Original Article

Evaluation of Salivary Biomarkers and Their Correlation to Periodontal Status and BMI

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Abstract

Objectives: This study aimed to determine the correlation between periodontal status and BMI by evaluating salivary biomarkers - Resistin, visfatin, and TNF-α.

Methods: Among 580 screened subjects, 86 subjects (30 male and 56 female) aged 30-60 years recruited and grouped into four groups, Group 1 – non-obese with healthy periodontium, group 2 – non-obese with periodontitis, group 3 - obese with healthy periodontium, and group 4 - obese with periodontitis, according to their clinical periodontal parameters and BMI. (ELISA) was used to measure the salivary concentration of Resistin, Visfatin, and TNF-α. (SPSS, v.20.0) applied for statistical analysis, Shapiro-Wilk used to test the Normality of distribution. One-way ANOVA and Mann-Whitney U test were used to analyze the outcome data.

Results: Significant differences in the clinical variables (PI, BI, PPD, and CAL) between test and control groups, with and with no periodontitis ($P<0.05$). However, no significant differences ($P>0.05$) between the level of Resistin were detected in the four groups tested. TNF-α was and periodontitis obese groups (P-value< 0.05). No significant correlations were found between clinical periodontal parameters and BMI and the three biomarkers' levels in the four groups tested in this study.

Conclusions: The study failed to detect significant positive correlations between the salivary biomarkers' levels and clinical periodontal and obesity parameters.

Keywords: *BMI, Obesity, Periodontitis, Salivary biomarker.*

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Introduction

Periodontitis is a chronic inflammatory disease of bacterial origin affecting tooth-supporting structures. The condition is triggered by toxic products of periodontal pathogens within the subgingival biofilm. Although host response to these products is a normal inflammatory response and part of the natural defense mechanism in the periodontium through producing local cytokines called pro-inflammatory mediators[1], it causes host tissue destruction by producing a high level of systemic and local pro-inflammatory cytokines. In the last few decades, the evidence-based correlation between periodontitis and systemic conditions, including; cardiovascular disease, adverse pregnancy outcomes, diabetes mellitus, respiratory disease, osteoporosis, rheumatoid arthritis, certain forms of cancer, and obesity was emerged[2].

In chronic periodontitis, Tumor Necrosis Factor- α (TNF-α), Interleukin-1 Beta (IL-1β), and IL-6, found to be increased and also in patients with chronic systemic diseases. Therefore, a bilateral link between periodontitis and these systemic conditions may be referred to as an imbalanced link between the pro-inflammatory host response and chronic periodontitis[3]. Periodontal disease as a chronic inflammatory disease influence both lipid and glucose metabolism, which may have a significant role in developing type 2 diabetes and cardiovascular disease[4]. Today, a three-way relationship emerged between the three most common global health problems; obesity, diabetes, and periodontitis, as shown in Figure 1. Obesity is a state of excessive fat accumulation at the different parts of the body; it is considered a risk to diabetes and periodontitis, causing insulin resistance through releasing adipokines such as Resistin, TNF-α, IL-6, visfatin, and adiponectin from the fatty masses in the body[5,6]. The relation, therefore, showing a remarkable interpretation between these three common conditions, and perhaps Resistin acts as a connecting molecular link between these conditions[7].

Growing research interest in finding the association between weight gain and chronic periodontitis came into view. Obesity has been identified as a potential risk indicator for chronic periodontitis in several cross-sectional studies[8,9] and was suggested to produce a hyper-inflammatory state due to the secretion of inflammatory adipokines[10]. This exacerbated individuals. Consensus has not been reached regarding the data on periodontal inflammation in obesity. Still, changes in inflammatory adipokines in periodontitis may be a part of the explanation of why periodontitis is associated with obesity in epidemiological studies[10,11].

