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ASSESSMENT OF SALIVARY LEVELS OF 8-OHDG IN PATIENTS WITH PERIODONTITIS AND/OR OBESITY

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Abstract

Background: Periodontitis is universally agreed to be an inflammatory disorder which arises as a consequence of periodontal pathogens interacting with the host's immune response. A significant and crucial element in the onset and advancement of periodontitis is oxidative stress. 8-OHdG is a molecule that's considered a significant biomarker of oxidative stress.

Aim of the study: Analyzing the salivary levels of 8-OHdG in patients with periodontitis/obesity in comparison to healthy controls with analysis of its correlation with various clinical periodontal parameters.

Materials and methods: 110 subjects (30 obese, 30 periodontitis, 30 obese periodontitis and 20 healthy subjects) took part in this study. Samples of saliva were acquired from all subjects before clinical examination. Full periodontal examination was conducted for each participant which entailed plaque index PLI, bleeding on probing BOP, probing pocket depth PPD and clinical attachment loss CAL. We assessed obesity utilizing body mass index BMI.

Results: It was showed that periodontitis patients and obese patients had significantly elevated concentrations of salivary 8-OHdG when compared with healthy subjects. Also these levels correlated positively with CAL in obese periodontitis patients.

Conclusions: This study revealed an association between 8-OHdG and periodontitis which implies that this biomarker serves a vital part in the periodontal disease process. Moreover, this study showed an association between this biomarker and obesity measures, which suggests that periodontitis and obesity are interconnected via oxidative stress.

Keywords: 8-OHdG, saliva, obesity, periodontitis, oxidative stress

Introduction

eriodontal disease is believed he an illness inflammatory origin which is caused by several factors that affect tissues that hold the teeth (alveolar bone and connective tissue). 1 Obesity is a diverse and multidimensional condition that has been connected to a number of medical conditions, such conditions include cancer, diabetes, and cardiovascular disease.2 The epidemic of obesity has been called a pandemic on a global scale.3 According to recent research, around 2.1 billion people worldwide are overweight or obese.⁴ Obesity was also shown to have a high prevalence in Iraq.⁵

A relationship exists between periodontal disease and obesity according to several systematic studies and meta-analyses.^{6, 7} Patients with obesity have a greater incidence of periodontal disease compared to

normal weight subjects, and this link becomes stronger as BMI rises. 8 A recent study on Iraqi population periodontitis that demonstrated severity is associated with obesity, although other risk factors could be implicated ⁹. The precise mechanism by which obesity affects periodontal disease is not yet fully understood. Obesity alters the immunological and inflammatory systems which influence the host's susceptibility. 10 Research implies that obesity may be linked to periodontitis through oxidative stress.¹¹ Hence, analysis of oxidative stress could be a beneficial technique comprehend the pathologic to pathways shared by two inflammatory disorders such as obesity and periodontal disease ¹².

A significant product of oxidative damage to DNA is 8-hydroxy-2'-deoxyguanosine (8-OHdG) which's mediated by (ROS) ¹³. It is a critical indicator of oxidative stress. ¹⁴ Several studies showed

greater concentrations of salivary 8-OHdG in individuals with periodontal disease when compared with healthy individuals, indicating an association between this biomarker and enhanced ROS generation during periodontal inflammation.¹⁵⁻¹⁷

The studies that inspected the relationship between this unique biomarker and periodontal disease are scarce. This paper evaluated this relationship and its association with obesity measures.

Patients and methods

Study design and patient population

The design of this study is observational case-control study, it was undertaken at the periodontics department of the Dental College of University of Baghdad. The University of Baghdad granted the ethical approval for this study (Ref. 454622 in January 19, 2022). Between March 2022 and June 2022, 110 subjects were

recruited to take part in this study, which comprised of 30 patients with Periodontitis, 30 patients with obesity $(BMI \ge 30)$, 30 patients with periodontitis and obesity, and 20 who were periodontally patients healthy with normal weight (BMI\u24.9). Informed consent was acquired from every participant before conducting this which research clarified the purpose of the study and the sampling procedure. The inclusion criteria for the enrollment into the study comprised of subjects aged between 30-50 years with no systemic diseases, not taking any medication in the last 3 months with 20 natural teeth at minimum, while subjects who were exempted from the study included smokers and alcohol drinkers, subjects with systemic disorders and subjects who have undergone periodontal therapy in the previous 3 months.

