tables 4 and 5.

**Table 3.** The mean percentages of ICDAS codes in the upper and lower second primary molars according to tooth surfaces.

| | ICDAS Codes | | | | | | |
|---|---|---|---|---|---|---|---|
| | **0** | **1** | **2** | **3** | **4** | **5** | **6** |
| 55/65 | | | | | | | |
| M | 80.75 | 0 | 0 | 0 | 2.25 | 3.35 | 13.65 |
| O | 59.55 | 0 | 11.2 | 3.35 | 4.55 | 8.9 | 12.45 |
| D | 74.1 | 0 | 0 | 0 | 0 | 5.6 | 20.3 |
| B | 89.85 | 0 | 0 | 0 | 0 | 3.35 | 6.8 |
| L | 78.65 | 0 | 2.25 | 3.35 | 0 | 6.65 | 9.1 |
| 75/85 | | | | | | | |
| M | 84.9 | 0 | 1.85 | 1.9 | 3.75 | 1.9 | 5.7 |
| O | 81.15 | 0 | 1.9 | 5.7 | 3.75 | 3.75 | 3.75 |
| D | 92.5 | 0 | 0 | 0 | 0 | 1.9 | 5.6 |
| B | 90.45 | 0 | 3.85 | 0 | 0 | 1.9 | 3.8 |
| L | 94.4 | 0 | 0 | 0 | 0 | 1.9 | 3.7 |

### 5.1.2 Association between ICDAS Scores in Primary and Permanent Teeth

The mean number of enamel caries surfaces was 0.14 (SD=0.5) and dentinal caries surfaces 0.90 (3.0) in the primary teeth. The respective means were 2.12 (2.5) and 1.07 (3.0) in the permanent teeth. The mean number of surfaces with the different ICDAS scores in primary teeth were: 1) 0.3, 2) 1.1, 3) 0.7, 4) 0.6, 5) 0.3, 6) 0.3 and in permanent teeth: 1) 0.0, 2) 0.1, 3) 0.0, 4) 0.1, 5) 0.2, 6) 0.5. The correlations between the mean numbers of enamel and dentinal caries surfaces between the primary and the permanent teeth were very low. The only statistically significant correlation (r=0.37) was between the number of enamel and dentinal caries lesions in the permanent dentition (Honkala et al., 2013).

**Table 4.** The mean percentages of ICDAS codes in the upper and lower permanent anterior teeth according to tooth surfaces.

| | ICDAS Codes | | | | | | |
|---|---|---|---|---|---|---|---|
| | **0** | **1** | **2** | **3** | **4** | **5** | **6** |
| 13/23 | | | | | | | |
| M | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| D | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| B | 98.4 | 0 | 0.6 | 0 | 1.1 | 0 | 0 |
| L | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12/22 | | | | | | | |
| M | 97.5 | 0 | 0 | 0 | 1.25 | 1.25 | 0 |
| D | 99.6 | 0 | 0 | 0 | 0 | 0.4 | 0 |
| B | 97.9 | 0 | 0 | 0 | 0.4 | 1.3 | 0.4 |
| L | 98 | 0 | 0.4 | 1.2 | 0 | 0.4 | 0 |
| 11/21 | | | | | | | |
| M | 97.5 | 0 | 0 | 0 | 1.2 | 1.2 | 0 |
| D | 98.4 | 0 | 0 | 0 | 1.2 | 0.4 | 0 |
| B | 96.8 | 0 | 0.8 | 0.4 | 0.8 | 1.2 | 0 |
| L | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| 33/43 | | | | | | | |
| M | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| D | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| B | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| L | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| 32/42 | | | | | | | |
| M | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| D | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| B | 99.6 | 0 | 0.4 | 0 | 0 | 0 | 0 |
| L | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| 31/41 | | | | | | | |
| M | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| D | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| B | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| L | 100 | 0 | 0 | 0 | 0 | 0 | 0 |

**Table 5.** The mean percentages of ICDAS codes in the upper and lower first permanent molars and second primary molars according to tooth surfaces.

| | ICDAS Codes | | | | | | |
|---|---|---|---|---|---|---|---|
| | **0** | **1** | **2** | **3** | **4** | **5** | **6** |
| 16/26 | | | | | | | |
| M | 97.2 | 0 | 0 | 0.8 | 0.8 | 0.8 | 0.4 |
| O | 74.2 | 3.25 | 10.65 | 3.7 | 6.55 | 0.8 | 0.8 |
| D | 98.4 | 0 | 0.4 | 0 | 0 | 0 | 1.2 |
| B | 96.7 | 0.4 | 0.4 | 0.4 | 0 | 0.4 | 1.6 |
| L | 89.35 | 0.4 | 4.5 | 1.25 | 1.7 | 1.2 | 1.6 |
| 15/25 | | | | | | | |
| M | 99.5 | 0 | 0.5 | 0 | 0 | 0 | 0 |
| O | 93.6 | 2 | 2.4 | 2 | 0 | 0 | 0 |
| D | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| B | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| L | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14/24 | | | | | | | |
| M | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| O | 93 | 2.6 | 3.1 | 0.4 | 0.9 | 0 | 0 |
| D | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| B | 99.1 | 0.45 | 0.45 | 0 | 0 | 0 | 0 |
| L | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| 36/46 | | | | | | | |
| M | 95.1 | 0.4 | 2.1 | 0.8 | 0.4 | 0 | 1.2 |
| O | 75.4 | 0 | 7.4 | 8.2 | 6.6 | 1.6 | 0.8 |
| D | 96.4 | 0 | 0.8 | 0 | 0.8 | 0.4 | 1.6 |
| B | 76.2 | 2.4 | 8.6 | 8.6 | 1.2 | 1.6 | 1.2 |
| L | 97.95 | 0 | 0.4 | 0 | 0 | 0 | 1.65 |
| 35/45 | | | | | | | |
| M | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| O | 97.8 | 0 | 1.1 | 0.5 | 0.5 | 0 | 0 |
| D | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| B | 99.5 | 0 | 0.5 | 0 | 0 | 0 | 0 |
| L | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| 34/44 | | | | | | | |
| M | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| O | 99.5 | 0.5 | 0 | 0 | 0 | 0 | 0 |
| D | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| B | 98.2 | 0 | 1.35 | 0 | 0.45 | 0 | 0 |
| L | 100 | 0 | 0 | 0 | 0 | 0 | 0 |

