ACTA ODONTOLOGICA SCANDINAVICA
2022, VOL. 80, NO. 5, 338–345
https://doi.org/10.1080/00016357.2021.2011400

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ORIGINAL ARTICLE

Systematic assessment of salivary inflammatory markers and dental caries in children: an exploratory study

Torunn Børsting[a,b], Vishwesh Venkatraman[c], Tone Natland Fagerhaug[a,b], Marit Slåttelid Skeie[a,d], Signe Nilssen Stafne[b,e], Astrid Jullumstrø Feuerherm[a] and Abhijit Sen[a,b]

[a]Center for Oral Health Services and Research, Mid-Norway (TkMidt), Trondheim, Norway; [b]Department of Public Health and Nursing, Norwegian University of Science and Technology (NTNU), Trondheim, Norway; [c]Department of Chemistry, Norwegian University of Science and Technology (NTNU), Trondheim, Norway; [d]Department of Clinical Dentistry, University of Bergen, Bergen, Norway; [e]Department of Clinical Service, Trondheim University Hospital (St. Olavs hospital), Trondheim, Norway

ABSTRACT

Objective: To investigate associations between a wide panel of salivary inflammatory markers and the presence of dental caries among children.

Material and methods: In this exploratory, cross-sectional study, 176 children, aged 7–9, underwent a dental examination. Information on the children's oral health habits and lifestyles was collected from their mothers. In addition, saliva samples were collected and analyzed using a multiplex immunoassay. Of 92 inflammatory markers measured, 56 were included in the statistical analyses. To identify potential inflammatory markers associated with caries, we applied low to advanced statistical analyses. First, we performed traditional logistic regression analysis followed by Bonferroni corrections. Thereafter, a more robust and less conservative statistical approach, i.e. Least Absolute Shrinkage and Selection Operator (LASSO), was applied. The models were adjusted for potential confounders.

Results: Of the 176 children in the study, 22.2% were affected by caries. Among the 56 salivary inflammatory markers, only macrophage colony-stimulating factor 1 (CSF1) was selected by the LASSO and found to be positively associated with the presence of caries.

Conclusions: The observed association between CSF1 and the presence of caries may be of clinical value in caries risk management and early diagnosis. Larger studies are warranted to assess the replicability of our findings.

ARTICLE HISTORY
Received 14 May 2021
Revised 10 September 2021
Accepted 19 November 2021

KEYWORDS
Dental caries; oral health; inflammatory markers; child health; public health

Introduction

Although largely preventable, dental caries continues to be one of the most common chronic diseases among children worldwide and at advanced stages it can have a considerable impact on children's quality of life [1,2]. Therefore, dental caries is a major public health concern, and it is important to continue to improve our understanding of the underlying factors influencing the initiation and progression of the disease, and ways in which to prevent or stagnate its development.

Dental caries is a dynamic, sugar-driven disease process characterized by repeated cycles of demineralization and remineralization of dental hard tissue [3]. Initiation or progression of lesions occurs when net demineralization prevails during a sufficient period, i.e. when pathological factors have shifted the balance and overcome the protective factors, with mineral loss as a consequence. Caries etiology is multifactorial and involves risk factors related to socioeconomic status, health behaviours, and lifestyle [4,5]. Dental caries-related risk factors have been associated with both composition and metabolism of the microbial biofilm on teeth, and their presence over time may result in aberrant colonization by cariogenic bacteria, creating an environment that encourages frequent demineralization [6]. Oral bacteria are key components of the oral microbiota, and several cariogenic bacteria have been identified, of which the acid-producing *Mutans streptococci* and *Lactobacilli* species are among those most commonly identified in children [7].

In the interplay between demineralization and remineralization of dental hard tissue, saliva plays an important caries protective role as part of the innate immune response for the oral cavity, for example, by buffering the oral environment at a neutral pH and by having anti-microbial effects [4,8]. Previous epidemiological studies among children have investigated potential associations between selected salivary inflammatory markers and dental caries, and have indicated that they have a role in regulating cariogenic bacterial growth and infection related to dental caries manifestation [9–16]. However, the detailed aspects of the inflammatory

CONTACT Torunn Børsting torbo@tkmidt.no Center for Oral Health Services and Research, Mid-Norway (TkMidt), Trondheim, Norway
Supplemental data for this article can be accessed here.

response to dental caries have not been extensively investigated, and to the best of our knowledge, no published studies have used wide panels of salivary inflammatory markers to explore this. More knowledge in this area is essential, as it may imply clinical value regarding caries risk management and early diagnosis. Hence, the present study aimed to perform a systematic assessment of associations between a wide panel of salivary inflammatory markers and the presence of dental caries among healthy 7–9-year-old children.

Material and methods

Study design and setting

A cross-sectional study design was used in the investigation. In brief, mother and child pairs who were invited to participate in a 7-year follow-up of the TRaining In Pregnancy (TRIP) study between 2014 and 2016, were also invited and eligible to participate in an oral health sub-study (a detailed description of the TRIP-study and follow-up can be found elsewhere [17,18]). The oral health sub-study (TRIP-tann) included a dental examination and saliva collection from the participating children who were 7–9 years old. The examinations were conducted at the Centres for Oral Health Services and Research in Trondheim and Stavanger between May 2016 and August 2017. Additionally, an electronic self-report questionnaire was filled in by the mothers at the examination using the software CheckWare.