Saliva collection and data analysis

Samples of whole unstimulated saliva were obtained from all prior participants oral any examination. Whole saliva was collected by using the drooling method into a plastic cup which then was transferred into a test tube in a cooling box 18, later we centrifuged the samples at 3000 rpm for 10 minutes and were put in a storage unit at -80° C freezer until the analysis day. We thawed the samples to room temperature on the day of laboratory analysis.

The levels of 8-OHdG in saliva were determined utilizing an ELISA assay (Catalog number MBS720604) suitable for the quantitative detection of Human concentrations of 8-OHdG following the manufacturer's directions. The competitive enzyme immunoassay approach is used in this kit, which includes a polyclonal anti-8-OHdG antibody and an 8-OHdG-HRP conjugate. The sample and buffer are incubated with the 8-OHdG-HRP

conjugate on a pre-coated plate for a period of one hour. HRP enzyme substrate is then incubated in the wells. A blue complex forms as a byproduct of the enzyme-substrate process. The process is finally halted by adding a stop solution, which turns the solution The vellow. spectrophotometric microplate reader at 450nm is used to determine the colour intensity. Since 8-OHdG from samples and 8-OHdG-HRP conjugate battle for the anti-8-OHDG antibody binding site, the intensity of the colour is inversely related to the levels of 8-OHdG.

Case definition and data collection

Prior periodontal to examination, the height and weight of every subject were determined by a measuring and tape. We scale classified participants into three weight groups with respect to their body mass index (BMI): Normal (BMI: 20–24.9), Overweight (BMI: 25–29.9) and Obese (BMI: ≥30). BMI

was calculated according to this equation: BMI = weight (Kg)/height(m2). 19

thorough periodontal examination by a calibrated examiner was performed using Michigan O probe, which consisted of plaque index (PI), bleeding on probing (BOP), Probing pocket depth (PPD) and Clinical attachment loss (CAL). All teeth surfaces were examined for all parameters except for PI where only four surfaces were examined. Subjects were classified as periodontitis when CAL was found at ≥ 2 non adjacent teeth Or buccal/oral CAL \geq 3mm with pocket>3mm at ≥ 2 teeth 20 . While healthy subjects were classified when BOP<10%, PPD<3mm, intact periodontium (no probing attachment loss) 21 .

SPSS (version 25) was utilised for both descriptive and inferential analysis. Checking the statistical normality of data was performed Utilizing Shapiro-Wilk test which revealed that all studied variables had a normal distribution among groups at p>0.05. To statistically evaluate the differences in gender distribution, we used chi-squared test as it is considered a categorical data. For the rest of the parameters, we found it to be normally distributed between healthy diseased patients and since this study had 4 groups, two-way ANOVA test for used the parameters' was evaluations. Correlations of 8-OHdG were analysed using Pearson's correlation test with the various clinical periodontal parameters. A significance level of α =0.05 was used for every test of this study.

Statistical analysis

Results

110 subjects took part in this study, they were divided into healthy control group (n=20), obese group (n=30), periodontitis group (n=30), and obese

periodontitis group (n=30). The age ranged between 30-50 years in all groups, the control group had a mean age of 37.55 ± 7.95 years. The obese group had a mean age of 36.30 ± 5.44 years and the mean age of periodontitis group was 37.13 ± 6.46 years while in obese periodontitis group it was 40.46 ± 6.64 years. Regarding gender, 51.82% of the total participants were females, while males constituted 48.18%. For age (p=0.083) and gender (p=0.692) there was no significant difference among all groups .

The mean level of 8-OHdG in saliva was 0.9 ng/ml in healthy control group, 1.29 ng/ml in obese group, 1.9 ng/ml in periodontitis group and 1.47 ng/ml in obese periodontitis group. The levels of 8-OHdG and all parameters in this study across all groups have been summarized in the table below Table 1.