The mechanism(s) whereby obesity may affect periodontal health is so far unclear; obesity interferes with the ability of the host immune system to appropriately respond to the periodontal pathogens infection (*Porphyromonas gingivalis*) in terms of TNF-α, IL-6, and serum amyloid response, this low-grade inflammation could be one of the mechanisms of increased bone loss after bacterial challenge. It might be responsible for the initiation and aggravation of non-transmissible chronic diseases[12]. TNF-α is secreted by adipose tissues, excessive production of TNF-α, causing periodontal tissue degradation. A study conducted on young adults showed that TNF-α in the gingival crevicular fluid is correlated with BMI in subjects with a BMI ≥ 40[13], and the level of TNF-α was positively associated with BMI in subjects without periodontal disease, suggesting that TNF-α in the gingival crevicular fluid is derived from adipose tissue in obese subjects. Studies suggest that Resistin plays a role in obesity, insulin resistance, cardiovascular diseases, rheumatoid arthritis, and periodontitis; Resistin derived its name from the original observation that it induced insulin resistance (resist-in: resist insulin)[14,15].

Visfatin is another adipokine, synthesized, and secreted primarily by visceral fat[16]. It has been indicated that visfatin synthesis is modulated by microbial infection. Nogueira et al. have demonstrated that periodontal ligament cells can produce visfatin, and this production is enhanced by Fusobacterium nucleatum. Therefore, it seems that microbial infection may influence visfatin levels and, consequently, clinical attachment loss[17].

Very few studies were conducted to confirm salivary biomarkers' role in the relationship between obesity and periodontitis. Although some studies confirmed the role of these pro-inflammatory proteins in determining a positive correlation between obesity and periodontitis; however, the results of some other studies are still controversial. Therefore, this study aimed to determine the correlation and strength of association of TNF-α, Resistin, and visfatin in saliva to obesity and periodontitis.

Patients and methods

Patient Recruitment

Patients were screened and recruited at Periodontics Clinic of Shorsh Dental Center from April to October 2019. Potential study participants were screened and assessed to fulfill the general inclusion criteria of the study, which included only healthy individuals within the age limit of the study. The exclusion criteria included individuals with a history of systemic disease that may affect the periodontal condition such as diabetes and long term use of medications such as

nifedipine and NSAID. Furthermore, pregnant and lactating women, smokers, and subjects on antibiotics in the last three months were excluded.

Full mouth clinical periodontal examination was performed, and saliva samples were collected. A structured case sheet was prepared included patients' demographic data such as name, age, gender, occupation, and smoking habit. An information sheet explaining all the study details was prepared to be read by every participant before signing informed consent. The study proposal was applied to the Scientific Committee of Faculty of Medicine, the University of Sulaimani, for registration and to obtain ethical approval, which was approved by the scientific committee on 07/02/2019, and was conducted according to the Helsinki Declaration of 1975 that revised in 2000.

Participants screening and recruitment.

The screening method involved the following examination on the participants to be recruited for this study and according to the Feasibility of a European Health Examination Survey (**FEHES**) measurement protocol[18]. Only obese BMI (30.0 ≥ 40.0) and normal BMI (18.5–24.9) were selected in this study, while overweight BMI (25.0-29.9) was considered non-obese subjects.

Periodontal status diagnosed according to consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions Criteria[19] as follow: Interdental CAL is detectable at ≥two non-adjacent teeth, or buccal or oral CAL ≥3 mm with pocketing ≥3 mm is detectable at ≥two teeth, but the observed CAL cannot be attributed to non-periodontitis-related causes.

Inter and intra examiner calibration was performed between the examiner and the examiner and between the examiner and an expert periodontist for all data capturing. The procedure was repeated frequently until a level of 85% accuracy was achieved.

Study Design

The patients were grouped as follow:

Group 1 (HNO), included ten subjects with healthy periodontium and non-obese (BMI <30).

Group 2 (PNO) included 25 subjects with periodontitis and non-obese (BMI <30)

Group 3 (HO) included 26 subjects with healthy periodontium and obese (BMI >30)

Group 4 (PO) included 25 subjects with periodontitis and obesity (BMI >30)

Saliva collection, storage and processing

Three milliliters of unstimulated whole saliva was collected from the participant by spitting method from 9:00 to 11:00 A.M[20]. Collected samples were placed immediately on ice, and then the saliva was placed into sterile Eppendorf and centrifuged at (3000 rpm for 15 minutes) then supernatant clear saliva placed into new Eppendorf and freeze at -80°C and stored until the total samples were collected, then the process of analysis started.

