

Effect of Obesity on the Levels of Salivary Matrix Metalloproteinase-8 (MMP-8) in Chronic Periodontitis Patients

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Abstract

Matrix metalloproteinase (MMP-8) is currently considered to be one of the most significant biomarkers for the development and early diagnosis of periodontitis. The aim of this study was to assess the salivary levels of MMP-8 in obese with chronic periodontitis patients.

The study involved 60 subjects visiting the School of Dental Sciences, Universiti Sains Malaysia, Kota Bharu, Kelantan, Malaysia. The participants were divided into two groups: group I consisted of 30 non-obese subjects with periodontitis while group II consisted of 30 patients with obesity and periodontitis. Chronic periodontitis was evaluated by several periodontal parameters, such as periodontal pocket depth (PPD), clinical attachment level (CAL), gingival bleeding index, plaque score, and obesity were measured by Body Mass Index (BMI). 3ml of stimulated saliva was collected for assessment of MMP-8 by enzyme-linked immunosorbent assay kit. Data were statistically analyzed by the independent t-test.

The salivary MMP-8 protein levels were slightly higher in group II as compared to group I, however, it was not statistically significant ($P=0.06$). Only PPD and CAL were found statistically significant ($P<.001$, $P=.005$) when compared between both groups. The findings of this study demonstrate no association between obesity and salivary MMP-8 levels in chronic periodontitis patients.

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Introduction

Obesity, a standout amongst the major wellbeing dangers of present-day society, is defined as a state of abnormal or extravagant fat accumulation in adipose tissue and able to impair the healthy condition of the body.¹ The worldwide epidemic of stoutness results from a mix of hereditary powerlessness, expanded accessibility of high vitality sustenance and diminished physical action in the present-day society. Worldwide, the prevalence of obesity and overweight increased by 27.5% for an adult and about 47.1% for children from 1980 to 2013.

From 1980 to 2013 the number of obese and overweight individuals increased from 857 million to 2.1 billion.² The prevalence of obesity among Malaysian adults was estimated at 27.2%.³ Obesity is multisystem condition and a vital risk factor for numerous adult diseases like type II polygenic disorder, hyperlipemia, arthritis and periodontitis.⁴

Periodontitis is a provocative condition that influences oral tissues encompassing the teeth. It is described by the devastation of periodontal connective tissues and tooth supporting bone leading to tooth loss.⁵ Globally, the prevalence of severe periodontitis in the population is described to be 11.2%.⁶

The link between obesity and periodontitis is the secretions of cytokines and hormones from adipose cells that are associated with the inflammation.⁷ Obesity would possibly represent a general condition, well known to control the onset and progression of periodontal disease.

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Recently, Han et al. studied that the visceral fat area is the foremost applicable indicator of obesity in reference to periodontal disease.⁸ Obesity plays a vital role to modulate the host immune response, causing increased sensitivity to different types of infections and increases the host immune responses of the body to them. Specifically what adipose tissue secretes are different inflammatory markers, involved in periodontal disease, including interleukin-6 (IL-6), Tumor Necrosis Factor- α (TNF- α) and chemokines, and can alter T-cell function.⁹ Among the host-determined biomarker, matrix metalloproteinases (MMPs) is a group of calcium-dependent, zinc-containing proteases involved in a periodontal disease condition.^{10,11} Type I collagen represents a large amount of periodontal extracellular matrix family; thus, extra focus is paid on collagenases and gelatinases like MMP-8, MMP-13, and MMP-9 in periodontal disease. MMP-8 is the principle collagenolytic MMP identified in the gingival tissue and oral liquids (80% of collagenases in GCF).¹² The MMP-8 has been depicted as one of the most grounded salivary biomarkers for distinguishing alveolar bone devastation, related with various clinical and radiological parameters, for example, developed periodontal pockets, increase in attachment and alveolar bone loss.¹³

It has been reported obese patients have increased circulating plasma MMP-8 levels in overweight/obese people,^{14,15,16} while the current study was investigating the level of MMP-8 in the saliva of the obese individual with regards to the one with the chronic periodontitis. To the best of our knowledge, there is no previous study that has studied those parameters in obese individuals with respect to periodontitis. This study is important because MMP-8 levels were positively associated with obesity and are an important biomarker for the degradation of the extracellular matrix and collagen. Therefore, in the present study, we aimed at comparing the salivary concentrations of MMP-8 in obese patients experiencing periodontitis with those who are non-obese and having periodontitis. Also, the periodontal parameters were compared between them.

Materials and methods

Ethical Guidelines

The research study protocol was reviewed and approved by the Human Research Ethics Committee of Universiti Sains Malaysia, Malaysia (USM/JEPeM/17100446). In this, institutionally approved study subjects were educated as to nature and aim for the study, written informed consent were given and signed.

