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Chemerin as a Biomarker for Periodontitis in Systemically Healthy and Type 2 Diabetic Patients

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Abstract

Aim of the study: The aim of the study was to evaluate the salivary levels of chemerin in patients with periodontitis and type 2 diabetes mellitus.

Materials and methods: A total of 88 subjects were divided into four groups; 13 systemically healthy individuals having a healthy periodontium (control group), 25 patients with type 2 diabetes mellitus and healthy periodontium (T2DM group), 25 patients with generalized periodontitis (P group), 25 patients with generalized periodontitis and type 2 diabetes mellitus (P-T2DM group). The clinical periodontal parameters including plaque index (PLI), bleeding on probing (BOP), probing pocket depth (PPD), and clinical attachment loss (CAL) were determined. Unstimulated saliva was gathered, and the concentration of chemerin was estimated utilizing an enzyme-linked immunosorbent assay.

Results: The results showed that the mean concentrations of chemerin were highest in the P-T2DM group with a mean (of 1.598±0.1499 ng/ml) followed by the P group (1.445±0.1021 ng/ml), T2DM group (1.368±0.096 ng/ml), and control group (1.229±0.152 ng/ml), respectively. There was a statistically significant difference among groups at ($p<0.05$). For the correlation of chemerin with clinical periodontal parameters, chemerin correlated with PPD in the P group at $p= 0.014$, and chemerin correlated with clinical periodontal parameters PLI, BOP, and PPD in the P-T2DM group at ($p=0.006$, $p< 0.000$, $p=0.035$), respectively.

Conclusion: Periodontitis and type 2 diabetes mellitus caused an increase in salivary chemerin concentration, which might be used as a marker for both diabetes and periodontitis.

Keywords: Chemerin, Periodontitis, Type 2 Diabetes Mellitus

INTRODUCTION

Periodontitis is the most widespread health problem that affects esthetic and social life and can lead to tooth loss. It is a multifactorial chronic inflammatory disease that is characterized by increased attachment loss, bone resorption, pocket formation, and gingival bleeding [(1)].

Periodontitis is a multifactorial disease, resulting from a mix of numerous variables such as inherited, environmental, and host immunological responses. The biofilm that accumulated on the tooth structure is considered as a primary etiologic factor of periodontitis. The host reaction, on the other hand, influences the disease progression [(2)].

Diabetes mellitus type II (T2DM) is a global disease characterized by insulin secretion defects and/or a decrease in insulin sensitivity [(3)]. Type 2 diabetes, formerly identified as "noninsulin-dependent diabetes" or "adult-onset diabetes," represents most diabetes cases [(4)].

Diabetes is a risk factor for periodontal disease, and it has been linked to an increase in the incidence, prevalence, and severity of periodontal disease [(5)]. Failure of the immune system to remove the cause of inflammation results in a persistent inflammatory response and overexpression of pro-inflammatory cytokines. This chronic inflammatory response is the crucial cause of periodontitis and DM coexistence [(6)].

Chemerin was originally known as tazarotene-induced gene 2 protein or retinoid acid receptor responder 2 [(7)]. Chemerin is secreted in an inactive form, as prochemerin, which is activated by different serine proteases that transform prochemerin into the complete active state. Chemerin is released from epithelial cells, fibroblasts, adipose tissue, endothelium, and keratinocytes. Chemerin binds to 3 receptors: chemokine-like receptor 1 (CMKLR1 or chemR23), chemokine CC motif receptor-like 2 (CCRL2), and G protein-coupled receptor 1 (GPCR1). ChemR23 is the sole receptor accountable for chemoattraction activity [(8)]. Chemerin binding stimulates chemR23 and causes chemoattraction of immune cells to the infected areas [(9)]. In addition to stimulating chemotaxis, chemerin has been shown to facilitate macrophage adherence to tissue endothelium [(10)]. Chemerin may contribute to periodontal tissue deterioration by increasing the expression of pro-inflammatory cytokines and matrix metalloproteinase. The effect of non-surgical periodontal therapy on chemerin levels was considerably decreased in levels of chemerin at 6 months than the baseline values [(11)]. The present study was conducted to evaluate the salivary levels of chemerin in patients with periodontitis and type 2 diabetes mellitus.