Clinical measurements

Full mouth periodontal examination was performed to collect the following clinical parameters using a periodontal probe (William's probe) and recorded in a data capture form. Plaque index (PI) was measured on six sites per tooth as absence (0) or presence (1) of plaque[21]. Gingival bleeding index (GBI) bleeding index was recorded at six sites per tooth within 30 seconds after probing for absence (0) or presence (1) of bleeding[21]. Probing Pocket Depth (PPD) was measured as the distance from the gingival margin to the location of the tip of the probe to the bottom of the pocket[22]. The clinical attachment level (CAL) was calculated for each site as the sum of PPD and gingival recession. The CAL was recorded at six sites per tooth for all teeth.

Enzyme-Linked Immunosorbent Assay

The Human TNF-alpha ELISA kit (MyBioSource/USA), Human Resistin ELISA kit (MyBioSource/USA), and Human Visfatin ELISA kit (MyBioSource/USA) were used to perform the enzyme-linked immunosorbent assays ELISA, which was performed according to the manufacturer's instruction to measure the concentrations of TNF, Resistin and Visfatin respectively in accordance. Concentrations of the biomarkers were determined using the standard curve.

Statistical analysis

Statistical analyses were performed using statistical software (SPSS, v.20.0 for Windows, IBM, Chicago, IL, USA). For all tests, the level of significance was set at

0.05. Data were expressed as means and standard deviations for parametric and median and IQR for non-parametric data. Shapiro-Wilk test was used to test the Normality of distribution of the variables. A one-way ANOVA test was carried out to determine the significant differences of parametric data (BI%) between the tested groups. LSD tests were done to make a pairwise comparison between groups. Mann-Whitney U test was used to find statistically significant differences of non-parametric data (PI %, PPD, CAL, BMI, RETN, VF, and TNF-α) between tested groups. Spearman's Rank Correlation analysis was done to investigate the correlation between clinical parameters (PPD, CAL, and BMI) and salivary biomarkers (RETN, VF, TNF-α). P≤ 0.05 was regarded as statistically significant.

Results

As demonstrated in Table 1, a total of 580 subjects were screened for eligibility to enter the study; among the examined subjects, 86 participants their ages ranged between 30 to 60 with a mean age of (39.85 ± 7.8) were recruited. The study sample comprised 56 females and 30 males that fulfilled the inclusion criteria of this study. Therefore, data from 86 subjects were included in the final analysis for clinical outcomes and salivary biomarker levels. The recruited participants were signed up into four groups according to their periodontal status and body weights (BMI) (Table 1).

Clinical data

Clinical parameters (plaque index, bleeding index, clinical attachment level, and probing pocket depth) were recorded along with the levels of biomarkers in the subjects' saliva. Variables were distributed as parametric or non-parametric according to the Shapiro Wilk test outcome, and the parametric data were (BI %). In contrast, non-parametric data were (PI %, CAL, PPD, BMI, RETN, VF, and TNF-α concentrations). Mean, standard error, and median with IQR values of the clinical periodontal parameters, BMI, and level of salivary biomarkers in the four tested groups of the study are demonstrated in Table 2. Whereas, Association between clinical data and the biomarker levels are shown in Table 3, by multiple comparisons of clinical parameters and biomarkers levels between examined groups. ANOVA test was used with normally distributed data as parametric tests, Whereas, Mann-Whitney U test was applied for non-parametric data.

Plaque index measured by PI% median value and interquartile range (IQR) for the control groups - Group 1 and group 3 were (45.2±20.1) and (32.73±18.7) respectively, and for the periodontitis groups – group 2 and group 4 were (64.08±19.96) and (66±23) respectively (Table 2). The Mann-Whitney comparison between groups showed significant differences (P-value<0.05) between the control (HO) and test groups (PO) and HNO and PNO (P-value >0.05) (Table 3).

The gingival bleeding index percentage (BI%) mean value and standard error for the control groups (HNO and HO) were (16.4 ±4.47) and (13.81±4.56) respectfully, It was significantly increased by multiple mean comparisons (ANOVA), (P-value = 0.00) at the periodontitis groups (PNO and PO), (44.16± 1.86) and (43.72±4.07) respectively (Table 2).

Probing pocket depth (PPD), Median, and IQR for the control groups (HNO, HO) were (1.39±1.74)) and (1.34±0.34) mm, respectively.