### 5.1.3 Caries Measurement by Caries Indices

Median (25th percentile, 75th percentile) DMFT/dmft of subjects was 1.0 (0. 0, 3.0) and the median ICDAS CI was 2 (0.0, 4.0). Subjects' caries status, experience and severity measured by different caries measures are summarised in Table 6.

**Table 6.** Median, 25th, 75th percentile of caries indices measured.

| Caries Measure | Median | 25th percentile | 75th percentile |
|---|---|---|---|
| DMFT/dmft | 1.0 | 0.0 | 3.0 |
| DMFS/dmfs | 2.8 | 0.0 | 4.5 |
| ICDAS Index | 2.0 | 0.0 | 4.0 |
| Total number of carious teeth | 2.0 | 0.0 | 4.0 |
| $D_{1-3}S$ | 2.0 | 0.0 | 4.0 |
| $D_{4-6}S$ | 0.0 | 0.0 | 2.0 |

## 5.2 Subjects' Oral Health Habits (I)

Less than half of the children visited the dentist during the last year. Three-fourths of all children brushed their teeth once a day or more and the majority did not use dental floss. All children used fluoridated toothpaste. More than half of the children consumed sweets more than once a day and 40 % drank soft drinks more than once a day. Subjects' oral health habits are summarised in Table 7.

**Table 7.** Oral health habits of subjects.

| Habit | N | % | Habit | N | % |
|---|---|---|---|---|---|
| **Last dental visit?** | | | **Use of mouthrinse?** | | |
| Less than a year | 56 | 45.9 | More than once a day | 15 | 12.3 |
| 1-2 years | 27 | 22.1 | Once a day | 19 | 15.6 |
| More than 2 years | 39 | 32.0 | Less than once a day | 88 | 72.1 |
| **Last pain episode?** | | | **Sugar use frequency?** | | |
| Never | 13 | 10.7 | More than once a day | 66 | 54.1 |
| Less than a year | 78 | 63.9 | Once a day | 31 | 25.4 |
| One to two years | 15 | 12.3 | Less than once a day | 25 | 20.5 |
| More than two years | 16 | 13.1 | | | |
| **Brushing frequency?** | | | **Use of soft drinks?** | | |
| More than once a day | 57 | 46.7 | More than once a day | 49 | 40.2 |
| Once a day | 36 | 29.5 | Once a day | 32 | 26.2 |
| Less than once a day | 29 | 23.8 | Less than once a day | 41 | 33.6 |
| **Flossing frequency?** | | | **Use of chewing gums?** | | |
| More than once a day | 2 | 1.6 | More than once a day | 63 | 51.6 |
| Once a day | 5 | 4.1 | Once a day | 26 | 21.3 |
| Less than once a day | 115 | 94.3 | Less than once a day | 33 | 27.0 |

## 5.3 Subjects' MS Levels in Plaque and Saliva (I, III, IV)

In study I, more than 50 % of subjects had salivary MS scores of 2 or more and 41.8 % had mean plaques scores of 2 or more. Study I subjects' MS scores in stimulated saliva are shown in Figure 2 and mean plaque scores in Figure 3 (I).

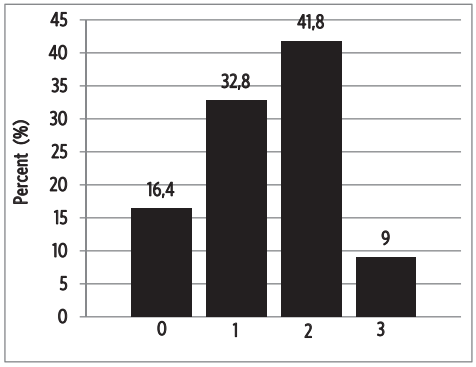



**Figure 2.** The percentages of subjects with different MS scores in saliva.

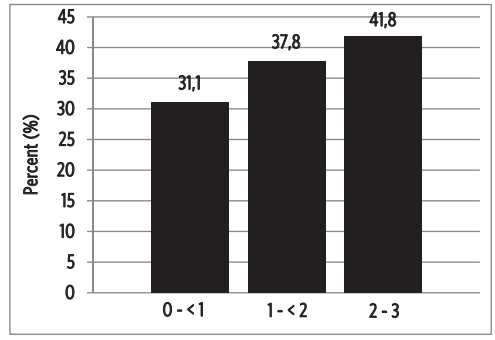



**Figure 3**. The percentages of subjects according to mean MS scores in plaque.

In study III, all subjects' mean MS counts (log CFU/ml) in unstimulated and stimulated saliva measured by MS plate culturing are summarised in Table 8. At the beginning of the study, no significant difference between xylitol and control group was found. Significant reduction in MS levels was seen in the xylitol group compared to control after 5-weeks used of xylitol gum ($p < 0.05$) (III).

