Caries Prevention in Patients Undergoing Orthodontic Treatment

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Cover illustration: Clinical photographs taken before, during and after orthodontic treatment with fixed appliances. The left- and right-hand photos were taken by Anna Westerlund. The middle photograph was taken by Sara Waldenström.

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ABSTRACT

Background: Scientifically based guidelines to predict and prevent caries during orthodontic treatment with fixed orthodontic appliances are lacking.

Aim: The overall aim of this thesis was to improve caries risk assessment before orthodontic treatment, as well as to evaluate caries risk and improve caries prevention during treatment.

Patients and methods: Patients (n=270) undergoing treatment with fixed appliance at the Specialist Clinic of Orthodontics in Mölndal, Sweden, were included. Studies I, III and IV were performed with an RCT design, with the subjects being randomly assigned to one of the following groups: i. Fluoride mouth rinse (FMR) group, 0.2 % sodium fluoride (NaF) mouth rinse plus 1450 ppm F toothpaste; ii. High-fluoride toothpaste (HFT) group, 5000 ppm F; and iii. Control (CTR) group, 1450 ppm F toothpaste. In study I, the effect of orthodontic treatment and the different fluoride regimens on caries risk and caries risk factors were evaluated. Study II was performed with a prospective design that evaluated the CRA programmes and the caries indices abilities to predict the outcome of caries during treatment. In studies III and IV, the impacts of the different fluoride regimens on caries incidence (through radiographs and clinical photographs) during orthodontic treatment were evaluated.

Results: The FMR and HFT groups showed an unchanged caries risk during treatment, while the caries risk increased significantly in the CTR group ($p<0.0001$). The DiFS index demonstrated the highest accuracy in predicting initial and manifest caries during treatment with fixed appliances, as compared to the multi-factorial CRA programmes. Radiographic analyses revealed no significant difference between the fluoride groups in terms of increased caries incidence during treatment. However, the numbers of patients with an increase of one or more white spot lesions (WSL) during

orthodontic treatment were significantly higher in the CTR group than in the FMR group (p=0.0097) or HFT group (p=0.018) when the data for incisors, lateral incisors and canines were included.

Conclusion: The DiFS index most-accurately predicts caries during orthodontic treatment. Furthermore, a mouth rinse or high-fluoride toothpaste can be recommended during orthodontic treatment to retain low caries risk and to reduce the numbers of WSLs in the aesthetic front.

Keywords: Caries, caries incidence, caries prevalence, caries risk, fluoride, mouth rinse, orthodontics, risk assessment, toothpaste.

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SAMMANFATTNING PÅ SVENSKA

Bakgrund: Evidensbaserade riktlinjer för riskbedömning och prevention av karies under behandling med fast tandställning saknas.

Mål: Det övergripande målet för studierna var att förbättra kariesriskbedömning före ortodontibehandling samt att utvärdera kariesrisk och förbättra kariesprevention under behandling.

Patient och metoder: Patienter (n=270) som behandlades med fast apparatur vid specialistkliniken för ortodonti i Mölndal, Sverige deltog. Studie I, III och IV genomfördes med RCT-design. Deltagarna randomiserades till en av följande grupper: i. Fluorskölj, 0,2% natriumfluorid (NaF) skölj + 1450 ppm fluortandkräm; ii. Högfluortandkräm, 5000 ppm F; och iii. Kontroll, 1450 ppm fluortandkräm. I studie I utvärderades effekten av ortodontibehandling samt olika fluorprodukter på kariesrisk och kariesrelaterade faktorer. Studie II utfördes med en prospektiv studiedesign där program för kariesriskbedömning samt kariesindex utvärderades avseende förmåga att prediktera kariesutfallet under tandregleringsbehandling. I studie III och IV utvärderades effekten av olika fluorprodukter på kariesutveckling (baserat på röntgen resp. kliniska foton) under ortodontibehandling.

Resultat: Kariesrisken ökade signifikant under ortodontibehandling i kontrollgruppen ($p<0.0001$), vilket skiljde sig från fluorskölj- och högfluortandkrämsgruppen som visade en statistiskt oförändrad kariesrisk under behandling. DiFS-index vid behandlingsstart visade sig vara den mest tillförlitliga prediktorn för utveckling av karies under ortodontibehandling. Ingen signifikant skillnad kunde ses mellan kontrollgrupp och fluorskölj- och högfluortandkrämsgruppen vad det gällde utvecklingen av initial och manifest karies under ortodontibehandling, baserat på röntgen. Däremot ökade antalet patienter med ≥1 kritkariesskador (WSL) på framtänder signifikant mer i kontrollgruppen jämfört med i fluorskölj- ($p=0.0097$) och högfluortandkrämsgruppen ($p=0.018$).

Konklusion: DiFS indexet uppvisade högst tillförlitlighet gällande prediktion av karies under tandregleringsbehandling. Vidare kan fluorsköljning eller högfluortandkräm rekommenderas under behandling med fast tandställning för att bibehålla en låg kariesrisk samt för att förhindra kritkaries i framtandsområdet.

LIST OF PAPERS

This thesis is based on the following studies, which are referred to in the text by their Roman numerals.

I. **Enerbäck H**, Möller M, Nylén C, Ödman Bresin C, Östman Ros I, Westerlund A. Effects of orthodontic treatment and different fluoride regimens on numbers of cariogenic bacteria and caries risk: a randomized controlled trial. Eur J Orthod. 2019; 41:59-66.

II. **Enerbäck H**, Lingström P, Möller M, Nylén C, Ödman Bresin C, Östman Ros I, Westerlund A. Caries risk assessment during orthodontic treatment. Am J Orthod Dentofacial Orthop. 2020; 158:92-101.e3

III. **Enerbäck H**, Lingström P, Möller M, Nylén C, Ödman Bresin C, Östman Ros I, Westerlund A. Effect of a mouth rinse and a high-fluoride toothpaste on caries incidence in orthodontic patients – a randomised controlled trial. Am J Orthod Dentofacial Orthop. 2022; 162:6-15.e3

IV. **Enerbäck H**, Lövgren M, Strömberg N, Westerlund A. Effect of high-fluoride toothpaste and mouth rinse on the prevention of white spot lesions during orthodontic treatment: a randomised controlled trial. In manuscript.

CONTENT

LIST OF ABBREVIATIONS V
DEFINITIONS IN BRIEF VI
1 INTRODUCTION 1
1.1 Dental caries 1
1.2 Epidemiology 1
1.3 Aetiology 2
1.3.1 Mechanisms 2
1.3.2 Diet, saliva and oral bacteria 3
1.3.3 Interacting factors 4
1.3.4 Emerging knowledge 4
1.4 Caries risk assessment 6
1.4.1 Caries risk 6
1.4.2 Risk factors 6
1.4.3 Caries risk assessment programmes 6
1.5 Caries index 12
1.6 Caries prevention strategies 12
1.6.1 Diet 12
1.6.2 Fluoride 13
1.6.3 Oral hygiene 15
1.6.4 Antimicrobial agents 15
1.6.5 Probiotics 15
1.6.6 Motivational interviewing 16
1.7 Caries in orthodontic patients 16
1.7.1 White spot lesions 16
1.7.2 Prevalence 17
1.7.3 Aggravating factors for increased caries risk during FOA . 17
1.8 Caries prevention in FOA patients 18

1.8.1 Meta-analysis and systematic reviews 18
1.8.2 RCT and cohort studies 18
1.9 The effect of fluoride on caries-related factors in FOA patients 19
1.10 Management of post-orthodontic WSLs 19
2 AIMS 20
3 PATIENTS AND METHODS 21
3.1 An overview of the studies 21
3.2 Study subjects 22
3.3 Study design 22
3.4 Interventions 24
3.5 Data collection 24
3.5.1 Caries risk factors 24
3.5.2 Radiographic caries registration 27
3.5.3 WSL registration 27
3.6 Compliance 28
3.7 Ethical considerations 28
3.8 Statistical analysis 29
3.8.1 Study I 29
3.8.2 Study II 29
3.8.3 Study III 30
3.8.4 Study IV 30
3.8.5 Sample size estimations 31
4 RESULTS 32
4.1 Study I 32
4.2 Study II 36
4.3 Study III 38
4.4 Study IV 41
4.4.1 Harms 42
5 DISCUSSION 43

5.1.1 Caries risk assessment 43
5.1.2 Caries risk during orthodontic treatment 45
5.1.3 Caries and fluoride interventions during orthodontic treatment 46
5.1.4 Studying caries in the orthodontic patients 48
5.1.5 Clinical implications 48
5.1.6 Methodological considerations 49
5.1.7 Limitations 49
5.1.8 Ethical considerations 49
6 CONCLUSIONS 50
7 FUTURE PERSPECTIVES 51
8 ACKNOWLEDGEMENTS 52
9 REFERENCES 53
10 APPENDIX 65
10.1 Questionnaire 65
10.2 Studies I-IV 67

LIST OF ABBREVIATIONS

CAMBRA	Caries management by risk assessment
CONSORT	Consolidated Standards of Reporting Trials
CRA	Caries Risk Assessment
DiFS	Decayed (initial and manifest) and Filled Surfaces
DFS	Decayed and Filled Surfaces
DFT	Decayed and Filled Teeth
DiMFS	Decayed (initial and manifest), Missing, and Filled Surfaces
DMFS	Decayed, Missing, and Filled Surfaces
DMFT	Decayed, Missing, and Filled Teeth
FOA	Fixed Orthodontic Appliance
LBC	Lactobacilli
MS	Mutans streptococci
NaF	Sodium fluoride
NBHW	National Board of Health and Welfare
QLF	Quantitative Light-induced Fluorescence
SiC index	Significant Caries Index
WSL	White spot lesion

DEFINITIONS IN BRIEF

Caries incidence	"The number/proportion of individuals with new or progressing caries at a specified threshold in a given population, detected during a given period (Machiulskiene et al., 2020)"
Caries prevalence	"The number/proportion of individuals with caries in a given population at a specified threshold, at a designated point in time (Machiulskiene et al., 2020)"
Caries risk	"The probability that caries lesions will appear or progress if conditions remain the same within a stated period of time. Caries risk is a proxy for the true outcome (new caries lesions or progression), which can only be validated over time" (Machiulskiene et al., 2020).
Prevented fraction	The difference between the incidence in the control and test group, divided by the incidence in the control group × 100
Reliability	Precision, how well the same test gives the same results for different measurements.
Sensitivity	Probability that a test result will be positive when the disease is present.
Specificity	Probability that a test result will be negative when the disease is not present.
Validity	Accuracy, how well you have in reality managed to measure what you intended to measure.

1 INTRODUCTION

1.1 DENTAL CARIES

Dental caries is one of the most prevalent health conditions of our time, affecting children and adults of all ages. Caries represents a serious public health problem, and the cost of dental care worldwide is substantial, corresponding to about 5% of the total global health expenditure (GBD 2016 Disease and Injury Incidence and Prevalence Collaborators, 2017; Listl et al., 2015). A caries lesion that is left untreated can lead to bacterial invasion of the pulp, followed by inflammation and necrosis of the pulp, and eventual apical infection of the tooth. The caries lesions can cause pain, discomfort while chewing, nutritional compromise, and tooth loss, thereby affecting negatively the patient's quality of life (Figueiredo et al., 2011).

In recent years, research has examined the association between oral health and its impact on general health. Caries and periodontitis are reported risk factors for stroke and cardiovascular disease (Vedin et al., 2015; Watanabe et al., 2016). Caries research attracts strong interest, with several clinical issues needing to be examined more thoroughly.

1.2 EPIDEMIOLOGY

Globally, caries within the permanent dentition is estimated to be the most common, chronic infectious disease (Kassebaum et al., 2015). Oral diseases affect approximately 3.58 billion people, entailing a great financial burden worldwide. (GBD 2016 Disease and Injury Incidence and Prevalence Collaborators, 2017).

Untreated caries in the permanent dentition appears in 2.4 billion adults, and the corresponding prevalence of untreated caries in the deciduous dentition is approximately 621 million children (Kassebaum et al., 2015). Furthermore, a recent systematic review demonstrated internationally the prevalence of caries in the permanent dentition to be 54% and in the primary dentition 46%, but with large regional differences (Kazeminia et al., 2020). Thus, the caries prevalence is skewed both within and between populations. Most of the dental diseases are found in the socioeconomically lowest quartile of the society (ten Cate, 2013). In general, in economically developed countries there has been a decline in the prevalence of caries during the last five decades, whereas in economically less-developed countries, the numbers of persons with caries have increased (Konig, 2004). The prevalence of dental caries increase in developing countries as the urbanization takes place, traditional diets is replaced with high sugar intake of fast food and lack of fluoride supplements (Diehnelt et al., 2001).

In Sweden, a clear reduction in caries prevalence has been seen since the 1960s (Hugoson et al., 2008). In particular, the use of fluoride toothpaste has played a major role in this decline, as have the use of fluoride rinses, fluoride varnishes and preventive measures. A clear increase in the number of caries-free children has occurred in recent decades, although this trend has slowed down and plateaued in recent years (Socialstyrelsen. National Board of Health and Welfare, 2020). The NBHW in Sweden presents every year caries prevalence data for children and adolescents in the age range of 3–19 years. The recent compilation for year 2021 presented a DFT value of 0.7 for12-year-olds, and DFT a value of 1.9 for 19-year-olds. In the same year, 69% of 12-year-olds and 46% of 19-year-olds were caries-free (Socialstyrelsen. National Board of Health and Welfare, 2022). The epidemiological compilation does not take into account caries lesions in the enamel, indicating an under-estimation of the prevalence of caries disease. To demonstrate the distribution of the skewness of caries disease, the Significant Caries Index (Sic index) is often used (D. Bratthall, 2000). In 2019, the Sic index values were 2.1 for 12-year-olds and 5.4 for 19-year-olds on a national level (Socialstyrelsen. National Board of Health and Welfare, 2021d). About 15% of the Swedish population are high-risk patients who have recurrent caries and are non-responders to standard prevention (Esberg et al., 2017). Furthermore, an increased prevalence of caries among the elderly population has been registered. This is due to increased life-span and that a higher proportion of patients retain their teeth throughout life (Selwitz et al., 2007). Recent immigration may also affect caries prevalence on the population level (Riggs et al., 2017).

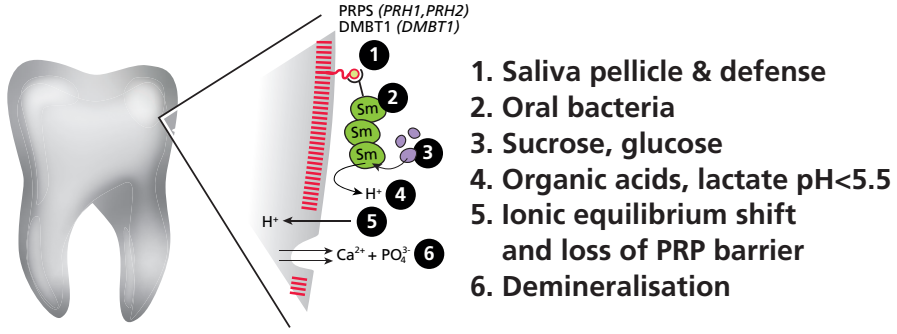
1.3 AETIOLOGY

1.3.1 MECHANISMS

Dental plaque is a biofilm that is naturally present on the teeth. The first step in the formation of a dental biofilm is the formation of a pellicle, which is a thin membrane of salivary proteins on the tooth surface, to which the oral bacteria and organic polymers subsequently attach (Fejerskov O, 2015). Bacteria in the biofilm grow slowly, express other genes and proteins, and are more stress- and antibiotic- resistant, as compared to non-biofilm-mediated bacteria in whole saliva. At neutral pH, the saliva is saturated in relation to hydroxyapatite, although as the pH decreases the solubility increases. Oral cariogenic bacteria, such as streptococci, lactobacilli (LBC) and actinomyces species, have the ability to produce organic acids, mainly lactic acid, from fermentable carbohydrates leading to a decreased in the pH within the biofilm (Aas et al., 2008; Beighton, 2005; Marsh, 2010). Protons and hydrogen ions diffuse in between the enamel and dentin calcium phosphate crystals. The ionic equilibrium in the liquid around the crystals shifts, and they become partially dissolved and the released calcium and phosphate crystals migrate out of the tooth, leading to demineralisation (Figure 1). While the enamel dissolves at about pH 5.5, corresponding demineralisation of the dentin occurs at about pH 6.2 (Fejerskov O, 2015). The access of the bacteria to fermentable carbohydrates affects the duration of the pH decrease, together with the saliva's flow rate, the saliva's buffering capacity, and access to fluoride, which shortens the time for the pH decrease (Marsh, 2010; van

Houte, 1994). Re-mineralisation occurs when there is an inward movement of re-mineralising agents. When the availability of fermentable carbohydrates decreases, the pH increases. The tooth substance is partially re-built from the calcium and phosphate that remain in the saliva around the tooth crystals.