Median and IQR for PNO & PO were (4.28±0.49) and (4.5±0.80) respectively (Table 2); on multiple comparisons, there was a statistically significant difference (P-value<0.05) between control and the test groups, but the non-significant difference (p-value >0.05) between periodontitis group in obese and non-obese groups. (Table 3).

For clinical attachment level CAL, median, and IQR were calculated for the control groups, but the control groups' values were zero for PNO & PO were (2.68±0.26) and (2.88±0.23) respectively (Table 2). On multiple comparisons, there was a statistically significant difference (p-value<0.05) between control and the two test groups PNO and PO but were not significant (p-value>0.05) between the periodontitis groups in obese and non-obese groups (Table 3).

For Body mass index: median and IQR for the BMI were significantly higher in obese groups than the non-obese groups, as shown in (Table 2 and 3).

Biomarkers level in the studied groups' saliva also investigated and their concentrations (pg/mL), as shown in Table 2, by presenting their median and IQR. Resistin was detected in all saliva samples. However, PNO recorded a higher salivary resistin level compared to other groups but did not reach significance (p>0.05) (Table 3). Furthermore, the obese groups (HO and PO) had higher salivary visfatin levels compared to non-obese groups, with the highest level at the PO group, which was statistically significant with HNO group (p<0.05), and as a general the periodontitis groups had higher salivary visfatin levels than control ones as shown in (Table 3). TNF-α concentrations (pg/mL) of study groups were demonstrated in Table 2. TNF-α was also detected in all saliva samples. Regarding salivary TNF-α concentration, the periodontitis non-obese group

(PNO) had the highest TNF-α concentration. During multiple comparisons, the HNO group recorded a significantly lower concentration of TNF-α than the other three groups. In contrast, the other comparisons were not significant (P>0.05), as shown in Table 3.

Correlation of salivary Resistin, Visfatin, and TNF-α with clinical parameters (BMI, CAL, PPD):

Resistin highest level was recorded in the PNO group. Correlation between the salivary biomarkers and the clinical parameters using Spearman's rank correlation test demonstrated that the Resistin levels showed a Negative weak, not significant correlation with PPD and CAL in periodontitis groups and BMI in periodontal health and disease, as shown in Table 4. Visfatin highest level was detected in the PO group, regarding correlation Visfatin levels showed the same result as Resistin except with BMI in which Visfatin levels showed a weak positive correlation with BMI in HNO and PO groups as shown in Table 5.

Regarding TNF-α levels, it showed the highest level in the PNO group; regarding correlation, it showed positive, non-significant weak correlation with PPD in periodontitis groups. In contrast, with CAL in the PNO group only, but with BMI, it was negative in the non-obese group and positively correlated with BMI in obese groups, as shown in Table 6.

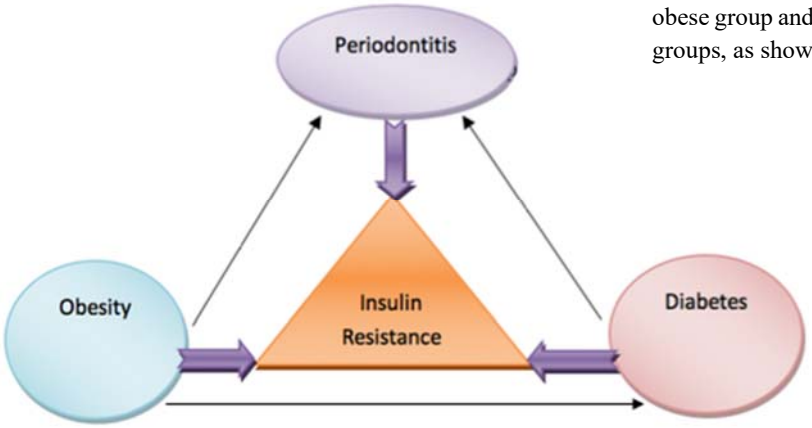

Figure 1: the three-way relationship between obesity, diabetes and their relation to periodontitis.

Table 1: Demographic characteristics median (IQR) for study populations.