**Table 8.** Mean (SD) stimulated and unstimulated salivary MS counts of all subjects.

| Sample | Mean (SD) Overall | Mean (SD) at Baseline | | Mean (SD) after 5 Weeks | |
|---|---|---|---|---|---|
| | | Xylitol | Control | Xylitol | Control |
| Stimulated saliva | 4.6 (1.5) | 4.86 (1.34) | 4.40 (1.71) | 4.29 (1.73) | 3.78 (1.83) |
| Unstimulated saliva | 3.8 (1.5) | 3.64 (1.73) | 3.92 (1.19) | 2.59 (2.03) | 2.92 (1.87) |

In study IV, subjects in both xylitol and control group had MS counts of 3 and 4 at the baseline. After 4 weeks of xylitol mouthrinse use, majority of subjects showed reduction in the MS scores in the xylitol group compared to control (Figure 4). Subjects' MS scores in stimulated saliva according to study groups at the baseline and after intervention are shown in Figure 4 (IV).

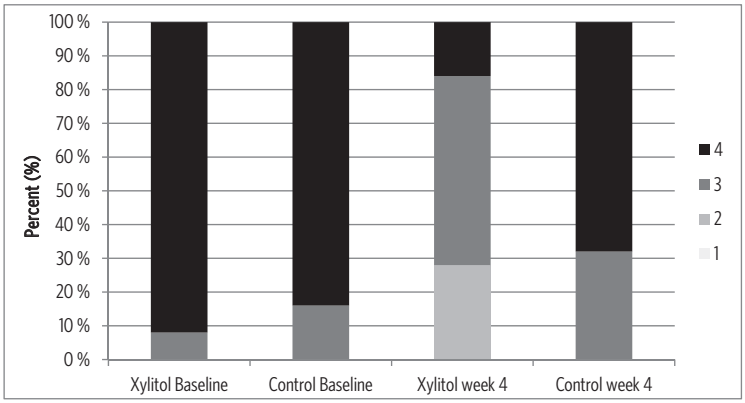



**Figure 4.** The distribution of MS scores in saliva of subjects in different groups at baseline and after 4-weeks intervention.

## 5.4 Caries and Oral Health Habits (I)

In study I, brushing, sweets and soft drinks consumption were the only habits associated with caries measured by the different indices. Lower ICDAS CI, DT/dt, $D_{1-3}S$, $D_{4-6}S$, DMFT/dmft and DMFS/dmfs were found in children who brushed once a day or more ($p < 0.01$). Consumption of sweets more than once a day was associated with significantly higher ICDAS CI, DT/dt, $D_{1-3}S$, $D_{4-6}S$, DMFT/dmft and DMFS/dmfs ($p < 0.05$). Use of soft drinks more than once a day was associated with higher ICDAS CI, DT/dt, $D_{1-3}S$, $D_{4-6}S$ ($p < 0.05$). No significant association was found between caries and flossing, use of mouthrinse, or gum chewing ($p > 0.05$).

## 5.5 Caries and MS Levels (I)

In study I, salivary and plaque levels of MS were significantly associated with higher DT/dt and $D_{1-3}S$. Children with salivary MS scores of 2 or more and/or plaque score of more than 1 had significantly higher DT/dt and $D_{1-3}S$ than children with lower scores ($p<0.05$) (Figures 5-8). The other caries indices showed no significant association.

