An Assessment of Salivary Leptin and Resistin Levels in Type Two Diabetic Patients with Chronic Periodontitis (A Comparative Study)

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ABSTRACT

Background: Type 2 diabetes mellitusand chronic periodontitis hold a close relationship that has been the focus of many researches. Currently there is an appreciation to the role of adipose tissue-derived substances "the adipokines" in immune-inflammatory responses; also, there is an interest in using the simple non-invasive saliva in diagnosing and linking oral and general health problems. The current study aims to determine the periodontal health status in the chronic periodontitis patients with and without poorly or well controlled type 2 diabetes mellitus, measure the salivary levels of two adipokines "leptin and resistin", pH and flow rate and then correlate between these clinical periodontal, biochemical and physical parameters in each study and control groups.

Materials and Methods: Seventy five males were recruited for the study, with an age range of (35-50) years. The subjects were divided into four groups: two non-diabetic groups: one of them with healthy periodontium and systemically healthy (Control, 15 subjects) and the other with chronic periodontitis (20 patients) and two type 2 diabetic groups: well controlled (20 patients) and poorly controlled (20 patients) both of them with chronic periodontitis.Unstimulated whole salivary samples were collected from all of the participants; salivary flow rate and pH were measured and then biochemically analyzed for assessment of resistin and leptin levels.Clinical periodontal parameters included: the plaque index, the gingival index, the bleeding on probing, the probing pocket depth and the clinical attachment level had been recorded for all subjects at four sites per tooth except for the third molars.

Results: The results of clinical periodontal examination revealed that the group of chronic periodontitis with poorly controlled type 2 diabetes mellitus had the worst periodontal health status. The biochemical analysis demonstrated that the lowest level of salivary leptin was foundin the chronic periodontitis with poorly controlled type 2 diabetes mellitus group. In addition, the highest level of salivary resistin was demonstrated in chronic periodontitis with well controlled type 2 diabetes mellitus group. When the salivary flow rate and pH were measured, it was found that they were decreased in the study groups as compared to the control group. A non-significant moderate negative correlation between salivary leptin with pH in the control group was found. While, salivary resistin demonstrated a high significant moderate positive correlation with the gingival index in the non-diabeticchronic periodontitis group and a non-significant moderate negative correlation with salivary flow ratein the control group. Finally, the study found that the correlation between salivary leptin and resistin was non-significant weak negative in each of the study and control groups.

Conclusion: It can be concluded that poorly controlled type 2 diabetic patients have more periodontal tissue destruction and less salivary flow rate than well controlled type 2 diabetic patients and non-diabetic patients all of them with chronic periodontitis. Salivary Resistin and Leptin hormones may be useful biochemical markers of periodontal tissue destruction and this will provide better opportunities in early diagnosis, monitoring and efficient management of periodontal diseases and T2DM.

Key words: T2DM, CP, resistin, leptin and saliva. (J Bagh Coll Dentistry 2015; 27(4):107-114).

INTRODUCTION

Diabetes is a group of metabolic diseases in which hyperglycemia results from defects in insulin secretion and/or action. The most prevalent type is type 2 diabetes mellitus (T2DM). The chronic hyperglycemia of diabetes adversely affects different body organs, particularly the eyes, kidneys, heart, blood vessels and nerves [(1)].

The periodontal disease (PD) is a chronic inflammatory process that affects the tooth supporting tissues and occurs as result of interaction between the periodontopathic bacteria and the host immune system. It can be broadly divided to gingivitis (which is a reversible form that isn't accompanied by attachment loss) and periodontitis (which is an irreversible form and results in attachment loss) [(2)].

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The most common form of periodontitis is the chronic periodontitis (CP) that typically affects adults between 40 to 50 years old and is characterized by its slowly progressing nature, but at some point undergoes exacerbation[(3)]. There is a close association between T2DM and PD that been well recognized in many clinical and epidemiological studies [(4-6)].

Resistin and leptin belong to the adipose tissue-derived adipokines which are molecules participate in the pathogenesis of both CP and T2DMvia their roles in immune-inflammatory responses, bone metabolism and insulin sensitivity [(7,8)].

Nowadays, there is a trend toward using the saliva as a diagnostic fluid for determination of systemic diseases because it is a non-invasive and cost-effective method [(9)] and this has motivated us to perform the current study which utilizes the salivary resistin and leptin levels for the purpose of determination of the effect of

glycemic control on the periodontal health status in patients with CP, since these two hormones are involved in the immune and inflammatory responses that comprise the basis of the cross-susceptibility between the CP and T2DM.

