Salivary interleukin-8 levels in children suffering from Type 1 diabetes mellitus

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Objective: *The aim of this study was to investigate the differences between the salivary levels of IL-8 in patients with Type 1 diabetes mellitus (DM) with (DM+P) or without (DM-P) concomitant periodontitis and healthy subjects. The correlations between the levels of these cytokines and clinical periodontal parameters were also established.* ***Methods****: Twenty children and adolescents with Type 1 DM (10 diagnosed with periodontitis, 10 presenting no signs of periodontitis) and a control group consisting of 20 healthy children and adolescents aged 7-18 years were recruited for this study.* ***Results****: The Salivary IL-8 level was statistically significantly ($p<0.005$) elevated in subjects with Type 1 DM (474.47 ± 716.76) compared to non-diabetic control group (101.99 ± 68.32). There was no difference ($p>0.05$) in the salivary IL-8 level when subjects with Type 1 DM with concomitant periodontitis were compared to diabetics without periodontitis. When the salivary IL-8 level in subjects with Type 1 DM was correlated with the clinical parameters, no statistical significance was found.* ***Conclusion****: An elevated salivary IL-8 level in subjects with Type 1 DM without concomitant periodontitis plays a major role in the development of diabetic micro and macroangiopathy and pathogenesis of atherosclerosis. Consequently, this may offer a basis for the assessment of risk, prophylaxis and treatment of diabetic complications.*

Keywords: *Type 1 diabetes mellitus, periodontitis, children, cytokines, interleukin-8*

INTRODUCTION

The pathological mechanisms of periodontitis are strikingly similar to those associated with complications of chronic diabetes mellitus (DM), suggesting that periodontitis should be considered as the sixth "classic" complication of diabetes.[1] Examination of the available data reveals evidence that DM is a risk factor for gingivitis and periodontitis and that the level of glycemic control appears to be an important determinant in this relationship.[2] However, the impact of periodontal disease on glycemic control in DM and the mechanisms through which these effects might occur are less clear.[3,4,5] The association between the two diseases is bidirectional, as periodontitis has been reported to adversely affect glycemic control in patients with DM and to contribute the development of diabetic complications.[6] However, intensive periodontal therapy reduces the level of HbA(1c) both in Type 1 DM patients and even more in Type 2 DM patients.[7] Conversely, diabetes prolongs inflammation and osteoclastogenesis in periodontitis and through TNF limits the normal reparative process by negatively modulating factors that regulate bone.[8]

Human saliva is a body fluid which can be used for the diagnosis and prognosis of various diseases, oral or systemic, because saliva collection and testing are both simple and safe.[9,10] Periodontal immune cells respond to dental plaque microorganisms by secreting a number of chemokines and inflammatory cytokines, especially tumor necrosis factor-α (TNF-α), prostaglandin E2 (PGE_2) and certain interleukins (IL-1β and IL-6).[11] Several investigations have found a correlation between the levels of cytokines in saliva and the severity of the periodontal disease, especially elevated levels of IL-6 in inflammatory periodontal lesions, which can be a useful indicator or a diagnostic marker for periodontitis. Interleukin-8 (IL-8), a chemotactic cytokine, can have atherogenic properties through its multiple actions, including the recruitment of neutrophils and T-lymphocytes into the subendothelial space, monocyte adhesion to endothelium and migration of vascular smooth muscle cells.[12-15] IL-8 is believed to offer a basis for the assessment of diabetic risk and treatment of diabetic complications.[16] However, data about systemic IL-8 levels in DM are limited and no change in diabetes patients has been recorded. There is little information about the role of IL-8 in the initiation and progression of periodontal disease, although there is substantial information concerning its role in inflammation within other tissues.[17] Macrophage-derived human foam cells contain high amounts of IL-8 which can also increase the instability of an atherosclerotic plaque through inhibition of the tissue inhibitor of metalloproteinase expression.[18,19] Concentrations

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of IL-8 in gingiva and gingival crevicular fluid are reported to be higher at sites of active periodontal disease than in healthy sites.[20,21]

The aim of the present study was to examine the concentration of salivary IL-8 in children and adolescents suffering from Type 1 DM with concomitant periodontal disease and to determine any possible correlation to the periodontal clinical parameters (pocket probing depth, clinical attachment level and bleeding on probing).