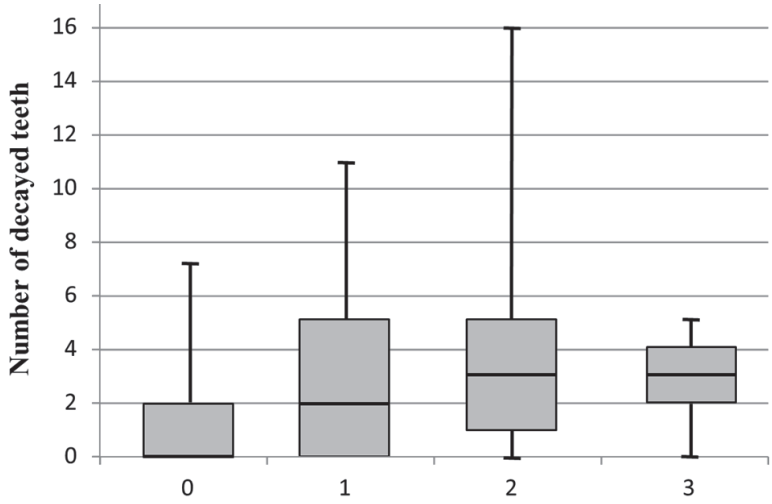



**Figure 5.** Box plots of DT/dt in relation to salivary MS counts.

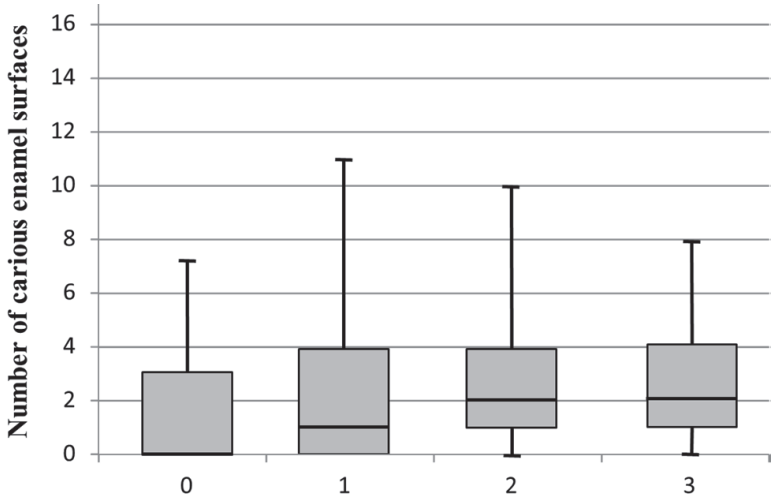



**Figure 6.** Box plots of D1-3S in relation to salivary MS counts.

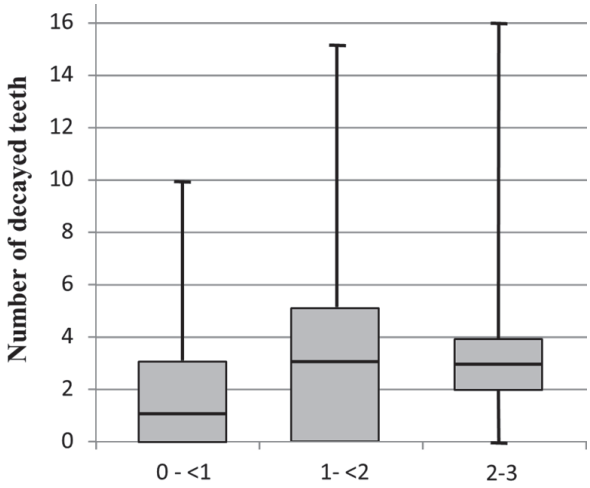



**Figure 7.** Box plots of DT/dt in relation to mean plaque MS counts.

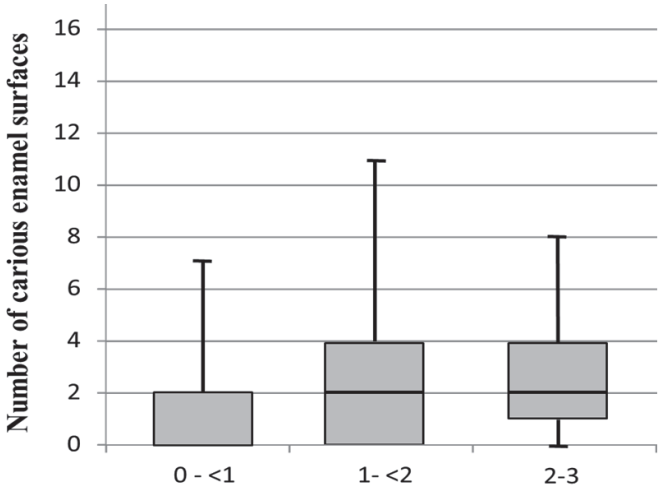



**Figure 8.** Box plots of $D_{1-3}S$ in relation to mean plaque MS counts.

## 5.6 HOMIM Oral Bacteria Profiles (II, III)

Ninety eight bacterial species and 9 bacterial clusters were detected. Bacterial species and cluster with frequent detection are shown in Figure 9.

