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Current Trends in Dental Epidemiology & Preventive Dentistry

Thesis for the attainment of the degree
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Submitted by
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from
Chirala (India)

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With permission from the medical faculty of

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This work is dedicated to my

supervisor,

parents,

wife

&

The Almighty.

Contents

List of Abbreviations

aOR	adjusted Odds Ratio
ASA	American Society of Anesthesiologists
BMI	Body Mass Index
CG	Control Group
CI	Confidence Interval
cm	centimeter
CONSORT	Consolidated Standards of Reporting Trials
CPI	Community Periodontal Index
CRP	C-Reactive Protein
dmf	decayed, missing, filled; in primary dentition
(DMF)T	(Decayed, Missing, Filled) Teeth; in permanent dentition
ECC	Early Childhood Caries
EG	Experimental Group
EQUATOR	Enhancing the QUAlity and Transparency Of health Research
ERF	Etch & Rise + adhesive + Flowable composite
ERFT	Etch & Rise + adhesive + Flowable & Traditional composite
ERT	Etch & Rise + adhesive + Traditional composite
g	gram
GCF	Gingival Crevicular Fluid
GEE	Generalized Estimating Equation
GI	Gingival Index
HR	Hazard Ratio
(hs)-CRP	(high sensitivity) C-Reactive Protein

ICDAS	International Caries Detection and Assessment System
ID	Identification number
IL6	Interleukin 6
ISO	International Organization for Standardization
kg	kilogram
LOD	Limit Of Detection
mg/L	milligram per litre
mm	millimeter
MPa	Megapascal
mW/cm^2	milliwatt per square centimeter
nm	nanometer
OHI	Oral Health Index
OR	Odds Ratio (unadjusted)
pg/L	picogram per litre
pH	potential of Hydrogen
PRISMA	Preferred Reporting Items for Systematic Review and Meta-Analyses
SBS	Shear Bond Strength
sd	standard deviation
SF	Self-etch adhesive + Flowable composite
SFT	Self-etch adhesive + Flowable & Traditional composite
s-SBI	simplified-Sulcus Bleeding Index
ST	Self-etch adhesive + Traditional composite
STROBE	The Strengthening the Reporting of Observational Studies in Epidemiology
UniViSS	Universal Visual Scoring System
WHO	World Health Organization

Chapter 1

Publication List

1.1 Ph.D. Topics

The topic of my Ph.D. thesis was "Current Trends in Dental Epidemiology and Preventive Dentistry". The research projects during this period focused on the fields of periodontology in terms of biomarkers; and cariology with respect to longevity of composite resin restorations, caries progression and caries prevention via pit and fissure sealants. As part of my Ph.D. requirements, it was considered to work on the following primary topics:

- **Project 1.** Influence of gingivitis and lifestyle on systemic inflammation in adolescents
- **Project 2.** Comparison of different workflows/protocols for performing adhesive restorations in primary teeth
- **Project 3.** Longitudinal study of caries progression in 2- and 3-year-old children
- **Project 4.** Shear bond strength and microleakage of a new pit and fissure sealant

From the primary Ph.D. topics and collaborations with other investigators, the following articles were published:

1.1.1 First-authorship articles

1. Pitchika V., Thiering E., Metz I., Rothmaier K., Willenberg A., Standl M., Hickel R., Heinrich J., Kocher T., and Kühnisch J.: Gingivitis and lifestyle influences on high-sensitivity C-reactive protein and Interleukin 6 in adolescents. J Clin Periodontol. 2017; 44(4):372-381

2. Pitchika V., Metz I., Rothmaier K., Crispin A., Hickel R., Bücher K., and Kühnisch J.: Comparison of different protocols for performing adhesive restorations in primary teeth - A retrospective clinical study. J Adhes Dent. 2016; 18(5):447-453

3. Pitchika V., Kokel C., Andreeva J., Laubender R., Hickel R., Garcia-Godoy F., Kühnisch J., and Heinrich-Weltzien R.: Longitudinal study on caries progression in 2/3-year old children. Community Dent Oral Epidemiol. 2016; 44(4):354-63

4. Pitchika V., Birlbauer S., Chiang M., Schuldt C., Crispin A., Hickel R., and Kühnisch J.: Shear bond strength and microleakage of a new self-etch adhesive pit and fissure sealant. Dent Mater J (26 June 2017) Accepted

1.1.2 Co-authored articles

Apart from these primary topics, I had contributed towards study design, data analysis, interpretation of results and preparation of manuscripts in the following projects:

1. Metz I., Rothmeier K., Pitchika V., Hickel R., Bücher K., and Kühnisch J.: Risk-factors for caries adjacent to direct composite restorations in primary teeth. Int J Paediatr Dent. 2015; 25(6):451-61

2. Bücher K., Metz I., Pitchika V., Hickel R., and Kühnisch J.: Survival characteristics of composite restorations in primary teeth. Clin Oral Investig. 2015; 19(7):1653-62

3. Schuldt C., Birlbauer S., Pitchika V., Crispin A., Hickel R., Ilie N., and Kühnisch J.: Shear bond strength and microleakage of a new self-etching/self-adhesive pit and fissure sealant. J Adhes Dent. 2015; 17(6):491-7

4. Kühnisch J., Söchtig F., Pitchika V., Laubender R, Neuhaus KW, Lussi A, and Hickel R.: In vivo validation of near-infrared light transillumination for interproximal dentin caries. Clin Oral Invest. 2016; 20(4):821-9

5. Kühnisch J, Lauenstein A, Pitchika V, McGlinn G, Staskiewicz A, Hickel R, and Gruppe G: Was Molar Incisor Hypomineralisation (MIH) present in archaeological case series? Clin Oral Investig. 2016; 20(9):2387-2393

6. Schuldt C., Pitchika V., Crispin A., Hickel R., and Kühnisch J.: Shear bond strength and microleakage of a self-etching adhesive for fissure sealing after different types of aging. Dent Mater J. 2016; 35(3):490-7

7. Heym R., Krause S., Hennessen T., Pitchika V., Ern C., and Hickel R: A New Model for Training in Periodontal Examinations Using Manikins. J Dent Educ. 2016; 80(12):1422-1429.

8. Chiang M., Birlbauer S., Lo Y., Pitchika V., Crispin A., Ilie N., Hickel R., and Kühnisch J.: Which factors influence the shear bond strength of sealant materials? J Adhes Dent. 2016;18(5):397-404

9. Rothmaier K., Bücher K., Metz I., Pitchika V., Hickel R., Heinrich-Weltzien R., and Kühnisch J.: Preventive and invasive treatment in special needs patients: a German retrospective study. Clin Oral Invest. 2017; 21:1343-1350

10. Bücher K., Metz I., Rothmaier K., Pitchika V., Hickel R., Heinrich-Weltzien R., and Kühnisch J.: Flowable composite as a direct restoration technique for primary molars. Eur J Paediatr Dent. (8 July 2016) Accepted

11. Birlbauer S., Chiang M.L., Schuldt C., Pitchika V., Hickel R., and Kühnisch J.: Shear bond strength and microleakage results for three experimental self-etching primer compositions for pit and fissure sealing. Clin Oral Investig. 2017; 21(5):1465-1473.

12. Heym R., Krause S., Hennessen T., Pitchika V., Ern C., and Hickel R: Development of a training approach for periodontal examination with models - A retrospective study of three consecutive semesters. J Dent Educ. (11 June 2017) Accepted

Chapter 2

Introduction

Dental caries and periodontitis are the most commonly occurring dental diseases in humans. Though their prevalence have reduced in the industrialised nations,[50,52] their occurrence is mostly polarised in some high risk populations due to many factors. Although the aetiology of these diseases are well studied and documented, there exists a number of influencing factors and confounders, which influence the relationship in different directions and magnitudes. Identification of all such risk factors can help in preventing the onset of disease at a very young age, thereby providing adequate oral health. In order to properly assess these factors and their relationships with the outcomes, a well designed study design is vital; and epidemiology is the branch of science which makes this possible.

2.1 Epidemiology

World Health Organization defines epidemiology as the study of the distribution and determinants of health-related states or events (including disease), and the application of this study to the control of diseases and other health problems.[37] Epidemiology is a multidisciplinary specialty, in which various clinical departments and the statistical team form a major component. Each of these units have equal importance that contributes towards a well performed research. Epidemiological data on all diseases is vital for the society in planning their preventive and treatment strategies among the population.

2.1.1 Epidemiology of dental caries in children

The average caries burden of the world in terms of DMFT was 2.11 ($\pm$1.32).[44] The global average for dental caries over 3 DMFT at 12 years of age classifies them under high risk population. The common age used to measure dental health among children in epidemiological studies worldwide is around 12 years of age. It is because at this age, the children leave their primary school and it might be the last time point when they could be accessed for data through school systems.[44] This is especially important in developing nations, where the school dropout ratio is higher after primary school. The caries experience among 12-year old children over different regions of WHO offices is shown in Figure 2.1.

Decline in caries prevalence is evident, particularly in industrialised nations and it

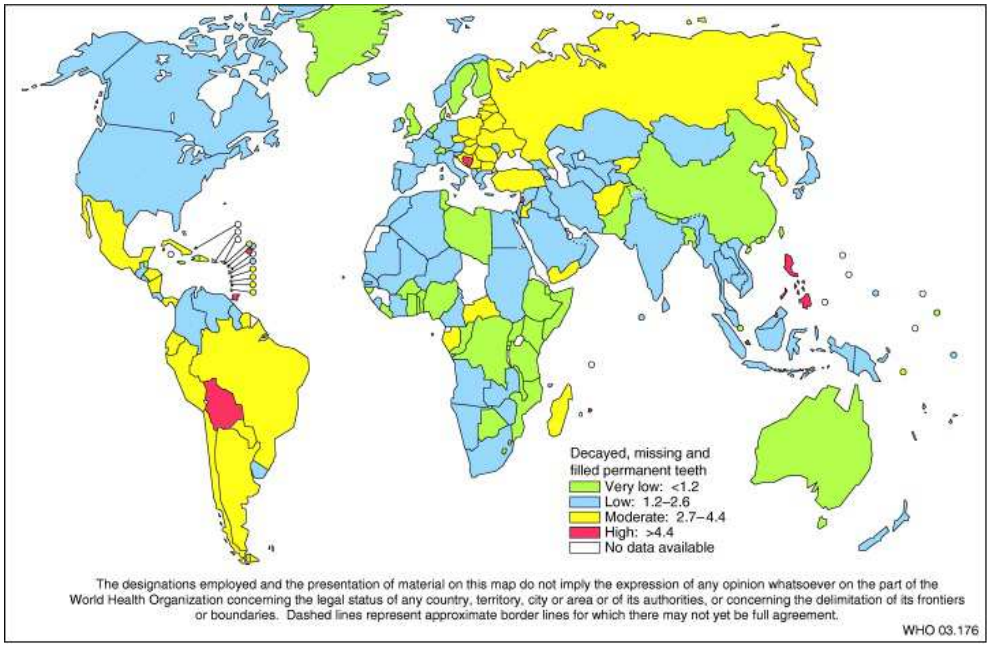


Figure 2.1: Caries experience (DMFT) of 12-year-old children worldwide[25,73]

has some implications on dental research. Furthermore, caries are commonly measured in epidemiological studies using dmf/DMF index according to the WHO standards,[71] which underestimates the caries burden. As the cavitated carious lesions begin to decline, the inclusion of non-cavitated carious lesions is critical to evaluate the caries burden with respect to the latest definitions. Studies have shown an increased caries experience under the inclusion of non-cavitated carious lesions. The research questions for the future studies have to be formulated focusing more towards prevention.

2.1.2 Epidemiology of gingivitis/periodontitis in children

Though it is a common perception that periodontal disease affects only in adulthood, it has been clearly demonstrated that gingivitis is already present during the early years of life.[47] Among younger adults, the prevalence of attachment loss ±4 mm ranged from 18-20% (Denmark, China and the USA) to more than 60% (Vietnam, France and Pomerania, Germany). Attachment loss prevalence universally increases with age.[21] Gingivitis is common in children and adolescents. Its prevalence, severity and extent increase with age until puberty, followed by a mild decline in adolescence.[29] Though the loss of periodontal support is difficult to measure in deciduous dentition due to

exfoliation and eruption of teeth, very limited data suggest it to be approximately 5% in children of European origin.[29] Over the last few decades, a reduction of gingivitis was observed in children and adolescents, predominantly from industrialised nations, which has been associated with improved oral hygiene. Whereas in other populations gingivitis levels have increased, which could be mainly attributed to poor oral hygiene.

2.2 Quality of dental research

A lot of above mentioned epidemiological information is obtained through various prestigious research conducted worldwide. Several studies have indicated the importance of study design and biostatistics in medical research.[60] The number of publications addressing dental research has increased significantly in the last two decades. However, the quality of study design and/or statistical methods used in most of the dental research are not of standard.[62] Though statistics forms a crucial part in the field of research, the very thought of this subject is dreadful among medical professionals. There is a common misconception that statistics is difficult and boring, which gives rise to health care professions showing lack of interest in the statistical part of the research. It is also known that the medical fraternity is more concerned about the significant associations in terms of p-value < 0.05 only, ignoring the other aspects of the analysis.[33] Some of the most commonly observed sources of errors in research are:

- Smaller sample size & over-stating the results
- Improper sampling strategy
- Data type errors
- Inappropriate statistical methods
- Management of outliers
- Adjustment of confounders
- Inconsistent reporting

2.2.1 Smaller sample size & over-stating the results

This might be the most common source of error in dental research. Lower sample size is an issue that can be very frequently encountered in dental literature. It has to be accepted that a huge proportion of research is conducted by the graduate and post-graduate students as part of their curriculum requirements. The main drawback students encounter is having limited resources, especially lack of time. For these reasons, most students opt to finalise the study design with considerably lower sample size. The same problem is also present in cases where experimental studies are performed which require specimens of human teeth. With dentistry focusing more towards prevention, it is getting difficult to acquire a significant number of human teeth. In addition to this, researchers rarely consult a statistician during the planning stages to determine the sample size required for studying the effect/association required. It is mandatory that the research team has to consult a statistician and to calculate the proper sample size with which all the hypotheses in the study can be answered without compromising on the power of the study.[17] However, large studies are being performed to bring out more than 1 publication out of it, each with a different hypothesis. Though it is not considered as "salami publishing", researchers should be aware if the study has enough power to carry out second or further publications from same study; especially when using the same dependent variable. Furthermore, it could be observed that researchers in their earlier stages of career are usually highly motivated and tend to overstate their results. A mere positive association would most commonly be interpreted as causation, ignoring other possibilities such as, reverse causation, influence of bias or confounders.

2.2.2 Improper sampling strategy

In addition to the lower sample size, the sampling strategy followed in some studies is inappropriate. Studies presenting skewed prevalence are not uncommon in dentistry; the main reason being non-representative selection of samples (selection bias),[32] in addition to information bias. Care should be taken to avoid generalisation of results to the overall population from such studies. In certain intervention studies, it is commonly observed that the participants are not randomly allocated to the groups.

2.2.3 Data type errors

Though this topic is underrated and least discussed, it is of utmost importance in data analysis. Most of the dental variables such as DMF index, plaque index, gingival index are usually considered as continuous variables by most researchers, even though they belong to the interval/scale data.[32] The main difference between the two types of variables is that the interval/scale data increases at a unit of 1 (ex. DMFT, UniViSS, ICDAS, OHI, GI, etc.), whereas the continuous/ratio data increases without any specific interval (ex. body weight, body height, all lab measurements such as hs-CRP, IL6, etc.). Though it is acceptable to calculate and present mean and standard deviations for normally distributed dental indices,[65] it is incorrect to treat some of these indices as continuous and to perform a linear regression.[32] The main problem encountered is the exponentially high number of *"zeros"* or healthy surfaces. Therefore, it is advisable to either categorise the indices into 2 or more categories and to perform a logistic regression or a zero-inflated Poisson regression analysis.

2.2.4 Inappropriate statistical methods

Another frequently observed sources of errors in dental/medical research is using inappropriate or wrong statistical methods.[32,17] As some researchers do not have access to statisticians, they tend to perform data analysis on their own without proper supervision. This has been possible in the last decades through the development of simplified statistical softwares such as SPSS, where a few clicks instantly produce results and the most sought-after p-values. Using such softwares without underlying statistical knowledge can lead to "Garbage in - Garbage out" phenomenon.[33,32,68] Student's t-test, Chi-square test and ANOVA are the most commonly used tests in dental studies, mainly because of their simplicity. The reason to choose one among these tests is that the previously conducted studies in the same line of research might have used the same methods. In most studies, information on normality of the data distribution is seldom mentioned. While performing Student's t-test, care has to be taken if outcome variable is normally distributed or if any log-transformation of the data is required. When the data cannot be transformed into a normal distribution, appropriate procedures such as non-parametric methods, regression models have to be performed. It is not uncommon to see studies

performing Student's t-test on a data which is not normally distributed. Furthermore, it is worth mentioning in the manuscript whether all the assumptions for any statistical tests were checked.

2.2.5 Management of outliers

As the scope of dental research is increasing, new and interesting hypotheses are being generated which require collection of some biomarkers apart from dental data. Body mass index (BMI), blood glucose level, inflammatory markers such as C-reactive protein (CRP), interleukins (IL) serve to be important variables in some studies. Some biomarkers are measured even orally from either saliva or gingival crevicular fluid (GCF). When measuring such continuous variables, there is always possibility to have extreme outliers, either positive or negative. An outlier is defined as an observation that lies at an abnormal distance from other values in a random sample from a population. This definition leaves the data analyst to decide what is a normal or an abnormal value. Because the outliers contain valuable information about the association under investigation, they should be investigated carefully. It is very important to visually analyse the data using scatter plots or box plots and to remove the highest or the lowest value in the dataset. For some continuous variables, it is possible to have more outliers, which shifts the mean value far away from the reality.[64] In these cases, the data can be treated by some definitions and the outliers can be removed effectively by using the formula:

$$Outlier = Mean_x \pm 4 * Standard\ deviation_x$$

where, x is the variable to be analysed.

This procedure leads to a truncated mean and can be repeated up to 3 times to remove almost all outliers effectively; although this procedure can be best used when the data is expected to fall into a symmetrical distribution. Researchers have to be careful not to use this formula for dental variables such as DMF index, gingivitis index, sulcus bleeding index, etc., as these variables are actually classified as interval data and not continuous as commonly thought so. When the outlier values are clinically practical and require to be included in the data, it is wise to present median and interquartile ranges

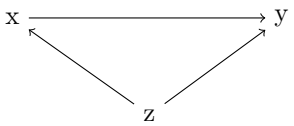
and perform the analysis using non-parametric approach or regression models.

2.2.6 Adjustment of confounders

In dental research, this is one of the most overlooked step. Though the popularity for utilising regression models in dental research is increasing, a vast majority of studies still rely on performing univariate analyses to show associations between two factors. The main problem with univariate analyses is they assume that the association is present only between two variables,[61] i.e.,

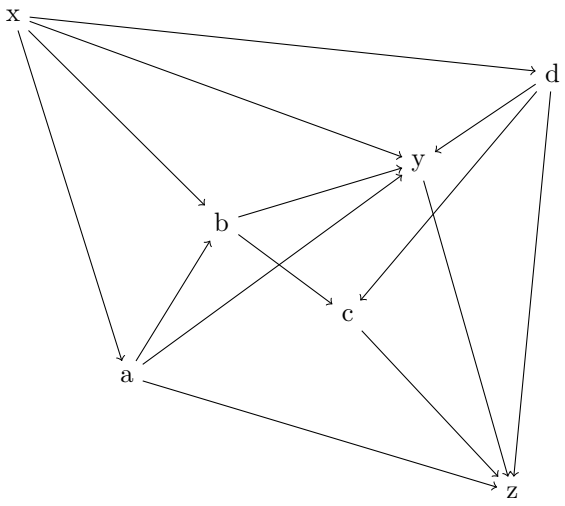
$$x \longrightarrow y$$

But, in reality, this is not the case. There are a number of extraneous factors that influence the relationship between two variables, which are called "confounders".



x: gingivitis y: systemic inflammation z: gender

Or sometimes, the association or causation hypothesis can be very complex such as:



These confounders have to be identified and adjusted while performing the data analysis; which leads to adjusted estimates. The quest for adjustment has to begin during the study design stage itself, as the researcher has to collect data regarding confounding

factors, so they could be adjusted later in the relevant models. However, caution should be exercised to have enough sample size from which adjustment could be made without compromising on the power of the study.

2.2.7 Inconsistent reporting

Though inconsistent reporting style is not a source of error in research, it is a minor factor that reduces the quality of research. From 2003 to 2012, the total number of publications in dental journals have more than doubled;[28] however, quality of reporting needs to be improved in the journal articles. A poor reporting could be prevented by conducting a thorough literature search on the topic of interest and by clearly formulating the findings. Additionally, there are a number of validated reporting guidelines for different types of study designs, such as, CONSORT, STROBE and PRISMA for reporting randomised controlled trials, observational studies, and systematic reviews, respectively. Accurate and complete reporting enables the journal's readers to fully evaluate research, replicate it, and use it;[45] therefore, the researchers have to make use of these checklists to report their research. The Enhancing the QUAlity and Transparency Of health Research (EQUATOR) network (`http://www.equator-network.org`) maintains an extensive database of all reporting guidelines required for medical research.

2.3 Objectives of the thesis

As a dental epidemiologist, one should conduct research by eliminating/ limiting the number of above discussed errors. Furthermore, it is imperative that a different study design relevant to the topic of interest is used to obtain the best possible information from the situation. For this reason, this thesis discusses the methodology in detail. The main objectives of this thesis were to:

- **Project 1:** Study the association between gingivitis and some lifestyle related factors such as BMI and smoking on systemic inflammatory marker hs-CRP among 10- and 15-year old children and IL6 on 10-year-olds
- **Project 2:** Compare the performance of different workflows/protocols for performing adhesive restorations in primary teeth

- **Project 3:** Study the progression of caries longitudinally in 2- and 3-year-old children from a district in Germany
- **Project 4:** Measure the shearbond strength and microleakage of a newly developed pit and fissure sealant material

Chapter 3

Research Projects

3.1 Project 1

Influence of gingivitis and lifestyle on systemic inflammation in adolescents

3.1.1 Introduction

Though it is well known that poor oral hygiene is the main cause for gingivitis/ periodontitis, various risk factors have been suggested for periodontitis, such as smoking, alcohol consumption, stress, obesity and diabetes. Most of these risk factors are related to lifestyle and may start during adolescence. In spite of being a local infectious disease, periodontitis also influences low-grade systemic inflammation, impacts ageing and increases the risk for diabetes, cardiovascular disease and, in the long run, mortality.[3,63] C-reactive protein (CRP) and interleukin 6 (IL6) are systemic inflammatory biomarkers at an early inflammatory stage.[39,38] Systemic inflammation is known to be triggered by being obese/overweight[14,8,57,31] and smoking.[7,72] Additionally, elevated levels of hs-CRP or IL6 are known to be predictors for cardiovascular diseases in later years of life.[56,19,6] Though there is evidence proving the influence of gingivitis and periodontitis on serum high-sensitivity (hs)-CRP and IL6 levels among adults; there is minimal information suggesting this association in young adults[59] and no information on this relationship in children and adolescents exists. Therefore, this study aimed to assess the influence of gingivitis, measured as sulcus bleeding, along with smoking and body mass index (BMI), on the serum levels of hs-CRP at the ages of 10 and 15 years and of IL6 at the age of 10 years.

3.1.2 Materials & Methods

Study population

The study sample came from the Munich centre of the two birth cohorts, GINIplus and LISAplus. After recruitment, the new-borns were followed-up at multiple time points. With respect to this study, we used a cross-sectional design that was repeated on the subjects from Munich centre at two time points (10 and 15 years after birth); during which the clinical and dental examinations were conducted. The two follow-ups were

synonymous with participants age. Children with severe acquired or congenital diseases, a birth weight < 2500 g, a gestation of < 37 weeks, or parents who were unable to complete the questionnaire were excluded from this study. The final analysis sample consisted of 806 and 846 subjects at 10- and 15-year follow-ups, respectively. The study had approximately 1:1 male to female ratio throughout the study period.

Questionnaire & clinical examination

All participants and their parents were given questionnaires to complete, from which data regarding the intake of any drugs, history of infections before clinical examination/blood withdrawal, pubertal status, smoking status and oral hygiene were obtained. All the subjects were clinically examined in each follow-up for body weight (kg) and body height (cm), from which BMI was calculated. Age- and gender-standardised BMI z-scores were calculated from the reference values for German children [34] and categorised into: underweight ($< 10^{\text{th}}$ percentile); normal weight ($\geq 10^{\text{th}}$ to $< 90^{\text{th}}$ percentile); and overweight/obese ($\geq 90^{\text{th}}$ percentile). At 10-year follow-up, pubertal onset information was obtained from parents by asking if they could notice any visible signs of puberty in their children and the answer obtained was binomial (Yes/No). In the 15-year follow-up, pubertal status was obtained from a self-rated pubertal development scale and classified into: i) pre-pubertal, ii) early pubertal, iii) mid-pubertal, iv) late pubertal and v) post-pubertal.[15] In the 15-year follow-up, participants were asked if they smoked and if so, the number of cigarettes smoked per week was obtained.

Serum hs-CRP levels were measured at both 10- and 15-years, whereas IL6 levels were measured only at the 10-year follow-up. The lower limit of detection (LOD) for hs-CRP and IL6 were 0.15 mg/L and 3.0 pg/L, respectively. Levels lesser than the LOD were set to 1/2 of the LOD.

Dental examinations were conducted during 10- and 15-year follow-ups. Two calibrated dentists working at the University hospital served as examiners in each follow-up. Different examiners performed the 10-year (JK, DH) and 15-year (YM, IM, KR) follow-up investigations. The dentists participating in the study were calibrated 2 weeks before the start of each follow-up examination schedule. The intra-rater and inter-rater reliability values ranged from 0.72 to 0.91 and 0.71 to 0.84, respectively. Participants brushed their teeth prior to the dental examination. Sulcus bleeding was measured using a

blunt CPI-probe (CP-11.5B6, Hu-Friedy, Chicago, IL, USA) and a dental mirror and scored according to Community Periodontal Index (CPI) criteria.[2] Using the CPI scores, binomial decisions were made for each sextant depending on whether sulcus bleeding was present, and the number of bleeding sextants (i.e., the s-SBI) was summed from 0 (no bleeding sextant) to 6 (all sextants affected).

Statistical analyses

All analyses were performed using R, version 3.2.1 (R Core Team, 2015). The outliers, which were defined as more than 4 times the standard deviation of the mean hs-CRP or IL6 levels were removed from the analysis of samples from both follow-ups. Other data cleaning steps could be seen from the attached publication *"(Figure 1 from the published pdf)"*. As the hs-CRP distribution was skewed and there was no linear relationship between gingivitis severity and hs-CRP values, smoother plots were constructed using the mean hs-CRP values at different cut-offs ranging from the 5^{th} to 95^{th} percentile for the 10- and 15-year follow-ups *"(Figure 2 from the published pdf)"*. A trend of association was observed between gingivitis and hs-CRP near the 85^{th} percentile; thus, this was used as the hs-CRP level cut-off, and the subjects were categorised binomially as 0 ($< 85^{\text{th}}$ percentile) and 1 ($\geq 85^{\text{th}}$ percentile). Similarly, the same cut-off was used for IL6 because it was the minimum cut-off at which a binomial distribution was present. The gingivitis scores were also categorised binomially (healthy and affected) as well into groups: 0 (healthy), 1 & 2 (mild), 3 & 4 (moderate), 5 & 6 (severe). A simple logistic regression was performed for hs-CRP and IL6 outcomes (85^{th} percentile cut-off) with gingivitis as an independent variable. Further, multiple logistic regression model was performed adjusting for study cohort, gender, presence of any chronic infections, parental education and BMI at 10-year follow-up. Additionally, oral hygiene and smoking status were also included as covariates in the 15-year follow-up. The results were presented as un-adjusted (OR), adjusted odds ratios (aOR) and their corresponding 95% confidence intervals *"(Table 1 & 2 from the published pdf)"*.

As part of the sensitivity analyses, longitudinal analysis of the participants present in both the follow-ups (N: 333) was performed using a generalised estimating equation (GEE) model. Furthermore, the time of blood collection and participants fasting status were analysed to assess their potential influence on hs-CRP values.