MATERIALS AND METHOD

This research was designed as a cross-sectional study. The sample consisted of two groups. The experimental group was composed of 20 children and adolescents, 7–18 years of age, with Type 1 DM treated only with multiple daily insulin injections. The subjects were chosen consecutively for inclusion in this study from patients attending the Outpatient Diabetes Clinic at the Mother and Child Healthcare Institute of Serbia, over a 5-month period. Out of these, 10 subjects were diagnosed with concomitant periodontitis (P) (at least one site at two non-adjacent teeth with any degree of clinical attachment level loss) and formed in DM+P subgroup. Ten subjects with no signs of periodontitis formed in DM-P subgroup. These patients were also part of a larger number of children included in a study of cutaneous lesions.[22] The control group consisted of 20 randomly chosen, healthy children and adolescents, aged 7–18 years, attending the Clinic of Dental Medicine, Military Medical Academy, Belgrade, for routine dental check-ups (Table 1). On average, diabetes had been diagnosed 5.78 ± 3.45 years before the commencement of the study. Children considered for inclusion in both groups had been excluded from the study if they were undergoing active orthodontic therapy, had other systemic diseases or had received systemic antibiotic therapy in the 6 months prior to the study.

Table 1. Demographic characteristics of the sampled subjects

	DM	Controls	t-value	*p*
n	20	20		
Male/female	9/11	8/12	0.312	0.75
Average age	12.84 ± 3.93	12.4 ± 3.3	0.383	0.7
HbA1c (%)	8.79 ± 0.84	5.21 ± 0.65	13.72	0.001

The study protocol was approved by the Institutional Review Boards of the Military Medical Academy, Belgrade, and the Mother and Child Healthcare Institute of Serbia, Belgrade. Both parents/legal guardians and school-aged children signed a written informed consent form.

Periodontal examination was performed in the presence of parents/legal guardians by only one experienced and calibrated periodontologist (D.D.) in order to avoid interexaminer inconsistencies. Pocket probing depth (PPD) and clinical attachment level (CAL) were measured by a graduated Williams periodontal probe only around fully erupted permanent teeth. PPD, defined as the distance between the gingival margin and the bottom of the probable pocket and rounded to the nearest millimetre, was measured at 6 sites per tooth (mesio-buccally, mid-buccally, disto-buccally, mesio-lingually, mid-lingually and disto-lingually). CAL was calculated by subtracting the distance from the cementoenamel junction to the gingival margin from the PPD. The examination also included bleeding on probing (BOP), which was recorded at 4 sites (mesio-buccally, disto-buccally, mesio-lingually and disto-lingually), and, after a mild probing, given a score of 0-4 (0-no bleeding, 1-bleeding from one point only, 2-multi-point bleeding, 3-confluent bleeding, 4-profuse bleeding).[23]

The subjects' glycosylated haemoglobin (HbA1) levels were reviewed for data on diabetes duration. Cumulative HbA1 values, as a measure of glucose control, were expressed as a mean of the yearly HbA1 levels (Table 1). HbA1 levels in whole blood were measured by a DCA 2000 analyser (Bayer Co.; Elkhart, USA) with HbA1c cartridges. This assay was based on latex immunoagglutination inhibition methodology.

Unstimulated saliva was collected in the morning from both groups. A 5-minute production of saliva was collected with a sterile syringe, placed into ice-chilled, graded test-tubes and brought immediately to the laboratory. The content of IL-8 in saliva was determined using a human Th1/Th2 Kit, according to the manufacturer's protocol (Bender Medsystems, Vienna, Austria). Briefly, capture beads and biotin conjugated detection antibodies were added to 25 µL of samples (standard or test), and the mixture was incubated, in the dark, for 2 hours, at room temperature. Incubation took place for one more hour after excess unbound antibodies had been washed off and Streptavidin-PE added. After washing off, samples were analysed on a flow cytometer. Two-colour flow cytometric analysis was performed using a flow cytometer (EPICS, XL-MCL, Beckman Coulter). A total of 1800 events was attained for the 5.5 µmol bead population. Analysis was performed using FlowCytomixPro software. The minimum detection level for IL-8 was 0.5 pg/mL. However, it was treated as undetectable in all samples where the calculated cytokine concentration was below the given sensitivity,.