Saliva collection and assessment

Stimulated whole saliva was collected from the children before the clinical dental examination, based on the guidelines for saliva collection from the University of Oslo [19]. All participants were instructed not to brush or rinse their teeth on the day of the examination, and not to eat or drink 60 min before the study visit. Any medications taken in the 24 h before the sample was taken, were noted. To stimulate saliva secretion, the participants chewed on a paraffin pellet for 30 s before commencing the saliva collection. The children were instructed to swallow once after 30 s, and then continue to chew on the pellet while collecting saliva in a 15 ml graded collection tube with a funnel for 5 min. The volumes of the saliva samples were recorded directly after collection. The saliva samples were then subjected to detection of *Streptococcus mutans* and *Lactobacilli* and graded independently by two of the authors (AJF and TB) according to the kit instructions (CRT Bacteria, Ivoclar Vivadent). The grading consisted of a range from 1–4, where grades 1–2 represented a low count ($<10^5$ colony forming units), and grades 3–4 represented a high count ($\geq 10^5$ colony forming units). The remaining saliva samples were aliquoted and stored at −80 °C.

Exposure assessment

A multiplex proximity enhanced extension assay (PEA) was conducted with remaining saliva samples using the Proseek Multiplex Inflammation I panel (Olink Bioscience) [20]. The panel included 92 high-quality assays for proteins related to inflammation, including chemokines, chemokine receptors, cytokines, cytokine receptors, enzymes, and growth factors. Data were expressed as normalized protein expression (NPX) values. An NPX value is an arbitrary unit in a Log2 scale, where one NPX corresponds to a doubling of the protein concentration. It is a relative quantification method, so different proteins with the same NPX values, may still differ in concentration. Therefore, we could not make comparisons of NPX values between different proteins, but it was possible to compare NPX values of the same protein between the study participants [21]. Of the 92 inflammatory markers measured, 36 markers had an NPX value below the limit of detection for more than 50% of our study samples and were excluded from the data analysis (see Supplementary Table S1 for a list of the excluded markers). The remaining 56 inflammatory markers were considered for the statistical analyses, and a summary of the included markers is presented in Supplementary Table S2. In addition, a heatmap plot is presented in Supplementary Figure S1 to illustrate the levels of the included inflammatory markers by dental caries status for each participant. The heatmap was produced using R version 4.0.2 (R Core Team 2020) and the Complex Heatmap package [22].

Outcome assessment

The dental examinations were performed by two experienced dentists according to a detailed 5-graded caries diagnostic tool [23]. Enamel lesions were denoted as grades 1 and 2, and dentin lesions as grades 3, 4, and 5. Before and during the study the two dentists both attended calibration courses and exercises. The mean weighted Cohen's kappa achieved from the calibration exercises was 0.95 for intra-examiner reliability, 0.63 for inter-examiner reliability when compared to the gold standard, and 0.90 for inter-examiner reliability between the two examiners (further details of the calibration procedure can be found in Supplementary Text S1). Caries data were collected by the use of the dmft/DMFT index, summarizing the number of decayed (including both enamel (d_{1-2}/D_{1-2}) and dentin (d_{3-5}/D_{3-5}) caries), missing due to caries (m/M) and filled (f/F) primary and permanent teeth (t/T) for each participant [24]. The examination protocol included detailed instructions on how the examiners were to distinguish missing teeth due to caries from missing due to exfoliation.

Covariates

The electronic questionnaire filled in by the mothers at the dental examination included items concerning the child's oral health habits and lifestyle, such as frequency of brushing teeth, use of fluoride tablets and vitamin D supplements, and intake of sugary beverages and milk-related desserts. The age and gender of the children were obtained from the TRIP-study records. Height in centimetres and weight in kilograms was also measured for most of the study participants

($n = 153$). Body mass index (BMI) was calculated as weight in kilograms divided by height in metres squared according to the BMI-for-age standard from the World Health Organization [25]. The participants' salivary volume was measured as part of the saliva collection procedure.

Statistical analyses

The statistical analyses were performed using Stata/IC 16 (Stata Corp). Descriptive variables were presented as mean (standard deviation (SD)) for continuous variables, and frequency (percentage) for categorical variables. Tests of significance were performed using Pearson's chi-square test for categorical variables and the Mann-Whitney U test for continuous variables. Caries presence ($d_{1-5}mft/D_{1-5}MFT>0$) and absence ($d_{1-5}mft/D_{1-5}MFT=0$) were used as a binary outcome variable, and the 56 salivary inflammatory markers (exposures) were considered as continuous variables. For the logistic regression analyses, one model for each salivary inflammatory marker was included at a time, and the models were adjusted for the potential confounders: age (continuously in years), use of vitamin D supplements (any use or non-users), saliva volume (continuously in ml), frequency of brushing teeth ($\leq$once a day or $\geq$twice a day), use of fluoride tablets (0–5 times a week or 6–7 times a week), intake of ice cream and milk-based desserts (never/rarely, 1–3 times a month, 1–2 times a week or 3–4 times a week), intake of soft drinks with sugar (never/rarely, 1–3 glasses a month, 1–3 glasses a week or 4–6 glasses a week), salivary *Streptococcus mutans* (high or low count) and *Lactobacilli* (high or low count). Results from the logistic regression models were presented as odds ratios (ORs) and 95% confidence intervals (95% CIs). We then applied a traditional multiple testing approach, i.e. Bonferroni corrections (the significance level of $\alpha = 0.05$ was divided by the number of tests). Further, to circumvent the issue of conservative approaches, and traditional variable selection methods when the number of covariates is large, we applied a shrinkage method, i.e. the Least Absolute Shrinkage and Selection Operator (LASSO) (including all confounders and inflammatory markers in one model) [26,27]. Ten-fold cross-validation was carried out to assess the robustness of the model. LASSO performs both variable selection and regularization to prevent overfitting by constraining the size of the regression coefficients and 'shrinking' the coefficients towards zero. This allows the least contributing variables to have a coefficient very close or equal to zero, equating to an odds ratio of '1', suggesting no association.