3.1.3 Results & Conclusions

57.2% of participants were affected by gingivitis in 10-year follow-up; whereas this proportion reduced to 22.7% in 15-year follow-up. The hs-CRP values ranged from 0.03 to 19.64 mg/L (mean: 0.59, sd: 1.50) and 0.15 to 8.42 mg/L (mean: 0.64, sd: 0.89) at 10- and 15-year follow-up, respectively. In 10-year follow-up, IL6 values ranged from 1.50 to 21.91 pg/L (mean: 1.84, sd: 1.39). Gingivitis was not associated with hs-CRP in 10-year olds, whereas gender and BMI played a significant role. 15-year-old subjects with gingivitis (aOR: 2.17) were associated with elevated hs-CRP levels. Additionally, daily smoking (aOR: 6.27) and being overweight/obese (aOR: 4.95) were significantly associated with elevated hs-CRP levels, and the observed effect was linear. Gender did not play a role in influencing hs-CRP in 15-year follow-up; whereas girls (aOR: 1.95) were significantly associated with higher hs-CRP levels at the 10-year follow-up. Neither gingivitis nor any of the included covariates had any effect on IL6 values.

This study investigated the associations between gingival health and lifestyle factors in relation to the systemic inflammatory blood markers hs-CRP and IL6 in adolescents. Within the limitations of our study, there was a significant positive association between hs-CRP and gingivitis, daily smoking, and overweight/obesity. Future studies on adolescents should address confirming the causal relationship between these factors.

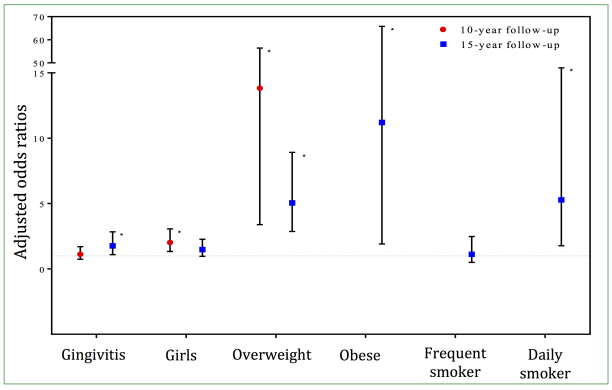


Figure 3.1: Association between significant lifestyle risk factors and hs-CRP represented as aOR and 95% CI adjusted for corresponding factors at 10- & 15-year follow-up

3.2 Project 2

Comparison of different workflows/protocols for performing adhesive restorations in primary teeth

3.2.1 Introduction

In the past couple of decades, adhesively bonded and tooth-coloured composite materials have become much more popular in dental practices,[13,42,46] and such materials are being constantly improved with better properties.[22,24,49] In addition to having lack of cooperation from children, restoring a tooth with composite material is technique sensitive.[40,41,66] The (paediatric) dentist often has to perform the restoration in a shorter time period. There are different self-etching adhesives and light-polymerising materials may help reduce the treatment time. Many studies have investigated the survival rates of individual materials such as different adhesives[53,58] and composite materials.[9–11,18,27,48] However, the clinical workflow/protocol is a crucial factor that might influence the longevity of the restoration. This provides essential information about the clinical usefulness of different strategies. Therefore, this study explored the survival of the most frequently used protocols in our department using retrospectively collected data from a university-based paediatric treatment setting. The null hypothesis was that no differences existed between the different protocols on the survival of composite resin restorations in primary teeth.

3.2.2 Materials & Methods

Study population

Dental records of patients who received a minimum of one direct adhesive restoration under local anaesthesia in the department between January 2004 and December 2012 and attended at least one recall appointment were included in the study. Restorations performed under general anaesthesia were excluded. The units of observation in this study were restorations, as some patients had multiple restorations. The endpoint measured in the study was loss of restoration. The study began with 676 patients (360 males and 316 females) and 2417 restorations. Seventy-five patients (271 restorations) were

removed from the database because they received restorations with less frequently used materials resulting in a final sample size of 601 patients (319 males and 282 females) with a mean age of 6.6 years (range: 1-13 years). A total of 2146 restorations were analysed with an average of 3.6 restorations per patient (range: 1-20). Of these restorations, 1778 remained intact, 192 were lost due to secondary caries, and 176 were lost for other reasons.

Restoration procedure

A total of six operators performed the restorations during the study period. Initial caries lesions were diagnosed via a visual examination and bitewing radiographs were performed in unambiguous cases. After preparation of the cavity on the tooth, defect-oriented carious dentine excavation was performed. A calcium hydroxide liner was indicated in deep and active carious lesions. Endodontic therapy was performed (N: 40) if pulp perforations were present. In proximal preparations, a retainer-less matrix system (AutoMatrix, Dentsply De Trey, York, PA, USA), along with wooden wedges, was used. Two different adhesive procedures were applied: 1) a self-etch adhesive (Clearfil SE Bond, Kuraray Noritake Dental Inc., Japan) or 2) an etch-and-rinse technique with phosphoric acid (Total Etch, Ivoclar Vivadent, Shaan, Liechtenstein, application time $\sim$ 10 seconds) combined with a total-bond adhesive (Syntac Classic, Ivoclar Vivadent, Shaan, Liechtenstein). The composite resin was mainly chosen according to the operators preferences and included: 1) a flowable hybrid composite resin (Tetric EvoFlow, Ivoclar Vivadent, Schaan, Liechtenstein), 2) a traditional hybrid composite resin (Tetric EvoCeram, Ivoclar Vivadent, Schaan, Liechtenstein), or 3) a combination of both, as described earlier.[12] All increments were polymerised for 20 seconds each, and the restoration was shaped and polished.

Recall and data acquisition

All patients treated with restorations were offered quarterly preventive recall examinations. During the recall examinations, all teeth and restorations were carefully checked by calibrated dentists in the dental diagnosis section. The restorations were followed until failure, if any, and the reasons for restoration loss were documented, which provided information about the survival time for each restoration. In case patients had multiple

restorations, they were recorded as different observations and were identified with the patient ID. Each restoration was treated as an observation and a unique observation ID was provided. The clinical restoration workflow/protocol was obtained from the patient records. The following variables were specifically considered for the clinical protocol: 1) the adhesive strategy, self-etch or etch-and-rinse, 2) the type of dentine/enamel adhesive used, and 3) the composite resin material. Using these possibilities, six frequent protocol patterns were identified: i) self-etch adhesive + traditional hybrid composite resin (ST), ii) self-etch adhesive + flowable hybrid composite resin (SF), iii) self-etch adhesive + flowable- and traditional hybrid composite resin (SFT), iv) etch-and-rinse + adhesive + traditional hybrid composite resin (ERT), v) etch-and-rinse + adhesive + flowable hybrid composite resin (ERF), and vi) etch-and-rinse + adhesive + flowable- and traditional hybrid composite resin (ERFT). Apart from the protocol variables, information about possible confounders such as, gender (male and female), age (0-3 years, 4-5 years, 6-7 years, 8-9 years and 10-13 years), type of tooth (incisors, canines and molars), lesion size (1, 2, 3 and $\geq$ 4 surfaces) and operator (6 operators) were collected and included in the dataset.

Statistical analyses

Data were entered into a spreadsheet programme (Excel 2010, Microsoft Corporation, Redmond, WA, USA) and exploratory data analyses were performed using SPSS Statistics for Windows, Version 21.0.1 (SPSS Inc., an IBM Company, Armonk, NY, USA). Restorations that were lost due to other reasons, such as exfoliations, extractions or endodontic treatment, were censored. Annual failure rates for all the protocols of performing composite restorations were calculated *"(Table 2 from the published pdf)"*. Kaplan-Meier curves were plotted for the different composite protocols *"(Figure 1 from the published pdf)"*. A step-wise Cox regression using backward elimination model was performed to determine whether differences existed between the different restoration workflows using the survival time in years as the time variable and loss of restoration as the outcome variable. The model was adjusted for the gender, age, tooth type, lesion size and operator. The hazard ratios for each variable were obtained from this analysis. For all analyses, a 95% confidence level and a two-tailed significance level of 0.05 were used. The overall model resulted in a p-value of <0.01.

3.2.3 Results & Conclusions

Of all the restorations retrospectively observed, 17.1% restorations failed within the observation period. Secondary caries was the most common cause of failure (52.2%), succeeded by total restoration loss (20%) and loss of retention (6%). Overall, 7.5% of restorations were lost in the first year. The most frequently used workflow was the ERF group, followed by the ERFT and SF groups *"(Table 2 from the published pdf)"*. The lowest hazard ratios were found for the ST (1.00), SFT (1.16), and SF (1.76) groups; these were followed by higher hazard workflows for the ERFT (1.80), ERT (2.35), and ERF (2.83) groups, in ascending order. There was no significant difference in the HR between females (1.00) and males (1.04). It was observed that larger lesions and younger age group (0-3 years) had higher risk towards restoration failure. *"Figure 1 from the published pdf"* shows the survival graphs for all the clinical workflows, where the self-etch groups exhibited better performance.

This study underlines the fact that analysis of clinical workflows is equally important as the individual materials or steps for determining the survival of a restoration. In our study, workflows employing self-etch adhesives combined with traditional hybrid composites had higher probabilities of survival. It was also noted that workflows with flowable composites had comparatively higher risks of failure.

3.3 Project 3

Longitudinal study of caries progression in 2- and 3-year-old children

3.3.1 Introduction

Early childhood caries (ECC) is defined as the presence of either a cavitated or non-cavitated carious lesion or missing/filled tooth surfaces on a primary tooth in a child <72 months of age.[5] On one hand, there is a reduction in caries prevalence and experience in industrialised nations;[43] and on the other hand, the burden of caries is concentrated in high-risk groups.[51] Many studies have identified the risk factors for caries, which highlight the multi-factorial nature of caries aetiology. Longitudinal studies were also performed to monitor caries preventive interventions, but predominantly in adults and children. To our knowledge, only limited longitudinal data on caries progression are available in pre-school children. Therefore, this prospectively designed longitudinal cohort study investigated the progression/regression rates of different types of carious lesions in 2- and 3-year-old kindergarten children over a 2-year observation period. Our hypothesis was that carious lesions at varying stages of progression exhibit different probabilities of either changing or remaining unchanged.

3.3.2 Materials & Methods

Population & study design

This study was a prospective longitudinal observational study in children attending kindergarten in the Kyffhäuser district, which is part of the German federal state Thuringia. The residents of this district were characterised in 2008 by a high unemployment rate (18.8%) and low socio-economic background. Only two-thirds of this age group attends kindergarten. Twelve kindergartens from seven towns agreed to participate and were included in this study. These towns provided a total of 467 children who were eligible to participate. An invitation letter was given to all parents of children who attended the participating kindergartens requesting their childrens participation in three follow-ups of annual dental examinations. A total of 400 healthy male and female 2-3year-old children

with American Society of Anaesthesiologists (ASA) status 1 whose parents consented to participate in this observational study and signed an informed consent were included. Additional information on study design and protocol were published elsewhere.[54] The children were divided into two groups: the fluoride group, which received a biannual application of a newly developed fluoride varnish, and the control group. The allocation of children to either group was not randomised, and the selection was made exclusively by the parents. These children were re-evaluated at 12 and 24 months after the baseline establishment by dentists who visited the participating kindergartens. Although the study sample was from a non-randomised trial on the effectiveness of fluoride varnish, the analysis sample consisted of an almost 1:1 fluoride to control group ratio (159:161) at both the baseline and the 2-year examinations.

Calibration of the study team

A 3-day training on the scoring of carious lesions according to WHO, ICDAS/UniViSS criteria was performed before the study began and before the 2-year follow-up exam. Training was held for the examiner (J.A.) under the supervision of experienced dentists (J.K. and R.H-W.). The kappa values at baseline for the inter-examiner and intra-examiner reliability were 0.78 and 0.86, respectively, and for 2-year follow-up they were, 0.84 and 0.88, respectively.

Clinical dental examination

Before their dental examination, children brushed their teeth using fluoride toothpaste under the supervision of the kindergarten staff. A calibrated dentist (J.A.) with 20 years of professional experience examined each child in the kindergarten at a previously made appointment, using standard equipment, such as dental mirror with a flat surface, a blunt CPI probe (CP-11.5B6, Hu-Friedy, Chicago, IL, USA) and a halogen lamp (Ri-Magic, Rudolf Riester GmbH, Jungingen, Germany). Cavitated carious status was measured at the surface level using the dmf index for primary dentition according to WHO standards.[71] Non-cavitated carious lesions were scored using criteria defined by ICDAS[55] and UniViSS.[36] The teeth were dried using cotton rolls, and no bitewing radiographs were taken for caries detection.

Statistical analyses

Descriptive and exploratory data analyses were performed using SPSS Statistics for Windows, Version 19.0.1 (SPSS Inc., an IBM Company, Chicago, IL, USA). As the data did not meet the conditions of normality, descriptive parameters of caries under WHO and ICDAS/UniViSS criteria were analysed between baseline and 1-year, baseline and 2-years, and 1-year and 2-years using the Wilcoxon-Signed-Rank-Sum test.

Among all the children present in both baseline and 2-year follow-up, all the surfaces of the teeth were followed up to observe if the caries lesions progress, reverse or remains same. Based on this principle, a categorical variable was created which consisted of 3 levels:

- Progression: When a carious lesion progressed to an advanced stage
- Reversal: When a carious lesion regressed one or more stages backward
- Unchanged: When a lesion did not progress or reverse but remained the same

As the proportion of reversals observed in the study were too low, this variable was re-coded as 0: Unchanged; 1: Change (either progression/reversal).

Because the 1-year data was similar to the baseline data, a regression analysis was performed only between the baseline and 2-year data to identify any significant changes in the behaviour of carious lesions. Behaviour of caries between baseline and 2-year examinations were analysed using a linear regression analysis employing a mixed-effects model with unbalanced design. The outcome variable for the regression model was any difference in the number of each type of carious lesions on different surfaces. The numbers of each lesion type on all surfaces at baseline and 2-year examination were added, and their difference was calculated. No change was scored as 0, and increases or decreases were scored as positive or negative difference scores, respectively. A difference in the number of lesions between baseline and 2-years was modelled using the type of lesion as the fixed effect. Because the patient ID was selected as a random effect, this mixed-effects model took into consideration the number of carious lesions per patient and modified the model according to the patient ID, resulting in adjusted estimates. Adjustments were made for gender, age, quadrant, and type of teeth. Because the analysis sample had approximately equal numbers of participants in the fluoride and

control groups, the observed effect was also adjusted for fluoride varnish usage. Because the difference in the number of teeth between baseline and the 2-year follow-up was minimal and statistically insignificant, we did not include it in the model as a factor for adjustment. The analysis was stratified for occlusal, proximal and smooth surfaces.

3.3.3 Results & Conclusions

The study began with a sample size of 400 children (195 girls and 205 boys), but some participants were not present in one or more examinations. To ensure a homogeneous sample and study the longitudinal caries progression, final data analyses comprised of children who attended both the baseline and 2-year examinations (n: 320; 155 girls and 165 boys). Caries prevalence at baseline according to WHO and ICDAS/UniViSS criteria were 16.6% and 52.5%, respectively. There was an increase of approximately 0.3% and 12.8% in overall caries prevalence over the 1-year and 2-year periods, respectively. Though the mean number of cavitated lesions after 2 years was comparable to the baseline, the number of restorations (f-component) and extractions (m-component) had increased. *"Table 2 from the published pdf"* lists the adjusted estimates from linear regression analysis using mixed effects model. In general, smooth surface lesions remained stable/unchanged than occlusal or proximal caries lesions in primary teeth. Non-cavitated caries lesions exhibited the greatest chance for change. Lesions with first visible signs on occlusal surfaces had the highest chance for change (0.38), and established lesions on proximal (1.05) and smooth surfaces (0.62) revealed the highest adjusted estimates. Proximal lesions showed a significant trend for change over the study period; the percentage of lesions that remained stable decreased as the hierarchy of lesions increased, but the trend to treat the lesions increased *("Figure 2 from the published pdf").* Established lesions were the most prone to reversal on all surfaces.

This study concludes that different types of carious lesions progress/regress at different rates, while some lesions remained stable. This information is crucial for dental practitioners during the decision-making process. Non-cavitated caries lesions were more prevalent in the 2-year observational period, and the majority of these lesions were observed on smooth surfaces. The probability of a carious lesion to change or receive treatment increased as the lesion severity increased.

3.4 Project 4

Shear bond strength and microleakage of a new pit and fissure sealant

3.4.1 Introduction

Preventive pit and fissure sealing is clinically recommended in young patients with caries activity[1] as caries occurs frequently in pits and fissures due to their anatomical characteristics and plaque-retentive nature.[35,26] Various sealing materials/procedures have been proposed for clinical use over the decades. As a measure of performance, the retention rates of resin-based materials are superior to those of other sealants, e.g., glass-ionomer cements.[1,67,70] Clinical usage requires simple and less-time consuming procedures such as, self-etching adhesives, particularly in children. Most recently, a new product (BeautiSealant; Shofu Inc., Kyoto, Japan) featuring reduced working time and fluoride-releasing and pH-buffering properties was released on the dental market.[20,69,30] As new materials are developed, stringent testing of such materials is imperative before formulating clinical recommendations. Shear bond strength (SBS) and microleakage assessment are frequently used to evaluate sealant materials in the laboratory.[16] No study has evaluated the adhesion properties of this new material. Therefore, this study aimed to study the SBS and microleakage of the new sealant in relation to acid etching. The null hypothesis of this study was that there is no difference in SBS and microleakage between the tested material and the conventional pit and fissure sealant, which served as a control group.

3.4.2 Materials & Methods

Specimen selection

This study follows an in-vitro testing design. 60 healthy, caries-free extracted human third molars that were free of development disorders, fillings, and fissure sealants and showed complete root development were used. The teeth were stored in sodium azide solution (0.2%) and all teeth were cleaned off gross debris and air-dried before using in this study. All roots were sectioned off 1 mm apical to the cemento-enamel junction, and the crowns

were sectioned into 4 surfaces (mesial, distal, lingual, and buccal) with a diamond disc (Dental Diamond Disc, H 340-F-300, HORICO, Berlin, Germany), resulting in 240 tooth surfaces. All tooth surfaces were embedded in cold-curing methyl methacrylate resin (Technovit 4004, Heraeus Kulzer GmbH, Wehrheim, Germany). Each tooth surface was strictly aligned horizontally in the embedding material. After embedding, all tooth surfaces were cleaned and rinsed with water spray. An unique randomisation table was charted for this study and all specimens were numbered according to the randomisation table. Only unprepared specimens (prismless enamel) were used in this study to simulate the clinical situation of fissure sealing. Specimens were randomly assigned to each group (n: 120) and stored in distilled water.

Experimental group (EG): Self-etch adhesive prior sealant application The application of the sealant material strictly followed the clinical recommendations of the manufacturer. The tooth surface was rinsed with water spray and dried with water- and oil-free air. The self-etching adhesive (BeautiSealant Primer, Shofu Inc., Kyoto, Japan) was brushed onto the tooth surface with an applicator tip and allowed to set for 5 seconds. Subsequently, the adhesive was dried thoroughly into a thin, smooth and glossy film using a gentle stream of air. A cylindrical plastic mould (Button Mould Insert, ISO 29022, Ultradent Products, South Jordan, UT, USA) in a bonding clamp (ISO 29022, Ultradent Products, South Jordan, UT, USA) was placed gap-free on a flat area of the tooth surface, and the fissure sealant was applied onto the surface. The fissure sealant was applied in the mould and light-cured for 20 seconds with a polymerization light (Bluephase, 1,200 mW/cm^2, wavelength 385515 nm, Ivoclar Vivadent, Schaan, Liechtenstein). The plastic mould and resin barrier were carefully removed. The final sealant cylinder on the tooth surface was 2.38 mm in diameter, equal to the standardised diameter of the cylindrical plastic mould required by ISO 2902210).

Control group (CG): Conventional fissure sealing with 30 second acid etching The tooth surface was rinsed with water spray and dried with water- and oil-free air. Next, the enamel surface was etched with 37% phosphoric acid gel (Total Etch, Ivoclar Vivadent, Schaan, Liechtenstein) for 30 seconds. The tooth surface was rinsed with water spray and dried with pressured air for 5 seconds until a chalky-white enamel

surface was visible. The fissure sealing material (Helioseal F, Ivoclar Vivadent, Schaan, Liechtenstein, LOT S03724) was applied through the plastic mould in a layer of 2.38 mm and light-cured as indicated above, resulting in a sealant cylinder on the tooth surface.

Alteration of the specimens

Each group (n: 120) was divided into 3 subgroups (n: 40), based on the alteration of the specimen employed:

- 1-day storage in distilled water at 37°C in a thermal oven
- 3 month storage in distilled water at 37°C in a thermal oven
- 1-day storage in distilled water at 37°C followed by a thermocycling bath between 5 (±2)°C and 55 (±2)°C for 5,000 cycles with a dwell time of 30 seconds and a transfer time of 5 seconds.

Shear bond strength (SBS) testing

A standardized Ultradent-method (Notched-edge SBS test, ISO 29022) was employed to test the SBS in a universal testing machine (MCE 2000ST, Quicktest Prüfpartner GmbH, Langenfeld, Germany) at a crosshead speed of 1 mm/minute. The specimens were aligned in a metal sample holder with the occlusal tooth surface facing down (crown down). The notched-edge shear fixture with the semi-circular moulded shear blade mounted to the universal testing machine and placed over the sealant cylinder on the aligned specimen. The semi-circular moulded shear blade was positioned exactly over the sealant cylinder and force-fitted without premature contact to ensure that the load was applied directly to the sealant cylinder. A constant crosshead speed of 1 mm/minute was applied until the material failed. The maximum force (N) until failure was recorded. The SBS was calculated based on the bonding area of the fissure sealant on the tooth surface and is expressed in MPa.

Failure mode analysis

The failure modes of all specimens were examined using a stereo-microscope with 20-fold magnification. Failures were classified as 1) adhesive failure (complete debonding of

material); 2) cohesive failure within the material; 3) mixed failure (partial adhesive and cohesive within material); and 4) enamel failure. The operator was blinded to the tested group, and the failure mode tests were performed and scored based on the randomisation number of the specimen.

Microleakage testing

Eight human third molars were selected and assigned to each of the 2 groups (EG: 80 sections; CG: 104 sections). All teeth were stored and cleaned as previously described. Each tooth was taken as a whole, and fissure sealing on the prismless enamel of the occlusal fissure pattern was performed in strict accordance with the manufacturers instructions. The specimens and were stored in distilled water at 37°C for 24 hours in a thermal oven and were aged in a thermocycling bath between 5 (±2)°C and 55 (±2)°C for 5,000 cycles, with a 30 second dwell time and a transfer time of 5 seconds. After the root surfaces were isolated with tacky wax, the entire tooth surface was covered with two layers of nail varnish, except the area within 1 mm of the fissure sealing. The specimens were then immersed in 0.5% basic fuchsine solution for 24 hours at 37°C. All specimens were rinsed with water, and the roots were sectioned off 1 mm below the cemento-enamel junction with a diamond disc. The tooth crowns were then fully embedded in cold-curing methyl methacrylate resin, which resulted in a rectangular block of approximately 2.5 x 1.2 x 0.8 cm for each tooth. The blocks were fixed in a sectioning saw with a diamond blade, and the crowns were sectioned (in a buccolingual direction) into at least 5 slices, each with a thickness of 1 mm. The front and back of each slice were inspected using a stereo-microscope, resulting at least 10 available section sides per tooth. Every side was photographed with a digital single-lens reflex camera and methodically separated all sides without dye penetration and then collected all section sides with dye penetration. Additionally, quality losses, such as dye penetration at sealant fractures, detachment of sealant, and defects of the fissure sealant, were recorded. If dye penetration was present, each side was quantitatively measured in relation to the total length of the interface between the enamel and the sealant. All measurements were performed with the imaging software ImageJ (Version 1.47, Wayne Rasband, National Institutes of Health, Bethesda, MD, USA). The percentage of microleakage was calculated. Microleakage was ruled out for dye penetration through enamel, dentine or fissure sealant cracks or along the

cemento-enamel junction.

Statistical analyses

A formal sample size calculation was performed, which resulted in a sample size of 40 specimens per group (10 pieces each from mesial, buccal, lingual and distal). To avoid the influence of some teeth on the results, we randomised the pieces such that no piece of a tooth was repeated more than once in a group. The descriptive and exploratory data analyses were performed using R, version 3.3.1 (R Core Team, 2016). Because the data were not normally distributed, pair-wise comparisons with respect to the material and the technique employed were performed using the Mann-Whitney U-Test. Furthermore, multiple linear regression was performed to study the influence of different factors such as material (EG and CG), alteration technique (1-day water storage, 3-month water storage and 5,000x thermocycling) and tooth surface (mesial, buccal, lingual and distal) on the SBS. The adjusted estimates, their standard errors and significance values are presented in Table 2. A two-tailed significance level of 0.05 and a 95% confidence level were used for all analyses. Another linear regression model was performed including an interaction term between the material and alteration technique; but the results were not significant and thus are not reported.

3.4.3 Results & Conclusions

The SBS decreased slightly in both groups after 3-month water storage and significantly after 5,000 cycles of thermocycling. The SBS in the EG was significantly lower and ranged between 3.2 MPa and 4.6 MPa, whereas in the CG, it ranged from 15.6 MPa to 19.1 MPa. The results from multiple linear regression models showing factors influencing the SBS were shown in *"Table 2 from the published pdf"*. The mean SBS estimate was 3.9 MPa for the EG but 17.5 MPa for the CG. The linear model indicated that 3-month water storage had no significant deteriorating effect compared to that of 1-day water storage. However, 5,000 cycles of thermocycling significantly altered the SBS by -2.5 MPa on average. Further, from failure mode analysis, it was observed that adhesive failure was the only cause of failure in the EG and the most predominant failure type in the CG (77.5% to 95%), followed by mixed failures (5% to 22.5%). The EG did

not fare well in the microleakage testing as well. EG (12.8%) had significantly higher microleakage than the CG (1.1%). The highest amount of microleakage observed was 95.9% in the EG but 36.1% in the CG.

The tested self-etch adhesive pit and fissure sealant had roughly four-fold lower SBS, and the microleakage was ten-fold higher compared to that of the control group. Reduced SBS coupled with increased microleakage, as observed in this study, is an indicator that the performance of a fissure sealant is doubtful. Within the limitations of this study, the tested material failed in the physical characteristics to that of the control group, and thus its usage in clinical practice cannot be fully recommended.

Chapter 4

Discussion

From the research projects that were performed during this PhD, the importance of choosing the right methodology for obtaining optimum results through research is clearly evident. Though there are various ways a good research can be developed, in this thesis we would like to classify them broadly under 2 stages:

- Planning stage
- Analytic stage

4.1 During planning stage

Research can be improved in its study design stage through the following means.

4.1.1 Extensive literature search

An elaborate research over the existing literature was performed before initiating to write the manuscripts. Our literature research consisted of forming well constructed search terms and to use them over different databases. Grey literature from the references section of relevant publications were not neglected. From the obtained literature, a clear line of argument was developed and care was taken to present both sides of the discussion and to weigh the contradictory arguments equally.

4.1.2 Choosing appropriate study design

Study design

In the research projects included in this thesis, each topic consisted of different study design, such as:

- Project 1: Cross-sectional analysis of data from prospective cohort study
- Project 2: Retrospective study
- Project 3: Longitudinal observation study
- Project 4: In-vitro material testing

Following good scientific practices, we took care to select the optimal study design that suits better in investigating their hypothesis. It has to be noted that certain study designs

such as cross-sectional observational study can only be used to measure associations, but not causation; therefore, selecting a right study design is critical.

Sampling and randomisation

Random sampling is critical to avoid sampling bias of the results. In the first project, a huge population-based cohort study from Munich centre was the basis of the topic. In the third project, we had analysed the longitudinal progression of caries among 2- and 3-year old German children. The data for this topic came from another study evaluating the effectiveness of a new fluoride varnish. As the study participants were of younger age, informed consent was obtained from their parents and they decided if their child receives the fluoride varnish or to be part of the control group. In this situation, randomisation was not possible. However, in the fourth project, we created a randomisation table to allocate the specimens of teeth so that no part of tooth is repeated twice in a group. This was done to avoid the influence of one tooth on the group.

