

Effect of Non-Surgical Periodontal Therapy on Interleukin-8(il-8) Level in Gingival Crevicular Fluid in Overweight and Obese Subjects with Chronic Periodontitis

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Abstract: Overweight and obesity have been suggested to be associated with periodontitis reported in different studies and narrative summaries. The aim of the present study to assess the association of overweight and obesity with chronic periodontitis and determine the interleukin-8(il-8) level before and after non-surgical periodontal therapy. This study included (14) obese subjects (body mass index ≥ 30 kg/m²) and (12) overweight subjects (body mass index = 25 kg/m²). All subjects in both groups had chronic periodontitis. Their range from (35 to 48) years. The periodontal parameters were measured before and 3 months after non-surgical periodontal therapy were: visible plaque index (PI), bleeding gingival index (GI), probing depth (PD). In addition, gingival crevicular fluid (GCF) sample was collected from both groups to determine interleukin-8(il-8) level using filter paper strip. The level of il-8 was determined using ELIZA Kits. The results show a statistical significant improvement after periodontal therapy in both obese and overweight groups with reduction in visible plaque index (p-value=0.451), bleeding gingival index (p-value=0.338) probing depth (p-value= 0.326) with significant at ($P \leq 0.05$). Circulatory il-8 showed decreased in both groups but still higher in obese subjects than overweight after non-surgical periodontal treatment. An improved clinical parameters response to non-surgical periodontal therapy is observed in obese and overweight patients, with decrease il-8 level in both obesity and overweight, but still higher in obese patients.

Key words: Overweight • Obesity • il-8 • Chronic periodontitis • Non-surgical periodontal therapy

INTRODUCTION

Obesity is a major public health problem, and it has been implicated as a risk factor for several chronic health conditions, including diabetes, hypertension and stroke, and is thus associated with increased mortality [1]. The World Health Organization (WHO) estimated that 1 billion people were overweight (body mass index (BMI) ≥ 25) or obese (BMI ≥ 30) in 2005 and the number will increase to 1.5 billion by 2015 if current trends continue [2]. Obesity is a risk factor for periodontitis with a higher prevalence of periodontitis instituted among obese patients. Obesity implies immunoinflammatory modifications and associated with increase susceptibility to periodontitis [3]. Clinical evidence seems to suggest

that obese individuals have an increased local inflammatory response as well as a potentially altered periodontal microflora because the host response to local bacterial challenge is a key factor in determining susceptibility to periodontitis. An increased inflammatory state, that found in obese individuals could predispose them to increased periodontal tissue destruction [4]. Obesity increases the host's susceptibility by modulating the immune and inflammatory systems in a manner that predisposes to inflammatory tissue destruction and leaves an individual at greater risk of periodontitis. A number of epidemiological studies have examined the association between obesity and periodontitis [5, 10]. The results of the studies have been inconsistent. For example reported an overall association, whereas found an association

among young subjects and found on among non-smoking women [5, 7]. It was revealed by Tomofuji *et al.* [11] that periodontitis and /or periodontal pathogens could induce hepatic inflammation. It also has been revealed that metabolic syndrome increases the risk of periodontitis, suggesting that patients exhibited metabolic syndrome should be encouraged to undergo regular periodontal examination follow-up [12]. Several publications described a relationship between periodontal disease and metabolic syndrome of which obesity, insulin resistance, dyslipidemia and hypertension represent components [13]. Meanwhile, periodontitis could be a source of oxidative stress perhaps through the alteration of levels of circulating adipocytokines such as leptin, which in turn, accelerate the onset of insulin resistance and metabolic syndrome [14,15]. Linden *et al.* [9] reported an association between a high body mass index (BMI) and periodontitis in group of 60-70-year-old men. Haffajee *et al.* [16] concluded an overgrowth of *T. forsythia* in subgingival biofilms of periodontally healthy, overweight and obese individuals that might put them at risk for initiation and progression of periodontitis. Obesity, could have the potential transforming the host's immunity and inflammatory system causing the patient to be more at risk to the effects of microbial plaque [17]. IL-8, is also enhanced in GCF subjects with periodontal disease, it is a powerful neutrophil chemoattractant produced in response to various inflammatory stimuli, including TNF- α . Obesity contributes to a proinflammatory cytokines, and the levels of TNF- α and il-8 in GCF are directly linked to the extent of obesity in relation to BMI [18]. Obese people may have different kinds of oral bacteria from normal-weight leading to periodontal disease [19]. IL-8 has been implicated recently as taking part in the pathogenesis of atherosclerosis [20]. Furthermore, the serum levels of il-8 were significantly increased in patients with both type I and type II diabetes as compared with healthy subjects [21]. IL-8 is highly expressed and produced in human adipose tissue [22], therefore we investigated in this study the association between between il-8 and overweight-obesity in patients with chronic periodontitis to obtain more information on the possible role of il-8 in obesity after non-surgical periodontal therapy.