Statistical analysis

Statistical tests were performed using SPSS version 10.0 for Windows (StatSoft Inc., 1999). Comparisons of the demographic and periodontal characteristics of the experimental and control subjects were made using Student's T-test. P values were based on a two-sided test and considered significant if less than 0.05. Mean values of PPD, CAL, BOP and salivary IL-8 were calculated for each subject and group, and comparisons between the two groups were made using a two-sample Mann-Whitney U-test. Mean values were chosen because medians of some inflammatory markers were undetectable. The correlation between salivary cytokines and clinical parameters was verified by calculating the Pearson´s Correlation Coefficient. A cut-off for significance was set at $p<0.05$ (two-sided).

RESULTS

The demographic characteristics of the Type 1 DM patients and glycosylated haemoglobin levels are summarised in Table 1. There were no statistically significant differences in demographic parameters between the groups. PPD and BOP (Table 2) were significantly higher in the experimental group (diabetic patients) compared to the control group (healthy patients) as calculated by Student's T-test. The CAL in the diabetic children with or without periodontitis (DM+P – 1.31 ± 0.49 and DM-P – 0.47 ± 0.22) was not statistically significantly higher ($p=0.95$) compared to the healthy children (0.89 ± 0.24). However, there was a statistically significant difference for PPD, CAL and BOP between the Type 1 DM+P patients when compared to the Type 1 DM-P patients ($p<0.05$) (Table 3).

Table 2. Periodontal measurements in Type 1 DM patients and healthy individuals (controls) (Mean ± SD)

	DM	Controls	t	*p*
PPD	1.69 ± 0.41	1.45 ± 0.32	2.02	0.05
CAL	0.89 ± 0.57	0.89 ± 0.24	0.06	0.95
BOP	0.65 ± 0.33	0.26 ± 0.28	4.12	0.0001

Table 3. Periodontal measurements in Type 1 DM patients with (DM+P) and without (DM-P) periodontitis (Mean ± SD)

	DM + P (n=10)	DM – P (n=10)	t-value	*P*
PPD	2.05 ± 0.18	1.33 ± 0.19	8.80	0.0001
CAL	1.31 ± 0.49	0.47 ± 0.22	4.96	0.0001
BOP	0.88 ± 0.33	0.43 ± 0.56	4.33	0.0004

The salivary IL-8 level was statistically significantly higher in diabetic patients compared to the control group ($p<0.005$). However, there were no differences in the level of salivary IL-8 between the Type 1 DM+P patients and Type 1 DM-P patients ($p=0.97$) (Figure 1). A correlation (Pearson's correlation test) between the levels of salivary IL-8 and clinical parameters (PPD, CAL and BOP) was not found (Table 4).

Table 4. Results of the Pearsons correlations test between clinical parameters and salivary IL-8 levels in children with Type 1 diabetes mellitus (n=20).

	PPD		CAL		BOP	
	r	p	r	p	r	p
IL-8	0.07	0.78	0.04	0.85	-0.19	0.43

PPD – pocket probing depth; CAL – clinical attachment level; BOP – bleeding on probing;

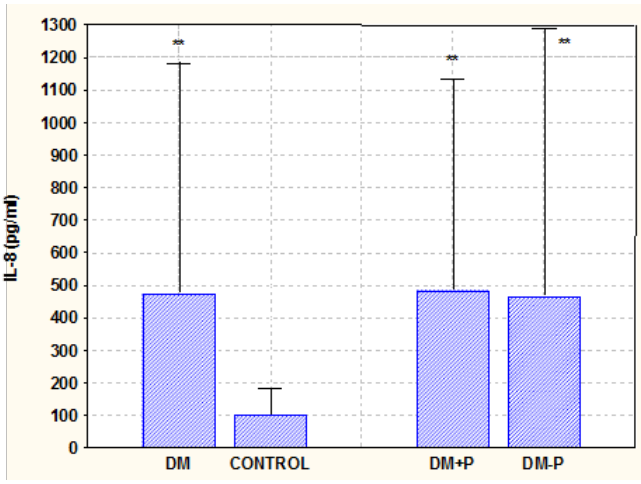

Figure 1. Salivary IL-8 levels in patients suffering from Type-1 DM with or without concomitant periodontitis and healthy controls.
** $p < 0.01$ vs control

