How to Cite:
Ramzan, R., Zainuddin, S. L., Ahmad, A., Taib, H., & Kassim, N. K. (2022). Periodontal parameters and salivary levels of receptor activator of NF-kB Ligand and osteoprotegerin in obese periodontitis patients. *International Journal of Health Sciences*, *6*(S6), 10738–10753. https://doi.org/10.53730/ijhs.v6nS6.12888

Periodontal parameters and salivary levels of receptor activator of NF-kB Ligand and osteoprotegerin in obese periodontitis patients

Raheem Ramzan
School of Dental Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia
Email: raheemrc3706@gmail.com

Siti LA Zainuddin
School of Dental Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia
*Corresponding author email: sitizainuddin@gmail.com, lailatul@usm.my

Azlina Ahmad
School of Dental Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia
Email: azlinakb@usm.my

Haslina Taib
School of Dental Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia
Email: haslinakk@usm.my

Nur Karyatee Kassim
School of Dental Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia
Email: karyatee@usm.my

***Abstract*---**This study aimed to assess levels of salivary receptor activator of nuclear factor kappa-B (RANKL) and osteoprotegerin (OPG) protein biomarkers in obese patients with periodontitis. Subjects were divided into two groups: group-I consisted of 30 non-obese subjects with periodontitis, while group-II consisted of 30 obese patients with periodontitis. The periodontal examination, including periodontal pocket depth (PPD), clinical attachment loss (CAL), plaque score (PS), and gingival bleeding index (GBI), were evaluated. Salivary RANKL and OPG were estimated in both study groups using an enzyme-linked immunosorbent assay. Levels of RANKL protein were significantly higher in the obese group than the non-obese group,

while levels of OPG protein were significantly higher in the non-obese group than the obese group. The periodontal parameters PPD, CAL, and PS, except for GBI, showed a significant difference between the two groups. The mean PPD scores were significantly higher in the obese group than the non-obese group, while the CAL scores in the obese group were significantly higher than the non-obese group. Only the salivary OPG levels were significantly associated with CAL. There is an association of OPG and CAL in both obese and non-obese with periodontitis.

***Keywords*---**periodontitis, obesity, osteoprotegerin, RANKL, periodontal parameters.

Introduction

Periodontitis is caused by the presence of bacteria in the dental plaque. The most associated bacteria to periodontitis are *Porphyromonas gingivalis, Tannerella forsythia and Treponema denticola* also known as the red-complex (da Silva-Boghossian et al., 2011). The immune system of the host is activated in response to these bacteria. The inflammatory response is initiated which causes an influx of macrophages, release of cytokines like interleukin (IL) -1 and IL-6 and activated T and B-lymphocytes into the affected area. As more cytokines are released, more cells move into the affected area, and this leads to more inflammation and damage to periodontat tissue (Marton & Kiss, 2000). According to World Health Organization (WHO) an adult with Body Mass Index (BMI) of 30 kg/m^2 or more is defined as obese. Obesity is a condition in which excessive body fat deposition leads to adverse effect on the body (Organization, 2000). Obesity is a risk factor for diseases like hypertension, cancers, diabetes and other inflammatory conditions leading to reduced life expectancy (Haslam & James, 2005).

The obese subjects have abnormal circulating levels of cytokines such as tumor necrosis factor-alpha (TNF-α), IL-6, C-reactive protein (CRP), adiponectin and leptin. These proinflammatory cytokines may have a harmful effect on the periodontium (Dahiya et al., 2012). Obesity increases bone resorption by the up regulation of IL-6 and TNF-α, which are proinflammatory cytokines and activate RANKL/RANK/OPG pathway which causes increase in osteoclast activity (Cao, 2011). Receptor activator of NF- κβ ligand (RANKL) is a member of tumor necrosis factor superfamily that causes osteoclastic differentiation and bone resorption (Fuller et al., 1998). Osteoprotegerin (OPG) is a soluble glycoprotein that belongs to tumor necrosis factor superfamily and its role is to stop RANKL from binding with its receptor RANK inhibiting osteoclastogenesis (Simonet et al., 1997). The RANKL/RANK/OPG pathway is activated by the action of cytokines (TNF-αand IL-6) and causes bone resorption. These cytokines are in elevated levels in obese individuals (Cao, 2011). RANKL/OPG ratio has been reported to be a good indicator for periodontitis-induced bone destruction (Belibasakis & Bostanci, 2012). Saliva is a good medium for the assessment of periodontal disease. Saliva contains constituents from glands and gingival crevicular fluid (GCF). Saliva can be collected easily without using any complex equipment and it is readily

available. Mediators from inflammation can be easily detected from saliva (Frodge et al., 2008).

