Original paper

Relationship between the salivary concentration of matrix metalloproteinases 8 and 20 and severe early childhood caries

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Abstract

Background. Dental caries is initiated through mineral dissolution by bacterial acids and collagen degradation by endogenous proteolytic enzymes, mainly collagenolytic matrix metalloproteinases (MMPs).

Objectives. The present research aimed to evaluate the relationship between severe early childhood caries (S-ECC) and salivary MMP-8 and MMP-20 concentrations.

Material and methods. Fifty children aged 36–60 months were assigned to either the caries-free (control) group or the S-ECC group. Standard clinical examinations were performed, and approx. 1 mL of expectorated unstimulated whole saliva was collected from all participants. In the S-ECC group, the sampling was repeated 3 months after restorative treatment. All samples were analyzed for the salivary concentrations of MMP-8 and MMP-20, using the enzyme-linked immunosorbent assay (ELISA). Statistical analysis employed the *t* test, the Mann–Whitney *U* test, the χ^2 test, Fisher's exact test, and the paired samples *t* test. The level of significance was set at 0.05.

Results. At baseline, the subjects in the S-ECC group presented with significantly elevated levels of MMP-8 as compared to the control group. However, the salivary concentration of MMP-20 did not exhibit a significant difference between the 2 groups. A significant reduction occurred in the levels of MMP-8 and MMP-20 3 months after restorative treatment in the S-ECC group.

Conclusions. The salivary levels of MMP-8 and MMP-20 were significantly affected by dental restorative treatment in children. Furthermore, MMP-8 was observed to be a better indicator of the dental caries status than MMP-20.

Keywords: dental caries, saliva, biomarker, matrix metalloproteinase 8, matrix metalloproteinase 20

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Introduction

Early childhood caries (ECC) is defined as the presence of one or more decayed (non-cavitated or cavitated lesions), missing (due to caries) or filled tooth surfaces in any primary tooth of a child aged 72 months or younger. However, any sign of smooth surface caries in children younger than 3 years is considered severe early childhood caries (S-ECC). From ages 3 through 5, one or more decayed, missing (due to caries) or filled smooth surfaces in primary maxillary anterior teeth, or a decayed-missing-filled teeth (dmft) score of ≥4 (age 3), ≥5 (age 4) or ≥6 (age 5) constitute S-ECC.[1] Pain is the most immediate consequence of ECC; it can disrupt the everyday activities of the child. The dental treatment of children with ECC is not always feasible due to their lack of cooperation, and sedation or general anesthesia might be required accordingly.[2] This implies the superiority of caries preventive strategies, which can be improved by determining the various factors responsible for the pathogenesis of dental caries.

The saliva makes a major contribution to the pathogenesis of dental caries, which is partly associated with various components of the immune system in its composition. Saliva sampling is a simple, economical and non-invasive method for oral health examinations. Therefore, in the case of oral diseases, the analysis of salivary biomarkers can be considered an appropriate method for an early diagnosis, as well as for the determination of the prognosis and the treatment success.[3–5]

Dentin is a biological composite of hydroxyapatite and organic compounds, with the organic material constituting 30% of its volume. A total of 90 wt% of dentinal organic material is composed of type I collagen, whereas the remaining 10 wt% consists of non-collagenous proteins. Following the dissolution of dentinal mineral content during the bacterial acidic attack, the extracellular matrix (ECM) is degraded by bacterial enzymes and host enzymes, such as matrix metalloproteinases (MMPs).[6,7]

Matrix metalloproteinases are calcium (Ca)-dependent, zinc (Zn)-containing endopeptidases isolated from dentin, the pulp tissue, odontoblasts, whole saliva, and the bacterial plaque. When the saliva penetrates the cavities created by dentinal caries, salivary MMPs might gain direct access to the demineralized dentin.[5,8–11]

Salivary MMP-8 (collagenase 2) can be derived from the gingival crevicular fluid (GCF) or secreted from the salivary glands.[9,12] This enzyme can convert collagen types I, II and III into one-quarter and three-quarter fragments.[13] Matrix metalloproteinase 20 is produced during primary dentinogenesis, becomes trapped in dentin and might be released during the progression of dental caries. Odontoblasts are the primary cell source responsible for the secretion of MMP-20 into the dentinal tubules.[14,15] Matrix metalloproteinase 20 is also released by ameloblasts in their secretion phase during amelogenesis and plays an essential role in the initial stages of enamel development by decreasing the levels of amelogenin.[12] According to the available literature, mild genetic variations in MMP-20 can alter an individual's susceptibility to caries,[14,16] though laboratory studies have shown that MMP-20 cannot degrade collagen types I and II. Therefore, dentin-bound MMP-20 might be involved in the early alteration of the non-collagenous components of the organic matrix during the progression of caries.[12]

Few studies have shown the relationship between the salivary levels of MMPs and dental caries. However, the confirmation of such a relationship by well-designed clinical studies could make the analysis of these salivary biomarkers a useful tool for determining the severity of dental caries and for evaluating the efficacy of caries preventive measures. Furthermore, if a sufficient number of clinical studies confirm the relationship between high concentrations of MMPs and dental caries, the use of MMP inhibitors might be recommended in children with S-ECC, in addition to emphasis on proper oral hygiene and dietary practices. The present research aimed to investigate the relationship between the salivary concentrations of MMP-8 and MMP-20 and S-ECC.