Figure 1. Schematic figure of tooth tissue demineralisation and key components involved: 1) saliva pellicle barriar and defences, 2) oral bacteria, including S. mutans (Sm), 3) sucrose exposure, 4) bacterial production of short chains fatty acids, such as lactate, and lowered pH 4) Ionic equilibrium shift and increased hydrogen, H+, activity, 5) enamel crystal disruption and demineralization. PRPs (PRH1, PRH2) and DMBT1 (DMBT1) are major saliva proteins (and associated genes) that influences caries development.



1.3.2 DIET, SALIVA AND ORAL BACTERIA

Saliva consists of 99.5% water and 0.5% of minerals and organic components including, salts, protein, glycoproteins, antibodies and antimicrobial substances. Human saliva has multiple functions, such as maintaining a neutral pH through its buffering capacity, which is attributed to the bicarbonate, phosphate and protein systems. In addition, the saliva exerts antimicrobial actions (antibodies, immunoglobulins), oral clearance, lubrication of the oral cavity, and digestion and transport of food (Pedersen et al., 2018).

The aetiological link between oral micro-organisms and caries has been discussed for centuries and has evolved over time. In the 1930s, the “nonspecific plaque hypothesis” was presented, which stated that the total oral microbiota can cause caries without discriminating between the virulence levels of bacteria. Plaque control and optimal oral hygiene became key principles. In 1976, the hypothesis was replaced by the “specific plaque hypothesis”, which proposed that a few oral bacteria species, including mutans streptococci (MS), are actively involved in causing disease (Loesche, 1976). However, the presence of MS and LBC in saliva as the sole predictor of caries was deemed to lack sufficient reliability (SBU. The Swedish Council on Technology Assessment in Health Care, 2007). In 1991, the “ecological plaque hypothesis” was presented, and this is the most widely accepted caries hypothesis today. It proposes that caries is manifested when a shift in the balance of the oral microflora occurs that

is driven by changes in local environmental conditions (ecological stress). For many individuals, this is caused by increased dietary intake of sugar. As a result, an imbalance in the composition (dysbiosis) of the biofilm is seen, which results in the enrichment of bacterial species with specific characteristics (Marsh, 1994). Micro-organisms that can adapt will survive and make better use of the available sugars. It is not necessarily the microbial composition that changes, but it is that the bacterial acid-resistant and acid-forming activities increase.

The dysbiotic communities in the onset of caries are mainly characterised by MS, LBC, aciduric non-MS and actinomyces species. MS, particularly *Streptococcus mutans* and *Streptococcus sobrinus* are associated with the initiation of caries and are characterised by their acidogenicity and acidurity (Loesche, 1986). Recent research has revealed that children and adolescents who demonstrate a high risk for caries carry more virulent variants of *S. mutans* with specific phenotypes (Esberg et al., 2017). LBC are able to lower significantly the biofilm pH and are more involved in the later stages of the caries lesion. However, numerous oral bacteria species and the interactions that occur between them contribute significantly to the caries process (Aas et al., 2008; Mira et al., 2017; Tanner et al., 2018).

1.3.3 INTERACTING FACTORS

The formation of caries involves a triad of indispensable factors; the microflora, the host, and diet, as traditionally presented in the Keye's circles triad (PH, 1962). Keye's circles have, over the years, been modified and extended to include numerous other factors to describe the multi-factorial nature of the caries disease. Factors such as saliva composition, saliva flow rate, and saliva buffering capacity, oral hygiene measures, fluoride, medications, frequency and types of diets, as well as genetic variations are only some of the factors affecting the rate of disease development (Selwitz et al., 2007). Socio-economics factors, level of education and socio-demographic status are other factors that affect the disease (Chapple et al., 2017).

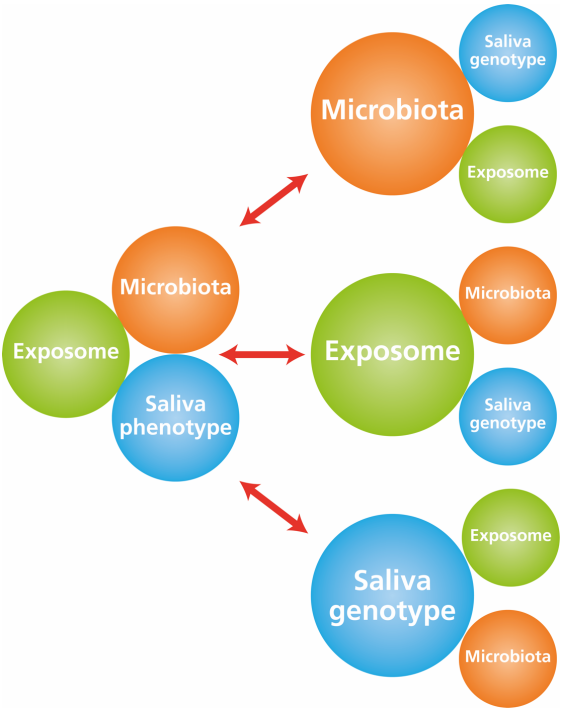
1.3.4 EMERGING KNOWLEDGE

In recent years it has been demonstrated that chronic caries infection is not only a common health risk but may also be a risk factor for cardiovascular disease later in life (Vedin et al., 2015). The linkage between specific subtypes of oral *S. mutans* that express collagen binding properties and the risk of cerebral micro-bleeds has been proposed as a risk for stroke and dementia (Watanabe et al., 2016). Furthermore, a link between caries and pneumonia, as well as between caries and Alzheimer's disease in elderly persons has been suggested (Holmer et al., 2018; Hong et al., 2018).

Recently, caries has been shown, among high-risk individuals, to be caused by genetic immunodeficiency and highly virulent strains of *S. mutans*. In about 15% of individuals who suffer from recurrent caries and are non-responsive to standard preventive treatment, the disease may be attributed to a genetic immunodeficiency aetiological type of caries (Esberg et al., 2017; Stromberg et al., 2017). Furthermore, it has been revealed that the high-risk children presented with a genetically defect set of saliva proteins do not transmit the same innate and adaptive immunity to the oral

flora as is the case in individuals with a medium or low risk of caries (Stromberg et al., 2017). A schematic of the different aetiological factors for caries and their interactions are presented in Figure 2.

Figure 2. Etiological causes (microbiota, genes encoding saliva proteins, exposome) for caries from early to today's models. To the left are early textbook models assuming that a number of microbial, saliva and exposome (e.g., dietary and oral hygiene habits and socio-economy) synergize in caries development. To the right are emerging novel models suggesting that specific commensal microbial pathogens, exposome or saliva genetic polymorphism may be primary and dominant causes for caries development in particular individuals.



1.4 CARIES RISK ASSESSMENT

1.4.1 CARIES RISK

There are various definitions of risk. There is currently no consensus as to a definition within and between different fields of Science. The World Health Organization (WHO), defined in 2009 a health risk as *"a factor that raises the probability of adverse health outcome*s" (World Health Organization (WHO), 2009). In epidemiology, risk also includes a time-span, defined as "*the probability of an 'unwanted' event occurring within a specified period of time*" (D Bratthall et al., 2004). Risk is a concept with several dimensions, probabilities and risks of outcome, as well as uncertain outcomes, including personal values that can differ between people.
Caries risk can be defined as the process of establishing "the probability that caries lesions will appear or progress if conditions remain the same within a stated period of time" (Machiulskiene et al., 2020). The purpose of a caries risk assessment is to prevent the development or progression of caries lesions through the implementation of individualised treatment and a recall interval.

1.4.2 RISK FACTORS

A risk factor implies causality on the aetiology of the disease. A risk factor can be congenital or acquired and represents a condition or trait. The variable indicates increased risk for an individual to develop a specific disease (Burt, 2005). A risk indicator, on the other hand, is a vaguer concept that applies when a direct causal relationship has not been demonstrated.

There are several caries-related risk factors for the development of caries. They can be divided into direct and indirect factors, as well as attack and defence factors (SBU. The Swedish Council on Technology Assessment in Health Care, 2007). Sugar intake and cariogenic bacteria species are directly involved in the biochemical process of caries and are, therefore, examples of direct attack factors. A direct defence factor, on the other hand, could be fluoride and saliva buffering capacity. Indirect factors are related to the development of caries but are not directly linked to the biochemical process. Past caries experience, educational level, socioeconomics, anxiety related to dental treatment and attitude to dentistry are all examples of indirect factors.

1.4.3 CARIES RISK ASSESSMENT PROGRAMMES

Caries risk assessment (CRA) is the cornerstone of caries management. The assessment of caries risk is complex, with several factors needing to be taken into account. Therefore, several CRA programmes have been developed to help the clinician to make a well-balanced assessment. As caries has reduced in prevalence, it has become increasingly important to assess patients caries risk individually and thereby target treatment and economic resources where they are needed (Domejean et al., 2017). Diagnoses are assessed and risk factors are identified through a clinical examination of the patient and in-depth anamnesis. Correct risk group assessment is important, as it is indicative for treatment, prevention and recall intervals (Twetman et

al., 2013). Since the risk may change over time, its evaluation requires regular updating, usually during follow-up or at the time of a new examination (G. H. Petersson et al., 2010b). An ideal CRA programme should present high validity and reliability and should be inexpensive and easy to perform in the daily dental practice (G. H. Petersson et al., 2010a).

The Swedish Agency for Health Technology Assessment and Assessment of Social Services concluded in their latest systematic review that there are good possibilities to identify children and adolescents who are at low risk for developing caries in the coming 2-3 years (SBU. The Swedish Council on Technology Assessment in Health Care, 2007). However, it is difficult to determine with good accuracy prediction of dental caries and which individuals are at risk for caries. Furthermore, past caries prevalence has been found to be the single best factor to predict new caries (SBU. The Swedish Council on Technology Assessment in Health Care, 2007). Other systematic reviews have shown that the validity of CRA varies between studies and is limited (Mejare et al., 2014; Tellez et al., 2013; Twetman, 2016; D. Zero et al., 2001). Currently, several different CRA programmes are used worldwide, taking into account a varying number of clinical and behavioural factors. In this thesis, three of these programmes will be evaluated: Cariogram, CAMBRA and R2 (Table 1).

Table 1. Overview of the three CRA programmes studied (Cariogram, CAMBRA and R2) and the factors included in the respective CRA programmes.

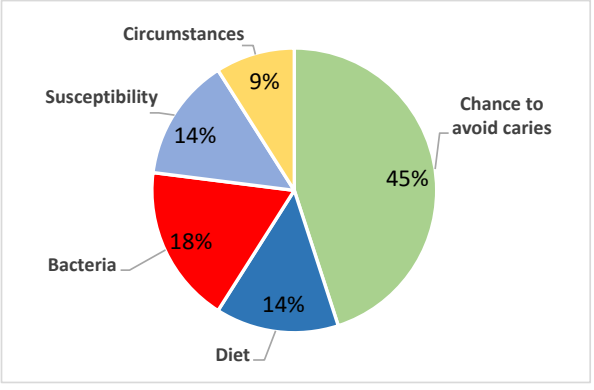
Factors and subfactors		Cariogram	CAMBRA	R2
Clinical status	Caries experience	✓	✓	✓
	WSL		✓	
	Orthodontic appliance		✓	
	Deep pits and fissures		✓	
	Exposed roots		✓	
	Oral hygiene	✓	✓	✓
Microbiological	Mutans streptococci	✓	✓	
	Lactobacilli	✓	✓	
Saliva	Flow rate	✓	✓	✓
	Buffering capacity	✓		
Behavioural	Diet	✓	✓	✓
	Fluoride usage	✓	✓	✓
	Self-rated oral health			✓
Health	Systemic diseases	✓	✓	✓
	Medication		✓	
	Smoking/snuff use			✓
Program feature	Possibility of own weighting	✓		✓
	Pedagogical picture	✓	✓	
	Suggested recall			✓
Risk parameters		Caries	Caries	Caries Periodontitis Technical Implants Malocclusion Erosion Other
Method of assessment		Algorithm, computer calculation	Checklist	Computer calculation
Caries risk prediction		Percentage to avoid caries, presented in a pie chart.	Low Moderate High Extreme-high	Low Moderate High

Cariogram

The Cariogram is a well-known computer- and logarithm-based CRA programme that was invented by Bratthall and colleagues at Malmö University in Sweden. The programme takes into account 10 different caries risk factors (D. Bratthall et al., 2005). The caries risk is displayed as a percentage "*chance of avoiding new cavities*" and is illustrated in a circle diagram (D Bratthall et al., 2004). The Cariogram also identifies the factors that are most-responsible for the caries risk, indicating where targeted treatment should focus (D Bratthall et al., 2004) (Figure 3).

The validity of the Cariogram in assessing the caries risk has been extensively studied and has shown acceptable degree of accuracy in schoolchildren (Campus et al., 2012; Hansel Petersson et al., 2002; Zukanovic, 2013), adolescents (Celik et al., 2012; G. H. Petersson et al., 2015) and the elderly (Hansel Petersson et al., 2003). However, the Cariogram has been found to have limited reliability in assessing caries risk in pre-school children in a community with a low prevalence of caries (Holgerson et al., 2009), as well as in low- or medium socio-economic areas (Birpou et al., 2019).

Figure 3. A Cariogram presenting an individual's caries risk and, at the same time, the possible impacts of various causal factors on this risk. The analysis is based on the caries-related factors listed to the right of the pie chart.



Caries related factors	
Caries experience	0-3
Related disease	0-2
Diet contents	0-3
Diet, frequency	0-3
Plaque amount	0-3
Mutans streptococci	0-3
Fluoride program	0-3
Saliva secretion	0-3
Buffer capacity	0-2
Clinical judgement	0-3

CAMBRA

The Caries Management by Risk Assessment (CAMBRA) was developed at the University of California in conjunction with the California Dental Association (J. Featherstone et al., 2019; J. D. Featherstone et al., 2007). CAMBRA is a reasoning-based CRA programme that contains a form with questions requiring yes/no answers concerning disease indicators, biological or environmental risk factors, and protective factors. A clinical examination together with balanced and summarised answers from the questionnaire determine the patient's caries risk as low, moderate, high, or extremely high (Figure 4). Thereafter, CAMBRA provides suggestions for therapy based on the observed risk level. Orthodontic treatment automatically places the patient in, at least, the moderate risk category (J. Featherstone et al., 2019).

Retrospective studies indicate that CAMBRA predicts caries well in adults (Chaffee et al., 2015; Domejean et al., 2011) and institutionalised children (Sudhir et al., 2016). A prospective clinical study has demonstrated that CAMBRA has high sensitivity (93.8%) and lower specificity (43.6%) in pre-school children (Gao et al., 2013).

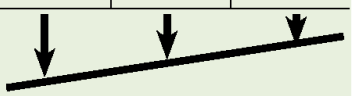
Figure 4. The CAMBRA form (J. D. Featherstone et al., 2007) including the disease indicators, risk factors and protective factors.