MATERIALS AND METHODS

The sample for the present study was recruited from obese and overweight patients for treatment at the National Research Center in Cairo, Egypt. The study was

conducted in September 2012. The study sample consisting of total 26 participants (14 obese and 12 overweight) in the age range from (35 to 48 years). The participants free from any systemic diseases, with chronic periodontitis were included in the study. The diagnosis of obesity and overweight was made by physician guidelines. Participants were recruited based on inclusion criteria: (i) the overweight group included 12 subjects with BMI (from 25-29.9 kg/m²) and obese group included 14 subjects with BMI (30 kg/m²) according to the WHO [2] (ii) no history of system disease (iii) no pregnancy at the time of the study; (iv) no three-fold elevation in liver enzymes; (v) no current hormone replacement treatment; (vi) no history of systemic antibiotic administration and antilipemic treatment with the past 3 months; and (vii) no periodontal treatment within the previous 6 months. A total of 14 obese and 12 overweight all affected with chronic periodontitis were included. The following data were collected for all participants: age, sex, medical history, BMI and medications used.

Clinical Periodontal Recordings Parameters: Dental examination was performed by only one dentist. All dental variables were assessed at four sites (mesio-buccal, mid-buccal, disto-buccal and mid-lingual) around each tooth. Complete periodontal evaluation data was collected that included the following: probing depth (PD) at six sites per tooth, gingival index (GI), plaque index (PI) according to Loe & Silness in 1964 [23]. Non-surgical periodontal therapy, consisting of scaling, root planning, and oral hygiene instructions, were performed after the periodontal examination, re-examination was carried out weekly for 3 months after non-surgical periodontal therapy was performed. Clinical measurements of periodontal parameters were recorded on a full-mouth basis and included plaque index (PI), probing depth (PD) and gingival index (GI) after non-surgical periodontal therapy in both obese and overweight groups.

Crevice Fluid Sampling: After removal of the supragingival plaque using sterile gauze, GCF was collected (at baseline) with a pre-weighed sterile filter paper strip (Periopaper) from both groups before and after non-surgical periodontal therapy. The individual tooth site was gently air-dried and isolated with cotton rolls and a saliva ejector was used to avoid salivary contamination of the samples. The filter papers were consecutively inserted into the crevice at the mesial or the distal midpoints until mild resistance was felt. The strips were left in situ for 30s. Samples were stored at -20°C for

assessment of (il-8) levels using Enzyme immunoassay (ELISA eBIOSCIENCE) in which an anti-human (il-8) coating antibody is adsorbed onto microwells. Human (il-8) present in the sample or standard binds to antibodies adsorbed to the microwells. A biotin-conjugated antihuman (il-8) antibody was added and binds to human (il-8) captured by the first antibody. Following incubation unbound biotin-conjugated antihuman (il-8) antibody was removed during a wash step. Streptavidin-HRP was added and binds to the biotin-conjugated anti-human (il-8) antibody. After incubation unbound Streptavidin-HRP was removed during a wash step and substrate solution reactive with HRP was added to the wells. A coloured product was formed in proportion to the amount of (il-8) present in samples. The reaction was terminated by addition stop solution and absorbance was measured at 450 nm.

