TURUN YLIOPISTON JULKAISUJA
ANNALES UNIVERSITATIS TURKUENSIS

SARJA - SER. D OSA - TOM. 979

MEDICA - ODONTOLOGICA

TARGETING OF CARIES PREVENTION AT PRESCHOOL CHILDREN

A practice-based study

by

Pia Meurman

TURUN YLIOPISTO
UNIVERSITY OF TURKU
Turku 2011

The Department of Community Dentistry, Institute of Dentistry
University of Turku, Finland

Supervisors

Professor Pentti Alanen DDS, PhD
Department of Community Dentistry
Institute of Dentistry
University of Turku

Docent Kaisu Pienihäkkinen DDS, PhD
Department of Community Dentistry
Institute of Dentistry
University of Turku

Reviewers

Docent Liisa Seppä DDS, PhD
Department of Pedodontics, Cariology and Endodontology
Institute of Dentistry
University of Oulu

Associate Professor, Docent Sisko Honkala DDS, PhD, MSc
Department of Developmental and Preventive Dentistry
Faculty of Dentistry
Kuwait University
Kuwait

Opponent

Professor Satu Alaluusua DDS, PhD
Department of Pediatric Dentistry and Orthodontics
University of Helsinki

ISBN 978-951-29-4732-4 (PRINT)
ISBN 978-951-29-4733-1 (PDF)
ISSN 0355-9483
Painosalama Oy – Turku, Finland 2011

ABSTRACT

Pia Meurman

Targeting of caries prevention at preschool children, a practice based study

Department of Community Dentistry, Institute of Dentistry, University of Turku
Annales Universitatis Turkuensis, Turku, Finland 2011

Aim and design: To evaluate an oral health program directed to expecting families and their children. The intervention was carried out in one of the four health care areas of the city of Turku. Another area acted as a control.

Subjects and methods: Children (n = 1217), born between January 1, 1998 and June 30, 1999, in the respective health care areas were screened for mutans streptococci bacteria (MS), and their caretakers were interviewed when the child was 18 months old. MS-colonization was used as the child's risk indicator. Intensified health education and the use of xylitol lozenges targeted at the children at risk were the main elements of the program. Controls and the non-MS-colonized children received routine prevention – examination and education at the ages of three and five years. Altogether 794 subjects were followed for 42 months after receiving consent from their caretakers. Associations of oral-health-related factors with MS colonization and caries increment were studied inside the control group.

Results: MS colonization associated with the occupation of the caretaker and ethnicity. The program was effective in white-collar families; prevented fraction being 67 %. In blue-collar families no effect was achieved. At the age of five years, caries increment was strongly related to the occupation of the caretaker, MS at 18 months, child's sugar use, night feeding, use of thirst quencher at the age of 18 months, and father's reported oral health.

Conclusions: Programs targeted at MS-colonized children can reduce caries in white-collar families. A program mainly based on activity at home seems to favor white-collar families, whereas different kind of support is needed for the blue-collar families.

Keywords: Caries, ECC, longitudinal study, prevention, preschool children, risk-assessment, xylitol.

TIIVISTELMÄ

Pia Meurman

Riskiperusteinen karieksen ehkäisyohjelma pikkulapsilla

Sosiaalihammaslääketiede, Hammaslääketieteen laitos, Turun yliopisto
Annales Universitatis Turkuensis, Turku, Suomi 2011

Tutkimuksen tarkoituksena oli arvioida varhaisen, riskinarviointiin perustuvan karieksen ehkäisyohjelman vaikutusta hampaiden reikiintymiseen pikkulapsilla. Parannusta ja tehoa reikiintymisen ehkäisyyn haettiin ohjelmalla, joka voidaan siirtää hammashoitoloista neuvolaan ja koteihin. Työnjakoa pyrittiin parantamaan delegoimalla ohjelma suuhygienisteille ja hammashoitajille sekä neuvolan terveydenhoitajille.

Materiaalit ja menetelmät: Tutkimus toteutettiin kahdella Turun neljästä terveydenhuollon alueesta. Aikavälillä 1.1.1998 – 30.6.1999 syntyneen 1217 lapsen mutansbakteeritartunta mitattiin 18 kuukauden iässä hammaspeitteestä yksinkertaisella testillä suun terveydentilan tarkastus- ja neuvontakäynnin yhteydessä. Lapsen huoltajaa haastateltiin suun terveyteen liittyvistä tottumuksista. Jos mutansbakteereja kasvoi testinäytteessä, koealueella asuva lapsi vanhempineen kutsuttiin suuhygienistin vastaanotolle ja ehkäisyohjelma aloitettiin vanhempien suostuessa. Ohjelmaan kuuluivat puolivuosittaiset neuvontakäynnit suuhygienistin vastaanotolla sekä kolmesti päivässä nautittavat ksylitolipastillit. Muille turkulaislapsille tarjottiin perinteistä ohjelmaa, johon kuului seuraavat tarkastus- ja neuvontakäynnit kolme- ja viisivuotiaana. Seurannassa oli 794 lasta viisivuotistarkastukseen asti, jolloin verrattiin koe- ja vertailualueen terveystuloksia.

Tulokset: Mutansbakteerien tarttuminen oli varhaisempaa lyhyemmän koulutuksen ammateissa toimivien perheiden lapsilla. Varhainen mutanstartunta ja perhetausta ennustivat lapsen hampaiden reikiintymistä viisivuotiaana. Ohjelma paransi pitemmän koulutuksen ammateissa toimivien koealueen perheiden lasten hammasterveyttä 67 %:lla vertailualueen vastaaviin lapsiin verrattuna. Lyhyemmän koulutuksen ammateissa toimivien perheiden lasten hammasterveydessä ei ollut eroa koe- ja vertailualueella.

Päätelmät: Pääasiassa kotona toteutetulla terveysohjelmalla saadaan vähennettyä hampaiden reikiintymistä niiden perheiden lapsilla, joiden vanhemmat toimivat pitemmän koulutuksen ammateissa. Muille perheille tarvitaan lisää tai toisenlaista tukea. Kotioloilla näyttää olevan tärkeä merkitys suunterveyden ylläpitämisessä alusta alkaen. Lasten hammasterveyden välisten erojen tasoittamiseksi tarvitaan tukea ja keinoja myös hammaslääketieteen keinojen ulkopuolelta.

Avainsanat: Kariespreventio, ksylitoli, pitkittäistutkimus, pikkulapset.

TABLE OF CONTENTS

ABBREVIATIONS

ARR	absolute risk reduction
BMI	body mass index
CA	Candida yeasts
CHX	chlorhexidine
CI	confidence interval
dmft	number of decayed, missing or restored primary teeth
dmfs	number of decayed, missing or restored primary tooth surfaces
DNA	deoxyribonucleic acid
ECC	early childhood caries
idmft	number of incipient caries lesion affected (active or arrested), decayed, missing or restored primary teeth
idmfs	number of incipient caries lesion affected (active or arrested), decayed, missing, or restored primary tooth surfaces
LB	lactobacilli bacteria
MS	mutans streptococci bacteria
MS+	mutans streptococci detected in the dental biofilm
MS-	mutans streptococci not detected in the dental biofilm
mo	month/months
NNT	number needed to treat
oh	oral hygiene
OHP	oral health program
OR	Odds ratio
p	value for significance
pH	scale for measuring acidity or basicity in solutions
SBU	The Swedish Council on Technology Assessment in Health Care
S-ECC	severe early childhood caries
SES	socioeconomic status
Sn, (Sp)	sensitivity, (specificity)
Xr, (Xs)	xylitol resistant, (sensitive) MS bacterium
yr	year/years
Δ	increment

LIST OF ORIGINAL PUBLICATIONS

The present study results are based on the original publications listed below. The copyright holders have kindly permitted the reproduction.

I Oral health programme for preschool children, a prospective, controlled study. Meurman P, Pienihäkkinen K, Eriksson A, Alanen P. Int J Paediatr Dent 19: 263-273; 2009.

II Xylitol-resistant Mutans streptococci Strains and Xylitol Consumption in Young Children. Meurman P, Meriläinen L, Pienihäkkinen K, Alanen P, Trahan L, Söderling E. Acta Odontol Scand 63: 314-316; 2005.

III Mutans streptococci colonization associates with the occupation of caretaker, a practise-based study. Meurman P, Pienihäkkinen K, Eriksson A, Alanen P. Int J Paediatr Dent 20: 144-150; 2010.

IV Factors associated with caries increment: a longitudinal study from 18 months to 5 years of age. Meurman P, Pienihäkkinen K. Caries Res 44: 519-524; 2010.

1. INTRODUCTION

Dental caries is widely spread throughout the world. It affects children and adults of all ages. Caries prevention has been conducted and studied for decades. In Finland, the focus in the Public Health law, in the early 1970's, was on preventive measures starting with young generations. As a consequence, health programs were established in the public dental health care centers. Dentists and, later towards the end of the century, dental hygienists and nurses, visited the child health clinics and schools giving education mainly about oral hygiene, the use of fluorides, and a healthy diet. In many cities and towns, the dental clinics were situated in major school buildings to facilitate the treating of children for caries and malocclusions. In those days, manifest dental caries in the permanent dentition was restored, whereas the primary dentitions often remained untreated. Only after research had revealed a number of predisposing or etiological factors of caries, was the health of primary teeth also considered important. The younger the child the more effort is needed for the handling of the child. Preventing or even postponing the disease could diminish the pain, the inconvenience, the health risks, and the costs of treatments.

In Finland, the prevailing health care system reaching practically all newborn infants with their caretakers is well suited for early-started oral health programs and studies. For decades, health programs have been conducted in both child health centers and dental clinics. The importance of health promotion has been recognized and accepted.

At the end of the 20th century, manifest caries affected approximately every tenth three-year-old child. This group of children, already at a young age and later in the following years, increasingly requires resources. If this group of cohorts could be identified early enough, preferably before any visible signs of caries can be detected, prevention could be targeted at it, and thus the measures could be more individual and intensive. Targeting the preventive measures at subjects or families at risk could be profitable. Risk-based programs had already been developed and promising results had been achieved in smaller communities. At the same time, all families were considered to be in need of basic education about favorable oral health habits.

2. REVIEW OF THE LITERATURE

2.1 Dental caries

Today, dental caries, an infectious, microorganism-bound disease, is considered to be an ecological destruction process on a tooth surface and its visible results (Marsh 2003). The microbes on tooth surfaces live as a microbial community, an ecosystem in the biofilm. The carious process is a consequence of a collapse of the equilibrium in the ecosystem (Marsh 2003). The equilibrium, the microbial homeostasis, is a complex interaction between the tooth surface, oral biofilm, salivary characteristics, immunity, dietary factors, and behavior (Marsh 2004). The disturbance in the equilibrium can occur, for instance, if the salivary secretion decreases or sugar intake increases. The disturbed, and hence unbalanced, equilibrium between the tooth surface and its environment can with time result in the initiation of a carious process.

The activity of microorganisms in caries was recognized already in the nineteenth century (Miller 1889). In the latter part of the twentieth century, especially the mutans streptococci (MS) have frequently been studied in connection with dental caries (Edwardsson et al. 1972, Hoerman et al. 1972, Tanzer and Freedman 1978). The actual process still engages researchers. The biofilm, dental plaque, is regarded as a prerequisite for the carious process. The biofilm bacteria attach to the tooth surface by producing glucans. The glucans, water-insoluble polysaccharides, can enhance the bacterial adhesion to other bacteria and their accumulation on the tooth surfaces. The biofilm bacteria, in their metabolism, use various sugars, and in consequence, produce organic acids which cause the pH to drop. In acidic conditions, the tooth surface minerals are dissolved, demineralized. Precipitation (= remineralization) follows when the pH rises again mainly due to the buffering effects of the saliva. The pH thus fluctuates continuously and if the minerals, during the acidic periods, are cumulatively lost (net mineral loss), the surface becomes porous and can be observed as a white spot carious lesion. When sugars are available, the bacterial activity accelerates, and thus the pH drops. An acidic environment again, favors the growth of the MS. Moreover, the streptococci bacteria are able to stock sugars as polysaccharides and use them as an energy source (Takahashi and Nyvad 2008).

2.2 Prevalence of early childhood caries

The prevalence of early childhood caries (ECC) differs considerably both in different parts of the world and within the countries and regions (Table 1). In the developed countries, the prevalence has slowly been decreasing as the standard of living has increased. The distribution of dental caries is skewed and, when decreasing, affects a diminishing proportion of the population. The underprivileged, the socially deprived, or families with a shorter educational background, seem to be most affected from the start of their infant's life (Radford et al. 2001, Beighton et al. 2004, Pine et al. 2004).

Table 1. Reported caries prevalences of young children

Country	n	Age	ECC, Dentinal caries, dmft/ dmfs >0	Caries + i >0	Reference
Brazil	602	5 years		55 %	Carvalho et al. 2009
Finland	828	5 years	28 %		Mattila et al. 2000
Finland /Tornio	200	5 years	34 %		Joensuu 2009
China	2014	3-5 years	55 %		Du et al. 2007
Germany	434	2-6 years	39 %		Robke 2008
Japan	3086	3 years	31 %		Aida et al. 2008
Lithuania	950	2.5-3.5 years	51 %		Slabsinskiene et al. 2010
Norway	1348	5 years	11 %		Wigen et al. 2010
Netherlands	435	5 years	49 %		Elfrink et al. 2006
Sweden	289	3 years		29 %	Wendt et al. 1996
Thailand	157	15-19 months		83 %	Vachirarojpisan et al. 2004

Key: ECC =early childhood caries, in dmft/dmfs d = decayed, m = extracted, f = restored, t = tooth / teeth, s = tooth surface / surfaces, i=incipient carious lesion / lesions

2.3 Risk assessment

Risk in health sciences is defined as the probability that an event will occur. The risk factor is present before the disease or other outcome occurs, and longitudinal studies to reveal them are needed (Burt 2001). In risk assessment programs, the risk is assessed using a method that reveals an anticipated risk factor or behavior. The more the early dental caries has been studied the more causal or associating factors have been suggested as being predictors or determinants of the disease.

The Swedish oral health authorities (SBU, The Swedish Council on Technology Assessment in Health Care) published a systematic, thorough literature review on dental caries, diagnosis, risk assessment, and non-invasive treatment in 2007 (Mejàre et al. 2007). The studies concerning risk assessment were sought from the electronic libraries of Medline and PubMed. The search included studies between 1966 and 2006. Finally, 63 articles remained for evaluation, 21 concerning preschool-aged subjects. In the final evaluation of 19 studies concerning preschool-aged children, three reached the high level of quality and relevance and three the medium level.