MATERIALS AND METHODS

The human sample consists of 75 males with age range of (35-50) years. The collection of patients with T2DM started in Erbil from family health care centers and then in Baghdad, were recruited from specialized center for Endocrinology and Diabetes in Baghdad /Al-Russafa, while the control and chronic periodontitis subjects were recruited from Periodontics Department, at the teaching hospital, in the College of Dentistry, University of Baghdad.

The subjects were divided into four groups:

A. CP with poorly controlled T2DM (CP+pT2DM): consisted of 20 males with CP and HbA1c > 9%.
B. CP with well controlled T2DM (CP+wT2DM): consisted of 20 males with CP and HbA1c < 7%.
C. Systemically healthy with chronic periodontitis (CP): consisted of 20 males. CP in patients was defined as the presence of minimally four sites with PPD ≥ 4 mm and clinical attachment loss of (1-2) mm or greater[10].
D. Systemically healthy with healthy periodontium (Control): consisted of 15 males apparently systemically healthy and with clinically healthy periodontium, this was defined by gingival index (GI) scores <0.5 [11] and without periodontal pockets or clinical attachment loss. This group represents a base line data for the levels of salivary Leptin and Resisitin.

Inclusion criteria include only males with T2DM (diabetic for ≥ 5 years) on oral hypoglycemic therapy only, at least 20 teeth present and body mass index within the normal range which is between 18.5-24.9 Kg/m^2 [12]. While, the exclusion criteria included: females, T1DM and T2DM administering insulin, smoking and alcohol consumption, presence of systemic diseases other than T2DM, presence of nephropathy, retinopathy and diabetic foot, patients who've undergone periodontal treatment oradministrated medications (anti-inflammatory, anti-microbial,anti-depressants and anti-lipidemic) in the three months prior to the study.

Unstimulated whole salivary samples were collected from all of the groups at 9-12 a.m.,[13]. Salivary flow rate (FR) was calculated by dividing the volume of the collected sample by the collection time. Then salivary pH was measured by using (DP universal test paper), then the samples were centrifuged at 4000 rpm for 15 min. and frozen at -20 ºC.

Clinical periodontal parameters examination was performed after collecting the salivary samples by using the Michigan O periodontal probe on four surfaces (mesial, buccal/ labial, distal and lingual/palatal) of all teeth except the third molar. These included:

1. Assessment of Soft Deposits by the PlaqueIndex System (PLI) [14].
2. Assessment of Gingival Inflammation by theGingival Index System (GI) [11].
3. Assessment of Gingival Bleeding on Probing (BOP) [15].
4. Assessment of Probing Pocket Depth (PPD).
5. Assessment of clinical attachment level (CAL).

For the purpose of biochemical analysis of salivary leptin hormone we used Demeditec Leptinenzyme-linked immunosorbent assay (ELISA) test kit (DEE007) and used (DEE050) Demeditec Resistin ELISA kit of salivary resistin hormone. Both hormones concentrations were determined by measuring the absorbance at 450 nm by the spectrophotometer.

Descriptive statistics in the form of median value and inferential statistics in the form of Kruskal-Wallis H test, Mann-Whitney U test and Pearson Correlation were used in this study.

The levels are accepted as significant (S) at (0.05 ≥ P-value > 0.01), highly significant (HS) at P-value ≤ 0.01 and non-significant (NS) at P-value > 0.05.

RESULTS

The highest mean of age parameterwas found in CP+pT2DM (46.30) followed by CP+wT2DM (45.40), CP group (42.10) and the least mean was found in Control group (37.53).

Clinical Periodontal Parameters Analysis:

The highest median values of the clinical periodontal parameters were recorded in CP+pT2DM, followed by CP+wT2DM then CP group except for BOP and PPD; the score 1 BOP was higher in CP than in CP+wT2DM and PPD was equal in these groups.

The comparisons between all pairs of the study groups revealed highsignificant differences between CP+pT2DM with both CP+wT2DM andCP; while, non-significant between CP+wT2DM with CP regarding (PLI,BOP, PPD and CAL). Hence, at GI they were high significant differencesbetween CP with both

diabetic groups but non-significant betweendiabetic groups with each other (table-1).

Biochemical Parameters Analysis:

The biochemical analysis (table-2) of the salivary resistin revealed that the highest concentration was in CP+wT2DM, followed by CP+pT2DM then CPand finallythe Control. Furthermore, salivary leptin revealed that the highest concentration was in the Control group followed by CP and CP+wT2DM equally and lastly the CP+pT2DM demonstrated the least concentration. Highly significant differencesin the median values of both leptin and resistin concentrations revealed among the study and control groups at $p < 0.01$.