Clinical examination

In the above mentioned research projects, appropriate and up-to-date methods of clinical examination or investigations were employed. For example, measurement of serum hs-CRP using high-sensitivity assay; inclusion of non-cavitated caries lesions in addition to the WHO criteria for caries assessment; and determination of SBS using notch-edge shear technique. It has been demonstrated how WHO criteria under estimated the caries experience, thereby supporting the inclusion of non-cavitated carious lesions.[54] However, it has to be noted that comparisons between studies can be made only when same methodology is used for measuring the outcome. For example, periodontitis could be measured by probing depths or bitewing radiography studies. Furthermore, the probe used to measure the probing depths could also be different between studies. It is researchers' duty to follow the standardised methods and to compare the studies using same methodologies.

4.1.3 Collect required information

All the four projects were designed with a lot of planning in its early stages. Only with this intention, a lot of variables considered to be confounders were collected or measured in each of these studies, which were later used in the analysis for adjustment. We also faced one such drawback in the study analysing the association between gingivitis and hs-CRP. As this study was planned many years ago, it was assumed at that time that surface level plaque, calculus, bleeding on probing and gingivitis collection is extensive and time consuming and just sextant based information was collected for these measures. However this drawback was discussed in the manuscript. Collecting wide information costs more time and funding. In such prospective studies extending to two decades, there is a possibility to have the methods used in first follow-up to become obsolete for final follow-up. Therefore, researchers must weigh the importance of variables and their reliability in future well in advance and to obtain them in the most efficient way.

4.2 During analytic stage

Research could also be improved to a certain extent after it has crossed the design and implementation stages. Based on our experience, below mentioned procedures could help improve the research.

4.2.1 Effective cleaning of data

In all the projects, we followed strict data management protocols to eliminate/minimise the data errors, implausibilities and missing data. Data errors and implausibilities were always checked by visualising the distributions of variables and identifying the outliers. Patient records or case report forms were checked for the correct information in case the errors were mere "typos". If the records had the same error, the data point in question was treated as missing. All plausible outliers were either eliminated or the variables were categorised into binary or categorical variable. Imputation of missing data was not performed in any of these projects. Missing information was treated as it is and were excluded analysis-wise and not case-wise. The missing information of all variables were made transparent in the results sections of each publication.

4.2.2 Appropriate statistical methods

Choosing the wrong statistical method is not just unethical, it can also have serious clinical consequences.[4,23] Appropriate statistical testing methods were used for all projects. All the statistical work was supervised by a qualified statistician. Univariate analyses were performed, but all conclusions were not based on the results from these analysis. Regression models were performed where plausible and the associations were adjusted for relevant confounders that were decided a priori. In case of doubt, statisticians from collaborating institutions were consulted to arrive at a better solution.

4.2.3 Effective reporting

The first point of initiating to write a manuscript was to thoroughly read through all the author guidelines. All the manuscripts were written strictly following the guidelines of the journals, both with respect to the content and the format. Relevant reporting guidelines from EQUATOR network were downloaded and the structure was followed to report the research findings. Additionally, the checklist of the reporting guideline was filled-in and attached as a supplementary material to facilitate the review process. Before finally submitting the manuscript, we also sent the articles to be edited by third-party English editors to improve the quality of the language and the writing style. In case of a manuscript being rejected by a journal, the manuscript was edited in accordance to the next preferred journal's guidelines and resubmitted.

Chapter 5

Summary

Despite dental research has been improving over the decades, the quality of research in this field still requires some improvements. One of the main pitfalls encountered in these studies are the methodology, ranging from insufficient sample size, inadequate data management, inappropriate statistical analyses and less standardised reporting. Following good epidemiological principles in dental research could improve the state of dental research and take it to the next level. For these reasons, this thesis consists of four research projects that were performed during the period of PhD in Oral Sciences, following good scientific practices. In these projects, we emphasised the importance of prevention of dental diseases such as, gingivitis and caries and the following conclusions were made:

- It was observed that local inflammation such as gingivitis in children and lifestyle factors could trigger the systemic inflammatory process, thereby elevating the levels of hs-CRP and IL6, which are considered early biomarkers for cardiovascular diseases at later age. Apart from gingivitis, 15-year olds who were overweight/obese or smoked daily also had elevated levels of hs-CRP.

- With respect to dental materials, we emphasized that analysing the workflow/protocol of performing a composite restoration is equally important as testing a single material/ method. In our study, we observed that the protocol utilising self-etch adhesive system and traditional composite resin had better survival rates in children.

- While observing the caries progression in Kindergarten children, the importance of including non-cavitated caries lesions in epidemiological studies was emphasised. Furthermore, these lesions showed highest probability to progress, while most of the cavitations were restored.

- On testing a newly developed self-etch adhesive based pit and fissure sealant, we observed that the tested material had poor physical characteristics such as shear bond strength and microleakage in comparison to the control group. The results were made transparent and we underlined that stringent testing of materials is important and manufacturers must improve their products before the material is brought on to the market.

Chapter 6

Publication I

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Journal of Clinical Periodontology

Gingivitis and lifestyle influences on high-sensitivity C-reactive protein and interleukin 6 in adolescents

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Abstract

Aim: This cross-sectional study was repeated at two time points and investigated the influence of gingivitis, smoking and body mass index (BMI) on the systemic inflammatory markers high-sensitivity C-reactive protein (hs-CRP) and interleukin 6 (IL6) in 10- and 15-year-olds.

Materials and Methods: The study sample of two birth cohorts, i.e. GINIplus and LISAplus, from the Munich centre consisted of 806 and 846 subjects who were evaluated at 10- and 15-year follow-ups respectively. Children and their parents completed questionnaires on participant-related lifestyle information. Gingivitis was measured at the sextant level using a simplified sulcus-bleeding index. Serum hs-CRP and IL6 levels were obtained from blood samples. Multiple logistic regressions adjusting for lifestyle-related factors and other confounders were performed to assess associations between the specified variables.

Results: There were no associations between gingivitis and the inflammatory markers hs-CRP and IL6 in 10-year-olds. In 15-year-olds, gingivitis (aOR: 2.17; 95% CI: 1.25–3.77); daily smoking (aOR: 6.27; 95% CI: 1.39–28.39); and being overweight/obese (aOR: 4.95; 95% CI: 0.73–33.68) were identified as significantly influencing factors for elevated hs-CRP values. Oral hygiene did not influence hs-CRP.

Conclusion: In this study, hs-CRP was positively associated with gingivitis, smoking daily and overweight/obesity among 15-year-olds.

Key words: body mass index; C-reactive protein; gingivitis; high-sensitivity C-reactive protein; inflammation; interleukin 6; obesity; periodontitis; smoking; sulcus bleeding

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Conflict of interest and sources of funding statement

The authors declare no potential conflicts of interest with respect to the authorship and publication of this article. This includes financial interests and the provision of study materials by the manufacturer for free or at a discount.

The Munich arm of the GINIplus/LISAplus studies was primarily supported by grants from the Federal Ministry for Education, Science, Research and Technology and from Helmholtz Zentrum Munich (formerly GSF). The dental investigations were funded by grants from the German Research Foundation (Deutsche Forschungsgemeinschaft, FKZ KU-2518/1-1, KU-2518/1-2, HE-3294/7-1 and HE-3294/7-2). The GABA GmbH, Lörrach, Germany, supported this study by providing oral health care packages for all participating children as incentives.

Periodontitis is a multifactorial inflammatory disease that affects the supporting structures of teeth (Williams 1990, Pihlstrom et al. 2005). Various risk factors have been suggested for periodontitis, including poor oral hygiene, smoking, consuming alcohol, stress, obesity and diabetes. Most of these risk factors are related to lifestyle and may start during adolescence. Although periodontitis is a local infectious disease, it also influences low-grade systemic inflammation (Ebersole & Cappelli 2000, Craig et al. 2003, Loos 2005). Currently, there is an increased interest in investigating the relationship between systemic inflammation and periodontal disease because elevated low-grade inflammation impacts ageing and increases the risk for diabetes, cardiovascular disease and, in the long run, mortality (Akbaraly et al. 2013, Stringhini et al. 2013). C-reactive protein (CRP) and interleukin 6 (IL6) are systemic inflammatory biomarkers at an early inflammatory stage (Loos et al. 2000, Loos 2005). Among adults, serum high-sensitivity (hs)-CRP and IL6 levels increase in the presence of local inflammatory conditions, such as gingivitis and periodontitis (Prabhu et al. 1996, Tüter et al. 2007, Paraskevas et al. 2008, Megson et al. 2010, Pradeep et al. 2010, Yucel-Lindberg & Båge 2013, Bansal et al. 2014). It is also known that serum (hs-)CRP levels increase with the progression of periodontitis (Pradeep et al. 2010, Bansal et al. 2014) and decrease when periodontal treatment is administered (Kumar et al. 2013, Jayaprakash et al. 2014, Mohan et al. 2014, de Souza et al. 2016). There is minimal information on the influence of periodontitis on the serum levels of (hs-)CRP and IL6 in young adults (Shaddox et al. 2011) and no information on this relationship in children and adolescents. Although periodontitis is a chronic disease that occurs later in life, gingivitis is presumed to precede the clinical manifestation of periodontitis (Hugoson & Norderyd 2008, Hugoson et al. 2008, Pari et al. 2014). Systemic inflammation in terms of (hs-)CRP is also known to be triggered by obesity or being overweight (Bulló et al. 2003, Berg & Scherer 2005, Sabiston et al. 2009, Kaneko et al. 2011) and by smoking (Azizi et al. 2015, Winning et al. 2015). In addition, elevated levels of high-sensitivity C-reactive protein (hs-CRP) or IL6 are known to be predictors for cardiovascular diseases in later years of life (Ridker et al. 2000, Danesh et al. 2004, Aulin et al. 2015). It would be interesting to know if being overweight/obese and smoking increase the levels of hs-CRP and IL6 at younger ages, as this knowledge might help prevent subsequent coronary diseases. Therefore, this study aimed to assess the influence of gingivitis, measured as sulcus bleeding, along with smoking and body mass index (BMI), on the serum levels of hs-CRP at the ages of 10 and 15 and of IL6 at the age of 10 years.

Materials and methods

The GINI-plus and LISA-plus protocols were approved by the ethics committee of the Bavarian General Medical Council, and written consent for the physical and dental examinations was obtained from all participating children and their guardians. The recommendations of the STROBE guidelines for observational studies were applied for reporting (von Elm et al. 2014).

Study population

The study sample was derived from two birth cohorts, i.e. GINIplus and LISAplus. After recruitment, the new-borns were followed up at the ages of 6 months (LISAplus only); 1 year; 18 months (LISAplus only); and 2, 3 (GINIplus only), 4, 6, 10 and 15 years. With respect to this study, we used a cross-sectional design that was repeated on the same subjects at two time points (10 and 15 years after birth); during which the clinical and dental examinations were conducted in the Munich centre. The two follow-ups were synonymous with participants' age. Children born in the Munich centre who had available dental examination data and hs-CRP values were included in this study. Children with severe acquired or congenital diseases, a birth weight <2500 g, a gestation of <37 weeks, or parents who were unable to complete the questionnaire were excluded from this study. Details concerning the study's background, representative recruitment strategy, follow-up schedules, inclusion and exclusion criteria have been described elsewhere (Heinrich et al. 2002, Zutavern et al. 2006, von Berg et al. 2013, Heitmüller et al. 2013, Kühnisch et al. 2014). At the 10-year follow-up, there were a total of 1066 cases (GINIplus: 650; LISAplus: 416), whereas the 15-year data consisted of 1206 cases (GINIplus: 806; LISAplus: 400) in total.

Questionnaire and clinical examination

All participants and their parents were given questionnaires to complete. Data regarding the intake of any drugs, e.g. antibiotics and non-steroidal anti-inflammatories (NSAIDs), history of infections before clinical examination/blood withdrawal, smoking status, pubertal status and oral hygiene were obtained from these questionnaires. The physician asked the children and their parents about the recent infections they had during the past one month or earlier, and categorized them into: (i) within the past week; (ii) within the past 1–2 weeks; (iii) within the past 2–4 weeks; and (iv) 4 weeks or more prior to the visit. Parental education, defined as the maximum paternal and maternal education combined, was obtained and grouped into high (either parent had a university degree), medium (secondary education) or low (primary education only). All subjects were clinically examined at each follow-up for body weight (kg), body height (cm) and vital signs. Age- and gender-standardized BMI *z*-scores were calculated from the reference values for German children (Kromeyer-Hauschild et al. 2001) and categorized into: underweight (<10th percentile); normal weight (≥10th to <90th percentile); and overweight/obese (≥90th percentile). At 10-year follow-up, pubertal onset information was obtained from parents by asking if they could notice any visible signs of puberty (acne or spots, pubic or axillary hair, breast development, menstruation, penis or testicle enlargement, etc.) in their children and the answer obtained was binomial (Yes/No). In the 15-year follow-up, pubertal status was obtained from a self-rated pubertal development scale and classified into: (i)

pre-pubertal, (ii) early pubertal, (iii) mid-pubertal, (iv) late pubertal and (v) post-pubertal (Carskadon & Acebo 1993). Participants were asked if they smoked and if so, the number of cigarettes smoked per week was obtained. In the 15-year follow-up, participants were asked, if they used any other oral hygiene methods in addition to daily toothbrushing, such as oral jets, mouth rinses, dental floss, or inter-dental brushes; but this variable was not included in the regression model.

Determination of hs-CRP and IL6 values

After the clinical examination, the subject's skin was disinfected, and venous blood was collected using a Multifly needle with a multiadapter (21G; Sarstedt, Nümbrecht, Germany) and SERUM-Monovette (7.5 ml; Sarstedt). Serum hs-CRP levels were measured at both the 10- and 15-year follow-ups, while IL6 levels were measured only at the 10-year follow-up. hs-CRP levels were measured using the Roche (Mannheim, Germany) Tina-quant CRP (latex) high-sensitivity assay. The lower limit of detection (LOD) for hs-CRP was 0.15 mg/l. Measurements of IL6 were obtained with the Modular System (Roche). The LOD for IL6 was 3.0 pg/l. Levels lower than the LOD were set to 1/2 of the LOD. Intra- and inter-assay coefficients of variation for the precision of quality controls varied between 3.5% and 4.5% for the concentrations of 206.1 and 35.2 ng/ml respectively.

Dental examination

The dental examination was performed following the clinical examination and blood withdrawal at both 10- and 15-year follow-ups. Two calibrated dentists working at the University hospital served as examiners in each follow-up. Different examiners performed the 10-year (JK, DH) and 15-year (YM, IM, KR) follow-ups. The dentists participating in the study were calibrated 2 weeks before the start of each follow-up examination schedule. Calibration was performed for periodontal examination, (non-) cavitated caries lesions, molar-incisor hypomineralization and fluorosis. The intra-rater and inter-rater reliability values ranged from 0.72 to 0.91 and 0.71 to 0.84 respectively. Participants brushed their teeth prior to the dental examination. The oral cavity was illuminated using a halogen lamp (Ri-Magic, Rudolf Riester GmbH, Jungingen, Germany). The surfaces of the teeth were dried with cotton rolls for improved visibility. Sulcus bleeding was measured using a blunt CPI-probe (CP-11.5B6, Hu-Friedy, Chicago, IL, USA) and a dental mirror and scored according to the CPI system (Ainamo et al. 1982). The probe was gently run along the gingival margin for the length of each sextant and the finding was recorded. Due to the young age of the population, pocket depth and attachment loss were not measured. Binomial decisions were made for each sextant depending on whether sulcus bleeding was present, and the number of bleeding sextants (i.e. the s-SBI) was summed from 0 (no bleeding sextant) to 6 (all sextants affected).

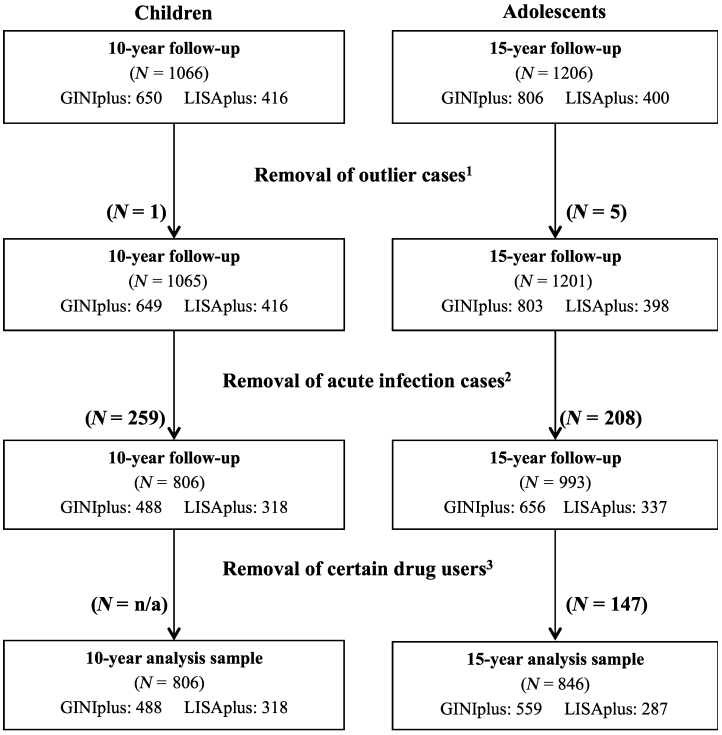
Statistical analyses

The data were entered into an Access database (Microsoft Office, Access 2010®, Unterschleißheim, Germany). All analyses were performed using R, version 3.2.1 (R Core Team, 2015). The outliers, which were defined as more than four times the standard deviation of the mean hs-CRP or IL6 levels, and participants with any infections within the past 1–2 weeks or earlier before the blood sample was drawn were removed from the analysis of samples from both follow-ups. In addition, those participants using NSAIDs, antibiotics or steroids were excluded from the 15-year analysis; this information was not available in the 10-year follow-up group. The samples used in the final analysis consisted of 806 and 846 subjects at the 10- and 15-year follow-ups respectively (Fig. 1). Descriptive statistics were calculated for each variable. Gingivitis was categorized binomially as healthy or diseased (1–6 sextants affected), or into four groups: 0 (healthy), 1 & 2 (mild), 3 & 4 (moderate) and 5 & 6 (severe). As the hs-CRP distribution was skewed and there was no linear relationship between gingivitis severity and hs-CRP values, smoother plots were constructed using the mean hs-CRP values at different cut-offs ranging from the 5th to 95th percentile for the 10- and 15-year follow-ups (Fig. 2). A trend of association was observed between gingivitis and hs-CRP near the 85th percentile; thus, this was used as the hs-CRP level cut-off, and the subjects were categorized binomially as 0 (<85th percentile) and 1 (≥85th percentile). Similarly, the same cut-off was used for IL6 because it was the minimum cut-off at which a binomial distribution was present. In addition, the median and interquartile ranges (IQR) were reported for all hs-CRP and IL6 measurements. Multiple logistic regression models were performed and adjusted for study cohort, gender, recent infections in the past 2–4 weeks or later, parental education, age/gender standardized BMI *z*-score categories and pubertal status at the 10-year follow-up. In addition, smoking status and number of cigarettes smoked per week were included in the model for the 15-year follow-up. The factors included in the model were decided a priori. The unadjusted odds ratio (OR), adjusted odds ratio (aOR), 95% confidence interval (95% CI) and their corresponding *p*-values were calculated. All analyses were performed separately for the GINIplus and LISAplus cohorts. Because there were no significant differences between the two cohorts, the datasets were merged together and the models were adjusted for the cohort variable (1: GINIplus; 2: LISAplus). All analyses were performed cross sectionally for the 10- and 15-year follow-ups.

As part of the sensitivity analyses, longitudinal analysis of the participants present in both the follow-ups (N: 333) was performed using a generalized estimating equation (GEE) model and the results are shown in supplementary material. Furthermore, the time of blood collection and participant's fasting status were analysed to assess their potential influence on hs-CRP values (results not shown).

Results

The mean ages of the participants at the 10- and 15-year follow-up visits



[1] Participants with very high hs-CRP values (> 4 times SD) were considered to be outliers and were eliminated from the data analysis.
[2] Participants who suffered from any infections in the 2 weeks prior to the exam were eliminated from the analysis.
[3] Participants taking any medications known to influence hs-CRP values, such as NSAIDs, antibiotics and steroids, were eliminated from the analysis. This information was unavailable at the 10-year follow-up.

Fig. 1. Overview of the flow of study participants at the 10- and 15-year follow-ups.

were 10.2 years (SD: ±0.2) and 15.3 years (SD: ±0.3) respectively (Table 1). The study had an approximately 1:1 male-to-female ratio throughout the study period. 461 (57.2%) and 192 (22.7%) participants were affected by gingivitis at 10- and 15-year follow-up respectively (Table 2). The proportion of overweight/obese children reduced from 9.9% to 8.9% from the 10- to the 15-year follow-up.

With respect to hs-CRP, there were 247 subjects (30.6%) below the LOD at the 10-year follow-up; whereas there were no subjects below LOD in 15-year follow-up. The hs-CRP values ranged from 0.03 to 19.64 mg/l (mean: 0.59, SD: ±1.50) and 0.15 to 8.42 mg/l (mean: 0.64, SD: ±0.89) at 10- and 15-year follow-up respectively. Girls had significantly higher hs-CRP levels than boys at the 10-year follow-up. Regarding IL6, there were 631 subjects (78.3%) below the LOD at the 10-year follow-up, and IL6 values ranged from 1.50 to 21.91 pg/l (mean: 1.84, SD: ±1.39). The time of blood collection and participant's fasting status did not have an influence on the hs-CRP and IL6 values.

The aORs for hs-CRP and IL6 in relation to gingivitis are shown in Table 3. There were no significant associations between hs-CRP and gingivitis at the 10-year follow-up. When the number of sextants affected with gingivitis was stratified, only 1–2 affected sextants were found to be significant (OR: 3.01) in 15-year follow-up; but there was no linear relationship. Neither gingivitis nor any of the included covariates had any effect on IL6 values (Table 3).

Table 4 illustrates the aORs from the multiple logistic regression for hs-CRP, and gingivitis. The 15-year-old subjects with gingivitis (aOR: 2.17) were associated with elevated hs-CRP levels. Furthermore, gender, BMI and smoking were found to be significantly associated with hs-CRP. Girls (aOR: 1.95) were associated with significantly higher hs-CRP levels at the 10-year follow-up, but this effect was not observed at the 15-year follow-up (aOR: 1.53). In 15-year-olds, daily smoking (aOR: 6.27) and being overweight/obese (aOR: 4.95) were significantly associated with elevated hs-CRP levels, and the observed effect was linear.

Discussion

This study assessed the influence of gingivitis, along with known periodontitis predictors, in 10- and 15-year-olds on their serum hs-CRP and IL6 levels. Our main finding suggests that there was an association between elevated hs-CRP values and gingivitis, factors associated with lifestyle, such as smoking daily and overweight/obesity in 15-year-olds. However, gingivitis and its predictors had no effect on IL6 values.

The main strength of our study was that we assessed a sample size of slightly over 800 individuals at 10- and 15-years after birth. Cross-sectional examinations were performed on the same study subjects at the 10- and 15-year follow-ups. This study also recorded the history of any recent infections in the past 1–2 weeks or earlier and any drug intake that could influence hs-CRP values, and positive cases were removed from the analyses (Fig. 1). Smoking status was included in the 15-year follow-up to see whether any associations developed with lifestyle changes. Because all individuals came from a single centre (Munich) with an elevated socio-economic status, it is likely that the study population was quite homogeneous and might not be representative of children or adolescents from other countries. This study had some limitations; for example gingivitis was assessed in a simplified binary manner (i.e. Yes or No) per sextant, and tooth level information was unavailable. In addition, IL6 values were not measured at the 15-year

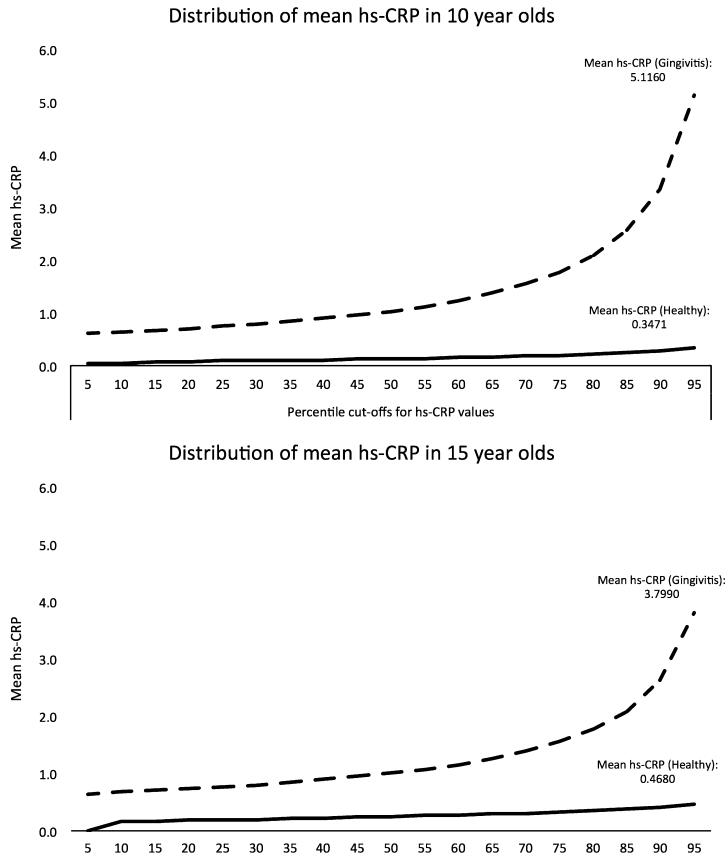


Fig. 2. Distribution of mean high-sensitivity C-reactive protein levels on a scale of various percentile cut-offs and stratified according to gingivitis (n = 61/192) and healthy gingival conditions (n = 345/654) in 10 and 15 year olds.

follow-up for financial reasons. The oral hygiene variable collected in this study was not an accurate measure for determining each individual's oral hygiene. Although carious status might give an estimate on an individual's oral hygiene status, it had no influence on the exposure or the outcome of the study. Therefore, oral hygiene and caries status were not included in logistic regression models. Furthermore, puberty status collected during the two follow-ups was different, which was unavoidable, as the questions asked during each follow-up were not optimal for the other follow-up. One of the main lifestyle-related factors recorded in our study was smoking status, which was available only in 15-year follow-up because it was too early to ask for such sensitive information at the 10-year follow-up. All these factors led to non-uniform confounding variables at each follow-up. In addition to all these limitations, the number of participants who were present in both the follow-ups was low (N: 333). As part of the sensitivity analysis, longitudinal analysis was performed with this reduced sample size by employing a GEE model, adjusting for the uniformly available variables. The results showed that there was no significant interaction with time (Table S1); thus, two cross-sectional analyses were performed for the 10- and 15-year follow-ups.

The proportion of participants with gingivitis at 10- and 15-year follow-ups was 57.2% and 22.7% respectively. The higher prevalence at the 10-year follow-up could be due to the presence of mixed dentition, in addition to active root resorption and bone remodelling in which the exfoliation of primary teeth and the eruption of permanent teeth might affect gingival health, particularly in the posterior sextants. This factor could not be measured and assessed within the limitations of our study, thus might have acted as a confounder. In addition, due to the young age of the participants, brushing skills required to maintain proper oral hygiene may not yet be well developed (Ainamo & Ainamo 1981, Ramberg et al. 1994). It is possible that participants brushing their teeth just before the dental examination might have introduced some bias. This could be especially relevant in the 10-year-olds. To counteract this bias, the participants were given toothbrushing training during the dental examinations, their brushing skills were supervised and mistakes were corrected. Different examiners at each follow-up might be a reason for the difference in the prevalence of gingivitis between the two time points; however, a 34.5% reduction observed might be less plausible among calibrated examiners.