Ethics

Ethical approval from the Norwegian Regional Committees for Medical and Health Research was acquired before the initiation of the original TRIP-study (4.2007.81), the 7-year follow-up (2014/618/REK midt), and the TRIP-tann oral health sub-study (2015/639/REK sør-øst). Parents of the children participating in the dental examination gave written consent.

Results

The recruitment of the participants into the TRIP-tann oral health sub-study is illustrated in Figure 1. A total of 176 children completed the dental examination and were included in the analyses for the present study. The mean age of the children was 8.1 years, and 48.3% were girls. Of the 176 study participants, 39 (22.2%) had at least one tooth with presence of caries ($d_{1-5}mft/D_{1-5}MFT>0$), and among those, 9 (5.1%) had at least one tooth with dentin caries ($d_{3-5}/D_{3-5}>0$). Seventeen (9.7%) children had at least one filling, and of those, 11 children had at least one filling without any other decayed teeth ($d_{1-5}/D_{1-5}=0$). Two of the children had experienced extractions of primary teeth due to caries (first-child: 1 tooth, and second child: 2 teeth). The mean caries experience including enamel caries ($d_{1-5}mft/D_{1-5}MFT$) was 0.44 (SD 1.05) for the total sample and 1.97 (SD 1.39) for those with caries experience. There were no significant differences found in the descriptive variables between the children with or without presence of caries, except *Lactobacilli* count, where those with presence of caries were more likely to have a higher count ($p = .01$) (Table 1).

The mean NPX value for each included salivary inflammatory marker among the children with and without caries is presented in Table 2, along with the logistic regression and LASSO analyses. The logistic regression analyses suggested that children with caries had a significantly higher mean NPX value of the salivary inflammatory marker macrophage colony-stimulating factor 1 (CSF1) than children without caries (OR 2.11, 95% CI 1.21–3.65). None of the other included inflammatory markers were found to be significantly associated with the presence of caries. As can be seen in Table 2, after adjusting for potential confounders, the results remained similar. Further, when correcting for multiple testing *via* Bonferroni (not included in Table 2), none of the inflammatory markers (including CSF1) were found to be associated with caries. However, when the more robust and less conservative statistical approach was applied, i.e. LASSO regression, CSF1 was again selected and found to be positively associated with the presence of caries.

Discussion

To the best of our knowledge, this is the first exploratory study that systematically assesses potential associations between a wide range of salivary inflammatory markers and the presence of caries among healthy, young children. The main result of the present study was that out of the 56 inflammatory markers analyzed by use of the LASSO approach, only CSF1 was selected and found to be positively associated with the presence of caries.

CSF1 is a pleiotropic cytokine that regulates macrophage formation, differentiation, and growth [28]. As such, CSF1 is a pro-inflammatory factor that modulates immune function and inflammation as part of the innate immune system [29]. CSF1 is also a regulator of osteoclastogenesis and induces osteoclast fusion, spreading, and survival, thereby regulating bone homeostasis [30]. In addition, CSF1 has been found to