Caries Risk Assessment Form — Children Age 6 and Over/Adults

Patient Name:_____Chart #:_____Date:_____

Assessment Date: Is this (please circle) base line or recall

Disease Indicators (Any one "YES" signifies likely "High Risk" and to do a bacteria test)**	YES = CIRCLE	YES = CIRCLE	YES = CIRCLE
Visible cavities or radiographic penetration of the dentin	YES		
Radiographic approximal enamel lesions (not in dentin)	YES		
White spots on smooth surfaces	YES		
Restorations last 3 years	YES		
Risk Factors (Biological predisposing factors)		YES	
MS and LB both medium or high (by culture**)		YES	
Visible heavy plaque on teeth		YES	
Frequent snack (> 3x daily between meals)		YES	
Deep pits and fissures		YES	
Recreational drug use		YES	
Inadequate saliva flow by observation or measurement (**If measured, note the flow rate below)		YES	
Saliva reducing factors (medications/radiation/systemic)		YES	
Exposed roots		YES	
Orthodontic appliances		YES	
Protective Factors			
Lives/work/school fluoridated community			YES
Fluoride toothpaste at least once daily			YES
Fluoride toothpaste at least 2x daily			YES
Fluoride mouthrinse (0.05% NaF) daily			YES
5,000 ppm F fluoride toothpaste daily			YES
Fluoride varnish in last 6 months			YES
Office F topical in last 6 months			YES
Chlorhexidine prescribed/used one week each of last 6 months			YES
Xylitol gum/lozenges 4x daily last 6 months			YES
Calcium and phosphate paste during last 6 months			YES
Adequate saliva flow (>1 ml/min stimulated)			YES
****Bacteria/Saliva Test Results: MS: LB: Flow Rate: ml/min. Date:**			



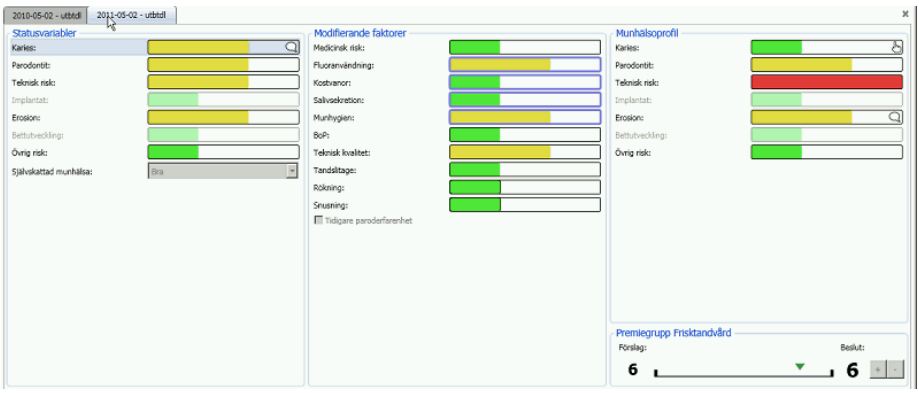
VISUALIZE CARIES BALANCE
(Use circled indicators/factors above)
(EXTREME RISK = HIGH RISK + SEVERE SALIVARY GLAND HYPOFUNCTION)
CARIES RISK ASSESSMENT (CIRCLE): EXTREME HIGH MODERATE LOW

Doctor signature/#:_____ Date:_____

R2

R2 (decision support R2) is a computer-based dental risk programme that was invented in Sweden in 2008. R2 was developed in a collaboration between the Public Dental Service in Jönköping County, the Department of Dentistry in Jönköping and the Public dental service in Västra Götaland. R2 is used primarily in the Public Dental Service in Sweden and is linked to the digital medical record. R2 was introduced when the dental health insurance "Frisktandvård" system was introduced in the medical record. R2 acts as a guide as to which risk- and insurance group the patient should be assigned. R2 presents an oral health profile, generated by a calculation based on status variables and modifying factors, and this acts as a guideline for the clinician to assess the caries risk as low, medium, or high (The Public Dental Service of Västra Götaland, 2013, [unpublished]) (Figure 5). The status variables are either imported directly from the medical records or stated by the clinician. The modifying factors are stated by the clinician and describe factors that may affect the risk of disease development and other oral conditions. The oral health profile can also be adjusted in the system by the clinician. Moreover, the programme assesses technical risk as well as the risk of developing other oral diseases, such as periodontitis. The validity of R2 has not been reported in the literature.

Figure 5. The figure presents the R2 risk assessment programme. The status variables (Statusvariabler) are presented to the left, the modifying factors (Modifierande faktorer) in the middle, and the oral health profile (Munhälsoprofil) is to the right.



1.5 CARIES INDEX

DMFT

DMFT, which is an index that assess caries prevalence, was introduced in 1938 by Klein and colleagues (Klein, 1937). The index describes an individual's caries prevalence and is widely used in epidemiological studies, as well as in health care planning. Furthermore, to assess caries in a more specific manner, the indices DMFS and DiMFS have been developed, the indexes take in to account caries lesions on surface level and initial caries lesions, respectively (World Health Organization (WHO), 2013). Subsequently, a severity grading diagnostic system for initial and manifest caries was developed (Amarante et al., 1998).

Other indices

The Gorelick index was developed in 1982 to assess WSLs, which are often seen following orthodontic treatment (Gorelick et al., 1982). The lesions on the buccal surfaces of the teeth are graded on a scale of 1–4, ranging from no visible WSL to visible cavitation. A modified version of the Gorelick index has been developed for assessment of the extension of WSLs, dividing the buccal tooth surface into thirds (Artun et al., 1986) and quarters (Banks et al., 1994).

The SiC index represents the mean DMFT of the one-third of the study population that presents with the highest caries scores (D. Bratthall, 2000). The SiC index was introduced in 2000 and is often used as a complement to the DMFT index.

The international Caries Detection and Assessment System (ICDAS) was developed in 2002 and updated to ICDAS II in 2005. Each tooth surface is assigned a code between 0 and 6, corresponding to the depth and the extension of the demineralisation (Ismail et al., 2007). The goal was to develop a universally standardised system for assessing dental caries and to advance understanding of the initiation and progression of caries. The Caries Assessment and Treatment (CAST) index also cover ranges of caries, from sound teeth enamel to caries progression and involvement of the pulp chamber (Frencken et al., 2011).

1.6 CARIES PREVENTION STRATEGIES

Prevention strategies or programmes for caries prevention generally entail a combination of different methods. Compliance and dose (frequency, concentration) are important components for a successful prevention.

1.6.1 DIET

Reducing sugar consumption is essential for caries prevention. The effects of fermentable carbohydrates, and especially of sucrose consumption on caries have been known for centuries and are well-established (Chapple et al., 2017; Gustafsson et al., 1954; Moore, 1983). The frequency of sugar intake is more crucial for the development and severity of caries than the total quantity of sugar consumed (Gustafsson et al.,

1954). However, recent studies have shown that both frequency and quantity of consumed sugars are important parameters in the occurrence of caries (World Health Organization (WHO), 2015). Moreover, different types of carbohydrates show different levels of cariogenicity, with starch being less-cariogenic than sucrose (Lingstrom et al., 2000). The properties of the fermentable carbohydrate, such as retentiveness, solubility, pH and buffering capacity, also affect the level of cariogenicity, as do the contents of caries-protective substances, such as protein, fats, calcium, phosphate and fluoride (Fejerskov O, 2015).

The influence of diet on caries is weaker in economically developed countries where fluoride is frequently used (Burt et al., 2001), whereas in developing countries, there is a linear relationship between the logarithm of DMT and sugar intake (Woodward et al., 1994). Recent guidelines from the WHO have recommended that for both children and adults the total intake of free sugars should be reduced to less than 10% or even 5% of total energy intake, so as to have a positive effect on caries prevalence (World Health Organization (WHO), 2015). Preventive strategies have recently been proposed that focus on dietary advice in terms of motivational interviewing (Wu et al., 2022).

1.6.2 FLUORIDE

The caries preventive effect of fluoride was discovered in the 1940s in Colorado, USA. Children who lived in geographical areas with high levels of natural fluoride in the domestic drinking water were to a high extent caries-free (Dean, 2006). Fluoride had its major caries-preventive breakthrough in dental care in the 1970s. The use of fluoride toothpaste has since then been one of the main contributors to the sharp decline in the caries disease in economically developed countries. Today, the preventive effects of fluoride on caries prevalence and incidence are well- documented in the literature (SBU. The Swedish Council on Technology Assessment in Health Care, 2002).

The cariostatic effect of fluoride is manifested through different modes of action. First, fluoride inhibits tooth demineralisation. Hydroxide ions (OH^-) that are replaced with fluoride ions generates a more insoluble fluoride hydroxyapatite, which is mainly found at low levels of fluoride concentrations in a neutral pH environment (Rolla, 1988; ten Cate, 2013). Calcium fluoride, on the other hand, predominates at high concentrations of fluoride and at low pH levels, also affecting the solubility of apatite (Rolla, 1988; Shellis et al., 1994). Second, fluoride ions enhance remineralisation of the teeth. The calcium- and phosphate crystals that diffused outwards during demineralisation return more easily to the tooth when the pH increases. Third, the fluoride ions inhibit the metabolism of the oral micro-organisms. For example, the aciduric ability of bacteria are being deteriorate by fluoride interfering with glycolysis via inhibition of the enzyme Enolase. Furthermore, fluoride ions change the tooth surface energy, making it difficult for the micro-organisms to attach to the surface and the microorganisms acidogenic ability is also affected (J. D. Featherstone, 1999; Hamilton, 1990; Hicks et al., 2004).

Fluoride toothpaste

There is a strong scientific evidence that daily use of a fluoride toothpaste is the most effective strategy for preventing caries in the permanent teeth of children and adolescents (Marinho et al., 2003) and in adults (Socialstyrelsen. National Board of Health and Welfare, 2021e). The frequency of tooth brushing affects the development of caries. A systematic review has shown a 14% increase in the prevented fraction when brushing with fluoride toothpaste twice daily instead of once daily (Marinho et al., 2003). The time duration of tooth brushing and the amount of dentifrice used for brushing are other factors that affect the development of caries (D. T. Zero et al., 2010). Sodium fluoride is the most frequent compound in toothpaste. The fluoride concentration in regular toothpaste has traditionally been limited to 1500 ppm F. A dose-response relationship has been observed whereby toothpaste with a higher concentration of F (1500 ppm) gives a greater anti-caries effect than toothpaste with a lower concentration of F (1000 ppm) (Stephen et al., 1988; Twetman et al., 2003; Walsh et al., 2019).

During the last two decades, a high-fluoride toothpaste containing 5000 ppm F has been developed, and it has been recommended to patients who have a high risk of caries. The high-fluoride toothpaste has shown preventive effects on proximal caries progression in adolescents with high caries risk (Nordstrom et al., 2010). High-fluoride toothpaste has also been shown to have an inhibitory effect on root caries (Baysan et al., 2001; Ekstrand et al., 2008) and on WSLs during orthodontic treatment (M. Sonesson et al., 2014).

Fluoride mouth rinse

Daily use of a mouth rinse that contains 0.2% NaF is one of the strongest recommendations for caries prevention, according to the Swedish National Guidelines (Socialstyrelsen. National Board of Health and Welfare, 2021b). A RCT has demonstrated that supervised, regular use of a fluoride mouth rinse, as a supplement to the daily use of fluoride toothpaste, has a positive effect of caries incidence on the approximal surfaces in adolescents with a low-to-medium risk of caries (Moberg Skold, Birkhed, et al., 2005). Rinsing frequency, fluoride concentration and rinsing time are factors that affect the caries-preventative effect of the mouth rinse (Mystikos et al., 2011; Songsiripradubboon et al., 2014). The latest Cochrane review has revealed an average reduction in DMFS of 27% when regular, supervised fluoride mouth rinsing was performed in children and adolescents (Marinho et al., 2016).

Fluoride varnish

Fluoride varnish is generally used in community-based caries prevention programmes for children and is applied to tooth surfaces with increased risk of caries (L. Petersson et al., 1997). One of the first RCTs of fluoride varnish was performed in Sweden. That study revealed that adolescents treated with fluoride varnish twice a year exhibited a significant reduction in caries incidence, as compared to a control group. The reduction was influenced by caries risk geographical areas, showing prevented fractions of 69% in high, 66% in medium, and 20% in low caries-risk areas (Moberg Skold, Petersson, et al., 2005). A more recent RCT showed that for adolescents living in a low caries

prevalence area there was no beneficial effect of school-based fluoride varnish applied twice yearly on approximal caries increments, as compared to a control group (Bergstrom et al., 2014). However, according to the Swedish National Guidelines, fluoride varnish applied twice a year, as a supplement to tooth brushing, is one of the strongest recommendations for caries prevention (Socialstyrelsen. National Board of Health and Welfare, 2021a). The latest Cochrane review evaluating the effect of using fluoride varnish has suggested substantial inhibition of caries, both in the primary and permanent dentition, with prevented fractions of 37% and 43%, respectively. However, conclusions should be drawn with some caution because the quality of the evidence was considered to be moderate (Marinho et al., 2013).

1.6.3 ORAL HYGIENE

Regular mechanical cleaning through tooth brushing prevents the build-up of a dysbiotic biofilm and is considered crucial for maintaining dental health. Self-care should focus on oral hygiene measures involving tooth brushing with a fluoride toothpaste twice a day, thereby preserving the oral microbiota in a favourable, balanced state (Carey, 2014; Chapple et al., 2017). In a systematic review, it was concluded that tooth brushing in conjunction with fluoride toothpaste twice a day instead of once a day increased by 14% the prevented fraction of caries (Marinho et al., 2003).

The deciduous dentition is more susceptible to infrequent tooth brushing in terms of the incidence of caries, as compared to the permanent dentition (Kumar et al., 2016). However, tooth brushing alone, without a fluoride toothpaste, does not significantly affect the development of caries (Figuero et al., 2017).

1.6.4 ANTIMICROBIAL AGENTS

Antimicrobial agents are sometimes used as supplemental therapy for caries prevention, with the aims of selectively reducing pathogens and controlling plaque accumulation. Chlorhexidine, which is a bisbiguanide, acts as an antibacterial agent and as a positively charged molecule that binds to teeth, plaque and the oral mucosa. The direct decrease of MS numbers in the saliva following treatment with chlorhexidine is certain. However, the long-term effect of this agent on oral biofilms remains uncertain and, as a consequence, the effect on caries management is also unclear (Twetman, 2018; Walsh et al., 2015). According to the Swedish National Guidelines, the use of chlorhexidine solution has a low preventive effect in adults who have a higher risk of coronal caries (Socialstyrelsen. National Board of Health and Welfare, 2012). Nevertheless, treatment with chlorhexidine gel in trays for adults with an increased risk of coronal caries and high numbers of MS constitutes a strong recommendation for caries prevention (Socialstyrelsen. National Board of Health and Welfare, 2021c).

1.6.5 PROBIOTICS

The most commonly used probiotic bacteria form part of the normal human flora and belong to the genera Lactobacillus and Bifidobacterium. Probiotic bacteria act both directly (locally) and indirectly (systemically). The local effect includes binding to the

biofilm, production of anti-bacterial toxins and bacterial inhibition of antagonists. The systemic effect involves stimulation of the immune system via the intestines. Cytokines and immunoglobulins (IgA) reach the oral cavity via the saliva and gingival fluid (Azad et al., 2018). Today there are some scientific supports for the fact that probiotic bacterial cultures given regularly to young children can prevent caries in the primary dentition. However, the evidence that probiotics can act as a preventative measure for caries in the permanent dentition in adolescents, adults and the elderly is currently insufficient (Cagetti et al., 2013).

1.6.6 MOTIVATIONAL INTERVIEWING

Motivational interviewing (MI) and the traditional dental health education are two established strategies for changing habits. MI, which is defined as "*a collaborative, person-centered form of guiding to elicit and strengthen motivation for change*", has been used and evaluated in various health promotion domains (Miller et al., 2009). Dental health education approaches assume that a patient is prepared to act upon the information imparted to them by a dental professional. MI, on the other hand, places patients in the role of action and allows the patient to decide how to interpret and integrate information in the context of their lives, social circumstances and whether it is relevant for them. Recent RCTs have verified the positive effects of MI on caries-related behaviours, such as tooth brushing frequency and snacking (Naidu et al., 2015; Wu et al., 2017; Wu et al., 2022). A recently published systematic review assessed the scientific evidence for the ability of MI to alter parents' risk-related behaviours and improve their knowledge of caries. A significant impact was seen on dental visit for fluoride varnish application, as well as an improvement of participants' oral health-related knowledge (Mortazavi et al., 2021).

1.7 CARIES IN ORTHODONTIC PATIENTS

1.7.1 WHITE SPOT LESIONS

As a result of the altered environment in the oral cavity, initial caries, white spot lesions (WSLs) is a frequent side-effect of orthodontic treatment with fixed appliances (Gorelick et al., 1982; Mitchell, 1992; Shungin et al., 2010). The incipient lesions are opaque white areas of enamel demineralisation. WSLs often occur on the buccal surfaces on the cervical or middle thirds of the teeth, which may impair the aesthetic outcome of the treatment (Gorelick et al., 1982; Shungin et al., 2010). WSLs are detectable by visual inspection and can be diagnosed by conventional clinical examination, photographs, Quantitative Light-induced Fluorescence (QLF) and Laser Fluorescence (DIAGNdent). The fluorescence radiance is decreased at sites of demineralisation, such that caries can be detected. The fluorescence-based diagnostic method has been demonstrated to be more sensitive for detecting demineralisation in the enamel than visual examination (Boersma et al., 2005).

1.7.2 PREVALENCE

The reported prevalence of WSL among patients undergoing fixed orthodontic appliance (FOA) treatment is in the range of 2%–96% (Chapman et al., 2010; Enaia et al., 2011; Gorelick et al., 1982; Richter et al., 2011; Shungin et al., 2010; Stecksén-Blicks et al., 2007; Tufekci et al., 2011). This wide range can be attributed in part to the variety of methods and prevention programmes applied and the types of population groups studied. In Sweden, approximately 25% of adolescents are treated with FOA, and the prevalence of WSLs at debonding is 30%–45%, based on reference groups without additional fluoride intervention (M. Sonesson et al., 2014; Stecksén-Blicks et al., 2007). The prevalence and severity of WSLs have been associated with longer treatment time (Marcusson et al., 1997). After bracket removal, the WSLs have a limited ability to reverse and remineralise (Mattousch et al., 2007).