In our analysis, there was no dose or linear relationship between gingivitis and hs-CRP levels, possibly due to the small number of participants with gingivitis affecting multiple sextants. The influence was observed only in the high-risk population, and therefore, the 85th percentile of hs-CRP was considered the best cut-off for determining the outcome of hs-CRP. The participants above this cut-off represented the group at high risk for elevated hs-CRP levels. The time of blood sampling and fasting status of the participant were expected to influence the hs-CRP values. These factors were analysed as part of the sensitivity analyses, and no influences were found. Therefore, these variables were not included in the logistic regression models. From the descriptive statistics, it was observed that girls had increased hs-CRP values in our study, which has been confirmed in the literature (Khera et al. 2005, Lakoski et al. 2006); but this association was non-significant after adjustment (Table 4).

Table 1. Crude associations between potential predictors for hs-CRP at the 10- and 15-year follow-ups, median hs-CRP (in mg/l) and IQR values

Variable	Groups	10-year follow-up			15-year follow-up		
		N (%)	hs-CRP		*N* (%)	hs-CRP	
			Median	IQR		Median	IQR
Gingivitis: Binomial	Healthy	345 (42.8)	0.23	0.37	654 (77.3)	0.36	0.35
	Affected	461 (57.2)	0.21	0.32	192 (22.7)	0.40	0.48
Gingivitis: No. of sextants affected	0	345 (42.8)	0.23	0.37	654 (77.3)	0.36	0.35
	1	101 (12.5)	0.21	0.33	85 (10.0)	0.41	0.20
	2	73 (9.1)	0.19	0.27	16 (1.9)	0.38	0.59
	3	83 (10.3)	0.27	0.56	18 (2.1)	0.35	0.21
	4	93 (11.5)	0.19	0.38	9 (1.1)	0.44	0.79
	5	54 (6.7)	0.21	0.23	10 (1.2)	0.35	0.38
	6	57 (7.1)	0.23	0.34	54 (6.4)	0.37	0.26
Gender	Boys	413 (51.2)	0.19	0.26	423 (50.0)	0.36	0.34
	Girls	393 (48.8)	0.27	0.42	423 (50.0)	0.39	0.42
Recent infection	<2–4 weeks	134 (16.6)	0.25	0.40	117 (13.8)	0.36	0.39
	>4 weeks	644 (79.9)	0.22	0.34	729 (86.2)	0.37	0.37
	Missing	28 (3.5)	–	–	–	–	–
Parental education	High	614 (76.2)	0.23	0.36	674 (79.7)	0.36	0.35
	Medium	148 (18.4)	0.19	0.34	143 (16.9)	0.38	0.40
	Low	43 (5.3)	0.29	0.31	27 (3.2)	0.48	0.44
	Missing	1 (0.1)	–	–	2 (0.2)	–	–
Body mass index (*z*-scores)	Normal	644 (80.0)	0.21	0.30	712 (84.1)	0.36	0.34
	Underweight	80 (9.9)	0.16	0.13	59 (7.0)	0.29	0.20
	Overweight/Obese	80 (9.9)	0.57	0.91	75 (8.9)	0.85	1.17
	Missing	2 (0.2)	–	–	–	–	–
Puberty onset	No	570 (70.7)	0.21	0.31	n/a	n/a	n/a
	Yes	226 (28.0)	0.25	0.48	n/a	n/a	n/a
	Missing	10 (1.3)	–	–	n/a	n/a	n/a
Puberty category scores	Pre-pubertal	n/a	n/a	n/a	1 (0.1)	0.17	0.00
	Early pubertal	n/a	n/a	n/a	15 (1.8)	0.37	0.19
	Mid-pubertal	n/a	n/a	n/a	137 (16.2)	0.31	0.29
	Late pubertal	n/a	n/a	n/a	489 (57.8)	0.36	0.37
	Post-pubertal	n/a	n/a	n/a	70 (8.3)	0.44	0.56
	Missing	n/a	n/a	n/a	134 (15.8)	–	–
Smoked today?	No	n/a	n/a	n/a	828 (97.9)	0.37	0.37
	Yes	n/a	n/a	n/a	18 (2.1)	0.49	0.82
Smoking status	No	n/a	n/a	n/a	728 (86.1)	0.36	0.35
	Infrequent	n/a	n/a	n/a	51 (6.0)	0.40	0.40
	Daily	n/a	n/a	n/a	16 (1.9)	0.61	1.39
	Missing	n/a	n/a	n/a	51 (6.0)	–	–

hs-CRP, high-sensitivity C-reactive protein; IQR, interquartile range.

Another important finding was that daily smokers had higher levels of hs-CRP than infrequent smokers or non-smokers (Tables 1 and 4). While this association has been investigated frequently in adult populations (Azizi et al. 2015, Winning et al. 2015), such studies are rare among adolescents. In the only available study, which was conducted in 13- to 16-year-olds (O'Loughlin et al. 2008), a similar finding was documented. Therefore, the lifestyle factor of smoking seems to have an influence on systemic inflammation. The number of cigarettes smoked per week in our study ranged between 0.5 and 182. Though this range seems to be extreme, the number remained moderate (mean: 18.6; median: 7) and was non-significant on hs-CRP values. One of the reasons that sulcus bleeding was observed in the smokers was perhaps that the number of cigarettes smoked was insufficient to cause the reduced gingival bleeding that is observed in adult smokers (Peruzzo et al. 2016); in addition to the high vascularity due to jaw remodelling. Furthermore, it was noted that the majority of the participants started smoking at the age of 14 or 15.

In our study, overweight/obese individuals had constantly higher hs-CRP levels, which was consistent with other studies (Bulló et al. 2003, Berg & Scherer 2005, Sabiston et al. 2009, Kaneko et al. 2011). Studies on children have also indicated that weight loss leads to a reduction in CRP levels (Garanty-Bogacka et al. 2011, Mustonen et al. 2012, Van Hoorenbeeck et al. 2012, Briggs et al. 2015, Lausten-Thomsen et al. 2015). Similar effect was observed in our study, where the underweight children had reduced probabilities for increased hs-CRP levels at both 10-year (aOR: 0.25) and 15-year follow-up (aOR: 0.48).

To the best of our knowledge, no published clinical investigations that studied lifestyle-related risk factors

Table 2. Crude associations between the exposure (gingivitis) and potential predictors at the 10- and 15-year follow-ups

Variable	Groups	10-year follow-up		15-year follow-up	
		Healthy *N* (%)	Gingivitis *N* (%)	Healthy *N* (%)	Gingivitis *N* (%)
Gender	Boys	165 (20.5)	248 (30.8)	313 (37.0)	110 (13.0)
	Girls	180 (22.3)	213 (26.4)	341 (40.3)	82 (9.7)
Recent infection	<2–4 weeks	55 (6.8)	79 (9.8)	97 (11.5)	20 (2.4)
	>4 weeks	279 (34.6)	365 (45.3)	557 (65.8)	172 (20.3)
	Missing	11 (1.4)	17 (2.1)	–	–
Parental education	High	266 (33.0)	348 (43.2)	523 (61.8)	151 (17.9)
	Medium	60 (7.4)	88 (10.9)	111 (13.1)	32 (3.8)
	Low	19 (2.4)	24 (3.0)	18 (2.1)	9 (1.1)
	Missing	–	1 (0.1)	2 (0.2)	–
Body mass index (*z*-scores)	Normal	274 (34.0)	370 (45.9)	557 (65.8)	155 (18.3)
	Underweight	34 (4.2)	46 (5.7)	43 (5.1)	16 (1.9)
	Overweight/Obese	36 (4.5)	44 (5.4)	54 (6.4)	21 (2.5)
	Missing	1 (0.1)	1 (0.1)	–	–
Puberty onset	No	247 (30.6)	323 (40.1)	n/a	n/a
	Yes	94 (11.7)	132 (16.4)	n/a	n/a
	Missing	4 (0.5)	6 (0.7)	n/a	n/a
Puberty category scores	Pre-pubertal	n/a	n/a	1 (0.1)	–
	Early pubertal	n/a	n/a	6 (0.7)	9 (1.1)
	Mid-pubertal	n/a	n/a	102 (12.1)	35 (4.1)
	Late pubertal	n/a	n/a	380 (44.9)	109 (12.9)
	Post-pubertal	n/a	n/a	58 (6.9)	12 (1.4)
	Missing	n/a	n/a	107 (12.6)	27 (3.2)
Smoked today?	No	n/a	n/a	639 (75.5)	189 (22.3)
	Yes	n/a	n/a	15 (1.8)	3 (0.4)
Smoking status	No	n/a	n/a	565 (66.8)	163 (19.3)
	Infrequent	n/a	n/a	35 (4.1)	16 (1.9)
	Daily	n/a	n/a	14 (1.7)	2 (0.2)
	Missing	n/a	n/a	40 (4.7)	11 (1.3)

Table 3. Adjusted odds ratios, 95% CI and *p*-values from the multiple logistic regression models for hs-CRP and IL6 values (using the 85th percentile as the cut-off) and gingivitis at the 10- and 15-year follow-ups. In the 10-year data, adjustments were made for study cohort, gender, infections within the past 2–4 weeks or earlier, parental education and body mass index. In the 15-year data, smoking status and number of cigarettes smoked per week were also included in the adjustment set

Groups	*N*	aOR	95% CI	*p*-value	*N*	aOR	95% CI	*p*-value
	10-year hs-CRP *versus* 10-year gingivitis				15-year hs-CRP *versus* 15-year gingivitis			
s-SBI = 0	345	1.00	–	–	654	1.00	–	–
s-SBI = 1–2 sextants	174	0.99	0.57–1.73	0.984	101	**2.25**	**1.28–3.95**	**0.005**
s-SBI = 3–4 sextants	176	1.27	0.75–2.13	0.371	27	1.87	0.64–5.46	0.255
s-SBI = 5–6 sextants	111	1.08	0.57–2.06	0.806	64	1.02	0.44–2.36	0.965
	10-year IL6 *versus* 10-year gingivitis				15-year IL6 was not measured			
Healthy/s-SBI = 0	345	1.00	–	–	654	n/a	n/a	n/a
Affected/s-SBI ≥ 1	461	0.96	0.64–1.44	0.844	192	n/a	n/a	n/a
s-SBI = 0	345	1.00	–	–	654	n/a	n/a	n/a
s-SBI = 1–2 sextants	174	0.79	0.46–1.38	0.407	101	n/a	n/a	n/a
s-SBI = 3–4 sextants	176	0.95	0.56–1.61	0.847	27	n/a	n/a	n/a
s-SBI = 5–6 sextants	111	1.27	0.71–2.28	0.428	64	n/a	n/a	n/a

aOR, adjusted odds ratios; CI, confidence intervals; hs-CRP, high-sensitivity C-reactive protein. Bold numbers indicate statistically significant effect ($p < 0.05$).

have considered sulcus bleeding or gingivitis as factors in adolescents. Interestingly, 15-year-olds with a higher BMI and/or who were daily smokers showed a more frequent occurrence of sulcus bleeding, which is understood to be an early marker of periodontitis. This raises the question of whether gingival health directly influences hs-CRP values or is merely a clinical symptom of lifestyle. With respect to the documented aOR (Table 4), daily smoking (aOR: 6.27) had the most significant influence, followed by being overweight/obese (aOR: 4.95). Therefore, the above-mentioned determinants seem to be integral components of the

Table 4. Adjusted odds ratios, 95% CI and *p*-values from the multiple logistic regression model for hs-CRP in relation to gingivitis in children and adolescents. In the 10-year data, adjustments were made for study cohort, gender, infections within the past 2–4 weeks or earlier, parental education and body mass index. In the 15-year data, smoking status and number of cigarettes smoked per week were also adjusted

Variable	Groups	10-year hs-CRP *versus* 10-year gingivitis			15-year hs-CRP *versus* 15-year gingivitis		
		aOR	95% CI	*p*-value	aOR	95% CI	*p*-value
Gingivitis binomial	Healthy/s-SBI = 0	1.00	–	–	1.00	–	–
	Affected/s-SBI ≥ 1	1.20	0.78–1.84	0.402	**2.17**	**1.25–3.77**	**0.006**
Gender	Male	1.00	–	–	1.00	–	–
	Female	**1.95**	**1.24–3.07**	**0.004**	1.53	0.53–2.69	0.855
Body mass index	Normal	1.00	–	–	1.00	–	–
	Underweight	**0.25**	**0.08–0.81**	**0.021**	**0.48**	**0.14–1.62**	**0.001**
	Overweight/Obese	**3.65**	**2.14–6.23**	**< 0.001**	**4.95**	**0.73–33.68**	**0.007**
Smoking status	Non-smoker	–	–	–	1.00	–	–
	Smokes frequently	–	–	–	1.03	0.53–2.78	0.986
	Smokes daily	–	–	–	**6.27**	**1.39–28.39**	**0.017**

aOR, adjusted odds ratios; CI, confidence intervals; hs-CRP, high-sensitivity C-reactive protein. Bold numbers indicate statistically significant effect ($p < 0.05$).

process of systemic inflammation, whereas gingivitis (aOR: 2.17) might play a minor role.

Conclusion

This study investigated the associations between gingival health and lifestyle factors in relation to the systemic inflammatory blood markers hs-CRP and IL6 in adolescents. Within the limitations of our study, there was a significant positive association between hs-CRP and gingivitis, daily smoking and overweight/obesity. Future studies of adolescents should address confirming the causal relationship between these factors.

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References

Ainamo, J. & Ainamo, A. (1981) Prevention of periodontal disease in the mixed dentition. *International Dental Journal* **31**, 125–132.

Ainamo, J., Barmes, D., Beagrie, G., Cutress, T., Martin, J. & Sardo-Infirri, J. (1982) Development of the World Health Organization (WHO) community periodontal index of treatment needs (CPITN). *International Dental Journal* **32**, 281–291.

Akbaraly, T. N., Hamer, M., Ferrie, J. E., Lowe, G., Batty, G. D., Hagger-Johnson, G., Singh-Manoux, A., Shipley, M. J. & Kivimäki, M. (2013) Chronic inflammation as a determinant of future aging phenotypes. *Canadian Medical Association Journal* **185**, E763–E770.

Aulin, J., Siegbahn, A., Hijazi, Z., Ezekowitz, M. D., Andersson, U., Connolly, S. J., Huber, K., Reilly, P. A., Wallentin, L. & Oldgren, J. (2015) Interleukin-6 and C-reactive protein and risk for death and cardiovascular events in patients with atrial fibrillation. *American Heart Journal* **170**, 1151–1160.

Azizi, A., Sarlati, F., Bidi, M., Mansouri, L., Azaminejad, S. M. & Rakhshan, V. (2015) Effects of smoking severity and moderate and severe periodontitis on serum C-reactive protein levels: an age- and gender-matched retrospective cohort study. *Biomarkers* **20**, 306–312.

Bansal, T., Dhruvakumar, D. & Pandey, A. (2014) Comparative evaluation of C-reactive protein in peripheral blood of patients with healthy gingiva, gingivitis and chronic periodontitis: a clinical and particle-enhanced turbidimetric immuno-analysis. *Journal of Indian Society of Periodontology* **18**, 739–743.

von Berg, A., Filipiak-Pittroff, B., Krämer, U., Hoffmann, B., Link, E., Beckmann, C., Hoffmann, U., Reinhardt, D., Grübl, A., Heinrich, J., Wichmann, H. E., Bauer, C. P., Koletzko, S. & Berdel, D. (2013) Allergies in high-risk schoolchildren after early intervention with cow's milk protein hydrolysates: 10-year results from the German Infant Nutritional Intervention (GINI) study. *The Journal of Allergy and Clinical Immunology* **131**, 1565–1573.

Berg, A. H. & Scherer, P. E. (2005) Adipose tissue, inflammation, and cardiovascular disease. *Circulation Research* **96**, 939–949.

Briggs, M. S., Spees, C., Bout-Tabaku, S., Taylor, C. A., Eneli, I. & Schmitt, L. C. (2015) Cardiovascular risk and metabolic syndrome in obese youth enrolled in a multidisciplinary medical weight management program: implications of musculoskeletal pain, cardiorespiratory fitness, and health-related quality of life. *Metabolic Syndrome and Related Disorders* **13**, 102–109.

Bulló, M., García-Lorda, P., Megias, I. & Salas-Salvadó, J. (2003) Systemic inflammation, adipose tissue tumor necrosis factor, and leptin expression. *Obesity Research* **11**, 525–531.

Carskadon, M. A. & Acebo, C. (1993) A self-administered rating scale for pubertal development. *The Journal of Adolescent Health* **14**, 190–195.

Craig, R. G., Yip, J. K., So, M. K., Boylan, R. J., Socransky, S. S. & Haffajee, A. D. (2003) Relationship of destructive periodontal disease to the acute-phase response. *Journal of Periodontology* **74**, 1007–1016.

Danesh, J., Wheeler, J. G., Hirschfield, G. M., Eda, S., Eiriksdottir, G., Rumley, A., Lowe, G. D. O., Pepys, M. B. & Gudnason, V. (2004) C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. *The New England Journal of Medicine* **350**, 1387–1397.

Ebersole, J. L. & Cappelli, D. (2000) Acute-phase reactants in infections and inflammatory diseases. *Periodontology 2000* **23**, 19–49.

von Elm, E., Altman, D. G., Egger, M., Pocock, S. J., Gøtzsche, P. C. & Vandenbroucke, J. P. (2014) The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *International Journal of Surgery* **12**, 1495–1499.

Garanty-Bogacka, B., Syrenicz, M., Goral, J., Krupa, B., Syrenicz, J., Walczak, M. & Syrenicz, A. (2011) Changes in inflammatory biomarkers after successful lifestyle intervention in obese children. *Endokrynologia Polska* **62**, 499–505.

Heinrich, J., Bolte, G., Hölscher, B., Douwes, J., Lehmann, I., Fahlbusch, B., Bischof, W., Weiss, M., Borte, M. & Wichmann, H. E. (2002) Allergens and endotoxin on mothers' mattresses and total immunoglobulin E in cord blood of neonates. *The European Respiratory Journal* **20**, 617–623.

Heitmüller, D., Thiering, E., Hoffmann, U., Heinrich, J., Manton, D., Kühnisch, J., Neumann, C., Bauer, C. P., Heinrich-Weltzien, R. & Hickel, R. (2013) Is there a positive relationship between molar incisor hypomineralisations and the presence of dental caries? *International Journal of Paediatric Dentistry* **23**, 116–124.

Hugoson, A. & Norderyd, O. (2008) Has the prevalence of periodontitis changed during the last 30 years? *Journal of Clinical Periodontology* **35**, 338–345.

Hugoson, A., Sjödin, B. & Norderyd, O. (2008) Trends over 30 years, 1973–2003, in the prevalence and severity of periodontal disease. *Journal of Clinical Periodontology* **35**, 405–414.

Jayaprakash, D., Aghanashini, S., Vijayendra, R. R., Chatterjee, A., Rosh, R. M. & Bharwani, A. (2014) Effect of periodontal therapy on C-reactive protein levels in gingival crevicular fluid of patients with gingivitis and chronic

periodontitis: a clinical and biochemical study. *Journal of Indian Society of Periodontology* **18**, 456–460.

Kaneko, H., Anzai, T., Nagai, T., Anzai, A., Takahashi, T., Mano, Y., Morimoto, K., Maekawa, Y., Itoh, H., Yoshikawa, T., Ogawa, S. & Fukuda, K. (2011) Human C-reactive protein exacerbates metabolic disorders in association with adipose tissue remodelling. *Cardiovascular Research* **91**, 546–555.

Khera, A., McGuire, D. K., Murphy, S. A., Stanek, H. G., Das, S. R., Vongpatanasin, W., Wians, F. H. Jr, Grundy, S. M. & de Lemos, J. A. (2005) Race and gender differences in C-reactive protein levels. *Journal of the American College of Cardiology* **46**, 464–469.

Kromeyer-Hauschild, K., Wabitsch, M., Kunze, D., Geller, F., Geiß, C. H., Hesse, V., von Hippel, A., Jaeger, U., Johnsen, D., Korte, W., Menner, K., Müller, G., Müller, M. J., Niemann-Pilatus, A., Remer, T., Schaefer, F., Wittchen, H.-U., Zabransky, S., Zellner, K., Ziegler, A. & Hebebrand, J. (2001) Perzentile für den body-mass-index für das Kindes- und Jugendalter unter Heranziehung verschiedener deutscher Stichproben [Percentiles of body mass index in children and adolescents evaluated from different regional German studies] (article in German). *Monatsschrift Kinderheilkunde* **149**, 807–818.

Kühnisch, J., Heitmüller, D., Thiering, E., Brockow, I., Hoffmann, U., Neumann, C., Heinrich-Weltzien, R., Bauer, C. P., von Berg, A., Koletzko, S., Garcia-Godoy, F., Hickel, R. & Heinrich, J. (2014) Proportion and extent of manifestation of molar-incisor-hypomineralizations according to different phenotypes. *Journal of Public Health Dentistry* **74**, 42–49.

Kumar, S., Shah, S., Budhiraja, S., Desai, K., Shah, C. & Mehta, D. (2013) The effect of periodontal treatment on C-reactive protein: a clinical study. *Journal of Natural Science, Biology, and Medicine* **4**, 379–382.

Lakoski, S. G., Cushman, M., Criqui, M., Rundek, T., Blumenthal, R. S., D'Agostino, R. B. Jr & Herrington, D. M. (2006) Gender and C-reactive protein: data from the Multiethnic Study of Atherosclerosis (MESA) cohort. *American Heart Journal* **152**, 593–598.

Lausten-Thomsen, U., Gamborg, M., Bojsoe, C., Hedley, P. L., Hagen, C. M., Christiansen, M. & Holm, J. C. (2015) Longitudinal changes in C-reactive protein, proform of eosinophil major basic protein, and pregnancy-associated plasma protein-A during weight changes in obese children. *Journal of Pediatric Endocrinology & Metabolism* **28**, 393–398.

Loos, B. G. (2005) Systemic markers of inflammation in periodontitis. *Journal of Periodontology* **76**, 2106–2115.

Loos, B. G., Craandijk, J., Hoek, F. J., Wertheim-van Dillen, P. M. & van der Velden, U. (2000) Elevation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients. *Journal of Periodontology* **71**, 1528–1534.

Megson, E., Fitzsimmons, T., Dharmapatni, K. & Bartold, P. M. (2010) C-reactive protein in gingival crevicular fluid may be indicative of systemic inflammation. *Journal of Clinical Periodontology* **37**, 797–804.

Mohan, M., Jhingran, R., Bains, V. K., Gupta, V., Madan, R., Rizvi, I. & Mani, K. (2014) Impact of scaling and root planing on C-reactive protein levels in gingival crevicular fluid and serum in chronic periodontitis patients with or without diabetes mellitus. *Journal of Periodontal & Implant Science* **44**, 158–168.

Mustonen, K., Keski-Nisula, L., Vaarala, O., Pfefferle, P. I., Renz, H., Riedler, J., Dalphin, J. C., Buechele, G., Lauener, R., Braun-Fahrlander, C., von Mutius, E. & Pekkanen, J. (2012) Few associations between high-sensitivity C-reactive protein and environmental factors in 4.5-year-old children. *Pediatric Allergy and Immunology* **23**, 522–528.

O'Loughlin, J., Lambert, M., Karp, I., McGrath, J., Gray-Donald, K., Barnett, T. A., Delvin, E. E., Levy, E. & Paradis, G. (2008) Association between cigarette smoking and C-reactive protein in a representative, population-based sample of adolescents. *Nicotine & Tobacco Research* **10**, 525–532.

Paraskevas, S., Huizinga, J. D. & Loos, B. G. (2008) A systematic review and meta-analyses on C-reactive protein in relation to periodontitis. *Journal of Clinical Periodontology* **35**, 277–290.

Pari, A., Ilango, P., Subbareddy, V., Katamreddy, V. & Parthasarthy, H. (2014) Gingival diseases in childhood – a review. *Journal of Clinical and Diagnostic Research*, **8**, Ze01–Ze04.

Peruzzo, D. C., Gimenes, J. H., Taiete, T., Casarin, R. C., Feres, M., Sallum, E. A., Casati, M. Z., Kantovitz, K. R. & Nociti, F. H. Jr (2016) Impact of smoking on experimental gingivitis. A clinical, microbiological and immunological prospective study. *Journal of Periodontal Research* **51**, 800–811.

Pihlstrom, B. L., Michalowicz, B. S. & Johnson, N. W. (2005) Periodontal diseases. *Lancet* **366**, 1809–1820.

Prabhu, A., Michalowicz, B. S. & Mathur, A. (1996) Detection of local and systemic cytokines in adult periodontitis. *Journal of Periodontology* **67**, 515–522.

Pradeep, A. R., Manjunath, R. G. & Kathariya, R. (2010) Progressive periodontal disease has a simultaneous incremental elevation of gingival crevicular fluid and serum CRP levels. *Journal of Investigative and Clinical Dentistry* **1**, 133–138.

Ramberg, P. W., Lindhe, J. & Gaffar, A. (1994) Plaque and gingivitis in the deciduous and permanent dentition. *Journal of Clinical Periodontology* **21**, 490–496.

R Core Team. (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL: http://www.R-project.org/.

Ridker, P. M., Hennekens, C. H., Buring, J. E. & Rifai, N. (2000) C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *The New England Journal of Medicine* **342**, 836–843.

Sabiston, C., Castonguay, A., Barnett, T., O'Loughlin, J. & Lambert, M. (2009) Body image and C-reactive protein in adolescents. *International Journal of Obesity* **33**, 597–600.

Shaddox, L. M., Wiedey, J., Calderon, N. L., Magnusson, I., Bimstein, E., Bidwell, J. A., Zapert, E. F., Aukhil, I. & Wallet, S. M. (2011) Local inflammatory markers and systemic endotoxin in aggressive periodontitis. *Journal of Dental Research* **90**, 1140–1144.

de Souza, A. B., Okawa, R. T., Silva, C. O. & Araújo, M. G. (2017) Short-term changes on C-reactive protein (CRP) levels after non-surgical periodontal treatment in systemically healthy individuals. *Clinical Oral Investigations* **21**, 477–484.

Stringhini, S., Batty, G. D., Bovet, P., Shipley, M. J., Marmot, M. G., Kumari, M., Tabak, A. G. & Kivimäki, M. (2013) Association of life-course socioeconomic status with chronic inflammation and type 2 diabetes risk: the Whitehall II prospective cohort study. *PLoS Medicine* **10**, e1001479.

Tüter, G., Kurtis, B. & Serdar, M. (2007) Evaluation of gingival crevicular fluid and serum levels of high-sensitivity C-reactive protein in chronic periodontitis patients with or without coronary artery disease. *Journal of Periodontology* **78**, 2319–2324.

Van Hoorenbeeck, K., Franckx, H., Debode, P., Aerts, P., Wouters, K., Ramet, J., Van Gaal, L. F., Desager, K. N., De Backer, W. A. & Verhulst, S. L. (2012) Weight loss and sleep-disordered breathing in childhood obesity: effects on inflammation and uric acid. *Obesity* **20**, 172–177.

Williams, R. C. (1990) Periodontal disease. *The New England Journal of Medicine* **322**, 373–382.

Winning, L., Patterson, C. C., Cullen, K. M., Stevenson, K. A., Lundy, F. T., Kee, F. & Linden, G. J. (2015) The association between subgingival periodontal pathogens and systemic inflammation. *Journal of Clinical Periodontology* **42**, 799–806.

Yucel-Lindberg, T. & Båge, T. (2013) Inflammatory mediators in the pathogenesis of periodontitis. *Expert Reviews in Molecular Medicine* **15**, e7.

Zutavern, A., Brockow, I., Schaaf, B., Bolte, G., von Berg, A., Diez, U., Borte, M., Herbarth, O., Wichmann, H. E. & Heinrich, J. (2006) Timing of solid food introduction in relation to atopic dermatitis and atopic sensitization: results from a prospective birth cohort study. *Pediatrics* **117**, 401–411.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Frequencies, adjusted odds ratios (aOR), 95% confidence intervals (CI) and the *p*-values for the longitudinal analysis performed for hs-CRP in relation to gingivitis for the subset population (N:333) using generalized estimating equation (GEE) model.

Data S1. Checklist of items according to the STROBE guideline [van Elm et al. 2008] that should be included in reports of observational studies.

