Salivary a-Amylase and Albumin Levels In Patients with Chronic Periodontitis and Poorly or Well Controlled Type 2 Diabetes Mellitus.

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ABSTRACT

Background: Recent studies suggest that chronic periodontitis (CP) and type2 diabetes mellitus (T2DM) are bidirectionally associated. Analysis of saliva as a mirror of oral and systemic health could allow identification of a amylase (a-Am) and albumin (A1) antioxidant system markers to assist in the diagnosis and monitoring of both diseases. The aims of study, compare the clinical periodontal parameters in chronic periodontitis patients with poorly or well controlled T2DM, salivary a-Am, A1, flow rate (FR) and pH then correlate between biochemical, physical and clinical periodontal parameters of each study and control groups.

Materials and Methods: 80 males, with an age range of (35-50) years were divided into four groups, (20 subjects at each): two groups had well or poorly controlled T2DM both of them with chronic periodontitis, group of patients with only chronic periodontitis and control group with healthy periodontium and systemically healthy. From all subjects unstimulated whole salivary samples were collected to measure FR, pH, Al and a – Am, then clinical periodontal parameters (plaque index, gingival index, bleeding on probing, probing pocket depth and clinical attachment level)were recorded.

Results: patients had chronic periodontitis with poorly controlled T2DM demonstrated the highest median values of all clinical periodontal parameters and highest increase in levels of salivary a-Am and Al with lowest median values of FR and pH, in addition to the highly significant differences among the study and control groups regarding biochemical and physical parameters. Positive correlation were revealed between a-Am with Al and both of them with all clinical periodontal parameters but, they were negative with FR and pH.

Conclusion: patients with poor glycemic control had more severe periodontal tissue break down with decrease in FR and pH also obvious increase in levels of A1 and a- Am so, these biochemical markers will provide an objective phenotype to allow practitioners for early diagnosis, which is essential for improved prognosis and effective delay of clinical complications associated with chronic periodontitis and DM and an important strategy to lower the incidence of both diseases world wide.

Keywords: periodontitis, T2DM, salivary albumin and α-amylase.

المستخلص

الخلفيه:الدراسات اثبت ان التهاب اللثه المزمن وداء السكري من النوع الثاني مرتبطان. تحليل اللعاب وتحديد مستوى اميليز و البومين للمساعده في تشخيص الامراض. اهداف الدراسه قياس مؤشرات اللثه السريريه في مرضى التهاب اللثه اللمزمن مع او بدون السكري من النوع الثاني المسيطر عليه او الغير مسيطر عليه و الفا اميليز و البومين اللعابي و حامضيه و معدل سريان اللعاب ثم تحديد العلاقه بينهم

المواد و الطرق: ٨٠ ذكر بعمر(٣٥-٥٠)سنه تم تقسيمهم لاربع مجاميع(٢٠ في كل مجموعه) اثنان لديهم التهاب اللثه المزمن مع السكري من النوع الثاني المسيطر او غير مسيطر عليه و مجموعه التهاب اللثه المزمن و المجموعه الضابطه.ثم جمع اللعاب منهم لقياس حامضيه ومعدل سريان اللعاب و اميليز و البومين اللعابي و مؤشرات اللثه السريريه.

النتائج:مرضى التهاب اللثه المزمن مع السكري من النوع الثاني الغير مسيطر عليه اظهروا اعلى قيم لمؤشرات اللثه السريريه و اعلى زياده في الاميليز و البومين و اقل قيم لحامضيه و معدل سريان اللعاب مع فروقات معنويه عاليه بين المجاميع.هناك علاقات موجبه بين اميليز و الالبومين و كلاهما مع مؤشرات انسجه ماحول الاسنان السريريه لكنها سالبه مع حامضيه و معدل سريان اللعاب

الاستنتاج: مرضى السكري النوع الثاني يعانون من تدمير اكثر لانسجة اللثة وانخفاض في حامضية وسريان اللعاب وزيادة في اميليز والبومين وهذين الأخيرين يساعدان في التشخيص المبكر لهذين المرضين لتقليل الاصابة بهما.