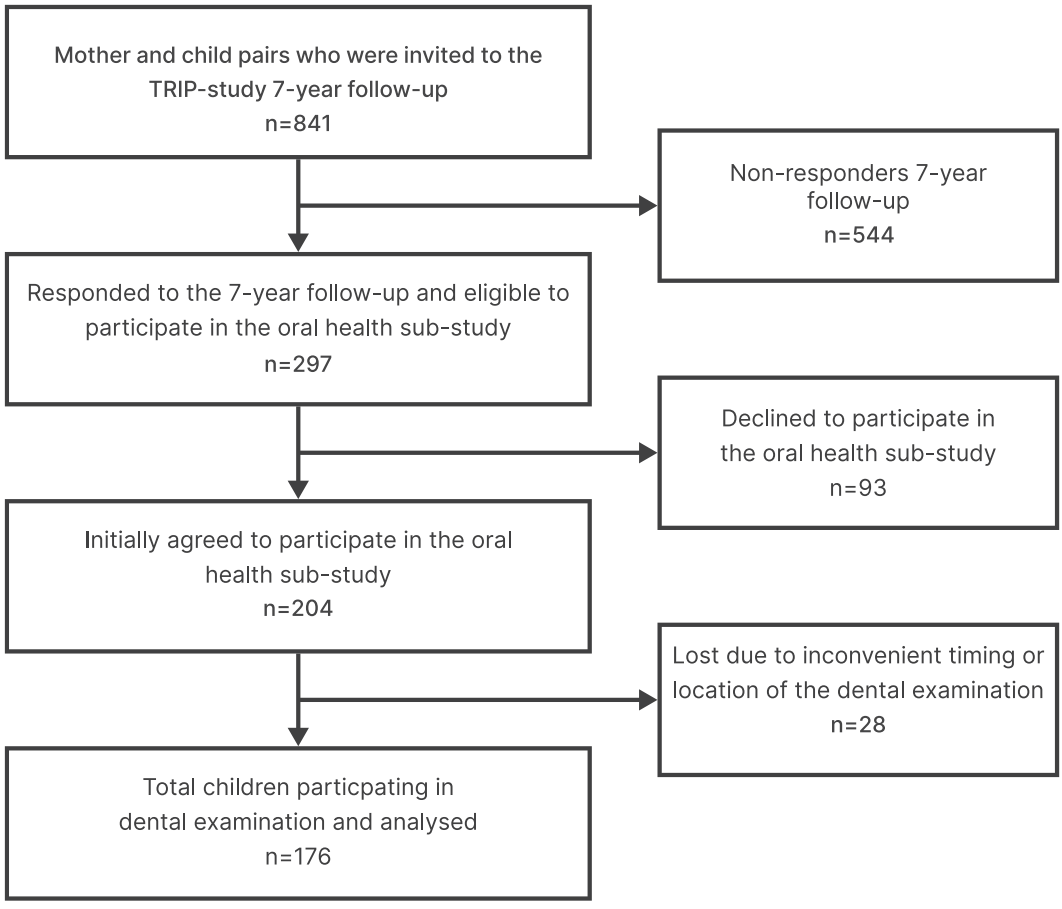

Figure 1. Flow chart of study recruitment.

Table 1. Characteristics of the study population.

	Absence of caries ($n = 137$)	Presence of caries ($n = 39$)	p-value
Categorical variables, *n* (%)			
Girls	67 (48.9)	18 (46.2)	.76
Any intake of vitamin D supplements	107 (78.1)	33 (84.6)	.37
Use of fluoride tablets*			.09
0–5 times a week	56 (41.2)	22 (56.4)	
6–7 times a week	80 (58.8)	17 (43.6)	
Frequency of brushing teeth			.53
$\leq$ once a day	46 (33.6)	11 (28.2)	
$\geq$ twice a day or more	91 (66.4)	28 (71.8)	
Intake of ice-cream and other milk-based desserts			.23
Never/rarely	2 (1.5)	1 (2.6)	
1–3 times a month	78 (56.9)	15 (38.5)	
1–2 times a week	48 (35.0)	20 (51.3)	
3–4 times a week	9 (6.6)	3 (7.7)	
Intake of soft drinks with sugar			.87
Never/rarely	33 (24.1)	7 (18.0)	
1–3 glasses a month	63 (46.0)	20 (51.3)	
1–3 glasses a week	38 (27.7)	11 (28.2)	
4–6 glasses a week	3 (2.2)	1 (2.5)	
*Streptococcus mutans***			.09
$\geq 10^5$ CFU/ml	22 (16.2)	11 (28.2)	
*Lactobacilli***			.01
$\geq 10^5$ CFU/ml	63 (46.3)	27 (69.2)	
Continuous variables, mean (SD)			
Age at dental exam	8.1 (0.4)	8.2 (0.4)	.34
Salivary volume (ml/5 min)	4.35 (2.54)	4.40 (2.10)	.66
BMI (WHO standardized)***	16.2 (1.6)	16.2 (2.0)	.74

*1 missing, **1 missing, ***23 missing.
Presence of caries ($d_{1-5}mft/D_{1-5}MFT>0$).
Absence of caries ($d_{1-5}mft/D_{1-5}MFT=0$).

have an important role in tooth development, where it is involved in tooth matrix formation and tooth eruption [31]. To the best of our knowledge, only one study has previously investigated the association between salivary levels of CSF1

Table 2. Mean normalized protein expression (NPX) value and standard deviation (SD) of salivary inflammatory markers among children with and without presence of caries, odds ratios (ORs) and 95% confidence intervals (95% CIs) from logistic regression models, and estimates from logistic LASSO regression (adjusted for confounders).