Statistical Analysis: Numerical data were presented as mean and Standard Deviation (SD) values. BMI data showed parametric distribution; so Student's t-test was used to compare between the two groups. Clinical parameters and il-8 level data showed non-parametric distribution; so Mann-Whitney U test was used to compare between the two groups. This test is the non-parametric alternative to Student's t-test. Qualitative data were presented as frequencies and percentages. Chi-square (χ^2) test was used to compare between the two groups. Spearman's correlation coefficient was used to determine significant correlation between il-8 level and different variables. The significance level was set at $P \leq 0.05$.

RESULTS

Clinical Results: The total participants of this study were twenty-six subjects (No=26) of both sexes with an age range of 35-48 years. Obese group included (14) subjects and overweight group included (12) subjects. The clinical periodontal condition of the subjects was assessed using PI, GI, PD. The statistically significant show no difference between gender distributions in the two groups. The mean standard deviation of BMI showing highly statistical significant difference in obese more than overweight group (P -value < 0.001) as shown in Table (1).

Demographic Data: No statistically significant difference between gender distributions in the two groups as shown in Fig (1).



Fig. 1: Bar chart representing gender distributions in the two groups

Table 1: Obese group showed statistically significantly higher mean BMI than overweight group. (P -value < 0.001) as shown :

Group Variables	Obese (n=14)	Overweight (n=12)	P-value
BMI (Kg/m ²)	36.6 ± 3.5	27.1 ± 1.9	<0.001*
Mean ± SD			
Gender			
Male	30%	70%	0.814
Female	25%	75%	

*: Significant at $P \leq 0.05$

Table 2: Comparison between clinical parameters on the two groups

Group Parameters	Obese (No=14)	Overweight (No=12)	P-value
Plaque index (Mean ± SD)			
Pre-operative	2.40 ± 0.52	0.20 ± 0.63	90.00 ± 31.62
Post-operative	1.97 ± 0.87	0.25 ± 0.71	79.17 ± 39.59
% reduction	0.429	0.871	0.451
Gingival index (Mean ± SD)			
Pre-operative	2.10 ± 0.74	0.20 ± 0.63	80 ± 63.25
Post-operative	1.88 ± 0.35	0.25 ± 0.71	87.5 ± 35.36
% reduction	0.425	0.871	0.338
Pocket depth (Mean ± SD)			
Pre-operative	3.15 ± 2.43	0.60 ± 1.29	53.33 ± 51.99
Post-operative	1.59 ± 2.34	0.75 ± 2.12	25.00 ± 46.29
% reduction	0.209	0.784	0.326
Pre-operative	48.4 ± 19	12.4 ± 5.2	70.3 ± 13.6
Post-operative	38.4 ± 13.7	7.2 ± 11.5	84.5 ± 23.5
% reduction	0.214	0.026*	0.021*

*: Significant at $P \leq 0.05$

Clinical Parameters: The mean standard deviation between clinical parameters in both obese and overweight groups show no statistical significant difference in PI, GI, PD, between the two groups pre-operatively, post-operatively as well as between the percentage reductions in PI, GI, PD, as shown in Table (2).

Table 4: Correlation coefficient for the correlation between il-8 level and different variables

Variables	Correlation coefficient	P-value
BMI and il-8 (Pre-operative)	0.415	0.025*
BMI and il-8 (Post-operative)	0.362	0.048*
PI and il-8 (Pre-operative)	0.228	0.362
PI and il-8 (Post-operative)	0.344	0.065
GI and il-8 (Pre-operative)	0.286	0.250
GI and il-8 (Post-operative)	0.307	0.215
PD and il-8 (Pre-operative)	0.265	0.601
PD and il-8 (Post-operative)	0.495	0.037*
PI and il-8 (Pre-operative)	0.228	0.362
PI and il-8 (Post-operative)	0.344	0.065
GI and il-8 (Pre-operative)	0.286	0.250
GI and il-8 (Post-operative)	0.307	0.215
PD and il-8 (Pre-operative)	0.265	0.601
PD and il-8 (Post-operative)	0.495	0.037*

*: Significant at $P \leq 0.05$

Effect of Obesity and Overweight on Il-8 Level: There was no statistically significant difference between the two groups pre-operatively (p-value=0.214) as shown in Table (3).