The results of the comparisons for all pairs of the study and control groups in (table-3) about both of leptin and resistin levels revealed: highly significant differences between Control group and all of the study groups, non-significant differences between CP+pT2DM and CP+wT2DM; as well as, between CP+wT2DM with CP group. Finally, the comparisons between CP+pT2DM with CP groups revealed a non-significant difference in leptin levels but the difference was significant in resistin levels.

Physical Parameters Analysis:

The highest median value (table-2) of salivary FR was in Control group, followed by CP then CP+wT2DM and lastly CP+pT2DM. The highest median value of salivary pH was found in the Control group while it was equal in the rest of the study groups.

The results revealed highly significant differences in the median values of both salivary pH and FR among the study and control groups at $p < 0.01$ as shown in table 2.

When comparing the salivary physical parametersin all pairs of study and control groups, the results showed that the salivary FR hadsignificant differences between Control with all of the study groups, but they were non-significant between CP with both diabetic groups as well as, between diabetic groups themselves.The intergroup comparisons of salivary pH revealed highly significant differences between Control with both diabetic groups; in addition, CP+pT2DM with CP, while they were non-significant between CP with Control and CP+wT2DM, as well as between diabetic groups. The results are shown in (table 3).

Correlations of Salivary Leptin and Resistin Hormones with Clinical Parameters and with Each Other:

As can be seen in table 4, leptin hormone generally, demonstratednon-significant weak correlations with all of the clinical parameters at all groups except for a non-significant moderate negative correlation with pH in Control group.

While the correlations of resistin hormone with clinical parameters (table-5) revealed a high significant moderate positive correlation existed with GI in CP group and a non-significant moderate negative correlation with FR in control group.

Finally, non-significant weak negative correlations were found between leptin with resistin hormones in the saliva at each of the study and control groups (table-6).

DISCUSSION

The highest mean of age parameter was found in CP+pT2DM while the lowest mean was found in control group, this can be attributed to the fact that the incidence of CP and T2DM is greater in older ages [16].

Clinical Periodontal Parameters Analysis:

The altered salivary FR and pH in the diabetic patients as well as altered oral flora and increased viscosity of the saliva[17], moreover, the increased glucose level in the gingival crevicular fluid(GCF) and saliva all contribute to the higher accumulation of plaque and calculus in the diabetic patients[18]. The DM causes and exacerbates the gingival inflammatory response to the bacterial plaque which means that there is an alteration in the response of periodontal tissue to local factors in diabetic patients. The inflammatory reactions are intensified during poor metabolic control, as the same amount of plaque causes more gingival bleeding in poorly controlled diabetic patients compared to the well-controlled ; hence, more plaque accumulation in CP+pT2DM leads to more gingival inflammation than CP+wT2DM group [5]. Moreover, the detrimental effects of advanced glycation end products and receptor for advanced glycation end products (AGEs-RAGEs) interactions in the periodontium of diabetic patients that include: increase vascular permeability, impaired wound healing and vascular changes contribute to more periodontal destruction[19]. The DM modifies periodontitis by dysregulating the immune and inflammatory responses in the periodontium, thus more cytokines are accumulated in the gingival tissues. Also, DM causes diminished function of the neutrophils and hyperactivity of macrophages

Table (1): Median Values of the Clinical Periodontal Parameters and the Intergroup Comparisons between all Pairs of the Study Groups

Periodontal parameters	Groups	Descriptive statistics	CP+pT2DM ×CP+wT2DM		CP+pT2DM×CP		CP+wT2DM×CP	
		Median	Mann-Whitney U test	P-value Sig.	Mann-Whitney U test	P-value Sig.	Mann-Whitney U test	P-value Sig.
PLI	CP+pT2DM	1.41	102.5	0.008 HS	72.5	0.001 HS	149	0.167 NS
	CP+wT2DM	1.26						
	CP	1.24						
	Control	0.196						
GI	CP+pT2DM	1.15	147.5	0.155 NS	70.5	0.000 HS	88	0.002 HS
	CP+wT2DM	1.08						
	CP	1						
	Control	0.05						
BOP Score 1	CP+pT2DM	62.20	74.5	0.001 HS	86.5	0.002 HS	199.5	0.989 NS
	CP+wT2DM	44.21						
	CP	44.78						
PPD	CP+pT2DM	6.19	84	0.002 HS	74	0.001 HS	186.5	0.715 NS
	CP+wT2DM	5.21						
	CP	5.21						
CAL	CP+pT2DM	3.13	83.5	0.002 HS	65.5	0.000 HS	153	0.203 NS
	CP+wT2DM	2.45						
	CP	2.28						