Inflammatory markers	Absence of caries ($n = 137$)		Presence of caries ($n = 39$)		Logistic regression (unadjusted)	Logistic regression (adjusted[#])	LASSO[#]
	NPX mean	SD	NPX mean	SD	OR (95% CI)	OR (95% CI)	Estimate
Chemokine receptor							
CD40	8.18	1.06	8.32	1.05	1.14 (0.81, 1.60)	1.24 (0.84, 1.83)	1.00
Chemokines							
IL8	12.28	0.90	12.44	0.88	1.23 (0.82, 1.84)	1.14 (0.74, 1.75)	1.00
MCP1	8.07	0.89	8.14	1.02	1.09 (0.74, 1.61)	1.12 (0.73, 1.74)	1.00
CXCL11	4.58	1.68	4.41	1.97	0.94 (0.77, 1.16)	0.93 (0.74, 1.17)	1.00
CXCL9	7.73	1.18	7.61	1.41	0.92 (0.69, 1.23)	0.87 (0.64, 1.17)	1.00
CXCL1	9.39	1.81	9.28	2.07	0.97 (0.80, 1.17)	1.01 (0.81, 1.27)	1.00
CCL4	2.97	1.56	3.31	1.73	1.14 (0.92, 1.41)	1.16 (0.91, 1.47)	1.00
SCF	2.06	0.80	2.13	0.86	1.11 (0.72, 1.72)	1.21 (0.73, 2.01)	1.00
CCL19	2.06	1.36	2.03	1.34	0.99 (0.76, 1.29)	1.06 (0.79, 1.41)	1.00
CXCL5	10.26	2.24	10.36	2.27	1.02 (0.87, 1.20)	1.02 (0.85, 1.23)	1.00
CCL23	1.06	0.42	1.11	0.64	1.21 (0.61, 2.39)	1.11 (0.53, 2.33)	1.00
CCL3	3.44	1.22	3.83	1.24	1.29 (0.97, 1.70)	1.20 (0.88, 1.63)	1.00
CXCL6	6.45	1.77	6.50	1.99	1.02 (0.84, 1.24)	1.01 (0.81, 1.26)	1.00
CXCL10	6.73	2.70	6.30	3.13	0.95 (0.83, 1.08)	0.98 (0.85, 1.14)	1.00
CCL28	6.00	1.30	6.06	1.34	1.03 (0.79, 1.36)	1.11 (0.80, 1.53)	1.00
MCP2	2.13	1.12	2.07	1.04	0.94 (0.68, 1.31)	0.97 (0.67, 1.40)	1.00
CX3CL1	5.15	1.32	5.39	1.48	1.14 (0.87, 1.50)	1.30 (0.93, 1.82)	1.00
CCL20	5.72	2.21	5.73	2.21	1.00 (0.85, 1.18)	0.99 (0.83, 1.17)	1.00
Cytokine receptors							
OPG	7.61	0.89	7.89	1.03	1.39 (0.94, 2.06)	1.22 (0.78, 1.92)	1.00
LIFR	2.76	1.35	2.72	1.38	0.98 (0.75, 1.27)	1.14 (0.82, 1.59)	1.00
IL10RB	3.04	0.80	3.18	0.86	1.24 (0.79, 1.94)	1.42 (0.82, 2.44)	1.00
IL18R1	6.03	1.26	6.17	1.31	1.09 (0.82, 1.44)	1.17 (0.84, 1.63)	1.00
TNFRSF9	2.77	1.04	3.01	1.18	1.22 (0.88, 1.70)	1.16 (0.81, 1.64)	1.00
Cytokines							
CSF1	5.42	0.63	5.75	0.75	**2.11 (1.21, 3.65)**	**2.22 (1.09, 4.53)**	**2.27**
IL7	3.32	0.54	3.37	0.69	1.17 (0.63, 2.17)	1.09 (0.56, 2.14)	1.00
IL6	4.18	1.97	4.58	1.75	1.11 (0.93, 1.33)	1.04 (0.84, 1.30)	1.00
IL17A	0.75	0.30	0.75	0.26	1.03 (0.31, 3.43)	0.92 (0.25, 3.40)	1.00
TRAIL	8.72	0.59	8.83	0.72	1.32 (0.75, 2.33)	1.41 (0.72, 2.75)	1.00
IL1alpha	8.16	2.75	8.19	2.85	1.00 (0.88, 1.14)	1.04 (0.89, 1.21)	1.00
IL18	8.42	1.64	8.45	1.61	1.01 (0.81, 1.26)	1.01 (0.78, 1.31)	1.00
TNFSF14	6.36	1.12	6.57	1.24	1.17 (0.86, 1.59)	1.14 (0.80, 1.63)	1.00
TRANCE	1.89	0.83	2.11	1.01	1.29 (0.89, 1.89)	1.18 (0.79, 1.77)	1.00
IL12B	1.27	1.05	1.37	1.03	1.09 (0.78, 1.52)	1.13 (0.80, 1.60)	1.00
IL10	1.32	0.61	1.43	0.63	1.32 (0.76, 2,31)	1.47 (0.78, 2.77)	1.00
Flt3L	1.90	0.61	2.00	0.71	1.27 (0.73, 2.20)	1.08 (0.56, 2.08)	1.00
LIF	1.67	0.57	1.81	0.68	1.44 (0.83, 2.49)	1.30 (0.71, 2.38)	1.00
TWEAK	6.69	0.78	6.84	0.90	1.26 (0.80, 1.97)	1.01 (0.60, 1.68)	1.00
Enzymes							
uPA	8.26	1.18	8.47	1.30	1.15 (0.86, 1.54)	1.12 (0.80, 1.57)	1.00
MMP1	8.75	1.99	9.18	1.72	1.13 (0.93, 1.36)	1.15 (0.94, 1.42)	1.00
MMP10	4.20	1.36	4.50	1.32	1.17 (0.90, 1.52)	1.20 (0.90, 1.60)	1.00
CASP8	4.59	1.58	4.80	1.67	1.08 (0.86, 1.36)	1.22 (0.93, 1.60)	1.00
ST1A1	1.88	1.04	1.99	1.12	1.11 (0.79, 1.55)	1.24 (0.85, 1.82)	1.00
STAMPB	3.09	0.90	3.20	0.94	1.14 (0.77, 1.68)	1.24 (0.77, 1.98)	1.00
ADA	4.44	1.16	4.73	1.16	1.24 (0.90, 1.69)	1.28 (0.89, 1.83)	1.00
Growth factors							
LAPTGFbeta1	3.66	0.93	3.98	0.97	1.43 (0.98, 2.09)	1.39 (0.90, 2.14)	1.00
TGFalpha	4.21	0.81	4.36	1.09	1.22 (0.81, 1.82)	1.21 (0.77, 1.91)	1.00
HGF	7.17	0.92	7.40	0.98	1.29 (0.89, 1.89)	1.16 (0.74, 1.81)	1.00
FGF19	0.96	0.58	0.88	0.57	0.78 (0.40, 1.51)	0.95 (0.46, 1.98)	1.00
Other							
CDCP1	6.02	0.98	6.30	0.96	1.34 (0.93, 1.94)	1.24 (0.80, 1.92)	1.00
CST5	8.19	0.86	8.39	0.76	1.35 (0.87, 2.08)	1.48 (0.89, 2.48)	1.00
OSM	6.01	1.32	6.35	1.43	1.22 (0.93, 1.59)	1.22 (0.91, 1.64)	1.00
CD5	3.22	1.29	3.41	1.37	1.12 (0.85, 1.47)	1.16 (0.86, 1.57)	1.00
EBP1	6.13	1.39	5.99	1.64	0.93 (0.73, 1.19)	0.97 (0.73, 1.28)	1.00
SIRT2	1.73	1.03	1.88	1.12	1.14 (0.82, 1.58)	1.32 (0.88, 1.97)	1.00
DNER	9.58	0.63	9.60	0.66	1.05 (0.60, 1.83)	1.36 (0.66, 2.76)	1.00
ENRAGE	6.98	0.89	7.11	0.83	1.20 (0.79, 1.83)	1.22 (0.76, 1.96)	1.00

