Salivary tissue factor activity and dental caries in 4-12 years old children

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Abstract

Backgroung: **The aim of this study was to evaluate the relationship between thromboplastic activity, salivary cells and caries experience in decidious and mixed dentition. Salivary pH, salivary flow rate and salivary thromboplastic activity of thirty-eight healthy children between ages 4-12 were determined.**

Methods: **Saliva pH was measured by using pH paper (Merck-pH indikatör papier, Neutralit pH 5.5-9.0). Salivary thromboplastic activity was evaluated according to Quick's one stage method using normal plasma.**

Results and conclusion: **Biochemical, cytological parameters and caries indexes did not significantly changed according to sex ($p>0.1$). Salivary flow rate was the lowest in decidious dentition ($p<0.05$). Salivary flow rate was significantly decreased (panova=0.038) and salivary thromboplastic activity was significantly increased (panova=0.0159) by the increasing DMF-T+dmf-t scores. Correlation analysis also showed a significant correlation ($p<0.05$) between salivary thromboplastic activity, epithelial cell counts (r=0.340) and DMF-T +dmf-t scores (r=0.381). It can be concluded that thromboplastic activity increases with caries increment.**

Key words : **Caries, Dentition, Salivary flow rate, Salivary pH, Salivary thromboplastic activity**

Introduction

Thromboplastin (tissue factor (TF) or Factor III) initiates the coagulation system and is a component of cell membrane but not found active in the blood [1-3]. Like various tissues and body fluids, saliva has also been known to have TF activity [2,4,5]. TF is one of the coagulation factors secreted to saliva when tissue damage has occurred in the oral cavity [6]. It is thought to supply the hemostasis when injury takes place in the mouth and it facilitates the barrier function of buccal mucosa [4]. TF is related to cells and cell fragments in saliva [4,5]. Seventy-eight percent of TF activity of saliva is attributed to the cells in the saliva [4]. TF also contributes to wound healing, inflammatory response, tumor growth, metastasis and angiogenesis [1,4]. The physiopathological importance of TF in human saliva is not clearly known. The aim of this study was to evaluate the relationship between TF activity, salivary cells and caries experience in decidious and mixed dentition. Salivary pH, salivary flow rate and salivary TF of thirty-eight healthy children between ages 4-12 were determined.

Material and method

Thirty-eight healthy children between 4-12 years old, who attended to Marmara University Faculty of Dentistry, were

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included in this study. Informed consent was obtained from each subject's family before saliva collection. The children were examined between 8-10 a.m. while sitting in upright position under sufficient light and with the help of a mirror and probe. World Health Organisation (WHO) criteria [7] were used for caries diagnosis and caries experience was reported as number of decayed (cavited lesions), missing, filled primary (dmf-t) and permanent teeth (DMF-T). DMF-T + dmf-t scores define the total number of tooth which is affected from caries and its results. Children were divided into 3 groups according to their ages: 4-6 years as deciduous dentition (14 children) , 7-9 years as early mixed dentition (15 children) and 10-12 years as late mixed dentition (9 children).

Saliva and salivary imprint samples were obtained after overnight fasting. The mouth had been rinsed with distilled water. Unstimulated mixed saliva samples were collected by spitting into a funnel in resting position. Saliva volume and collection time (10 minutes) were recorded to calculate salivary flow rate. Saliva samples were analysed for the pH by using pH paper (Merck-pH indikatör papier, Neutralit pH 5.5- 9.0). TF activity of saliva samples was evaluated according to Quick's one stage method using normal plasma [8]. This was performed by mixing 0.1 ml saliva with 0.1 ml of 0.02 M CaCl2, with the clotting reaction being started on addition of 0.1 ml of plasma. All reagent were brought to the reaction temperature (37oC) before admixture.

For the cytological examination, one drop of saliva was dried over a slide. The slides were kept first in concentrated May Grünwalde solution for 7 minutes then in Giemsa solution diluted 1/20 with water for 13 minutes. The slides were washed with distilled water for 1 minute and left to dry at room temperature. Dried slides were observed under light microscope [9].

The results were evaluated using Kruskal-Wallis, Mann-Whitney-U tests, Chi square tests, Anova variance analysis, and Spearman's Correlation analysis using Unistat 5.0 Statistical package programme.

Table 1: Biochemical and caries index results of 38 children

	Mean ± SD (n=38)
Age (year)	7.71 ± 2.52
Salivary pH	7.30 ± 0.38
Salivary flow rate ($mL\ min^{-1}$)	0.42 ± 0.41
Salivary thromboplastic activity (sec)	156.89 ± 77.55
DMF-T	1.48 ± 1.55
dmf-t	4.86 ± 3.93
DMF-T+dmf-t	5.61 ± 3.97

DMF-T: Decayed, missing, filled permanent teeth.
dmf-t: Decayed, missing, filled primary teeth

Results

Caries indexes and biochemical results of 38 children were given in Table 1 and the cytological results in Table 2.