The best combination of predictors, among the high quality studies, were sociodemographic and dietary factors, together with the occurrence of MS in the Swedish study of one-year-olds (Grindefjord et al. 1995). The best single predictor of caries was an immigrant background (Grindefjord et al. 1995). A Finnish study found the best predictive value in the combination of incipient caries lesions, dietary habits and MS, the latter being also the best single predictor of future caries (Pienihäkkinen et al. 2004). The caries predictive ability of frequent consumption of sweets, although a significant risk factor, was limited

in both studies. Several research groups studied the occurrence of MS as a single predictor of caries. In a Swedish study, no association was found between salivary MS at the age of one year and caries at the age of three years (Wendt et al. 1996). A limited value of MS as a single predictor of caries was found in two studies (Grindefjord et al. 1995, Pienihäkkinen et al. 2004). As predictors, the occurrence of lactobacilli and salivary characteristics reached a low value in caries prediction. Visible plaque and oral hygiene as caries predictor lacked relevance in several studies, or else the factor was not unequivocally graded and studied. In three studies, a combination of two or three predictors reached an average of 80 % in accuracy (Demers et al. 1992, Grindefjord et al. 1995, Pienihäkkinen et al. 2004). Birth weight showed no association with caries (Burt and Pai 2001).

The SBU reviewers commented that there is no evidence of gender-bound differences in prediction of caries. The populations, study criteria, study designs, result measures, diagnostic criteria, analyses etc vary substantially among the individual studies, thus complicating the comparison. The results are not necessarily comparable in different socioeconomic or caries prevalence conditions. Concerning preschool children, the conclusions of the review were that caries experience was the best single predictor of future caries. Adding predictors improves the prediction accuracy up to an average of 80 %, being higher in preschool children compared with school children. MS colonization at the age of one to two years as a single predictor was not found accurate enough. The use of sugary products or candies as predictors had a relatively low sensitivity (Table 2).

Table 2. Conclusions in the review of risk assessment by The Swedish Council on Technology Assessment in Health Care (Mejàre et al. 2007)

Risk predictor	Age	Prediction interval	Sn/ Sp %	Comment	Level of evidence
Caries experience	preschool	-	-	Best single predictor	1
MS	1-2 years	2-3 years	-	As single predictor not reliable	1
Use of sugary products, candies > once a week	1-2 years	-	72-84 / 45-55	As single predictor Sp relatively low	1

Key: Sn = sensitivity, Sp = specificity. Level of evidence: 1 = evidence from at least two studies of high evidential value, (-) = information not given or counted in the review

2.3.1 Literature search

For the present study, using the same inclusion criteria, a systematic search in the PubMed (MEDLINE) library was made in November 2009. Reports published after the SBU reports, between the beginning of 2006 and November 2009, were sought. The search query is shown in the Appendix, page 47. It yielded originally 814 publications. After excluding studies of subjects older than six years, studies concerning disabled or

sick children (e.g. diabetes, cancer), studies in other languages than English or German, cross-sectional and in vitro studies, a total of 22 studies met with the inclusion criteria for the final evaluation. The quality of 12 studies was ranked high, or relatively high and seven were ranked medium by the present author. The ranking criteria (Table 3) were in line with the Swedish review (Mejàre et al. 2007). The high or relatively high quality 12 studies met with a majority of the inclusion criteria (Oliveira et al. 2006, Oscarson et al. 2006, Petti and Hausen 2006, Yonezu et al. 2006a, Marshall et al. 2007, Sakuma et al. 2007, Ismail et al. 2008, Minah et al. 2008, Nishimura et al. 2008, Skeie et al. 2008, Holgerson et al. 2009, Tamaki et al. 2009). Seven studies of medium quality lacked more than the previous reports in terms of the criteria used in their design, inclusion criteria, analyses, reports methods, or follow-up time (Seki et al. 2006, Ollila and Larmas 2007, Scavuzzi et al. 2007, Teanpaisan et al. 2007, Ollila and Larmas 2008, Yonezu and Yakushiji 2008, Warren et al. 2009). Three studies lacked more criteria than the above-mentioned reports, and thus were ranked as low quality studies (Zhan et al. 2006, Yonezu et al. 2006b, Cogulu et al. 2008).

Table 3. Criteria for inclusion and evaluation of studies in the SBU review, unofficial translation of the original criteria by The Swedish Council on Technology Assessment in Health Care (Mejàre et al. 2007)

Design	Longitudinal cohort, randomized study Same outcome, different prediction model Children: only one aetiological factor or caries experience as a predictor
Inclusion	Defined inclusion criteria Defined population $n \geq 70$, representative with no selection bias Clinical and demographic criteria described Heterogenic cohorts included if stratified in the analyses All intended individuals should be included in the analyses
Method	Diagnostic criteria described Only one examiner accepted if the same examiner at baseline and follow-up Defined prediction variables Defined validity variable(s)
Follow-up	Minimum 2 yr for permanent teeth and 1 yr for deciduous teeth
Result & Analysis	Caries as outcome variable: teeth, surfaces, dentin, enamel or combination Result measured: sensitivity and specificity, relative risk or Odds ratio or ROC Studies on posteruptive age as risk factor included if incidence or survival analysis was done or could be calculated

Of the selected high or medium ranked studies, the main caries predictors, the number and age of subjects, and the follow-up time, together with the main outcome measure are presented in Table 4. Low ranked studies, studies with a follow-up time less than one year were left out of the table as were studies evaluating predictive tools.

Table 4. High or medium ranked caries risk assessment studies with a minimum follow-up time of one year

Author, year country	n	Age	Follow-up time	Main outcome measure	Main caries predictor
Ismail et al. 2008 USA	788	0-5 yr	24 mo	ECC, S-ECC	age, gender, carer's age, religiosity (protective), fatalistic belief, soft drinks
Marshall et al. 2007 USA	427	0	4.5-6.9 yr	caries experience and BMI	low SES, mother's education, age, overweight
Minah et al. 2008 USA	219	6-15 mo	26 mo	caries dmfs	plaque, MS in low SES subjects
Oliveira et al. 2006 Brazil	228	12-36 mo	36 mo	ECC, S-ECC	enamel defects, night breastfeeding, poor oh
Ollila and Larmas 2007 Finland	183	2 yr	7 yr	time between birth and molar restoration	candies x2 week, poor oh (prolonged night bottle, pacifier)
Ollila and Larmas 2008 Finland	166	0.7-4.3 yr	7 yr (range 2.0-8.9 yr)	time between birth and molar restoration	salivary LB and CA
Oscarson et al. 2006 Sweden	118	2 yr	2 yr	main outcome measure MS colonization, caries development secondary	MS

Table 4 continued

Author, year country	n	Age	Follow-up time	Main outcome measure	Main caries predictor
Petti and Hausen 2006 Italy	121	3 yr	18 mo	mean dft increment	salivary MS level
Sakuma et al. 2007 Japan	5107	18 mo	18 mo	caries increment	sugar consumption, breastfeeding, caries experience
Scavuzzi et al. 2007 Brazil	186	12-30 mo	1 yr	dmft and idmft	incipient lesions, plaque
Skeie et al. 2008 Norway	304	3-5 yr	2 yr	'severe caries increment'	immigrant status, attitude to diet, caries experience
Teanpaisan et al. 2007 Thailand	169	3 mo	9-24 mo	number of ds	salivary MS, LB, early, persistently high
Warren et al. 2009 USA	128	6-12 mo	18 mo	caries incidence rate	salivary MS, sugary drink
Yonezu et al. 2008 Japan	401	18 mo	18 mo	caries at 36 mo	non nutritive sucking habits

Key: CA = candida, ECC = early childhood caries (S = severe), LB = lactobacilli, MS = mutans streptococci, mo = month, oh = oral hygiene, yr = year, SES = socioeconomic status, BMI = body mass index

A variety of indicators or risk factors were found to associate with ECC, in line with the SBU review (Mejàre et al. 2007). Previous caries experience and incipient, white spot lesions, actually the early stages of the disease, were regarded as good or the best predictors of future caries development (Seki et al. 2006, Scavuzzi et al. 2007, Ismail et al. 2008, Warren et al. 2009).

In three high ranked reports, MS were found to be a significant risk factor of caries in young children. A high specificity of 0.98 and a sensitivity of 0.64 of MS was reported among one-year-old low-income residents (Minah et al. 2008). According to Holgerson and co-workers oral MS in two-year-olds were the strongest single factor associated with caries development at the age of seven in a community with low caries prevalence (Holgerson et al. 2009). Furthermore, the initially 3-year-olds, whose salivary MS levels were high, showed significantly more dentinal caries than those with low MS levels (Petti and Hausen 2006). The risk group classification, based on MS levels was reported to predict caries. The researchers also reported that chlorhexidine (CHX) gel applications, although they lowered the salivary MS counts, had no effect on caries incidence (Petti and Hausen 2006). Furthermore, early MS colonization was reported to associate with caries (Seki et al. 2006, Teanpaisan et al. 2007, Cogulu et al. 2008, Warren et al. 2009). A single test result of MS is relatively strongly and significantly associated with future caries and thus useful in risk assessment, although unreliable for screening purposes. This is in line with the SBU conclusions.

Lactobacilli (LB) were detected in three-month-olds and, together with MS, associated with prevalence and severity of caries. Furthermore, LB were reported as being markers of caries progression, whereas MS indicated a risk of the initiation of caries (Teanpaisan et al. 2007). In a follow-up study, LB together with Candida yeasts at baseline were reported to associate significantly with the mean net caries increment in primary teeth (Ollila and Larmas 2008).

Socioeconomic factors, ethnicity, as well as the age or education level of the caretakers, have largely been investigated as predictors of ECC. In fact, several recent studies have been conducted among socially deprived subjects (Oliveira et al. 2006, Ismail et al. 2008, Minah et al. 2008, Warren et al. 2009). Low socioeconomic status (SES) (Marshall et al. 2007) and the parents' dental attitudes and beliefs (Skeie et al. 2008) were reported as being a strong predictor of caries. An immigrant background, in line with the SBU review, was reported as predicting 'severe caries increment' together with caries experience and the attitude to diet (Skeie et al. 2008). In addition, religiosity (a protective factor) or fatalistic beliefs, the older age of the caretaker and child, together with dietary factors were reported to associate with caries in young children (Ismail et al. 2008).

Feeding habits, sugar use, soft drinks and overweight were found to be strongly connected with ECC (Marshall et al. 2007, Ismail et al. 2008, Nishimura et al. 2008, Skeie et al. 2008, Holgerson et al. 2009, Warren et al. 2009). Early weaning (Yonezu et al. 2006a,

Nishimura et al. 2008) was found protective, whereas prolonged breastfeeding before the deciduous dentition is fully erupted was found to be a risk factor for ECC (Sakuma et al. 2007). On the other hand, finger sucking was not reported to increase the caries risk (Yonezu and Yakushiji 2008). Nursing bottle at night, inappropriate oral hygiene and the use of candies at the age of two years were reported as significant caries risk indicators in a seven-year follow-up (Ollila and Larmas 2007). Early weaning, less sucrose intake by the child, and tooth brushing by the parents lowered the child's caries risk in the study by Nishimura and co-workers (Nishimura et al. 2008). The early started use of F-toothpaste, early weaning, and sugar control were found to be effective in reducing the risk of caries (Sakuma et al. 2007).

Three of the analysed studies included a prevention program (Oscarson et al. 2006, Petti and Hausen 2006, Minah et al. 2008). A high level of MS counts was reported to be a caries risk-assessment tool in the study by Minah and co-workers. Petti and Hausen also reported high salivary MS levels to be significant risk factors of caries development (Petti and Hausen 2006). In the study by Oscarson and co-workers, the level of MS colonization was the main outcome measure, and the only significant relation was found in MS levels between the child and the mother (Oscarson et al. 2006).

For the prediction of caries, a variety of prediction tools have been developed. Among data tools (data mining) the decision analysis was the most reliable tool compared with regression and neural network (Tamaki et al. 2009). A modified Cariogram test (a computer program assessing the risk profile by 10 caries-related factors) was validated, but was not found useful in revealing high risk of young children in a low caries-prevalence community (Holgerson et al. 2009). A third test, the Cariostat, a caries- activity test based on two pH indicators, was able to predict caries status at the age of 42 months and risk at the age of both 18 months and 24 months (Nishimura et al. 2008).

2.4 Prevention of early childhood caries

Primary prevention is prevention at a phase where there are no visible signs of the disease. Prevention of dental caries can be achieved either by focussing on the tooth or on the near or more distant environment around it. Basic caries prevention is based on either an influence on microorganisms, nutrients, or the tooth itsef. Prevention can be individually targeted or population-based. The individual prevention can be achieved by means of reinforcing the tooth surface with fluorides or, for instance, by improving the salivary function. Individual prevention can be achieved by restricting the availability of nutrients, mainly sugars, or by either weakening or inhibiting the microorganisms or their transmission. However, also more distant elements connected with the living conditions or parenting are possible.

The main elements of prevention in a health program can be mixed to suit the target individuals or populations. Population-based programs like water, milk or food fluoridation can reach the whole population regardless of compliance. Worldwide, about

300 million people, in 39 countries, live in areas where water is fluoridated (Pizzo et al. 2007).

The availability and every-day use of fluorides is universally considered important. In recent review articles, the individual use of fluoride supplements, such as fluoride toothpaste, was found effective in preventing caries among children (Rasines 2010, Walsh et al. 2010). In primary dentition, however, the evidence of the preventive effect of fluoride supplements was found to be weak or inconsistent in a previous review (Ismail and Hasson 2008). On the other hand, in Japan, the early use of fluoridated toothpaste was reported as an effective caries prevention method (Sakuma et al. 2007). Adequate use of fluoride toothpaste and good oral hygiene were recently recommended in the guidelines on children's fluoride use given by the European Academy of Paediatric Dentistry (EAPD 2009).

In Finland, practically all communities have programs and guidelines for oral health promotion. The combination of supervised tooth brushing and fluoride toothpaste has traditionally been the backbone of all dental health education for caretakers of young and older children. Additionally, professional applications of fluoride products have been used. However, if the level of basic prevention is high and the prevalence of caries is low in a community, focussing mainly on education and proper toothpaste use rather than professional application of fluoride gels or varnishes has been considered reasonable (Forss 1999, Seppä 2001).

Several reports have been published concerning associations of ECC with the infant's close environment, living conditions, socioeconomic status, dietary factors, and the caretakers' health, education, and oral health habits (Caufield et al. 1993, Wendt et al. 1996, Mattila et al. 2000, Tanner et al. 2002, Pine et al. 2004, Vachirarojpisan et al. 2004, Takahashi and Nyvad 2008, Mobley et al. 2009, Warren et al. 2009). Oral health programs have been designed to promote oral health by trying to tackle these factors. Conventionally, dental personnel have carried out the programs. However, reports of other professionals promoting oral health have been published. In the UK, primary caries prevention was effective, when trained educators regularly visited the homes of infants for a period of three years, and educated the mothers (Kowash et al. 2000). Also training pediatric doctors and clinic nurses in counseling caretakers about decreasing the ECC risks has been reported (Kressin et al. 2009). In consequence, a reduction of 77 % in the risk of developing ECC was reported (Kressin et al. 2009). In addition, interesting results have been reported concerning the use of probiotics in caries prevention in day-care centers (Näse et al. 2001). The group had suggested a favorable effect on general health of probiotics in milk (Hatakka et al. 2001).