Material and methods

Participants

A total of 50 children aged 36–60 months were included in the study. They were assigned to either the caries-free (control) group (n = 25) or the S-ECC group (n = 25). The sample size was calculated to be 25 in each group based on previous studies, assuming a type-one error $\alpha = 0.05$ ($Z_{1-\alpha/2} = 1.96$) and a type-two error $\beta = 0.10$ (power of 90%) ($Z_{1-\beta} = 1.28$), and the extraction of the parameters $\mu1 = 55$, $\mu2 = 32$, $\sigma1 = 40$, and $\sigma2 = 20$, using an appropriate statistical formula.[17] The participants were selected from among the children referred to the Department of Pediatric Dentistry at Shahid Beheshti University of Medical Sciences, Tehran, Iran, and from randomly selected kindergartens. The inclusion criteria were as follows: the absence of systemic or gingival diseases; no consumption of medications during the previous 2 months; and the absence of any exfoliating primary teeth or erupting permanent teeth at the time of the study.

Each participant was enrolled in the study after their parent read, understood and completed the informed consent document. Also, a questionnaire was completed to examine the parental level of education, and the type (breast milk or formula milk) and duration of child nighttime feeding. The research was designed and performed in accordance with the Declaration of Helsinki,

and was independently reviewed and approved by the Institutional Committee for Ethics in Research at Shahid Beheshti University of Medical Sciences, Tehran, Iran (IR.SBMU.RIDS.REC.1395.216).

Clinical examinations

The presence of dental caries was evaluated based on the World Health Organization (WHO) diagnostic criteria.[1] After being dried with sterile gauze, the teeth were clinically examined using disposable dental mirrors and explorers (Shiang Shin Corporation, Taiwan) under sufficient light. Only children with open tooth contacts were included in the study to ensure the absence of interproximal caries. Children without dental caries were assigned to the caries-free group, and those affected by S-ECC were diagnosed according to the American Academy of Pediatric Dentistry definition of S-ECC. The definition includes the presence of one or more decayed, missing (due to caries) or filled smooth surfaces in primary maxillary anterior teeth, or a dmft score of ≥4 at the age of 3 years, ≥5 at the age of 4 years or ≥6 at the age of 5 years.[1] The dmft index was recorded for each child, as was the plaque index (PI), using the simplified oral hygiene index (OHI-S), which consists of the simplified debris (plaque) and calculus indices (DI-S and CI-S). The present study used the index version modified for primary teeth, as defined by Miglani et al.,[18] for the evaluation of the buccal surfaces of second primary molars and central incisors in the upper right and lower left quadrants.[18,19]

Saliva sampling

The children were asked to refrain from eating, drinking, toothbrushing, and using dental floss for 2 h before sampling, which was carried out between 11:00 a.m. and 12:00 a.m. to minimize the effects of the circadian rhythm on the saliva composition. Afterward, approx. 1 mL of unstimulated whole saliva was collected by passive drooling for 5 min. The samples were collected into sterile, capped, pre-chilled microtubes and coded. The microtubes were immediately transferred to the immunology laboratory in a container with dry ice to preserve salivary proteins and prevent their hydrolysis. The saliva samples were centrifuged (Eppendorf® microcentrifuge 5415; Merck, Darmstadt, Germany) at 3,000 g for 15 min. The supernatant from each sample was carefully transferred to a new microtube with a sampler and stored at –80°C until further use.

After completing the required dental treatment and the provision of oral hygiene and nutrition instructions for the patients in the S-ECC group by an experienced post-graduate student of pediatric dentistry, the participants were recalled for post-treatment saliva sampling 3 months later.[5] The salivary samples were collected and stored under the same conditions as outlined above.