Materials and Methods

Study Design

This was a cross sectional study carried out at school of Dental Sciences, Universiti Sains Malaysia (USM) over a period of 6 months. The study was divided into 2 groups: each group consisted of 30 subjects:

- Group 1: Patients with periodontitis.
- Group 2: Patients with obesity and periodontitis

Sample size calculation

The sample size for this study was calculated by using reference values from a study done on levels of salivary RANKL and OPG. The salivary levels of RANKL of chronic periodontitis patients was 0.2004 ng/ml (Behfarnia et al., 2016). The mean salivary level of OPG for chronic periodontitis patients was 0.6431 ng/ml (Behfarnia et al., 2016). But the values of RANKL and OPG have not been established in saliva of obese patients with periodontitis. The calculated sample size for RANKL and OPG came out to be 27 for each group. After accounting for 10% drop out rate the final sample size was 30 (n).

Method of collection of saliva sample

Saliva is collected from both groups to be used for the estimation of RANKL and OPG. To stimulate saliva the patients were given some citrus to lick. The patients were instructed to rinse there moth properly for 1 min. After rinsing the subjects are requested to tilt head forward and allow saliva to pool at the floor of the mouth (Navazesh, 1993). Saliva those pools at the floor of the mouth can be collected either by a straw or by passing directly into falcon tube. 3-4 ml saliva is collected from the subjects in 3-5 minutes. Samples are centrifuged at 3,000g, 4 °C for 15 min. Supernatant is separated from the rest of saliva and was frozen at -80 °C until assays were performed (Cutando et al., 2014).

ELISA assay procedure

The procedure starts with 100 µl of standard solution is added side by side in first two columns of the well plate. The 100 µl of standard solution is prepared in different concentration 1.25 ng/mL to 0.08 ng/ mL. 100 µl of saliva sample were added on each well respectively in all the remaining wells. The plate was sealed and incubated for 90 min at 37°C with a shaker. In the next step 100 µl of prepared biotinylated detection antibody working solution is added in all the well sides after the liquid is removed from the plate. 300 µl of wash buffer solution is used in the next step to was the plate. The wash buffer solution is placed in each well for 1 to 2 mins and removed. The plate is dried and sealed again for incubation for 1 hour at 37°C degrees within a shaker. After the working time,

plate was removed and solution was removed out of each well followed by additional backside of the plate. This washing step was repeated for 3 times.

HRP conjugate working solution was prepared and added in each of the well side. The plate was covered again with sealer and incubated for 30 min at 37°degrees. Plate was removed after the given time and the solution was aspirated from wells and the washing process was repeated for 5 times. In the next step, 90 µl of substrate reagent was added directly to each well side and was covered with a new seal cover. Addition of substrate reagent changed the color of the samples into blue. The mixture was incubated for about 15-30 minutes approximately at 37°C in the shaker plus incubator. This whole step was very light senistive so it was carried out in a dark room with minimum light. The plate must be protected from light after addition of substrate reagent. In the final step prior to plate reading, 50 µl of stop solution was poured in each well side in order to stop the enzymatic reaction to prevent exaggerated color development in each well. When the stop solution was added the samples turn their colour from blue to yellow and optical density (OD) was determined with micro plate reader was already checked and set at 450 nm.

Results and Discussions

A total of 60 subjects with periodontitis, equally distributed in the obese and non-obese group, who attended the dental clinics of Hospital Universiti Sains Malaysia (HUSM) for dental treatment or routine dental checkup had participated in this study. In group 1 non obese with periodontitis (otherwise systemically healthy with no medical problem), in group 2 obese with periodontitis. A description of the demographic characteristics of all the patients by age, gender, ethnicity and BMI for both the groups is provided in Table 1.

Table 1
Demographic Data of Subjects for Incidence study

Cumulative Samples (n = 60)	Nonobese group (n=30)	Obese group (n=30)	*p*-value
Age (years) mean ± SD	44.06 ± 9.05	48.86 ± 8.94	
Gender			
Male n (%)	14 (46%)	12 (44%)	
Female n (%)	16 (54%)	18 (56%)	
Race			
Malays n (%)	25 (83.4	25 (83.4 %)	
Chinese n (%)	5 (16.6%)	5 (16.6%)	
Body mass index (kg/m^2)	21.29 ± 1.31	32.01 ± 1.39	<0.05*

The participants in group 2 have higher mean age as compared to the group 1 (mean difference = -4.80, p <0.04). The mean age for group 1 is 44.06 ± 9.051, while the mean age for group 2 is 48.86 ± 8.947. With respect to the ethnicity there were 25 (83.4 %) Malays and 5 (16.6%) Chinese participants in both groups.

Based on the gender there are marginally higher number of females than males in both the groups. Body mass index (kg/m^2) for group 1 and group 2 was analysed using Independent t-test with $p < 0.05$. The mean BMI for group 1 is 21.29 ± 1.31 and for group 2 is 32.01 ± 1.39. Table 2 shows analysis of periodontal parameter between group 1and group 2. Participants in the group 2 have more adversely affected periodontal status as compared to group 1 with deeper pocket (mean dif=-0.682) and more clinical attachment loss (mean dif =-0.790). The mean PPD for the group 1 is 4.6 ± 0.61 and for the group 2 is 5.2 ± 0.67 with p <0.00. CAL for group 1 is 4.8 ± 0.64 and for the group 2 is 5.6 ± 1.21 with p <0.03. The analysis indicates significant difference in PPD and CAL in the obese and non-obese group.