Table I Mean values of all parameters and comparisons between all study groups

Parameters	Mean values				P values			
	Healthy	Obese	Periodontitis	Obese	Healthy vs	Healthy vs	Obese vs	Periodontitis
	n=20	n=30	n=30	Periodontitis	Obese	Periodontitis	obese	vs obese
				n=30			periodontitis	periodontitis
PI (%)	20.25±7.50	21.7±10.39	40.4±24.39	44.6±21.22	0.78^{NS}	<0.001**	<0.001**	0.37^{NS}
BOP (%)	2.5±2.35	4.53±2.27	36.93±26.29	34.1±20.4	0.68^{NS}	<0.001**	<0.001**	$0.53^{\rm NS}$
PPD (mm)	-	-	4.62±0.55	4.61±0.49	-	-	-	$0.93^{\rm NS}$
CAL (mm)	-	-	3.46±0.98	3.69±1.02	-	-	-	0.40^{NS}
8-OHdG	0.9±0.16	1.29±0.52	1.9±0.12	1.47±0.24	<0.001**	<0.001**	0.031**	<0.001**
(ng/ml)								

^{*}Significant

Regarding plaque index (PLI) and bleeding on probing (BOP), a significant difference was found between healthy control group and periodontitis group, also a significant difference between obese group and obese periodontitis group; however, we did not find any significant difference between periodontitis group and obese periodontitis group in terms of PLI and BOP. On the other hand, when we compared

^{**}Highly significant

NS Non-significant

periodontitis and obese periodontitis groups in terms of (PPD) and (CAL), there was no significant difference noticed between these groups.

Regarding the levels of salivary 8-OHdG, a significant difference was noted between healthy control group and periodontitis group as between healthy control group and obese group, also a significant difference was found between obese group and obese periodontitis group.

On correlating the salivary levels of 8-OHdG with various clinical parameters as shown in Table II, we found a significant moderate positive correlation between this biomarker and CAL in obese periodontitis group as illustrated in [Figure 1].

Table II Correlations of the clinical parameters with 8-OHdG salivary levels in all study groups

Groups		PLI	BOP	PPD	CAL
Healthy	r value	0.32	-0.11	-	-
Treating	p value	0.15 ^{NS}	0.64 ^{NS}	-	-
Obese	r value	0.08	0.002	-	-
· Coese	p value	0.66 ^{NS}	0.99 ^{NS}	-	-
Periodontitis	r value	0.08	0.01	0.24	0. 2
1 Chodonerus	p value	0.65 ^{NS}	0.94 ^{NS}	0.24 ^{NS}	0.2 6 ^{NS}
Obese	r value	0.25	0.14	0.22	0.5
periodontitis	p value	0.17 ^{NS}	0.45^{NS}	0.31 ^{NS}	0.00 4**

^{*}Significant

^{**}Highly significant

NS Non-significant

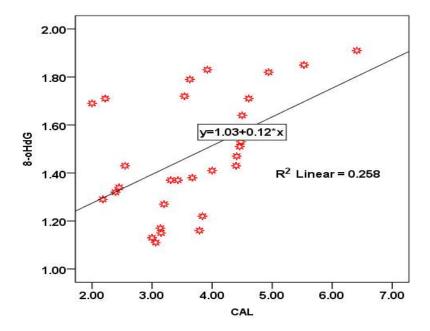


Fig. 1 Correlation of salivary concentrations of 8-OHdG with CAL in obese periodontitis group

Discussion

This paper sought to assess the levels of 8-OHdG in saliva and its relation to periodontal disease. We also investigated its association with obesity. 8-OHdG is the most prevalent outcome of oxidative DNA repair and researchers have relied on this stable oxidatively modified DNA product as a benchmark of the extent of oxidation damage of DNA. 13 Periodontal disease is an illness of inflammatory origin which can be considered as primary etiological factor for tooth loss, leading to destruction to all structures supporting the teeth which include mainly periodontal ligament, root cement, and alveolar bone. Many studies have shown that oxidative stress, as well as an individual's

total antioxidant capability, play a substantial part in the etiology of periodontal disorders. Lower levels of antioxidants in gingival crevicular fluid (GCF) were linked to exacerbated gingival and structural damage induced by neutrophils. ¹⁶ Similarly, a second research demonstrates association between periodontal disease and hyperreactive neutrophils that have enhanced generation of (ROS).²² According to several studies, higher concentrations of 8-OHdG in saliva were noticed in individuals with periodontal disease compared to those in healthy individuals, indicating that the aforementioned biomarker is associated with enhanced (ROS) generation during periodontal inflammation. 15-17 Similarly,

when periodontitis patients get effective antiinflammatory medication, its levels decrease. ¹⁷ Recent research revealed that the presence of periodontal bacteria is strongly associated with the salivary 8-OHdG concentrations, and they were much greater than other oxidative stress indicators. ²³