During recent decades, risk-based programs have been designed. Especially in low caries-prevalence communities, prevention can be targeted at those with known or anticipated risk factors. The programs may contain education and motivation in oral hygiene, proper use of fluorides, restricting and substituting sugary products, and recommending

regular, healthy meals. Professionally, the dental biofilm can be either mechanically or chemically removed or altered. Targeted programs are either based on risk assessment or targeted at an age group or otherwise related group, for instance, a kindergarten, or at a group with similar living conditions.

Among oral health programs, training in plaque control and education on oral health have been extensively practiced in Nexø, Denmark (Ekstrand and Christiansen 2005). The program is based on education, training in plaque control at home, mechanical plaque removal, and fluoride applications.

The evaluated and published Finnish oral health programs have mostly been targeted at schoolchildren or adolescents (Seppä et al. 1991, Hausen et al. 2000, Hausen et al. 2007). Some reports and evaluations have been published on the oral health programs for young children in Finland, e.g. a risk assessment study and its evaluation in the community of Vanha Korpilahti (Pienihäkkinen and Jokela 2002, Pienihäkkinen et al. 2004). In another report from Kemi, a longitudinal, controlled study compared the costs of two prevention models. It was concluded that early started prevention and caries control resulted in better or at least as good dental health with lower cumulative costs compared with a conventional prevention model (Joensuu 2009). Both latter reports stress the early risk assesment and control of caries.

The current Finnish care guidelines for managing and controlling dental caries were published in 2009 (Hausen et al. 2009). The main message was emphasizing of self-care, controlling and maintaining good oral health by means of healthy meals and nourishment with proper intervals, restriction of sugary snacks or drinks, good oral hygiene, and the use of fluoride-containing toothpastes at home. Additional professional preventive measures were recommended for persons at known increased risk of caries or with visible incipient carious lesions.

2.5 Mutans streptococci

The mouth, being warm and moist, offering diverse surfaces, niches and nutrients, is a suitable environment for microorganisms. A multitude of microorganisms live in symbiosis with human beings. Bacteria start to colonize the infant´s mouth from birth on, transmission starting in the birth canal. The early colonizers attach to the soft tissues and can be detected within some hours after birth. With time, the number of bacterial species increases to several hundreds (Aas et al. 2005, Marsh and Nyvad 2008). Several reports have suggested high counts of MS as determinant of dental caries (Alaluusua and Renkonen 1983, Köhler et al. 1988, Alaluusua and Malmivirta 1994). Of the studied oral bacteria, especially *Streptococcus mutans* and *Streptococcus sobrinus* have been considered important caries-associated microorganisms (Tanzer et al. 2001) together with lactobacilli (Kanasi et al. 2010) and *Actinomyces* species (Beighton et al. 2004, Beighton 2005).

In the 1990's, Caufield and co-workers introduced the idea of a window of infectivity where the MS colonization was reported occurring mostly between the ages of 19 and 31 months (Caufield et al. 1993). The MS counts coincided with the erupting tooth surfaces, hard surfaces enabling the proliferation of MS (Berkowitz et al. 1975, Tanzer et al. 2001). The early colonization on the tooth surfaces was considered crucial for rapid caries development. Since then, it has been suggested that the window of infectivity remains open for a longer period of time (Straetemans et al. 1998, Söderling et al. 2001). Contacts, especially frequent salivary contacts with the mother, were found to be the main source of MS (Li and Caufield 1995, Alves et al. 2009). Although the mothers have been the main transmitters of MS, also horizontal transmission has been reported (Kozai et al. 1999, Mattos-Graner et al. 2001, Alves et al. 2009). The bacterial colonization on the teeth is of prime interest, but the surface of the tongue has been regarded as a reservoir of several bacteria species and one of the earliest sites where the MS have been detected (Nyvad 1993, Wan et al. 2001, Tanner et al. 2002, Teanpaisan et al. 2007, Kanasi et al. 2010). From the saliva or mucous membrane, the MS have been isolated during the child's first year (Karn et al. 1998, Wan et al. 2001) and even as early as at the age of two months (Tankkunnasombut et al. 2009).

2.6 Prevention of mutans streptococci transmission

Since salivary contacts have been found to be the main transmitters of MS, several oral health programs have been targeted at highly MS-colonized mothers. Oral health programs for mothers have been reported to reduce the MS transmission and caries occurrence in their children (Köhler et al. 1984, Günay et al. 1998, Köhler and Andréen 2010). The programs included chlorhexidine rinses, along with counseling and professional tooth cleaning. Chlorhexidine gel was used with promising results in another trial to inhibit or postpone the MS transmission (Tenovuo et al. 1992). Long-lasting effects on inhibiting or postponing the MS transmission have been achieved by xylitol products used by the mothers of infants (Isokangas et al. 2000, Söderling et al. 2000, Söderling et al. 2001, Thorild et al. 2003, Laitala 2010). The effects of xylitol on the mother – child transmission of MS, have been reported to decrease first the child's MS colonization (Söderling et al. 2000, Thorild et al. 2003, Nakai et al. 2010) and subsequently, the child's caries experience for years (Isokangas et al. 2000, Laitala 2010).

2.7 Mutans streptococci and xylitol

Xylitol, a naturally occurring pentitol, and its effects on the oral cavity have been studied since the early 1970's and found to be beneficial for oral health (Scheinin et al. 1974). Regular use of xylitol decreases not only the transmission of MS, but also their quantity and growth in the dental plaque and thus, prevents caries (Mäkinen and Scheinin 1976, Söderling et al. 1989, Mäkinen et al. 1996, Söderling 2009).

The effect of xylitol both in caries prevention and in decreasing the MS has been found to be dose-dependent. In caries prevention, a low daily dose (0.5 to 1 g) of xylitol was not found effective in preschool children (Oscarson et al. 2006), whereas a 4 - 5 g daily dose of xylitol gum significantly reduced the MS counts in dental plaque (Autio 2002, Mäkinen et al. 2005). The effect of xylitol lozenges on dental caries has been reported to correspond to that of xylitol gums (Alanen et al. 2000). In a recent study, a daily dose of 8 g xylitol, in syrup form, effectively prevented caries in toddlers (Milgrom et al. 2009). The regular use of xylitol gums or lozenges requires adult supervision in children, and compliance or a tradition of oral self-care in adults. For instance, no significant differences in the microbial plaque composition of infants were achieved after the mothers' xylitol gum use compared with sorbitol gum or no gum (Fontana et al. 2009).

The MS in habitual xylitol users have shown a tendency towards the selection of so called "xylitol resistant" (Xr) strains of MS. These strains are adapted to the presence of xylitol although they are not able to ferment it and are thus presumed to be less virulent (Trahan 1995). Reports concerning the virulence of the "xylitol-resistant" strains have, however, been conflicting (Söderling et al. 1998, Assev et al. 2002). Anyhow, in habitual users of xylitol, the MS counts decrease and remain low (Söderling 2009). In addition, xylitol-sweetened gum has been reported to decrease the amount and acidogenicity of dental plaque (Söderling et al. 1989, Holgerson et al. 2007, Campus et al. 2009). The results were achieved using daily doses between 6 and 11 g of xylitol.

The early MS colonization, along with oral health habits, seem to be the most powerful predictors of ECC. The early MS colonization in the primary dentition can thus be used as an indicator of an elevated risk of caries. Screening toddlers for MS and targeting an effective oral health program at the children at risk and their caretakers could decrease the incidence of dental caries. In caries prevention, xylitol has been found effective in schoolchildren. Xylitol, regularly used by mothers of infants, decreases MS transmission and gains caries-free years in their children (Söderling et al. 2001, Laitala 2010). In newly MS-colonized young children, little is known about the effects of xylitol. It might be speculated that soon after MS colonization, the use of xylitol could weaken or even reverse the MS colonization. Regular use of xylitol as part of early started risk-assessment programs in newly colonized toddlers could thus enhance the prevention of ECC.

2.8 Initiation of the present studies

On assignment from the authorities in Turku Health Center, an approach with work division and a risk-based health program was sought and a trial organized. It was planned that the dental hygienists or nurses would carry out the program as a part of the oral health care system. The aim was to improve the caries prevention practices using a multiprofessional approach in collaboration with health nurses, dental hygienists and dentists. A prevention program should increase the proportion of healthy primary dentitions, and thus decrease the need for restorative treatment.

3. AIMS OF THE STUDY

The aim of the study was to evaluate an oral health program (OHP) intended to maintain children's good oral health.

Specific objectives were

- to find factors associating with MS colonization
- to evaluate, in MS-colonized children, the preventive effect of a new OHP compared with the traditional OHP
- to find out whether the proportion of the so-called "xylitol-resistant" strains of MS in the oral biofilm associate with xylitol use in order to develop a tool for evaluating the compliance of xylitol lozenges use as a part of an OHP
- to determine caries predictors, factors associating with caries increment.

4. SUBJECTS AND METHODS

4.1 Designs and characteristics of the studies

Turku is divided into four health care areas. Two of these areas, one for intervention and the other for control, were appointed for the study purposes by the health authorities in the city of Turku. Study I, performed in both appointed health care areas, was a longitudinal, controlled intervention study comparing the preventive effect of the traditional oral health program (OHP) and the risk-based OHP. Study II was a cross-sectional, explorative study investigating associations of xylitol use and selection of MS-strains in a group of preschool subjects. Study III with a cross-sectional design investigated associations of MS colonization with background factors. In a longitudinal risk-assessment study (IV), associations of background factors with caries increment were studied in the control group. Studies III and IV were performed in one health care area in Turku where the OHP was traditional. Studies I, III, and IV were conducted as a part of public dental health care. The subjects in study II participated in their local prevention programs. The research set-ups of the studies are presented in Table 5. The study plan was approved by the local ethics' committee (City of Turku, Health Office, November 16, 1997).

Table 5. Characterization of the studies

Study	Type	n	Outcome measure	MS detected	Indicator	caries status	Analysis
I	follow-up intervention/ control	794	- caries at 5 yr - caries reduction - NNT	site-test	group gender collected data	yes	chi square log reg ARR NNT
II	cross-sectional	64	- proportion of Xr	site-test laboratory	frequency of xylitol use	no	chi square
III	cross-sectional	512	- MS colonization	site-test	collected data	no	chi square log reg
IV	follow-up/ control	366	- caries 42 mo increment	site-test	collected data	yes	chi square log reg

Key: ARR = absolute risk reduction, log reg = logistic regression analysis, mo = months, MS = mutans streptococci, n = number, NNT = number needed to treat, Xr = xylitol resistant MS, yr = years

4.2 Study program

4.2.1 Study subjects and prevention groups

In two of the four health care areas in Turku, all 1275 children born between the 1st of January 1998 and the 30th of June 1999 formed the study group. At the age of 18 months, 1217 children (1128 Finnish and 89 ethnic children) were screened for MS. 1148 children were attainable in either of the the studies I, III and IV. The inclusion criteria for the present studies were the available screening test result, the interview, the information about the child's gender, and for the longitudinal studies the caries status at the age of 5 years ± 6 months.

Among the screened children, 69 in the intervention area and 20 in the control area were of ethnic origin (mother tongue other than Finnish or Swedish). The ethnic groups participated in the corresponding prevention programs. The written material in the intervention OHP was translated into their languages. Although interpreters were of help, they were not available during all visits. The instructions and education in the ethnic groups were thus not comparable to those communicating in their mother tongue. The ethnic groups were therefore left out of the analyses in study I, but included in studies III and IV (Figure 1). In the longitudinal study I, 334 children (30 %) had dropped out. 77 % of the drop-outs had moved out of town.

A total of 794 children met the inclusion criteria for the oral health program study, 446 in the intervention group and 348 in the control group (I). The study program (I) was directed to the MS-positive children in the intervention group. In the MS colonization study, 512 children in the control group met the inclusion criteria (III). The caries increment study (IV) comprised 366 subjects in the control group (Figure 1).

In the xylitol resistance study (II), the study group consisted of 64 screened MS-positive children (age between 19 and 46 months), 39 from Turku, and additionally 25 from the communities of Maaninka and Siilinjärvi, near the city of Kuopio.

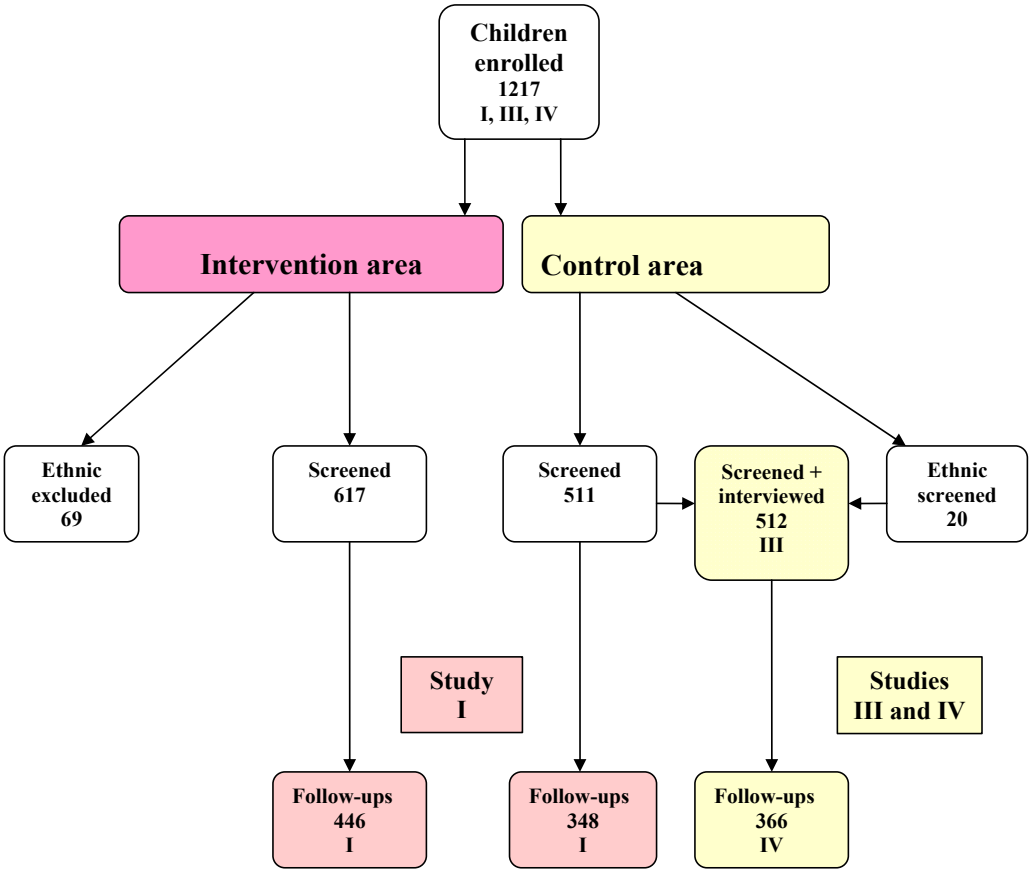

Figure 1. Number and living area of subjects in the longitudinal studies I and IV and the cross-sectional study III

4.2.2 Study regimen

At the age of 18 months, the child along with the caretakers, met the dental hygienist in the child health clinic. For the MS-colonized subjects in the intervention group, the second invitation to the hygienist's office was due after three months, and thereafter every six months until the age of 5 years (Table 6). During the following visits, the caretakers received repeated motivation, xylitol lozenges for the child, as well as information on fluorides, tooth brushing, healthy meals, snacks, and drinks. The hygienists were encouraged and advised to create a supportive, relaxed atmosphere during the OHP visits (Study I).