Table 2
Clinical parameters of the subjects (n = 60) PPD, CAL, gingival bleeding index, plaque score, and BMI

Clinical parameters	Non-Obese group n = 30	Obese group n = 30	p-value	Mean difference	Standard error
PPD % (mm) mean ± SD	4.6 ± 0.61	5.2 ±0.67	0.000*	-0.68	0.16
CAL % (mm) mean ± SD	4.8 ± 0.64	5.6 ± 1.21	0.003*	-0.79	0.25
Plaque score % mean ± SD	60.9 ± 18.8	70.5 ± 17.3	0.045*	-9.57	4.66
Gingival bleeding Index % mean ± SD	48.7 ± 15.9	59.7 ± 25.7	0.051*	-11.0	5.52

Independent t-test used
PPD = Periodontal pocket depth
CAL = Clinical attachment level

PS is higher for the group 2 as compared to the group 1 ($p < 0.045$). Mean PS for group 1 is 60.9 ± 18.8 and for group 2 is 70.5 ± 17.3. There is no significant difference in GBI in both groups. Mean GBI for group 1 is 48.7 ± 15.9 and group 2 is 59.7 ± 25.7 with $p > 0.05$. Table 3 shows the analysis between group 1 and group 2. The analysis showed the levels of OPG were significantly higher in the group 1 as compared to group 2 (mean dif=.247). And the RANKL level is significantly lower in the group 1 compared to the group 2 (mean dif= -.017). The level of the salivary protein OPG for group 1 is 2.23 ± 0.51 ng/ml and group 2 is 1.86 ± 0.62 ng/ml with $p < 0.046$. RANKL for group 1 is 0.033 ± 0.02 ng/ml and group 2 is 0.045 ± 0.026 ng/ml with $p < 0.015$. Independent t-test showed significant difference between the two groups.

Table 3
Mean ± SD values of salivary RANKL and OPG levels in obese and non-obese group

Salivary parameters (Mean ± S.D [ng/ml])	Non-obese group		Obese group		Mean difference	Standard error difference	p-value (independent t-test)
OPG	2.23 0.51	±	1.86 0.62	±	0.2470	0.122	0.015*
RANKL	0.033 0.02	±	0.045 0.026	±	-0.017	0.0052	0.048*

*Represents a statistically significant difference.

In the correlation analysis between RANKL/OPG and CAL, one observation from the obese group was removed because the value of CAL was too high compared to other observations. The analysis showed that there was a low but significant inverse correlation between OPG levels and CAL (r = -0.112, $p < 0.01$). There was no significant correlation between RANKL and CAL ($p > 0.05$) Scatterplot (bivar) graph demonstrating statistical correlation between salivary OPG (*Y*-axis) and periodontal parameter CAL (*X*-axis) in Figure 1 and salivary RANKL (*Y*-axis) and CAL (*X*-axis) in Figure 1 Black dots represent subjects in both groups.

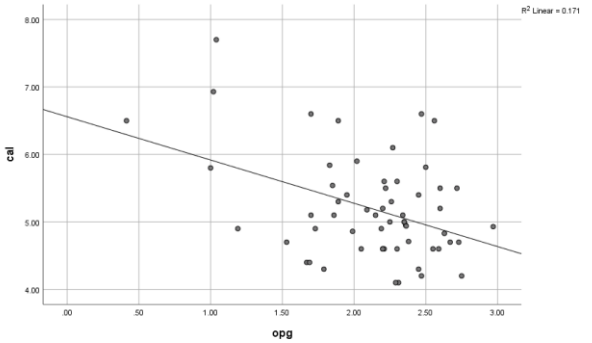

Figure 1. Scatterplot graph between OPG and CAL

In the present work, we assess clinical periodontal factors and salivary proteins RANKL and OPG in obese patients with periodontitis. A comparison of the outcomes for obese patients with periodontitis was done against non-obese patients with periodontitis. The variation in periodontal parameters OPG and RANKL for both groups and the correlation between CAL and OPG was assessed. The study was conducted for eight months (July 2019 to February 2020) at the Hospital Universiti Sains Malaysia. To the best of our knowledge, this is the first work which makes a comparison of the levels of salivary proteins RANKL and OPG among obese and non-obese subjects with periodontitis along with the periodontal parameters.

For this work, a total of 60 patients with chronic periodontitis were enrolled. The patients were split into two groups, with 30 patients in every group. Group 1

comprised non-obese patients with periodontitis and group 2 had obese patients with periodontitis. The mean age of patients was 44.06 ± 9.05 in group 1 and 48.86 ± 8.94 years in group 2. The mean age of patients with periodontitis was 45.2 ± 2.2 years as per the annual report of the National Health and Nutrition Examination Survey on prevalence and severity of periodontitis in the United States, which is consistent with a study (Eke et al., 2015).