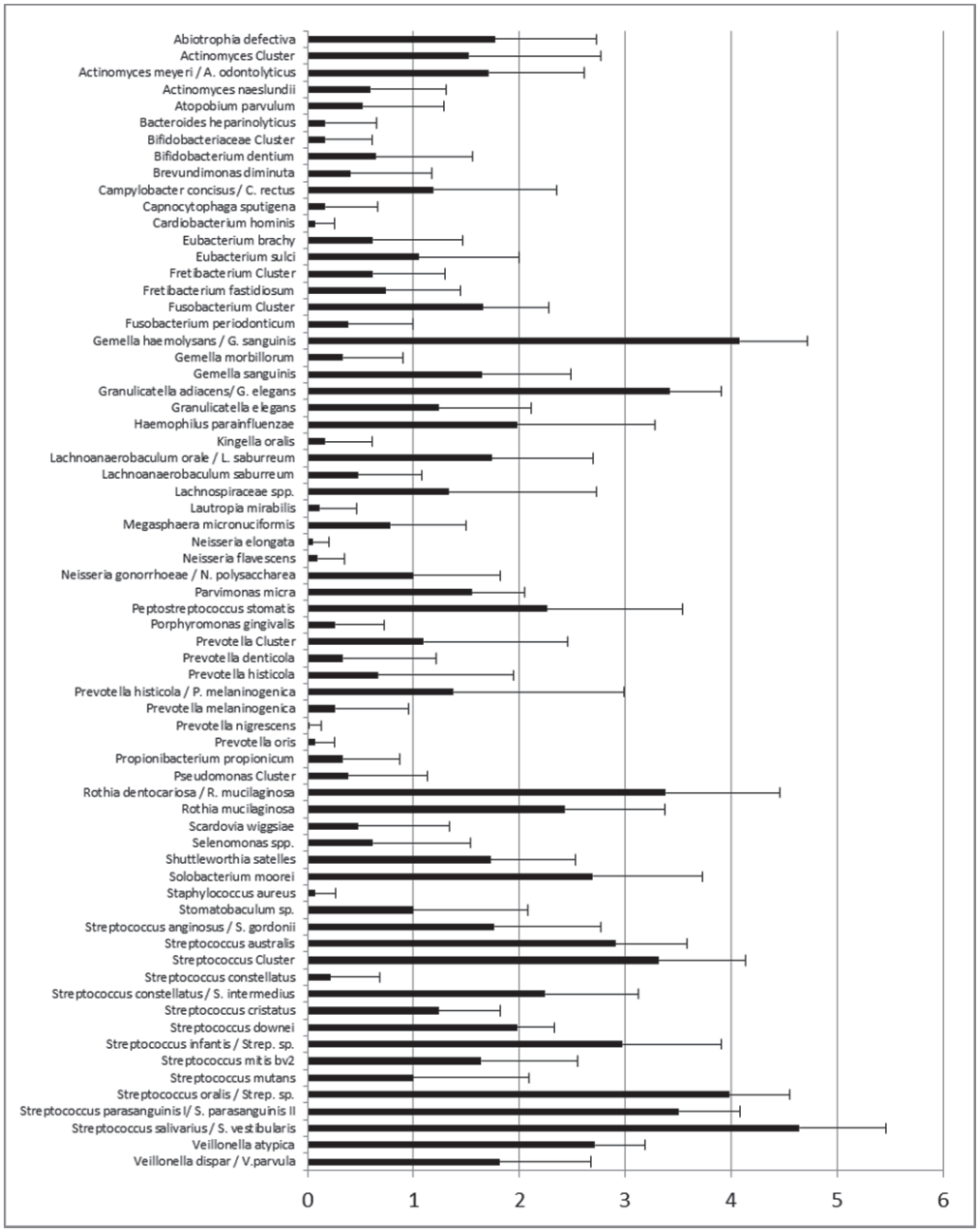



**Figure 9.** The means and standard deviations of bacteria detected by HOMIM (n=28).

## 5.7 Caries and Oral Microbiota (II)

Levels of most detected bacterial taxa according to caries groups are shown in Study II Figure 1. Bacteria that were significantly higher in caries affected children include *Abiotrophia defectiva* and *Actinomyces meyeri/ Actinomyces odontolyticus* while *Shuttleworthia satelles* was significantly higher in the caries-free children. Bacteria with significant correlations with different caries indices are shown in Study II Table 1.

## 5.8 Effects of Xylitol on MS Distribution in Stimulated and Unstimulated Saliva (III)

Significant correlations between MS (log CFU/ml) from unstimulated (US) and stimulated (SS) saliva in the xylitol and control group were detected at baseline ($r = 0.43$, $p = 0.007$; $r = 0.60$, $p < 0.001$, respectively) and after the 5-week use of the chewing gum ($r = 0.62$, $p < 0.001$; $r = 0.62$, $p < 0.001$, respectively).

## 5.9 Effects of Xylitol on Oral Microbiota (III)

In study III, there was no significant difference in the levels of bacteria at the beginning of the study between xylitol and sorbitol groups. HOMIM analysis showed that xylitol consumption did not affect the salivary microbiota in stimulated saliva except for a decrease in salivary MS.

## 5.10 Effects of Xylitol Mouthrinse on Salivary MS (IV)

At the beginning of study IV, all subjects in both xylitol (mean = 3.9, SE = 0.03) and control group (3.9, 0.04) has MS levels of more than $10^5$ CFU/ml with no significant difference in MS distribution between the groups ($p = 0.713$). Significant reduction in the level of MS was observed in the xylitol group (2.8, 0.13) compared to control (3.8, 0.07). Changes were observed in 76 % of the xylitol and only 28 % the control group.

## 5.11 Adverse Effects

No adverse effects were identified during the study and no complaints received from the subjects.

# 6. DISCUSSION

The present study examined the relationship between daily habits, oral microbiota and caries and evaluated the effect of xylitol on oral microbiota. Caries was measured by ICDAS and ICDAS CI was introduced as a new measure of caries severity. Brushing, the comsumption of sweets and soft drinks were the habits mostly associated with caries occurrence and severity. Levels of *A. defectiva* and *A. meyeri/A. odontolyticus* were significantly higher in caries-affected subjects. Multiple bacterial taxa were significantly associated with the number of enamel caries lesions. Habitual use of xylitol as a chewing gum or a mouthrinse was associated with reduction in MS counts. Xylitol use had no effect on the overall composition of oral microbiota.

## 6.1 Measurement of Caries

In study I, we introduced a new measure for caries severity calculated from ICDAS scores named ICDAS CI. ICDAS CI was calculated by counting all caries scores dived by a total number of carious teeth. ICDAS CI was significantly correlated with DMFT/dmft, DMFS/dmfs, number of enamel and dentine caries surfaces and total number of carious teeth. Using ICDAS in caries examination overcomes the deficiencies in using DMF system which includes only counts of dentine caries and includes missing and filled teeth in addition to caries.

Multiple caries indices were used. Measuring the total number of enamel caries lesions is also a measure of caries activity, although ICDAS codes 1-3 also includes arrested caries lesions. This is a disadvantage of ICDAS. Enamel caries lesions are the most important to be monitored in caries prevention programs and increase in their number is an alarm for a shift in caries activity. Dentine caries lesions reflect caries history as these are established lesions and require treatment. Most of these can be open carious lesions, and their number may affect the level of cariogenic bacteria in saliva. DMFT/dmft and DMFS/dmfs are measures of caries experience.