1.7.3 AGGRAVATING FACTORS FOR INCREASED CARIES RISK DURING FOA

The risk of caries during orthodontic treatment increases due to multiple factors. First, the level of plaque increases during treatment with FOA (Chang et al., 1999; Naranjo et al., 2006). The appliances with their associated brackets and arch-wires complicate oral hygiene measures during orthodontic treatment (Naranjo et al., 2006). The cleaning interactions of the tongue, cheeks and saliva are affected negatively by the devices of the FOA owing to the impaired access to the tooth surfaces (Stromberg et al., 2017). The treatment is also associated with pain, which may further impair the oral hygiene processes (Scheurer et al., 1996). A qualitative change in the microbiota is also seen. The number of Gram-positive, acidogenic bacterial species in the saliva and in the dental plaque, such as MS and LBC, increase during orthodontic treatment (Jing et al., 2019; Topaloglu-Ak et al., 2011). Second, the increased plaque accumulation hinders the access of saliva, thereby decreasing the delivery of immune responses and leading to a more cariogenic plaque (Stromberg et al., 2017). The pH levels of the plaque are reduced to a greater extent and the progression of caries is faster compared to patients without orthodontic treatment (Chatterjee et al., 1979).

Besides other caries-prone surfaces and higher availability, time is a factor that differentiates the development of caries in orthodontic patients from that in the general population. Demineralisation associated with orthodontic treatment occurs to rapidly. Both micro-radiographic and scanning electron microscopy examinations have shown surface softening of the enamel surface within 4 weeks of bonding, in the absence of fluoride (Ogaard et al., 1988). Another *in vivo* study demonstrated reduced micro-hardness of the enamel around the bracket within 4 weeks after bonding, despite the usage of a toothpaste that contained 1100 ppm NaF (O'Reilly et al., 1987). The formation of regular WSLs, detected by visual examination, has been observed within 6 months of bonding (Tufekci et al., 2011).

1.8 CARIES PREVENTION IN FOA PATIENTS

1.8.1 META-ANALYSIS AND SYSTEMATIC REVIEWS

Recent systematic reviews and meta-analyses have evaluated the effects of various methods to prevent the development of WSL during orthodontic treatment. Active patient reminders, fluoride-releasing materials, flat surface sealant, fluoride varnish around orthodontic brackets, fluoride foam (12300 ppm F), and high-fluoride toothpaste (5000 ppm F) may all be associated with a reduced incidence of WSLs (Benson et al., 2019; Nascimento et al., 2016; Sardana et al., 2019; Tasios et al., 2019). However, the quality of evidence of the findings were mostly considered low or insufficient due to the risk of bias. More high-quality studies are needed to assess evidence-based guidelines for caries prevention during orthodontic treatment.

1.8.2 RCT AND COHORT STUDIES

High-fluoride toothpaste

A greater anti-caries potential has been demonstrated in orthodontic patients using a 5000 ppm F toothpaste in combination with no post-brushing water rinsing, as compared to a conventional toothpaste (1450 ppm F) with three sessions of post-brushing water rinsing (Al-Mulla et al., 2010). Recently, this was confirmed in an RCT, where the preventative effect on the development of WSL was significant lower in the test group (35%) that used high-fluoride toothpaste (5000 ppm F), as compared to the control group (45%) that used ordinary toothpaste (1450 ppm F) (M. Sonesson et al., 2014). Alexander and co-workers have shown a significantly higher caries-protective effect of using a high-fluoride dentifrice (5000 ppm F) during orthodontic treatment, as compared to using a mouth rinse (0.05% NaF) combined with tooth brushing with a fluoride toothpaste (1000 ppm F) (Alexander et al., 2000).

Mouth rinse

Geiger and colleagues have demonstrated a significant reduction in WSLs during orthodontic treatment in patients who used a fluoride mouth rinse (0.05% NaF); a dose-response relationship was demonstrated for those who rinsed at least once every other day, in that they showed significantly fewer WSLs (21%) compared to those who rinsed less frequently (49%) (Geiger et al., 1992). Furthermore, a recently published RCT confirmed the positive effect of mouth rinse on WSLs and revealed less demineralisation in patients who used a fluoride rinse (150 ppm NaF and 100 ppm amine fluoride) compared to those who used a placebo rinse (van der Kaaij et al., 2015).

Fluoride varnish

The effect of regularly applied fluoride varnish on brackets during treatment with FOA was evaluated in a placebo-controlled RCT. The incidence of WSL was significantly higher in the control group at 25%, than the 7% in the fluoride varnish group (Stecksén-Blicks et al., 2007). Another recently published RCT investigated the effectiveness of a fluoride varnish that contained 1.5% ammonium, and showed a similar prevalence of WSL for the two groups on the subject level (test group, 42% versus placebo group, 44%). However, the number of patients with severe WSLs differed significantly between the groups (Mikael Sonesson et al., 2019).

1.9 THE EFFECT OF FLUORIDE ON CARIES-RELATED FACTORS IN FOA PATIENTS

Few clinical trials have evaluated the effects of fluoride on caries-related factors (e.g., oral bacteria and dental plaque) during orthodontics. However, a randomised prospective clinical study demonstrated a positive effect of fluoride varnish on caries-related factors. Significantly higher levels of plaque and gingival bleeding and higher numbers of MS in the plaque at debonding were seen in the control group (fluoride toothpaste only), as compared to the intervention group (fluoride varnish and toothpaste) (Ogaard et al., 2001). Furthermore, a double-blinded clinical study demonstrated a significant decrease in plaque levels during orthodontics for the fluoride rinse group (0.05% NaF) and the placebo group; a significant reduction in the number of MS ($p < 0.05$) was seen in the fluoride mouth rinse group, as compared to the placebo group (Dehghani et al., 2015).

1.10 MANAGEMENT OF POST-ORTHODONTIC WSLS

A second preventative measure consists of interventions that enhance remineralisation and improve aesthetics after bracket removal. In this context, many methods have been evaluated, including topical fluoride, self-assembling peptide and casein phosphopeptide amorphous calcium phosphate (CPP-AcP). The prevalence of WSL following FOA can be expected to decrease spontaneously by approximately 50% on the patient level after 1 year (M. Sonesson et al., 2021).

Slightly more invasive techniques to improve the appearance of the lesions are resin infiltration, bleaching and micro-abrasion, and these should preferably be initiated after at least 6 months, to allow spontaneous remineralisation of the lesions (Mattousch et al., 2007; Shungin et al., 2010). Mattousch and co-workers have reported statistically significant lesion improvement measured by QLF loss at 6 months (median 48%) compared to the time-point of debonding (median 56%) (Mattousch et al., 2007). A recently published systematic review recorded that monthly use of fluoride varnish seems to be the most effective supplement for reducing post-orthodontic WSLs (Hochli et al., 2017).

2 AIMS

Overall aim

The overall aim of this thesis was to improve caries risk assessment before orthodontic treatment, as well as evaluate caries risk and improve caries prevention during treatment.

Specific aims

I. To evaluate the effects of orthodontic treatment on caries risk and caries risk factors. Furthermore, to evaluate the effects of high-fluoride toothpaste and mouth rinse on caries risk and caries risk factors during orthodontic treatment.

II. To evaluate the validity of different caries risk assessment programmes and prevalence indexes for predicting caries outcomes during orthodontic treatment.

III. To evaluate the effect of a fluoride mouth rinse and a high-fluoride toothpaste on caries incidence, based on dental radiographs, in patients undergoing orthodontic treatment with fixed appliances.

IV. To evaluate the effect of a fluoride mouth rinse and a high-fluoride toothpaste on WSLs, based on clinical photographs, in patients undergoing orthodontic treatment with fixed appliances.

3 PATIENTS AND METHODS

3.1 AN OVERVIEW OF THE STUDIES

The present thesis is based on four studies. Study I, III and IV were designed as randomised controlled trials, and study II is a prospective longitudinal study. An overview of the studies is presented in Table 2.

Table 2. Characteristics of the four studies in this thesis.

	Study I	**Study II**	**Study III**	**Study IV**
Study design	RCT	Prospective longitudinal	RCT	RCT
Hypothesis	Orthodontic treatment and different fluoride regimens do not affect the caries risk or the caries risk factors.	There is no difference between the methods regarding CRA before orthodontic treatment.	The intervention groups would show a lower caries incidence than the control group during orthodontic treatment.	There is no difference between the fluoride methods in preventing WSL during orthodontic treatment.
Included participants	n=270	n=270	n=270	n=270
Completed participants	n=255	n=255	n=255	n=248
Mean age (years)	15.4 (± 1.7)	15.4 (± 1.7)	15.4 (± 1.7)	15.4 (± 1.6)
Females	n=165 (64.7%)	n=165 (64.7%)	n=165 (64.7%)	n=164 (66.1%)
Time-points for data collection	• Before treatment • 1 year into treatment	• Before treatment • After treatment	• Before treatment • After treatment	• Before treatment • After treatment
Predictor variable	Fluoride interventions and FOA	Caries risk parameters and FOA	Fluoride interventions and FOA	Fluoride interventions and FOA
Primary outcome variable(s)	• Caries risk • The numbers of cariogenic bacteria (counts of MS and LBC)	Caries outcome after treatment, as compared to the assessment made by CRA programmes and caries indices at baseline	Incidence of DiFS (approximal and occlusal caries, based on radiographs)	Incidence of WSL (buccal tooth surface, based on clinical photographs)
Secondary outcome variable(s)	• General health status • Plaque amount • Food intake frequency		Incidence of: DFT, DFS, initial caries, manifest caries	

3.2 STUDY SUBJECTS

The study population for all the studies (I–IV) were patients who presented at one of the Swedish Public Dental Service Clinics in Mölnlycke, Lindome, Nödinge, Krokslätt, Öckerö, Älvängen, Partille, Landvetter and Mölndal. Data were collected during the period of October 2010 to December 2012.

Inclusion criteria: Patients who were referred to the Specialist Clinic of Orthodontics, Public Dental Service, Mölndal for FOA in both arches [MBT™ (McLaughlin, Bennett, Trevisi), pre-adjusted with 0.022-inch slots; 3M Unitek Orthodontic Products, Monrovia, CA, USA], in the age range of 12–20 years, and with expected treatment time of at least 1 year.

Exclusion criteria: Removable appliances, lingual fixed appliances, suffering from severe disease.

The criterion of 'severe disease' included patients who did not receive general dental care at Public Dental Service clinics due to their health condition.

3.3 STUDY DESIGN

Studies I–IV all included the same study population. In studies I, II and III, the same participants completed the study, although, the reasons applied for exclusion from the analysis differed between the studies (Figure 6).

Randomisation

The RCTs had a three-armed parallel group design. The randomisation was performed in blocks of 30 to ensure an equal distribution of all three groups. Paper sheets with the group affiliation were folded and placed in a basket. Each participant selected a paper sheet from the basket for randomisation. The allocation concealment aimed to eliminate bias during randomisation and the process of recruitment. The allocation sequence was concealed from those assigning participants to the intervention groups, until the moment of assignment. Thereafter, the orthodontists and the orthodontic assistants registered the participants according to their respective group affiliation.

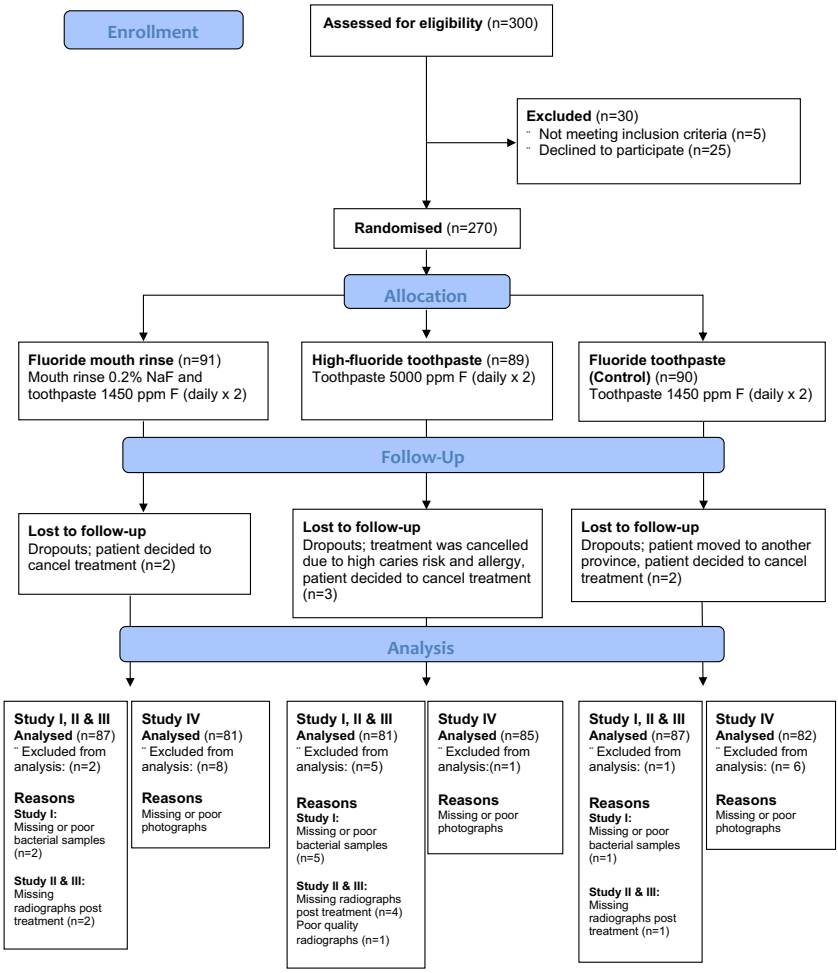
Blinding

Blinding was performed as much as possible to reduce performance and ascertainment biases after randomisation. The caries registration, and the sorting of anamnestic data, bacterial data and other clinical data (H.E.), were conducted in a blinded fashion with the author being unaware of the patient's group affiliation. The author (H.E.) was not involved in the treatment of the participants. The participants' group affiliation was revealed after completion of the data analysis.

Patient flow

Assessed for eligibility were 300 patients, 30 of whom were excluded because they either did not meet inclusion criteria (n=5) or they declined to participate in the study (n=25). Therefore, 270 patients were randomised to the intervention groups and control group. During treatment, 7 patients were lost to follow-up (FMR group: n=2, patient decided to cancel treatment; HFT group: n=3, treatment was cancelled due to high caries risk and allergy, patient decided to cancel treatment; CTR group: n=2, patient moved to another geographical area, patient decided to cancel treatment). Overall, the number of included participants in Studies I, II and III was 255 (FMR group, n=87; HFT group n=81; and CTR group n=87) and there were 248 participants in Study IV (FMR group, n=81; HFT group, n=85; and CTR group, n=82). An overview of the patients excluded from analysis (with corresponding reasons) for all four studies (I–IV) is shown in the flow chart in Figure 6.

Figure 6. Flow chart of the trials, adapted from CONSORT.



3.4 INTERVENTIONS

In all four studies and after eligibility for inclusion and acceptance to participate in the study were established, the participants were randomly assigned to one of the following groups:

- **Fluoride mouth rinse (FMR) group.** Participants were instructed to rinse their teeth with a fluoride mouth rinse (0.2% NaF, Flux; Actavis, Stockholm, Sweden) twice per day 1 hour after brushing their teeth with a fluoride toothpaste (1450 ppm F, Colgate Caries Control; Colgate-Palmolive, Lyngby, Denmark).

- **High-fluoride toothpaste (HFT) group.** Participants were instructed to brush their teeth with a high-concentrated fluoride toothpaste (5000 ppm F, Duraphat; Colgate-Palmolive) twice per day.

- **Control (CTR) group.** Participants were instructed to brush their teeth with a fluoride toothpaste (1450 ppm F, Colgate caries control; Colgate-Palmolive) twice per day.

The subjects were instructed to brush their teeth for 2 minutes, every morning after breakfast and every night before going to bed. The amount of toothpaste used was instructed to be approximately 1g, corresponding to a string of 2 cm, in accordance with the manufacturer's instructions. The fluoride mouth rinse procedure was instructed to be carried out with 10 mL of the solution for 2 minutes. The participants were told to avoid drinking and eating for at least 1 hour after tooth brushing and mouth rinsing. Furthermore, the participants were informed not to use water during or after brushing and rinsing. Tooth brushes, toothpastes and mouth rinses were provided to the patients free of charge on a regular basis throughout the study period.

