

# Oral Health Status, Salivary MMP-8 & Secretory Leukocyte Peptidase Inhibitor (SLPI) Among Uncontrolled Type-I Diabetes Mellitus In Iraqi Patients

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## ABSTRACT

**Back ground:** diabetes is a metabolic disease characterized by hyperglycemia that results in deficiency or absence of insulin production. The dental caries and gingivitis/periodontitis are widespread chronic diseases in diabetes. The aim of the present study was determined the salivary matrix metalloproteinase (MMP-8), Secretory Leukocyte Peptidase Inhibitor (SLPI) and oral health status among uncontrolled diabetic group in comparison with healthy control group.

**Materials and Methods:** The total sample composed of 90 adults aged (18-35) years. Divided into 60 uncontrolled diabetic patients (HbA1c >7%) and 30 healthy control group. Unstimulated saliva was collected from each subject with type-I DM, BMI, duration of diabetes, HbA1c%, DMFT, gingival index (GI) and periodontal disease index were recorded during clinical visit. Level of salivary MMP-8 and SLPI was measured by using ELISA immunoassay analysis.

**Results:** The DMFT was highly significant higher among type-I DM group than control group ( $p < 0.001$ ), GI and pocket depth was significant difference ( $p = 0.002$ ,  $p < 0.001$  respectively) between two groups except the attachment loss with no significant difference ( $p = 0.06$ ). The Salivary MMP-8 was elevated, whereas SLPI was lowered in individuals with type I diabetes mellitus in comparison to the healthy controls, but statistically was non-significant. Analysis among uncontrolled diabetic patients revealed that the HbA1c% correlate positively significant with salivary MMP-8 ( $r = 0.321$ ,  $p = 0.012$ ), SLPI ( $r = 0.276$ ,  $P = 0.033$ ) and attachment loss ( $r = 0.353$ ,  $p = 0.006$ ); however the correlation between MMP-8 & SLPI was a significant in negative direction ( $r = -0.395$ ,  $p = 0.002$ ).

**Conclusion:** The DMFT, pocket depth and gingival index was higher in uncontrolled diabetes group. HbA1c% was positively correlation with MMP-8, SLPI and attachment loss in uncontrolled diabetic group. Salivary SLPI associated inversely with significant correlation with salivary MMP-8.

**Keywords:** Diabetes mellitus, Salivary MMP-8, SLPI, oral health status. (J Bagh Coll Dentistry 2017; 29(2):65-70)

## INTRODUCTION

Diabetes mellitus (DM) is one of the most serious diseases of metabolism and produces a developing medical irregularity, with concomitant morbidity and mortality that involve people of all ages<sup>(1)</sup>. It has been known to affect the salivary function and composition, eventually effecting oral cavity and dental health<sup>(2)</sup>. In the American Diabetes Association<sup>(3)</sup>, HbA1c has been referred to as A1C. It is consider as a good indicator of average glycemic concentrations during the previous 90 to 120 days and it is the standard method for assessing long-term glycemic control.

The diagnosis of type 1 diabetes may be occur at any age, at birth the individuals with a genetic susceptibility have normal beta cell mass but start to lose beta cells secondary to autoimmune destruction that occurs over months to years so the main cause of type 1 diabetes is immune-mediated nature, and the beta cell loss is a (T-cell) mediated autoimmune attack<sup>(4)</sup>.

The most consistent finding in poorly controlled diabetic patients is the periodontal disease.

Approximately 75% of these patients have periodontal disease that characterized by increased alveolar bone resorption and inflammatory gingival changes and the dental caries considers being a major oral health problem affecting children, adolescents, adults as well as elderly people<sup>(5,6)</sup>.

According to Pucher & Stewart<sup>(7)</sup> the periodontal disease is a group of chronic inflammatory disorder that associated with damage the periodontal attachment apparatus (cementum, collagen fibrils and a layer of calcified inter fibrillar matrix on the root surface of the tooth). The destruction of periodontal tissues starts early in young people with type-I, but it is more evidently expressed in pre-pubertal and pubertal periods, depending on the duration of disease, glycemic control and the presence of gingivitis<sup>(8)</sup>.