Total	participants	Male	female	Mean age group
580	86 (14.8%)	30 (5.2%)	56 (9.6%)	30-60 (39.8±7.8)
Study Groups				
	Group 1 HNO-*Control 1*	Group 2 PNO-*Test 1*	Group 3 HO-*Control 2*	Group 4 PO-*Test 2*
Patients – M/F	10-3/7	25- 8/17	26- 7/19	25- 12/13
BMI(Kg/m²) Median (IQR)	25.5(3)	25(4.7)	34(2)	32(3)

Table 2: Clinical periodontal and anthropometric parameters with salivary concentrations of (Resistin, Visfatin, TNF-α) of the tested groups.

Variables	NON-OBESE		OBESE	
	Health	Periodontitis	Health	Periodontitis
PI (%) Median (IQR)	45.2(6.39)	64.8(3.99)	32.73(3.66)	66(23)
BI (%) Mean (SE)	16.4(4.47)	44.16(4.56)	13.81(1.86)	43.72(4.07)
PPD (mm) Median (IQR)	1.39(1.74)	4.28(0.48)	1.34(0.62)	4.5(0.8)
CAL (mm) Median (IQR)	0	2.68(0.26)	0	2.88(0.23)
BMI (kg/m²) Median (IQR)	25.54(0.92)	25(4.7)	33.68(0.43)	32(3.2)
Resistin (ng/mL) Median (IQR)	0.31(0.047)	0.33(0.031)	0.22(0.32)	0.24(0.028)
Visfatin (ng/mL) Median (IQR)	13.2(1.61)	13.6(0.84)	13.64(1.29)	14.11(0.32)
TNF-α (pg/mL) Median (IQR)	8.75(1.95)	10.98(2)	9.9(1.59)	10.25(2.9)

Table 3: Multiple comparisons of clinical parameters and biomarkers levels between examined groups, *ANOVA test, ** Mann-Whitney U test between every two groups.

Variables	Group 1 vs. group 2	Group 1 vs. group 3	Group 1 vs. group 4	Group 2 vs. group 3	Group 2 vs. group 4	Group 3 vs. group 4
BI (%)[A]	0.000*	0.699	0.000*	0.000*	0.931	0.000*
PI (%)[B]	0.015*	0.077	0.053	0.000*	0.345	0.000*
PPD (mm)[B]	<0.0001*	>0.9999	<0.0001*	<0.0001*	>0.9999	<0.0001*
CAL (mm)[B]	0.000*	1.000	0.000*	0.000*	0.448	0.000*
BMI (kg/m²)[B]	0.464	<0.0001*	0.0008*	<0.0001*	<0.0001*	0.014*
Resistin (ng/mL)[B]	0.971	0.447	0.125	0.224	0.050	0.932
Visfatin (ng/mL)[B]	0.170	0.069	0.036*	0.341	0.093	0.381
TNF-α (pg/mL)[B]	0.005*	0.017*	0.007*	0.203	0.419	0.699

Table 4: Spearman's rank correlation test to correlate salivary (RETN) with (PPD, CAL, BMI) within the groups.

Groups	Salivary biomarker	PPD	p-value	CAL	P-value	BMI	p-value
G1: HNO N=10	Resistin	-0.15	0.69	.	.	- 0.055	0.881
G2: PNO N=25	Resistin	-0.282	0.172	-0.62	0.768	- 0.179	0.393
G3: HO N=26	Resistin	0.363	0.07	.	.	-0.177	0.39
G4: PO N= 25	Resistin	-0.18	0.93	-0.22	0.29	-0.008	0.97

Table 5: Spearman's rank correlation test to correlate salivary (VF) with (PPD, CAL, BMI) within the four groups.

Groups	Salivary biomarker	PPD	p-value	CAL	p-value	BMI	p-value
G1: HNO N=10	Visfatin	-0.61	0.06	.	.	0.162	0.656
G2: PNO N=25	Visfatin	-0.014	0.95	-0.216	0.3	-0.162	0.44
G3: HO N=26	Visfatin	0.127	0.54	.	0.77	-0.136	0.507
G4: PO N= 25	Visfatin	-0.35	0.09	-0.003	0.99	0.077	0.71

Table 6: Spearman's rank correlation test correlates salivary (TNF-α) with (PPD, CAL, BMI) within the four groups.