3.5 DATA COLLECTION

3.5.1 CARIES RISK FACTORS

In Studies I and II, clinical data were collected before initiating the orthodontic treatment, and in Study I the same types of data were collected also after 1 year of treatment. In Study I, the clinical parameters were used to assess caries risk based on the Cariogram, as well as to investigate the effects of high-fluoride toothpaste and mouth rinse on individual risk factors. In Study II, the clinical parameters were used to assess caries risk according to the CRA programmes at baseline. Assessments of the caries indices, DFT and DiFS, were also performed at baseline, based on radiographs taken before the start of orthodontic treatment (see *Caries prevalence* section below).

Orthodontists and orthodontic assistants at the specialist clinic in Mölndal collected the following data at baseline and 1 year into treatment:

Related Diseases. Participants answered a questionnaire concerning diseases, medications, antibiotic use, and tobacco habits (Appendix).

In CAMBRA, saliva-reducing factors due to medications or systemic disease are considered under "Risk factors".

In the Cariogram, the following disease scoring scale is used:
0=No disease related to caries.
1=Disease/conditions, mild degree that can influence the caries process and contribute to higher caries risk.
2=Disease/conditions, severe degree that can influence the caries process and contribute to a higher caries risk.

The numbers of MS and LBC per mL of saliva were assessed in whole saliva samples. The participants chewed on a piece of paraffin wax for 5 minutes and the saliva was collected in a test tube. One mL of the saliva was placed in VMG II medium. At the laboratory, the transport medium (6.7 mL) was mixed with 3.3 phosphate-buffered saline (PBS), to dilute and quantify the bacteria in the solution. Serial dilutions were carried out, with the concentration being reduced by 10^{-1} for each step. The samples were plated in duplicate on MSB agar and Rogosa SL agar. After incubation, the colony-forming units (CFUs) were counted.

In CAMBRA, for MS and LBC, both medium and high levels are considered as risk factors.

In the Cariogram, the MS levels are scored on the following scale:
0=Very low or zero levels of MS in the saliva.
1=Low levels of MS in the saliva.
2=High levels of MS in the saliva.
3=Very high levels of MS in the saliva.

In the Cariogram, the numbers of LBC are associated with the intake of fermentable carbohydrates and scored as follows:
0=Very low intake of fermentable carbohydrate.
1=Low intake of fermentable carbohydrate, 'non-cariogenic' diet.
2=Moderate intake of fermentable carbohydrate.
3=High intake of fermentable carbohydrate, inappropriate diet.

The thresholds for the numbers of bacteria were defined as follows for Cariogram and CAMBRA:
MS:
Very low level, $<10^4$ CFU/mL saliva;
Low level, 10^4–10^5 CFU/mL saliva;
Medium-to high-level, $>10^5$–10^6 CFU/mL saliva;
Very high level, $>10^6$ CFU/mL saliva.
LBC:
Very low level, $<10^3$ CFU/mL saliva;

Low level, 10^3–10^4 CFU/mL saliva;
Medium-to-high level, >10^4–10^5 CFU/mL saliva; and
Very high level, >10^5 CFU/mL saliva.

Diet intake frequency. Participants answered a questionnaire concerning their intake frequency of food, snacks and drinks containing sugar (Appendix).

In CAMBRA, frequent snacking on fermentable carbohydrates, at least 3 times/daily apart from meal times, constitutes a risk factor.

In the Cariogram, diet intake frequency is divided as follows:
0=Maximum 3 meals/day including snacks. Very low diet intake frequency.
1=Maximum 5 meals/day. Low diet intake frequency.
2=Maximum 7 meals/day. High diet intake frequency.
3≥7 meals/day. Very high diet intake frequency.

Plaque. Optimal lighting, a mirror and a dental explorer were used to assess the amount of plaque. Before the start of treatment, the care-givers were calibrated to ensure consistency in the assessment of the plaque index score. A modification of the Silness and Löes index (Silness et al., 1964) was used, including an overall judgement of all the teeth, registered as follows:
0=No plaque, extremely good oral hygiene
1=Film of plaque adhering to the free gingival margin and adjacent area of the tooth, good oral hygiene.
2=Moderate accumulation of soft deposits in the gingival pocket or on the tooth gingival margins, poorer oral hygiene. Can be seen with the naked eye.
3=Abundance of soft matter within the gingival pocket and/or on the tooth gingival margins, unsatisfactory oral hygiene.

In CAMBRA, “heavy plaque” constitutes a risk factor. Number 3 in the plaque index was set to correspond to “heavy plaque”. The Silness and Löes index is used in the Cariogram to assess the amount of plaque.

Fluoride and oral hygiene. Participants answered a questionnaire regarding tooth brushing frequency and their usage of toothpaste and other additional fluoride products (Appendix).

In CAMBRA, the following fluoride use is considered a “Protective factor”: fluoride toothpaste at least 1–2 times/daily, fluoride mouth rinse daily, 5000 ppm fluoride toothpaste daily.

Cariogram assess fluoride use according to the following scores:
0=Maximum fluoride use. Regular use of fluoride toothpaste plus additional measures.
1=Infrequent use of additional fluoride measures, besides regular use of fluoride toothpaste.
2=Fluoride toothpaste only, no additional fluoride products.
3=Avoiding fluorides, not using fluoride toothpaste or other fluoride products.

Caries prevalence. Four bitewing radiographs were taken for each participant to assess caries prevalence (DFT) before and after treatment. In Study I, the same DFT value was used 1 year into treatment.

In CAMBRA, the following caries prevalence-related factors are assessed as "Disease indicators": visible cavities or radiographic penetration of the dentin, radiographic approximal enamel lesions (not in dentin), white spots on smooth surfaces, restorations in the last 3 years.

In the Cariogram the caries prevalence score is set as follows:
0=Caries free and no fillings.
1=Better status than normal, for the specific age groups and area.
2=Worse status than normal or several new caries lesions.
Epidemiological caries data from the region of Västra Götaland (assessed from the dental journal system, T4) were retrieved to assess the appropriate caries prevalence score, according to the Cariogram.

The factors of salivary buffering capacity and saliva flow rate were excluded from the Cariogram risk assessment. The Cariogram variable "clinical judgement" was set as normal for all participants. The factors "deep pits and fissures" and "saliva flow rate" were excluded from the CAMBRA risk assessment. Caries risk assessments were made for Cariogram and CAMBRA based on the respective programme's instructions (D Bratthall et al., 2004; J. D. Featherstone et al., 2007). The R2 (The Public Dental Service of Västra Götaland, 2013, [unpublished]) CRA (oral health profile) was performed by the patient's regular dental team, as extracted from the dental journal.

3.5.2 RADIOGRAPHIC CARIES REGISTRATION

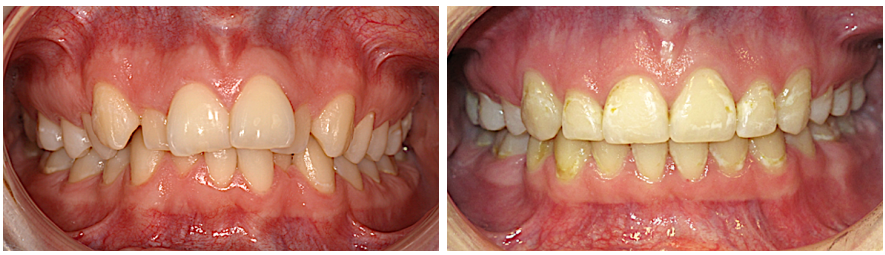
In Study III, four radiographs were taken for each participant before and after orthodontic treatment. Based on these radiographs, the DiFS, DFT, DFS, initial caries and manifest caries were registered for each patient. All permanent teeth from the distal surface of the second molars to the distal surface of the canines were included in the caries registration. Manifest caries also included secondary caries. Teeth extracted due to orthodontic treatment were excluded from the pre- and post- caries prevalence values. Caries was registered radiographically as follows:
Initial caries: radiolucency of the enamel with or without reaching/ penetrating the enamel-dentin border, without obvious spread within the dentin.
Manifest caries: radiolucency with broken enamel-dentin border and with obvious spread within the dentin.

3.5.3 WSL REGISTRATION

In Study IV, three intra-oral close-up photographs (one frontal photograph, two lateral photographs) were taken before and after treatment. The photographs were taken with a digital camera (Canon Powershot G7X; Canon Inc, Tokyo, Japan). Before treatment, all the teeth were polished with a rubber cup and pumice paste and gently dried with air before the photographs were taken. At debonding, the composite material was carefully removed with a slowly rotating carbide bur, and thereafter the teeth surfaces

were polished with pumice paste on a rubber cup. After gently air drying, a new series of photographs was taken (Figure 7).

Figure 7. Left panel: Dental status before treatment. Right panel: Dental status after treatment (note the WSLs on the teeth after treatment).



The photographic images were stored in the participant's digital journal (Edward) and projected onto a screen (Elite Display E222; Hewlett Packard, Palo Alto, CA, USA) in a dark room, enabling WSL registration. WSLs were assessed by the author (H.E.) according to the Gorelick scoring system (Gorelick et al., 1982):
1=No white spot formation.
2=Slight white spot formation (thin rim).
3=Excessive white spot formation.
4=White spot formation with cavitation.
Tooth surfaces with developmental or environmental alterations, such as fluorosis, stains and hypoplasia, were distinguished from WSL caries based on clinical appearance. If there was uncertainty regarding the correct diagnosis, the matter was discussed with the second author (M.L.) until consensus was reached.

3.6 COMPLIANCE

To evaluate how the fluoride interventions were used according to the instructions given, the participants answered a questionnaire 1 year after the initiation of treatment (Appendix 1). The following questions were posed in the questionnaire:

How often do you brush your teeth?
How often do you use toothpaste?
Do you use any additional fluoride product?

The term "additional fluoride" included high-fluoride toothpaste and fluoride mouth rinse.

3.7 ETHICAL CONSIDERATIONS

The studies were approved by the Regional Ethical Review Board in Gothenburg, Sweden (Dnr: 321-09). The research protocol followed the Helsinki Declaration of Human Rights (World Medical Association, 2013). In all the studies (I–IV), the

adolescents and their guardians received both verbal and written information about the study plan. Patients were also informed that withdrawing their participation was possible at any time without giving any reason. Participants gave their informed consent, if the subjects were aged <18 years, assent was obtained from the participant and informed consents were obtained from their guardian. The total dosage of radiation obtained from the examination was equivalent to approximately 1 week of natural background radiation, and was evaluated as an insignificant radiation risk. Ethical approval for the radiation procedure was obtained from the Swedish Radiation Protection Institute (Dnr: 290-09).

3.8 STATISTICAL ANALYSIS

For categorical variables, *n* (%) is presented. For continuous and count variables, the frequencies (%), mean, standard deviation, median, quartiles and number of values are presented. Statistical tests were used to test if the data were normally distributed. Statistical significance was set at a p-value of $p<0.05$. All statistical tests were performed in a two-sided manner. The statistical analyses were performed using the IBM-SPSS ver. 27 software (IBM Inc., Chicago, IL, USA) and the SAS ver. 9.4 software (SAS Institute Inc., Cary, NC, USA).

3.8.1 STUDY I

The Kruskal–Wallis test was used for continuous variables for comparisons between groups. The Sign test and the Wilcoxon Signed-Rank test were used for comparisons within groups. For pairwise comparisons between groups, the Mann–Whitney U-test was used for continuous variables and the Mantel–Haenszel Chi Square test was used for ordered categorical variables. Logarithmic values were used for the numbers of MS and LBC, to obtain more normally distributed values, enabling comparisons regarding the numbers of bacteria before and during orthodontic treatment. Caries on teeth being extracted during the treatment was registered with the same caries status before and after treatment.

Intra- and inter-examiner reliabilities were determined using an intra-class correlation coefficient (ICC), based on a single measurement/rater, absolute agreement, and a two-way mixed-effects model.

3.8.2 STUDY II

To enable a comparison with CAMBRA and R2 which are divided into low, medium and high risks, the following distribution of Cariogram and DFT were assessed: low risk, Cariogram 61%–100% chance to avoid caries, DFT better than normal status for the corresponding age group; medium risk, Cariogram 41%–60% chance to avoid caries, DFT normal status for the corresponding age group; and high risk, Cariogram 0%–40% chance to avoid caries, DFT worse than normal status for the corresponding age group. Moreover, the values for Cariogram and DFT were also analysed without any group categorisation. Caries on teeth that were being extracted during the treatment was registered with the same caries status before and after treatment.

Univariable logistic regression was performed to calculate the odds ratio (OR) with 95% confidence interval (95% CI), p-values, area under the receiver operating characteristic (ROC) curve (AUC), and estimated probability, based on the original values and not on stratified groups. The OR, which is the ratio of the odds for an increase of the predictor of 1 unit, reveals the associations between the CRA methods and caries increase.

A ROC curve is a graph that displays the accuracy of a diagnostic programme. The ROC curve presents the true-positive rate (sensitivity) on the *y*-axis and the false-positive rate (1-specificity) on the *x*-axis, for all possible cut-off values. The AUC is a measure of the overall performance of a diagnostic test, and is measured as the area under the ROC curve. An area of 1 represents an optimal test, whereas an area of 0.5 (coinciding with the diagonal) represents a useless test, with no discriminant capability.

Cohen's kappa correlation was used to calculate the inter- and intra-individual agreements regarding caries registration. The inter-individual agreement analysis was performed on 50 patients (200 bitewing radiographs) by the author (H.E.) and an oral radiologist. The intra-individual agreement analysis was performed by re-assessing a random sample of 50 patients 1 month later (H.E.).

3.8.3 STUDY III

The Kruskal–Wallis test was used for continuous variables for comparisons between groups. The Mann–Whitney U-test was used for pairwise comparisons between groups for continuous variables. The Wilcoxon Signed-Rank test was used for comparisons within groups. The risk ratio (RR) with 95% CI was calculated to compare the increases in caries between the groups (pair-wise), based on clinically relevant cut-offs. To adjust the RR for baseline values, the Cochran-Mantel-Haenszel method was used. Teeth that were extracted during treatment, as a part of the orthodontic treatment, were excluded from the pre- and post-treatment caries values.

Cohen's kappa correlation was used to calculate the inter- individual (oral radiologist and H.E.) and intra-individual agreement levels (H.E.) regarding caries registration. The same procedure protocol as in Study II was performed followed.

3.8.4 STUDY IV

For comparisons between groups, the Kruskal-Wallis test was used for continuous variables. For pair-wise comparisons between groups, the Mann-Whitney U-test was used for continuous variables. For comparisons between groups, Fisher´s exact test was used for dichotomous variables and the Mantel-Haenszel Chi Square test was used for ordered categorical variables. Logistic regression was used to calculate an adjusted p-value, with adjustments for treatment time (months) and baseline level. Teeth that were extracted during treatment, as a part of the orthodontic treatment, were excluded from the pre- and post-treatment caries values.

Cohen's kappa coefficient was calculated to check for inter-examiner reliability. A random collection of photographs for 30 patients was registered by the author (H.E.) and by another experienced dentist (M.L.). The same collection of photographs was re-assessed 1 month later by the author (H.E.) to check for intra-examiner reliability.

3.8.5 SAMPLE SIZE ESTIMATIONS

Sample size calculations were performed for Studies I, III and IV (table 3).

Table 3. Overview of the power calculations made for Studies I, III and IV. All power calculations were based on a significance level of 5 % ($p < 0.05$) and 80 % power.