All MMPs have the following characteristics: they degrade proteins of the extracellular matrix (ECM); they require calcium for their stability; they contain zinc in the active site that can have a significant effect in a relevant pathophysiological end point<sup>(9)</sup>; Periodontal MMP-8 (collagenase 2) expression is associated with periodontal disease but the information concerning the periodontal

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MMP-8 expression in type-I diabetic patients with periodontal disease is insufficient<sup>(10,11)</sup>.

The Secretory Leukocyte Peptidase Inhibitor (SLPI) is an 11.7-kD a protein which is naturally present in saliva, suggesting that this protein is likely a major antiviral and antimicrobial component of oral secretions<sup>(12)</sup>. Although the level of glycemic control plays a central role with respect to periodontal status, the two chronic diseases (diabetes and periodontal diseases) are considered to be biologically linked, in addition to that the combination of diabetes with other risk modifiers for periodontal diseases such as cigarette smoking or genetic polymorphisms may confer cumulative risks not yet elucidated<sup>(13)</sup>.

## MATERIALS AND METHODS

The participants were 90 adult aged 18-35 years of both genders. The patients were examined at the Diabetic Clinic in Baghdad Teaching Hospital and AL- Kindy Teaching Hospital (Diabetic - Endocrinology Center) in Baghdad city during the period from (November 2014 to April 2015).

Unstimulated whole saliva sample were collected for 10 mints by the spitting method at the same day of blood sample aspiration for HbA1c measurements and after informed consent was obtained from all individual. They were all with confirmed diagnosis of Insulin Dependent Diabetes Mellitus (IDDM) with duration more than 3 years. Adult individual with any other systemic diseases, taking any medications like antihypertensive, anti-lipid and Aspirin and subjects less than 18 years of age were excluded. The samples were divided into two groups: 60 patients with uncontrolled type 1 diabetes mellitus (HbA1c >7%) and non-diabetic subjects as a control group were included 30 healthy subjects who did not suffer from any systemic disease and matching with the study group.

The Decayed-Missing-Filled teeth (DMFT) index which was introduced according to World Health Organization criteria<sup>(14)</sup> to measure the prevalence of dental caries/teeth, gingival index (GI) and periodontal disease index<sup>(15, 16)</sup> were all recorded for all participants. In the laboratory the salivary samples were centrifuged at (4000 rpm for 10 minutes) to remove any unwanted particles; then the supernatant has been taken by micropipette, aliquot into Eppendorf tubes (500 $\mu$ l) and stored at - 20°C and -70°C for protein until analysis. The immunoassay analyses of salivary sample were doing to measure the concentration of MMP-8 and SLPI by using an enzyme-linked immunosorbent assay (ELISA technique) according to the manufacturer's instructions. The

saliva sample was diluted by using phosphate buffer 150 fold; the concentration read from the standard curve must be multiplied by dilution factor.

Statistical analyses were done using SPSS version 21 computer software (Statistical Package for Social Sciences) in association with Excel version 5. The statistical significance of difference in mean between 2 groups was assessed using the independent samples t-test. P value less than 0.05 was considered statistically significant and highly significance when  $P < 0.01$ .

## RESULTS

The (mean  $\pm$  SD) age in patients with diabetes was (24.8 $\pm$ 5.4) years, while the (mean  $\pm$  SD) age in healthy controls subjects was (23.8 $\pm$ 5). Dental caries was significantly higher in diabetic patient than healthy control group ( $p < 0.001$ ). The mean DMFT in diabetic individual was (9) in comparison to the control (5.8).

In table 1, the median pocket depth and gingival index were significantly lower in healthy control group (0.92, 1.06 respectively) in comparison to the diabetic group (1.48, 1.25 respectively). The difference was significant between two groups, also the median attachment loss was lowest among healthy control group, but the difference was non-significant when compared to the uncontrolled diabetic group ( $P = 0.06$ ).