Table 6. Planned events for the study groups (I)

Group	18 mo	21 mo	27 mo	3 yr	3.5 yr	4 yr	4.5 yr	5 yr
Interv. MS+	Screening	visit	visit	Exam	visit	visit	visit	Exam
Interv. MS-	Screening			Exam				Exam
Control MS+MS-	Screening			Exam				Exam

Key: mo = months, yr = years, visit = oral health visit to the hygienist, Interv = intervention group, Control = control group, MS+ mutans streptococci-colonized, MS- no detected mutans streptococci bacteria in the dental biofilm, Exam = clinical examination

4.2.3 Clinical examinations

The children were examined at the age of 18 months in the child health clinics by one of three hygienists or three dentists (I, III, IV). The examinations in the child health clinics were done in well-lit rooms, the caretaker holding the child. An air syringe was not available. The oral health status, including the caries status on all available tooth surfaces, was recorded using the idmfs index. The symbol i stood for incipient, active or arrested carious lesion, d for dentinal carious lesion, m for extracted, f for filled/restored, and s for tooth surface. At the ages of three and five years, one of 54 dentists examined the children in one of 21 public dental health care clinics on a dental chair with all the necessary equipment. Radiographs were not included in the examinations.

The oral status was recorded and the children treated according to need. The children in study II were examined according to the schedule of their local prevention programs.

4.2.4 Interview

The examining professional carried out the interview with caretakers using a structured form, on a four-graded Lickert scale, designed for the collection of information on oral-health-related factors (Appendices, pages 48-49). The interview was carried out at the child health center when the child was 18 months old. In the control group, 97 % of the forms were successfully completed (IV).

The caretakers of the children in the Xr-study (II) were interviewed concerning the child's health and xylitol use, as well as the use of xylitol by other family members. The caretakers reported the frequency of their child's xylitol use on four-graded scale. Xylitol use of other family members was registered as use or no use.

4.2.5 Personnel and training

The prevention program was carried out partly by health nurses but mainly by dental hygienists, all of whom had experience in health education and were additionally trained

for the purposes of the present program. The training of the two responsible hygienists in the intervention area consisted of a minimum of five rehearsal sessions (15 h) and ten meetings (20 h) with two authors of the original publications (KP and PM). In the control area, all professionals involved in sampling were trained in two rehearsing sessions (4h). All dentists also attended a one-day supplemental educational session in caries detection and prevention. How to record the oral status was specified orally and in writing. Study II was mainly carried out by one dentist (PM) in Turku and one in Siilinjärvi, near the city of Kuopio, Finland. The dentist in Siilinjärvi was trained by the writer of the present thesis (PM).

4.2.6 Oral health counseling and education

In the intervention area, during the third trimester of pregnancy, the local maternity health nurse gave information concerning the importance of the mother's good oral health for the expected infant. A paediatric health nurse, a dental hygienist or a dentist met the infant at 6 - 8 months of age and gave education to all caretakers. Health-related topics were discussed also during other appointments. All caretakers received education on oral health during the child's 18-month examination. The progression of events in the intervention and control areas is illustrated in Figure 2.

The MS-colonized intervention group children, along with their caretakers, were invited to the hygienist's office and the study plan was explained. The informed consent or opinion of the caretakers was recorded in the patient record. Thereafter, they were invited to the hygienist's office for motivation and education every six months. The education contained motivation in tooth brushing and advice on the proper use of fluorides. Healthy meals, snacks, and thirst quenchers, as well as avoiding sugar was stressed. If the caretakers were not willing to participate in the study program, they participated in the traditional prevention program. No actual refusals were recorded but in the drop-out group, during the follow-up period, reasons mentioned for absence were e.g. logistic problems, unsuitable working hours, newborn baby at home, or no reason mentioned. Among some of these families, there might be refusals. The MS-negative children and the MS-colonized children in the control group received routine prevention on the judgement of the dentist or hygienist: education on healthy habits and the proper use of fluorides. During possible restorative sessions, advice was repeated if considered useful.

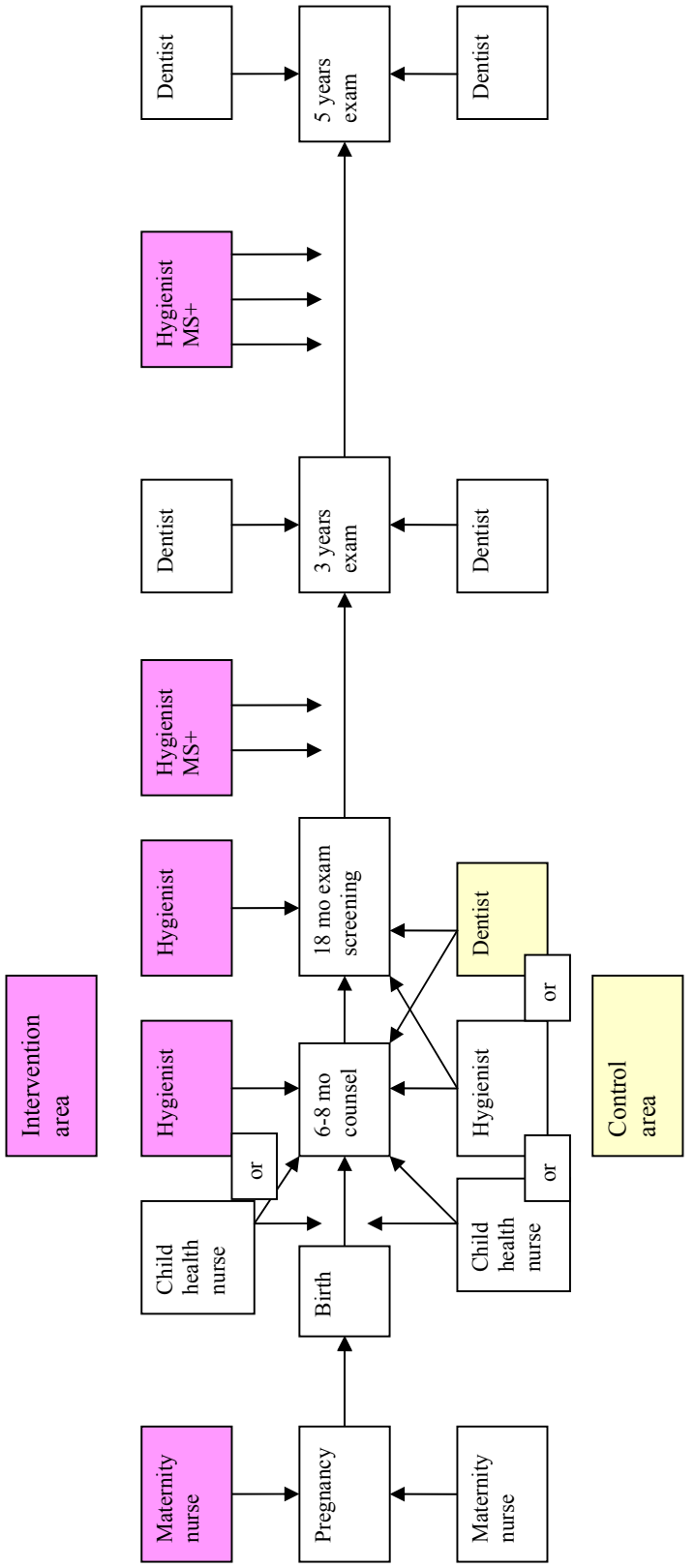

Figure 2. Flow of events and acting professionals in "Oral health programme " study (I)

Key: Counsel = counseling, child health nurse = paediatric health nurse, exam = examination, mo = months, yr = years, pink coloring = guided counseling

All caretakers received education upon request and during the child's examination visits in the public dental clinics at the ages of three and five years (I, III, IV). The subjects in study II participated in their local prevention programs.

4.2.7 MS screening and Xr determination

For all studies the dental biofilm samples of the 18-month-old children were gathered from the interproximal spaces of the upper central incisors using dental floss (Oral-B Flossette® Oral-B Laboratories, Belmont, USA, or Hager® floss, Hager & Werken, Taiwan). The strip of the Dentocult-SM strip mutans test® (Orion Diagnostica Espoo, Finland) kit was immediately inoculated by the biofilm and incubated at 35-36 °C for three days. The strips, after been dried in room temperature, were observed with the naked eye by either of two specially trained dental hygienists. The classification of the strips was done according to the manufacturer´s classification chart. No growth of MS colonies was considered negative (0). If solitary round, spherical, typical MS colonies were noticed, the strip was considered positive and given a value 1. Values 2 and 3 equaled the values in the chart. Any other possible stained spots or lines on the strip were considered negative (0).

To measure the proportions of the so called "xylitol-resistant" MS-strains, plaque samples of newly colonized children were gathered from surfaces with visible plaque using sterile scalers or dental sticks (II). The samples were stored in their vials at -70 ºC and sent for analyses in Québec City, Canada. The MS in the plaque samples were further cultivated in a laboratory, and the proportions of the labeled Xr strains were counted using published methods (Trahan and Mouton 1987).

4.2.8 Xylitol lozenges

The caretakers of the MS-colonized children were offered and delivered specially made xylitol / maltitol lozenges for the children. The recommendation was to use two lozenges each containing 0.25 g xylitol (Leaf, Turku Finland) after the meals three times daily which equals 1.5 g xylitol. The lozenges were delivered until the child's third birthday. Three mothers reported laxative effects of the lozenges and their children discontinued the use of the lozenges. These children were not excluded from the study.

4.2.9 The role of child health care

During pregnancy, the mother and the father met the maternity health nurse and the obstetrician. A reminder about motivating them to good oral health habits had previously been sent by the researchers to the maternity health nurses in the intervention area. The infant was in the care of the paediatric health nurse and a pediatrician. During the first year, the child was scheduled to undergo a thorough health program of ten visits to the child health center. At the age of six months, the paediatric health nurse or a dental hygienist gave education about oral health.

All visits and observations were documented in the patient records along with the personal data, including the occupation of the caretakers.

4.3 Outcome measures and indicators

In the oral health program study (I), caries at the age of five years was the main outcome measure. The independent variables were the MS colonization, the study group, gender, the occupation of the primary caretaker in the family, the number of the child's antibiotic treatments, the child's appetite, the night-feeding frequency, the child's use of added sugar, the child's sweet-snack consumption, the child's use of thirst-quenchers other than water, and the reported oral health of the caretakers.

For study II, the proportion of the labeled Xr strains was the outcome measure, and the frequency of xylitol use was the independent variable. For study III, the MS colonization was the outcome measure, and for study IV, the 42-month caries increment. For the studies III and IV, the independent variables were the occupation of the primary caretaker in the family, the child's mother tongue (ethnicity), the number of the child's antibiotic treatments, the child's appetite, the night-feeding frequency, the child's use of added sugar, the child's sweet-snack consumption, the child's use of a thirst-quencher other than water, and the reported oral health of the caretakers.

4.4 Data handling and statistical analyses

The data were retrieved from the patient records and interview forms. The values of MS colonization test and caries indices were dichotomized as 0 for the value 0 of the MS-screening test and 0 for idmf = 0 and dmf = 0. All other values were represented by 1. The differences in the distribution of the interviewed background categories were analysed using the 2-sided Chi-square test and the statistical significance level was selected as being $p < 0.05$ (I, III, IV). The cut-off points were set combining the highest sum of sensitivity and specificity with a minimum category size of 5 % to ensure clinical relevance. The same principle was used for dichotomizing the occupation of the primary caretaker, the occupations being classified using the official Finnish statistical classification (Tilastokeskus 1983). The classification was based on the need of education and the degree of independence of the occupation. The parent with the higher socioeconomic status was considered to be the primary caretaker if the classification differed between the parents and neither one of them was mentioned in the patient record as being the primary caretaker.

In order to measure the clinical effectiveness of the OHP, the absolute risk reduction (ARR) and, subsequently, the number needed to treat (NNT) with their 95 % confidence intervals were counted using the method described by Sackett (Sackett et al. 2000).

A univariate logistic regression model was used to estimate the effect of the background factors and the oral-health-related habits on the MS colonization and caries increment

(I, III, IV). The variables were further included in a multifactorial, stepwise logistic regression model (I, III, IV). The results of the analyses were presented using Odds ratios (OR) with the 95 % confidence intervals and p-values.

In study II, the frequency of the child's xylitol use during the preceding three months, was reported by the parents and further categorized into four classes: several times a day, once a day, weekly, and seldom. To measure the strength of the association between xylitol use and the proportion of Xr, the statistical analysis was carried out using the original Xr percentages of MS, applying the Spearman correlation coefficient and one-way analysis of variance and the Pearson Chi Square-test. The program SPSS 13.0 (Chicago, IL, USA) for Windows was used in all analyses.

5. RESULTS

The MS colonization was found to be associated with the occupation of the caretaker. The oral health program, targeted at MS-colonized children in the intervention group, was effective in children of white-collar families, but was not successful in children of blue-collar families. Xylitol use seemed to have some influence on the selection of the MS-strains. Caries increment in five-year-old children was strongly associated with early MS colonization, as well the family background, such as the socioeconomic status, feeding practices, and the oral health of the caretaker.

5.1 Oral health program

The MS colonization percentage was 20 in the intervention group and 25 in the control group (I, III, IV). At baseline, among the follow-up children in study I, dental caries (dmft > 0) was detected in one subject in the intervention group and one subject in the control group. After the follow-up period, the numbers and proportions of caries- affected subjects in relation to MS colonization, occupation of caretaker and study group are presented in Table 7.

Table 7. The proportion of children with caries (dmft > 0 at the age of 5 years) in relation to the occupation of caretaker and group. The absolute risk reduction (ARR) and the number needed to treat (NNT) values are given as a measure of the preventive effect of the OHP targeted at MS-colonized (MS+) children in the intervention group. As reference, the corresponding data of non-MS- colonized (MS-) children.