In this study, a significant difference was found when we compared healthy control group with periodontitis group, also a significant difference was found between obese group and obese periodontitis group. Salivary concentrations of 8-OHdG in saliva are always increasing in the periodontitis group whether normal weight or obese (more than double in periodontitis group in comparison to control group). This result agrees with many previous studies which found that the levels of 8-OHdG in saliva to be high and sometimes very high in periodontitis group with a significant difference.²³⁻²⁵ Periodontitis is an irreversible illness caused by microbial plaque.²⁶ Large amounts of neutrophils, enzymes, and (ROS) are released in reaction to microbial plaque, and this is thought to be the primary cause of tissue damage.²⁷ (ROS) levels have been found to rise during periodontitis.²⁸ Damage to DNA could occur as a consequence of (ROS) accumulation; a hallmark of damaged

DNA damage is 8-OHdG, which is secreted in bodily fluids. A variety of chronic inflammatory disorders, such as periodontitis, have been associated with the levels of 8-OHdG. ²⁹

In relation to obesity, clinical, animal, epidemiological investigations and established the relevance of oxidative stress in the development of obesity and its associated risk factors.30 Oxidative stress may promote obesity through enhancing white adipose tissue formation modifying intake of food; it has been shown that oxidative stress may enhance preadipocyte expansion and adipocyte differentiation.³¹ It has been reported that (ROS) can be engaged in regulating body weight by affecting hypothalamic neurons, which govern satiety and hunger behavior. ³²

A significant difference was revealed in this study in the levels of salivary 8-OHdG between healthy and obese groups (both periodontally healthy) and these levels increased in the obese group. This result is consistent with a study that found significantly higher concentrations salivary 8-OHdG in obese patients compared to normal weight subjects ³³. This rise in 8-OHdG concentrations in obese group can be explained on the basis of oxidative stress

which is indicated by 8-OHdG levels. DNA, proteins and lipids are all prone to oxidative damage when exposed to high levels of (ROS).³⁴ Several biochemical pathways, including superoxide formation by NADPH oxidases (NOX) and oxidative phosphorylation are triggered by obesity to induce a state of systemic oxidative stress ³⁰. Consistent with our findings, a previous research discovered that the levels of 8-OHdG rose with the severity of obesity and a positive correlation between 8-OHdG and BMI was found.³⁵. Surprisingly, another investigation found lower concentrations of 8-OHdG in plasma of obese patients compared to normal weight people. This reduction in obese people may be related to the base excision repair (BER) pathway repairing certain 8-OHdG damages. ³⁶

This study revealed a significant moderate positive correlation between 8-OHdG and CAL in obese periodontitis group. Currently, the available literature is very limited. However; a previous study did not find any correlation between 8-OHdG and CAL in obese periodontitis patients and only

revealed a significant positive correlation between GCF 8-OHdG and GI (gingival index).³⁷ 8-OHdG has been shown to correlate positively with clinical periodontal parameter (CAL), This may be associated with the duration of the disease, and indirectly, the severity of the illness since prolonged cytokines stimulation such TNF-α causes both an increase in extracellular (ROS) generation through PMN activation and an increase in mitochondrial (ROS production). This idea is consistent with periodontitis features. ³⁸

Conclusion

This study illustrated an association between the salivary levels of 8-OHdG and periodontitis, this study also suggested an association between salivary levels of 8-OHdG and obesity. 8-OHdG is a significant indicator and biomarker of oxidative stress that reflects levels of DNA damage. Obesity and periodontitis may be linked through their shared association with oxidative stress, which reflects an importance in maintaining a healthy lifestyle and reducing exposure to risk factors for these conditions.

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The corresponding author is prompt to supply datasets generated during and/or analyzed during the current study on wise request.

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