Study	Primary outcome variable	A clinically meaningful difference between the groups		Group size estimation
I	Cariogram and Oral bacteria	Cariogram: 20 % (SD=20%)	Oral bacteria: 2.0 units log MS (SD=4.5 units log MS)	84
III	DiFS	The distribution of DiFS increase in the CTR group: 0 (50%), 1 (25%), 2 (20%) and 3 (5%) In the FMR and HFT groups: 0 (75%), 1 (10%), 2 (10%) and 3 (5%)		62
IV	WSL	On the patient level, with an increase of ≥1 WSL, disclosing a proportion difference of 25% between the groups.		66

4 RESULTS

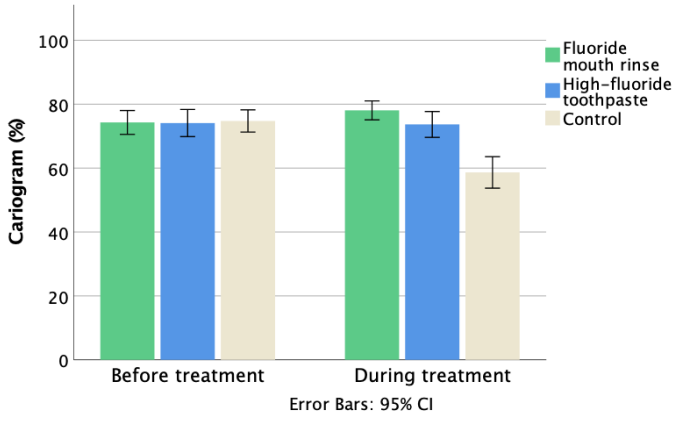
In Studies I, II and III, the participants had a baseline mean age of 15.4 ± 1.7 years. Of the participants, 64.7% were female and 35.3% were male. Overall, 8% of the subjects stated that they had a disease, with asthma being the most common. Similar baseline findings were seen for Study IV, (see the *Results* for Study IV). The mean orthodontic treatment duration was 25.9 ± 9.1 months. The treatment times for the groups were as follows: FMR group, 25 ± 8.9 months; HFT group, 28.1 ± 10.5 months; and CTR group, 24.6 ± 7.3 months. No statistically significant difference in treatment time was noted between the groups (p=0.09). In total, 498 teeth were extracted as a part of the orthodontic treatment, and the mean number of extracted teeth per person was 2±1.9. In total, 113 (44.3%) patients had no teeth extracted during treatment, 26 (10.2%) patients had 1 or 2 teeth extracted, and 116 (45.5%) patients had 3–4 teeth extracted. Between the three groups, no statistically significant difference was seen in the numbers of extracted teeth (p=0.4). At baseline, the mean DFT of the participants was 0.9 ±1.5 and 64% were caries-free (DFT=0). The DFT baseline values for the groups were as follows: FMR group, 0.8 ± 1.5; HFT group, 1.0± 2.7; and CTR group, 0.9± 1.5; there was no statistically significant differences between the groups.

4.1 STUDY I

The effect of orthodontic treatment

The caries risk described as "percentage chance to avoid caries", based on the Cariogram, increased significantly in the CTR group from a mean of 74.8% (95% CI 71.3-78.2) at baseline to 58.6% (95% CI 53.7-63.6) during orthodontic treatment ($p < 0.0001$) (Figure 8).

Figure 8. Mean caries risk (95% CI) as "percentage chance of avoiding new cavities", based on the Cariogram, for the three groups studied, before and during the orthodontic treatment.



Statistically significant increases ($p < 0.0001$) were also seen for the logarithmic mean numbers of MS and LBC in the CTR group (Figure 9 and 10, Table 4).

Figure 9. Logarithmic mean levels of MS (95% CI) before and during orthodontic treatment for the three groups.

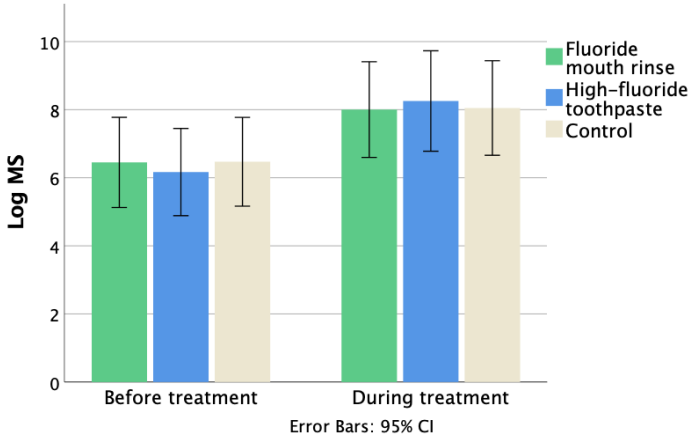


Figure 10. Logarithmic mean levels of LBC (95% CI) before and during orthodontic treatment for the three groups.

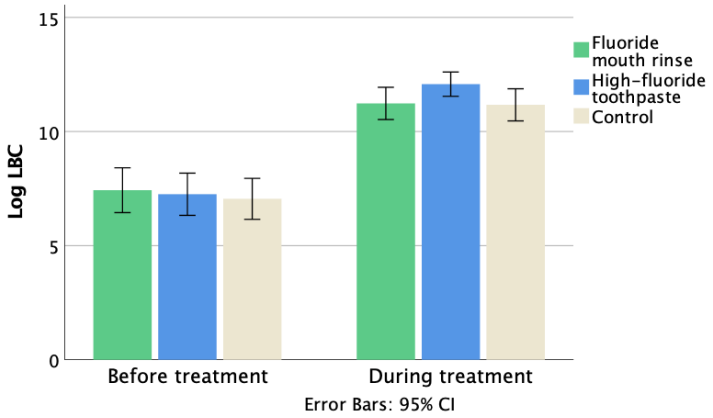


Table 4. The distribution of MS and LBC numbers before and during orthodontic treatment. Colony-forming-units (CFU) are measured per mL of saliva. The data are not logarithmised in order to give a clear presentation of the distribution of the numbers of bacteria.

	Before treatment			**During treatment**		
	FMR (n=87)	HFT (n=81)	CTR (n=87)	FMR (n=87)	HFT (n=81)	CTR (n=87)
MS (CFU/mL)						
<10 000	42 (48.3%)	44 (54.3%)	40 (46.0%)	40 (46.0%)	33 (40.7%)	34 (39.1%)
>10 000-100 000	16 (18.4%)	15 (18.5%)	18 (20.7%)	9 (10.3%)	7 (8.6%)	12 (13.8%)
>100 000-1 000 000	16 (18.4%)	17 (21.0%)	23 (26.4%)	19 (21.8%)	18 (22.2%)	23 (26.4%)
>1 000 000	13 (15.0%)	5 (6.2%)	6 (6.9%)	19 (21.8%)	23 (28.4%)	18 (20.7%)
LBC (CFU/mL)						
<1000	31 (35.6%)	28 (34.6%)	33 (37.9%)	8 (9.2%)	1 (1.2%)	4 (4.6%)
>1000-10 000	20 (23.0%)	25 (30.9%)	22 (25.3%)	5 (5.7%)	6 (7.4%)	15 (17.2%)
>10 000-100 000	21 (24.1%)	17 (21.0%)	23 (26.4%)	31 (35.6%)	24 (29.6%)	19 (21.8%)
>100 000	15 (17.2%)	11 (13.6%)	9 (10.3%)	43 (49.4%)	50 (61.7%)	49 (56.3%)

The levels of dental plaque and food intake frequency were unchanged during the treatment in the CTR group (Figure 11 and Table 5).

Figure 11. Distributions of dental plaque scores (0–3) in the three groups studied, according to the Cariogram, before and during orthodontic treatment.

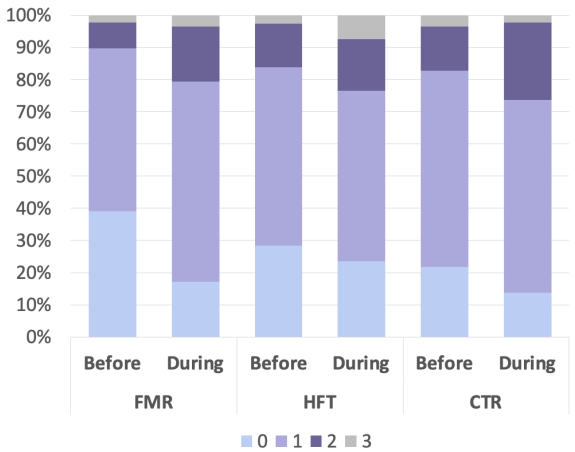


Table 5. Food intake frequency before and during orthodontic treatment, measured in meals/day for each of the groups.

	Before treatment			**During treatment**		
	FMR (n=87)	HFT (n=81)	CTR (n=87)	FMR (n=87)	HFT (n=81)	CTR (n=87)
Food intake frequency, mean (95% CI)	4.9 (4.6-5.1)	4.5 (4.2-4.8)	4.6 (4.4-4.9)	4.4 (4.2-4.6)	4.2 (4.0-4.4)	4.6 (4.4-4.8)

The effect of high-fluoride toothpaste and mouth rinse

The caries risk was statistically unchanged in the HFT- and FMR groups during treatment. The FMR group presented a mean baseline value for caries risk of 74.3% (95% CI 70.6-78.0) before treatment and 78.1% (95% CI 75.1-81.0) during treatment. The corresponding values for the HFT group were 74.1% (95% CI 69.9-78.4) and 73.7% (95% CI 70.0-77.7), respectively. The FMR and HFT groups differed significantly ($p<0.05$) from the CTR group during treatment, the CTR group demonstrated statistically increased caries risk during treatment (Figure 8).

The logarithmic mean levels of MS and LBC increased statistically significantly in the HFT and FMR groups during treatment ($p<0.0001$), and showed no statistical differences compared to the CTR group (Figure 9 and 10, Table 4). The levels of plaque remained statistically unchanged within and between the fluoride intervention groups and the CTR group during orthodontic treatment (Figure 11). The food intake frequency decreased significantly in the HFT group and FMR group during treatment ($p<0.05$). The change in frequency of food intake differed in a statistically significant manner between the CTR group and the FMR group during treatment ($p<0.05$), such that the FMR group showed a significantly greater decrease compared to the CTR group (Table 5). A hypothetical calculation was performed to determine whether the differences in caries risk between the groups was mainly due to differences in food intake frequency. However, the statistically significant differences between the groups persisted even after adjusting the food intake frequency to the same values in all the groups. The null-hypothesis was rejected because the orthodontic treatment increased both the caries risk and the bacterial levels. Furthermore, the caries risk was unchanged in the HFT and FMR groups.

Error of the method

The intra-examiner reliability ICC for the examiner (H.E.) regarding caries registration on the intra-oral radiographs was 0.97, and the inter-examiner reliability ICC for the oral radiologist and the examiner (H.E.) was 0.95.

4.2 STUDY II

The participants' mean DiFS value at baseline was 2.9±3.7 and the mean DFT value was 0.9±1.5. The mean increase in DiFS during treatment was 0.9±1.4 and 108 participants (42.4%) showed an increase in DiFS (ΔDiFS >0) during treatment. The mean increase in DFT during treatment was 0.2±0.7. During orthodontic treatment with FOA, 43 participants (16.9%) demonstrated an increase in DFT (ΔDFT >0).

The null-hypothesis could be rejected because the DiFS at baseline presented the highest AUC values for both initial caries at 0.71 (95% CI 0.64–0.77) and manifest caries at 0.77 (95% CI 0.7–0.85), as compared to the other CRA programmes and indices (Figure 12). In other words, DiFS manifested the highest overall performance of a diagnostic test in terms of predicting caries, as illustrated by the ROC-curve (Figure 13).

*Figure 12. AUC-values for initial caries and manifest caries for each CRA programme and caries index. *Risk categorised as low, medium, or high.*

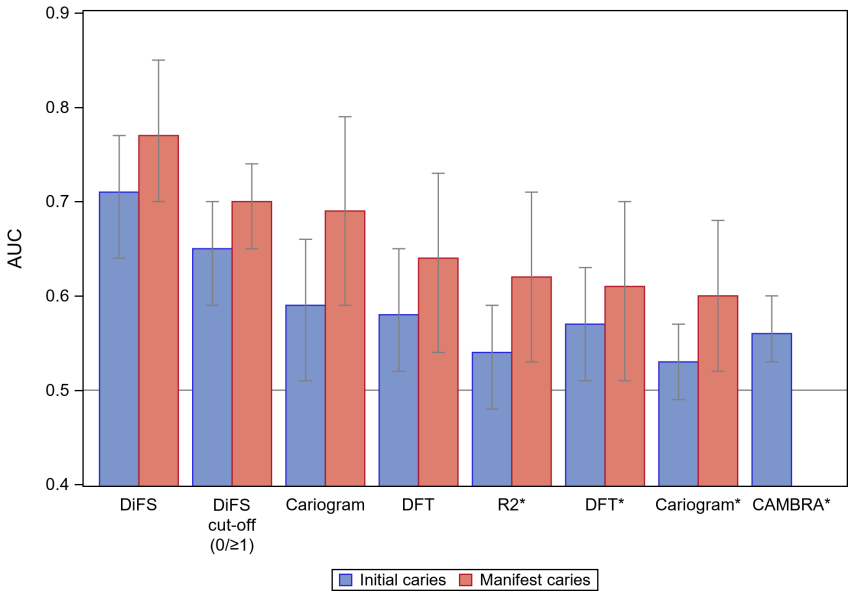
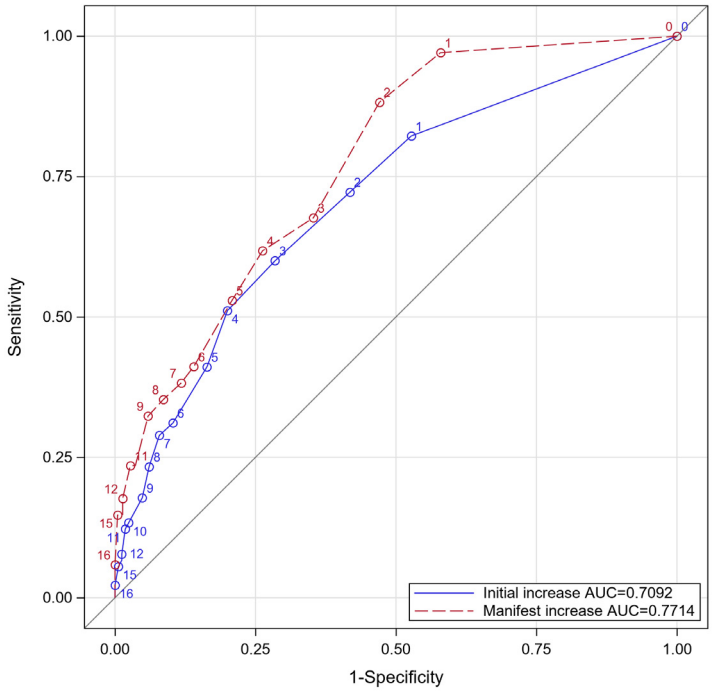


Figure 13. ROC curves for manifest caries (red line) and initial caries (blue line), with DiFS as the predictor. Each point on the ROC curve represents a DiFS baseline value.



For predicting initial caries, in general, the abilities of the CRA programmes and caries indices were weaker than those for predicting manifest caries (Figures 12 and 13). The AUC-values for the CRA programmes and caries indices for the prediction of initial caries are presented as follows, in descending order: DiFS, 0.71 (95% CI 0.64–0.77); Cariogram, 0.59 (95% CI 0.51–0.66); DFT, 0.58 (95% CI 0.52–0.65); CAMBRA (divided into low, medium, high risk), 0.56 (95% CI 0.53–0.60); and R2 (divided into low, medium, high risk), 0.54 (95% CI 0.48-0.59) (Figure 12).

The corresponding AUC-values for the CRA programmes and caries indices, for the prediction of manifest caries are presented as follows, in descending order: DiFS, 0.77 (95% CI 0.7–0.85); Cariogram, 0.69 (95% CI 0.59–0.79); DFT, 0.64 (95% CI 0.54–0.73); and R2 (divided into low, medium and high risk), 0.62 (95% CI 0.53–0.71) (Figure 12). The AUC-value could not be calculated for CAMBRA when predicting manifest caries because no participant was distributed into the low caries risk group and no patient in the medium caries risk group developed manifest caries.

The highest OR values for manifest caries (23.97) and initial caries (4.15) were seen for DiFS using a cut-off (0/ ≥1). The DiFS cut-off was introduced with the aim to simplify the prediction of caries during orthodontic treatment. However, DiFS with a

cut-off presented lower AUC-values (initial caries, 0.65; manifest caries, 0.7), as compared to DiFS without a cut-off (initial caries, 0.71; manifest caries, 0.77). A linear trend was seen for DiFS and the estimated probability of an increase in initial caries. A similar tendency was seen for the estimated probability of an increase in manifest caries.

Error of the method

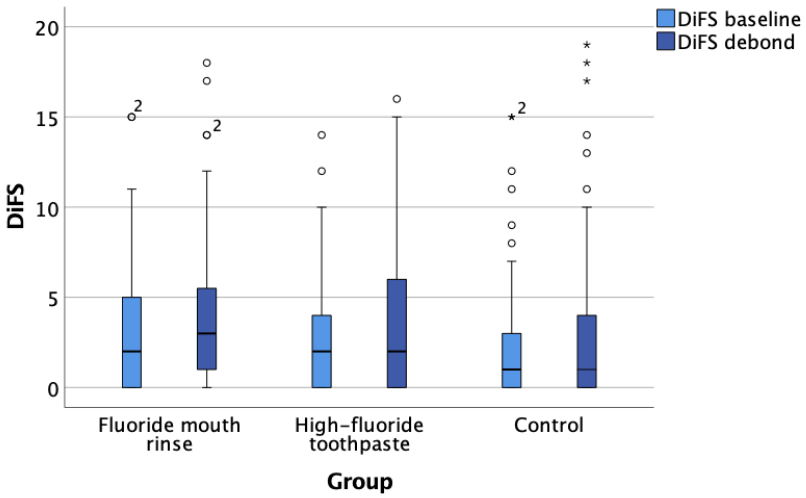
The inter-examiner kappa values were 0.83 (very good) for initial caries and 0.89 (very good) for manifest caries. The intra-examiner values were 0.92 (very good) for initial caries and 0.89 (very good) for manifest caries.