Group	Occupation of caretaker	MS status	Subjects in subgroup	Subjects with dmft > 0	
			n	n	%
Intervention	White-collar	MS+	29	4	**13.8**
	Blue-collar	MS+	60	25	**41.7**
	White-collar	MS-	138	15	**10.9**
	Blue-collar	MS-	219	46	**21.0**
			446	**90**	
Control	White-collar	MS+	37	16	**43.2**
	Blue-collar	MS+	51	19	**37.3**
	White-collar	MS-	154	12	**7.8**
	Blue-collar	MS-	106	24	**22.6**
			348	**71**	

Key: MS+ = MS-colonized subjects, MS- = no detected MS bacteria in dental biofilm
White-collar families MS+: ARR 0.29 (95% CI 0.09 - 0.50), NNT 3 (95% CI 2.01 – 10.94)
Blue-collar families MS+: ARR -0.04 (95% CI -0.23 – 0.14)

The risk-based oral health program was evaluated in MS-colonized children (I). The program was effective in MS-colonized white-collar children in the intervention group, the prevented fraction being 67 %, ARR 0.29 (95% CI 0.09 - 0.50), and the NNT 3 (95% CI 2 – 11) (Table 7). In children of the blue-collar families, no risk reduction was found in comparison with

those in the control group. However, regarding all MS-colonized children, the proportion of children with at least one caries lesion at the age of five years was relatively low, 33 % in the intervention group and 40 % in the control group (Table 7). The corresponding percentages among the MS-negative subjects were 17 and 14, respectively.

There was no group difference in the prevalence of caries, including incipient carious lesions (idmft > 0), in the 794 children followed up to the age of five years. However, a strong association of caries including incipient lesions was found with MS colonization, the occupation of the caretaker, and with gender (Table 8; I). The boys in blue-collar families had more often initial and dentinal carious lesions.

Of the white-collar children, 52% and of the blue-collar children, 50% had regularly used the xylitol lozenges in the study. Regarding caries, no significant differences were found between regular and irregular xylitol users.

Table 8. Prevalence of carious lesions (idmft>0 at the age of 5 years) in relation to MS colonization at 18 months, gender, occupation of caretaker and study group. Logistic regression analysis with carious lesions (idmft>0 at 5 years) as dependent variable, and study group, occupation of caretaker, gender and MS colonization at 18 months as independent variables (retrieved from study I)

Group	Occupation of caretaker	Gender and MS	Subjects in subgroup	Subjects with idmft>0	
			n	n	%
Intervention group 446	White-collar	girls +	11	4	36.4
		girls-	68	14	20.6
		boys+	18	8	44.4
		boys-	70	23	32.9
			167	**49**	**29.3**
	Blue-collar	girls +	22	13	59.1
		girls-	108	29	26.9
		boys+	38	24	63.2
		boys-	111	48	43.2
			279	**114**	**40.9**
Control group 348	White-collar	girls +	18	11	61.1
		girls-	88	21	23.9
		boys+	19	11	57.9
		boys-	66	14	21.2
			191	**57**	**29.8**
	Blue-collar	girls +	29	13	44.8
		girls-	52	19	36.5
		boys+	22	13	59.1
		boys-	54	23	42.6
			157	**68**	**43.3**
Total			**794**	**288**	**36.3**

Key: Girls+ = MS-colonized girls, girls- = no detected MS bacteria in dental biofilm; boys correspondingly
Group: p= 0.87, OR 1.0 (95% CI 0.8 – 1.4)
Occupation of caretaker: p= 0.002, OR 1.6 (95% CI 1.2 - 2.2)
Gender: p=0.009, OR 1.5 (95% CI 1.1 - 2.0)
MS colonization: p< 0.001, OR 2.6 (95% CI 1.8 - 3.6)

5.2 "Xylitol resistance"

The proportion of so-called "Xylitol resistant" (Xr) MS strains showed a trend towards an association with the reported xylitol use of the child (Table 9; II). The study subjects had been screened for MS and were found to be highly colonized. However, in the laboratory analyses, the MS were not detected in a third of the samples. In children reported to have used xylitol several times daily, the proportion of no growth samples was 47 %, but 29 % in less frequent users of xylitol (Table 9).

Table 9. Relation between reported xylitol use and the percentage of xylitol-resistant (Xr) strains of MS. Number of subjects in Xr categories and the number of subjects with no MS growth in laboratory analyses (retrieved from study II)

	Proportions of Xr strains						
Frequency of xylitol use	**0 %**	**1-25 %**	**6-75 %**	**76-100 %**	**MS growth**	**No MS growth**	**Total**
Several times daily	2	-	1	5	8	7	
Once a day	5	3	-	5	13	4	
Weekly	7	-	2	3	12	6	
Seldom	6	1	1	2	10	4	
Total					43	21	64

Spearman Correlation coefficient 0.297 (p = 0.053)

5.3 Associations of MS colonization

Approximately one fourth of the screened children harboured MS in their oral biofilm at the age of 18 months (20-25%; III, IV). In univariate analyses, the MS colonization at the age of 18 months associated significantly with the socioeconomic status and the ethnicity of the child (Table 10). No other significant associations with the MS colonization were found (II).

Table 10. Reported background factors and their associations with MS colonization using univariate analyses. Dichotomized MS colonization (MS+ for MS-colonized subjects) as dependent variable and dichotomized background factors as independent variables; logistic regression, univariate analyses (retrieved from study III; typing error corrected)

Background information	Total	MS+ subjects		Log reg OR	
	n	n	%	95% CI	p-value
Gender:					
girl	268	60	22		
boy	244	60	25	1.1 0.8-1.7	0.557
Occupation of caretaker:					
white-collar	278	51	18		
blue-collar	234	69	30	**1.9** 1.2-2.8	**0.003**
Ethnicity:					
Finnish	495	111	22		
Non-Finnish	17	9	50	**3.9** 1.5-10.3	**0.006**
Antibiotic treatment:					
≤ 1	254	57	22		
≥ 2	258	63	24	1.0 0.6-1.3	0.597
Appetite:					
good/usually good	444	104	23		
poor/medium	68	16	24	1.0 0.5-1.8	0.985
Night feeding:					
never/seldom	473	109	23		
nightly	39	11	28	0.8 0.4-1.6	0.466
Sugar added:					
never/seldom	479	110	23		
daily	33	10	30	0.7 0.3-1.5	0.338
Sweet snacks:					
never/seldom	458	106	23		
daily	54	14	26	0.9 0.5-1.6	0.648
Drink other than water:					
never/seldom	399	86	22		
daily	113	34	30	0.6 0.4-1.0	0.060
Maternal xylitol use:					
never/seldom	304	66	22		
daily	208	54	26	1.3 0.8-1.9	0.265

Key: Log reg = logistic regression, OR = Odds ratio

5.4 Caries risk indicators

In the univariate analyses, the MS colonization at the age of 18 months, the occupation of the caretaker and several interviewed factors associated with the 42-month caries increment (Table 11). In the final model, significant predictors of caries were the MS colonization, blue-collar occupation of the caretaker, the child's sugar use, night feeding, use of other thirst-quenchers than water, and the father's reported poor oral health (IV).

The strongest predictor of future caries was the MS colonization at the age of 18 months, showing a tripled risk for caries, and the blue-collar occupation of the caretaker showing a doubled risk (Table 11).

Table 11. A multivariate stepwise forward logistic regression model with 42-month caries increment as dependent factor and background factors (at 18 months) as independent factors; n =337 (retrieved from study IV)

Background information	**OR; 95% CI**	**p**
MS + *vs* MS-	**3.4**; 1.9-6.1	**<0.001**
Blue-collar *vs* white-collar	**1.9**; 1.0-3.4	**0.034**
Finnish *vs* non-Finnish		ns
Nightly feeding *vs* never/seldom	**2.8**; 1.0-8.2	**0.052**
Sugar added sometimes *vs* never	**2.2**; 1.1-4.5	**0.024**
Drink other than water daily/weekly *vs* never/seldom	**2.0**; 1.1-3.5	**0.021**
Reported oral health of mother poor *vs* good		ns
Reported oral health of father poor *vs* good	**2.4**; 1.0-5.1	**0.022**

Key: OR = Odds ratio, CI = confidence interval, ns = not significant

6. DISCUSSION

The present longitudinal study emphasizes the importance of the early circumstances on the developing child. The family background, the socioeconomic status, oral-health related behavior, and feeding patterns in early childhood influences the child's oral health for years. If the caretakers of an infant are motivated and have enough knowledge, the early childhood caries can be prevented. The present oral health program was based on activities at home, and the prevention effect on caries was achieved in white-collar families. The results, thus, indicate that prevention programs can have an effect on the oral health of a child.

6.1 Methods; validity and reliability

A longitudinal, controlled cohort study in two separate areas in a city was considered a relatively valid setting for testing the effect of an oral health program (OHP). Randomization of the areas was not carried out. For practical reasons, the area with two dental hygienists was chosen to be the area of intervention. The control area employed one hygienist only. The study population comprised the whole cohort born during a restricted period of 1.5 years. Practically all families, irrespective of their socioeconomic background, use the services of the child health clinics and the public dental clinics. The studied groups, thus, represented well the cohort and the different types of families. In the Nordic countries, where cohorts are reached through the health care system, longitudinal cohort studies can be conducted even on a larger scale (Grindefjord et al. 1996, Mattila et al. 2000, Skeie et al. 2008, Joensuu 2009, Wigen et al. 2010).

The group sizes can be considered large enough to draw conclusions on the studied matters despite the low caries prevalence of the children. The number of drop-outs was relatively large, but reflects the trend of young families to move to larger homes often in the neighbouring communities rather than a refusal to participate in the study. All oral health records were available if the family moved out of the study area, but inside the city borders. The drop-out rates are unlikely to have caused bias in the study. Neither did the lack of blinding of the examiners to the screening test result probably have any effect on the caries diagnoses made at the end of the follow-up time. The screening-test result was available in the patient records.

The follow-up time of 42 months was considered to be long enough in terms of caries development in primary dentitions (I, IV). However, the groups were discovered not to be identical regarding the socioeconomic background. The occupation of the caretaker was thus controlled for in the analyses, which improved the reliability of the results.

The character and goals of the OHP were discussed and the guidelines emphasized to the responsible professionals in the intervention area. The maternity health nurses were requested to remind the expecting parents about adequate oral health care and avoiding

salivary contacts with the expected baby. The paediatric health care nurses were requested to repeat the message. A multiprofessional approach and repetition should strengthen the acceptance of the message. The education and awareness of the caretakers alone may decrease the colonization rate (Taipale 2010). The awareness, in addition, may come from friends or the media. Discussions in the social media have also become an important source of information to the caretakers.

The dental hygienists were encouraged to form pleasant, responsive relations to the child and the caretakers. The interview not only gathered information on general health and the health-related habits in the family, but also guided the discussion by bringing up issues from the questionnaire. The structured interview form gave the interview situations a frame and similarity. Although the interview information was collected during an ordinary child health clinic visit in a supportive atmosphere, some embellishment of the answers may have occurred. About 3 % of the forms (study IV) lacked details or answers which resulted in a reduced number of cases in the analyses. This small proportion was not considered to have an influence on the results.

The dental examinations were performed both in the child health clinics and the public dental clinics by experienced professionals. An air syringe was not available in the child health clinics. Hence, the detecting of incipient carious lesions was problematic, and thus, the incipient lesions were left out of the longitudinal analyses. The five-year examinations were done on a dental chair with all the necessary equipment. Radiographs were not included in the examinations. This apparently somewhat reduced the number of detected carious lesions, but most probably similarly in both groups**.** All examining dentists had attended a one-day supplemental education and motivation session on caries detection and prevention. Oral and written instructions on recording the oral health status had additionally been given to those involved. These procedures were meant to reduce the differences in the diagnoses. Formally calibrated investigators could have added to the accuracy of the diagnoses although in larger settings also diagnoses made by experienced health care dentists can be considered reliable (Hausen et al. 2001).

The risk assessment by screening for MS can be considered valid and reliable although only one plaque sample was collected. In young children, reports have found MS detected in the biofilm to be stronger predictors of caries than the salivary MS (Thenisch et al. 2006). The targeting proved to be reasonable: the MS colonization (at the age of 18 months) was the strongest predictor of caries increment.

The collection and cultivation processes of the biofilm samples were done with well known and documented equipment. The processes were both shown to the dental hygienists and dentists involved in the study and practiced by them to ensure the reliability of the procedure. The methods used in screening were proven and of good quality. Of the available chair-side tests, the Dentocult SM Strip test was well suited for the practice. Dental plaque, rather than stimulated saliva, was properly, quickly and easily collected using dental floss. The used method detects both *Streptococcus mutans*

and *Streptococcus sobrinus,* whereas another possible chair-side test, the CRT® test (Ivoclar Vivadent, FL-9494, Schaan Liechtenstein) detects both LB and MS. Yet a third test, the Saliva-check SM test (GC, Tokyo Japan), does not detect *S. sobrinus.* Besides, both the CRT® test and the Saliva-check SM need stimulated saliva, which is difficult or impossible to get from toddlers. In addition, the plaque MS level has been found to have a stronger correlation with caries development with or without caries experience at baseline when compared with salivary MS from unstimulated saliva (Seki et al. 2006). The chair-side test correlates well with laboratory testing (Karjalainen et al. 2004). The interpretation of all samples (strips) was delegated to two specially trained dental hygienists and thus was comparable in both the intervention group and the controls. The hygienists were blinded to the clinical data when interpreting the control group tests.

Unfortunately, the presence of biofilm/plaque on the tooth surfaces at baseline was not recorded comprehensively enough in the control group to be used in the analyses on caries prediction.

The non-parametric statistical analyses used were adequate for analysing differences between groups and the associations of various factors with either MS colonization or caries. The dichotomization of these factors was based on clinical relevance and was suitable for an epidemiological study measuring caries risk and caries prevalence in a group. It thus helps the authorities to estimate the needs and requirements in planning the dental services of the community**.**

6.2 Effect of the oral health program

The OHP had an effect on caries occurrence in children of white-collar families. Counseling during pregnancy and infancy may have influenced the oral health habits and consequently diminished the colonization process in the intervention group. The MS-colonized white-collar-family children formed only 6.5 % of the intervention group and 10.6 % of the control group. Differences between the entire intervention and control groups were not seen.

The oral health program required compliance at home and visits to the hygienist's office. The topics of the health education and motivation were structured for the interview. In other respects, the hygienists acted according to their personal feelings although a relaxed, supporting atmosphere and delicate handling of the meetings were recommended. The counseling procedure, thus, was a traditional mode of operation. The targeted OHP was found to be effective in children of the white-collar families but not of the blue-collar families. This indicates that motivating with knowledge and using measures emphasizing self-care, is more effective in white-collar families. These families may have more potential and motivation to improve their oral health habits or their lifestyle may be healthier. In a Finnish survey, the oral self-care (tooth brushing frequency) correlated positively with the level of education. The use of sugar was lower the higher the subject's level of education was (Vehkalahti and Knuuttila 2008). In the

same survey, the reported use of sweet or fizzy drinks was highest in the age group 30-44 years. To-day the majority of caretakers belong to that age group.