## 6.2 Daily Habits and Caries

Last dental visit, brushing, flossing, sugar and soft drinks consumptions, and use of mouthrinses and chewing gums are all daily habits evaluated in the present study. The frequency of dental visits was low. This can be due to lack of knowledge of the importance of dental visits regardless of the dental treatment need. In the present study,

a high percentage of children consumed sweets and soft drinks more than once a day. The results are consistent with an earlier larger study (Honkala et al., 2012). The use of dental floss or mouthrinses was generally very low.

In the present study, consumption of sweets more than once a day was associated with the higher caries experience than consumption of sweets only once a day or less. This was very consistent with previous studies (Garcia-Closas et al., 1997; Vanobbergen et al., 2001; Johnson et al., 2009; Guido et al., 2011).

The association between soft drinks and caries has been well documented (Garcia-Closas et al., 1997; Marshall et al., 2003; Sohn et al., 2006; Lim et al., 2008; Llena and Forner, 2008; Slater et al., 2010). Our results support this association, as more than once a day consumption of soft drinks was associated with significantly higher ICDAS CI, DT/dt, $D_{1-3}S$, $D_{4-6}S$, and plaque MS counts than consumption of soft drinks once a day or less. Our data showed that the use of soft drinks more than once a day was associated with higher numbers of enamel and dentine caries lesions compared to once a day or less. The lack of significant association with DMFT/dmft and DMFS/dmfs can be explained by the lack of enamel caries measured by DMF index. As new lesions may appear due to the behavioural changes, these changes cannot be observed when WHO criteria and DMF were used as a measure of caries. Continuous exposure to high acidic drinks containing fermentable sugars creates a favourable environment for caries formation (Johansson et al., 2007; Bartlett et al., 2011; Jager et al., 2012; Jawale et al., 2012).

In this study, brushing once a day or more was associated with lower caries indices. These results are consistent with the current literature (Liu et al., 2010; Chankanka et al., 2011; Guido et al., 2011). High toothbrushing frequency has been shown to associate consistently with fewer new non-cavitated caries surfaces and fewer newly cavitated caries surfaces (Chankanka et al., 2011). Although the efficiency of fluoride is well established (Marinho et al., 2003; Twetman et al., 2003; Mannaa et al., 2014), the effect of using fluoridated toothpaste on caries wasn't studied in this study since all participants were using fluoridated toothpaste as it is commonly available in the market.

Both flossing and use of mouthrinse had no significant association with any caries indices used in this study. This was most probably due to the low number of children who flossed or used mouthrinse. Although high percentages of children used chewing gum once a day or more, no significant association with caries was found. This is mainly due to lack of anti-cariogenic substances in the gum used as the Kuwaiti market lack xylitol products.

Children of the studied age group are known for their high consumption of sweets, soft drinks and other sugar products (Honkala et al., 2006) as well as high caries rate (Al-Mutawa et al., 2006). Our results support these finding and highlight the deficiency

in dental visits and hygiene care. Although these children receive educational knowledge by the national School Oral Health Program (SOHP), our results highlighted the need for more extensive behaviour changing approaches. SOHP provides children with oral health education activities, prevention, and treatment. Children receive fluoride varnish bi-annually and fissure sealants at school. All treatments in centres as well as school clinics are free of charge (Ariga et al, 2014). With all the efforts and resources provided, the effectiveness still seems to be questionable. The effectiveness of the SOHP has to be evaluated and the educational approaches have to be modified for better behavior changes.

## 6.3 Oral Microbiota and Caries

High salivary and plaque MS counts measured by chair-side test and culture plating were associated with higher DT/dt and $D_{1-3}S$ with no association with higher DMF index. This can be explained by enamel lesions being arrested by fluoride varnish received twice a year as part of the national school oral health program and not progressing to dentinal caries, which is counted in the DMF index.

In the present study, higher levels of *A. defectiva* and *A. meyeri/A. odontolyticus* were detected in caries-affected children. Previous studies detected *A. defectiva* more frequently in plaque samples of caries-free children (Becker et al., 2002; Corby et al., 2005; Kanasi et al., 2010a). However, this discrepancy can be due to different type of samples used and requires further investigations. The association of *Actinomyces* species with caries is well studied (Tanner et al., 2002; Aas et al., 2008; Kanasi et al., 2010b). *Actinomyces* species were detected at high levels in caries initiation compared to those of cavitated and dentine plaque samples (Aas et al., 2008). Previous HOMIM analyses of whole saliva found the *Actinomyces* cluster to be present only in caries-affected subjects which also supports our results (Luo et al., 2012).

Caries-free children compared to caries-affected children had significantly higher levels of *S. satelles* in their saliva. Nothing is known about the association of *S. satelles* and caries. Thus, it seems to be an interesting bacteria to study as its level was negatively correlated with ICDAS CI and the number of enamel lesions in the present study.

Multiple bacterial taxa were correlated with the number of enamel caries lesions. *Lachnospiraceae sp.*, *Capnocytophaga granulosa*, *Campylobacter concisus, Campylobacter rectus, Eubacterium yurii, Catonella morbi, Catonella sp., Gemella haemolysins/Gemella sanguinis, Prevotella histicola, and Prevotella melaninogenica* were positively correlated with the number of enamel caries lesions. In addition, *G. haemolysans/G. sanguinis* was positively correlated with ICDAS CI and $D_{4-6}S$.