4.3 STUDY III

The increases in DiFS, DFT, DFS, initial caries and manifest caries were statistically significant ($p<0.05$) within each group during orthodontic treatment.

In the FMR group, 48.3% showed an increase in DiFS during orthodontic treatment (mean 1.03, 95% CI 0.75–1.32). The corresponding values for the HFT group and CTR group were 42.0% (mean 0.89, 95% CI 0.60–1.18) and 35.6% (mean 0.78, 95% CI 0.45–1.12), respectively. There was no significant difference between the groups in terms of increased DiFS ($p=0.17$) (Table 6). The distributions of DiFS before and after orthodontic treatment, for the three groups, are presented in a box plot (Figure 14).

Figure 14. Boxplot of the distributions of DiFS values before and after orthodontic treatment for each group. Adapted from Study III.



In the FMR group, 20.7% showed an increase in DFT during orthodontic treatment (mean 0.32, 95% CI 0.14–0.50). The corresponding figures for the HFT group and

CTR group were as follows: 16.0% (mean 0.17, 95% CI 0.08–0.26), and 13.8% (mean 0.21, 95% CI 0.08–0.33), respectively. There was no significant difference between the groups in terms of increased DFT (p=0.46) (Table 6). The distributions of DFT before and after orthodontic treatment, for the three groups, are presented in a box plot (Figure 15).

Figure 15. Box plot of the distributions of DFT values before and after orthodontic treatment for each group. Adapted from Study III.

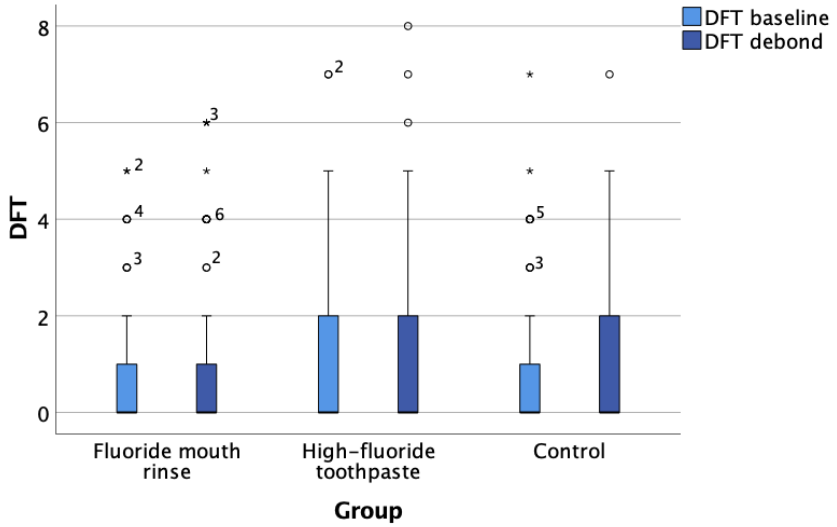


Table 6. Distribution of ΔDiFS and ΔDFT, during orthodontic treatment, for each group.

	FMR group	**HFT group**	**CTR group**
ΔDiFS			
0	45 (51.7%)	47 (58.0%)	56 (64.4%)
1	15 (17.2%)	13 (16.0%)	15 (17.2%)
2	13 (14.9%)	11 (13.6%)	9 (10.3%)
≥3	14 (16.1%)	10 (12.4%)	7 (8.0%)
Mean (95% CI)	1.03 (0.75;1.32)	0.89 (0.60;1.18)	0.78 (0.45;1.12)
p within groups	<0.001	<0.001	<0.001
p between groups	0.17		
	FMR vs HFT 0.41	HFT vs CTR 0.32	CTR vs FMR 0.06
ΔDFT			
0	69 (79.3%)	68 (84.0%)	75 (86.2%)
1	13 (14.9%)	12 (14.8%)	8 (9.2%)
2	3 (3.4%)	1 (1.2%)	2 (2.3%)
≥3	2 (2.3%)	0 (0%)	2 (2.3%)
Mean (95% CI)	0.32 (0.14;0.50)	0.17 (0.08;0.26)	0.21 (0.08;0.33)
P within groups	<0.001	<0.001	0.002
P between groups	0.46		
	FMR vs HFT 0.38	HFT vs CTR 0.77	CTR vs FMR 0.24

Regarding caries increase for DFS, initial caries or manifest caries, no statistically significant differences were noted between the groups (p=0.39, p=0.46, and p=0.78, respectively). Comparison between the groups regarding increase in caries was also calculated for all outcome variables using the RR with 95% CI, based on clinically relevant cut-offs, even then, no significant difference was seen between the groups (Table 7). The hypothesis was rejected on the basis that there was no significant difference in caries increment between the three groups.

Table 7. Between-group comparisons regarding increase in caries during orthodontic treatment. The risk ratios (RRs) are presented, based on clinically relevant cut-offs. The p-values and RR:s are adjusted for baseline (*). Adapted from Study III.

Caries measure and cut-offs	Group comparison (A vs B)	Caries increase in group A	Caries increase in group B	RR* (95% CI)	p-value*
DiFS ≥2	FMR vs CTR	27 (31.0%)	16 (18.4%)	1.4 (0.8- 2.3)	0.23
	HFT vs CTR	21 (25.9%)	16 (18.4%)	1.2 (0.7- 2.1)	0.51
	HFT vs FMR	21 (25.9%)	27 (31.0%)	0.9 (0.6- 1.5)	0.76
DFS ≥1	FMR vs CTR	19 (21.8%)	12 (13.8%)	1.6 (0.9- 3.1)	0.13
	HFT vs CTR	14 (17.3%)	12 (13.8%)	1.2 (0.6- 2.3)	0.63
	HFT vs FMR	14 (17.3%)	19 (21.8%)	0.7 (0.4- 1.3)	0.29
DFT ≥1	FMR vs CTR	18 (20.7%)	12 (13.8%)	1.6 (0.8- 3.1)	0.19
	HFT vs CTR	13 (16.0%)	12 (13.8%)	1.1 (0.6- 2.3)	0.75
	HFT vs FMR	13 (16.0%)	18 (20.7%)	0.8 (0.4-1.4)	0.41
Initial caries ≥2	FMR vs CTR	16 (18.4%)	10 (11.5%)	1.4 (0.7- 2.9)	0.41
	HFT vs CTR	16 (19.8%)	10 (11.5%)	1.5 (0.7- 3.3)	0.26
	HFT vs FMR	16 (19.8%)	16 (18.4%)	1.2 (0.7- 2.2)	0.48
Manifest caries ≥1	FMR vs CTR	13 (14.9%)	10 (11.5%)	1.3 (0.6- 2.6)	0.56
	HFT vs CTR	11 (13.6%)	10 (11.5%)	1.0 (0.5- 2.2)	0.93
	HFT vs FMR	11 (13.6%)	13 (14.9%)	0.8 (0.4- 1.8)	0.65

Error of method

The inter-examiner kappa values were 0.83 (very good) for initial caries and 0.89 (very good) for manifest caries. The intra-examiner values (H.E.) were 0.92 (very good) for initial caries and 0.89 (very good) for manifest caries.

4.4 STUDY IV

In total, 248 patients were included in the study, with a dropout rate of 22 patients (8.1%). At baseline, the mean age was 15.4 years (SD 1.6). The gender distribution was 164 females (66.1%) and 84 males (33.9%). Thirty patients stated they had a disease, with asthma and/or allergy being the most commonly reported disease. In total, 491 teeth were extracted as part of the orthodontic treatment. The mean number of extracted teeth was 2 (±1.9), with no statistically significant difference between the

groups (p=0.2). The mean duration for orthodontic treatment was 25.9 (±9.1) months. The three groups showed the following treatment durations: FMR group, 25.0 (±9.3) months; HFT group, 27.8 (±10.3) months; and CTR group, 24.7 (±7.3) months. There was no statistically significant difference between the groups with respect to treatment duration (p=0.12).

WSL on the subject level

Before orthodontic treatment, 17 patients (21.0%) in the FMR group showed no WSL, with 22 patients (25.9%) in the HFT group and 15 (18.3%) patients in the CTR group. On the patient level, no statistically significant difference between the groups was seen regarding the prevalence of WSL at baseline (Study IV). In total, 171 patients (69.0%) demonstrated an increase of ≥1 WSL during orthodontic treatment. The number of patients with an increase of ≥1 WSL did not differ significantly between the groups during orthodontic treatment, when all teeth were included in the analysis. However, the number of patients with an increase of ≥1 WSL in the "aesthetic zone" (including all central incisors, lateral incisors and canines in the upper and lower arches) during orthodontic treatment was significantly higher in the CTR group compared to the HFT group (p=0.011) and in the CTR group compared to the FMR group (p=0.006) (Study IV). The WSLs were favoured by the high-fluoride interventions, so the null-hypothesis could, therefore, be partly rejected.

WSLs on the tooth level

In total, 5952 surfaces were diagnosed with respect to WSLs. The mean numbers of new WSLs during treatment were as follows: FMR group, 2.6±3.2; HFT group, 2.8±3.1; and CTR group, 3.3±3.3; there was no statistically significant difference between the groups (p=0.28). The percentage distribution of the degree of WSL (1–4) after debonding is presented in Study IV. No statistically significant differences were seen between the groups. At baseline, the tooth that was most frequently affected by WSL was the first molar. During treatment, the central incisor, lateral incisor and canine in the maxillary arch showed the highest increase of WSL (Study IV).

Error of the method

The inter-examiner kappa value for caries registration on photographs was 0.78 (good) and the intra-examiner value was 0.81 (very good).

4.4.1 HARMS

No patient reported any allergic reaction or other harm or adverse event in relation to the use of fluoride products during any of the four studies.

5 DISCUSSION

The levels of scientific support for risk assessment, prevention of caries, and how caries-related factors are affected by high-fluoride preventive measures during orthodontic treatment are rather low. Therefore, the current thesis focuses on evaluating and addressing these issues during orthodontic treatment with FOA.

5.1.1 CARIES RISK ASSESSMENT

In Study II, the caries risk and caries index were assessed before treatment and DiFS was found to be the most-accurate predictor of caries. This is in line with other clinical trials that have presented previous caries prevalence as the most accurate predictor of future caries (Mejare et al., 2014; Twetman et al., 2013; D. Zero et al., 2001), which is far from ideal, as the caries disease is already established. However, a clinical study and a systematic review showed the opposite, that multi-factorial programmes are the most powerful explanatory variable of caries incidence (Hansel Petersson et al., 2002; Twetman, 2016). In line with the findings of Study II, Lif Holgersson and co-workers have demonstrated, in a study of pre-school children, that a modified Cariogram is not especially useful for identifying patients with high caries risk in a low-caries community (Holgerson et al., 2009). In this thesis, the participants showed low caries prevalence, as compared to the general population (Socialstyrelsen. National Board of Health and Welfare, 2021d). The study population had a mean DFT at baseline of 0.9 ±1.5, with 64% of the participants being caries-free at a mean age of 15.4 years. The NBHW reveals a similar caries prevalence for children aged 12 years, with a mean DFT of 0.7 and 67% being caries-free (Socialstyrelsen. National Board of Health and Welfare, 2021d). Nonetheless, identifying patients with an increased risk of caries so as to provide them with individualised treatment is crucial for the prevention of caries that would otherwise jeopardise the outcome of the orthodontic treatment. Multi-factorial CRA programmes require additional time and resources. To justify the extra resources, the predictive power of the multi-factorial programmes must be significantly higher than the predictive abilities of the individual variables. It remains a challenge to identify patients who are at high risk in clinical trials involving populations with a low caries prevalence.

In Study II, CRA was assessed before orthodontic treatment, which is a time-point that is especially important because decisions must be made as to whether or not the treatment should be performed. CRA must be performed regularly and with a shorter time interval when a higher risk of caries is expected. Such circumstances could entail, for instance, impeding cancer treatment or orthodontics, extensive prosthetic work, and medical disease or factors that significantly affect saliva flow, such as Sjögren's syndrome. Systematic reviews have demonstrated that the validities of CRA programmes in children and adolescents differ between studies and are limited (Mejare et al., 2014; Twetman, 2016; D. Zero et al., 2001). There is a lack of scientific guidelines regarding caries risk assessments for patients prior to orthodontic treatment.

CRA programmes should be seen as a help and support tool for the clinician to make well-balanced decisions. It is important that the programmes are evidence-based. The

lack of evidence-based CRAs causes dentists to rely on best practice and clinical experience, also known as “gut feeling” and “clinical feeling”. A major consequence of dentists' trusting their different clinical experiences, in the absence of evidence-based guidelines, is varying treatment regimens for patients with the same diagnosis and symptoms (Bader et al., 1995). In the broader perspective, the lack of evidence-based guidelines for caries risk assessment can result in different modes of utilisation of dental care resources and un-even dental care. According to “the Dental Care Act” in Sweden, it is every human’s right to receive equal dental treatment and dental care based on the patient’s needs (Statens offentliga utredningar, 2011). Therefore, evidence-based CRAs play an important role in ensuring that risk assessment is correctly conducted and unanimously approved by clinicians, which in the long run will contribute to a more equal dental care system.

The choice of prophylaxis on a population level can also be based on how risk is assessed or anticipated in the population. In populations with skewed caries prevalence, as in the present study, the relevance of targeting costly additional caries preventative strategies to an entire population can be questioned. The high-risk strategy (also called “individual-based strategy”) is commonly recommended for populations that demonstrate a caries prevalence that is not normally distributed, with mainly a low caries population. The approach aims to identify high-risk individuals and give them individualised treatment with the appropriate prescribed amount of fluoride. Another strategy discussed in the literature is the population-based strategy, which consists of general caries prevention in an entire population. This is often the choice when the entire population demonstrates a high caries prevalence. The population-based prevention strategy can, for instance, consist of fluoride toothpaste, fluoridation of water supplies and public education in oral hygiene (Rose, 2001). Hausen and colleagues identified patients with high caries risk in a population that had a low prevalence of caries, although a negligible difference was seen between high-risk patients treated with an intensive prevention programme compared to the same group of patients treated with basic prophylaxis (Hausen et al., 2000). The strategies are sometimes said to be contradictory. Nevertheless, both strategies are often, as in this thesis, used together in the same programme (Burt, 1998). Economic resources, the time and effort required, as well as the accuracy with which the applied methods identify patients with increased caries risk are other factors to consider when planning for caries prevention during orthodontic treatment.

The results of Study II, together with those of other clinical risk assessment studies (Tellez et al., 2013; D. Zero et al., 2001), emphasise that a reliable, evidence-based CRA programme is needed to predict high caries risk patients. A novel approach to tackling the problem would be to classify patients into different risk categories at baseline. The variable propensities of patients to develop caries due to genetics and microbiological variations, including bacterial and salivary factors, should be considered from the outset. It would be interesting to genetically test the patients presented in this thesis, to see if the patients who presented with outlier values for caries incidence also show defect genes expression. The results might partly explain the weak effect of high-fluoride toothpaste and mouth rinse had on caries incidence, as seen in Study III.

5.1.2 CARIES RISK DURING ORTHODONTIC TREATMENT

In Study I we showed that caries risk, according to the Cariogram, increase during orthodontic treatment with fixed appliances and that high-fluoride toothpaste and mouth rinse can modulate the caries risk by maintaining it at a constant level. Several clinical trials have evaluated and confirmed the accuracy of the Cariogram in predicting caries, both in school-children (Campus et al., 2012; Hansel Petersson et al., 2002; Zukanovic, 2013) and young adults (Celik et al., 2012). The fact that orthodontic treatment leads to an increased risk of developing caries is well-known (Gorelick et al., 1982; Richter et al., 2011; Shungin et al., 2010), and is verified in the present study. However, the effects of the high-fluoride toothpaste and mouth rinse on caries-related variables during orthodontic treatment with FOA have not been extensively studied previously. The different risk variables are important to assess, in order to understand the interplay between the factors that influence the disease profile and that have direct effects on treatment decisions, as well as on future disease prognosis.