Grouping of Study Participants

The study consisted of 60 subjects aged 35-65 who had visited the out-patient clinic of the School of Dental Sciences, Hospital Universiti Sains Malaysia. The subjects were divided into two groups; group I consisted of 30 patients with periodontitis only, while, group II consisted of 30 obese patients with periodontitis. A detailed medical and dental history was taken. The periodontal parameters such as periodontal pocket depth (PPD), clinical attachment level (CAL), gingival bleeding index, and plaque score were examined. All volunteer participants were directed to the oral health department for the periodontal examination and collection of saliva.

Inclusion and Exclusion Criteria

Individuals with BMI >30 were included in group II. However, for the group I all participants was with BMI <24.9. For both groups, the periodontal pocket of selected patients was ≥ 3 mm and clinical attachment level was ≥ 2 mm and had not undergone any periodontal treatment for the last 6 months. All the patients that included in the study are systemic healthy. Pregnant and lactating females, subjects having chronic inflammation, patients on smoking, oral medications and other medical conditions such as type II diabetes mellitus were excluded.

Periodontal and Body Mass Index (BMI) examination

Periodontal disease status (PPD, CAL) was assessed at six sites of every mandibular and maxillary tooth (i.e., distobuccal, buccal, mesiobuccal, distolingual/palatal, lingual/palatal, mesiolingual/palatal) excluding the third molars and was performed by the same examiner using the same periodontal probe (University of North Carolina -15 probes). To determine intra-examiner reliability for PPD and CAL, repeated measurements on teeth were performed by the same examiners on 150 sites. The intraclass correlation coefficient test was used to estimate the measured values, i.e., 0.78 & 0.68

respectively. The gingival bleeding index and plaque score was calculated in percentage by evaluating the presence of bleeding and plaque on each surface of tooth dividing by a total number of surfaces of all present teeth.

Obesity was determined by Body Mass Index (BMI) according to the protocol of the World Health Organization (WHO). In this method, the weight of the patients in kilograms was measured by using a standard physician's scale and the height of the patients in centimeters was measured using stadiometer. BMI was calculated, and the patients were classified as obese (BMI >30) or non-obese (BMI < 24.9).

Collection of Saliva Samples

The saliva samples required for evaluation of MMP-8 were taken from all the groups. The subjects were directed to sit straight comfortably with eyes open and head tilted forward and were instructed to pool the saliva at the base of the mouth. Sterile 15mL of saliva collecting tubes were used to collect about 3mL of saliva. The collected saliva was centrifuged instantly to remove cell debris (1,000 × g for 15 min at 4°C). Following that, the supernatant was stored in small aliquots at -80°C.

MMP-8 levels in saliva were analyzed in duplicate for all subjects using Quantikine Human Total MMP-8 Immunoassay kit, based on the enzyme-linked immunosorbent assay method as recommended by manufacturer's (Elabscience, China). Absorbance was tested at 450 nm of wavelength.

Statistical Analysis

Data were analyzed using SPSS (version 24) software, comparison between salivary MMP-8 concentration levels and the periodontal parameters between both groups was presented in mean standard deviation and were assessed by independent t-test.

Results

There was a total of 30 obese and 30 non-obese chronic periodontitis patients (mean age = 49.0, SD = 11.2) who participated in the study. The mean age and, gender and ethnic distributions were found to be similar in both

groups ($P > .05$) (Table 1). The BMI in the obese group was significantly greater than the non-obese group (mean difference = -9.3).

Variables	Non-obese n=30	Obese n=30
Age (years)	47.06 ± 12.57	50.92 ± 9.79
Gender		
• Male n(%)	14 (46.6%)	16 (57.10%)
• Female n(%)	16 (53.4%)	12 (42.90%)
Ethnic		
• Malay n(%)	25 (81.48%)	24 (85.18%)
• Chinese n(%)	5 (18.52%)	4 (14.82%)

Table 1. Demographic characteristics of the study subjects (n = 60).

The clinical periodontal parameters between group I (n=30) and group II (n=30) are shown in Table 2. The obese group had poorer periodontal and oral hygiene status than the non-obese. The PPD and was significantly greater in the obese group than those of group I ($P < .001$). Similarly, the CAL values for a group I were also significantly lower than group II ($P = .005$).

Gingival bleeding index and plaque scores showed no significant difference between both groups ($P = 0.98$, $P = 0.44$). The gingival bleeding index for the group I (35.96 ± 24.13) was slightly lower than that of group II (35.85 ± 26.74). Similarly, the plaque score for the group I (37.85 ± 24.90) was non-significantly lower than that of group II (42.90 ± 25.49).

The salivary MMP-8 concentration levels in group I was 0.12 ± 0.21 ng/mL as compared to group II 0.15 ± 0.83 ng/mL (Figure 1). The results show no significant differences in salivary MMP-8 concentration level when compared between group I and group II ($P = 0.06$).