Table (2): Median Values of Salivary Leptin, Resistin, FR and pH and the Significance of Difference among the Study and Control Groups

Parameters	CP+pT2DM	CP+wT2DM	CP	Control	Kruskal-Wallis H test	
	Median	Median	Median	Median	X^2	P- value Sig.
Leptin ng/ml	2.24	2.34	2.34	2.564	16.295	0.001 HS
Resistinng/ml	8.96	9.82	8.35	4.74	18.079	0.000 HS
FR ml/min	0.33	0.36	0.39	0.41	13.411	0.004 HS
pH	6	6	6	7	17.080	0.001 HS

Table (3): Intergroup Comparisons of the Median Values of Salivary Leptin, Resistin, 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 Sig.	Mann-Whitney U test	P-value Sig.	Mann-Whitney U test	P-value Sig.	Mann-Whitney U test	P-value Sig.	Mann-Whitney U test	P-value Sig.	Mann-Whitney U test	P-value Sig.
Leptin	189	0.753 NS	166.5	0.343 NS	42.5	0.000 HS	176.5	0.495 NS	50.5	0.001 HS	74.5	0.009 HS
Resistin	198	0.957 NS	118.5	0.027 S	39.5	0.000 HS	144	0.130 NS	53	0.001 HS	73	0.010 HS
FR	138	0.071 NS	136	0.058 NS	59.5	0.002 HS	197	0.927 NS	89	0.028 S	86	0.019 S
pH	138.5	0.090 NS	104.5	0.009 HS	41.5	0.000 HS	160	0.273 NS	69	0.006 HS	97	0.075 NS

Table (4): Correlations between the Levels of Leptin Hormone with the Clinical Parameters at Each Study and Control Groups

Parameters	Statistical analysis	CP+pT2DM	CP+wT2DM	CP	Control
PLI	r	-0.175	0.198	0.006	0.107
	P	0.460	0.402	0.981	0.704
GI	r	0.018	-0.067	-0.287	0.223
	P	0.940	0.780	0.220	0.424
BOP Score 1	r	-0.142	-0.295	0.237	X
	P	0.551	0.207	0.314	X
PPD	r	-0.205	0.036	0.131	X
	P	0.387	0.880	0.582	X
CAL	r	0.245	0.182	-0.190	X
	P	0.299	0.442	0.423	X
FR	r	-0.065	0.253	-0.149	-0.077
	P	0.787	0.281	0.532	0.786
pH	r	-0.132	-0.349	0.006	-0.421
	P	0.578	0.132	0.981	0.118

Table (5): Correlations between the Levels of Resistin Hormone with the Clinical Parameters at Each Study and Control Groups

Parameters	Statistical analysis	CP+pT2DM	CP+wT2DM	CP	Control
PLI	r	-0.301	-0.168	0.292	-0.195
	P	0.197	0.479	0.212	0.487
GI	r	-0.303	0.189	0.645	-0.345
	p	0.195	0.424	0.002	0.208
BOP Score 1	r	0.138	-0.276	0.115	X
	p	0.563	0.239	0.629	X
PPD	r	0.068	0.037	-0.076	X
	p	0.774	0.876	0.750	X
CAL	r	0.131	0.222	-0.235	X
	p	0.581	0.347	0.319	X
FR	r	-0.014	-0.173	-0.073	-0.442
	p	0.954	0.465	0.760	0.099
pH	r	0.165	-0.293	-0.178	-0.071
	p	0.487	0.210	0.454	0.801

Table (6): Correlation between Salivary Levels of (Leptin with Resistin) Hormones at Each Study and Control Groups

Parameter	Statistical analysis	CP+pT2DM	CP+wT2DM	CP	Control
Resistin	r	-0.006	-0.177	-0.330	-0.142
	P	0.980	0.454	0.156	0.613

and monocytes which will result in further periodontal destruction [20], so diabetic patients have greater prevalence and extent of periodontal pockets [21]. Poorly controlled diabetics had three-fold increase in risk of having periodontitis compared to non-diabetics; furthermore, are prone to more severe periodontitis [22] and increases the risk of progressive bone loss and attachment loss over time [23].