For blue-collar families, different kinds of approaches and support are needed in the future – perhaps more contacts and more professional preventive measures in the clinics. Guided group meetings with families in the same neighborhood or families sharing other common interests might be one acceptable way to strengthen the families, especially to empower the mothers, in their every-day problems. The systematic "motivational interview" method for mothers suggested by Weinstein might be one possible novel method of tackling the practical obstacles in the family (Weinstein et al. 2006). The mothers in the program by Weinstein were interviewed and systematically led to solve their oral-health-related behavior problems. Similarly, in a Finnish study, a sophisticated theoretical framework with stepwise change and motivational interview has been suggested to be useful in the oral health consultation of schoolchildren in their early teens (Kasila et al. 2006). Furthermore, in the program by Kressin and co-workers, the systematic education program for pediatricians and clinic nurses resulted in a reduction of 77 % in the risk of developing ECC (Kressin et al. 2009). In the present study, the impact of the role of the maternity health nurses was not separately evaluated. It is, however, very likely that all the information concerning the well being of the infant, given to the expecting families, would come to fruition. It naturally strengthens the message if information and support are given by all the professionals.

6.3 Effects of xylitol use

The regular daily use of xylitol products was planned to improve the oral health of the MS-colonized children (I). Products sweetened by xylitol have been found beneficial for oral health (Scheinin et al. 1974, Isokangas et al. 2000, Söderling et al. 2000, Mäkinen 2010). The element of sweet xylitol/maltitol lozenges was anticipated to be acceptable and an easily adopted pleasant way of preventing ECC. The lozenges were delivered until the child's third birthday. Half of the children used the lozenges according to the recommendations. Although the use of xylitol in the program cannot be separately evaluated, the dosage, being only 1.5 g per day, was probably too small. Hence, no significant effect of xylitol use on caries was found (I). This finding is in line with a study in Sweden, where low doses of xylitol were not found effective in children's caries prevention (Oscarson et al. 2006).

In study II, the so-called xylitol-resistant (Xr) MS-strains from the plaque of the newly colonized children were counted in order to develop a tool for measuring the compliance in the study and, further, for evaluation of the OHP. The study subjects were locally screened for MS and found to be MS-colonized. One third of the plaque samples, however, showed no growth when cultivated. An interesting finding was that among the frequent xylitol users, the growth of MS was negative in almost twice as many plaque samples compared with that among the seldom or never users of xylitol. Whether this

finding really reflected the connection of MS growth and xylitol use, or whether it was connected to plaque characteristics or transport wastage in the process deserves to be further studied. The finding is interesting because xylitol is known to prevent MS transmission. The use of xylitol has been reported to reduce the MS levels (Söderling 2009). Thus, the early started use and the appropriate quantity and frequency of xylitol products might even reverse the colonization process in its early phases.

The xylitol resistance study, nevertheless, showed some xylitol-dependent differences in the Xr levels in the oral biofilm. This is in accordance with a previous study (Trahan 1995). It had been hypothesized on the grounds of previous reports that even in young children the proportion of the Xr strains of MS in the biofilm would reflect the regularity of xylitol use (Trahan et al. 1992, Tanzer et al. 2006), and thus could be a tool for evaluating the OHP. The results of our xylitol resistance study, however, could not confirm the hypothesis. In fact, the proportion of Xr strains may equally well reflect that of the caretaker, the source of MS. Therefore, the tool intended for measuring the compliance in study I was not used.

6.4 Caries predictors

The occupation of the caretaker associated significantly with MS colonization at the age of 18 months, as well as with caries at the age of five years. The whole age cohorts, including all social classes, of the health care areas were included in the study and the sample size was large enough. This is a strength of the present study. Many previous prediction studies have been performed in deprived areas, where the socioeconomic profile of the study subjects was predetermined, which may have caused some kind of selection bias.

The socioeconomic aspects in the colonization process can reflect the family situation and parenting as a whole. Any disturbance in the family dynamics may cause parenting stress and have an effect on the child care (Paunio et al. 1993, Mattila et al. 2000, Wigen et al. 2010). At the age of 18 months, many of the studied factors (appetite, night feeding, sweet snacks, or thirst quenchers) traditionally connected to ECC, were not found to associate with MS colonization in study III. Whether the main etiological factors of MS colonization are social, hereditary, or mainly depend on the high MS counts of the caretaker, bad feeding habits, or some other factors, was not cleared up in the present study. Since the MS colonization seems to be a powerful risk predictor of ECC, this deserves to be more widely studied.

The carious process, also, seems to be closely associated with the occupation of the caretaker, the MS colonization and even the male gender of the child. This could be noticed when the incipient lesions were included in the outcome measure (idmft). The independent variables, MS colonization at 18 months, gender, and the occupation of the caretaker all remained significant in the same final model. This shows the strength of the association.

In several recent reports, it has been suggested that also the attitudes or beliefs are important and noteworthy factors in the prediction or prevention of future caries (Weinstein et al. 2006, Ismail et al. 2008, Skeie et al. 2008, Wigen et al. 2010). Early childhood is an important period in the development of general health, and oral health in particular. The aspects concerning dental caries are of great interest. In the present study, the results concerning ECC seem to correspond with the reports and results from different kinds of communities and caries prevalence conditions (Beighton et al. 2004, Pine et al. 2004, Skeie et al. 2006). Of the studied factors the early MS colonization was found to be significantly associated with ECC. This is also in accordance with the fairly recent literature (Petti and Hausen 2006, Minah et al. 2008, Holgerson et al. 2009, Tamaki et al. 2009, Warren et al. 2009). Of the interviewed oral-health-related habits at the age of 18 months, several associated with caries increment at the age of five years**.** Added sugar, sugar use, candies or sugary drinks, or sugar in another form, have fairly recently been reported as associating with ECC or predicting it (Ollila and Larmas 2007, Sakuma et al. 2007, Ismail et al. 2008, Nishimura et al. 2008, Warren et al. 2009). Feeding practices, especially prolonged nightly feeding (breast or bottle) have been reported to be a risk factor for ECC (Yonezu et al. 2006a, Ollila and Larmas 2007, Sakuma et al. 2007, Tiberia et al. 2007, Nishimura et al. 2008). This is in line with the present study.

6.5 Clinical relevance

In the present study population, the prevalence of dentinal caries in the control group children was less than 1 % at the age of 18 months, but 40 % at the age of 5 years (IV). Generalisation of the results may be restricted in different cultures, because of dental care systems, prevention strategies, fluoride concentrations, and caries prevalence rates.

The results of caries prediction in this study are in line with those from other countries almost worldwide. The early MS colonization, frequent sugar use and the socioeconomic background, together with parental habits and attitudes, associate significantly with ECC in the present study, which is in accordance with the results from several countries (Grindefjord et al. 1995, Marshall et al. 2007, Ismail et al. 2008, Skeie et al. 2008, Warren et al. 2009, Wigen et al. 2010). The result of significant association between family background and ECC is also in line with a recent report from dissimilar areas in China by Li and co-workers. They reported significant associations of caries with living and day-care conditions and eating habits (Li et al. 2010).

The prevention programs targeted at preschool children in different countries have been based on clinical measures combined with counseling. The study protocol of Minah and co-workers included counseling and fluoride varnish applications either every three or every six months in a group of one hundred low-SES children, the follow-up time being 26 months (Minah et al. 2008). They reported a decrease in cavitated lesions in the intervention group, whereas the precavitated lesions corresponded to those of

the controls. The results seem to be in line with the present results concerning caries including incipient lesions.

In the Italian program, counseling took place every four months with a CHX-gel application series at three-month intervals for the high-risk group, the follow-up time being 18 months. The measures could not decrease the caries increment of the high-risk group in comparison with the low-risk group although the salivary MS-levels were lowered (Petti and Hausen 2006). The Swedish program, carried out in a fairly small sample was based on counseling, low-dose xylitol tablets and a follow-up time of two years. At the age of four, the MS levels were not lowered, but the proportion of caries affected children in the intervention group was 18 %, and in the control group 25 % (Oscarson et al. 2006). The difference was not significant although the finding suggests some effect of the program on caries. A decrease in the caries proportions was in line with the present results. The intensive prevention in the earlier Finnish program for preschool children was successful. The program was based on controlling caries by clinical measures including professional cleaning of the dentition, fluoride varnish, or CHX varnish applications, and counseling every three months (Pienihäkkinen et al. 2005).

These results suggest that lowering the risk of caries is very difficult. Predefining the risk at an early age or even before birth has recently been suggested, enabling early started health promotion (Wigen et al. 2010). This enhances the possibilities of primary prevention of dental caries.

7. CONCLUSIONS

The results indicate that family-dependent factors play a vital role in the MS colonization process.

MS colonization at the age of 18 months and the family-dependent factors associate strongly with caries at the age of five years. Both the early MS colonization and the occupation of caretaker are indicators of elevated risk of ECC.

The present oral health program, based on education and activity at home, reduced the caries risk of MS-colonized children in white-collar families, but not in blue-collar families. It can be concluded that in blue-collar families, measures targeted at the family dependent factors should be more effective in the prevention of ECC.

The proportion of Xr strains of MS does not unequivocally reflect only the child´s xylitol use. The compliance can thus not be evaluated by measuring the Xr strains of MS.

7.1 Recommendations

The feeding habits of a young child and the early implemented good oral health habits of the family should be stressed to all families early enough, preferably before the child is born.

MS colonization, indicating a risk of dental caries should be further investigated to find the actual causes of colonization and dental caries. The caries predictive value of the MS colonization test could be improved by combining it with some additional factors, for instance, incipient carious lesions and/or socioeconomic status (occupation of the caretaker). Other oral-health-related factors could be combined as well.

Families with children at risk could benefit from tailored oral health programs and new approaches should be taken. Building up support networks, educating professionals outside dentistry, or laymen, or also changing the legislation to support the families could improve attitudes towards healthy oral habits. As the socioeconomic background seems to associate so vitally with both the MS colonization and the caries increment of young children, solid measures to improve the living conditions might improve the control of life in these families. We should challenge all health care professionals in different positions and the society as a whole to contribute to building up a healthy environment for all children.

8. ACKNOWLEDGEMENTS

The present study was carried out at the Department of Community Dentistry at the Institute of Dentistry and the Oral Health Care Division in the Health Center of Turku. The Deans of the Institute, Professors Pentti Kirveskari and Pekka Vallittu are thanked for the opportunity to study and use the facilities at the institute. The PeGaSOS postgraduate school is acknowledged for enabling the participation in the postgraduate program.

I am greatly thankful for Professor Pentti Alanen, my highly respected teacher and supervisor for accepting me to be a part of the study group and guiding me in the scientific world. You are the brains behind a unique series of prevention studies. Thank you for your wise opinions and clear-sighted intellect.

My dear supervisor and friend, positive, encouraging teacher, Docent Kaisu Pienihäkkinen, I owe you my deepest gratitude and admiration for the expertise, guidance and patience during all these years. You were always helpful and available. Kaisu, thank you! Without you this thesis could not have been realized.

I wish to thank Doctor Anna-Leena Eriksson, the former Chief Dental Officer of the Oral Health Care Division of Turku. You were actively involved with the study planning and gave a chance and personnel to perform the project.

Docent Eva Söderling, our Lady Xylitol, I admire your knowledge, your helpful attitude, and always happy smile. Thank you so much for your support, supervision and help in research as well as in the practical issues.

Professor Luc Trahan is acknowledged for his input, cooperation, and great experience especially in matters concerning Xylitol resistance.

I wish to cordially thank the reviewers of the study, Docent Liisa Seppä and Docent Sisko Honkala for their valuable and precise comments and notes.

Professor Eino Honkala and Acting Professor Liisa Suominen in our department have kindly encouraged and helped me with ideas and references, thank you.

Dr Mauno Ruokola, the former Chief in the Oral Health Care Division of Turku, one of the initiators of the present study and Doctor Minna Kinnarinen are acknowledged for their understanding and support. Doctor Marina Merne-Grafström, my immediate superior, is acknowledged for her creative and positive spirit.

During this period, I have had wonderful colleagues and professionals working in the Faculty, Dentalia, and the Health Care Center. I am grateful for all the help and support from my teachers and tutors: Doctor Varpuleena Kirstilä, Docent Sára Karjalainen, Professors Jorma Tenovuo, and Juha Varrela. My important coworkers during the sample collection for the project Ms Leila Lahti, Ms Marja Larmasto, and later, Ms Leena

Konkka, Dr Arna Laajos, Dr Ritva Lehtinen, Dr Ulla Suni, and Dr Marianne Vigren are thanked for their important and well accomplished input. All colleagues and coworkers in the Oral Health Care are thanked for their cooperation in examining and providing care for the children and education to their parents. Dr Leena Meriläinen is thanked for her positive attitude and contribution in the sample collection.

My colleagues and roommates at the Institute of Dentistry: Aaro, Ami, Anna, Anne, Arzu, Eva, Hanna, Helena H, Helena Y, Jaana, Jaana, Jari, Katja, Mani, Marika, Marja, Merja, Mervi, Riikka, Riina, and Tiina are thanked for their friendship and rousing conversations, usually over luncheon or coffee.

For their fine technical support and contribution, I want to thank Ms Sirpa Laakso, Ms Annukka Wallenius, Ms Päivi Mäkimattila, and Ms Annikki Vaisto. Ms Oona Hällfors is thanked for her guidance with the cultivation of the bacteria. The problems with the computer, programs and network have well been solved by Mr Jaro Saarikoski and his colleagues. In the Medical Library, Helena Tähtinen, MSc, has helped me with planning of a search query and the Library staff has provided me with the rare references. Thank you. The English language was revised by Jacqueline Välimäki, MA. My best thanks for her rapid and thorough work.

My personal friends and relatives are thanked for the love, forbearance and support in the past years. Together we have experienced so many invigorating conversations, meetings and excursions. The “Eagle Patrol”, thank you for our refreshing gatherings. You are my very special friends.

Last but not least, my precious and understanding family, I love you.

The supporters of the studies: The Research Fund of the Finnish Dental Organizations, The Finnish Dental Society Apollonia, Suomen Naishammaslääkärit r.y., and the Leaf Company, Turku providing the xylitol lozenges for the study, are warmly acknowledged.