The table 2 showed the average of salivary MMP-8 & SLPI levels (ng/ml) among study groups. The highest salivary MMP-8 value was represented in the saliva of uncontrolled diabetic group, whereas the SLPI was lowest, but the difference was failed to reach the significant ( $p = 0.14$ ,  $p = 0.09$ , respectively). Among uncontrolled diabetic type-I the mean DMFT was lowest (6.9) among diabetic with youngest age group (<20 year) and increase gradually with increasing age to reach its maximum value (11.9) among oldest age group (30+ year), also the median pocket depth among diabetic with youngest age groups was lowest (1.3, 1.73 and 1.32 respectively) compared to the oldest age group (2.12). The difference was statistically significant ( $p = 0.007$ ,  $p = 0.044$ , respectively) as shown in table 3.

The mean DMFT was highest (9.4) among diabetic with acceptable weight group (<25) and decrease gradually with increasing weight to reach its minimum value (7) among obese group (30+), whereas the attachment loss median was highest in overweight group in comparison to other categories, but statistically no significant differences, on other hand the median pocket depth and gingival index was highest among

diabetic with overweight (1.88 & 1.37 respectively) and acceptable weight (1.44 & 1.25 respectively) in comparison to the obese group (0.93 & 1 respectively). This difference was statistically significant as shown in table 3 (P=0.03, P=0.041, respectively) as shown in table 3.

The correlation of HbA1c with salivary MMP-8, attachment loss and SLPI were

significant in positive direction among uncontrolled diabetic type-I group (r=0.321,p=0.012; r=0.353, p=0.006;r=0.276, p=0.033; respectively) as shown in table 4., in contrast the correlation between salivary MMP-8 and SLPI was statistically in negative direction (r=-0.395, p=0.002) (table 5).

**Table 1: Clinical parameters (mean/median) and Standard deviation among study groups**

Clinical and oral health parameters	Uncontrolled diabetic (type-I)		Healthy control		Statistical analysis P-value
	mean/median		mean/median		
Age (years)	24.8 (±5.4)		23.8 (±5)		0.44[NS]***
BMI	24.3 (± 4.3)		24.4 (±4.4)		0.91[NS]
DMFT	9 (±4.4)		5.8 (± 2.6)		P<0.001*
Loss of attachment	0.6		0.24		0.06[NS]
Pocket depth	1.48		0.92		P<0.001*
Gingival index	1.25		1.06		0.002**

\*(p<0.01) highly significant, \*\* (p<0.05) significant, \*\*\* (p>0.05) non-significant

**Table 2: Salivary MMP-8 and SLPI levels (ng/ml)(Median/Mean) among study groups**

Salivary parameter	Uncontrolled diabetic (type-I)		Healthy control		Statistical analysis P-value
	Median/Mean	Mean rank	Median/Mean	Mean rank	
MMP-8	67.1	48.4	41.1	39.7	0.14[NS]*
SLPI	31.5		36.8		0.09[NS]*

\* (p>0.05) non-significant

**Table 3: the difference in average (mean/median) of oral health status parameter between age and BMI categories among uncontrolled diabetic type-I**

Clinical parameters	Uncontrolled diabetic type-I(Mean/ Median)			
	DMFT	Attachment loss	Pocket depth	Gingival index
Age				
<20	6.9	0.82	1.3	1.03
20-24	8.4	0.42	1.73	1.27
25-29	9.4	0.36	1.32	1.06
30+	11.9	1.53	2.12	1.33
P-value	0.007*	0.32[NS]**	0.044*	0.12[NS]**
BMI				
Acceptable(<25)	9.4	0.42	1.44	1.25
Overweight (25-29.9)	9.1	1.13	1.88	1.37
Obese(30+)	7	0.21	0.93	1
P-value	0.23[NS]**	0.24[NS]**	0.03*	0.041*

\* (p<0.05) significant \*\* (p>0.05) non-significant

**Table 4: Correlation coefficients between HbA1c and salivary parameters (MMP-8, SLPI) and attachment loss among diabetic type-I group.**

Uncontrolled diabetic type-I						
Glycated Hemoglobin	Salivary MMP-8		Attachment loss		Salivary SLPI	
	r	p	r	p	r	p
HbA1c%	0.321	0.012	0.353	0.006	0.276	0.033

**Table 5: Correlation coefficients between salivary parameters (MMP-8, SLPI) among diabetic type-I group.**

Uncontrolled diabetic type-I		
Salivary SLPI	Salivary MMP-8	
	r	p
	-0.395	0.002

## DISSUSION

Diabetes mellitus has been linked with an increased risk of oral diseases. Saliva has a major role in maintaining the health of oral cavity. The recent consensus of the American Diabetes Association and the European Association for the study of diabetes advocate glycated hemoglobin as the main parameter to assess the metabolic control prior to dental treatment.