INTRODUCTION

Periodontitis is irreversible inflammatory disorder of the supporting structures of the tooth leading to progressive attachment loss and destruction of alveolar bone. Chronic periodontitis (CP) is the most prevalent form of periodontitis, hence affects about 10%-15% of adult population world wide. Furthermore in the presence of systemic disease (e.g.DM),which modify the host response to plaque accumulation, the disease progression may become more aggressive[1]. The DM, is a metabolic disorder characterized by hyperglycemia and T2DM which is the most common type is linked to insulin resistance and patients with DM are prone to oral complications such as periodontal disease (PD), dry mouth and abscesses [2]. Hence, today various researches are being conducted to evaluate possible compound in the oral fluids through which it may possible to assess the presence and severity as well as, to identify the patients at risk for these diseases thus, analysis of saliva which is a complex secretory fluid that can be easily collected through non-invasive means for the screening of large samples in addition, saliva contains locally produced microbial and host response mediators, as well as, systemic (serum) markers [3]. Thus the investigation of salivary proteins such as Al and α- Am in patients with CP and DM may be useful to enhance the knowledge of their roles in these diseases. So, this study was designed to determine the effect of glycemic control in T2DM on periodontal health status as well as, on the levels of salivary Al, α-Am, FR and pH.

MATERIAL & METHODS

The participants in this study was 80 males with age range (35-50) years, recruited from specialized center for endocrinology and Diabetes in Baghdad and from periodontics Department, at the teaching hospital in the College of Dentistry, University of Baghdad. They were divided into four groups.

1. Study group of 20 males suffer from CP with well controlled T2DM HbA1c < 7%[4] (CP+wT2DM).
2. Study group of 20 males suffer from CP with poorly controlled T2DM,HbA1C > 9%[4](CP+pT2DM).
3. Study group of 20 males suffer from CP but systemically healthy (CP).
4. Control group of 20 males with clinically healthy periodontium and apparently systemically healthy. Healthy periodontium defined by the absence of any signs and symptoms of gingival inflammation, without periodontal pockets or clinical attachment loss. This group represented a base line data for the salivary Al and α-Am levels.

Patients with CP demonstrated the presence of at least four sites with PPD (≥4mm) and clinical attachment loss of (1-2) mm or greater [5].

The inclusion criteria were only males with at least 20 teeth present, T2DM ≥ 5 years on oral hypoglycemic therapy only and body mass index within the normal range (18.5-24.9 kg/m^2) [6]. The exclusion criteria were females, presence of systemic diseases other than T2DM, patients administered medications (anti-inflammatory and anti-microbial) or undergone periodontal treatment in the 3 months prior to the study, smoking, alcohol consumption, T1DM and T2DM administring insulin, presence of nephropathy, retinopathy and diabetic foot. Unstimulated whole salivary samples were collected from all participants [7]. During that salivary (FR) was measured through dividing the volume of the collected sample by the collection time. After this by using DP universal test paper, the salivary pH was measured, then samples were centrifuged for 15min. at 4000 rpm and frozen, at -20 ^{o}C. By using Michigan O periodontal probe, the examination of clinical periodontal parameters was performed on four surfaces (mesial, buccal /labial, distal and lingual / palatal) of all teeth except the 3rd molar, which included:

1. Plaque index system (PLI)[8].
2. Gingival Index system (GI) [9].
3. Bleeding on probing (BOP) [1].
4. Probing pocket Depth (PPD).
5. Clinical Attachment level (CAL).

For biochemical analysis of salivary A1, Protein U.S / Syrbio kit was used. While for salivary α-Am, (single Reagent GALG2-CNP) /SPECTRUM kit was used, hence the activities were determined by measuring the absorbace at 598 nm and 405 nm respectively both by the spectrophotometer. Descriptive statistics that include mean and median values and inferential statistics which include kruskal – Wallis H test, Mann- Whitney U test and pearson correlation (r) were used. The level of significance (S) was accepted at P ≤0.05, highly significance (HS) at P < 0.01 and non-significant (NS) at P > 0.05. We certify that this study involving human subjects is in accordance with the Helsinky declaration of 1975 as revised in 2000 and that it has been approved by the relevant institutional ethical committee.