According to the 2010 Malaysian National Health Survey, the 45-54 years age group tends to have deeper periodontal pockets and gingival health worsens as age rises (Mohd Dom et al., 2016). Aging is linked to the loss of periodontal attachment and bone (Huttner et al., 2009). According to a study, there is acute periodontal disease and higher plaque development in elderly individuals in comparison to younger people (Abdellatif & Burt, 1987). It has been indicated that cumulative tissue destruction in the aged is the reason behind the acute periodontal disease (Genco, 1996). The cumulative tissue destruction could be because of plaque related periodontitis and trauma from tooth brushing (Garg et al., 2013).

In this work, group 1 comprised 16 females and 14 males and group 2 comprised 18 females and 12 males. Both groups had more female patients with periodontitis. A 2010 national health survey conducted in Malaysia indicated that Malaysian females (55.7%) had more periodontal problems in comparison with Malaysian males (Mohd Dom et al., 2016). According to Mohd Dom *et al.*, the rise in periodontal disease is because of urbanisation and espousal of new habits like high-fat and high-sugar diet, smoking, and stress-inducing routines (Mohd-Dom et al., 2013). Another study noted that in Malaysia, females are remarkably more obese (36.7%, CI; 35.4-37.8) than males (30.4%, CI; 29.1-31.7) ($p<0.001$) (Yasin, Daher, Nasir, Ramli, Miskan, Keat, Razak, Krishnapillai, Ariffin, Hamid, et al., 2012).

According to 2013 statistics (Ng et al., 2014), obesity appears to be rising globally in advanced as well as emerging nations, and the prevalence of obesity is greater in women than in men in emerging nations. In adults, the prevalence of obesity has risen by 50% in females in Libya, Kuwait, Kiribati, Qatar, Tonga, and the Federated States of Micronesia (Ng et al., 2014). Globally, females are 3 percent more likely to be obese or overweight compared to males. In the United States, females are 3.3 percent more likely to be compared to males (Hallam et al., 2016). The differences in food cravings could be one of the reasons for obesity in men and women. According to research, females have greater appetite for sweet foodstuffs like pastries, chocolates, and ice creams, whereas men crave more for savoury foodstuffs like fish, meat, and eggs (Weingarten & Elston, 1991; Zellner et al., 1999).

Most subjects in this work were Malay (83.4%) and the remaining were Chinese (16.6%). There were 25 Malays and 5 Chinese in both sets. There is a difference in ethnicity because the Kelantan state exhibits a high density of Malay populace (95% of the total populace in this Malaysian state) and the Chinese community makes around 3.8%, making it the second highest ethnicity (Swee-Hock, 2015). In our work, it was noted that Malays was more obese compared to the Chinese. In an earlier research carried out in Malaysia, the results indicated that obesity was

highest in Malay, followed by Indians, and it was the lowest among Chinese (Yasin, Daher, Nasir, Ramli, Miskan, Keat, Razak, Krishnapillai, Ariffin, & Hamid, 2012). According to another research, the Malay populace has a greater risk of obesity compared to the Chinese populace in Malaysia (Richard A Dunn et al., 2012). The main reasons impacting BMI distribution in various ethnic groups in Malaysia might be diet and the socioeconomic status (SES) (Richard A. Dunn et al., 2012). Research has proved that there is a rise in obesity in emerging nations and in populaces with lower SES. This could be due to the lower degree of health-linked knowledge and education, and challenges in acquiring high-quality foodstuffs (like vegetables, fruits, and whole-grain cereals), as well as dearth of exercise (Monteiro et al., 2004).

In the present work, post statistical analysis, a significant difference is observed in the periodontal parameters of CAL and PPD between group 1 and group 2. The mean PPD for group 2 (5.2 ± 0.67) was significantly greater as against group 1 (4.6 ± 0.61). The mean CAL for group 2 (5.6 ± 1.21) was significantly greater as against group 1 (4.8 ± 0.64). The observations specify that subjects with obesity have higher likelihoods of periodontal destruction. Periodontitis is a disease that causes inflammation of the connective tissue that supports the bones neighbouring the teeth. Several studies concerning periodontitis have indicated that the disease causes higher periodontal pocket creation and also clinical attachment loss (Albandar, 1993; Botero et al., 2007; Queiroz et al., 2008). Research conducted concerning patients having periodontitis and healthy subjects indicated that the individuals suffering from periodontitis had higher clinical attachment loss and higher periodontal pocket formation (Balli et al., 2016; Queiroz et al., 2008).