The present study was carried out to elucidate the effect of hyperglycemia on salivary parameter like MMP-8, SLPI and oral health status among uncontrolled diabetes mellitus type-I in Baghdad city in Iraq, in addition the study groups selected aged 18-35 years, as at these ages the type 1 diabetes mellitus are predominate, the duration of disease at least three years.

Data of the present study revealed the diabetic patient had significant higher mean DMFT than control healthy ( $P < 0.001$ ). The elevation in the severity of dental caries among uncontrolled diabetic group may be related to changes in the salivary secretion that reduce in diabetic patient, this a long with poor oral hygiene, decrease salivary flow rate, susceptibility to infection by microorganism and decrease immune response to inflammation or due to impaired neutrophil chemotaxis result in the development of dental caries<sup>(17)</sup>. This result is in agreement with<sup>(18, 19)</sup> and dis agreement with other studies, some found lower<sup>(20)</sup>, while other reported no difference in dental caries<sup>(1)</sup>.

On other hand, Eduardo Bernabé and Aubrey Sheiham<sup>(21)</sup> were found the level of caries increased through to adolescence and become a larger increase in DMFT in adulthood, in this study the DMFT among uncontrolled diabetic was lowest with youngest age group and increase gradually among oldest age group, significant differences was found between age groups.

Individual with type 1 diabetes mellitus and poor glycemic control have highest pocket depth median in compared to the healthy controls, in addition there is no differences in attachment loss median between diabetic and healthy controls groups. This may be due to the hyperglycemia that has a negative impact on the anti-inflammatory response and increases the oxidative stress of microvascular disorder in periodontal tissues, another explanation about the tissue change (periodontium) among DM due to the advanced glycation end-products (AGE) synthesized as a result of hyperglycemia, can convert macrophages into cells with a destructive phenotype, producing high levels of interleukin-1B, interleukin-6 and tumor necrosis factor-alpha (TNF-alpha). This AGE

have the ability to increase the permeability of endothelium and express high levels of molecular adhesion receptors that lead to high susceptibility to infections and delayed wound healing in diabetic patients<sup>(22)</sup>. The result is in agreed with other studies<sup>(23, 24)</sup>.

In this study there is a significant difference in GI median between study groups, the microflora in the dental plaque that forms daily adjacent to the teeth cause this inflammatory process<sup>(25)</sup>. This result is in agreed with other study<sup>(26)</sup> who found the subject with poor glycemic control has more sever gingival inflammation by the higher score of gingival index, also the pocket depth and gingival index become high in overweight category and decreased gradually in the obese category. The possible explanation may be related to the circulating Secretary Leukocyte Peptidase Inhibitor (SLPI) that express in subcutaneous white adipose tissue, in addition to the macrophage, neutrophil and mucous membrane epithelial cell<sup>(27)</sup>. This SLPI have antibacterial effect and play a significant role in the host defense by maintain the balance between inflammation and protective response, this result is disagreement with study<sup>(28)</sup> who found no increase in the circulating SLPI in diabetic patients and in agreement with other study<sup>(29)</sup> who reported the circulating SLPI has been correlated with metabolic dysfunction.

The highest salivary MMP-8 and lowest SLPI values were represented in the saliva of uncontrolled diabetic group, but the difference was failed to reach the significant this may be due to small size of sample. The positive correlation between salivary MMP-8, attachment loss and HbA1c% showed that the poor glycemic control patients (HbA1c >10%) has higher blood glucose level which make the salivary glucose level is increased and enters the oral cavity through saliva and gingival crevicular fluid, soaks the biofilm and causes an increase in total biofilm accumulation and started the inflammatory process which causes the alteration in the balance between activated MMPs and Tissue Inhibitors of Metalloproteinases (MMPs/TIMPs) that control the extracellular matrix, this imbalance result in elevated (MMP-8) level due to increase the neutrophils number migration into the periodontitis lesion which is correlated closely to increase MMP-8 level in GCF and saliva that mainly related to the connective tissue destruction and the periodontitis process will began, also the highly level of salivary glucose result in lowered the salivary SLPI and higher the elastase level in saliva and gingival crevicular fluid<sup>(30, 31)</sup>. These



results are in agreement with other researchers<sup>(26, 32,33)</sup>.