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Clinical Relevance

Scientific rationale for the study: While there is sufficient evidence of the association between periodontal disease, overweight/obese and smoking on hs-CRP and IL6 levels in adults; information on gingivitis and its association with inflammatory markers in children and adolescents is lacking.
Principal findings: In 15-year-olds with gingivitis, we found significantly elevated hs-CRP levels in those who smoked daily and who were overweight/obese.
Practical implications: This study indicates that in addition to oral hygiene, lifestyle-related factors seem to have equal importance in counteracting systemic inflammation.

Chapter 7

Publication II

Comparison of Different Protocols for Performing Adhesive Restorations in Primary Teeth – A Retrospective Clinical Study

Vinay Pitchika[a] / Isabel Metz[b] / Katrin Rothmaier[c] / Alexander Crispin[d] / Reinhard Hickel[e] / Katharina Bücher[f] / Jan Kühnisch[g]

Purpose: To examine the influence of different adhesive restoration protocols on the survival of composite resin restorations in primary teeth.

Materials and Methods: This study included 601 patients at risk of caries (319 males and 282 females), with a mean age of 6.6 years (range: 1 to 13 years) and an average need for 3.6 restorations per patient (range: 1 to 20). The mean observation period was 1.3 years (± 1.4), with a maximum of 7 years. Six different adhesive restoration protocols with respect to etching, adhesives, and composite materials were analyzed. The statistical analyses included descriptive analyses and a Cox regression model from which hazard ratios (HR with 95% CI) for protocols and possible predictors were calculated.

Results: The mean dmft was 6.6 (± 4.0), which indicates a high risk population. Secondary caries was the most frequent reason for loss of restoration in this study (52.2%). Out of 2146 restorations, 368 failed; the mean annual failure rate was 13.2%. Adhesive restoration protocols that employed a self-etching system performed significantly better (HR range: 1.0 to 1.8) than did the etch-and-rinse system (HR range: 1.8 to 2.8). Protocols using only flowable materials had a moderately increased probability of failure.

Conclusion: With respect to the practice-based study design, young age, and high risk of caries in this population, comparatively high failure rates were observed for direct composite restorations in primary dentition, but adhesive restoration protocols using self-etching adhesives in combination with universal composites yielded a higher probability of survival.

Keywords: retrospective, composite resin, adhesive restoration protocol, restoration, longevity, deciduous dentition.

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Over the last two decades, adhesively bonded and tooth-colored restorations have become much more popular in dental practices than conventional amalgam and cement restorations.[6,20,23] Additionally, adhesives and the functional properties of composite resin materials have constantly improved; as a result, good adhesive systems with little wear and low failure probability have been reported.[13,14,26] Typically, the relevant steps of the adhesive restoration protocol are removal of caries-affected enamel/dentin, isolation and cleaning of the cavity, the bonding procedure, placement of the restorative material, light polymerization, and polishing.[8] It has been reported that placing direct adhesive restorations is a technique-sensitive procedure.[18,19,32] This situation is further complicated by lack of cooperation in children. In situations when the patients are not compliant, the (pediatric) dentist often has to perform the restoration in a shorter time period. Therefore, different self-etching adhesives and light-polymerizing materials may help reduce the treatment time. Although several clinically controlled studies have investigated the influence and survival rates of different adhesives[28,31] and composite materials,[2-4,10,11,15-17,25,33] few published studies have compared the survival probability of different adhesive restoration protocols. This provides essential information about the clinical utility of different strategies. Therefore, this study explored the survival of the most

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frequently used procedures in our department using retrospectively collected data from a university-based pediatric treatment setting. Using a practice-based study design, the purpose of this study trial was to provide information about the influence of different adhesive restoration protocols on the survival of composite resin restorations in primary teeth. The null hypothesis was that no differences existed between the different adhesive restoration protocols in terms of the survival of composite resin restorations in primary teeth.

MATERIALS AND METHODS

The reporting in this study followed the recommendations of the ISPOR guidelines for retrospective studies.[1] The ethics committee of the medical faculty of Ludwig Maximilian University (LMU) of Munich approved the study design (project number 030-13).

Study Population

Dental records of patients who received a minimum of one direct adhesive restoration under local anesthesia in the Department of Conservative Dentistry and Periodontology at LMU between January 2004 and December 2012 and attended at least one recall appointment were included in the study. Restorations performed under general anesthesia were excluded. The endpoint of our study was loss of restoration. The study began with 676 patients (360 males and 316 females) and 2417 restorations. A total of six operators performed the restorations during the study period. Seventy-five patients (271 restorations) were removed from the database because they received restorations with less frequently used materials such as Ceram and Venus composite resins. This resulted in a final sample size of 601 patients (319 males and 282 females) with a mean age of 6.6 years (range: 1 to 13 years). A total of 2146 restorations were analyzed with an average of 3.6 restorations per patient (range: 1 to 20). Of these restorations, 1778 remained intact, 192 were lost due to secondary caries, and 176 were lost for other reasons. Thirteen intact restorations were right-censored for survival analyses because these teeth either had an endodontic event, were exfoliated, or the tooth was extracted for other reasons. The study sample had a mean dmft value of 6.6 (± 4.0). The units of observation in this study were restorations, and some patients had multiple restorations.

Restoration Procedure

Following tooth cleaning with a bristle brush, a diagnosis of the caries lesion was performed via visual examination. If a carious lesion was visually suspected, bitewing radiographs were taken to identify any radiolucency in the dentin. After preparation of the access cavity on the clean tooth, defect-oriented carious dentin excavation was performed. Soft, wet, and leathery dentin was removed. A calcium hydroxide liner was indicated in deep preparations of active carious lesions. If pulp perforations were present, endodontic treatment (N: 40) was performed. A rubber-dam was used when feasible. In proximal preparations, a retainerless matrix system (AutoMatrix, Dentsply De Trey; York, PA, USA) was used along with wooden wedges. Two different adhesive procedures were applied: a self-etching adhesive (Clearfil SE Bond, Kuraray Noritake; Osaka, Japan), or an etch-and-rinse technique using phosphoric acid (Total Etch, Ivoclar Vivadent; Schaan, Liechtenstein, application time ca 10 s) combined with a three-step adhesive system (Syntac Classic, Ivoclar Vivadent). The composite resin was mainly chosen according to the operator's preferences and included a flowable hybrid composite resin (Tetric EvoFlow, Ivoclar Vivadent), a traditional hybrid composite resin (Tetric EvoCeram, Ivoclar Vivadent), or a combination of the two, which was described earlier.[5] All increments were polymerized for 20 s each, and the restoration was shaped and polished.

Recall and Data Acquisition

All patients requiring caries-related restorations were considered to be at high risk and were therefore offered quarterly preventive recall examinations. During the recall examinations, all teeth and restorations were carefully checked by calibrated dentists in the dental diagnosis department. The restorations were followed until failure, if any, and the reasons for restoration loss were documented, which provided information about the survival time for each restoration. In case patients had multiple restorations, they were recorded as different observations and were identified with the patient ID. Each restoration was treated as an observation and a unique observation ID was provided. Information such as patient characteristics, caries experience, direct adhesive restoration materials used, and the status of the restoration were obtained from the patient case report forms. The adhesive restoration protocol was obtained from the patient records. The following variables were specifically considered: 1. the adhesive strategy, self-etching or etch-and-rinse, 2. the type of dentin/enamel adhesive used, and 3. the composite resin material. Using these possibilities, six frequent adhesive restoration protocols were identified: 1. self-etching adhesive + traditional hybrid composite resin (ST); 2. self-etching adhesive + flowable hybrid composite resin (SF); 3. self-etching adhesive + flowable and traditional hybrid composite resin (SFT); 4. etch-and-rinse + adhesive + traditional hybrid composite resin (ERT); 5. etch-and-rinse + adhesive + flowable hybrid composite resin (ERF); 6. etch-and-rinse + adhesive + flowable and traditional hybrid composite resin (ERFT). Apart from the adhesive restoration protocol variables, information about possible confounders, such as gender (male and female), age (0–3 years, 4–5 years, 6–7 years, 8–9 years, and 10–13 years), type of tooth (incisors, canines and molars), lesion size (1, 2, 3 and ≥4 surfaces) and operator (6 operators) were collected and included in the dataset.

Statistical Analysis

Data were entered into a spreadsheet program (Excel 2010, Microsoft; Redmond, WA, USA). Descriptive and explorative data analyses were performed with Microsoft Excel 2010 and SPSS Statistics for Windows, Version 21.0.1

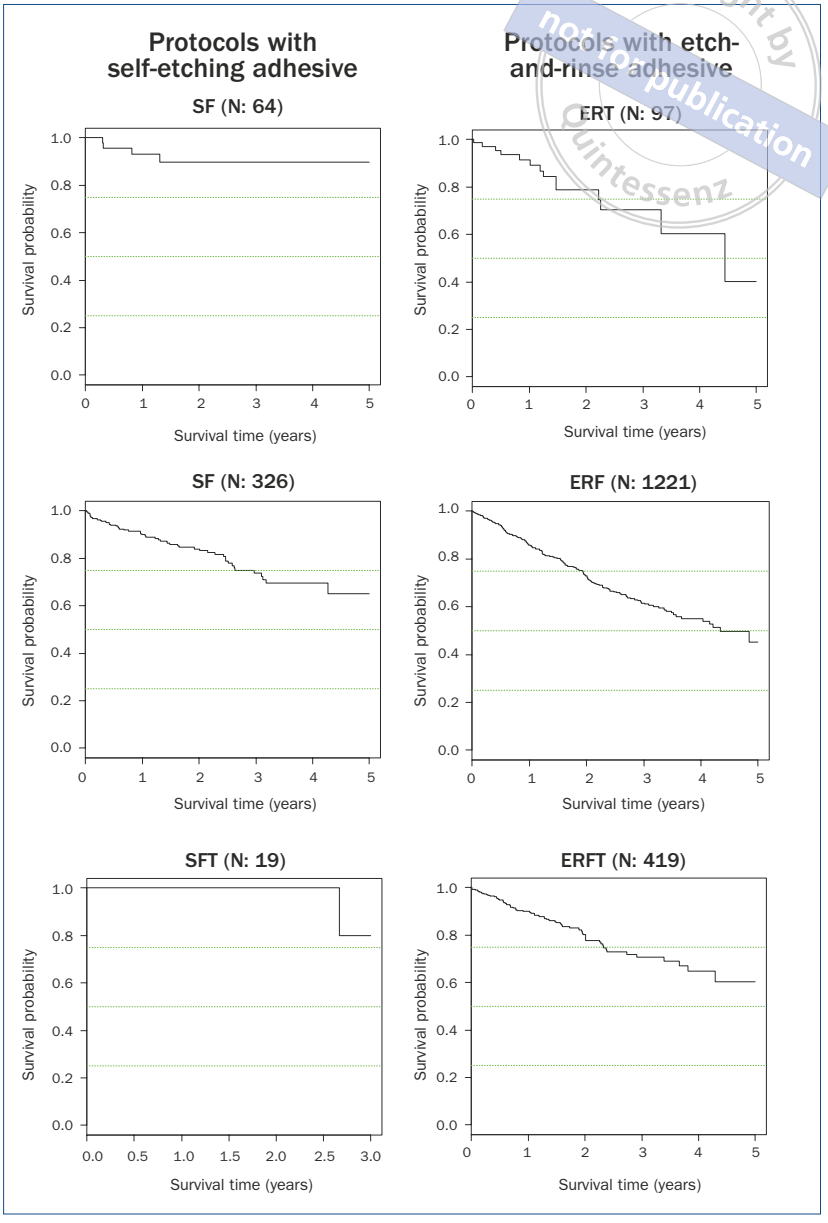

Fig 1 Kaplan-Meier curves for six different adhesive restoration protocols for performing a composite resin restoration. ST: self-etching adhesive + traditional hybrid composite resin; SF: self-etching adhesive + flowable hybrid composite resin; SFT: self-etching adhesive + flowable and traditional hybrid composite resin; ERT: etch-and-rinse + adhesive + traditional hybrid composite resin; ERF: etch-and-rinse + adhesive + flowable hybrid composite resin; ERFT: etch-and-rinse + adhesive + flowable and traditional hybrid composite resin.

(SPSS IBM; Armonk, NY, USA). Restorations that were lost due to other reasons, such as exfoliations, extractions or endodontic treatment, were right-censored. The adhesive restoration protocols used for the composite restorations were considered a separate variable comprised of six combinations, and the annual failure rates were calculated (Table 2). Kaplan-Meier curves were plotted for the different adhesive restoration protocols (Fig 1). A stepwise Cox regression using a backward elimination model was performed to determine whether differences existed between the adhesive restoration protocols using the survival time in years as the time variable and loss of restoration as the outcome variable. The model was adjusted for gender, age, tooth type, lesion size and operator. The hazard ratios (HR) for each variable were obtained from this analysis. For all analyses, a 95% confidence interval (95% CI) and a two-tailed significance level of 0.05 were used. The overall model resulted in a p-value of <0.01.

Table 1 Reasons for failure and their corresponding annual failure rates as absolute numbers and cumulative percentages

	0–1 year		1–2 years		2–3 years		3–4 years		4–5 years		>5 years		Total	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Healthy restorations	968	84.3	363	78.9	219	78.5	126	88.1	57	85.1	45	91.8	1778	82.9
Failures	180	15.7	97	21.1	60	21.5	17	11.9	10	14.9	4	8.2	368	17.1
Reasons for failure														
Secondary caries	72	37.5	63	32.8	32	16.7	13	6.8	9	4.7	3	1.5	192	100
Primary caries	1	20.0	–	–	2	40.0	2	40.0	–	–	–	–	5	100
Loss of retention	13	59.1	5	22.7	3	13.6	–	–	–	–	1	4.6	22	100
Total filling loss	53	71.6	11	14.9	9	12.2	–	–	1	1.4	–	–	74	100
Fractured filling	7	63.6	1	9.1	2	18.2	1	9.1	–	–	–	–	11	100
Tooth fracture	1	100.0	–	–	–	–	–	–	–	–	–	–	1	100
Wear and marginal loss	2	33.3	2	33.3	1	16.7	1	16.7	–	–	–	–	6	100
Other reasons	31	54.4	15	26.3	11	19.3	–	–	–	–	–	–	57	100

RESULTS

Of the total composite restorations, 368 (17.1%) restorations failed within the observation period (mean 1.3 ± 1.4 years, maximum 7 years). Table 1 shows the reasons for restoration failure and their annual failure rates. Secondary caries was the most common cause of failure, accounting for 52.2% of lost restorations, which was followed by total restoration loss (20%) and loss of retention (6%). Among the restorations lost, most (92%) of the losses occurred during the first 3 years. Overall, 7.5% of the restorations were lost in the first year. The mean dmft of males was significantly higher than that of females (6.21 vs 5.04).

The most frequently used adhesive restoration protocol was ERF, followed by ERFT and SF (Table 2). Adhesive restoration protocols were analyzed separately using the Cox regression model adjusted for gender, age, tooth type, lesion size, and operator (Table 3), which resulted in higher HRs for restorations constructed with etch-and-rinse techniques than with self-etching adhesives. The lowest HRs were found for ST, SFT, and SF; these were followed by ERFT, ERT, and ERF, in ascending order. There was no significant difference in the HR between males and females. It was observed that larger lesions and younger age (0–3 years) increased the risk of restoration failure. Figure 1 shows the survival graphs for all the adhesive restoration protocols; the self-etching combinations performed better.

DISCUSSION

This retrospective study analyzed the survival probability of composite restorations made according to different adhesive restoration protocols in deciduous dentition in a population with a high caries risk (mean dmft of 6.6). Of a total of 2146 restorations qualified for analysis, 1778 (82.9%) remained healthy/intact, with 17.1% of all composite restorations failing within the observation/follow-up period. This seems to be an acceptable overall failure rate and agrees with similar studies.[7,9] Considering that composite restorations are technique-sensitive procedures, six different types of adhesive restoration protocols employing various etching methods, bonding agents, and composite materials (Table 2) were employed. These clinical protocols were primarily used to achieve maximum cooperation from the patient, although operator preference clinically played a minor role. Lesion size may not have played a major role, as only 3.3% of the restorations (N: 71) were large and consisted of ≥ 4 surfaces. The Cox regression model revealed that the adhesive restoration protocols with self-etching adhesives yielded a higher probability of survival; the literature has suggested the same for primary teeth.[12,14,27,30] It is worth noting that because these restorations were performed in pediatric patients, it was more difficult to obtain optimum cooperation than in adult patients.

Among the adhesive restoration protocols, those using flowable materials had significantly higher risks of failure, which indicates that the viscosity of materials might influence the survival of composite restorations in primary teeth. However, flowable composites possess some advantages, such as lining the cavity, ease of adaptability, reducing the time required for placing a restoration, and minimizing voids during application. In our study, 52.5% of the restorations lost were due to secondary caries. Although this proportion seems to be large, the overall sec-

Table 2 Descriptive statistics showing the absolute numbers (N), percentages, mean observation time in years (MOT) and annual failure rates (AFR) for the adhesive restoration protocols and the potential predictors included in the analysis

Variable	Groups	Healthy restorations		Failed restorations		Total restorations			
		N	%	N	%	N	%	MOT	AFR
Restorations	Healthy	–	–	–	–	1778	82.9	–	–
	Failed	–	–	–	–	368	17.1	–	–
Adhesive restoration protocols	ST	58	3.3	6	1.6	64	3.0	1.17	8.0%
	SF	273	15.4	53	14.4	326	15.2	1.29	12.6%
	SFT	17	1.0	2	0.5	19	0.9	1.16	9.1%
	ERT	83	4.7	14	3.8	97	4.5	0.79	18.3%
	ERF	994	55.9	227	61.7	1221	56.9	0.78	23.8%
	ERFT	353	19.9	66	17.9	419	19.5	1.00	15.8%
Gender	Female	821	46.2	156	42.4	977	45.5	0.90	17.7%
	Male	957	53.8	212	57.6	1169	54.5	0.93	19.5%
Age	0–3 years	125	7.0	48	13.0	173	8.1	1.20	23.1%
	4–5 years	424	23.8	134	36.4	558	26.0	1.18	20.4%
	6–7 years	564	31.7	116	31.5	680	31.7	1.03	16.6%
	8–9 years	462	26.0	55	14.9	517	24.1	0.64	16.6%
	10–13 years	203	11.4	15	4.1	218	10.2	0.33	20.9%
Tooth type	Incisor	182	10.2	51	13.9	233	10.9	0.62	35.3%
	Canine	175	9.8	21	5.7	196	9.1	0.96	11.2%
	Molar	1421	79.9	296	80.4	1717	80.0	0.95	18.1%
Lesion size	1 surface	583	32.8	106	28.8	689	32.1	1.08	14.2%
	2 surfaces	1014	57.0	207	56.3	1221	56.9	0.86	19.7%
	3 surfaces	126	7.1	39	10.6	165	7.7	0.81	29.2%
	≥ 4 surfaces	55	3.1	16	4.3	71	3.3	0.66	34.1%
Operator	1	750	42.2	171	46.5	921	42.9	0.87	21.3%
	2	622	35.0	137	37.2	759	35.4	0.97	18.6%
	3	87	4.9	19	5.2	106	4.9	0.79	22.7%
	4	56	3.1	8	2.2	64	3.0	0.77	16.2%
	5	193	10.9	29	7.9	222	10.3	1.27	10.3%
	6	70	3.9	4	1.1	74	3.4	0.28	19.3%

ondary caries rate in this high risk population was lower.[21] Previously, composite resin restorations were believed to carry a higher risk of failure and be more prone to developing secondary caries, but recent studies have suggested that such evidence is of low quality.[29] Furthermore, in pediatric cases in which a large cavity has to be restored, it could be filled using bulk-fill composites, which were not readily available when the restorations in this study period were performed.

Our study had some advantages, such as the fact that the mean age of the study population was 6.6 years (range: 1 to 13 years), which addresses the entire spectrum of deciduous dentition. The patients had a suitable follow-up time, with a maximum of 7 years (mean: 1.3 years; few

Table 3 Hazard ratios (HR) and their corresponding 95% confidence intervals (95% CI) using the Cox regression model for different adhesive restoration protocols

Variable	Groups	HR	95% CI	p
Adhesive restoration protocols	ST	1	–	0.005*
	SF	1.76	0.68–4.57	
	SFT	1.16	0.22–6.04	
	ERT	2.35	0.82–6.68	
	ERF	2.83	1.11–7.21	
	ERFT	1.80	0.69–4.67	
Gender	Female	1	–	0.70††
	Male	1.04††	0.84–1.29††	
Age	0–3 years	1	–	<0.001*
	4–5 years	0.87	0.60–1.25	
	6–7 years	0.60	0.41–0.90	
	8–9 years	0.46	0.30–0.73	
	10–13 years	0.35	0.19–0.67	
Tooth type	Incisor	1	–	0.07†
	Canine	0.60	0.34–1.04	
	Molar	1.03	0.71–1.48	
Lesion size	1 surface	1	–	0.004*
	2 surfaces	1.43	1.09–1.86	
	3 surfaces	1.95	1.30–2.92	
	≥ 4 surfaces	1.80	1.03–3.15	
Operator	1	1	–	0.03*
	2	1.23	0.93–1.63	
	3	1.73	1.03–2.90	
	4	0.70	0.33–1.47	
	5	0.80	0.51–1.24	
	6	0.45	0.17–1.23	

The model was adjusted for gender, age, tooth type, lesion size, and operator. *Statistically significant ($p < 0.05$). †Although the p-value of tooth type was not significant as an individual variable, the p-value of the overall model was significant even after inclusion of this variable in the stepwise model. ††Gender was not a significant factor in the model. This factor was eliminated in the stepwise model, but the estimates reported belong to step 1, where it was included. ST: self-etching adhesive + traditional hybrid composite resin; SF: self-etching adhesive + flowable hybrid composite resin; SFT: self-etching adhesive + flowable and traditional hybrid composite resin; ERT: etch-and-rinse + adhesive + traditional hybrid composite resin; ERF: etch-and-rinse + adhesive + flowable hybrid composite resin; ERFT: etch-and-rinse + adhesive + flowable and traditional hybrid composite resin.

cases were followed-up longer than 5 years), and the sample size was sufficiently large (601 patients and 2146 restorations). Few studies have attained equal or larger sample sizes.[17,25] In this study, the effects of individual factors such as etching, bonding agents, or composite materials on the survival of restorations were not analyzed, which provided no information about the effects of combinations of factors that are observed in clinical scenarios. These results have been presented elsewhere.[5] Additionally, the risk of one factor outweighing the others is always present when modelling individual factors. Ideally, researchers would prefer to perform randomized controlled trials, where all forms of bias and confounders are avoided, but this situation is not encountered in real life. Although numerous clinically controlled trials exist, a retrospective study was chosen because it provides practice-based information regarding different adhesive restoration protocols in caries-associated, direct adhesive restorations in primary teeth, irrespective of potential limitations such as limited cooperation or younger age, which can hinder inclusion in a (randomized-) controlled trial. Therefore, conducting practice-based research from a specialized dental practice or pediatric dentistry departments from universities or hospitals is suggested.[24] One of the limitations of this study was that the follow-up time was different for each restoration, which is commonly observed in retrospective study designs. As in typical retrospective studies, the data were collected by various dentists at various times, making it difficult to obtain perfectly calibrated and reliable data, compared to a controlled trial undertaken by highly calibrated investigators. Limitations are present in both retrospective and prospective studies. To overcome this, future studies should employ mixed methods[22] so that different characteristics from various setups can be optimally implemented.

CONCLUSION

This study underlines the fact that the analysis of different clinical protocols is as important as evaluating individual materials or steps for determining the survival of a restoration. In our study, adhesive restoration protocols employing self-etching adhesives combined with packable composites yielded higher probabilities of survival. It was also noted that restoration protocols with flowable composites had comparatively higher risks of failure.

REFERENCES

1. Berger ML, Mamdani M, Atkins D, Johnson ML. Good research practices for comparative effectiveness research: defining, reporting and interpreting nonrandomized studies of treatment effects using secondary data sources: the ISPOR Good Research Practices for Retrospective Database Analysis Task Force Report–Part I. Value Health 2009;12:1044-1052.
2. Bohaty BS, Ye Q, Misra A, Sene F, Spencer P. Posterior composite restoration update: focus on factors influencing form and function. Clin Cosmet Investig Dent 2013;5:33-42.
3. Brunthaler A, König F, Lucas T, Sperr W, Schedle A. Longevity of direct resin composite restorations in posterior teeth. Clin Oral Investig 2003;7:63-70.

4. Bryant RW. Long-term implications for composite resin restorations. Ann R Australas Coll Dent Surg 1989;10:84-90.
5. Bücher K, Metz I, Pitchika V, Hickel R, Kühnisch J. Survival characteristics of composite restorations in primary teeth. Clin Oral Investig 2014;19:1653-1662.
6. Buerkle V, Kuehnisch J, Guelmann M, Hickel R. Restoration materials for primary molars-results from a European survey. J Dent 2005;33: 275-281.
7. Busato AL, Loguercio AD, Reis A, Carrilho MR. Clinical evaluation of posterior composite restorations: 6-year results. Am J Dent 2001;14: 304-308.
8. Carlen A, Nikdel K, Wennerberg A, Holmberg K, Olsson J. Surface characteristics and in vitro biofilm formation on glass ionomer and composite resin. Biomaterials 2001;22:481-487.
9. Casagrande L, Dalpian DM, Ardenghi TM, Zanatta FB, Balbinot CE, García-Godoy F, De Araujo FB. Randomized clinical trial of adhesive restorations in primary molars. 18-month results. Am J Dent 2013;26: 351-355.
10. Da Rosa Rodolpho PA, Donassollo TA, Cenci MS, Loguercio AD, Moraes RR, Bronkhorst EM, Opdam NJ, Demarco FF. 22-year clinical evaluation of the performance of two posterior composites with different filler characteristics. Dent Mater 2011;27:955-963.
11. Demarco FF, Correa MB, Cenci MS, Moraes RR, Opdam NJ. Longevity of posterior composite restorations: Not only a matter of materials. Dent Mater 2012;28:87-101.
12. Frankenberger R, Lohbauer U, Roggendorf MJ, Naumann M, Taschner M. Selective enamel etching reconsidered: Better than etch-and-rinse and self-etch? J Adhes Dent 2008;10:339-344.
13. García-Godoy F, Donly KJ. Dentin-enamel adhesives in pediatric dentistry: An update. Pediatr Dent 2015;37:133-135.
14. Giannini M, Makishi P, Ayres AP, Vermelho PM, Fronza BM, Nikaido T, Tagami J. Self-etch adhesive systems: A literature review. Braz Dent J 2015;26:3-10.
15. Heintze SD, Rousson V. Clinical effectiveness of direct class II restorations - a meta-analysis. J Adhes Dent 2012;14:407-431.
16. Hickel R, Manhart J. Longevity of restorations in posterior teeth and reasons for failure. J Adhes Dent 2001;3:45-64.
17. Kopperud SE, Tveit AB, Gaarden T, Sandvik L, Espelid I. Longevity of posterior dental restorations and reasons for failure. Eur J Oral Sci 2012;120:539-548.
18. Mackenzie L, Burke FJ, Shortall AC. Posterior composites: A practical guide revisited. Dent Update 2012;39:211-212, 215-216.
19. Mackenzie L, Parmar D, Shortall AC, Burke FJ. Direct anterior composites: A practical guide. Dent Update 2013;40:297-299, 301-292, 305-298 passim.
20. Manhart J, Chen H, Hamm G, Hickel R. Buonocore Memorial Lecture. Review of the clinical survival of direct and indirect restorations in posterior teeth of the permanent dentition. Oper Dent 2004;29:481-508.
21. Metz I, Rothmaier K, Pitchika V, Crispin A, Hickel R, García-Godoy F, Bücher K, Kühnisch J. Risk factors for secondary caries in direct composite restorations in primary teeth. Int J Paediatr Dent 2015;25:451-461.
22. O'Cathain A, Murphy E, Nicholl J. The quality of mixed methods studies in health services research. J Health Serv Res Policy 2008;13:92-98.
23. Opdam NJ, Bronkhorst EM, Loomans BA, Huysmans MC. 12-year survival of composite vs. amalgam restorations. J Dent Res 2010;89:1063-1067.
24. Opdam NJ, Bronkhorst EM, Loomans BA, Huysmans MC. Longevity of repaired restorations: A practice based study. J Dent 2012;40:829-835.
25. Pallesen U, van Dijken JW, Halken J, Hallonsten AL, Holgaard R. A prospective 8-year follow-up of posterior resin composite restorations in permanent teeth of children and adolescents in Public Dental Health Service: Reasons for replacement. Clin Oral Investig 2014;18:819-827.
26. Perdigão J. New developments in dental adhesion. Dent Clin North Am 2007;51:333-357, viii.
27. Perdigão J, Frankenberger R, Rosa BT, Breschi L. New trends in dentin/enamel adhesion. Am J Dent 2000;13:25d-30d.
28. Peumans M, De Munck J, Mine A, Van Meerbeek B. Clinical effectiveness of contemporary adhesives for the restoration of non-carious cervical lesions. A systematic review. Dent Mater 2014;30:1089-1103.
29. Rasines Alcaraz MG, Veitz-Keenan A, Sahrmann P, Schmidlin PR, Davis D, Iheozor-Ejiofor Z. Direct composite resin fillings versus amalgam fillings for permanent or adult posterior teeth. Cochrane Database Syst Rev 2014;3:Cd005620.
30. Rosa WL, Piva E, Silva AF. Bond strength of universal adhesives: A systematic review and meta-analysis. J Dent 2015;43:765-776.
31. Sampaio CS, Rodrigues RV, Souza-Junior EJ, Freitas AZ, Ambrosano G, Pascon FM, Rontani RP. Effect of restorative system and thermal cycling on the tooth-restoration interface - OCT evaluation. Oper Dent 2015; 41:162-170.
32. Thompson VP, Watson TF, Marshall GW, Jr., Blackman BR, Stansbury JW, Schadler LS, Pearson RA, Libanori R. Outside-the-(cavity-prep)-box thinking. Adv Dent Res 2013;25:24-32.
33. van de Sande FH, Opdam NJ, Rodolpho PA, Correa MB, Demarco FF, Cenci MS. Patient risk factors' influence on survival of posterior composites. J Dent Res 2013;92:78s-83s.