Groups	Salivary biomarker	PPD	p-value	CAL	p-value	BMI	p-value
G1: HNO N=10	TNF-α	0.34	0.34	.	.	-0.367	0.297
G2: PNO N=25	TNF-α	0.131	0.53	0.075	0.72	-0.146	0.49
G3: HO N=26	TNF-α	-0.178	0.39	.	.	0.153	0.46
G4: PO N= 25	TNF-α	0.33	0.1	-0.113	0.6	0.183	0.38

Discussions

Saliva has been screened as a non-invasive, relatively stress-free, and easily assessed diagnostic alternative to blood. Currently, saliva testing is used for clinical assessment of hormonal perturbations, detection of HIV antibodies, DNA analysis, alcohol screening, and drug testing[23]. Recently, saliva has been used for direct detection of the SARS-CoV-2 virus and provides useful clinical information about the disease and could be potentially included in guidelines for sample collection for the diagnosis, disease management, and control of Covid-19[24]. Today, there is an increasing interest in evaluating the diagnostic potential of saliva in obesity, inflammation, and insulin resistance[25].

This case-control study is based on an analysis of three biomarkers in the saliva of 86 subjects assigned into four groups according to clinical periodontal parameters and BMI of the participants. The study was designed and conducted to identify changes in these biomarkers' levels (TNF-α, Resistin, and Visfatin) as dependent variables to obesity and periodontitis status. It was expected to recognize at least some positive correlations between the concentration of these salivary biomarkers and the status of obesity and periodontitis of the subjects involved in this study. Therefore, it would be possible to assume that obese individuals perhaps suffer from periodontitis or are expected to be at risk of developing periodontitis forward in life. This could be performed by measuring these salivary biomarkers (TNF-α, Resistin,

and visfatin). The rationale behind this was the relationship between periodontitis and obesity based on the assumption that adipose tissues release numbers of cytokines and hormones that are involved in inflammatory processes, pointing to similar pathways involved in the pathophysiology of obesity and periodontitis[9].

The outcome of the current study could be summarized as follow, no significant differences in the levels of BI and PI in obese and non-obese groups (P>0.05). Although deeper PPD and CAL were detected in the periodontitis group within the obese group than the non-obese group statistically, their mean differences were non-significant (P>0.05). Furthermore, all salivary biomarker's level was higher in periodontitis groups than control groups, and Resistin and TNF-α were found to be higher in non-obese groups than obese groups. Visfatin was higher within both obese groups than the non-obese groups. However, the correlation test, Resistin, showed a weak negative correlation with (PPD, CAL, and BMI) almost in all the groups (P >0.05). Visfatin also showed a weak negative correlation with PPD in almost all the groups, whereas with CAL only in the periodontitis groups and BMI in two groups – PNO and HO are negative correlation (P >0.05). TNF-α was positively correlated with PPD (P >0.05), whereas CAL in PNO and with BMI was positive in the two obese groups and negative in the other two groups (P >0.05). The only significant mean differences were reported for Visfatin. Its level was significantly higher (p<0.05) in PO (G4) than HNO (G1). And the level of TNF-α in the control group (HNO) vs. PNO, HO, and PO.

Furthermore, no significant differences were found in periodontal parameters in obese and non-obese groups; this result was consistent with other studies[26,27]. The reason behind that could be due to the lack of a wide margin or band of the difference of BMI between obese and non-obese subjects; in other words, overweight individuals (BMI =25-29.9) were not excluded from non-obese groups, so they considered non-obese.

Periodontitis is a low-grade infection in which the periodontal tissues are infiltrated by the inflammatory cells that will act as a source of production for Resistin[28]. Lipopolysaccharides (LPSs) produced by periodontal pathogens are shown to induce Resistin by macrophages through a cascade involving pro-inflammatory mediators' production results in the destruction of periodontal tissues[29].

Obesity has emerged as a risk marker for periodontitis. Several studies suggested that Resistin, visfatin, and TNF-α are involved in the etiology of obesity, insulin resistance, and periodontitis. Furthermore, Resistin could provide a link of association between obesity and periodontitis[14,25].