RESULTS

The highest mean of age was found in CP + pT2DM group (45.85), followed by CP + wT2DM (44.95), then CP group (41.7) while, the least mean of age was detected in control group (38). Patients with CP + pT2DM demonstrated the highest median values of the clinical periodontal parameters, then patients suffer from CP + wT2DM, after that CP patients. Inter study groups comparisons regarding all clinical periodontal parameters revealed, HS differences between CP + pT2DM with both CP + wT2DM and CP groups while, they were NS differences between CP + wT2DM with CP groups (Table -1).

From (Table -2), the biochemical analysis of both Al and α – Am presented that highest increase in median values were revealed in CP + pT2DM group after that patients with CP + wT2DM, then CP group as compared to the control group hence, HS differences were demonstrated among the four groups. On the other hand, the physical parameters analysis showed decrease in median values of both FR and PH in study groups when compared to control group and the lowest median values demonstrated in CP + pT2 DM group. Again, HS differences among the study and control groups were found.

The comparisons between all pairs of the study and control groups regarding α-Am, A1, FR and pH demonstrated HS and S differences except the NS differences between CP+wT2DM with CP groups concerning α-Am, A1 and pH(Table -3).

The results of correlations (Table -4&5) between α-Am and A1 with clinical periodontal parameters were positive but they were negative with FR and pH at all groups, although α-Am revealed moderate positive correlations with PLI and GI at CP + pT2 DM and CP + wT2DM groups respectively.

The correlations between α-Am with Al were

positive at all groups (table -6).

DISCUSSION

The CP + T2DM patients revealed higher mean of age, this can be explained by the greater incidence of both diseases in adults[10].

In diabetic patients, the vascular changes, neutrophil dysfunction, altered collagen synthesis, accumulation of advanced glycation end products leading to impaired tissue repair capacity [1], as well as increased glucose level in gingival crevicular fluid (GCF) and saliva [11], decrease FR that disrupt the cleaning and buffering capacities and clearance of bacterial substrate which then increase accumulation of plaque and calculus [12], in addition increased levels of α – Am and Al,in which the former favored proliferation of both aerobic and anerobic bacteria in plaque, while the latter considered potential energy sources and enable the attachment of pathogenic bacteria thus alter the composition of plaque [13]. So, diabetics had 3fold increase in risk of having periodontitis compared to non-diabetics,hence adults with an HbA1c level 0f 9%had significantly higher prevalence of severe periodontitis thus, the gingival inflammation and bleeding are intensified, greater prevalence and extent of pockets with twice as likely a non- diabetics to have attachment loss [2,10,12].

Saliva contains numerous defense antioxidant proteins e.g. Al and α- Am which able to inhibit the generation of free radicals [14]. The highly significant increase in α-Am level in CP patients as compared to control group revealed by this study are in accordance with other studies [15-19], the same result was found when comparing CP + T2 DM groups with control group, hence different researchers had reported that salivary α-Am concentrations from T2DM patients were higher [11,20-22] or lower [23-26] than its levels in non-diabetics. The response of salivary gland to inflammatory diseases, resulting in enhanced synthesis and secretion of defense proteins [15]. The increased basement membrane permeability of salivary glands in diabetics leads to increased passage of proteins into the saliva, moreover the sialosis in the parotid gland in T2 diabetics, hence most of α -Am being synthesized in this gland, could result in variations in the salivary composition [22]. Studies showed that α – Am is a major lipopolysaccharide binding protein of *Agri, gatibacter actinomycetem comitans* and *Porphyromonas gingivalis (P.gingivalis)* and interfere with bacterial adherence and biofilm formation also performs a direct inhibitory effect on the growth of *Neisseria Gonorrhoea* and *P.gingivalis* [13]. The notable increase in Al level in CP patients in comparison to control subjects in this study was in consistent with findings of previous studies [27-29], while others [14,30]demonstrated decrease in Al levels with deterioration of periodontal tissue condition. Although the significant increase of Al in T2 diabetics found by researchers [31,32] were in agreement with this study, but disagree with other results [33,34]. Al accounting for more than 50% of all plasma proteins, thus is regarded as markers for plasma protein leakage occurring as a consequence of inflammatory process, so the high salivary Al level in CP patients due to ulceration in sulcular epithelium confirming the sulcular origin of Al from GCF, thus 4-5 times rise in Al level was noted during periodontal tissue destruction when compared with that of the control [28],moreover the presence of *Treponema Denticola* seemed to increase Al in periodontitis patients [35]. On the other hand, disregulation in the factors that regulate Al synthesis during DM occur which include nutrition, hormonal balance and osmotic pressure and the inflammation of salivary gland causing increased leakage of serum proteins into the saliva [33]. Finally, studies measured A1 and α – Am levels in T2 diabetics, they ignor their periodontal health status.