Comparison according to dentition:

According to the dentition salivary flow rate significantly increased (panova= 0.010). Salivary TF activity tended to increase (shorter clotting time means higher TF activity) and the salivary pH tended to decrease, however the differences were not significant. (Table 3).

In the cytological evaluation of the salivary imprint samples, bacterial density in early mixed dentition was significantly different than the other groups (p=0.026, Table 4), however no significant differences were found in epithelial and lymphocycte cell counts and keratinization according to dentition.

Comparison according to sex:

Biochemical, cytological parameters and caries indexes did not differ significantly between boys and girls (p>0.1).

Comparison according to caries risk:

When the children were groupped according to caries risk, significant differences were found in salivary flow rate and salivary TF activity (panova=0.0159, Table 5). Children having DMF-T + dmf-t scores between 1-5 had significantly higher TF activity than those free of caries (DMF-T+ dmf-t=0). Children having DMF-T+dmf-t scores higher than 10, had lower salivary flow rates than those with lower scores. The children who were free of caries had significantly lower TF activity than those who had caries.

According to DMF-T+ dmf-t scores, cytological evaluation revealed no significant difference in the related epithelial cells, lymphocytes, keratinization and bacteria.

Correlation analysis:

Correlation analysis also showed a significant correlation (p<0.05) between salivary TF activity, epithelial cell counts (r=0.340) and DMF-T +dmf-t scores (r=0.381). TF activity increases with the increase in DMF-T+ dmf-t scores and epithelial cell count.

Discussion

Analysis of saliva may be useful for the diagnosis of hereditary disorders, autoimmune diseases, malignant and infectious diseases, and endocrine disorders, as well as in the assessment of therapeutic levels of drugs and the monitoring of illicit drug use [10,11]. Salivary TF may establish the hemostasis following oral traumas and facilitates barrier functions of oral mucosa [6,11]. The physiopathological importance of TF in human saliva is not clearly known

Table 2 : Cytological evaluation of saliva imprint samples

Epithelial cells	There were 0-15 epithelial cells in 16 children; 16-30 in 9 children and over 30 in 13 children.
Lymphocytes	Lymphocytes were seen only in the salivary imprint samples of 3 children.
Keratinization	There was no keratinization in 16 children, in 10 of them it was very low, in 7 of them it was moderate and in 5 of them it was high.
Bacteria	No bacteria was found in 3 children, in 13 children bacteria density was low, in 12 children moderate and in 10 children high.

Table 3: Comparison of the parameters according to the dentition

	Decidious Mean SD	Early mixed Mean SD	Late mixed Mean SD	P anova
Age (year)	5.10 ± 0.83 (n=14)	8.10 ± 0.88 (n=15)	11.22 ± 0.83 (n=9)	0.0001
Salivary pH	7.36 ± 0.41 (n=14)	7.30 ± 0.41 (n=15)	7.11 ± 0.22 (n=9)	0.3370
Salivary flow rate (ml min^{-1})	0.23 ± 0.15 (n=14)	0.37 ± 0.27 (n=15)	0.81 ± 0.60 (n=9)	0.010
Salivary thromboplastic activity (sec)	180.93 ± 102.03 (n=14)	150.13 ± 53.15 (n=15)	130.78 ± 63.96 (n=9)	0.4668
DMF-T	0.60 ± 0.89 (n=5)	1.20 ± 1.47 (n=15)	2.44 ± 1.59 (n=9)	0.0824
dmf-t	5.07 ± 5.45 (n=14)	5.14 ± 2.32 (n=14)	3.86 ± 3.13 (n=7)	0.7240

and few studies have been conducted on salivary TF activity and its relations with biochemical, cytological factors in saliva [4,5]. The influence of some local etiologic and systemic factors on salivary TF activity in diabetics had been investigated and the only significant difference within the diabetic group has been found to be due to antibiotic usage [5].

In the present study, the relationships between salivary TF activity and caries and dentition were investigated for the first time. When the children were groupped according to the dentition, although salivary TF activity tended to increase, no significant difference was found between groups.