Biochemical Parameters Analysis:

In the light of the present study, resistin (which serves as a proinflammatory mediator) is found in the saliva in both health and disease, but its concentration increases with presence of inflammation that is involved in both CP and T2DM, which assures it's involvement in the inflammatory process. Human resistin is derived from the infiltrating immune cells [24].

Inflammatory cytokines as interleukin-1(IL-1), IL-6 and tumor necrosis factor-α (TNF-α) which are involved in pathogenesis of CP were found to affect the resistin expression in vitro [25]. It was found that lipopolysaccharides (LPS) of *Escherichia coli (E. coli)* and leukotoxinof Aggregatebacteractinomycetemcomitans A.a.

(which are both periodontal pathogens) increase the production of resistin [26]. Resistin binds to human leukocytes and induces the cytokines production by peripheral blood mononuclear cells[27].

Also, resistin suppressed the neutrophils chemotaxis and reduces the oxidative burst provoked by E.coli [28]. Moreover, a potential role for resistin in bone metabolism was suggestedby increased resistin levels that coincided with osteoclast differentiation [29]. It was demonstrated that salivayresistin levels in T2DM patients were significantly higher than non-diabetic patients [30]. Also, it was found that resistin expression in the adipose tissue and its levels in the serum are increased in response to hyperinsulinemia and hyperglycemia [31].

From the present study, it can be observed that the differences in Leptin hormone levels between all pairs of the study groups were non-significant, however when comparing each one of the study groups with the Control group, it was found that the differences were highly significant. These results come in agreement with [32,33], but, disagree with the result of Thanakun et, al., [34] who demonstrated that salivary leptin level did not differ between healthy controls and patients with metabolic syndrome.

Leptin hormone has a role in pathogenesis of DM since it exerts a regulatory effect on food intake as well as on hyperinsulinemia and hyperglycemia [35]. Moreover, it has a role in pathogenesis of CP via its direct effect on innate immunity (organizes phagocytosis and cytokines production from macrophages, oxidative capacity of polymorphonuclear leukocytes and natural killer cells cytotoxicity) [36] and adaptive immunity (stimulates pro-inflammatory cytokines production by T and B lymphocytes which include: IL-6, IL-10, TNF-α [37].

Kim [38] found that leptin has the ability to enhance the TNF-α production that is induced by PrevotellaIntermedia LPS; thus result in chronic lesion and osseous tissue destruction which are both involved in inflammatory PD. However, it was demonstrated that leptin levels in the saliva are low and inversely correlated with the progression of the periodontium from health to disease [32, 34].

Physical Parameters Analysis:

The DM is associated with chronic complications such as neuropathies and deterioration of microcirculation which can lead to salivary glands hypofunction [39] and altered salivary FR and xerostomia [40] which will unfavorably influence the diluting and cleaning capacities of the saliva as well [41]. Hence, acidic pH can be attributed to the diminished salivary FR as in DM [42] or due to CP [43].

Correlations of Salivary Leptin and Resistin Hormones with Clinical Parameters and with Each Other:

The current study revealed that salivary leptin had non-significant weak correlations with the clinical parameters except for the non-significant moderate negative correlation with pH in the control group.The leptin is produced by the salivary glands [44], however, its levels in the saliva are decreased as the periodontal disease progresses which might indicate that leptin is down regulated within the salivary glands themselves and gingival tissues in one way or another in accordance to the degree of the gingival inflammation [32], however, a study by Sattari et, al., [24] disagree with the results of this study. Karam [32] found that salivary leptin showed a significant negative correlation with the PLI and GI in healthy controls, while in CP group no significant correlations with the clinical periodontal parameters.

Concerning resistin correlation, the results of this study disagree with Karam [32] who found a significant positive correlation between resistin and both of PLI and GI in Control group, while no correlations were found between resistin and the clinical periodontal parameters in the CP group.Another study [8] found that salivary resistin levels were significantly and positively correlated with GCF levels,in addition salivary resistin level was significantly and positively correlated to the percentages of BOP sites, mean (PPD and CAL) as well as periodontal inflamed surface area and suggested that the elevated levels of resistin in saliva reflect the intensity of local inflammation in the periodontium and not related to T2DM; also, suggested that the resistin was derived from immune cells that respond to periodontopathic microorganisms and then this resistin seeps from GCF into the oral fluid.

No study that addresses the correlation between these two hormones in saliva was performed before.The possible explanation of the weak correlation is the limited human sample size.The correlation between leptin and resistin was found to be negative which coincides with the fact that, the increased inflammation in the study groups was associated with increased resistin concentration and decrcreased leptin concentration.

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