[#]Adjusted for age, vitamin D supplements (any vs. non-users), frequency of brushing teeth (≤once a day vs. ≥twice a day), intake of ice cream and milk-based desserts (never/rarely, 1–3 times a month, 1–2 times a week, 3–4 times a week), intake of soft drinks with sugar (never/rarely, 1–3 glasses a month, 1–3 glasses a week, 4–6 glasses a week), use of fluoride tablets (0–5 times a week vs. 6–7 times a week), saliva volume, salivary *Streptococcus mutans* and *Lactobacilli* count (low vs. high). One participant was missing from the use of fluoride tablets variable and was excluded from the adjusted analyses.

Logistic Regression models with statistically significant results (p-value ≤.05) and salivary markers estimated and selected by LASSO are presented in bold.

Absence of caries ($d_{1-5}mft/D_{1-5}MFT=0$).

Presence of caries ($d_{1-5}mft/D_{1-5}MFT>0$).

For full names of inflammatory markers see https://www.olink.com/products/inflammation/biomarkers/

and dental caries, but among adults. In line with our findings, this study suggested that an increased salivary level of CSF1 was associated with dental caries [15]. However, when this former study excluded individuals with periodontitis from their analysis, the association between CSF1 and dental caries did no longer remain significant. Thus, it was concluded that the periodontal status may partly explain the observed association between CSF1 and dental caries. Comparatively, it may be that the involvement of periodontal disease can be disregarded from our study, as the participants consisted of healthy and young children with favorable oral hygiene (mean oral hygiene index score of 0.81, SD 0.63) [32].

Contrary to our study findings, previous studies have suggested significant positive associations between salivary levels of other cytokines, which were also included in our study, and dental caries among children. For instance, salivary levels of interleukin (IL)-6, IL-8 and tumor necrosis factor-alpha (TNF-α) were reported to be elevated, and also positively correlated with the severity of caries in children with early childhood caries (ECC) [11] and adolescents with caries experience [10]. Another study of mother and child pairs suggested positive associations between salivary levels of vascular endothelial growth factor (VEGF) and IL-6, and caries severity in both the mother and child (aged between 24 and 71 months) [12]. Some of the factors that may explain these discrepancies compared to our study could be that previous studies included children more severely affected by dental caries (e.g. ECC), included a wider age span (representing different dentitions), or that they used different procedures for saliva collection or different bioassays for salivary inflammatory marker measurement. Interestingly, in line with our findings, a study from Turkey, conducted among 6–12-year-olds, also reported no significant association between salivary levels of IL-10 and dental caries [14].

Although the role of cytokines in the etiology of dental caries is not fully understood, the colonization of cariogenic bacteria is a known aspect of caries pathogenesis and plays a role in activating various innate immune system responses [16]. Interestingly, in our analysis, the association between CSF1 and the presence of caries remained after adjusting for salivary levels of *Streptococcus mutans* and *Lactobacilli,* which are two of the bacteria that have commonly been associated with dental caries in children [7]. However, we cannot exclude that other cariogenic bacteria that were not assessed in the present study, may also have played a role in the present association.

Another possible explanation for the observed association between CSF1 and the presence of caries could be that CSF1, in addition to its pro-inflammatory role, also is involved in responding to the tissue damage caused by the caries lesion. For example, previous studies have suggested that dentin matrix components, such as dentin sialoprotein and dentin phosphoprotein, which are released as a result of the tissue injury caused by the bacterial acid demineralization, can activate pro-inflammatory markers, such as TNF-α and IL-1β [33,34]. It is plausible that CSF1 is another cytokine that is activated as a response to the release of these components. Furthermore, studies have shown that pro-inflammatory cytokines are reactionary to the stages of caries disease [33]. With persistent or chronic inflammation their main purpose is to respond to the bacterial infection, and in these conditions, they can also have a detrimental effect on dental and oral tissues. On the other hand, at the early stages of the caries process or later stages, after the bacterial infection has been cleared, they can have a more regenerative function in responding to tooth injury [33]. This is in line with our finding of CSF1 being associated with the presence of caries, which included both enamel and dentin caries. In addition, previous studies have found that CSF1 is involved in the process of tooth eruption, and elevated levels could therefore be related to this [31,35]. In the present study, the participants were at a similar age (with a mixed dentition), and we therefore assumed that variation in eruption status would be reasonably similar between the two outcome groups (i.e. presence or absence of caries). However, in future studies it would be interesting to further explore whether our finding is replicable in a group of children with a fully erupted permanent dentition. Moreover, genetic contributions influencing the immune system response to caries could be part of the explanation for the association between CSF1 and the presence of caries [36]. It has previously been shown that Single Nucleotide Polymorphisms (known as SNPs) in IL-6 and IL-1β genes were associated with dental caries susceptibility [37,38]. However, due to the limited number of previous studies that have investigated the association between CSF1 and dental caries, further studies are needed to clarify these potential mechanisms. This could add to our understanding of the underlying factors involved in caries disease itself, and may also be of clinical value in caries risk assessment and early diagnosis.