The results of Study I are in line with those of other clinical trials demonstrating increases in the numbers of MS and LBC during orthodontic treatment (Jing et al., 2019; Maret et al., 2014; Topaloglu-Ak et al., 2011). In addition, in the present study, it is revealed that the numbers of bacteria increase during orthodontic treatment, regardless of group affiliation. The impact of fluoride on the numbers of bacteria during orthodontic treatment appears to be limited. In Study I, 96 patients (37.6%) showed no MS during orthodontic treatment, indicating a rather healthy study group. The bacterial levels and the difference between the groups might have been higher and more pronounced, respectively, in a population with a higher caries prevalence. High numbers of MS have been associated with higher caries outcomes (Pannu et al., 2013; Salonen et al., 1990). However, fluoride has an anti-microbial effect on oral bacteria in deteriorating their aciduric and acidogenic abilities (J. D. Featherstone, 1999; Hamilton, 1990; Hicks et al., 2004). Therefore, it is reasonable to assume that the additional fluoride would have impacts of the on the physiological features and metabolic functions of the bacteria. Recent research has shown that cell-surface components (facilitating adhesion to other cells) and different genotypic characteristics of the bacterial species may be crucial for caries development (Stromberg et al., 2017). Furthermore, a recently published study has shown that different adhesin subtypes of *Streptococcus mutans* predict individual caries development (Esberg et al., 2017).

In general, the literature indicate that plaque levels increase during orthodontic treatment, with bands and brackets retaining food debris and complicating oral cleaning (Chang et al., 1999; Naranjo et al., 2006). A RCT demonstrated a significantly lower visible plaque index during orthodontic treatment when using fluoride varnish and toothpaste, as compared to a control group using only toothpaste (Ogaard et al., 2001). These outcomes for plaque are discrepant with the results of Study I, which demonstrate consistent plaque levels in all the groups during treatment. The frequency of food intake during orthodontic treatment has sometimes been found to decrease, albeit only initially in some cases (Johal et al., 2013; Wysocka et al., 2014), and sometimes been shown to be constant (Chang et al., 1999). The improved diets in the

intervention groups may be linked to the fact that orthodontic patients can be restricted in terms of eating, due to sore teeth (Johal et al., 2013; Wysocka et al., 2014). However, in the control group, the patients showed an unchanged food intake frequency. Good oral hygiene for all participants and improved dietary habits for the majority of the patients during orthodontic treatment in Study I, as well as a low caries prevalence and a low caries incidence in the study population, support the notion that the participants in the present studies are a selected group of patients. All the participants were accepted for treatment with FOA, for which low caries risk and good oral hygiene are pre-requisites.

Having a predominance of defensive factors, or at least maintaining a balance between attack and defence mechanisms, is crucial for the prevention of caries. In Study I, it seems as though the differences in caries risk between the intervention groups and the control group are mainly due to statistically significant differences between the groups with respect to food intake frequency and fluoride intake. A calculation was performed to determine whether the differences in caries risk are mainly due to differences in food intake frequency. The statistically significant differences between the groups persisted even after adjusting the food intake frequency to the same value in all the groups. The results confirm the importance of additional fluoride prevention in keeping the caries risk low during orthodontic treatment. Both intervention groups benefitted from additional fluoride in terms of caries risk.

5.1.3 CARIES AND FLUORIDE INTERVENTIONS DURING ORTHODONTIC TREATMENT

Study III demonstrates that, based on examinations of approximal and occlusal caries, high-fluoride toothpaste and mouth rinse do not significantly alter the caries incidence compared to ordinary toothpaste, during orthodontic treatment. In line with the findings of Study III, Study IV demonstrates that the effect of ordinary toothpaste is statistically equivalent to that of high-fluoride toothpaste and mouth rinse, in terms of WSL incidence when all teeth were included. The weak effect of additional fluoride products in patients presented with low caries prevalence has been confirmed in other clinical studies (Axelsson et al., 1987; Bergstrom et al., 2014; Skold et al., 2001). Few studies have appraised the effect of additional fluoride on approximal and occlusal caries during orthodontic treatment, which makes Study III unique but the caries outcome difficult to compare. However, a retrospective study found statistically fewer number of approximal initial and manifest caries lesions in patients who were treated with FOA, as compared to a non-treated control group (Baumgartner et al., 2013). Another clinical study showed that orthodontically treated patients demonstrated no significant differences in relation to the development of dentine lesions compared to a non-orthodontically treated control group (Hadler-Olsen et al., 2012). Orthodontic patients, at least in Sweden, constitute a select patient material with respect to low caries prevalence, and this seems to influence the impacts of high-fluoride products.

A positive effect of additional fluoride on the development of WSL was seen when studying teeth in the aesthetic zone. This is in line with the results of a RCT conducted by Sonesson and colleagues, in which they demonstrated a significant increase in WSL

incidence in a control group compared to a HFT group during orthodontic treatment (M. Sonesson et al., 2014). The reported prevalence rates of WSL during orthodontic treatment vary greatly (in the range of 2–96%) between studies (Chapman et al., 2010; Enaia et al., 2011; Gorelick et al., 1982; Richter et al., 2011; Shungin et al., 2010; Stecksén-Blicks et al., 2007; Tufekci et al., 2011). In Study IV, the number of patients who exhibited at least one new lesion during treatment were: in total, 171 (69.0%); 53 (65.4%) in the FMR group; 58 (68.2%) in the HFT group; and 60 (73.2%) in the CTR group. When comparing the WSL incidence in Study IV during treatment with the studies of Sonesson et al. and Stecksén et al., which involved similar study populations, the latter two studies showed a lower baseline WSL prevalence, as well as s lower WSL incidence (M. Sonesson et al., 2014; Stecksén-Blicks et al., 2007). However, the numbers of teeth included in the studies of Stecksén et al. and Sonesson et al. were lower and the teeth in the mandible were not included, therefore, a direct comparison should be approached with caution. Nevertheless, the prevented fraction described by Sonesson et al. for control versus HFT was 31.9%, corresponding reasonably well to the prevented fraction derived from the same comparison in Study IV (23.1%).

In Study IV, the maxillary front teeth (canine-to-canine) show the highest incidence of WSL during orthodontic treatment, where the central- and lateral incisors demonstrate the highest incidence. In similarity to Study IV, the lateral incisor has been shown in other clinical studies to be the tooth that is most frequently affected by WSLs during orthodontic treatment (Chapman et al., 2010; Gorelick et al., 1982; M. Sonesson et al., 2014; Stecksén-Blicks et al., 2007). Explanatory factors may be higher plaque accumulation due to palatinal positioned lateral incisors at baseline, which complicate oral hygiene measures. Another possible explanation is the reduced access of saliva. Teeth with a higher caries incidence seem to derive greater benefit from prophylaxis with high-concentration fluoride. This could also be true on the patient level. Nordström et al. described a significant positive effect of high-fluoride toothpaste on occlusal and proximal caries in teenagers, as compared to the use of ordinary toothpaste on patients presenting with high caries activity. This was particularly evident in subjects who showed a low level of compliance (Nordstrom et al., 2010). As in Study I, it is likely that the high-concentration fluoride products used in Studies III and IV would have had greater impacts on the caries incidence if the included population had a higher caries incidence, as well as a low level of compliance. On the other hand, if the patients presented with a high caries prevalence in this thesis are presented with genetic immunodeficiency the positive effect of additional fluoride is more doubtful.

A simplified caries index that includes only the canine-to-canine group of teeth saves time for clinicians and is, from the patient's perspective, more interesting in terms of aesthetics. Future research might focus on developing further indices in the aesthetic zone, including and weighting those buccal areas of the teeth that are more susceptible to demineralisation, such as the cervical surfaces.

5.1.4 STUDYING CARIES IN THE ORTHODONTIC PATIENTS

Studying the development of caries is both costly and time-consuming. High dental costs, high-risk populations with recurrent caries that are non-respondent to standard preventive measures and an increased caries prevalence among specific patient groups are all issues that underline the importance of carrying out high-quality studies of caries. To study caries in the orthodontic patient, i.e., using orthodontic treatment as a caries model, can facilitate the conduct of clinical studies of caries. This is because the prevalence rates of high-risk subjects and the responses to high-fluoride prophylaxis during orthodontics correspond to those in the general population.

In Study III, a total of 21 (8.2%) patients before and 16 (6.3%) patients after orthodontic treatment displayed DFT outlier values. These numbers correspond quite well with the approximately 15% of children who show a high risk of caries regardless of dietary and oral hygiene habits (Esberg et al., 2017). The finding that orthodontically treated patients show broadly the same skewed caries prevalence distribution as the general public speaks in favour of the use of orthodontics as a caries model. That the percentage in Study III is somewhat lower (8% versus 15%) could be explained by the fact that the patients in that study were already selected as manifesting low caries risk and good oral hygiene, as these are pre-requisite for initiating orthodontic treatment. The high caries prevalence among the patients who presented with outlier values for DFT may be attributed to genetic variations, defective salivary proteins, and extra-virulent *S. mutans* (Esberg et al., 2017; Stromberg et al., 2017). Another factor that supports the usage of orthodontics as a caries model is the observation of a lack of effect of high-fluoride products on caries, based on radiographic diagnoses of low-caries-prevalence populations, as shown in Study III as well as in other prospective clinical studies without orthodontic treatment (Axelsson et al., 1987; Bergstrom et al., 2014; Skold et al., 2001). It is a recognized fact that conducting clinical caries studies on populations with a low caries prevalence is a challenge, with a long time-span (up to 5 years) often being required for such clinical studies. The use of orthodontics as a caries model could benefit from shorten time span with up to 2-3 years. Furthermore, patient exposure to radiation can be reduced, as the caries-prone surfaces are accessible and, therefore, other caries diagnostic methods, such as QLF, can be used. Other advantages of this model are better discrimination of low versus high caries phenotypes and simplified biological sampling from visible, easily accessible buccal surfaces. Moreover, the model is safe because the orthodontic patient visits the orthodontist regularly, which means that treatment can be terminated if WSLs become apparent.

5.1.5 CLINICAL IMPLICATIONS

As there is currently no optimally reliable method to predict initial and manifest caries during orthodontic treatment, the DiFS index is recommended. For orthodontic treatment with fixed appliances, the use of high-concentration fluoride toothpaste or fluoride mouth rinse in combination with regular toothpaste is recommended twice daily to prevent an increase in the risk of caries during the treatment. Furthermore, WSLs in the aesthetic zone (canine-to-canine) benefit from the high-concentration

fluoride products, resulting in significantly lower numbers of lesions. These clinical implications are true during orthodontic treatment for a select group of patients who have a low caries prevalence and good oral hygiene.

5.1.6 METHODOLOGICAL CONSIDERATIONS

Three out of four of the quantitative studies presented in this thesis have an RCT design. RCT is considered to be the gold standard for clinical trials. The control of the methodology and the inclusion of control groups are strong advantages of RCTs in terms of minimising the risk of bias.

5.1.7 LIMITATIONS

It would have been interesting to include a control group with no orthodontic treatment and to compare that group with the control group presented in this thesis. A certain increase in caries can be expected over a 2-year period, even without FOA. The RCTs do not have a double-blinded design, which must be regarded as a limitation. The type of randomisation involving sealed papers is not optimal, ideally, it should be done using a computer-based programme, thereby minimising the risk of bias. The patient compliance being followed up during the studies should preferably be tested regularly and digitally by using cell phones, and posing more specific questions to ensure reliability.

5.1.8 ETHICAL CONSIDERATIONS

In contrast to the positive effects of orthodontic treatment, such as the provision of aligned teeth and good occlusion, alteration of the oral environment is associated with the development of caries. The teeth can be damaged and lost, which may severely affect the patient both psychologically and physically. On the one hand, the patients in the control group may be suffering from a higher risk of developing caries due to the lack of additional fluoride prophylaxis. On the other hand, a general consensus regarding optimal fluoride prophylaxis during orthodontics has not yet been reached. All patients in the study received base prophylaxis including toothpaste (1450 ppm F) twice daily. The patients in the present study were controlled for caries, oral hygiene and caries risk regularly throughout the study, more thoroughly than usual during orthodontic treatment. Treatment was cancelled if a patient exhibited a risk of severe caries injury, just as with regular orthodontic treatment. Furthermore, there are strict requirements related to good oral hygiene and low caries activity for the initiation of orthodontic treatment, and the patients included in this thesis presented with a lower caries prevalence (DFT) than is average for their age group. From an ethical point of view, it is considered desirable to establish evidence-based guidelines for caries prevention during orthodontics, so as to avoid caries.

6 CONCLUSIONS

Currently, the DiFS index most accurately predict caries in orthodontic patients. The use of high-fluoride toothpaste or mouth rinse can be recommended during orthodontic treatment, to limit the caries risk and to reduce the number of WSLs in the aesthetic front. Furthermore, the present work suggest that caries advantageously can be studied in the orthodontic patients.

The specific conclusions from this thesis are as follows:

- Orthodontic treatment with fixed appliances increases the risk of caries. Regular use of fluoride mouth rinse (0.2% NaF) in combination with a fluoride toothpaste (1450 ppm F) or) or a high-fluoride toothpaste (5000 ppm F) reduces caries risk during orthodontic treatment with FOA, as compared to using ordinary fluoride toothpaste (1450 ppm F).
- To predict initial and manifest caries during orthodontic treatment with FOA, DiFS is recommended. DiFS demonstrates higher accuracy in predicting caries compared to DFT, Cariogram, CAMBRA or R2.
- The caries incidence during orthodontic treatment, based on dental radiograph-based diagnosis, does not seem to be significantly affected by the use of a mouth rinse (0.2% NaF) in combination with a fluoride toothpaste (1450 ppm F) or high-fluoride toothpaste (5000 ppm F), as compared to using ordinary toothpaste (1450 ppm F).
- Fluoride mouth rinse (0.2% NaF) in combination with a fluoride toothpaste (1450 ppm F) or high-fluoride toothpaste (5000 ppm F) significantly reduces the incidence of WSL in the aesthetic zone during orthodontic treatment.

7 FUTURE PERSPECTIVES

Effective caries preventive care encompasses accurate caries diagnosis and risk assessment. This thesis emphasises the need for a more-accurate caries risk assessment before orthodontic treatment. It would be interesting to examine in greater detail the patients in this thesis who presented with a high caries incidence during treatment, to define the type of MS and genetic variations. Research has shown that approximately every fifth individual suffers from caries that is difficult to treat, for whom dietary advice and oral hygiene instructions do not help significantly due to genetic variations. It is possible that a large proportion of patients belonging to the high-risk genetic group are never offered orthodontic treatment due to their extensive caries history. In a future risk assessment programme, patients should be categorised based on aetiological factors, such as genetic and microbiological variations at baseline.

The fact remains that currently there are many young patients who meet the functional requirement for orthodontic treatment but are denied treatment due to increased risk of caries and/or a lack of adequate oral hygiene. The use of a risk programme with high accuracy during orthodontic treatment would facilitate resource optimisation and, in a broader perspective, provide orthodontic care that is primarily guided by the patients' functional needs.

Furthermore, clinical caries research in industrialised countries with populations that present with low caries prevalence is a challenge. More-sensitive methods for evaluating the effects of caries (WSLs) prevention are needed, to measure more effectively the impacts of prophylaxis and to identify more precisely those patients, teeth and surfaces that are at increased risk for caries. Diagnosing WSLs on the surface level in the frontal dentition (canine-to-canine) seems promising in terms of developing a new and more accurate, time-efficient tooth index.

This thesis suggests that orthodontic treatment can function as a caries model from which future clinical caries studies can benefit. The time-span for clinical caries trials can be significantly reduced, as caries develops more rapidly during orthodontic treatment. The amount of radiation to which the patient is exposed can also be decreased, as the caries-prone surfaces are accessible and can be detected with other methods, such as QLF. Other advantages of this model are more-accurate discrimination of low versus high caries phenotypes and simplified biological sampling on easily accessible buccal surfaces. Moreover, the model supports more-regular caries controls for patients.

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10 APPENDIX

10.1 QUESTIONNAIRE

Name: National ID number:

General
Do you have any disease? If yes, please describe:
Do you take any medicines? If yes, please describe:
Have you taken antibiotics the past month?
Have you used snuff or smoked cigarette the past hour?

Diets
How many times a day do you eat? (i.e., main course and snack)
How many times each day do you eat food and snacks containing sugar?
How many times each week do you eat sweets or crisps?
How many times each week do you drink cordial, soft drink or sport drink?
How many times each week do you drink juice?
How many times each week do you eat cookies or ice cream?
What do you drink with your meal when you are thirsty?

Tooth brushing and fluoride habits
How often do you brush your teeth?
How often do you use toothpaste?
Do you use any additional fluoride product?