MATERIALS AND METHODS

This study was an observational case-control study that included 88 subjects with an age range between (35 - 65) years old. The subjects recruited for the study were patients attending Al-Kindi Teaching Hospital, and Al-Elwya Specialist Dental Center from January 2022 to April 2022. Each subject sign to a written consent for their acceptance to participate after fitting the inclusion criteria. The consent thoroughly describes the goals of the research. The protocol was approved by the ethical committee of the College of Dentistry/University of Baghdad and followed the guidelines of Helsinki and Tokyo for humans (Reference no. 456 in 19-1-2022). Sample size calculators were used to calculate sample size at a 95% confidence interval and a 5% error margin.

Grouping of the study sample

Control group: Consisted of 13 subjects without any systemic disease and having a healthy periodontium.

Type 2 diabetes mellitus (T2DM) group: Consisted of 25 subjects diagnosed to have T2DM with HbA1c ≥ 7% and healthy periodontium.

Periodontitis (P) group: Consisted of 25 subjects who have generalized periodontitis without any systemic disease.

Periodontitis - Type 2 diabetes (P-T2DM) group: Consisted of 25 subjects who have generalized periodontitis and were diagnosed to have T2DM with HbA1c ≥ 7%.

Inclusion criteria

- Body mass index BMI ranges between 18.50 kg/m² - 24.99 kg/m² [(12)].
- Presence of at least 20 teeth for each subject.
- For the control group: Bleeding on probing less than 10% and Probing pocket depth ≤ 3 mm [(13)].
- For the periodontitis group: A patient is considered periodontitis if interdental CAL is detectable at ≥2 non-adjacent teeth, or buccal or oral CAL ≥3 mm with pocketing >3mm is detectable at ≥2teeth. All cases were generalized (>30% of

teeth involved) and unstable (PPD ≥ 4mm with BOP or PPD ≥ 5mm) [14].

Exclusion criteria: Patients with systemic diseases rather than T2DM, pregnant women, smoker patients with, a history of periodontal therapies during the last 3 months, antibiotics treatment during the last 3 months, patients receiving insulin treatment, and patients with diabetic complications.

Saliva collection**:** Unstimulated saliva was collected from participants between 9 a.m. – 11 a.m. The participants must not eat or drink one hour before the sample collection. The patients were instructed to swallow saliva and then tilt their heads to allow saliva to drool passively. All samples were centrifuged at 3000 rpm for 20 minutes by a centrifuge machine (Hettich, Germany). Then saliva was stored at - 20 C until analyzed by the Enzyme-Linked Immunosorbent Assay (ELISA)

Clinical periodontal parameter

The clinical periodontal parameters were assessed by using William's periodontal probe

- Assessment of Soft Deposits by using the Plaque Index (PLI), no plaque (0) score, presence of plaque (1) score [15].
- Assessment of Gingival Bleeding on Probing (BOP), if there is bleeding within 15-30 seconds, the surface was given a score of 1, and a score of 0 for the non-bleeding surface [16].
- Assessment of Probing Pocket Depth (PPD)
- Assessment of Clinical Attachment Level (CAL)

The calibration was made to achieve examiner accuracy and repeatability by measuring the clinical periodontal parameters (BOP, PPD, and CAL) for five volunteers until reach the level of agreement (>0.75) by using the Interclass Correlation Coefficient test.

The concentration of chemerin was estimated utilizing an enzyme-linked immunosorbent assay. Laboratory steps were done according to the manufacturer's instructions (MyBioSource). Absorbance and concentration were determined using a spectrophotometry plate reader (HumaReader HS, Germany) at 450 nm.

Statistics

Description of data, analysis, and presentation was achieved utilizing Statistical Package for Social Science (SPSS version 24). Mean, standard deviation, and percentage were used to describe data and the inferential statistics applied were One Way Analysis of Variance (ANOVA), Games-Howell or Tukey HSD post hoc tests, independent sample t-test, and Pearson correlation (r). The level of significance at ($P < 0.05$ = significant and $P > 0.05$ = non-significant).