Turku, September 2011

Pia Meurman

9. APPENDICES

Literature search query

PubMed November 2009

	Most Recent Queries	Result
12	**#10 not #11**	814
11	**#10** Limits: Editorial, Addresses, Biography, Historical Article, Lectures, Legal Cases, News, Newspaper Article, Patient Education Handout, Retracted Publication	8
10	**(#4 or #8)** Limits: ("2006/01/01"[Publication Date] : "3000"[Publication Date]), Humans	822
9	**#4 or #8**	4618
8	**#5 and (#6 or #7)**	192
7	**Cohort[tw] or Longitudinal[tw] or Sensitivity[tw] or Specificity[tw] or "Follow up"[tw] or Followup[tw] or Compared[tw] or Comparison[tw] or Evaluate[tw] or Evaluated[tw] or Evaluation[tw] or "Odds-ratio"[tw] or "Odds-ratios"[tw]**	4512403
6	**"Sensitivity and Specificity" or "Comparative Study"[MeSH Terms]**	226018
5	**Dental Caries Susceptibility[MeSH Major Topic]**	722
4	**#1 and (#2 or #3)**	4495
3	**"predict*"[Text Word]**	127880
2	**(Risk or Prognosis or Forecasting or Socioeconomic Factors or Saliva/secretion or Saliva/microbiology or Hydrogen Ion or Concentration[MeSH Terms]) AND ((dental caries[MeSH Major Topic]))**	4476
1	**(dental caries[MeSH Major Topic])**	22187

Tietojenkeruulomake 18 kk, Questionnaire at 18 months

Nimi _____ Sotu _____

Ensimmäisen hampaan puhkeamisikä
Perheessä asuvien lasten lukumäärä
Tutin käyttö
Tuttipullon käyttö / mitä pullossa

Antibioottikuureja tähän asti	0-1 0	2-4 1	n5 2	yli5 3
Yösyönnin kerrat	ei 0	harv 1	1/yö 2	us/yö 3
Muu janojuoma kuin vesi	ei 0	1/vko 1	>1/vko 2	päivitt 3
Sokerinlisäys ruokaan	ei 0	1/vko 1	>1/vko 2	päivitt 3
Makeisten, pullien, keksien rusinoiden ym käyttö	ei 0	kuukausitt 1	viikottain 2	päivitt 3
Ruokahalu	hyvä 0	yl hyvä 1	kohtal 2	huono 3
Vanhempien ksylitolin käyttö	3-5/pv	päivitt	1-3/vko	ei
äiti	0	1	2	3
isä	0	1	2	3
Sisarusten ksylitolin käyttö	3-5/pv	päivitt	1-3/vko	ei
1	0	1	2	3
2	0	1	2	3
3	0	1	2	3
Vanhempien suuterveys	hyvä	koht hyvä	melko huono	huono
äiti	0	1	2	3
isä	0	1	2	3
Sisarusten suuterveys	hyvä	koht hyvä	melko huono	huono
1	0	1	2	3
2	0	1	2	3
3	0	1	2	3

Questionnaire at 18 months (unofficial translation)

Name _____ ID _____

First primary tooth: age
Number of siblings
Use of pacifier
Use of baby bottle / its contents

Antibiotic treatments	0-1 0	2-4 1	5 2	>5 3
Nightly feedings	no 0	seldom 1	1/night 2	several 3
Thirst quencher other than water	no 0	1/week 1	>1/week 2	daily 3
Added sugar	no 0	1/week 1	>1/ week 2	daily 3
Use of sweets, buns, cookies, raisins etc	no 0	monthly 1	weekly 2	daily 3
Appetite	good 0	usually good 1	moderate 2	poor 3
Xylitol use	3-5 / day	daily	1-3 / week	no
mother	0	1	2	3
father	0	1	2	3
Xylitol use of siblings	3-5 / day	daily	1-3 / week	no
1	0	1	2	3
2	0	1	2	3
3	0	1	2	3
Oral health				
	good	fairly good	fairly poor	poor
mother	0	1	2	3
father	0	1	2	3
Oral health of siblings				
	good	fairly good	fairly poor	poor
1	0	1	2	3
2	0	1	2	3
3	0	1	2	3

10. REFERENCES

Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. J Clin Microbiol 43: 5721-32; 2005.

Aida J, Ando Y, Oosaka M, Niimi K, Morita M. Contributions of social context to inequality in dental caries: A multilevel analysis of japanese 3-year-old children. Community Dent Oral Epidemiol 36: 149-56; 2008.

Alaluusua S, Malmivirta R. Early plaque accumulation--a sign for caries risk in young children. Community Dent Oral Epidemiol 22: 273-6; 1994.

Alaluusua S, Renkonen O. Streptococcus mutans establishment and dental caries experience in children from 2 to 4 years old. Scand J Dent Res 91: 453-7; 1983.

Alanen P, Isokangas P, Gutmann K. Xylitol candies in caries prevention: Results of a field study in estonian children. Community Dent Oral Epidemiol 28: 218-24; 2000.

Alves A, Nogueira R, Stipp R, Pampolini F, Moraes A, Gonçalves R, Höfling J, Li Y, Mattos-Graner R. Prospective study of potential sources of streptococcus mutans transmission in nursery school children. J Med Microbiol 58: 476-81; 2009.

Assev S, Stig S, Scheie AA. Cariogenic traits in xylitol-resistant and xylitol-sensitive mutans streptococci. Oral Microbiol Immunol 17: 95-9; 2002.

Autio JT. Effect of xylitol chewing gum on salivary streptococcus mutans in preschool children. ASDC J Dent Child 69: 81-6, 13; 2002.

Beighton D. The complex oral microflora of high-risk individuals and groups and its role in the caries process. Community Dent Oral Epidemiol 33: 248-55; 2005.

Beighton D, Brailsford S, Samaranayake L, Brown J, Ping F, Grant-Mills D, Harris R, Lo E, Naidoo S, Ramos-Gomez F, Soo T, Burnside G, Pine C. A multi-country comparison of caries-associated microflora in demographically diverse children. Community Dent Health 21: 96-101; 2004.

Berkowitz RJ, Jordan HV, White G. The early establishment of streptococcus mutans in the mouths of infants. Arch Oral Biol 20: 171-4; 1975.

Burt B. Definitions of risk. J Dent Educ 65: 1007-8; 2001.

Burt BA, Pai S. Does low birthweight increase the risk of caries? A systematic review. J Dent Educ 65: 1024-7; 2001.

Campus G, Cagetti MG, Sacco G, Solinas G, Mastroberardino S, Lingström P. Six months of daily high-dose xylitol in high-risk schoolchildren: A randomized clinical trial on plaque ph and salivary mutans streptococci. Caries Res 43: 455-61; 2009.

Carvalho J, Figueiredo M, Vieira E, Mestrinho H. Caries trends in brazilian non-privileged preschool children in 1996 and 2006. Caries Res 43: 2-9; 2009.

Caufield PW, Cutter GR, Dasanayake AP. Initial acquisition of mutans streptococci by infants: Evidence for a discrete window of infectivity. J Dent Res 72: 37-45; 1993.

Cogulu D, Ersin N, Uzel A, Eronat N, Aksit S. A long-term effect of caries-related factors in initially caries-free children. Int J Paediatr Dent 18: 361-7; 2008.

Demers M, Brodeur J, Mouton C, Simard P, Trahan L, Veilleux G. A multivariate model to predict caries increment in montreal children aged 5 years. Community Dent Health 9: 273-81; 1992.

Du M, Luo Y, Zeng X, Alkhatib N, Bedi R. Caries in preschool children and its risk factors in 2 provinces in china. Quintessence Int 38: 143-51; 2007.

EAPD EAoPD. Guidelines on the use of fluoride in children: An eapd policy document. Eur Arch Paediatr Dent 10: 129-35; 2009.

Edwardsson S, Koch G, Obrink M. Strep. Sanguis, strep. Mutans and strep. Salivarius in saliva. Prevalence and relation to caries increment and prophylactic measures. Odontol Revy 23: 279-96; 1972.

Ekstrand KR, Christiansen ME. Outcomes of a non-operative caries treatment programme for children and adolescents. Caries Res 39: 455-67; 2005.

Elfrink ME, Veerkamp JS, Kalsbeek H. Caries pattern in primary molars in dutch 5-year-old children. Eur Arch Paediatr Dent 7: 236-40; 2006.

Fontana M, Catt D, Eckert G, Ofner S, Toro M, Gregory R, Zandona A, Eggertsson H, Jackson R, Chin J, Zero D, Sissons C. Xylitol: Effects on the acquisition of cariogenic species in infants. Pediatr Dent 31: 257-66; 2009.

Forss H. Efficiency of fluoride programs in the light of reduced caries levels in young populations. Acta Odontol Scand 57: 348-51; 1999.

Grindefjord M, Dahllöf G, Nilsson B, Modéer T. Prediction of dental caries development in 1-year-old children. Caries Res 29: 343-8; 1995.

Grindefjord M, Dahllöf G, Nilsson B, Modéer T. Stepwise prediction of dental caries in children up to 3.5 years of age. Caries Res 30: 256-66; 1996.

Günay H, Dmoch-Bockhorn K, Günay Y, Geurtsen W. Effect on caries experience of a long-term preventive program for mothers and children starting during pregnancy. Clin Oral Investig 2: 137-42; 1998.

Hatakka K, Savilahti E, Pönkä A, Meurman JH, Poussa T, Näse L, Saxelin M, Korpela R. Effect of long term consumption of probiotic milk on infections in children attending day care centres: Double blind, randomised trial. BMJ 322: 1327; 2001.

Hausen H, Alaluusua S, Alanen P, Anttonen V, Hiiri A, Jokela J, Järvinen S, Keskinen H, Laitinen J, Pienihäkkinen K, Seppä L. Karieksen hallinta; controlling dental caries [online]. Available at: http://www.terveysportti.fi/xmedia/hoi/hoi50078.pdf.

Hausen H, Kärkkäinen S, Seppä L. Application of the high-risk strategy to control dental caries. Community Dent Oral Epidemiol 28: 26-34; 2000.

Hausen H, Kärkkäinen S, Seppä L. Caries data collected from public health records compared with data based on examinations by trained examiners. Caries Res 35: 360-5; 2001.

Hausen H, Seppä L, Poutanen R, Niinimaa A, Lahti S, Kärkkäinen S, Pietilä I. Noninvasive control of dental caries in children with active initial lesions. A randomized clinical trial. Caries Res 41: 384-91; 2007.

Hoerman K, Keene H, Shklair I, Burmeister J. The association of streptococcus mutans with early carious lesions in human teeth. J Am Dent Assoc 85: 1349-52; 1972.

Holgerson P, Twetman S, Stecksèn-Blicks C. Validation of an age-modified caries risk assessment program (cariogram) in preschool children. Acta Odontol Scand 67: 106-12; 2009.

Holgerson PL, Sjöström I, Stecksén-Blicks C, Twetman S. Dental plaque formation and salivary mutans streptococci in schoolchildren after use of xylitol-containing chewing gum. Int J Paediatr Dent 17: 79-85; 2007.

Ismail A, Hasson H. Fluoride supplements, dental caries and fluorosis: A systematic review. J Am Dent Assoc 139: 1457-68; 2008.

Ismail A, Lim S, Sohn W, Willem J. Determinants of early childhood caries in low-income african american young children. Pediatr Dent 30: 289-296; 2008.

Isokangas P, Söderling E, Pienihäkkinen K, Alanen P. Occurrence of dental decay in children after maternal consumption of xylitol chewing gum, a follow-up from 0 to 5 years of age. J Dent Res 79: 1885-9; 2000.

Joensuu T. Cumulative costs of caries prevention and treatment in children. In: Faculty of Medicine, Community Dentistry; Thesis. Turku, Finland: University of Turku; 2009: 104.

Kanasi E, Johansson I, Lu S, Kressin N, Nunn M, Kent RJ, Tanner A. Microbial risk markers for childhood caries in pediatricians' offices. J Dent Res 89: 378-83; 2010.

Karjalainen S, Söderling E, Pienihäkkinen K. Validation and inter-examiner agreement of mutans streptococci levels in plaque and saliva of

10-year-old children using simple chair-side tests. Acta Odontol Scand 62: 5; 2004.

Karn TA, O'Sullivan DM, Tinanoff N. Colonization of mutans streptococci in 8- to 15-month-old children. J Public Health Dent 58: 248-9; 1998.

Kasila K, Poskiparta M, Kettunen T, Pietilä I. Oral health counselling in changing schoolchildren's oral hygiene habits: A qualitative study. Community Dent Oral Epidemiol 34: 419-28; 2006.

Kowash M, Pinfield A, Smith J, Curzon M. Effectiveness on oral health of a long-term health education programme for mothers with young children. Br Dent J 188: 201-5; 2000.

Kozai K, Nakayama R, Tedjosasongko U, Kuwahara S, Suzuki J, Okada M, Nagasaka N. Intrafamilial distribution of mutans streptococci in japanese families and possibility of father-to-child transmission. Microbiol Immunol 43: 99-106; 1999.

Kressin N, Nunn M, Singh H, Orner M, Pbert L, Hayes C, Culler C, Glicken S, Palfrey S, Geltman P, Cadoret C, Henshaw M. Pediatric clinicians can help reduce rates of early childhood caries: Effects of a practice based intervention. Med Care 47: 1121-8; 2009.

Köhler B, Andréen I. Mutans streptococci and caries prevalence in children after early maternal caries prevention: A follow-up at eleven and fifteen years of age. Caries Res 44: 453-458; 2010.

Köhler B, Andréen I, Jonsson B. The effect of caries-preventive measures in mothers on dental caries and the oral presence of the bacteria streptococcus mutans and lactobacilli in their children. Arch Oral Biol 29: 879-83; 1984.

Köhler B, Andréen I, Jonsson B. The earlier the colonization by mutans streptococci, the higher the caries prevalence at 4 years of age. Oral Microbiol Immunol 3: 14-7; 1988.

Laitala M-L. Dental health in primary teeth after prevention of mother-child transmission of mutans streptococci. A historical cohort study on restorative visits and maternal prevention costs. In: Faculty of Medicine, Department of Community Dentistry; Thesis. Turku, Finland: University of Turku; 2010: 111.

Li Y, Caufield P. The fidelity of initial acquisition of mutans streptococci by infants from their mothers. J Dent Res 74: 681-5; 1995.

Li Y, Zhang Y, Yang R, Zhang Q, Zou J, Kang D. Associations of social and behavioural factors with early childhood caries in Xiamen city in China. Int J Paediatr Dent 21: 103-11; 2011.

Marsh P. Are dental diseases examples of ecological catastrophes? Microbiology 149: 279-94; 2003.

Marsh P. Dental plaque as a microbial biofilm. Caries Res 38: 204-11; 2004.

Marsh PD, Nyvad B. The oral microflora and biofilms on teeth. In: O Fejerskov, E Kidds eds. Dental caries; the disease and its clinical management. Oxford, UK: Blackwell Publishing Company; 2008; 163-185.

Marshall T, Eichenberger-Gilmore J, Broffitt B, Warren J, Levy S. Dental caries and childhood obesity: Roles of diet and socioeconomic status. Community Dent Oral Epidemiol 35: 449-58; 2007.

Mattila M, Rautava P, Sillanpää M, Paunio P. Caries in five-year-old children and associations with family-related factors. J Dent Res 79: 875-81; 2000.

Mattos-Graner R, Li Y, Caufield P, Duncan M, Smith D. Genotypic diversity of mutans streptococci in brazilian nursery children suggests horizontal transmission. J Clin Microbiol 39: 2313-6; 2001.

Mejàre I, Axelsson S, Dahlén G, Espelid I, Norlund A, Tranaeus S, Twetman S. Riskbedömning. In: I Mejàre, S Axelsson, G Dahlén, I Espelid, A Norlund, S Tranaeus, S Twetmans eds. Karies - diagnostik, riskbedömning och icke-invasiv behandling a systematic review. Stockholm: SBU The Swedish Council of Technology Assessment in Health Care; 2007; 199-326.