The more acidic pH in CP patients was in line with some studies [29,36],hence significant correlation did exist between pH and PPD on the other hand increase in pH was found [28,37] in CP patients. From the present study the decrease in pH of diabetics was coincide with other reports [12,25,38], hence significant decrease in pH was demonstrated when comparing uncontrolled T2DM with healthy and controlled T2DM as well as, healthy with controlled T2DM [39]. The decrease in salivary FR and bicarbonate content consequently contributed to the more acidic saliva [38]. The higher concentrations of hydrogen ions (from salivary glands or oral microbiota), the lowest the pH, since pH level negatively correlated with proportion of periodontal pathogens, that grow in mildly acidic pH, either utilize or create products that are mild to moderately acidic in nature [29].

The decrease in salivary FR in this study coincide with others concerning CP [15,19,29], and DM [12, 24,25,40] but diverge with previous studies about CP [37] and DM [41] who reported increased of FR, on the other hand some researchers found that FR levels not affected by periodontal health status [28] or presence of DM [42]. There are multiple causes of salivary hypofunction including inflammation e.g. periodontal disease [19], hydrogen concentration, aging [40] or systemic disease e.g. DM [24], so in this case the

decrease in pH, medication givin for diabetics, poly urea and dehydration, neuropathies, microvascular changes, metabolic disturbances also, hypertrophy of salivary glands can be attributed to decrease in FR [12, 25].

Positive correlations of α –Am and A1 with each other and with clinical periodontal parameters, but they were negative with FR and pH, this can be explained by the presence and increased inflammation with periodontal tissue destruction due to CP and DM which lead to increased levels of α-Am and Al but decrease in FR and pH. These results were in concurrent with other results [16,17,19] who found significant positive correlations between α-AM with PPD and CAL, while significant negative correlation with FR[19] in CP patients. In general, there were correlations between α – Am with glycemic control [23,24,26], but non significant with FR at controlled and uncontrolled T2DM[23]. Significant positive correlation was detected between Al levels with GI in T2 diabetes [43].

Finally, the results may differ from one study to another these may be due to e.g. the diversity in selection criteria of samples, metabolic control, wide range of age, different types of saliva,that can limit direct comparison.

Table (1): Median values of the clinical periodontal parameters and the inter groups comparisons between all pairs of the study groups

Clinical periodontal parameters	*Groups*	*Median*	*CP+ pT2DM & CP+ wT2DM*		*CP+ pT2DM & CP*		*CP & CP+ wT2DM*	
			Mann Whitney U test	*P-value Sig.*	*Mann Whitney U test*	*P-value Sig.*	*Mann Whitney U test*	*P-value Sig.*
PLI	CP+ pT2DM	2.682	4.735	0.00 HS	5.411	0.00 HS	1.948	0.51 NS
	CP+ wT2DM	1.815						
	CP	1.341						
	Control	0.232						
GI	CP+ pT2DM	2.553	5.410	0.00 HS	5.42	0.00 HS	0.677	0.499 NS
	CP+ wT2DM	1.556						
	CP	1.5						
	Control	0.108						
BOP score1	CP+ pT2DM	60.5	4.390	0.00 HS	3.993	0.00 HS	1.233	0.217 NS
	CP+ wT2DM	46						
	CP	42						
PPD	CP+ pT2DM	6.67	4.363	0.00 HS	4.255	0.00 HS	0.825	0.409 NS
	CP+ wT2DM	6.13						
	CP	5.945						
CAL	CP+ pT2DM	4.4	4.372	0.00 HS	4.749	0.00 HS	0.989	0.323 NS
	CP+ wT2DM	3.08						
	CP	2.435						

***P<0.01 High significant**

Table (2): Median values of salivary α-Amylase , Albumin ,FR and pH and the significance of differences among the study and control groups.