Obese group showed statistically significantly higher mean il-8 level than overweight group post-operatively (p-value=0.026) as shown in Table (3).

Obese group showed statistically significantly lower mean % reduction in il-8 level than overweight group (p-value=0.021) as shown in Table (3).

Correlation Between Il-8 Level and Different Variables: There was a statistically significant positive (direct) correlation between il-8 level and BMI pre- and post-operatively.

There was a statistically significant positive (direct) correlation between il-8 level and PD post-operatively.

There was non-statistically significant correlation between il-8 level and other parameters pre- and post-operatively.

DISCUSSION

Obesity as an independent or aggravating risk factor for several diseases, including coronary heart disease, osteoarthritis and type 2 diabetes mellitus [5]. Obesity and overweight are potential confounders in association between periodontitis and other diseases as a risk factor for atherosclerotic cardiovascular diseases [24,25]. Studying the relationship between obesity and periodontal disease is therefore important since this association could further contribute to increase morbidity of these diseases in overweight or obese individuals [26]. It was evaluated by Ekuni *et al.* [27] on young 618 subjects aged 18-24

years with BMI <30kg/m² found that age and BMI were associated to periodontal index. Several literatures suggested that metabolic syndrome may be triggered and/or exacerbated by adverse social factors and certain psychological pathologies trait and behaviours [28]. Ozier *et al.* [29] revealed that metabolic syndrome and obesity linked to several psychological factors, body dissatisfaction, self-esteem, depression, stress, positive and negative effect. It was also been demonstrated that human leptin is present within healthy and marginally inflamed gingival, and decreased in concentration with increase pocket depth [30]. The relationship between overweight/obesity and inflammatory processes (periodontitis), the adipose cells secreted by the adipose tissue, such as adipocytes, preadipocytes, and macrophages secrete adipokines locally or in systemic circulation of liver, muscle and endothelium. It appears possible that periodontitis may stimulate inflammatory change in adipose tissue, creating a triangular self-generating cycle of morbidity linking obesity, diabetes and periodontal disease [31]. Also, adipokines act as hormones (e.g. leptin and adiponectin) as cytokines (e.g. tumor necrosis factor- α , interleukin-6 (il-6), interleukin-8 (il-8)) as proteins participating in vascular hemostasis (e.g. plasminogen activator inhibitor-1 (PAI-1)) [32]. Several studies suggested that chemokines such as il-8 monocyte chemoattractant and activating protein-1, may be involved in the pathogenesis of atherosclerosis and thereby in cardiovascular disease [33]. This study was designed with inclusion criteria applied to eliminate the confounding factors that affect both diseases. The physician attempted to find participants without any systemic diseases through elimination of potential shared confounders, such as impaired glucose tolerance, diabetes mellitus, and cardiovascular disease, which are all conditions believed to be involved in the development and/or progression of both periodontal disease and obesity. When taking into account all of the study population the gender distribution showed no statistical significance in both groups as shown in Fig (1). There was statistical significant between obese groups with higher mean BMI than overweight group these findings have also been supported by Dalla *et al.* [9] who emphasize that obesity was significantly associated with occurrence of periodontitis. Our findings demonstrate that non-surgical periodontal therapy promotes a significant improvement in all periodontal clinical parameters such as PI, GI, PD, in both obese and overweight groups as shown in Fig (2). Thus, it can be suggested that obesity does not act as a negative modifying factor on periodontal clinical