*Lachnospiraceae sp, C. granulosa, G. haemolysins, P. melaninogenica* and *G. sanguinis* were bacterial taxa previously found to be associated with caries (Kanasi et al. 2010a; Luo et al. 2012). Gomar-Vercher et al. (2014) found that the levels of *Porphyromonas* and *Capnocytophaga* were associated with worst caries status. However, *E. yurii* was detected more often in plaque samples of caries-free children by Kanasi et al. (2010a), while it was detected only in the saliva samples of the caries-active group by Luo et al. (2012). There is a lack of knowledge on how these bacteria could contribute in caries formation.

## 6.4 Effects of Xylitol on MS and the Oral Microbiota

Consumption of xylitol chewing gum (III) or mouthrinse (IV) was associated with lower MS levels. The effect of xylitol chewing gum on MS levels is well established in the literature (Söderling, 2009; Milgrom et al., 2012). However, the mouthrinse was an alternative method of delivery of xylitol. Few previous studies showed conflicts on its effect as a mouthrinse (Giertsen et al., 1999; Hildebrandt et al., 2010; Arunakul et al., 2011). Our results reinforced the fact that a mouthrinse is an effective method for xylitol delivery, for example, for subjects who may suffer from laxative side-effects of xylitol. We used 20 % concentration of xylitol to evaluate the effect. However, 12.5% showed a significant effect after 10 weeks use on MS levels (Arunakul et al., 2011). It seems that its efficiency was comparable to chlorhexidine when mixed with sodium fluoride and triclosan (Subramaniam and Nandan, 2011). In addition, some reduction in plaque formation was also observed after rinsing with xylitol in addition to the reduction in MS (Lingström et al., 1997).

No effect was found after 5 weeks xylitol consumption on the composition of oral microbiota assessed by HOMIM except its effect on MS assessed with plate culturing. This is consistent with a previous pilot study in which 14 species were evaluated by DNA-DNA hybridisation after 4 weeks use of xylitol (Söderling et al., 2011). In addition, no effect of xylitol on *S. sanguinis* or lactobacilli was reported previously (Loesche et al., 1984).

## 6.5 Methodological Considerations

All children participated in this study were males. The main reason was that no mixed gender government schools or community centres exist in Kuwait. As choosing two different schools with different genders may have added confounders, like compliance differences between the schools and between genders. However, this should not have

affected the results of this study as the earlier oral microbiota studies showed no effect of gender on the microflora (Kanasi et al., 2010a, b). In addition, no gender effect on caries prevalence in the Kuwaiti population (Al-Mutawa et al., 2006; 2010) and no significant difference in sweets and sugary products consumptions between male and female school children in Kuwait was found (Honkala et al., 2006). However, this study reported the relationship between daily habits and caries as observation in schoolboys (I).

In the present study, caries was measured using ICDAS system. The advantages of using the ICDAS system are that it detects early enamel caries lesions according to the stage of their progression, as well as categorise dentine caries lesions according to their progression (Pitts, 2009a; b). By measuring non-cavitated and cavitated caries lesions as well as sealants and recurrent caries lesions, ICDAS overcomes the deficiency in the WHO examination criteria. Codes 1 to 3 were used to present enamel caries and codes 4 to 6 were used to describe dentine caries lesions. All examinations were done at school by one trained examiner (EH) using a mobile dental unit and light, oral mirror, and air/water syringe.

All subjects were interviewed regarding their daily oral health habits by using a standardized interview form (Appendix 2). The interviews were performed by a dentist (ME) and a research assistant, both fluent in Arabic and English. The interview form consisted of questions about frequency of brushing (type of toothpaste), flossing, use of mouthrinse, consumption of sweets, soft drinks (frequency and type) and chewing gum (frequency and type). They were also asked about their possible use of any antibiotic during the last two weeks.

Samples were collected by experienced professionals, and all the procedures were performed according to standardised methods reported in the literature. Saliva was collected from children in a standardized manner at the same time in both pre- and post-intervention. Children were trained how to salivate during the screening period. Plaque samples also were collected in a standardized way by a dentist (ME) and from same sites of all children using sterile mini brushes. Chair-side tests were performed on the spot and according to the manufacturer's instructions. Samples were managed and stored properly before culturing or DNA analysis. The chair-side test and plate culturing of MS were correlated. The storage and transportation procedure of bacteria were according to previously tested protocols. DNA extraction and HOMIM was done under supervision of experts in that field.

Chair-side MS tests were used for MS screening in study I and IV. These tests measure the combination of *S. mutans* and *S. sobrinus*. As only one subject showed a combination of *S. mutans* and *S. sobrinus* by plate culturing, we presented the MS counts

as *S. mutans* counts in Study I and IV. In the papers II and III, as well as in the summary, we use the term mutans streptococci (MS).

All children received a code. All oral examination sheets, interview sheet, saliva, plaque, and DNA samples were coded. Groups were also coded and blinded for the participants and the examiners of the study until the final analysis. Participant sample and data were managed in full accordance with ethics.

# 7. CONCLUSIONS

The following conclusions were drawn from the results presented in this thesis:

**1.** ICDAS CI calculated from ICDAS caries scores correlated with other caries indices and can be used as a measure of caries severity. The consumption of soft drinks and sugar in Kuwait schoolboys was generally high and correlated with caries severity. Brushing habits were the only OH habits associated with reduced caries severity.

**2.** New bacterial species like *A. defectiva*, *A. meyeri/A. odontolyticus,* and *S. satelles,* were associated with caries occurrence/absence.

**3.** Apart from decreasing MS, xylitol had no effects on other members of the oral microflora.

**4.** A xylitol mouthrinse is effective in reducing salivary MS in children with high MS and its effects are comparable to other xylitol products.