A strength of the present study was the homogeneity of the study sample in terms of many sociodemographic and health-related factors, which to a certain extent may have reduced the possibility of residual confounding (e.g. most of the study participants' mothers were highly educated and non-smokers and most of the participants lived in two-parent families, had a Norwegian ethnic background, and had similar BMIs) [39]. Another strength of the study was the wide range of potential confounders adjusted for when assessing the association between inflammatory markers and the presence of caries. Also, the meticulous standardization related to the dental examination and saliva collection should be considered valuable in reducing the chance of misclassification bias. Finally, we included a wide panel of inflammatory markers, many of which to our knowledge has not previously been investigated in association with caries, and we used the LASSO approach, which is more robust and less conservative than other statistical approaches traditionally used when the number of covariates is large. In recent years, the LASSO has become a very common and popularly used method in the field of biomarker assessment [40–42].

Our study has several limitations. First, we had a relatively small sample size with reasonable statistical power. Second, the low presence of caries in the sample restricted our ability to perform further subgroup analyses, for example, by caries

severity. Third, due to the unavailability of information, we could not adjust for potential confounders, such as gingival inflammation. Although, the number of children in our study with gingivitis may be negligible as the oral hygiene status observed was mostly favourable. Fourth, among the children with caries presence, eleven had fillings without other decayed (d_{1-5}/D_{1-5}) lesions, and this may to some degree have biased the results. Another limitation was that some of the questions reported by the mother might be tied to recall and social desirability bias. Lastly, due to the cross-sectional nature of the study design, we cannot rule out the possibility of reverse causality.

Conclusively, by using a wide panel of inflammatory markers, this exploratory study found that the salivary level of CSF1 was positively associated with the presence of caries among 7–9-year-old, healthy children. Further studies are warranted to investigate whether our findings are replicable in larger studies, and preferably in child populations more affected by caries.

Acknowledgments

The authors want to thank Annemarie A. Schuller, Marie Sager, Gunnar Lyngstad, and Line Cathrine Nymoen for their invaluable efforts related to the data collection in this study. We also want to thank the mothers and children who participated in this study.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

The current study was financially supported by the Norwegian Directorate of Health, through their support for research at the Norwegian Centres for Oral Health Services and Research (https://www.helsedirektoratet.no/tilskudd/etablering-og-drift-av-regionale-odontologiske422kompetansesentre).

ORCID

Torunn Børsting http://orcid.org/0000-0002-8807-3807
Vishwesh Venkatraman http://orcid.org/0000-0001-7609-2245
Tone Natland Fagerhaug http://orcid.org/0000-0001-6492-4075
Marit Slåttelid Skeie http://orcid.org/0000-0002-7888-4868
Signe Nilssen Stafne http://orcid.org/0000-0003-4922-477X
Astrid Jullumstrø Feuerherm http://orcid.org/0000-0001-5559-2072
Abhijit Sen http://orcid.org/0000-0001-9560-4226

Data availability statement

The data that support the findings of this study are available from the corresponding author, TB, upon reasonable request.

References

[1] Ramos-Jorge J, Alencar BM, Pordeus IA, et al. Impact of dental caries on quality of life among preschool children: emphasis on the type of tooth and stages of progression. Eur J Oral Sci. 2015; 123(2):88–95.

[2] Petersen PE. The world oral health report 2003. Geneva: World Health Organization; 2003.

[3] Featherstone JD. The continuum of dental caries – evidence for a dynamic disease process. J Dent Res. 2004;83 Spec No C:C39–42.

[4] Pitts NB, Zero DT, Marsh PD, et al. Dental caries. Nat Rev Dis Primers. 2017;3:17030.

[5] Selwitz RH, Ismail AI, Pitts NB. Dental caries. Lancet. 2007; 369(9555):51–59.

[6] Lamont RJ, Koo H, Hajishengallis G. The oral microbiota: dynamic communities and host interactions. Nat Rev Microbiol. 2018; 16(12):745–759.

[7] Becker MR, Paster BJ, Leys EJ, et al. Molecular analysis of bacterial species associated with childhood caries. J Clin Microbiol. 2002; 40(3):1001–1009. Mar

[8] Van Nieuw Amerongen A, Bolscher JG, Veerman EC. Salivary proteins: protective and diagnostic value in cariology? Caries Res. 2004;38(3):247–253.

[9] Laputková G, Schwartzová V, Bánovčin J, et al. Salivary protein roles in oral health and as predictors of caries risk. Open Life Sci. 2018;13(1):174–200.

[10] Gornowicz A, Bielawska A, Bielawski K, et al. Pro-inflammatory cytokines in saliva of adolescents with dental caries disease. Ann Agric Environ Med. 2012;19(4):711–716.

[11] Sharma V, Gupta N, Srivastava N, et al. Diagnostic potential of inflammatory biomarkers in early childhood caries – a case control study. Clin Chim Acta. 2017;471:158–163. Aug

[12] Ribeiro CCC, Pachêco CJB, Costa EL, et al. Proinflammatory cytokines in early childhood caries: salivary analysis in the mother/children pair. Cytokine. 2018;107:113–117. Jul

[13] Zhao A, Blackburn C, Chin J, et al. Soluble toll like receptor 2 (TLR-2) is increased in saliva of children with dental caries. BMC Oral Health. 2014;14:108.