TF is related to the cells and cell fragments of saliva, in that, high cell content (epithelial and leukocyte cells) of saliva increases TF activity [5, 6, 11]. Normally, leukocyte cells do not have TF activity, they can only secrete TF when they are exposed to vein media or collagene [12, 13, 14]. In the present study, no significant differences were found between dentition groups

Table 4 : Significant Chi square results according to the dentition in cytological evaluation

	Decidious	Early mixed	Late mixed	P anova
0 Bacterium 1 2 3	% 0 (n=0)	% 20.0 (n=3)	% 0 (n=0)	0.026
	% 42.9 (n=6)	% 33.3 (n=5)	% 22.2 (n=2)	
	% 42.9 (n=6)	% 33.3 (n=5)	% 11.1 (n=1)	
	% 14.3 (n=2)	% 13.3 (n=2)	% 66.7 (n=6)	

0= No bacterium, 1:low bacterium, 2:Moderate bacterium, 3:high bacterium

Table 5: Comparison of the parameters according to DMF-T+dmf-t

	DMF-T+dmf-t (0) Mean SD	DMF-T+dmf-t (1-5) Mean SD	DMF-T+dmf-t (6-10) Mean SD	DMF-T+dmf-t (10→) Mean SD	P
Salivary pH	7.36 ± 0.38 (n=7)	7.27 ± 0.41 (n=11)	7.28 ± 0.41 (n= 16)	7.13 ± 0.23 (n=4)	0.7813
Salivary flow rate	0.43 ± 0.67 (n=7)	0.42 ± 0.13 (n=11)	0.39 ± 0.26 (n=16)	0.12 ± 0.08 (n=4)	0.038
Salivary thromboplastic	265.57 ± 97.82 (n=7)	123.00 ± 42.44 (n=11)	140.00 ± 45.85 (n=16)	127.50 ± 63.80 (n=4)	0.0159

according to the epithelial and other cell counts. Only bacterial density was significantly lower in early mixed dentition. Since seventy-eight percent of TF activity of saliva is attributed to the cells in the saliva [4], our finding, therefore, was consistent with literature.

DMF-T+dmf-t scores define the number of tooth which is affected from caries and its results. In the present study, as these scores increased, the TF activity increased (panova = 0.0159, Table 5). Correlation analysis also showed a significant correlation ($p<0.05$) between salivary TF activity, epithelial cell counts ($r = 0.340$) and DMF-T+dmf-t scores ($r = 0.381$).

The relationship between salivary pH and salivary flow rate is well known [15,16]. Fluctuations in salivary pH may change the secretion of TF from the cells present in saliva. Salivary pH and TF activity can be affected by the changes of salivary flow rate.

The negative correlation between salivary TF activity and salivary flow rate has been shown in healthy subjects [5]. In the present study, there was no significant correlation between TF activity and salivary flow rate ($p>0.1$).

In contrast to salivary thromboplastic activity, the effect of salivary flow rate on caries status of individuals has been discussed widely in the literature. Ferguson reports no correlation between salivary flow rate and dentition [17], whereas, Shern et al reported an increase in the stimulated saliva with age [18] and Crossner reported the increase of salivary flow rate with age, until age 15 [19].

In our study, the salivary flow rate in the decidious dentition was significantly lower than in the mixed dentition ($p=0.010$, Table 3). Since salivary flow rate increases proportionally with the mass of salivary glands and salivary volume [20], our findings which indicated low salivary flow rate in the decidious dentition is consistent with this study. Russel et al. reported no correlation between salivary flow rate and dental caries thus supporting our results [21]. Anderson et al. reported that they have found the salivary flow rates of girls between 5-13 years significantly lower than boys [22].

Watanabe and Dawes found similar unstimulated salivary flow rates in a group of 5 year old boys and girls [23], Rotteveel et al. also reported the same result for 6-11 years old [24]. These findings are consistent with ours.

In the present study, children having DMF-T+dmf-t scores higher than 10, had lower salivary flow rates than those with lower scores. This result clearly underlines preventative and washing property of saliva in respect of caries.

Salivary pH increases with salivary flow rate. The critical pH for caries initiation is 5.5 and people who are more resistant to caries have higher pH values than

those susceptible to caries [25,26]. In some studies salivary flow rate was found to be higher in caries free people when compared to those having caries. Our findings about salivary pH, salivary flow rate and caries indexes in children with deciduous, mixed dentition are consistent with the literature [24]. It has been assumed that higher caries scores are usually observed in people with salivary flow discrepancies [17, 25, 26)].

Conclusion

It can be concluded that TF activity increases with caries increment (higher DMF-T+dmf-t scores, lower pH and lower salivary flow rate). To our knowledge, this is the first study which investigates the relationship between salivary TF activity and dental caries. Therefore further investigations are necessary in order to clarify its role and the mechanism in the oral cavity.

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