Numerous studies suggest obesity being correlated with periodontal conditions (Chaffee & Weston, 2010; Yousef Saleh Khader et al., 2009; Östberg et al., 2009). Research concerning young adults from Iran was conducted and the periodontal condition of normal and obese patients was compared. It was found that obese individuals had a significantly higher rate of CAL and PPD compared to the others (Sarlati et al., 2008). Another research also indicated a connection between obesity and periodontal disease (Y. S. Khader et al., 2009). Research conducted concerning healthy and obese subjects indicated that obese subjects had higher CAL and PPD as compared to the to healthy subjects (Suvan et al., 2015). Periodontitis-caused PPD causes inflammation of the periodontal tissue, which is responsible for higher penetrability of the periodontal instrument (Listgarten, 1980). Research indicates that inflammation level and age are determinants of CAL occurrence. It was noted that after the age of 40, the chances of having CAL significantly increase (Schätzle et al., 2003).

Plaque score for both the groups (Group 1 and 2) was compared and a significant difference was observed. The plaque score was determined by calculating the affected sites as a fraction of the total number of sites. The results were presented using the plaque score. Group 2 had a mean plaque score of 70.5 ± 17.3 that was significantly higher compared to the score for Group 1 (60.9 ± 18.8). Dental plaque affects the generation of gingival inflammation and causing periodontitis (Seneviratne et al., 2011). Plaque is a film comprising bacteria that sticks to the soft and hard tissues and is the primary aetiologic aspect of periodontal disease

(Fine, 1988). Prakash *et al.* (2012) suggested higher PS for periodontitis patients compared to healthy individuals. At the same time, PS is directly related with periodontal disease severity (Prakash et al., 2012). Research concerning healthy and obese individuals indicated statistically significant increase in PS in obesity (Franchini et al., 2011). Research conducted in Turkey also indicated statistically significant increase in PS values for obese individuals compared to their non-obese counterparts (Buduneli et al., 2014).

Inadequate oral hygiene leads to higher PS, which causes higher bacterial growth and increased severity of periodontal conditions (Seneviratne et al., 2011). Some researchers opine that inadequate knowledge of oral health, improper oral hygiene and lack of healthy lifestyle concerning obese individuals leads to periodontal conditions (Saito & Shimazaki, 2007; Ylöstalo et al., 2008). Dental plaque consists of several substances that are produced by bacteria. These substances cause the host cells to release inflammatory mediators (Page, 1991) as a consequence of which there is local inflammation and an associated decrease in connective tissue (Bernimoulin, 2003). In the context of the two groups, there was no statistically significant difference between the gingival bleeding index (GBI). Group 1 had a mean GBI of 48.7 ± 15.9, while for Group 2, the score was 59.7 ± 25.5, thereby indicating a higher score for Group 2. The clinical diagnosis of periodontitis considers GBI as a crucial indicator. If bleeding is observed during probing, it indicates inflammation of the periodontal tissues (Lang et al., 1986). The severity of the disease at the periodontal sites is indicated by the GBI.

Research indicates that individuals suffering from periodontitis, as compared to their healthier counterparts, have higher bleeding upon probing (Haffajee et al., 1998; Queiroz et al., 2008; Teles et al., 2009). Research conducted in Turkey presented similar observations where probing-associated bleeding was higher for obese individuals (Buduneli et al., 2014). Furthermore, a study by (Zuza et al., 2016) indicates that among patients having periodontitis, those having obesity had comparatively higher bleeding upon probing. Gingival bleeding is an indication of inflammation and is associated to chronic plaque. Individuals lacking adequate oral hygiene are susceptible to higher plaque accumulation and gingival bleeding (Löe et al., 1965). In obese patients, the tissues affected due to periodontal inflammation lead to the production of inflammatory markers (Pischon et al., 2007). Pro-inflammatory cytokines such as TNF-a, adipokines (leptin, adiponectin), IL-1, and IL-6 are associated with an increase in BMI and may result in periodontal breakdown and gingival hyperinflammation (Scorzetti et al., 2013). Research indicated that inflammation of the gingival tissue caused higher bleeding on probing compared to healthy gingival tissue (Greenstein et al., 1981).

In this study, analysis was carried out on the two proteins OPG and RANKL that are responsible for osteoclastogenesis. RANKL is found to have a relationship with osteoclastic activation and differentiation, and persistent increased levels of RANKL can cause bone resorption in periodontitis (Mogi et al., 2004). As per the results, the mean RANKL for group 2 (0.045 ± 0.026 ng/ml) was considerably more than that of group 1 (0.033 ± 0.02 ng/ml). At the same time, the protein level of RANKL for group 2 was also substantially higher than that of group 1. It

was further observed that the obese group had higher value of salivary RANKL and showed more severity in periodontal disease.

According to a study on obese mice conducted by Halade *et al.* (2011), it was observed that osteoclastogenesis was linked with a reduction of OPG and a rise of RANKL, which eventually becomes the cause of bone loss (Halade et al., 2011). It was further observed that obese human subjects had higher levels of RANKL in serum as against their non-obese counterparts (Corbo et al., 2019) and in GCF (Saloom et al., 2019). In the present study involving saliva, the results reveal identical levels of RANKL. In several studies carried out on subjects related to periodontitis, it was found that the salivary RANKL levels were considerably higher as against the levels observed with a healthy group (Buduneli et al., 2008; Ochanji et al., 2017; Tobón-Arroyave et al., 2012). Likewise, in comparison to the healthy group, the levels of RANKL in GCF samples and serum of periodontitis patients were found to be relatively high (Sufaru et al., 2016).