Clinical relevance: This study emphasizes the importance of analyzing the overall adhesive restoration protocol along with traditional analyses of individual components of restoration procedures when investigating the long-term survival of restorations.

Chapter 8

Publication III

Community Dent Oral Epidemiol 2016; 44; 354–363

COMMUNITY DENTISTRY AND ORAL EPIDEMIOLOGY

Longitudinal study of caries progression in 2- and 3-year-old German children

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Abstract – *Objective:* This 2-year longitudinal study in 2- and 3-year-old kindergarten children investigated lesion progression on different surfaces of primary teeth. *Methods:* The study was conducted between September 2008 and September 2010 on a sample of 400 children from the Kyffhäuser district (Thuringia, Germany). A calibrated investigator recorded (non)cavitated caries lesions according to World Health Organization (WHO), International Caries Detection and Assessment System (ICDAS) and Universal Visual Scoring System (UniViSS) criteria. Nonparametric methods and linear regression using a mixed-effects model with an unbalanced design were used for data analysis. *Results:* There was a significant increase in the prevalence of noncavitated caries lesions during the 2-year period, with the highest chance for change on all surfaces compared to cavitated lesions. First visible sign lesions on occlusal surfaces had the highest chance for change (estimate 0.38), whereas established lesions revealed the highest chance for change on proximal (estimate 1.05) and smooth surfaces (estimate 0.62). Proximal lesions exhibited the greatest chance for change irrespective of severity level. *Conclusion:* Our study demonstrated that each type of carious lesion had different changing rates. Greater lesion severity correlated with greater chances to change and receive treatment. This information is crucial for dental practitioners in decision-making processes.

Key words: caries progression; dental caries; epidemiology; longitudinal study; noncavitated caries lesions

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Early childhood caries (ECC) is defined as the presence of either a cavitated or a noncavitated carious lesion or missing/filled tooth surfaces on a primary tooth in a child <72 months of age[1]. Epidemiological studies suggest that caries prevalence and experience are lower in industrialized nations, compared to developing nations, primarily because of better oral hygiene practices and preventive measures[2,3]. Additionally, the burden of caries is concentrated in high-risk groups[4]. However, recent studies show an increase in caries prevalence in developing and industrialized nations, likely because of the immigration of populations from rural to urban centres and the increased consumption of bottled water instead of fluoridated tap water[5]. Dental research in the past decades tried to identify reliable caries risk factors using longitudinal studies[6–9]. High numbers of *Streptococcus mutans*, excessive dietary intake of cariogenic/erosive drinks, sweets and foodstuffs, poor oral hygiene and low socioeconomic status were identified as relevant risk factors, and these factors highlight the multifactorial nature of caries aetiology[10]. The core of successful caries risk assessment tools, such as Caries Management by Risk Assessment (CAMBRA) or Cariogram[11,12], is the longitudinal recording of carious lesions, optimally with the inclusion of noncavitated caries lesions. Longitudinal studies have also been performed to monitor preventive interventions[13–18].

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Several studies used cavitations or the dmf/DMF index as a reference standard[19–21], but the importance of noncavitated carious lesions[22,23] should not be underestimated. Noncavitated carious lesions should be included in longitudinal studies, particularly those in the primary dentition. These aspects suggest the examination of recently published visual criteria for caries detection and diagnosis, for example the International Caries Detection and Assessment System (ICDAS)[24] or the Universal Visual Scoring System (UniViSS)[25,26], in different age groups is required. To our knowledge, only limited longitudinal data are available in preschool children. However, a connection between the probability to change (i.e. reverse or progress) or to remain unchanged (stagnate) in different types of noncavitated caries lesions is unknown because only a few studies have investigated this issue[19–21,27,28]. This lack of information seems remarkable because the probability to change, particularly to progress, is accepted as an indicator for lesion activity[2], which is also a relevant factor in the decision-making process to distinguish between different treatment strategies[29]. Therefore, longitudinal studies are essential to provide a dental practitioner with important information about the transition of a lesion. This prospectively designed longitudinal cohort study investigated the transition rates of different types of carious lesions in 2- and 3-year-old kindergarten children during a 2-year observation period. Our hypothesis was that carious lesions at varying stages of progression exhibit different probabilities of changing or stagnating.

Materials and methods

The recommendations of STROBE guidelines for observational studies were used to report this study[30]. The ethics committee of the University Hospital Jena approved the study design (registration number 2376-09/08).

Population and study design

This study was a prospective longitudinal observational study in children attending kindergarten in the Kyffhäuser district. This county district is part of the German federal state Thuringia, which is located in middle-eastern Germany. The residents of this district were characterized in 2008 by a high unemployment rate (18.8%) and low socioeconomic background[31]. Data from annual dental examinations of the community public dental health service indicate a high caries prevalence and experience in preschool children relative to children of other Thuringian districts. Only two-thirds of this age group attend kindergarten. The drinking water in the Kyffhäuser district has a fluoride content of approximately 0.2 parts per million (ppm).

The basic preventive programme in the Kyffhäuser District includes daily supervised tooth brushing with fluoride toothpaste (500 ppm) in all kindergartens, dietary counselling on healthy meals for the kindergarten staff and one visit to a dental practice annually to reduce dental anxiety. This programme is based on the legal right of preschool children in Germany to receive free preventive care from public health dentists and their teams. The participation rate of preschool children between 2 and 6 years of age in the Thuringian basic preventive programme was close to 100%. Additional population characteristics have been previously published[32]. Participation in annual dental examinations provided by the community public dental health service is voluntary for preschool children under the Thuringian education act § 55 (2010). In addition to the consent obtained from the children's parents to participate in the Thuringian basic preventive programme, an additional consent to take part in our study was provided by parents at the beginning of the study. This consent was needed only once, and annual follow-ups did not require additional consent. Therefore, only preschool children whose parents sign an informed consent can be included in the study.

The local public dental health service offered municipal kindergartens (n = 58) participation in a 2-year examination in preparation for an intensified preventive programme, including biannual fluoride varnish application, to gather more detailed information on caries experience at the surface level in preschool children. Twelve kindergartens from seven towns agreed to participate and were included in this study. These towns provided a total of 467 children who were eligible to participate. An invitation letter was given to all parents of children who attended the participating kindergartens requesting their children's participation in three follow-ups of annual dental examinations. The children were divided into two groups: the fluoride group, which received a biannual application of a newly developed fluoride varnish, and the control group. The allocation of children to either group was not randomized, and the selec-

tion was made exclusively by the parents. Operative treatments were recommended for cavitated teeth and performed by local dental practitioners. In cases where the parents declined operative care, they were reinformed about caries and its ill-effects, and carious lesions were registered during the study period.

A total of 400 healthy male and female 2- to 3-year-old children with American Society of Anesthesiologists (ASA) status 1 whose parents consented to participate in this observational study and signed an informed consent were included. These children were re-evaluated at 12 and 24 months after the baseline establishment by dentists who visited the participating kindergartens. Although the study sample was from a nonrandomized trial on the effectiveness of fluoride varnish, the analysis sample consisted of an almost 1:1 fluoride to control group ratio (159:161) at both the baseline and the 2-year examinations. The effects observed in prior publication[32] were adjusted for various factors, such as age, gender, investigation group, fluoride application frequency and socioeconomic status. Parents of a small number of children did not provide information on family income, which was used to calculate socioeconomic status. Therefore, the sample size was smaller as only those participants with complete data were included in the analyses. However, in this study, we observed only real-time caries progression, leading to a slightly larger sample size.

Clinical dental examination

Standard dental examination equipment, which consisted the following instruments, was used: a dental mirror with a flat surface, a blunt CPI probe (CP-11.5B6, Hu-Friedy, Chicago, IL, USA) and a halogen lamp (Ri-Magic, Rudolf Riester GmbH, Jungingen, Germany). A calibrated dentist (J.A.) with 20 years of professional experience examined each child in the kindergarten at a previously made appointment. Cavitated carious status was measured at the surface level using the dmf index for primary dentition according to WHO standards[33]. Noncavitated carious lesions were scored using criteria defined by ICDAS[24] and UniViSS[25,26]. Before their dental examination, children brushed their teeth using fluoride toothpaste under the supervision of the kindergarten staff. The teeth were dried using cotton rolls, and no bitewing radiographs were taken for caries detection.

Calibration of the study team

A 3-day training on the scoring of carious lesions according to WHO, ICDAS/UniViSS criteria was performed before the study began and before the 2-year follow-up examination. Training included the theoretical and practical calibration module, and it was held for the examiner (J.A.) under the supervision of experienced dentists (J.K., R.H-W.). Before the study began, the kappa values for the interexaminer and intra-examiner reliability were 0.78 and 0.86, respectively, and they were 0.84 and 0.88, respectively, at the 2-year follow-up. At baseline, the inter- and intra-examiner reliabilities for the dmf criteria on occlusal surfaces were 0.78 and 0.84, respectively, whereas on smooth surfaces, the reliabilities were 0.83 and 0.91, respectively. When using the ICDAS/UniViSS criteria, the inter- and intra-examiner reliabilities were 0.76 and 0.83 on occlusal surfaces and 0.79 and 0.86 on smooth surfaces, respectively.

Statistical analysis

Data obtained from dental examinations were entered into an SQL database (Access 2007, Microsoft Corporation, Redmond, WA, USA) and transferred to an Excel sheet (Excel 2003, Microsoft Corporation, Redmond, WA, USA). Descriptive and explorative data analyses were performed using SPSS Statistics for Windows, Version 19.0.1 (SPSS Inc., an IBM Company, Chicago, IL, USA). The mean and standard deviation of all components of $d_{3\text{-}4}$mft/s and $d_{1\text{-}2}$s scores were calculated and used in further analyses.

When a carious lesion progressed from one stage to an advanced stage, it was defined as 'progression'. If a lesion regressed one or more caries stages backward, it was defined as 'reversal'. Either a progression or a reversal was coded as 'change'. When a lesion did not progress or reverse but stagnated at the same stage, it was called 'unchanged'. If a carious lesion underwent either a restoration or an extraction, it was defined as 'treated'. As the data did not meet the conditions of normality, descriptive statistics were analysed between baseline and 1 year, baseline and 2 years and 1 year and 2 years using the Wilcoxon signed-rank-sum test. The data were grouped as occlusal, proximal (mesial & distal) and smooth surfaces (buccal & lingual). Because the 1-year data were similar to the baseline data, a regression analysis was performed only between the baseline and 2-year data to identify any significant changes in carious lesions.

Cross-sectional data of the baseline and 2-year examinations were analysed using a linear regression analysis that employed a mixed-effects model with unbalanced design[29]. The outcome variable for the regression model was any difference in the number of each type of carious lesions on different surfaces. The numbers of each lesion type on all surfaces at baseline and 2-year examination were added, and their difference was calculated. No change was scored as 0, and increases or decreases were scored as positive or negative difference scores, respectively. A difference in the number of lesions between baseline and 2 years was modelled using the type of lesion as the fixed effect. Since the patient ID was selected as a random effect, this mixed-effects model took into consideration the number of carious lesions per patient and modified the model according to the patient ID, resulting in adjusted estimates. Adjustments were made for gender, age, quadrant and type of teeth. As the analysis sample had approximately equal numbers of participants in the fluoride and control groups, the observed effect was also adjusted for fluoride varnish usage. Because the difference in the number of teeth between baseline and the 2-year follow-up was minimal and statistically insignificant, we did not include it in the model as a factor for adjustment. The analysis was stratified for occlusal, proximal and smooth surfaces.

Results

The study began with a sample size of 400 children (195 girls and 205 boys), but a percentage of this group did not attend the baseline examination. However, these missing cases participated in the 1-year examination. In addition, some of the participants missed the 2-year examination. To ensure a homogenous sample and allow analysis of the longitudinal caries progression, children who attended both the baseline and 2-year examinations ($n = 320$; 155 girls and 165 boys) were included for the final data analyses. The reasons for the reduced sample size in the study were either migration of the children to different places, absence on the date of examination or incomplete data sets due to poor compliance. The mean age of the entire study group at baseline was 2.98 years (2.95 ± 0.9 years and 3.01 ± 0.8 years for girls and boys, respectively). Gender and fluoride varnish usage did not play a significant role in altering the distribution of the variables of interest.

Caries prevalence at baseline was 16.6% according to WHO criteria and 52.5% based on ICDAS/UniViSS criteria (Table 1). There were increases of approximately 0.3% and 12.8% in overall caries prevalence over the 1-year and 2-year periods, respectively. This trend was accompanied by a significant twofold increase in mean noncavitated caries lesions after 2 years. In contrast, the mean number of cavitated lesions after 2 years was comparable to the baseline mean, but the number of restorations (f-component) and extractions (m-component) increased (Table 1). Figure 1 shows the frequency of each visual type of caries lesion in relation to tooth surface and the time point of investigation over the 2-year study period. Figure 2 presents the absolute numbers and percentages of lesion reversal, stagnation and progression, and Table 2 lists the adjusted estimates from the linear regression analysis. In general, smooth surface lesions were more unchanged than occlusal or proximal caries lesions in primary teeth. Noncavitated caries lesions exhibited the greatest chance for change. Lesions with first visible signs on occlusal surfaces had the highest chance for change (0.38), and established lesions on proximal (1.05) and smooth surfaces (0.62) revealed the highest adjusted estimates. All types of proximal lesions showed a significant trend for change over the study period. The percentage of lesions that remained stable decreased as the hierarchy of lesions increased, but the trend to treat the lesions increased (Figure 2). A distinct number of cases with cavitations were untreated. Established lesions were the most prone to reversal on all surfaces.

Discussion

This 2-year longitudinal observational study of 2- to 3-year-old children assessed different stages of carious lesions in the primary dentition. Our study demonstrated that different types of caries lesions exhibited different probabilities for change in 2- and 3-year-old children over the 2-year observation period (Table 2). Significant differences were observed in new and established caries lesions on all surfaces, which may be attributed primarily to a higher probability of progression (Figure 2) and supports our initial hypothesis.

Our study is unique because it observed the clinical progression of caries in a young population over a 2-year period and included noncavitated

Table 1. Caries experience scored by ICDAS/UniViSS and WHO criteria on tooth surface level at baseline and 2-year follow-up in 2- to 3-year-old children

Caries experience	Baseline examination	1-year examination	2-year examination
No. of participants, *N* (girls:boys ratio)	*N* = 320 (155:165)	*N* = 374 (179:195)	*N* = 320 (155:165)
No. of participants, *N* (fluoride:control ratio)	159:161	197:177	159:161
No. of teeth examined, *N*	6271	7476	6396
Caries prevalence – WHO standard *N* (%), [95%CI]	53 (16.6) [12.5–20.6]	77 (20.6) [16.5–24.7]	89 (27.8) [22.9–32.7]
Caries prevalence – Noncavitated level *N* (%), [95%CI]	168 (52.5) [47.0–58.0]	234 (62.6) [57.7–67.5]	205 (64.1) [58.8–69.3]
Overall caries prevalence – WHO + ICDAS/UniViSS N (%), [95% CI]	177 (55.3) [49.8–60.8]	208/374 [50.2–61.0]	218/320 [63.0–73.2]
Total caries experience according to ICDAS/UniViSS (surface level)			
Noncavitated caries lesions Mean (SD)	2.4 (3.8)[a,b]	3.1 (4.2)[a,c]	3.9 (6.3)[b,c]
First visible sign, mean (SD)	1.6 (3.3)[a,b]	2.1 (3.3)[a]	2.1 (3.1)[b]
Established lesion, mean (SD)	0.4 (1.0)[a,b]	0.5 (1.2)[a]	1.5 (5.2)[b]
Microcavitation, mean (SD)	0.3 (1.0)	0.5 (1.1)[a]	0.4 (1.0)
Total caries experience according to WHO criteria (surface level)			
Cavitated caries lesion (d-component of dmf index) Mean (SD)	1.9 (6.1)[a,b]	2.0 (5.8)[a]	1.8 (4.7)[a,b]
Dentin exposure, mean (SD)	0.4 (1.8)[a,b]	0.5 (1.8)[a]	0.8 (1.9)[b]
Large cavity, mean (SD)	1.2 (4.1)	1.1 (3.7)	0.7 (2.9)
Pulp exposure, mean (SD)	0.3 (2.2)[a]	0.4 (2.6)[a]	0.4 (2.3)
Restorations (f-component) Mean (SD)	0.3 (1.3)[a,b]	0.9 (3.2)[a,c]	1.4 (3.3)[b,c]
Extractions (m-component) Mean (SD)	0.3 (2.1)[b]	0.3 (1.9)[c]	1.2 (5.2)[b,c]
dmfs Mean (SD)	2.5 (6.9)[a,b]	3.3 (8.0)[a,c]	4.4 (8.6)[b,c]

[a,b,c]Indicates statistical significance for pairwise comparisons under Wilcoxon signed-rank test (*P*-value <0.05).

caries lesions. This study also calculated adjusted estimates for different types of caries lesions on all surfaces of primary teeth. Despite these advantages, the study had its limitations, such as the difficulty in investigating young children, the challenge of recording noncavitated carious lesions in a field setting in such a young population. The population came from lower socioeconomic status, which might be associated with the cases lost to follow-up, as previously reported[32]. In addition to the consent provided by the children's parents to participate in the Thuringian basic preventive programme, consent to take part in the fluoride varnish study was provided by parents at the beginning of the study. No additional consent was required for the study-related follow-up examinations. In addition, the visiting dentist examined the children while they were at kindergarten. At a later point in time, if parents no longer wanted their children to be a part of this study, they could withdraw from the study by not sending their children to kindergarten on the day of the follow-up examinations. However, this was unlikely, as the parents were unaware of the dates of the follow-up visits. Furthermore, visual caries diagnostics were only used to detect proximal lesions, which are not reliable at these sites[10,34,35]. No additional diagnostic methods, for example bitewing radiographs, laser fluorescence or near-infrared fluorescence, were used because it would be nearly impossible to perform extensive investigations in young preschool children in a field setting. A wider confidence interval range for the estimates (Table 2) among the proximal surfaces might explain the reduced accuracy in these surfaces as there was no tooth separation involved in this study. Several potential confounding factors should be mentioned, such as the difficulty of ensuring an excellent cleaned primary dentition in all 2- to 3-year-olds, motivating each child to cooperate at their best during the dental examination and performing an error-free clinical investigation under field conditions.

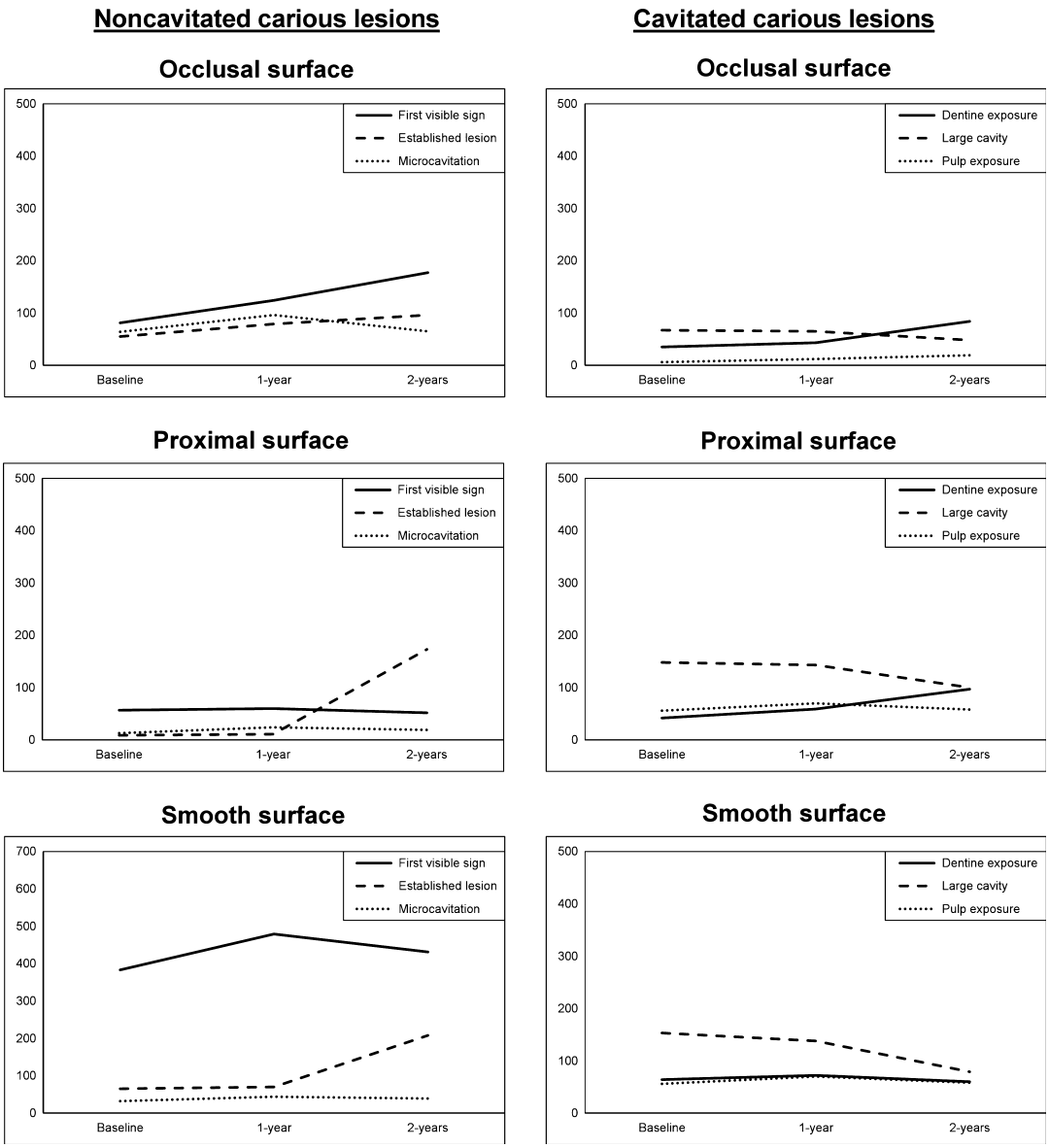

Fig. 1. Distribution of caries lesions on surface level in primary dentition of 2/3-year-old children during 2-year follow-up.

The calibration training emphasized the safeguarding of the quality of recordings, but the previously mentioned source of errors cannot be ignored and may have influenced our findings.

Recently, longitudinal studies have been performed on caries progression among children and young adults[13,15,16,23,34–37], with some targeting the ECC age group[5,10,38,39]. Dirks[22] described that the incidence of caries in mixed dentition could be challenging to evaluate due to teeth erupting at different time points, whereas there are not as many new teeth erupting between the ages of 2 and 3 years (baseline) or during a follow-up 2 years later. We observed that only 125 new teeth erupted during this time frame; therefore, new teeth did not pose any difficulties in the calculation and interpretation of the proportion of caries at each time point. In this study, a total of 72.2% of cavita-

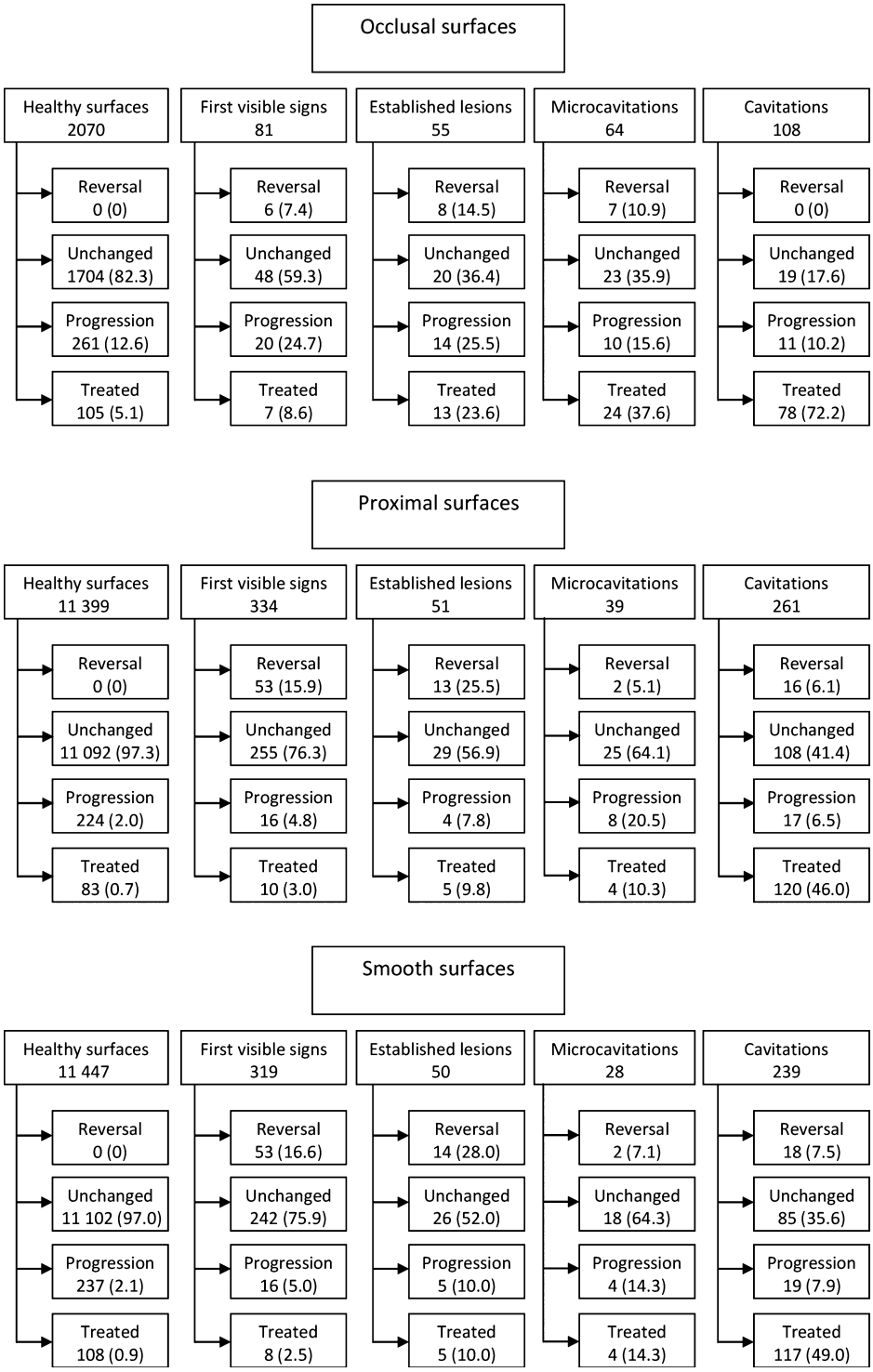

Fig. 2. Surface-related caries transition (absolute number and percentages) in 2/3-year-old children during 2-year follow-up.