RESULTS

Using the shapiro-Wilks test for testing the normality of data, these data were normally distributed. The total mean and standard deviation of the age were (52.863±8.028) ranging between (35 to 65) years old. Regarding gender distribution, the participants were composed of 51 males and 37 females. The participants were distributed according to age and gender throughout the groups as shown in (table1).

The results showed that the highest means of PLI and BOP were recorded in the P-T2DM group, (73.731±13.314) and (64.068±9.965), respectively. ANOVA test revealed a statistically significant difference among groups for both PLI and BOP at ($p<0.05$) (table 2). Intergroup multiple comparisons for PLI by utilizing Tukey HSD post hoc test a statistically significant difference between all pairs of groups except between control and T2DM group were recorded. Regarding BOP, intergroup multiple comparisons using the Games-Howell post hoc test showed a statistically significant difference between all pairs of study and control groups (table 3).

PPD and CAL mean in P-T2DM were (5.0970.601) and (3.9920.978), respectively, and were considered higher than those in the P group (4.7920.501) and (3.5790.918), respectively. Independent Sample T-test

showed a non-significant difference between the P groups and the P-T2DM group for both PPD and CAL (table 2).

The result showed that the mean concentrations of salivary chemerin were highest in P-T2DM (1.598±0.1499 ng/ml), followed by the P group (1.445±0.1021 ng/ml), T2DM group (1.368±0.096 ng/ml), and control group (1.229±0.152 ng/ml), respectively. ANOVA showed a statistically significant difference in concentrations of chemerin among groups at ($p<0.05$) (table 2). Following intergroup multiple comparisons using the Games-Howell post hoc test, a statistically significant difference in concentrations of chemerin were found between all pairs of study and control group (table 3).

Using Person's Correlation Coefficient (r) for the correlation between the salivary levels of chemerin with clinical parameters, there was a significant moderate positive correlation with PPD in the P group at $p= 0.014$. While for the P-T2DM group there was a significant moderate positive correlation with PLI at $p=0.006$, BOP at $p< 0.000$, and PPD at $p=0.035$ (table 4).

DISCUSSION

Chemerin levels in the periodontitis group were higher than those of the control group with statistically significant differences between them ($P<0.05$). This result is in agreement with Özcan et al [17] Which is the first study that investigated the level of chemerin in the saliva of periodontitis patients and suggested that chemerin levels gives more specificity than other adipokines in distinguishing destructive periodontal disease. Also in agreement with Özcan et al [12] who found a significantly different in salivary chemerin between the control and periodontitis group with a p-value ($P<0.05$).

Chemerin's main function in inflammation is chemotaxis. It retrieves inflammatory cells to the site of inflammation, such as macrophages and polymorphonuclear leukocytes [18]. Another function is that it stimulates pro-inflammatory cytokines like ILIβ and TNF-α [19], which may play a crucial role in periodontitis by stimulating bone resorption and increasing prostaglandin E2 (PGE2) and secretion of collagenases [20]. Chemerin causes permanent tissue damage by raising MMP levels [21].

Salivary chemerin level in the T2DM group was higher than the control group with a statistically significant difference between them. Diabetes is thought to be an inflammatory disease, with high levels of proinflammatory mediators [22]. A previous study by Bobbert et al [23] suggested that chemerin can be applied to predicting diabetes mellitus.

The current study revealed that the salivary chemerin level in P-T2DM was higher than the P group with a statistically significant difference. This result was in agreement with previous studies that also found a higher chemerin level in both saliva and GCF of the periodontitis diabetes group than in only the periodontitis group [24-26]. Chemerin may be involved in the etiology of periodontitis and diabetes mellitus and the relationship between T2DM and periodontitis may also alter chemerin production. Because diabetes affects the immunologically active molecule, increasing cytokines in periodontal tissues [27].