In conclusion the salivary SLPI associated inversely with significant correlation with salivary MMP-8 that expressed in periodontal tissues in type I DM and it considers to be a key mediators of the irreversible tissue destruction that associated with periodontitis and correlated positively with poor glycemic control (HbA1c >7%). If regular oral screenings and periodontal treatment programs are considered as a standard of care for young patients with Type 1 DM, the periodontal diseases can be prevented in these individuals especially at an early stage of diseases.

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### المستخلص

المقدمة: مرض السكري هو مرض أيضي يتميز بارتفاع السكر في الدم والذي يؤدي الى نقص او غياب الانسولين ويعتبر تسوس الاسنان، التهاب اللثة وامراض اللثة و ما حول الاسنان من الامراض المزمنة على نطاق واسع في مرض السكري. ان الغرض من الدراسة هو تحديد انزيم (MMP-8) وبروتين (SLPI) في اللعاب والحالة الصحية للفم بين مجموعة مرضى السكري غير المنضبط ومقارنتهم بالاشخاص الاصحاء الغير مصابين بداء السكري.

المواد وطرائق العمل: اشتملت الدراسة 90 شخص بلغ تتراوح اعمارهم بين 18-35 سنة. مقسمة الى 60 مريض بداء السكري غير المنضبط نسبة ( $HbA1c > 7\%$ ) و 30 شخصا سليما غير مصابين بداء السكري كمجموعة ضابطة. وتم جمع عينات اللعاب غير المحفز من كل شخص مصاب بالنوع الاول من داء السكري وتم تحديد مؤشر كتلة الجسم، مدة الاصابة بمرض السكري، اختبار الكلايكتيد هيموغلوبين، ونسبة التسوس من خلال مؤشر DMFT، ومؤشر اللثة، ومؤشر امراض اللثة وما حول الاسنان من خلال الزيارة السريرية. وقد تم قياس مستوى انزيم (MMP-8) وبروتين (SLPI) في اللعاب باستخدام طريقة التحليل المناعية

النتائج: اظهرت النتائج ان نسبة التسوس لدى مرضى النوع الاول لداء السكري مرتفعة وبفرق احصائي معنوي عال مقارنة بالمجموعة الضابطة، ووجد ان الحالة الصحية للفم والتي تشمل مؤشر التهاب اللثة وعمق الجيب ايضا مرتفعة وبفروق معنوية بين المجموعتين باستثناء فقدان النسيج الرابط حيث لا يوجد فرق ذات دلالة احصائية مهمة. واطهرت النتائج ايضا ان مستوى انزيم (MMP-8) في اللعاب لدى الافراد المصابين بالنوع الاول لداء السكري عالي بالمقارنة مع الاصحاء وبروتين (SLPI) منخفض لكن لا يوجد فرق معنوية. وكشفت النتائج ان نسبة ( $HbA1c$ ) ترتبط بعلاقة احصائية معنوية مع انزيم (MMP-8) وبروتين (SLPI) والعلاقة مع فقدان النسيج الرابط كبيرة وفي اتجاه ايجابي عند مجموعة المصابين بداء السكري غير المنضبط.

بالاضافة لذلك كان الارتباط بين (MMP-8) و (SLPI) معنوي وباتجاه سلبي.

الاستنتاج: كانت نسبة DMFT وعمق الجيب والتهاب اللثة عالي لدى مجموعة مرضى السكري النوع الاول غير المنضبط. ( $HbA1c$ ) % لديه ارتباط ايجابي مع (MMP-8) ، (SLPI) ، فقدان النسيج الرابط بينما يرتبط انزيم (MMP-8) في اللعاب ارتباطا معنويا وباتجاه عكسي مع بروتين (SLPI).