[14] Cogulu D, Onay H, Ozdemir Y, et al. Associations of interleukin (IL)-1β, IL-1 receptor antagonist, and IL-10 with dental caries. J Oral Sci. 2015;57(1):31–36. Mar

[15] Lira-Junior R, Åkerman S, Gustafsson A, et al. Colony stimulating factor-1 in saliva in relation to age, smoking, and oral and systemic diseases. Sci Rep. 2017;7(1):7280.

[16] Meyle J, Dommisch H, Groeger S, et al. The innate host response in caries and periodontitis. J Clin Periodontol. 2017;44(12): 1215–1225.

[17] Stafne SN, Salvesen KÅ, Romundstad PR, et al. Regular exercise during pregnancy to prevent gestational diabetes: a randomized controlled trial. Obstet Gynecol. 2012;119(1):29–36.

[18] Ellingsen MS, Pettersen A, Stafne SN, et al. Neurodevelopmental outcome in 7-year-old children is not affected by exercise during pregnancy: follow up of a multicentre randomised controlled trial. BJOG. 2020;127(4):508–517.

[19] Universitetet i Oslo. Kliniske rutiner – Kariologi: Saliva sekresjonshastigehet: UiO: Det odontologiske fakultetet; 2011 [cited 2015 November 09]. Available from: https://www.odont.uio.no/studier/ressurser/kariologi/Kariesutredning/Salivatester/saliva_sekresjon-shastighet.html

[20] Assarsson E, Lundberg M, Holmquist G, et al. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. PLOS One. 2014;9(4):e95192.

[21] Olink. What is NPX?: Olink; 2020 [cited 2020 September 01]. Available from: https://www.olink.com/question/what-is-npx/

[22] Gu Z, Eils R, Schlesner M. Complex heatmaps reveal patterns and correlations in multidimensional genomic data. Bioinformatics. 2016;32(18):2847–2849.

[23] Amarante E, Raadal M, Espelid I. Impact of diagnostic criteria on the prevalence of dental caries in Norwegian children aged 5, 12 and 18 years. Community Dent Oral Epidemiol. 1998;26(2):87–94.

[24] World Health Organization. Oral health surveys: basic methods. 5th ed. Geneva, Switzerland: WHO; 2013.

[25] World Health Organization. BMI-for-age: WHO; 2020 [cited 2020 January 15]. Available from: https://www.who.int/toolkits/child-growth-standards/standards/body-mass-index-for-age-bmi-for-age

[26] Tibshirani R. Regression shrinkage and selection via the lasso. J R Stat Soc Series B (Methodol). 1996;58(1):267–288.
[27] Armstrong RA. When to use the Bonferroni correction. Ophthalmic Physiol Opt. 2014;34(5):502–508.
[28] Stanley ER, Cifone M, Heard PM, et al. Factors regulating macrophage production and growth: identity of colony-stimulating factor and macrophage growth factor. J Exp Med. 1976;143(3):631–647.
[29] Chitu V, Stanley ER. Colony-stimulating factor-1 in immunity and inflammation. Curr Opin Immunol. 2006;18(1):39–48.
[30] Cecchini MG, Hofstetter W, Halasy J, et al. Role of CSF-1 in bone and bone marrow development. Mol Reprod Dev. 1997;46(1):75–83.
[31] Werner SA, Gluhak-Heinrich J, Woodruff K, et al. Targeted expression of csCSF-1 in op/op mice ameliorates tooth defects. Arch Oral Biol. 2007;52(5):432–443.
[32] Malmö University. Methods and indices: Oral Hygiene Index: Malmö University; 2020 [cited August 13]. Available from: https://capp.mau.se/methods-and-indices/
[33] Cooper PR, Takahashi Y, Graham LW, et al. Inflammation-regeneration interplay in the dentine-pulp complex. J Dent. 2010;38(9):687–697.
[34] Silva TA, Lara VS, Silva JS, et al. Dentin sialoprotein and phosphoprotein induce neutrophil recruitment: a mechanism dependent on IL-1beta, TNF-beta, and CXC chemokines. Calcif Tissue Int. 2004;74(6):532–541.
[35] Richman JM. Shedding new light on the mysteries of tooth eruption. Proc Natl Acad Sci U S A. 2019;116(2):353–355.
[36] Vieira AR, Modesto A, Marazita ML. Caries: review of human genetics research. Caries Res. 2014;48(5):491–506.
[37] Reis CLB, Barbosa MCF, Machado B, et al. Genetic polymorphisms in interleukin-6 and interleukin-1-beta were associated with dental caries and gingivitis. Acta Odontol Scand. 2021;79(2):96–102.
[38] Hu XP, Song TZ, Zhu YY, et al. Association of ENAM, TUFT1, MMP13, IL1B, IL10 and IL1RN gene polymorphism and dental caries susceptibility in Chinese children. J Int Med Res. 2019;47(4):1696–1704.
[39] Gustafsson MK, Romundstad PR, Stafne SN, et al. Alterations in the vitamin D endocrine system during pregnancy: a longitudinal study of 855 healthy Norwegian women. PLOS One. 2018;13(4):e0195041.
[40] Avalos M, Adroher ND, Lagarde E, et al. Prescription-drug-related risk in driving: comparing conventional and lasso shrinkage logistic regressions. Epidemiology. 2012;23(5):706–712.
[41] Lu M, Zhou J, Naylor C, et al. Application of penalized linear regression methods to the selection of environmental enteropathy biomarkers. Biomark Res. 2017;5(1):9.
[42] Bränn E, Fransson E, White RA, et al. Inflammatory markers in women with postpartum depressive symptoms. J Neurosci Res. 2020;98(7):1309–1321.