Table 2. Adjusted estimates and their corresponding 95% confidence intervals obtained from the linear regression analysis using mixed-effects model for the chance of caries lesions on different surfaces of primary teeth to change

	Occlusal surface		Proximal surface		Smooth surface	
Lesion type	Estimate	95% CI	Estimate	95% CI	Estimate	95% CI
Constant (Intercept)	−0.08	−0.22 to 0.06	−0.49	−0.76 to −0.22	−0.13	−0.43 to 0.16
First visible sign	0.38*	0.20 to 0.57	0.53*	0.17 to 0.88	0.33	−0.06 to 0.71
Established lesion	0.21*	0.02 to 0.40	1.05*	0.70 to 1.41	0.62*	0.23 to 1.01
Microcavitation	0.09	−0.10 to 0.28	0.56*	0.21 to 0.91	0.20	−0.19 to 0.59
Dentin exposure	0.24*	0.05 to 0.43	0.71*	0.36 to 1.06	0.16	−0.23 to 0.55
Large cavity	0.03	−0.16 to 0.21	0.39*	0.04 to 0.74	−0.06	−0.45 to 0.33
Pulp exposure	0.13	−0.06 to 0.31	0.53*	0.18 to 0.89	0.16	−0.23 to 0.55
Fluoride varnish usage	−0.009	−0.11 to 0.09	−0.10	−0.29 to 0.09	−0.09	−0.30 to 0.12

Linear regression analysis was performed employing mixed-effects model with unbalanced design, using type of lesion as the fixed effect, and the patient ID treated as a random effect.
*Indicates statistical significance (P-value <0.05).

tions were treated, which is higher than expected because of the possible inclusion of occlusal surfaces that were filled while restoring proximal lesions. We observed some reversals in cases of noncavitated carious lesions, which could be plausible, but the extent to which such reversals represent misclassifications is unknown. However, there were some reversals of cavitations, and most of these implausible reversals could have been the result of rating highly aesthetic tooth-coloured restorations as healthy surfaces, which highlights the difficulty of scoring caries lesions in field settings using only visual diagnostic methods. Identifying tooth-coloured restorations in 2- to 3-year-old children in a field setting is difficult. In our study, tooth-coloured restorations may have been missed altogether at baseline and at both follow-ups. Although we were unable to quantify the proportions of tooth-coloured restorations that would have been missed altogether, at the baseline and follow-up examinations, the likelihood for this to happen is expected to be very minimal. These issues suggest that sufficient care has to be taken in evaluating tooth-coloured restorations in future research.

The majority of previous studies on this topic assessed either the incidence rates and distributions among the populations or the risk factors for ECC. Although these study designs were sufficient, they did not employ stringent statistical methods to analyse the data, therefore underestimating the presence of any confounders. Many studies have presented the prevalence and incidence rates for caries. In contrast to this popular approach, we used a mixed-effects linear regression model by adjusting for various factors, such as gender, age, quadrant, type of teeth and fluoride varnish usage. Although the sample was derived from a nonrandomized trial, the group (fluoride or control) did not influence the caries progression under the mixed-effects model. Because the difference in the number of teeth between baseline and the 2-year follow-up appointment was minimal (125 new teeth erupted overall) and insignificant and because age and the number of teeth are colinear, we adjusted only for age. We used cross-tabulations between the baseline and 2-year data and present the flow chart of caries transition in Fig. 2, which is a modified version of a previously described matrix approach[40]. This differs from older methods[11,18] in which an increase in the number of caries was calculated by subtracting the number of reversals, which are implausible for studies using the dmf index.

Our findings demonstrate the caries risk of our study population and show a considerable need for preventive efforts in these young children. This study highlights the importance of longitudinal studies and scoring noncavitated caries lesions as early markers in the primary teeth during epidemiological studies. More studies are necessary to study the progression of caries among children in the ECC group.

Conclusion

This study indicated that different types of caries lesion exhibited different rates of either reversal or progression, and some lesion types remained unchanged. This information is crucial for dental practitioners during the decision-making process. Noncavitated caries lesions were the most prevalent type in the 2-year observational period, and

the majority of these lesions were observed on smooth surfaces. The probability of a carious lesion to change or receive treatment increased as the lesion severity increased.

References

1. American Academy of Pediatric Dentistry. Definition of Early Childhood Caries (ECC). Pediatr Dent 2014;36:50–2.
2. Marthaler TM, O'Mullane DM, Vrbic V. The prevalence of dental caries in Europe 1990-1995. Caries Res 1996;30:237–55.
3. Marthaler TM. Changes in dental caries 1953-2003. Caries Res 2004;38:173–81.
4. Petersen PE. World Health Organization global policy for improvement of oral health–World Health Assembly 2007. Int Dent J 2008;58:115–21.
5. Peltzer K, Mongkolchati A, Satchaiyan G, Rajchagool S, Pimpak T. Sociobehavioral factors associated with caries increment: a longitudinal study from 24 to 36 months old children in Thailand. Int J Environ Res Public Health 2014;11:10838–50.
6. Chankanka O, Cavanaugh JE, Levy SM, Marshall TA, Warren JJ, Broffitt B et al. Longitudinal associations between children's dental caries and risk factors. J Public Health Dent 2011;71:289–300.
7. Meurman PK, Pienihakkinen K. Factors associated with caries increment: a longitudinal study from 18 months to 5 years of age. Caries Res 2010;44:519–24.
8. Alm A, Wendt LK, Koch G, Birkhed D, Nilsson M. Caries in adolescence – influence from early childhood. Community Dent Oral Epidemiol 2012;40:125–33.
9. Chankanka O, Marshall TA, Levy SM, Cavanaugh JE, Warren JJ, Broffitt B et al. Mixed dentition cavitated caries incidence and dietary intake frequencies. Pediatr Dent 2011;33:233–40.
10. Chaffee BW, Feldens CA, Rodrigues PH, Vítolo MR. Feeding practices in infancy associated with caries incidence in early childhood. Community Dent Oral Epidemiol 2015 Aug;43:338–48.
11. DePaola PF. Measurement issues in the epidemiology of dental caries. In: Bader JD, editor. Risk assessment in dentistry. Chapel Hill: University of North Carolina Dental Ecology, 1990; 19–26.
12. Jenson L, Budenz AW, Featherstone JD, Ramos-Gomez FJ, Spolsky VW, Young DA. Clinical protocols for caries management by risk assessment. J Calif Dent Assoc 2007;35:714–23.
13. Makhija SK, Gilbert GH, Funkhouser E, Bader JD, Gordan VV, Rindal DB et al. Twenty-month follow-up of occlusal caries lesions deemed questionable at baseline: findings from the National Dental Practice-Based Research Network. J Am Dent Assoc 2014;145:1112–8.
14. Leong PM, Gussy MG, Barrow SY, de Silva-Sanigorski A, Waters E. A systematic review of risk factors during first year of life for early childhood caries. Int J Paediatr Dent 2013;23:235–50.
15. Mao C, Li X, Hu D. [Two-year longitudinal investigation of 733 twelve-year-old children's caries status in Sichuan Province] (article in Chinese). Hua Xi Kou Qiang Yi Xue Za Zhi 2014;32:363–6.
16. Fontana M, Platt JA, Eckert GJ, Gonzalez-Cabezas C, Yoder K, Zero DT et al. Monitoring of sound and carious surfaces under sealants over 44 months. J Dent Res 2014;93:1070–5.
17. Beck JD, Lawrence HP, Koch GG. A method for adjusting caries increments for reversals due to examiner misclassification. Community Dent Oral Epidemiol 1995;23:321–30.
18. Slade GD, Caplan DJ. Methodological issues in longitudinal epidemiologic studies of dental caries. Community Dent Oral Epidemiol 1999;27:236–48.
19. Tubert-Jeannin S, Leger S, Manevy R. Addressing children's oral health inequalities: caries experience before and after the implementation of an oral health promotion program. Acta Odontol Scand 2012;70:255–64.
20. Masood M, Yusof N, Hassan MI, Jaafar N. Longitudinal study of dental caries increment in Malaysian school children: a 5-year cohort study. Asia Pac J Public Health 2012;26:260–7.
21. Wong MC, Lu HX, Lo EC. Caries increment over 2 years in preschool children: a life course approach. Int J Paediatr Dent 2012;22:77–84.
22. Dirks OB. Longitudinal dental caries study in children 9–15 years of age. Arch Oral Biol 1961;6:94–108.
23. Broffitt B, Levy SM, Warren J, Cavanaugh JE. Factors associated with surface-level caries incidence in children aged 9 to 13: the Iowa Fluoride Study. J Public Health Dent 2013;73:304–10.
24. Schroth RJ, Halchuk S, Star L. Prevalence and risk factors of caregiver reported Severe Early Childhood Caries in Manitoba First Nations children: results from the RHS phase 2 (2008-2010). Int J Circumpolar Health 2013;72:21167–76.
25. Kühnisch J, Goddon I, Berger S, Senkel H, Bücher K, Oehme T et al. Development, methodology and potential of the new Universal Visual Scoring System (UniViSS) for caries detection and diagnosis. Int J Environ Res Public Health 2009;6:2500–9.
26. Kühnisch J, Bücher K, Henschel V, Albrecht A, Garcia-Godoy F, Mansmann U et al. Diagnostic performance of the Universal Visual Scoring System (UniViSS) on occlusal surfaces. Clin Oral Investig 2011;15:215–23.
27. Ferreira Zandoná A, Santiago E, Eckert GJ, Katz BP, Pereira de Oliveira S, Capin OR et al. The natural history of dental caries lesions: a 4-year observational study. J Dent Res 2012;91:841–6.
28. Ghazal T, Levy SM, Childers NK, Broffitt B, Cutter GR, Wiener HW et al. Factors associated with early childhood caries incidence among high caries-risk children. Community Dent Oral Epidemiol 2015 Aug;43:366–74.
29. Hedeker D. A mixed-effects multinomial logistic regression model. Stat Med 2003;22:1433–46.
30. Vandenbroucke JP, von Elm E, Altman DG, Gotzsche PC, Mulrow CD, Pocock SJ et al. Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. Int J Surg 2014;12:1500–24.
31. Arbeitslose und Arbeitslosenquote im Jahresdurschnitt nach Kreisen in Thüringen [Unemployed and unemployment rate on average by districts in

Thuringia] (article in German) [internet]. 2008 [cited 2015 Nov 23]. Available from: http://www.statistik.thueringen.de/datenbank/TabAnzeige.asp?tabelle=kr000304%7C%7C

32. Pitchika V, Kokel C, Andreeva J, Crispin A, Hickel R, Kühnisch J et al. Effectiveness of a new fluoride varnish for caries prevention in pre-school children. J Clin Pediatr Dent 2013;38:7–12.
33. WHO. Oral Health Surveys. Basic methods 4th edn. Geneva: World Health Organization, 1997.
34. Ha DH, Spencer AJ, Slade GD, Chartier AD. The accuracy of caries risk assessment in children attending South Australian School Dental Service: a longitudinal study. BMJ Open 2014;4:e004311–8.
35. André Kramer AC, Skeie MS, Skaare AB, Espelid I, Östberg AL. Caries increment in primary teeth from 3 to 6 years of age: a longitudinal study in Swedish children. Eur Arch Paediatr Dent 2014; 15:167–73.
36. Rui T, Yisi Z, Xue L, Deyu H, Tao H. [Three years follow-up observation and analysis of caries status of primary and permanent teeth among 652 6-year-old children in Sichuan Province] (article in Chinese). Hua Xi Kou Qiang Yi Xue Za Zhi 2015; 33:46–9.
37. Kopycka-Kędzierawski DT, Billings RJ. A longitudinal study of caries onset in initially caries-free children and baseline salivary mutans streptococci levels: a Kaplan-Meier survival analysis. Community Dent Oral Epidemiol 2004;32:201–9.
38. Lim S, Tellez M, Ismail AI. Dental caries development among African American children: results from a 4-year longitudinal study. Community Dent Oral Epidemiol 2015;43:200–7.
39. Guedes RS, Piovesan C, Ardenghi TM, Emmanuelli B, Braga MM, Ekstrand KR et al. Validation of visual caries activity assessment: a 2-yr cohort study. J Dent Res 2014;93:101S–7S.
40. Ismail AI, Lim S, Sohn W. A transition scoring system of caries increment with adjustment of reversals in longitudinal study: evaluation using primary tooth surface data. Community Dent Oral Epidemiol 2011;39:61–8.

Chapter 9

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Shear bond strength and microleakage of a new self-etch adhesive pit and fissure sealant

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This study aimed to evaluate the shear bond strength (SBS) and microleakage of a new self-etch adhesive-based fissure sealant (EG) on aprismatic enamel in comparison to conventional fissure sealing with 30 s acid etching (CG). The fissures were sealed according to the manufacturer's instructions. Each group was divided into 3 subgroups: 1-day water storage, 3-month water storage, and 5,000× thermocycling. After measuring SBS using the Ultradent method, failure mode was analyzed. In additional 16 teeth, microleakage was tested using dye penetration method. Pairwise comparisons were analyzed using Mann-Whitney *U*-Test. Multiple linear regression was performed to assess the factors influencing on SBS. EG had significantly lower mean SBS (4.1 MPa±2.1) than the CG (17.6 MPa±6.4). CG (1.1%) performed significantly better than the EG (12.8%) in microleakage analysis. The tested EG yielded significantly inferior results and its clinical use should be decided after weighing its pros and cons.

Keywords: Pit and fissure sealant, Shear-bond test, Self-etch adhesive, Acid etching, *In vitro* testing

INTRODUCTION

Caries is diagnosed frequently in pits and fissures on permanent molars due to their anatomical characteristics and plaque-retentive nature[1,2]. Therefore, preventive pit and fissure sealing is clinically recommended, particularly in young patients with caries activity, and have been assessed as caries-preventive procedures[3]. Various sealing materials/procedures have been proposed for clinical use over the decades. As a measure of performance, the retention rates of resin-based materials are superior to those of other sealants such as glass-ionomer cements[3-5]. Clinical usage requires simple and less-time consuming procedures, particularly in children. The application of self-etching adhesives instead of acid etching might simplify the clinical workflow, reduce application times and achieve acceptable cooperation from children. Most recently, a new product (BeautiSealant, Shofu, Kyoto, Japan) featuring reduced working time and fluoride-releasing and pH-buffering properties was released on the dental market[6-8]. Stringent testing of new materials is imperative before formulating clinical recommendations. Shear bond strength (SBS) and microleakage assessment are frequently used to evaluate sealant materials in the laboratory[9]. No study has evaluated the adhesion properties of this new material to predict the survival of the material clinically. Therefore, this study aimed to study the SBS and microleakage of the new sealant in relation to acid etching. The null hypothesis of this study was that there is no difference in SBS and microleakage between the tested material and the conventional pit and fissure sealant, which served as a control group.

MATERIALS AND METHODS

For this study, 60 healthy, caries-free extracted human third molars were used. The teeth were free of development disorders, fillings, and fissure sealants and showed complete root development. After extraction, the teeth were stored in sodium azide solution (0.2%). Before using the teeth for this study, all teeth were cleaned of gross debris and air-dried. All roots were sectioned off 1 mm apical to the cemento-enamel junction, and the crowns were sectioned on 4 surfaces (mesial, distal, lingual, and buccal) with a diamond disc (Dental Diamond Disc, H 340-F-300, HORICO, Berlin, Germany), resulting in 240 tooth surfaces. Specimens were randomly assigned to each group (n=120) and stored in distilled water. All tooth surfaces were embedded in cold-curing methyl methacrylate resin (Technovit 4004, Heraeus Kulzer, Wehrheim, Germany). Each tooth surface was strictly aligned horizontally in the embedding material. All specimens were numbered according to the randomization table. After embedding, all tooth surfaces were cleaned and rinsed with water spray. Only unprepared specimens (prismless enamel) were used in this study to simulate the clinical situation of fissure sealing (Fig. 1).

Experimental group (EG) —Self-etch adhesive prior sealant application

The *in vitro* application of the sealant material strictly followed the clinical recommendations of the manufacturer. This self-etching/self-adhesive sealant

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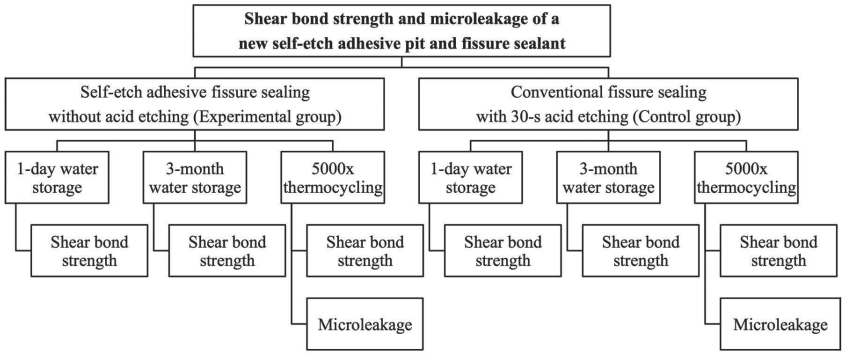

Fig. 1 An overview of the investigated sealants for the applied methods.

(BeautiSealant Paste, Shofu, LOT 021301) obviates additional acid etching. In this case, the tooth surface was rinsed with water spray and dried with water- and oil-free air. Care was taken to ensure that the enamel surface was not over-dried. The self-etching adhesive (BeautiSealant Primer, Shofu) was brushed onto the tooth surface with an applicator tip and allowed to set for 5 s. Subsequently, the adhesive was dried thoroughly into a thin, smooth and glossy film using a gentle stream of air. In the next step, a cylindrical plastic mould (Button Mould Insert, ISO 29022, Ultradent Products, South Jordan, UT, USA) in a bonding clamp (ISO 29022, Ultradent Products) was placed gap-free on a flat area of the tooth surface, and the fissure sealant was applied onto the surface. To avoid outflow of the fissure sealing material, a light-cured resin barrier (OpalDam, Ultradent Products) was applied at the outside of the plastic mould before applying the material. Then, the fissure sealant was applied in the mould and light-cured for 20 s with a polymerization light (Bluephase, 1,200 mW/cm^2, wavelength 385–515 nm, Ivoclar Vivadent, Schaan, Liechtenstein). The plastic mould and resin barrier were carefully removed. The final sealant cylinder on the tooth surface was 2.38 mm in diameter, equal to the standardized diameter of the cylindrical plastic mould required by ISO 29022[10].

Control group (CG) —Conventional fissure sealing with 30-s acid etching

The tooth surface was initially rinsed with water spray and air-dried with water- and oil-free air. Next, the enamel surface was etched with 37% phosphoric acid gel (Total Etch, Ivoclar Vivadent) for 30 s. The tooth surface was rinsed with water spray and dried with pressured air for 5 s until a chalky-white enamel surface was visible. The fissure sealing material (Helioseal F, Ivoclar Vivadent, LOT S03724) was applied through the plastic mould in a layer of 2.38 mm and light-cured as indicated above, resulting in a sealant cylinder on the tooth surface.

All specimens were checked for proper configuration, and specimens with bubbles and/or outflow sealing material were discarded. There were no quality deficits after treatment and no initial loss of sealant cylinders prior to SBS testing.

Alteration of the specimens

Each group was divided into 3 subgroups (n=40), which were altered using different protocols: a) 1-day storage in distilled water at 37°C in a thermal oven (Jouan EU3, INNOVENS Ovens, ThermoFisher Scientific, Waltham, MA, USA); b) 3 months storage in distilled water at 37°C in a thermal oven; and c) 1-day storage in distilled water at 37°C followed by a thermocycling bath (Haake W15, Thermo Haake, Karlsruhe, Germany) between 5(±2) and 55(±2)°C for 5,000 cycles with a dwell time of 30 s and a transfer time of 5 s.

SBS

A standardized Ultradent-method (Notched-edge SBS test, ISO 29022) was employed to test the SBS. After specimen storage, the SBS test was performed in a universal testing machine (MCE 2000ST, Quicktest Prüfpartner, Langenfeld, Germany) at a crosshead speed of 1 mm/min. The specimens were aligned in a metal sample holder (Test base clamp, ISO 29022, Ultradent Products) with the occlusal tooth surface facing down ("crown down"). The notched-edge shear fixture (Notched-edge crosshead assembly, ISO 29022, Ultradent Products) with the semi-circular moulded shear blade (Notched-edge shear blade, ISO 29022, Ultradent Products) mounted to the universal testing machine and placed over the sealant cylinder on the aligned specimen. The semi-circular moulded shear blade was positioned exactly over the sealant cylinder and force-fitted without premature contact to ensure that the load was applied directly to the sealant cylinder. A constant crosshead speed of 1 mm/min was applied until the material failed. The maximum force (N) until failure was recorded. The SBS was calculated based on the bonding area of the fissure sealant on the tooth surface and is expressed in MPa.

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Failure mode analysis
The failure modes of all specimens were examined using a stereomicroscope (Stemi SV11, Carl Zeiss, Jena, Germany) with 20-fold magnification. Failures were classified as 1) adhesive failure (complete debonding of material); 2) cohesive failure within the material; 3) mixed failure (partial adhesive and cohesive within material); and 4) enamel failure. The operator was blinded to the tested group, and the failure mode tests were performed and scored based on the randomization number of the specimen. The data management personnel reorganized the values into the investigated groups based on their identification numbers.

Microleakage
Eight human third molars were assigned to each of the 2 groups (EG: 80 sections; CG: 104 sections). All teeth were stored and cleaned as previously described. Each tooth was taken as a whole, and fissure sealing on the prismless enamel of the occlusal fissure pattern was performed in strict accordance with the manufacturer's instructions (see above). All specimens were stored in distilled water at 37°C for 24 h in a thermal oven (Jouan EU3, INNOVENS Ovens, ThermoFisher Scientific). In the next step, the specimens were aged in a thermocycling bath (Haake W15, Thermo Haake) between 5(±2) and 55(±2)°C for 5,000 cycles, with a 30 s dwell time and a transfer time of 5 s. After thermocycling, the root surfaces were isolated with tacky wax (Boxing Wax Sticks, Kerr, Romulus, MI, USA). Afterwards, the entire tooth surface was covered with two layers of nail varnish, except the area within 1 mm of the fissure sealing. The varnish was applied to avoid dye penetration to other parts of the tooth. The specimens were then immersed in 0.5% basic fuchsine solution for 24 h at 37°C. All specimens were rinsed with water, and the roots were sectioned off 1 mm below the cemento-enamel junction with a diamond disc (Dental Diamond Disc, H 340-F-300, HORICO). The tooth crowns were then fully embedded in cold-curing methyl methacrylate resin (Technovit 4004, Heraeus Kulzer). This treatment resulted in a rectangular block of approximately 2.5×1.2×0.8 cm for each tooth. The blocks were fixed in a sectioning saw (Isomet Low Speed Saw, Buehler, Lake Bluff, IL, USA) with a diamond blade (Diamond Blade, Leco, St. Joseph, MI, USA), and the crowns were sectioned (in a buccolingual direction) into at least 5 slices, each with a thickness of 1 mm. The front and back of each slice were inspected, resulting at least 10 available section sides per tooth. The side analysis was performed using a stereomicroscope (Stemi SV11, Carl Zeiss) with a 20-fold magnification. Every side was photographed with a digital single-lens reflex camera. The following picture analysis methodically separated all sides without dye penetration and then collected all section sides with dye penetration. Additionally, quality losses, such as dye penetration at sealant fractures, detachment of sealant, and defects of the fissure sealant, were recorded. If dye penetration was present, each side was quantitatively measured in relation to the total length of the interface between the enamel and the sealant. All measurements were performed with the imaging software ImageJ (Version 1.47, Wayne Rasband, National Institutes of Health, Bethesda, MD, USA). The percentage of microleakage was calculated. Microleakage was ruled out for dye penetration through enamel, dentine or fissure sealant cracks or along the cemento-enamel junction.

Statistical analyses
A formal sample size calculation was performed using 80% power, a 95% confidence level and a two-tailed significance level of 5%. The least expected mean difference in the SBS between the two groups was set at 3 MPa with a variance of 20 and 25 for the two groups, respectively. This resulted in a sample size of 40 specimens per group (10 pieces each from mesial, buccal, lingual and distal). Furthermore, to avoid the influence of some teeth on the results, we randomized the pieces such that no piece of a tooth was repeated more than once in a group. The descriptive and explorative data analyses were performed using R, version 3.3.1 (R Core Team, 2016). Descriptive statistics for SBS and microleakage were calculated and presented as the mean and standard deviation or as percentages, respectively. Because the data were not normally distributed, pair-wise comparisons with respect to the material and the technique employed were performed using the Mann-Whitney *U*-Test. Furthermore, multiple linear regression was performed to study the influence of different factors such as material (EG and CG), alteration technique (1-day water storage, 3-month water storage and 5,000× thermocycling) and tooth surface (mesial, buccal, lingual and distal) on the SBS. The adjusted estimates, their standard errors and significance values were calculated. A two-tailed α significance level of 0.05 and a 95% confidence level were used for all analyses. Another linear regression model was performed including an interaction term between the material and alteration technique; but the results were not significant and thus are not reported.

RESULTS

The results of the SBS test are shown in Table 1. The SBS decreased slightly in both groups after 3-month water storage and significantly after 5,000 cycles of thermocycling. The SBS in the EG was significantly lower and ranged between 3.2 and 4.6 MPa, whereas the SBS in the CG ranged from 15.6 to 19.1 MPa. Further analysis using multiple linear regression models revealed significant influences of both factors on the SBS (Table 2). The mean SBS estimate was 3.9 MPa for the intercept and 13.6 MPa for the CG. The linear model indicated that 3-month water storage had no significant deteriorating effect compared to that of 1-day water storage. However, 5,000 cycles of thermocycling significantly reduced the SBS by −2.5 MPa on average. The surface was a significant factor but had minimal influence on the SBS. The evaluation of the failure mode analysis is presented in Table 3. Adhesive failure

Table 1 Shear bond strength for the tested sealant materials (EG and CG)

Tested sealant	Shear Bond Strength in MPa Mean (standard deviation) Min–Max		
	1-day water storage	3-month water storage	5,000 cycles of thermocycling
Fissure sealing with self-etch adhesive (EG)	4.6 (1.6)[1,a] 2.0–8.4	4.4 (2.2)[b] 0.2–11.8	3.2 (2.0)[1,c] 0.1–7.4
Fissure sealing with 30 s acid etching (CG)	19.1 (6.2)[1,a] 8.8–35.3	18.2 (7.5)[b] 5.1–30.3	15.6 (4.4)[1,c] 6.1–22.3

[1] indicates statistically significant difference between 1-day water storage and 5,000×thermocycling ($p<0.05$)
[a, b, c] indicates statistically significant difference between the linked groups ($p<0.05$)

Table 2 Multiple linear regression results for the influence of material, alteration and surface on the shear bond strength of the fissure sealants

Factor	Factor level	Estimate for SBS in MPa	Standard error	p
Reference	—	3.9	0.8	—
Material	CG	13.6	0.6	<0.001*
Alteration	3-month water storage	−0.6	0.7	0.002*
	5,000 cycles of thermocycling	−2.5		
Surface	Buccal	0.6	0.8	0.01*
	Lingual	2.6		
	Distal	1.5		

The estimate at the reference level corresponds to the EG under 1-day water storage on the mesial surface
* indicates significance under multiple linear regression analysis ($p<0.05$)

Table 3 Failure mode analysis of the tested sealant materials (EG and CG) following the shear bond strength measurements

Tested sealant	Type of failure	Failure mode analysis N (%)		
		1-day water storage	3-month water storage	5,000 cycles of thermocycling
Fissure sealing with self-etch adhesive (EG)	Adhesive	40 (100)	40 (100)	40 (100)
	Cohesive	—	—	—
	Mixed	—	—	—
	Enamel	—	—	—
Fissure sealing with 30 s acid etching (CG)	Adhesive	31 (77.5)	32 (80.0)	38 (95.0)
	Cohesive	—	—	—
	Mixed	9 (22.5)	7 (17.5)	2 (5.0)
	Enamel	—	1 (2.5)	—

was the only cause of failure in the EG and the most predominant failure type in the CG, ranging between 77.5 and 95%, followed by mixed failures, which ranged between 5 and 22.5%.