Upregulating proinflammatory cytokines such as IL-6 and TNF-α may be responsible for the increased bone resorption in people with obesity (Cao, 2011; Pfeilschifter et al., 2002). These proinflammatory cytokines have the potential to stimulate osteoclast activity by enabling control of the RANKL/RANK/OPG pathway (Khosla, 2001). As reported by Ochanji et al. (2017), as a result of the building up of plaque, there occurs an enhancement in the local periodontal tissue inflammation. Consequently, various cellular productions, and T and B lymphocytes are augmented as well. The activated T and B lymphocytes are regarded as the key cellular sources of RANKL. Hence, a surge in the production of RANKL leads to an increase in the relative RANKL/OPG ratio, instigating tissue destruction and causing more rapid clinical attachment loss with increased periodontal pocket depth and more intense bone loss (Ochanji et al., 2017).

As per the study, there is a noteworthy difference in the levels of salivary OPG for both the groups. It was found that the mean level of the salivary protein OPG for group 1 (2.23 ± 0.51 ng/ml) was considerably higher than that of group 2 (1.86 ± 0.62 ng/ml). Also, the level of OPG was higher for group 1 as against that of group 2. OPG functions as a decoy receptor by binding with RANKL and preventing RANK activation, and therefore, in turn prevents the activation and differentiation of the osteoclast (Lacey et al., 1998). In periodontitis patients, it is found that low levels of OPG can cause osteoclastic bone resorption (Mogi et al., 2004). For the obese group, the circulating OPG levels were found to be lower than that of the normal-weight group (Ashley et al., 2011). As concluded by Halade *et al.*, there were lower levels of OPG in obese mice as against those in non-obese mice (Halade et al., 2011). This is according to the expectations because OPG functions as an antagonist to the RANKL, where lower concentrations of OPG can result in bone resorption.

A study conducted in recent times showed that the serum levels of OPG were high in healthy individuals, while the same levels were low in the obese group (Corbo et al., 2019). Other studies conducted on OPG levels indicate that there are substantially low levels of OPG in periodontitis as against those in healthy individuals (Crotti et al., 2003; Dereka et al., 2010). The results imply that there is downregulation of OPG levels and upregulation of RANKL levels in periodontitis

(Bostanci et al., 2007). It is observed that OPG is an antagonist to the RANKL and low levels of OPG can cause bone resorption (Belibasakis & Bostanci, 2012). These observations are in agreement with our findings, which indicate that OPG levels are strongly present in non-obese individuals with periodontitis as against obese individuals with periodontitis. In this study, the OPG levels in the saliva reveal a major correlation with the periodontal parameter CAL. According to Buduneli *et al.*, there exists low levels of OPG and high levels of RANKL in periodontitis patients, along with a substantial correlation between CAL and OPG. Indicating that OPG may be associated with periodontal tissue attachment loss (Buduneli et al., 2008).

Conclusion

The values for the periodontal parameters (except gingival bleeding index) in obese individuals with periodontitis were significantly higher as compared to the patients with periodontitis. There was significant increase in the average salivary RANKL levels of obese periodontitis patients compared to the periodontitis patients. OPG level were higher in non-obese periodontitis patients as compared to obese periodontitis patients. Increase RANKL to OPG levels are responsible for net bone loss and degradation in advanced periodontal lesion. There is negative correlation between OPG and periodontal parameter CAL.

Acknowledgments

This work was supported by the Research Fund provided by Universiti Sains Malaysia Research University Grant (1001/PPSG/8012353).

References

Abdellatif, H., & Burt, B. (1987). An epidemiological investigation into the relative importance of age and oral hygiene status as determinants of periodontitis. Journal of Dental Research, 66(1), 13-18.

Albandar, J. M. (1993). Juvenile periodontitis–pattern of progression and relationship to clinical periodontal parameters. Community dentistry and oral epidemiology, 21(4), 185-189.

Ashley, D. T., O'Sullivan, E. P., Davenport, C., Devlin, N., Crowley, R. K., McCaffrey, N., Moyna, N. M., Smith, D., & O'Gorman, D. J. (2011). Similar to adiponectin, serum levels of osteoprotegerin are associated with obesity in healthy subjects. Metabolism, 60(7), 994-1000.

Balli, U., Ongoz Dede, F., Bozkurt Dogan, S., Gulsoy, Z., & Sertoglu, E. (2016). Chemerin and interleukin-6 levels in obese individuals following periodontal treatment. Oral Diseases, 22(7), 673-680.