In this study, clinical data showed that salivary resistin level was not significantly different between obese and non-obese groups. In contrast, a comparable difference was found between the periodontitis group and control groups, suggesting that Resistin may be, to some extent, related to periodontal status, independent of obesity. Furthermore, the HNO group had a higher Resistin level than the PO group, following other studies[11,30]. This variation can be explained firstly by improper BMI application within non-obese groups. In other words, overweight individuals (BMI=25-29.9) were included in the non-obese group as there is a study considered overweight as obesity and found a correlation with increased salivary Resistin levels[31]. Secondly, gingivitis was included in control (no periodontitis) groups, as BI% were (16.4) in HNO group and (13.8) in HO group, and according to the Consensus report of workgroup 1 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions, clinical features of gingival health on (intact periodontium and reduced & stable periodontium) defined as < 10% bleeding sites[32], as there is studies found that salivary RETN, TNF-α, and Visfatin levels unable to differentiate between health, gingivitis and periodontitis and in some are higher in gingivitis and periodontitis than healthy periodontium[33,34].

Another reason could be due to the periodontitis cases selected for this study with no regards to severity and or distribution (localized or generalized) or staging and grading; perhaps the majority of subjects recruited were mild, localized form of periodontitis with a minimal degree of inflammation and these salivary biomarkers are pro-inflammatory molecules and their level increase subsequently with the severity of the periodontal disease[11].

Earlier study evidence that both local and systemic visfatin levels were found to be higher in individuals with gingivitis and periodontitis and also with high BMI values[35], and their salivary levels decreased after non-surgical periodontal treatment[36]. In our study, visfatin levels comparable between the groups with both obese groups were higher than non-obese groups, and periodontitis groups than control groups and PO were significantly higher than the HNO group (P<0.05) which is similar to previously mentioned studies. The same explanation given for Resistin may be suggested for the comparable not significant difference of visfatin concentration between other groups.

TNF-α is a well-recognized pro-inflammatory cytokine produced by adipocytes and immune cells. Evidence

indicates that the local levels of TNF-α are increased in individuals who are obese[11,37] and decreased after weight loss[38].

In this study, the salivary concentration of TNF-α was higher in the HO group (9.9) group than individuals in the HNO (8.7) group (P<0.05), suggesting that levels of TNF-a maybe, to some extent, related to obesity, independent of the periodontal status. Intergroup comparisons of TNF-α reveals a significant difference (P<0.05) between the HNO group and PNO, HO, and PO groups, which is following another study[30]. In contrast, no significant difference was found between obese groups (HO & PO), which is similar to[39]; this could be due to the small sample size of the studied population and absence of class II & III of obesity, which may carry higher risks.

The possible explanation of our findings is that there are several methods to assess obesity; BMI is common. However, BMI is a determinant of overweight. Still, it does not truly reflect the body fat in our study; BMI has been used to measure obesity. Besides BMI, waist circumference (WC) and waist to hip ratio (WHR) have been determinants of obesity which measure abdominal obesity. Upper body obesity (abdominal adiposity) is believed to have greater ill-effects on general health than lower body obesity.

Therefore, further studies are required with larger sample sizes and special consideration to grade and stages of periodontists (severity and extension) to achieve a wide band of differences between periodontally healthy subjects and periodontitis subjects. Furthermore, gingivitis should also be considered as being a chronic source of inflammation that might affect the level of salivary biomarkers. Furthermore, proper application of BMI, including the higher classes of obesity (Cl II and Cl III) to have a wide band of differences between obese and non-obese subjects. Therefore, and under these circumstances, we would be able to correlate the level of salivary biomarkers appropriately with BMI, and Periodontitis attributes and to identify the strength of association between the level of these salivary biomarkers and periodontitis and obesity.

Conclusions

Within the limitations of this study, results indicate no significant differences in the level of the salivary biomarkers used in this study between the two control groups (Group 1 – HNO and group 3 – HO) and the two test groups (Group 2 – PNO and Group 4 – PO). Therefore, the study was unable to correlate obesity to periodontitis based on the level of the biomarkers applied in the saliva, as no positive correlations were identified between the clinical periodontal parameters and BMI according to the level of Resistin and Visfatin and TNF-α in the saliva of the recruited sample. However, a significant mean difference was detected between TNF-α in control group HNO and PNO, HO, and PO groups.

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