# ACKNOWLEDGEMENTS

I owe my deepest gratitude and admiration to Dr Eino Honkala, the person who taught me everything I know in research and own everything I achieve so far. Ten years ago, he adopted me as a student in my fifth-year at dental school and invested his time and effort creating the person who I am now. He was always beside me with his continuous guidance, encouragement, and positive attitude. When I face an ethical or professional dilemma, I always deal with it with the thought of "How Dr Eino would deal with such dilemma" as he is the best reference of professionalism anybody would ever refer to. You, Dr Honkala, are the ideal example of the researcher who dedicated his life for research. Words are not enough to express how grateful and thankful I am.

I wish to express my deepest gratitude to my second supervisor, Dr Eva Söderling, her guidance, time, advice, and support during these years. It was an honor working with her. Her insightful criticism and valuable comments in preparing manuscripts and her superior expertise, especially in the field of microbiology, are reflected in this work.

I'd like to thank Dr. Sisko Honkala for being a supportive supervisor, advocate and friend who is always willing to listen. Her kind words of encouragement, support and valuable advice were a push every time I was pulled backwards.

Professor Jorma Virtanen, University of Oulu, and Professor Walter A. Bretz, University of New York, are warmly thanked for reviewing this thesis, and providing valuable comments, ideas and criticism, which greatly improved this work at its final stages. Also, I would like to thank Mrs. Sharon Wood for language checking the thesis.

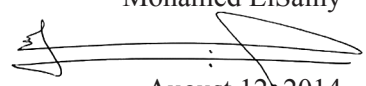
I am greatly thankful for Miss Anisha Varghese for her great help during the projects especially in sample collections and preparations. I also wish to express my sincere gratitude to all my co-authors for their work and improvements of the publications: Margherita Fontana, Susan Flannagan, Alexis Kokaras, and Bruce J. Paster.

I would like to thank my parents, brothers, and friends for their unconditional love and support.

I wish to express my deepest respect for the participants of this study. Without volunteers, this thesis work would not have been possible.

This study was supported by Kuwait University grants DD02/10, GD1/11, SRUL02/13.

Mohamed ElSalhy



August 12, 2014

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# APPENDICES

## Appendix 1 – ICDAS Form

### Kuwaiti Xylitol Clinical Trial

**Number:** I___I___I___I **Date of Birth:** I___I___I___I

| Surface | | | 55 | 54 | 53 | 52 | 51 | 61 | 62 | 63 | 64 | 65 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 17 | 16 | 15 | 14 | 13 | 12 | 11 | 21 | 22 | 23 | 24 | 25 | 26 | 27 |
| M | | | | | | | | | | | | | | |
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| B | | | | | | | | | | | | | | |
| L | | | | | | | | | | | | | | |
| ⌾ | | | | | | | | | | | | | | |

| Surface | | | 85 | 84 | 83 | 82 | 81 | 71 | 72 | 73 | 74 | 75 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 47 | 46 | 45 | 44 | 43 | 42 | 41 | 31 | 32 | 33 | 34 | 35 | 36 | 37 |
| M | | | | | | | | | | | | | | |
| O | | | | | □ | □ | □ | □ | □ | □ | | | | |
| D | | | | | | | | | | | | | | |
| B | | | | | | | | | | | | | | |
| L | | | | | | | | | | | | | | |
| ⌾ | | | | | | | | | | | | | | |

| **Restoration and Sealant Codes**: | **Caries Codes** |
|---|---|
| 0 = Not sealed or restored | 0 = Sound tooth surface. |
| 1 = Sealant (partial) | 1 = First visual change in enamel. |
| 2 = Sealant (full) | 2 = Distinct visual change in enamel. |
| 3 = Tooth colour restoration | 3 = Enamel break down. No dentin visible |
| 4 = Amalgam restoration | 4 = Dentinal shadow (not cavitated into dentin) |
| 5 = Stainless steel crown | 5 = Distinct cavity with visible dentin. |
| 6 = Porcelain, gold, PMF crown or veneer | 6 = Extensive distinct cavity with visible dentin. |
| 7 = Lost or broken restoration | **Missing Teeth** |
| 8 = Temporary restoration | 97 = Extracted due to caries |
| | 98 = Missing for other reason |
| | 99 = Un-erupted |
| | P = Implant |

## Appendix 2 – Interview Form

### Kuwaiti Xylitol Trial Questionnaire

**Number:** I___I___I___I

How long time ago did you visit a dentist?

- o Less than one year ago
- o 1-2 years ago
- o More than 2 years ago

Have you ever experienced toothache?

- o No
- o Yes, less than 1 year ago
- o Yes, 1-2 years ago
- o More than 2 years ago

How often do you brush your teeth?

- o More than once a day
- o Once a day
- o Less than once a day

brand of toothpaste_____

How often do you use dental floss?

- o More than once a day
- o Once a day
- o Less than once a day

Do you use a mouthrinse?

- o More than once a day
- o Once a day
- o Less than once a day
- o If yes, Brand_____

How often do you use regular sweets (sugarfree do not count)?

- o More than once a day
- o Once a day
- o Less than once a day

How often do you use soft drinks/juices (light drinks do not count)?

- o More than once a day
- o Once a day
- o Less than once a day

Do you use chewing gum?

- o More than once a day
- o Once a day
- o Less than once a day
- o If yes, brand_____

Do you use any medication just now or few weeks ago?

- o No
- o Yes, what?_____