Behfarnia, P., Saied-Moallemi, Z., Javanmard, S. H., & Naseri, R. (2016). Serum, saliva, and GCF concentration of RANKL and osteoprotegerin in smokers versus nonsmokers with chronic periodontitis. Advanced Biomedical Research, 5.

Belibasakis, G. N., & Bostanci, N. (2012). The RANKL-OPG system in clinical periodontology. Journal of Clinical Periodontology, 39(3), 239-248.

Bernimoulin, J. P. (2003). Recent concepts in plaque formation. Journal of Clinical Periodontology, 30, 7-9.

Bostanci, N., İlgenli, T., Emingil, G., Afacan, B., Han, B., Töz, H., Atilla, G., Hughes, F. J., & Belibasakis, G. N. (2007). Gingival crevicular fluid levels of RANKL and OPG in periodontal diseases: implications of their relative ratio. Journal of Clinical Periodontology, 34(5), 370-376.

Botero, J. E., Parra, B., Jaramillo, A., & Contreras, A. (2007). Subgingival human cytomegalovirus correlates with increased clinical periodontal parameters and bacterial coinfection in periodontitis. Journal of Periodontology, 78(12), 2303-2310.

Buduneli, N., Bıyıkoğlu, B., İlgenli, T., Buduneli, E., Nalbantsoy, A., Saraç, F., & Kinane, D. F. (2014). Is obesity a possible modifier of periodontal disease as a chronic inflammatory process? A case–control study. Journal of Periodontal Research, 49(4), 465-471.

Buduneli, N., Bıyıkoğlu, B., Sherrabeh, S., & Lappin, D. F. (2008). Saliva concentrations of RANKL and osteoprotegerin in smoker versus non-smoker chronic periodontitis patients. Journal of Clinical Periodontology, 35(10), 846-852.

Cao, J. J. (2011). Effects of obesity on bone metabolism. Journal of Orthopaedic Surgery and Research, 6(1), 30.

Chaffee, B. W., & Weston, S. J. (2010). Association between chronic periodontal disease and obesity: a systematic review and meta-analysis. Journal of Periodontology, 81(12), 1708-1724.

Corbo, F., Brunetti, G., Crupi, P., Bortolotti, S., Storlino, G., Piacente, L., Carocci, A., Catalano, A., Milani, G., & Colaianni, G. (2019). Effects of sweet cherry polyphenols on enhanced osteoclastogenesis associated with childhood obesity. Frontiers in Immunology, 10, 1001.

Crotti, T., Smith, M. D., Hirsch, R., Soukoulis, S., Weedon, H., Capone, M., Ahern, M. J., & Haynes, D. (2003). Receptor activator NF κB ligand (RANKL) and osteoprotegerin (OPG) protein expression in periodontitis. Journal of Periodontal Research, 38(4), 380-387.

Cutando, A., López-Valverde, A., de Diego, R. G., de Vicente, J., Reiter, R., Fernández, M. H., & Ferrera, M. J. (2014). Effect of topical application of melatonin to the gingiva on salivary osteoprotegerin, RANKL and melatonin levels in patients with diabetes and periodontal disease. Odontology, 102(2), 290-296.

da Silva-Boghossian, C. M., do Souto, R. M., Luiz, R. R., & Colombo, A. P. V. (2011). Association of red complex, A. actinomycetemcomitans and non-oral bacteria with periodontal diseases. Archives of Oral Biology, 56(9), 899-906.

Dahiya, P., Kamal, R., & Gupta, R. (2012). Obesity, periodontal and general health: Relationship and management. Indian Journal of Endocrinology and Metabolism, 16(1), 88.

Dereka, X. E., Markopoulou, C. E., Fanourakis, G., Tseleni-Balafouta, S., & Vrotsos, I. A. (2010). RANKL and OPG mRNA level after non-surgical periodontal treatment. Inflammation, 33(3), 200-206.

Dunn, R. A., Tan, A. K. G., & Nayga, R. M. (2012). Obesity inequality in Malaysia: decomposing differences by gender and ethnicity using quantile regression. Ethnicity & Health, 17(5), 493-511.

Dunn, R. A., Tan, A. K., & Nayga, R. M. (2012). Obesity inequality in Malaysia: decomposing differences by gender and ethnicity using quantile regression. Ethnicity & Health, 17(5), 493-511.

Eke, P. I., Dye, B. A., Wei, L., Slade, G. D., Thornton-Evans, G. O., Borgnakke, W. S., Taylor, G. W., Page, R. C., Beck, J. D., & Genco, R. J. (2015). Update on prevalence of periodontitis in adults in the United States: NHANES 2009 to 2012. Journal of Periodontology, 86(5), 611-622.

Fine, D. (1988). Mouthrinses as adjuncts for plaque and gingivitis management. A status report for the American Journal of Dentistry. American Journal of Dentistry, 1(6), 259-263.

Franchini, R., Petri, A., Migliario, M., & Rimondini, L. (2011). Poor oral hygiene and gingivitis are associated with obesity and overweight status in paediatric subjects. Journal of clinical Periodontology, 38(11), 1021-1028.