Investigating the salivary concentrations of MMP-8 and MMP-20

On the day of the experiment, the microtubes were retrieved from the freezer and kept at room temperature to be thawed. The enzyme-linked immunosorbent assay (ELISA) was performed using human saliva matrix metalloproteinase 8, 20 ELISA kits (Zellbio, Lonsee, Germany). The plates containing the samples were then transferred to an ELISA microplate reader (Anthos 2020; Anthos Mikrosysteme, Friesoythe, Germany) for spectrophotometric analysis at a wavelength of 450 nm. All analyses were performed by 2 experienced immunologists blinded to the sample groups. Inter-examiner reliability was determined using the kappa agreement coefficient ($\kappa = 0.8$). Subsequently, the final salivary concentrations of MMP-8 and MMP-20 were quantified and recorded in nanograms per milliliter (ng/mL).

Statistical analysis

The data was analyzed using IBM SPSS Statistics for Windows, v. 21.0 (IBM Corp., Armonk, USA). The *t* test compared age, the duration of nighttime feeding, PI, and the initial MMP-8 and MMP-20 concentrations between the 2 groups. The Mann–Whitney *U* test compared the dmft scores, and the χ^2 test and Fisher's exact test compared gender distribution, the parental level of education, and the type of nighttime feeding between the 2 groups. Finally, the paired samples *t* test determined the significance of the treatment effect on the salivary concentrations of MMP-8 and MMP-20 in the S-ECC group. The significance level was set at 0.05.

Results

A total of 50 children aged 36–60 months were included in the study, and assigned to either the caries-free ($n = 25$) or S-ECC ($n = 25$) groups. The mean dmft scores in the control and S-ECC groups were 0 and 15.2 ±7.0, respectively. The Mann–Whitney *U* test showed a significant difference between the 2 groups in this regard ($p < 0.001$). Furthermore, the independent *t* test revealed a significant difference between the groups in terms of PI ($p < 0.001$), with the mean values of 0.91 ±0.28 and 1.56 ±0.32 in the control and S-ECC groups, respectively (Table 1).

Table 1. Mean plaque index (PI) values, decayed-missing-filled teeth (dmft) scores, and initial salivary concentrations of matrix metalloproteinases 8 and 20 (MMP-8 and MMP-20) [ng/mL] in the caries-free and severe early childhood caries (S-ECC) groups

Group	PI	dmft	MMP-8	MMP-20
Caries-free	0.91 ±0.28	0	0.336 ±0.047	4.218 ±1.403
S-ECC	1.56 ±0.32	15.2 ±7.0	0.698 ±0.388	3.801 ±0.692

Data presented as mean ± standard deviation (*M* ±*SD*).

The statistical analysis of the data showed no significant differences in age, gender distribution, the parental level of education, and the type of nighttime feeding (breast milk or formula milk) between the 2 groups. However, there was a significant difference in the maternal education levels ($p = 0.010$). The results of the independent t test regarding the duration of nighttime feeding also showed a significant difference between the 2 groups ($p = 0.003$), with the mean duration in the S-ECC group being approx. 6 months longer as compared to the caries-free group.

As presented in Table 1, the independent t test showed that the initial mean salivary concentration of MMP-8 in the S-ECC group was approx. 2 times higher than that in the caries-free group, which was statistically significant ($p < 0.001$). However, there was no significant difference between the 2 groups in the initial mean salivary concentration of MMP-20 ($p = 0.189$). According to the results of the paired samples t test, the salivary concentrations of MMP-8 ($p < 0.001$) and MMP-20 ($p = 0.024$) in the S-ECC group decreased significantly 3 months after restorative treatment (Table 2).

Table 2. Mean pre-treatment and post-treatment (3 months after restorative treatment) salivary concentrations of MMP-8 and MMP-20 [ng/mL] in the S-ECC group

MMP	Saliva sampling	MMP concentration [ng/mL]	*p*-value
MMP-8	pre-treatment	0.698 ±0.388	<0.001*
	post-treatment	0.331 ±0.080	
MMP-20	pre-treatment	3.801 ±0.692	0.024*
	post-treatment	3.438 ±0.309	

Data presented as *M* ±*SD*. * statistically significant (paired samples *t* test; $p < 0.05$ at 95% confidence interval (*CI*)).

Discussion

Salivary components play an essential role in the incidence and development of dental caries due to their continuous contact with the teeth.[20] The degradation of the dentinal organic matrix during the caries process is initiated by endogenous proteolytic enzymes, mainly MMPs.[6,7] Assessing the presence of various MMPs in the saliva by identifying the association between these enzymes and the development or progression of dental caries in children could provide suitable non-invasive diagnostic and prognostic biomarkers.

The present study investigated the salivary concentrations of MMP-8 and MMP-20 in caries-free and S-ECC-affected children, and revealed significantly higher initial salivary concentrations of MMP-8 in the S-ECC group as compared to the caries-free group. In addition, the salivary concentrations of MMP-8 significantly decreased following the completion of dental treatment in the S-ECC group.