Table 4 illustrates the results from the microleakage analyses. Although the observed number of sites with any quality loss was similar in the EG and the CG, the mean microleakage was significantly higher in the EG (12.8%) than that in the CG (1.1%). The highest amount of microleakage observed was 95.9% in the EG but 36.1% in the CG.

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Table 4 Microleakage of the tested sealant materials (EG and CG) after 5,000 cycles of thermocycling

Microleakage	Fissure sealing with self-etch adhesive (EG)	Fissure sealing with 30 s acid etching (CG)
Number of teeth, N	8	8
Number of available tooth specimens, N	80	104
Surfaces with no dye penetration, N	58	83
Surfaces with dye penetration, N	22	19
Surfaces with dye penetration at sealant fractures, N	5	2
Detachment of fissure sealant	—	—
Sites with any quality loss, N (%)	27 (33.8%)	21 (20.2%)
Microleakage, mean % (SD)	12.8% (±26.8)*	1.1% (±3.9)*
Range of microleakage, min–max	0–95.9%	0–36.1%

* indicates statistically significant difference ($p<0.05$)

DISCUSSION

Based on our findings, the hypothesis that the tested sealant performs equally well is rejected. Within the limitations of this study, the performance of the newly developed self-etching fissure sealant in the SBS and microleakage tests was significantly inferior compared to that of conventional fissure sealing with 30 s of phosphoric acid etching. This result was consistent for different alteration procedures (Tables 1 and 2), and 5,000 cycles of thermocycling degraded the SBS most aggressively, by nearly −2.5 MPa. The SBS for the EG ranged from only 3.2 to 4.6 MPa, whereas in the CG, values were registered between 15.6 and 19.1 MPa. These findings are somewhat consistent with previously published studies of self-etching/self-adhesively bonded sealant materials. Several studies have reported results within the same order of magnitude or lower SBS values[11-13]. To further confirm the results, multiple linear regression was performed and yielded similar estimates for the two alteration methods (Table 2). Although these new self-etching/self-adhesive sealants have the potential to simplify the clinical workflow, the bonding ability under laboratory conditions suggests reduced longevity in clinical practice, particularly with this experimental material. Therefore, the primer component of the experimental sealant material might be replaced by conventional acid etching. An *in vitro* study of a similar material observed improved performance when the acid etching step was included prior to fissure sealant application[13], but such a modification of the protocol was not assessed in the EG in the present study. Moreover, 100% of all failures in the EG were due to adhesive failures, which is an indicator of reduced adhesive performance.

Similar to the results of the SBS analysis, the microleakage results in the EG were inferior (12.8%); in contrast, in the CG, little dye infiltration was observed (1.1%). A similar trend has been reported in the literature, in which a few studies have observed microleakage for the self-etch adhesive sealants in a range closer to that of acid-etch sealants[14,15]. However, extremely high microleakage values greater than those observed in our study have also been reported[16]. Few studies have assessed its additional fluoride-releasing property[6] and pH buffering mechanism[7,8]. Though these cariostatic properties were not measured in this study, its use in clinical settings has to be decided based on weighing both its physical and cariostatic properties.

The methodological strengths of our study include the use of an equal sample size of 40 specimens per group and randomization within a group to avoid repeating analysis of the same specimen. To simulate the clinical situation of teeth for fissure sealing, we used aprismatic enamel because ground prismatic surface would lead to a flat surface, which is ideal for bonding but does not reflect the conditions of fissure sealing. All groups were consistently subjected to three types of alterations, such as 1-day water storage, 3-month water storage and 5,000 cycles of thermocycling. With this approach, we tested the material under various types of stress exposures that might be encountered in the oral cavity. 1-day (frequently used control) and 3-month water storage helps to assess the hydrolytic degradation. Thermocycling was performed to assess the effect of thermal stress which is usually encountered in the oral cavity. To test this physical characteristic in adhesive materials, a range of 250 to 2,500 thermocycling cycles is generally used[9,17,18]. Contrary to this "standard", we followed the stringent method of subjecting the material to 5,000 cycles of thermocycling, which might indicate a long-term perspective on the longevity of the investigated sealant material. Further, we used a notch-edge shear technique, the most the recently recommended standardized method according to the International Organization for Standardization[10].

CONCLUSION

New materials in the field of dentistry are essential, as are stringent quality control and testing of these materials. The tested self-etch adhesive pit and fissure sealant had roughly four-fold lower SBS, and the microleakage was ten-fold higher compared to that of the control group. Reduced SBS coupled with increased microleakage, as observed in this study, is an indicator that the performance of a fissure sealant is doubtful. Within the limitations of this study, the tested material failed in the physical characteristics to that of the control group, and thus its usage in clinical practice cannot be fully recommended.

REFERENCES

1) Kühnisch J, Galler M, Seitz M, Stich H, Lussi A, Hickel R, Kunzelmann KH, Bücher K. Irregularities below the enamel-dentin junction may predispose for fissure caries. J Dent Res 2012; 91: 1066-1070.
2) Hannigan A, O'Mullane DM, Barry D, Schäfer F, Roberts AJ. A caries susceptibility classification of tooth surfaces by survival time. Caries Res 2000; 34: 103-108.
3) Ahovuo-Saloranta A, Forss H, Hiiri A, Nordblad A, Mäkelä M. Pit and fissure sealants versus fluoride varnishes for preventing dental decay in the permanent teeth of children and adolescents. Cochrane Database Syst Rev 2016; 1: Cd003067.
4) Tinanoff N, Coll JA, Dhar V, Maas WR, Chhibber S, Zokaei L. Evidence-based update of pediatric dental restorative procedures: Preventive strategies. J Clin Pediatr Dent 2015; 39: 193-197.
5) Welbury R, Raadal M, Lygidakis NA. Eapd guidelines for the use of pit and fissure sealants. Eur J Paediatr Dent 2004; 5: 179-184.
6) Dionysopoulos D, Sfeikos T, Tolidis K. Fluoride release and recharging ability of new dental sealants. Eur Arch Paediatr Dent 2015; 17: 45-51.
7) Ushimura S, Nakamura K, Matsuda Y, Minamikawa H, Abe S, Yawaka Y. Assessment of the inhibitory effects of fissure sealants on the demineralization of primary teeth using an automatic ph-cycling system. Dent Mater J 2016; 35: 316-324.
8) Kaga M, Kakuda S, Ida Y, Toshima H, Hashimoto M, Endo K, Sano H. Inhibition of enamel demineralization by buffering effect of s-prg filler-containing dental sealant. Eur J Oral Sci 2014; 122: 78-83.
9) Chiang ML, Birlbauer S, Lo YF, Pitchika V, Crispin A, Ilie N, Hickel R, Kühnisch J. Which factors influence the shear bond strength of sealant materials? J Adhes Dent 2016; 18: 397-404.
10) International Organisation for Standardisation. Iso 29022. Dentistry-adhesion-notched-edge shear bond strength test; 2013.
11) Wadenya RO, Yego C, Blatz MB, Mante F. Bond strength and microleakage of a new self-etch sealant. Quintessence Int 2009; 40: 559-563.
12) Dhillon JK, Pathak A. Comparative evaluation of shear bond strength of three pit and fissure sealants using conventional etch or self-etching primer. J Indian Soc Pedod Prev Dent 2012; 30: 288-292.
13) Margvelashvili M, Vichi A, Carrabba M, Goracci C, Ferrari M. Bond strength to unground enamel and sealing ability in pits and fissures of a new self-adhering flowable resin composite. J Clin Pediatr Dent 2013; 37: 397-402.
14) Javadinejad S, Razavi M, Birang R, Atefat M. In vitro study of microleakage of different techniques of surface preparation used in pits and fissures. Indian J Dent Res 2012; 23: 247-250.
15) Bassir L, Khanehmasjedi M, Nasr E, Kaviani A. An in vitro comparison of microleakage of two self-etched adhesive and the one-bottle adhesive used in pit and fissure sealant with or without saliva contamination. Indian J Dent Res 2012;23: 806-810.
16) Schuldt C, Birlbauer S, Pitchika V, Crispin A, Hickel R, Ilie N, Kühnisch J. Shear bond strength and microleakage of a new self-etching/self-adhesive pit and fissure sealant. J Adhes Dent 2015; 17: 491-497.
17) Leloup G, D'Hoore W, Bouter D, Degrange M, Vreven J. Meta-analytical review of factors involved in dentin adherence. J Dent Res 2001; 80: 1605-1614.
18) Heintze SD, Zimmerli B. Relevance of in vitro tests of adhesive and composite dental materials. A review in 3 parts. Part 3: In vitro tests of adhesive systems. Schweiz Monatsschr Zahnmed 2011; 121: 1024-1040.

Bibliography

1. Ahovuo-Saloranta A., Forss H., Hiiri A., Nordblad A., and Mäkelä, M. Pit and fissure sealants versus fluoride varnishes for preventing dental decay in the permanent teeth of children and adolescents. The Cochrane Database of Systematic Reviews 2016;1:Cd003067.

2. Ainamo, J., Barmes, D., Beagrie, G., Cutress, T., Martin, J., and Sardo-Infirri, J. Development of the World Health Organization (WHO) Community Periodontal Index of Treatment Needs (CPITN). International Dental Journal 1982;32:281–91.

3. Akbaraly, T.N., Hamer, M., Ferrie, J.E., Lowe, G., Batty, G.D., Hagger-Johnson, G., Singh-Manoux, A., Shipley, M.J., and Kivimki, M. Chronic inflammation as a determinant of future aging phenotypes. Canadian Medical Association Journal 2013;185:E763–70.

4. Altman D.G. Statistics and ethics in medical research. VIII-Improving the quality of statistics in medical journals. British Medical Journal 1981;282:44–7.

5. American Academy of Pediatric Dentistry. Policy on Early Childhood Caries (ECC): Classifications, consequences, and preventive strategies. Pediatric Dentistry 2016;38:52–54.

6. Aulin J., Siegbahn A., Hijazi Z., Ezekowitz M.D., Andersson U., Connolly S.J., Huber K., Reilly P.A., Wallentin L., and Oldgren J. Interleukin-6 and C-reactive protein and risk for death and cardiovascular events in patients with atrial fibrillation. American Heart Journal 2015;170:1151–60.

7. Azizi, A., Sarlati F., Bidi M., Mansouri L., Azaminejad S.M., and Rakhshan V. Effects of smoking severity and moderate and severe periodontitis on serum C-

reactive protein levels: an age- and gender-matched retrospective cohort study. Biomarkers 2015;20:306–12.

8. Berg, A.H., and Scherer, P.E. Adipose tissue, inflammation, and cardiovascular disease. Circulation Research 2005;96:939–49.

9. Bohaty B.S., Ye Q., Misra A., Sene F., and Spencer P. Posterior composite restoration update: Focus on factors influencing form and function. Clinical, Cosmetic and Investigational Dentistry 2013;5:33–42.

10. Brunthaler A., Konig F., Lucas T., Sperr W., and Schedle A. Longevity of direct resin composite restorations in posterior teeth. Clinical Oral Investigations 2003;7:63–70.

11. Bryant R.W. Long-term implications for composite resin restorations. Annals of the Royal Australasian College of Dental Surgeons 1989;10:84–90.

12. Bücher K., Metz I., Pitchika V., Hickel R., and Kühnisch J. Survival characteristics of composite restorations in primary teeth. Clinical Oral Investigations 2014;19:1653–62.

13. Buerkle V., Kühnisch J., Guelmann M., and Hickel R. Restoration materials for primary molars-results from a European survey. Journal of Dentistry 2005;33:275–81.

14. Bulló, M., García-Lorda, P., Megias, I., and Salas-Salvadó, J. Systemic inflammation, adipose tissue tumor necrosis factor, and leptin expression. Obesity Research 2003;11:525–31.

15. Carskadon M.A., and Acebo C. A self-administered rating scale for pubertal development. The Journal of Adolescent Health 1993;14:190–195.

16. Chiang M.L., Birlbauer S., Lo Y.F., Pitchika V., Crispin A., Ilie N., Hickel R., and Kühnisch J. Which Factors Influence the Shear Bond Strength of Sealant Materials? Journal of Adhesive Dentistry 2016;18:397–404.

17. Clark G.T., and Mulligan R. Fifteen common mistakes encountered in clinical research. Journal of Prosthodontic Research 2011;55:1–6.

18. Da Rosa Rodolpho P.A., Donassollo T.A., Cenci M.S., Loguercio A.D., Moraes R.R., Bronkhorst E.M., Opdam N.J., and Demarco F.F. 22-Year clinical evaluation of the performance of two posterior composites with different filler characteristics. Dental Materials 2011;27:955–63.

19. Danesh J., Wheeler J.G., Hirschfield G.M., Eda S., Eiriksdottir G., Rumley A., Lowe G.D.O., Pepys M.B., and Gudnason V. C-Reactive Protein and Other Circulating Markers of Inflammation in the Prediction of Coronary Heart Disease. The New England Journal of Medicine 2004;350:1387–1397.

20. Dionysopoulos D., Sfeikos T., and Tolidis K. Fluoride release and recharging ability of new dental sealants. European Archives of Paediatric Dentistry 2015.

21. Dye B.A. Global periodontal disease epidemiology. Periodontology 2000 2012;58:10–25.

22. García-Godoy F., and Donly K.J. Dentin-enamel adhesives in pediatric dentistry: an update. Pediatric Dentistry 2015;37:133–5.

23. Gardenier J.S., and Resnik D.B. The misuse of statistics: Concepts, tools, and a research agenda. Accountability in Research 2002;9:65–74.

24. Giannini M., Makishi P., Ayres A.P., Vermelho P.M., Fronza B.M., Nikaido T., and Tagami J. Self-etch adhesive systems: a literature review. Brazilian Dental Journal 2015;26:3–10.

25. Global Oral Health Data Bank. 2002. URL: `http://www.who.int/oral_health/databases/en/`.

26. Hannigan A., O'Mullane D.M., Barry D., Schäfer F., and Roberts A.J. A caries susceptibility classification of tooth surfaces by survival time. Caries Research 2000;34:103–8.

27. Hickel R., and Manhart J. Longevity of restorations in posterior teeth and reasons for failure. Journal of Adhesive Dentistry 2001;3:45–64.

28. Jayaratne Y.S., and Zwahlen R.A. The evolution of dental journals from 2003 to 2012: A bibliometric analysis. PLoS One 2015;10:e0119503.

29. Jenkins W.M., and Papapanou P.N. Epidemiology of periodontal disease in children and adolescents. Periodontology 2000 2001;26:16–32.

30. Kaga M., Kakuda S., Ida Y., Toshima H., Hashimoto M., Endo K., and Sano H. Inhibition of enamel demineralization by buffering effect of S-PRG filler-containing dental sealant. European Journal of Oral Sciences 2014;122:78–83.

31. Kaneko H., Anzai T., Nagai T., Anzai A., Takahashi T., Mano Y., Morimoto K., Maekawa Y., Itoh H., Yoshikawa T., Ogawa S., and Fukuda K. Human C-reactive protein exacerbates metabolic disorders in association with adipose tissue remodelling. Cardiovascular Research 2011;91:546–55.

32. Kim J.S, Kim D-K, and Hong S.J. Assessment of errors and misused statistics in dental research. International Dental Journal 2011;61:163–167.

33. Krithikadatta J., and Valarmathi S. Research methodology in dentistry: Part II - The relevance of statistics in research. Journal of Conservative Dentistry 2012;15:206–13.

34. Kromeyer-Hauschild K., Wabitsch M., Kunze D., Geller F., Geiss C.H., Hesse V., von Hippel A., Jaeger U., Johnsen D., Korte W., Menner K., Müller G., Müller M.J., Niemann-Pilatus A., Remer T., Schaefer F., Wittchen H.-U., Zabransky S., Zellner K., Ziegler A., and Hebebrand J. Perzentile fuer den Body-mass-Index fr das Kindes- und Jugendalter unter Heranziehung verschiedener deutscher Stichproben [Percentiles of body mass index in children and adolescents evaluated from different regional German studies] (article in German). Monatsschrift Kinderheilkunde 2001;149:807–818.

35. Kühnisch J., Galler M., Seitz M., Stich H., Lussi A., Hickel R., Kunzelmann K.H., and Bücher, K. Irregularities below the enamel-dentin junction may predispose for fissure caries. Journal of Dental Research 2012;91:1066–70.

36. Kühnisch J., Goddon I., Berger S., Senkel H., Bücher K., Oehme T., Hickel R., and Heinrich-Weltzien R. Development, methodology and potential of the new Universal Visual Scoring System (UniViSS) for caries detection and diagnosis. International Journal of Environmental Research and Public Health 2009;6:2500–9.

37. Last J.M. In: *Dictionary of Epidemiology*. 4th ed. New York: Oxford University Press, 2001.

38. Loos, B.G. Systemic markers of inflammation in periodontitis. Journal of Periodontology 2005;76:2106–15.

39. Loos, B.G., Craandijk, J., Hoek, F.J., Wertheim-van Dillen, P.M., and van der Velden, U. Elevation of systemic markers related to cardiovascular diseases in the peripheral blood of periodontitis patients. Journal of Periodontology 2000;71:1528–34.

40. Mackenzie L., Burke F.J., and Shortall A.C. Posterior composites: A practical guide revisited. Dental Update 2012;39:211–2, 215–6.

41. Mackenzie L., Parmar D., Shortall A.C., and Burke F.J. Direct anterior composites: a practical guide. Dental Update 2013;40:297–9, 301–2, 305–8 passim.

42. Manhart J., Chen H., Hamm G., and Hickel R. Buonocore Memorial Lecture. Review of the clinical survival of direct and indirect restorations in posterior teeth of the permanent dentition. Operative Dentistry 2004;29:481–508.

43. Marthaler T.M. Changes in dental caries 1953-2003. Caries Research 2004;38:173–81.

44. Moreira R. Epidemiology of Dental Caries in the World. In: *Oral Health Care - Pediatric Research, Epidemiology and Clinical Practices*. Ed. by Virdi MS. Rijeka: InTech, 2012. Chap. 8:149–168.

45. Nichani A.S. Reporting guidelines for research. Journal of Indian Society of Periodontology 2015;19:485.

46. Opdam N.J., Bronkhorst E.M., Loomans B.A., and Huysmans M.C. 12-year survival of composite vs. amalgam restorations. Journal of Dental Research 2010;89:1063–7.

47. Oredugba F., and Ayanbadejo P. Gingivitis in Children and Adolescents. In: *Oral Health Care - Pediatric Research, Epidemiology and Clinical Practices*. Ed. by Virdi MS. Rijeka: InTech, 2012. Chap. 4:69–86.

48. Pallesen U., van Dijken J.W., Halken J., Hallonsten A.L., and Hoigaard R. A prospective 8-year follow-up of posterior resin composite restorations in permanent teeth of children and adolescents in Public Dental Health Service: Reasons for replacement. Clinical Oral Investigations 2014;18:819–27.

49. Perdigão J. New developments in dental adhesion. Dental Clinics of North America 2007;51:333–57, viii.

50. Petersen P.E. The World Oral Health Report 2003: Continuous improvement of oral health in the 21st century - The approach of the WHO Global Oral Health-Programme. Community Dentistry and Oral Epidemiology 2003;31(Suppl 1):3–24.

51. Petersen P.E. World Health Organization global policy for improvement of oral health–World Health Assembly 2007. International Dental Journal 2008;58:115–21.

52. Petersson G.H., and Bratthall D. The caries decline: a review of reviews. European Journal of Oral Sciences 1996;104:436–443.

53. Peumans M., De Munck J., Mine A., and Van Meerbeek B. Clinical effectiveness of contemporary adhesives for the restoration of non-carious cervical lesions. A systematic review. Dental Materials 2014;30:1089–103.

54. Pitchika V., Kokel C. J., Andreeva J., Crispin A., Hickel R., Kühnisch J., and Heinrich-Weltzien R. Effectiveness of a new fluoride varnish for caries prevention in pre-school children. Journal of Clinical Pediatric Dentistry 2013;38:7–12.

55. Pitts, N.B. Detection, assessment, diagnosis and monitoring of caries. Monographs in Oral Science. Vol 21. Basel (Switzerland): Karger, 2009.

56. Ridker P.M., Hennekens C.H., Buring J.E., and Rifai N. C-Reactive Protein and Other Markers of Inflammation in the Prediction of Cardiovascular Disease in Women. The New England Journal of Medicine 2000;342:836–843.

57. Sabiston, C., Castonguay, A., Barnett, T., O'Loughlin, J., and Lambert, M. Body image and C-reactive protein in adolescents. International Journal of Obesity 2009;33:597–600.

58. Sampaio C.S., Rodrigues R.V., Souza-Junior E.J., Freitas A.Z., Ambrosano G., Pascon F.M., and Rontani R.P. Effect of restorative system and thermal cycling on the tooth-restoration interface - OCT evaluation. Operative Dentistry 2015.

59. Shaddox, L.M., Wiedey, J., Calderon, N.L., Magnusson, I., Bimstein, E., Bidwell, J.A., Zapert, E.F., Aukhil, I., and Wallet, S.M. Local inflammatory markers and systemic endotoxin in aggressive periodontitis. Journal of Dental Research 2011;90:1140–4.

60. Shetty A.C. Essentials of Statistics in Dental Research: Part 1. IJSS Case Reports & Reviews 2015;2:35–38.

61. Skelly A.C., Dettori J.R., and Brodt E.D. Assessing bias: the importance of considering confounding. Evidence-Based Spine-Care Journal 2012;3:9–12.

62. Strasak A.M., Zaman Q., Marinell G., Pfeiffer K.P., and Ulmer H. The use of statistics in medical research: A comparison of "The New England Journal of Medicine" and "Nature Medicine". The American Statistician 2007;61:47–55.

63. Stringhini, S., Batty, G.D., Bovet, P., Shipley, M.J., Marmot, M.G., Kumari, M., Tabak, A.G., and Kivimäki, M. Association of lifecourse socioeconomic status with chronic inflammation and type 2 diabetes risk: the Whitehall II prospective cohort study. PLoS Medicine 2013;10:e1001479.

64. Thiese M.S., Arnold Z.C., and Walker S.D. The misuse and abuse of statistics in biomedical research. Biochemia Medica 2015;25:5–11.

65. Thomas E. An introduction to medical statistics for health care professionals: Describing and presenting data. Musculoskeletal Care 2004;2:218–28.

66. Thompson V.P., Watson T.F., Marshall G.W.Jr., Blackman B.R., Stansbury J.W., Schadler L.S., Pearson R.A., and Libanori R. Outside-the-(cavity-prep)-box thinking. Advances in Dental Research 2013;25:24–32.

67. Tinanoff N., Coll J.A., Dhar V., Maas W.R., Chhibber S., and Zokaei L. Evidence-based update of pediatric dental restorative procedures: Preventive strategies. Journal of Clinical Pediatric Dentistry 2015;39:193–7.

68. Tu Y.K., and Gilthorpe M.S. Key statistical and analytical issues for evaluating treatment effects in periodontal research. Periodontology 2000 2012;59:75–88.

69. Ushimura S., Nakamura K., Matsuda Y., Minamikawa H., Abe S., and Yawaka Y. Assessment of the inhibitory effects of fissure sealants on the demineralization of primary teeth using an automatic pH-cycling system. Dental Materials Journal 2016;35:316–24.

70. Welbury R., Raadal M., and Lygidakis N.A. EAPD guidelines for the use of pit and fissure sealants. European Journal of Paediatric Dentistry 2004;5:179–84.

71. WHO. In: *Oral Health Surveys. Basic Methods*. 4th ed. Geneva: World Health Organization, 1997:39–44.

72. Winning L., Patterson C.C., Cullen K.M., Stevenson K.A., Lundy F.T., Kee F., and Linden G.J. The association between subgingival periodontal pathogens and systemic inflammation. Journal of Clinical Periodontology 2015.

73. World Health Organization. WHO Oral Health: Oral health information systems. Available on the Internet from: `http://www.who.int/oral_health/action/information/surveillance/en/`. Accessed: 2017-04-01.

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Chapter 10

Appendix

Vinay Pitchika

Dental Researcher & Epidemiologist

PROFILE

A post-graduate with 5 years of work experience in the field of dental epidemiology and biostatistics; which was used to obtain a PhD in Oral Sciences. Secured research grants from external funding organizations. Completed the requirements for PhD and awaiting thesis defense.

WORK EXPERIENCE

1.) Research Associate (Mar 2012 – Dec 2017)

Dept. of Operative Dentistry & Periodontology

Ludwig-Maximilians-Universität München, Germany

- Given lectures on research methodology, epidemiology and biostatistics
- Supervision of post-graduate and graduate dental students
- Planning, designing and execution of clinical/ epidemiological/ in-vitro studies
- Preparation of SOPs, SAPs and CRFs
- Clinical data analysis using R, SPSS and SAS
- Preparation of manuscripts for peer-reviewed journals with impact factor
- Presentation of research results at international conferences

2.) Internship – Data Analyst (Feb 2015 – Jun 2015)

Institute of Epidemiology I

Helmholtz Zentrum München, Germany

- Preparation of SOP and SAP
- Analysis of longitudinal data from GINIplus/ LISAplus cohorts using R
- Performed analysis to study the influence of gingival bleeding on hs-CRP values
- Assessed the effect of food intake on the prevalence and incidence of early childhood caries

3.) Site Monitor (Feb 2014 – Nov 2014)

Harvard Medical School

- Co-ordinated in conducting the online course "Principles and Practice of Clinical Research (PPCR 2014)" in the Munich centre via video-conferencing
- Explaining statistical procedures and clarifying students' doubts in classroom

4.)Associate Researcher (Jun 2010 – Oct 2012)

Institute for Medical Informatics, Biometrics & Epidemiology

Ludwig-Maximilians-Universität München, Germany

- Creating CRFs using Cardiff Teleform Designer and MS Access
- Writing codebooks for variables used in analyses
- Data entry and management using Cardiff Teleform, SAS, SPSS and MS Access

EDUCATION

1.) PhD in Oral Sciences (Oct 2013 – Dec 2017)

School of Dentistry, Ludwig-Maximilians-Universität München (LMU Munich), Germany

Research topics:

- Influence of gingivitis & lifestyle on hs-CRP among adolescents
- Assessment of clinical workflows of performing composite restorations
- Longitudinal caries progression in kindergarten children
- Efficacy of a new pit and fissure sealant

2.) M.Sc. Epidemiology (Oct 2009 – Sep 2011)

Institute for Medical Informatics, Biometrics & Epidemiology, LMU Munich, Germany

Specializations:

- Clinical epidemiology
- Advanced (Infectious, Pharmaceutical & Cancer) epidemiology

3.) Bachelor of Dental Surgery (Sep 2003 – Mar 2009)

Ragas Dental College & Hospital, Chennai, The Tamilnadu Dr. M.G.R. Medical University, India

Majors:

- Basic Medical Sciences
- Clinical Dentistry

PROFESSIONAL MEMBERSHIPS & OTHER DUTIES

International Association for Dental Research **(IADR)**
Ambassador for **CED-IADR**
Indian Dental Association (**IDA**)

AWARDS WON

1.) Dental Education Award 2015 (1st Prize)
Kurt Kaltenbach Stiftung, KaVo, Frankfurt, Germany

2.) IADR-Colgate Research in Prevention Travel Award 2016
IADR in association with Colgate-Palmolive, Seoul, Korea

3.) Oral-B blend-a-med Prophylaxepreis 2016 (1st Prize)
Deutschen Gesellschaft für Kinderzahnheilkunde, Ludwigsburg, Germany

PUBLICATIONS

No. of accepted international publications:	**18**
No. of international publications under review:	**2**
No. of articles under preparation:	**3**

NON-TECHNICAL SKILLS

- Leadership qualities: Representative of the batch for 5 years during undergraduate course. Chairman (elect) of Student Council for organizing month-long cultural, literary and sport events for approx. 600 students. Played a significant role in conducting workshops, farewell meetings & annual excursions.
- Managing multiple projects to a timeline.
- Making presentations to students, project teams, superiors and/or those in other functions.