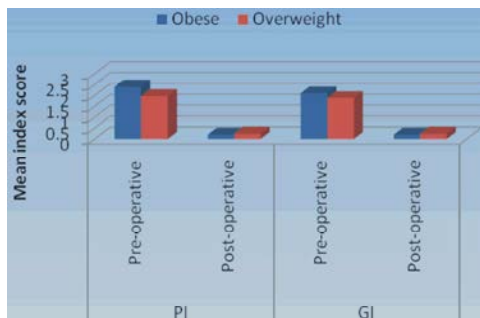


Fig. 2: Bar chart representing PI and GI findings in the two groups

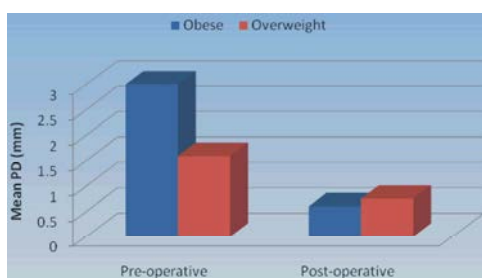


Fig. 3: Bar chart representing mean PD in the two groups

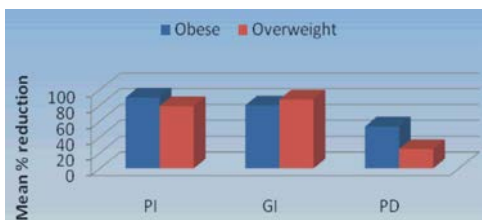


Fig. 4: Bar chart representing mean % reduction in clinical parameters in the two groups

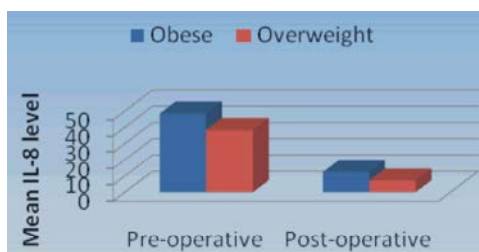


Fig. 5: Bar chart representing mean il-8 levels in the two groups

response after non-surgical periodontal treatment. Table (3) show the results of an immunologic data demonstrate show a positive correlation between il-8 and BMI in both groups before and after non-surgical periodontal therapy,opposing pattern to this coassociation.No statistical difference in pocket depth (PD) in both obese and overweight groups as shown in Fig (3), these results opposed by Saito *et al.* [10] that

declared obesity is extensively associated with deep probing depths which is a sign of destruction associated with periodontal disease.In the study, there is a perpetuation of higher level of il-8 in obese subjects after non-surgical periodontal therapy compared to overweight subjects,as shown in Fig (5) this is because the local periodontal inflammation was treated and the obese condition was not altered throughout the course of the study so this elevation could have been related to the subject's obesity;the perpetuation of adiposity was an important factor verifying the influence of the obese condition.A similar study shows that obesity doesn't seem to have a prominent effect on clinical periodontal parameters but it does have correlations with circulatory inflammatory present results supported several investigators who reported that there was a relationship between periodontal disease and BMI[7].Recent reports focused on the serum lipids in the association between periodontitis and systemic diseases.The potential inflammatory mechanisms play a potential role in this association have also been explored in this point of view.Inflammatory and immune mediators that are established in gingival crevicular development of periodontitis,and there is increase level of TNF- α and il-8 found in GCF enhance the periodontal disease [34].The adipose tissue produces cytokines and hormone-like substances(e.g.tumor necrosis factor- α (TNF- α , interleukin- 6(il-6), interleukin-8(il-8), leptin and plasminogen activator inhibitor-1),these substances are most prevalent in the inflammatory response.These adipokines affect the metabolism of the entire body and contribute to lower grade systemic and vascular inflammation due to accumulation of gram-negative bacteria and inflammatory mediators[35,36].Increase body fat may stimulate a hyperinflammatory response in periodontal disease.It was also stated by Morita *et al.*[37] that serum TRG(triglycerides) levels may play a potential role in periodontitis.Tandon *et al.*[38] reported that patients of chronic generalized periodontitis who were offered periodontal therapy showed improvement in various lipid parameters except high density lipoproteins cholesterol, which was significantly altered, these patients could be contributing to systemic inflammatory burden and adversely altering lipid profile.Conclusion: The level of il-8 was higher in obese subjects than overweight subjects after non-surgical periodontal therapy,although obesity doesn't seem to play a negative role in the improvement of the periodontal clinical response, and it could be useful to include evaluation of BMI in oral health

examination. Further researches could be necessary regarding the relationship among obesity and periodontal disease with other systemic and chronic diseases.

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