There are few clinical studies on the relationship between MMP-8 and dental caries. According to a study by Hedenbjörk-Lager et al., a salivary MMP-8 concentration in adults significantly correlated with the caries rate.[6] In another study by Yang et al., it was concluded that the salivary levels of MMP-8 in children with dental caries were higher than in caries-free individuals.[21] Rabelo Buzalaf et al. reported that individuals with higher salivary concentrations of MMPs were more likely to have dental caries; the authors also showed an inhibitory effect of salivary MMP-8 on the remineralization of the demineralized dentin.[22] Similarly, in another recent study by Ashwini et al., dentin degradation and caries progression were positively correlated with salivary MMP-8 levels.[23] The results of the abovementioned studies are consistent with those of the present study.

The salivary source of MMP-8 is, at least partly, the demineralized decayed dentin.[24] Some amount of MMP-8 remains in the demineralized dentin and is expected to be involved in its degeneration process.[25] Therefore, MMP-8 can be released during the process of the complete or relatively complete degeneration of the demineralized dentin.[6] The MMPs derived from GCF and/or the salivary glands are also involved in the degeneration of the dentinal matrix undergoing full or relative demineralization.[26] Salivary MMPs can gain direct access to dentin through caries-induced cavities.[12]

While considering the effect of caries treatment on the concentrations of MMPs, Chibinski et al. observed an increased expression of MMPs 60 days after sealing the cavity with a glass-ionomer sealant, which is associated with dentin repair rather than the development of caries.[27] The difference between the results of the above study and the present research can be attributed to differences in the treatment procedure, study participants and the experimental method. However, according to another study by the same research group led by Kuhn, sealing the affected dentin of primary molars with glass-ionomer cement reduced the concentration of MMP-8 in dentin, paving the way for the initiation of the repair process by reducing the degenerative effects of this enzyme on the dental tissue.[28] This is consistent with the results of the present research, suggesting a significant decrease in the concentration of MMP-8 in the post-treatment phase. This concentration change is probably due to the reduction of bacterial load following restorative treatment, which significantly decreased the production of chemotactic products and the number of neutrophils in the oral cavity, subsequently leading to a reduction in the concentrations of neutrophil derivatives, such as MMP-8.

The results of the present study showed no significant difference in the initial salivary concentrations of MMP-20 between the 2 groups ($p = 0.189$). However, the concentrations of MMP-20 were significantly reduced in the S-ECC group after the completion of restorative treatment ($p = 0.024$).

Matrix metalloproteinase 20 plays an essential role in the formation of normal enamel, and mutations in this enzyme are related to amelogenesis imperfecta.[16,29] There are few studies on the effect of MMP-20 on dental caries, though Filho et al. reported that the presence of specific MMP-20 genotypes in children was associated with resistance to dental caries.[30] In a recent study by Okamoto et al., MMP-20 was shown to stimulate tertiary dentin formation, facilitating the wound-healing processes in the dentin–pulp complex.[31] Since MMP-20 is found in enamel, the dentinal matrix and the dentinal tubule fluid, the post-treatment reduction in the salivary concentration of this enzyme in the S-ECC group can be attributed to the removal of these sources.[32]

The role of endogenous enzymes of salivary and dentinal origin in the development of dental caries was shown in previous studies.[8,33,34] The current study findings are of great importance, as they propose new ideas for the prevention and treatment of caries. Reducing or preventing the degradation of the organic matrix enables natural lesion repair through remineralization. Mazzoni et al. reported that changes in collagenous and non-collagenous proteins impaired the physical properties of dentin, as well as its remineralization ability.[7] Considering the role of MMPs in collagen degradation, the secretion of these enzymes from the dentin–pulp complex leads to a vicious circle and causes further degeneration of dentinal collagens. Therefore, using MMP inhibitors can be an effective therapeutic intervention for MMP-dependent oral diseases.[7] Several industrial MMP inhibitors have been manufactured, with their inhibitory activity mostly based on their Ca-Zn chelating groups. Ethylenediaminetetraacetic acid (EDTA) is one of the most effective substances.[8] Chlorhexidine can also inhibit MMPs through the Ca-Zn chelation mechanism.[7,9,14]

Considering the limited number of clinical studies on the relationship between MMPs and dental caries, further research should be conducted with larger sample sizes and a more extended follow-up duration.

Conclusions

In conclusion, the results of the present study revealed that dental restorative treatment in children significantly affected salivary MMP-8 and MMP-20 levels. Furthermore, MMP-8 was shown to be a better indicator of the dental caries status than MMP-20.

Ethics approval and consent to participate

The research was designed and performed in accordance with the Declaration of Helsinki, and was independently reviewed and approved by the institutional committee for ethics in research at Shahid Beheshti University of Medical Sciences, Tehran, Iran (IR.SBMU.RIDS.REC.1395.216). The informed written consent was obtained from the participants' parents.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

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