parameters	*CP+ pT2DM*	*CP+ wT2DM*	*CP*	*Control*	*Kruskal-Wallis H test*	
	Median	*Median*	*Median*	*Median*	*Chi square*	*P-value Sig.*
α-Amylase U/L	162.14	99.25	90.86	65.47	43.62	0.00 HS
Albumin mg/dl	104.8	79.18	75.72	56.51	30.568	0.00 HS
FR ml/min	0.23	0.725	0.75	1.2	65.6	0.00 HS
pH	5	6	6	7	24.96	0.00 HS

Table(3): Inter groups comparisons of the median values of salivary α-Amylase , Albumin ,FR and pH between all pairs of the study and control groups

parameters	CP+ pT2DM&CP+ wT2DM		CP+ pT2DM&CP		CP+ pT2DM&-Control		CP+ wT2DM&CP		CP+ wT2DM&-Control		CP& Control	
	Mann Whitney U test	P-value	Mann Whitney U test	P-value	Mann Whitney U test	P-value	Mann Whitney U test	P-value	Mann Whitney U test	P-value	Mann Whitney U test	P-value
α-Amylase U/L	3.354	0.001 S	3.517	0.00 HS	5.410	0.00 HS	0.352	0.725 NS	4.436	0.00 HS	4.003	0.00 HS
Albumin mg/dl	2.998	0.021 S	2.976	0.03 S	4.816	0.00 HS	0.864	0.322 NS	2.332	0.044 S	4.275	0.00 HS
FR ml/min	4.998	0.00 HS	5.437	0.00 HS	5.444	0.00 HS	4.870	0.00 HS	5.278	0.00 HS	4.809	0.00 HS
pH	4.275	0.00 HS	4.925	00.00 HS	5.231	0.00 HS	1.274	0.203 NS	3.213	0.01 S	2.453	0.014 S

Table (4): Correlations between the levels of α-Amylase with the clinical parameters of each study and control groups.

parameters	Statistical analysis	CP+ pT2DM	CP+ wT2DM	CP	Control
PLI	r	0.56	0.188	0.254	0.248
	p	0.816 NS	0.427 NS	0.281 NS	0.292 NS
GI	r	0.164	0.57	0.222	0.290
	p	0.489 NS	0.012 S	0.348 NS	0.214 NS
BOP score1	r	0.207	0.227	0.003	-
	p	0.381 NS	0.330 NS	0.990 NS	-
PPD	r	0.173	0.039	0.199	-
	p	0.466 NS	0.871 NS	0.400 NS	-
CAL	r	0.154	0.201	0.068	-
	p	0.516 NS	0.395 NS	0.775 NS	-
FR	r	-0.268	-0.442	-0.009	-0.156
	p	0.254 NS	0.049 S	0.969 NS	0.511 NS
pH	r	-0.131	-0.035	-0.144	-0.096
	p	0.582 NS	0.884 NS	0.543 NS	0.687 NS

Table(5): Correlations between the levels of Albumin with the clinical parameters of each study and control groups.

parameters	Statistical analysis	CP+ pT2DM	CP+ wT2DM	CP	Control
PLI	r	0.148	0.134	0.085	0.131
	p	0.533 NS	0.573 NS	0.721 NS	0.581 NS
GI	r	0.327	0.186	0.070	0.224
	p	0.159 NS	0.434 NS	0.771 NS	0.343 NS
BOP score1	r	0.378	0.157	0.186	-
	p	0.100 NS	0.508 NS	0.434 NS	-
PPD	r	0.121	0.268	0.255	-
	p	0.611 NS	0.253 NS	0.277 NS	-
CAL	r	0.482	0.189	0.107	-
	p	0.032 S	0.424 NS	0.653 NS	-
FR	r	-0.321	-0.214	-0.151	-0.046
	p	0.167 NS	0.365 NS	0.526 NS	0.847 NS
pH	r	-0.197	-0.273	-0.045	-0.235
	p	0.406 NS	0.245 NS	0.849 NS	0.318 NS

Table (6): Correlations between salivary levels of(α-Amylase and Albumin of each study and control groups.

Parameters	*Statistical analysis*	*CP+ pT2DM*	*CP+ wT2DM*	*CP*	*Control*
α-amylase and albumin	r	0.291	0.103	0.195	0.511
	p	0.214 NS	0.665 NS	0.411 NS	0.831 NS

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