Clinical parameters	Non-obese (n=30)	Obese (n=28)	Mean difference 95%CI	p value
*PPD (mm)	4.62±0.71	5.35±0.79	- 0.73 (-1.1, - 0.3)	< 0.001
Plaque score	37.85±24.90	42.90±25.49	- 5.04 (-18.0, 7.9)	0.441
**CAL (mm)	4.66±0.31	5.21 ± 0.95	- 0.55 (- 0.9, - 0.1)	0.005
Gingival bleeding index	35.96±24.13	35.85±26.74	0.10 (-13.0, 13.2)	0.987
Body mass index (kg/m ²)	23.33± 1.67	32.70±2.44	-9.36 (-10.4, -8.2)	< 0.001

Table 2. Clinical parameters of the study subjects (n=60).

*Periodontal pocket depth **Clinical attachment level

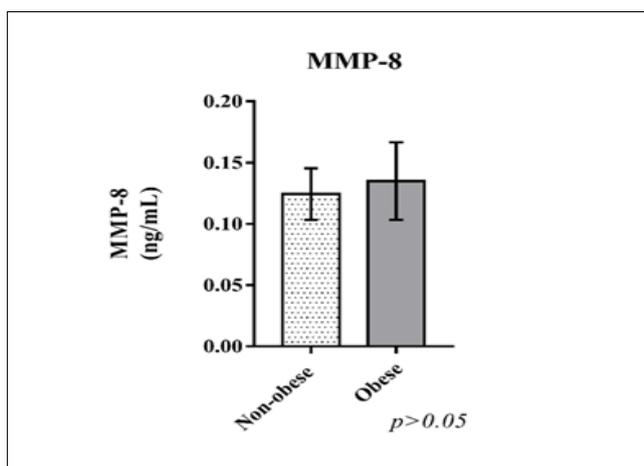


Figure 1. Human Salivary MMP-8 concentration between group I and group II.

Discussion

Human saliva is effectively available natural fluid that contains a different inflammatory biomarker, which helps the researchers to screen and diagnose the diseases. Saliva could be an alternative to plasma/serum and other body fluid to identify inflammatory mediators that release from the different cell during the inflammatory condition. Inflammatory biomarkers, such as MMP-8, TNF- α , IL-6, and IL-8, and TIMP-1 associated with different oral diseases: gingivitis, periodontitis, and dental caries have been detected in saliva.^{17,18,19} To the best of our

knowledge the current study is first of its kind that compared the levels of salivary MMP-8 in obese and non-obese individuals with chronic periodontitis.

MPP-8 are also known as neutrophil collagenase that is produced by endothelial cells and neutrophils. They might be engaged with type I and type III collagen decomposition.^{20, 21} The previous study has reported that there is an increase in levels of circulating plasma MMP-8 concentration in obese adults, and higher MMP-8/TIMP-1 than the non-obese group, thereby increasing the chance for tissue remodelling.²² Another study by Lauhio et al in 2016 was conducted among obese (n = 34), overweight (n = 76) and normal weight (n = 130) twin individuals using time-resolved immunofluorometric assay revealed that serum MMP-8 increased in obese subjects as compared to non-obese patients.²³

In contrast, this study found no differences in MMP-8 concentration in saliva between the obese and non-obese group with periodontitis. In support to our study, a previous study performed on the obese tissue of mice showed the presence of several types of MMPs, except MMP-8.²⁴ An important finding in a previous study also indicated lower levels of plasma MMP-8 in obese women as compared to lean women.²⁵

A microarray study revealed that while many MMP family members were dynamically upregulated in adipose tissue in a culture medium, however, MMP-8 levels were unnoticed.²⁶ These data support our findings and proposed that adipose tissue most probably does not produce MMP-8 in amounts enough to interfere with periodontitis although the levels of MMP-8 might be high in plasma. This difference in results may be due to the discrepancy in ethnicity, population, genetic makeup, and methodological difference.

The results for gingival bleeding index and plaque score were statistically not significant. However, CAL and PPD showed significant results between both groups that are an effective indicator of inflammations. Some studies have described that MMP-8 acts as a vital marker in chronic periodontitis and has been highly associated with periodontal pocket, clinical attachment level, and bleeding on probing.^{27, 28} This study represents that obesity had a positive association with periodontitis and was in support of studies conducted in other regions like Japan and Jordan, where increased in obesity was found related with periodontitis.²⁹ Al Zahrani et al described that obesity has a positive association with increased periodontitis incidence³⁰ however, the biological mechanism for the association between obesity and periodontitis is yet to be established.

Here, the limitations of the current study are important to be recognized. Firstly, the levels of tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) in the saliva of both groups were not assessed and the levels of MMP-8 in other mediums like GCF and blood were also not analyzed as well. Secondly, we only used ELISA method to compare the levels of salivary MMP-8, however there are other methods that could also be used to analyze the levels of salivary MMP-8 such as time-resolved immunofluorometric assay and dento-analyzer device. Finally, this study involved a single type of population, but the variety in population might yield variation in the results.

Conclusions

From the above study, it might be concluded that obesity does not affect the levels of salivary MMP-8 of patients with chronic periodontitis. However, the concentration of

MMP-8 seemed to show an association with periodontal inflammation. Thus, change in the levels of salivary MMP-8 should be taken into account in diagnoses of periodontal disease in the future.

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Declaration of Interest

The authors proclaim no conflict of interests.

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