Milgrom P, Ly KA, Tut OK, Mancl L, Roberts MC, Briand K, Gancio MJ. Xylitol pediatric topical oral syrup to prevent dental caries: A double-blind randomized clinical trial of efficacy. Arch Pediatr Adolesc Med 163: 601-7; 2009.

Miller WD. Ursache der zahncaries. In die microorganismen der mundhöhle. Die örtlichen und allgemeinen erkrankungen, welche durch

dieselben hervorgerufen werden. Leipzig: Verlag von Georg Thieme; 1889.

Minah G, Lin C, Coors S, Rambob I, Tinanoff N, Grossman L. Evaluation of an early childhood caries prevention program at an urban pediatric clinic. Pediatr Dent 30: 499-504; 2008.

Mobley C, Marshall T, Milgrom P, Coldwell S. The contribution of dietary factors to dental caries and disparities in caries. Acad Pediatr 9: 410-4; 2009.

Mäkinen KK. Sugar alcohols, caries incidence, and remineralization of caries lesions: A literature review. Int J Dent 2010: 981072; 2010.

Mäkinen KK, Chen CY, Mäkinen PL, Bennett CA, Isokangas PJ, Isotupa KP, Pape HR. Properties of whole saliva and dental plaque in relation to 40-month consumption of chewing gums containing xylitol, sorbitol of sucrose. Caries Res 30: 180-8; 1996.

Mäkinen KK, Isotupa KP, Mäkinen PL, Söderling E, Song KB, Nam SH, Jeong SH. Six-month polyol chewing-gum programme in kindergarten-age children: A feasibility study focusing on mutans streptococci and dental plaque. Int Dent J 55: 81-8; 2005.

Mäkinen KK, Scheinin A. Turku sugar studies. Vii. Principal biochemical findings on whole saliva and plaque. Acta Odontol Scand 34: 241-83; 1976.

Nakai Y, Shinga-Ishihara C, Kaji M, Moriya K, Murakami-Yamanaka K, Takimura M. Xylitol gum and maternal transmission of mutans streptococci. J Dent Res 89: 56-60; 2010.

Nishimura M, Oda T, Kariya N, Matsumura S, Shimono T. Using a caries activity test to predict caries risk in early childhood. J Am Dent Assoc 139: 63-71; 2008.

Nyvad B. Microbial colonization of human tooth surfaces. APMIS Suppl 32: 1-45; 1993.

Näse L, Hatakka K, Savilahti E, Saxelin M, Pönkä A, Poussa T, Korpela R, Meurman JH. Effect of long-term consumption of a probiotic bacterium, lactobacillus rhamnosus gg, in milk on dental caries and caries risk in children. Caries Res 35: 412-20; 2001.

Oliveira A, Chaves A, Rosenblatt A. The influence of enamel defects on the development of early childhood caries in a population with low socioeconomic status: A longitudinal study. Caries Res 40: 296-302; 2006.

Ollila P, Larmas M. A seven-year survival analysis of caries onset in primary second molars and permanent first molars in different caries risk groups determined at age two years. Acta Odontol Scand 65: 29-35; 2007.

Ollila P, Larmas M. Long-term predictive value of salivary microbial diagnostic tests in children. Eur Arch Paediatr Dent 9: 25-30; 2008.

Oscarson P, Lif Holgerson P, Sjöström I, Twetman S, Stecksén-Blicks C. Influence of a low xylitol-dose on mutans streptococci colonisation and caries development in preschool children. Eur Arch Paediatr Dent 7: 142-7; 2006.

Paunio P, Rautava P, Helenius H, Alanen P, Sillanpää M. The finnish family competence study: The relationship between caries, dental health habits and general health in 3-year-old finnish children. Caries Res 27: 154-60; 1993.

Petti S, Hausen H. Caries-preventive effect of chlorhexidine gel applications among high-risk children. Caries Res 40: 514-21; 2006.

Pienihäkkinen K, Jokela J. Clinical outcomes of risk-based caries prevention in preschool-aged children. Community Dent Oral Epidemiol 30: 143-50; 2002.

Pienihäkkinen K, Jokela J, Alanen P. Assessment of caries risk in preschool children. Caries Res 38: 156-62; 2004.

Pienihäkkinen K, Jokela J, Alanen P. Risk-based early prevention in comparison with routine prevention of dental caries: A 7-year follow-up of a controlled clinical trial; clinical and economic aspects. BMC Oral Health 5: 2; 2005.

Pine C, Adair P, Nicoll A, Burnside G, Petersen P, Beighton D, Gillett A, Anderson R, Anwar S, Brailsford S, Broukal Z, Chestnutt I, Declerck D, Ping F, Ferro R, Freeman R, Gugushe T, Harris R, Lin B, Lo E, Maupomé G, Moola M, Naidoo S, Ramos-Gomez F, Samaranayake L, Shahid S, Skeie M, Splieth C, Sutton B, Soo T, Whelton H. International comparisons of health inequalities in childhood dental caries. Community Dent Health 21: 121-30; 2004.

Pizzo G, Piscopo M, Pizzo I, Giuliana G. Community water fluoridation and caries prevention: A critical review. Clin Oral Investig 11: 189-93; 2007.

Radford J, Ballantyne H, Nugent Z, Robertson M, Longbottom C, Pitts N, Beighton D, Brailsford S. Does social deprivation in 1, 2, 3 and 4-year-old scottish infants influence the frequency isolation of caries-associated micro-organisms? J Dent 29: 325-32; 2001.

Rasines G. Fluoride toothpaste prevents caries in children and adolescents at fluoride concentrations of 1000 ppm and above. Evid Based Dent 11: 6-7; 2010.

Robke FJ. Effects of nursing bottle misuse on oral health. Prevalence of caries, tooth malalignments and malocclusions in north-german preschool children. J Orofac Orthop 69: 5-19; 2008.

Sackett D, Straus S, Richardson W, Rosenberg W, Haynes R. Evidence-based medicine. How to practice and teach ebm. London: Harcourt Publishers; 2000.

Sakuma S, Nakamura M, Miyazaki H. Predictors of dental caries development in 1.5-year-old high-risk children in the japanese public health service. J Public Health Dent 67: 14-9; 2007.

Scavuzzi A, De França Caldas Junior A, Couto G, De Vasconcelos M, De Freitas Soares R, Valença P. Longitudinal study of dental caries in brazilian children aged from 12 to 30 months. Int J Paediatr Dent 17: 123-8; 2007.

Scheinin A, Mäkinen KK, Ylitalo K. Turku sugar studies. I. An intermediate report on the effect of sucrose, fructose and xylitol diets on the caries incidence in man. Acta Odontol Scand 32: 383-412; 1974.

Seki M, Yamashita Y, Shibata Y, Torigoe H, Tsuda H, Maeno M. Effect of mixed mutans streptococci colonization on caries development. Oral Microbiol Immunol 21: 47-52; 2006.

Seppä L. The future of preventive programs in countries with different systems for dental care. Caries Res 35 Suppl 1: 26-9; 2001.

Seppä L, Hausen H, Pöllänen L, Kärkkäinen S, Helasharju K. Effect of intensified caries prevention on approximal caries in adolescents with high caries risk. Caries Res 25: 392-5; 1991.

Skeie M, Espelid I, Riordan P, Klock K. Caries increment in children aged 3-5 years in relation to parents' dental attitudes: Oslo, norway 2002 to 2004. Community Dent Oral Epidemiol 36: 441-50; 2008.

Skeie M, Riordan P, Klock K, Espelid I. Parental risk attitudes and caries-related behaviours among immigrant and western native children in oslo. Community Dent Oral Epidemiol 34: 103-13; 2006.

Slabsinskiene E, Milciuviene S, Narbutaite J, Vasiliauskiene I, Andruskeviciene V, Bendoraitiene E, Saldūnaite K. Severe early childhood caries and behavioral risk factors among 3-year-old children in lithuania. Medicina (Kaunas) 46: 135-41; 2010.

Straetemans MM, van Loveren C, de Soet JJ, de Graaff J, ten Cate JM. Colonization with mutans streptococci and lactobacilli and the caries experience of children after the age of five. J Dent Res 77: 1851-5; 1998.

Söderling E, Isokangas P, Pienihäkkinen K, Tenovuo J. Influence of maternal xylitol consumption on acquisition of mutans streptococci by infants. J Dent Res 79: 882-7; 2000.

Söderling E, Isokangas P, Pienihäkkinen K, Tenovuo J, Alanen P. Influence of maternal xylitol consumption on mother-child transmission of mutans streptococci: 6-year follow-up. Caries Res 35: 173-7; 2001.

Söderling E, Mäkinen KK, Chen CY, Pape HR, Loesche W, Mäkinen PL. Effect of sorbitol, xylitol, and xylitol/sorbitol chewing gums on dental plaque. Caries Res 23: 378-84; 1989.

Söderling E, Trahan L, Lenander-Lumikari M. Growth of xylitol-resistant versus xylitol-sensitive streptococcus mutans strains in saliva. Acta Odontol Scand 56: 116-21; 1998.

Söderling EM. Xylitol, mutans streptococci, and dental plaque. Adv Dent Res 21: 74-8; 2009.

Taipale T. *Personal communication*. 2010.

Takahashi N, Nyvad B. Caries ecology revisited: Microbial dynamics and the caries process. Caries Res 42: 409-18; 2008.

Tamaki Y, Nomura Y, Katsumura S, Okada A, Yamada H, Tsuge S, Kadoma Y, Hanada N.

Construction of a dental caries prediction model by data mining. J Oral Sci 51: 61-8; 2009.

Tankkunnasombut S, Youcharoen K, Wisuttisak W, Vichayanrat S, Tiranathanagul S. Early colonization of mutans streptococci in 2- to 36-month-old thai children. Pediatr Dent 31: 47-51; 2009.

Tanner A, Milgrom P, Kent RJ, Mokeem S, Page R, Riedy C, Weinstein P, Bruss J. The microbiota of young children from tooth and tongue samples. J Dent Res 81: 53-7; 2002.

Tanzer J, Freedman M. Genetic alterations of streptococcus mutans' virulence. Adv Exp Med Biol 107: 661-72; 1978.

Tanzer J, Thompson A, Wen Z, Burne R. Streptococcus mutans: Fructose transport, xylitol resistance, and virulence. J Dent Res 85: 369-73; 2006.

Tanzer JM, Livingston J, Thompson AM. The microbiology of primary dental caries in humans. J Dent Educ 65: 1028-37; 2001.

Teanpaisan R, Thitasomakul S, Piwat S, Thearmontree A, Pithpornchaiyakul W, Chankanka O. Longitudinal study of the presence of mutans streptococci and lactobacilli in relation to dental caries development in 3-24 month old thai children. Int Dent J 57: 445-51; 2007.

Tenovuo J, Häkkinen P, Paunio P, Emilson CG. Effects of chlorhexidine-fluoride gel treatments in mothers on the establishment of mutans streptococci in primary teeth and the development of dental caries in children. Caries Res 26: 275-80; 1992.

Thenisch N, Bachmann L, Imfeld T, Leisebach Minder T, Steurer J. Are mutans streptococci detected in preschool children a reliable predictive factor for dental caries risk? A systematic review. Caries Res 40: 366-74; 2006.

Thorild I, Lindau B, Twetman S. Effect of maternal use of chewing gums containing xylitol, chlorhexidine or fluoride on mutans streptococci colonization in the mothers' infant children. Oral Health Prev Dent 1: 53-7; 2003.

Tiberia MJ, Milnes AR, Feigal RJ, Morley KR, Richardson DS, Croft WG, Cheung WS. Risk factors for early childhood caries in canadian preschool children seeking care. Pediatr Dent 29: 201-8; 2007.

Tilastokeskus. Sosioekonominen asema – luokitus. Ammattiasemaluokitus. (The Finnish Official Statistical Classification). 1983.

Trahan L. Xylitol: A review of its action on mutans streptococci and dental plaque--its clinical significance. Int Dent J 45: 77-92; 1995.

Trahan L, Mouton C. Selection for streptococcus mutans with an altered xylitol transport capacity in chronic xylitol consumers. J Dent Res 66: 982-8; 1987.

Trahan L, Söderling E, Dréan M, Chevrier M, Isokangas P. Effect of xylitol consumption on the plaque-saliva distribution of mutans streptococci and the occurrence and long-term survival of xylitol-resistant strains. J Dent Res 71: 1785-91; 1992.

Vachirarojpisan T, Shinada K, Kawaguchi Y, Laungwechakan P, Somkote T, Detsomboonrat P. Early childhood caries in children aged 6-19 months. Community Dent Oral Epidemiol 32: 133-42; 2004.

Vehkalahti M, Knuuttila M. Oral self-care. Helsinki, Finland: National Public Health Institute; 2008.

Walsh T, Worthington HV, Glenny AM, Appelbe P, Marinho VC, Shi X. Fluoride toothpastes of different concentrations for preventing dental caries in children and adolescents. Cochrane Database Syst Rev: CD007868; 2010.

Wan A, Seow W, Purdie D, Bird P, Walsh L, Tudehope D. Oral colonization of streptococcus mutans in six-month-old predentate infants. J Dent Res 80: 2060-5; 2001.

Warren J, Weber-Gasparoni K, Marshall T, Drake D, Dehkordi-Vakil F, Dawson D, Tharp K. A longitudinal study of dental caries risk among very young low ses children. Community Dent Oral Epidemiol 37: 116-22; 2009.

Weinstein P, Harrison R, Benton T. Motivating mothers to prevent caries: Confirming the beneficial effect of counseling. J Am Dent Assoc 137: 789-93; 2006.

Wendt L, Hallonsten A, Koch G, Birkhed D. Analysis of caries-related factors in infants and

toddlers living in sweden. Acta Odontol Scand 54: 131-7; 1996.

Wigen TI, Espelid I, Skaare AB, Wang NJ. Family characteristics and caries experience in preschool children. A longitudinal study from pregnancy to 5 years of age. Community Dent Oral Epidemiol; 2010.

Yonezu T, Ushida N, Yakushiji M. Longitudinal study of prolonged breast- or bottle-feeding on dental caries in japanese children. Bull Tokyo Dent Coll 47: 157-60; 2006a.

Yonezu T, Yakushiji M. Longitudinal study on influence of prolonged non-nutritive sucking habits on dental caries in japanese children from 1.5 to 3 years of age. Bull Tokyo Dent Coll 49: 59-63; 2008.

Yonezu T, Yotsuya K, Yakushiji M. Characteristics of breast-fed children with nursing caries. Bull Tokyo Dent Coll 47: 161-5; 2006b.

Zhan L, Featherstone J, Gansky S, Hoover C, Fujino T, Berkowitz R, Den Besten P. Antibacterial treatment needed for severe early childhood caries. J Public Health Dent 66: 174-9; 2006.