DISCUSSION

Comparison of our clinical and laboratory research conducted on children suffering from Type 1 DM showed that they more frequently also had gingivitis and periodontitis compared to the healthy children of the same age. When compared to the control group, gingival and periodontal parameters (PPD, CAL, BOP) were statistically significantly higher in the diabetics, regardless of their periodontal status. Similar results were recorded previously in Serbia and other authors recorded the same results in their research implying that Type 1 DM is a risk factor for gingivitis and periodontitis.[24-29]

The second major finding of this study was the evidence that the level of salivary IL-8 was statistically significantly higher in the diabetic group than in the control group. However, we did not find any significant difference when the levels of salivary IL-8 in children with DM+P were compared to the levels in children with DM-P. To our knowledge, this is the first research on salivary inflammatory mediators and periodontitis in children with Type 1 DM. Fully developed periodontal disease can cause elevated salivary IL-8 levels. In this study the average measured PPD in the Type 1 DM+P children was 2.05 mm, while the average CAL was 1.31 mm (Table 2), which suggests that periodontitis was in its initial stage. Therefore, the elevation of the salivary IL-8 level was attributed to the presence of DM metabolic changes, rather than the presence of periodontitis. Furthermore, this was accentuated by the lack of correlation between the clinical parameters and the levels of salivary IL-8. Erbağci *et al* reported similar results by measuring the level of IL-8 in serum. In their study, the IL-8 serum level was elevated in children with Type 1 DM compared to the non-diabetic control group and remained significantly higher after adjustment for age, Body Mass Index, lipids, apolipoproteins and glycemic control.[30]

The elevation of IL-8 may be a result of its specific upregulation.[31] Increased serum levels of IL-8 have been reported in both adults and children with Type 1 DM.[29,32] It is assumed that the level of IL-8 was increased because hyperglycemia can induce transcription of the IL-8 gene in human endothelial cells and that hyperglycemia and ketosis regulate the production of IL-8 in cultured monocytes.[33,34]

The finding of an increase in the levels of IL-8 could suggest that the degree of metabolic control has a more specific influence on the regulation of a particular inflammatory mediator rather than on the general, pro-inflammatory state associated with Type 1 DM.[31] Increased IL-8 level could also be explained by the development of the diabetic macroangiopathy and pathogenesis of atherosclerosis and may offer a basis for further research related to the role of IL-8 for the assessment of risk, prophylaxis, and treatment of diabetic complications.[35]

The results of the Pearson's correlations test between the clinical parameters and the levels of salivary cytokines in the children with Type 1 DM did not show any statistically significant correlation. One possible explanation for these discrepancies is the fact that periodontitis was in its early stage in the children with Type 1 DM. Our findings are consistent with some, and conflicting with other, studies that found also good correlations of gingival bleeding but no correlation between attachment loss alone and diabetic control.[36]

It is also worth noting that the methodology used in this study has its limits. The dilution of gingival crevicular fluid components in saliva could mask the existing differences in the levels of interleukins at the site level.[37] Furthermore, the same authors reported that the differences in the methods of saliva collection (stimulated and unstimulated), processing (speed and time of centrifugation), storage (time, temperature and addition or not of protease inhibitors) and in the methodology used for the quantification of the biomarkers (enzyme-linked immunosorbent assay vs. Luminex) might also have had an impact on the difference in the results.[37]

CONCLUSION

The level of salivary IL-8 was significantly higher in the children with Type 1 diabetes mellitus compared to the healthy controls regardless of their periodontal status. Since no correlation was found between clinical periodontal parameters of disease and the levels of salivary IL-8, we concluded that the increase in the level of salivary IL-8 could be attributed to the presence of diabetes. This stresses the need for periodontal screening and timely prophylaxis and treatment of periodontal disease in the diabetic population. Periodontitis, as a complication of diabetes mellitus, should be given more attention and its treatment must be included early in life in the treatment plan for children suffering from diabetes mellitus.

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