Regarding clinical periodontal parameters, there was a significant difference between the P group and the P-T2DM group concerning PLI, and BOP. These results agree with previous studies by Abdul-wahab and Ahmed; Hassan and Salman [28,29] who also found a signfcant difference. The possible explanation is that diabetes is usually correlated with increasing gingival inflammation in response to bacterial plaque, suggesting that periodontal tissue reacts differently to local factors [28].

Regarding PPD and CAL, the result showed a higher mean value in the P-T2DM group than in the P group with non-significant differences between them. This result is in agreement with Serrano et al [30]

who found a non-significant difference in PPD, and CAL between diabetes patients with periodontitis and non-diabetic patients with periodontitis. Many explanations were proposed to explain the diabetes-related periodontal disease and increase in PPD such as a change in the activity of the polymorphonuclear cell, altered host defenses [(31)], and alterations in tissue homeostasis. This is linked to an increase in collagen degradation owing to increased synthesis of MMP [(32)]. Furthermore, Hyperglycemia may cause an alteration in the subgingival microenvironment which favor the growth of the utmost prevalent pathogenic microbial species [(33)].

Conclusion

Chemerin could be used as an indicator of both diabetes and periodontitis as found that these conditions caused an increase in salivary chemerin levels. The limitation of this study did not consider the severity of periodontitis. For future research suggested measuring the level of chemerin concerning the severity of periodontitis.

Conflicts of Interest

The authors reported that they have no conflicts of interest

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Table 1: Distribution of age and gender among the study group

	Control	T2DM	P	P-T2DM	Total	P-value
Age (Mean±SD)	49.000±8.621	55.280±7.797	51.440±7.730	53.880±7.655	52.863±8.028	0.089 [a]
Gender						
Male (N)	7	15	16	13	51	0.832 [b]
Percentage	53.85%	60%	64%	52%	57.95%	
Female (N)	6	10	9	12	37	
Percentage	46.15%	40%	36%	48%	42.05%	

[a] One Way ANOVA, [b] Chi-Square

Table 2: Mean and standard deviation of clinical periodontal parameters and biomarker for study groups

	Control	T2DM	P	P-T2DM	df	P-value
Clinical parameter						
	Mean±SD	Mean±SD	Mean±SD	Mean±SD		
PLI	24.641±11.864	32.374±10.213	64.112±15.046	73.731±13.314	3	**0.000** [a]
BOP	4.531±2.506	7.111±1.419	55.922±10.449	64.068±9.965	3	**0.000** [a]
PPD			4.792±0.501	5.097±0.601	48	0.058 [c]
CAL			3.579±0.918	3.992±0.978	48	0.130 [c]
Biomarker						
Chemerin ng/ml	1.229±0.152	1.368±0.096	1.445±0.1021	1.598±0.1499	3	**0.000** [a]

[a] One Way ANOVA, [c] Independent Sample T-test

Table 3: Inter groups multiple comparisons of chemerin and clinical periodontal parameters (PLI, BOP) between all pairs of study groups by using post hoc tests

		PLI [d]		BOP [e]		Chemerin [e]	
		P	**Sig**	**P**	**Sig**	**P**	**Sig**
Control	**T2DM**	.300	NS	.016	S	.037	S
	P	.000	S	.000	S	.001	S
	P-T2DM	.000	S	.000	S	.000	S
P	**T2DM**	.000	S	.000	S	.044	S
	P-T2DM	.047	S	.034	S	.001	S
P-T2DM	**T2DM**	.000	S	.000	S	.000	S

[d] Tukey HSD, [e] Games-Howell, S = significant, NS = non-significant

Table 4: Person's Correlation Coefficient (r) between clinical periodontal parameters and the salivary levels of chemerin for each study group

	PLI		BOP		PPD		CAL	
	r	**p**	**r**	**p**	**r**	**p**	**r**	**p**
Control	0.214	0.482 NS	0.020	0.948 NS				
T2DM	0.284	0.169 NS	-0.042	0.840 NS				
P	0.194	0.352 NS	0.196	0.348 NS	0.483	**0.014 S**	0.353	0.084 NS
P-T2DM	0.530	**0.006 S**	0.650	**0.000 S**	0.424	**0.035 S**	0.260	0.210 NS

S = significant, NS = non-significant