Frodge, B. D., Ebersole, J. L., Kryscio, R. J., Thomas, M. V., & Miller, C. S. (2008). Bone remodeling biomarkers of periodontal disease in saliva. Journal of Periodontology, 79(10), 1913-1919.

Fuller, K., Wong, B., Fox, S., Choi, Y., & Chambers, T. J. (1998). TRANCE is necessary and sufficient for osteoblast-mediated activation of bone resorption in osteoclasts. The Journal of Experimental Medicine, 188(5), 997-1001.

Garg, A., Bhickta, S., Gupta, R. K., & Sharma, A. (2013). Aging and Periodontium. Dental Journal of Advance Studies, 1(01), 026-029.

Genco, R. J. (1996). Current view of risk factors for periodontal diseases. Journal of Periodontology, 67, 1041-1049.

Greenstein, G., Caton, J., & Polson, A. (1981). Histologic characteristics associated with bleeding after probing and visual signs of inflammation. Journal of Periodontology, 52(8), 420-425.

Haffajee, A., Cugini, M., Tanner, A., Pollack, R., Smith, C., Kent Jr, R., & Socransky, S. (1998). Subgingival microbiota in healthy, well-maintained elder and periodontitis subjects. Journal of Clinical Periodontology, 25(5), 346-353.

Halade, G. V., El Jamali, A., Williams, P. J., Fajardo, R. J., & Fernandes, G. (2011). Obesity-mediated inflammatory microenvironment stimulates osteoclastogenesis and bone loss in mice. Experimental Gerontology, 46(1), 43-52.

Hallam, J., Boswell, R. G., DeVito, E. E., & Kober, H. (2016). Focus: sex and gender health: gender-related differences in food craving and obesity. The Yale journal of Biology and Medicine, 89(2), 161.

Haslam, D., & James, W. (2005). Obesity Lancet. 366: 1197–1209. Google Scholar, Crossref, Medline.

Huttner, E. A., Machado, D. C., De Oliveira, R. B., Antunes, A. G. F., & Hebling, E. (2009). Effects of human aging on periodontal tissues. Special Care in Dentistry, 29(4), 149-155.

Khader, Y. S., Bawadi, H. A., Haroun, T. F., Alomari, M., & Tayyem, R. F. (2009). The association between periodontal disease and obesity among adults in Jordan. Journal of Clinical Periodontology, 36(1), 18-24.

Khader, Y. S., Bawadi, H. A., Haroun, T. F., Alomari, M., & Tayyem, R. F. (2009). The association between periodontal disease and obesity among adults in Jordan. Journal of Clinical Periodontology, 36(1), 18-24.

Khosla, S. (2001). Minireview: The opg/rankl/rank system. Endocrinology, 142(12), 5050-5055.

Lacey, D., Timms, E., Tan, H.-L., Kelley, M., Dunstan, C., Burgess, T., Elliott, R., Colombero, A., Elliott, G., & Scully, S. (1998). Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. Cell, 93(2), 165-176.

Lang, N. P., Joss, A., Orsanic, T., Gusberti, F. A., & Siegrist, B. E. (1986). Bleeding on probing. A predictor for the progression of periodontal disease? Journal of Clinical Periodontology, 13(6), 590-596.

Listgarten, M. (1980). Periodontal probing: what does it mean? Journal of Clinical Periodontology, 7(3), 165-176.

Löe, H., Theilade, E., & Jensen, S. B. (1965). Experimental gingivitis in man. The Journal of Periodontology, 36(3), 177-187.

Marton, I., & Kiss, C. (2000). Protective and destructive immune reactions in apical periodontitis. Oral Microbiology and Immunology: Mini-review, 15(3), 139-150.

Mogi, M., Otogoto, J., Ota, N., & Togari, A. (2004). Differential expression of RANKL and osteoprotegerin in gingival crevicular fluid of patients with periodontitis. Journal of Dental Research, 83(2), 166-169.

Mohd Dom, T. N., Ayob, R., Abd Muttalib, K., & Aljunid, S. M. (2016). National economic burden associated with management of periodontitis in Malaysia. International Journal of Dentistry, 2016.

Mohd-Dom, T. N., Abdul-Muttalib, K., Ayob, R., Lan, Y. S., Mohd-Asadi, A. S., Abdul-Manaf, M. R., & Aljunid, S. M. (2013). Periodontal status and provision of periodontal services in Malaysia: trends and way forward. Journal of Public Health Medicine, 13(2), 38-47.

Monteiro, C. A., Moura, E. C., Conde, W. L., & Popkin, B. M. (2004). Socioeconomic status and obesity in adult populations of developing countries: a review. Bulletin of the World Health Organization, 82, 940-946.

Navazesh, M. (1993). Methods for collecting saliva. Annals of the New York Academy of Sciences, 694(1), 72-77.

Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C., Mullany, E. C., Biryukov, S., Abbafati, C., & Abera, S. F. (2014). Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. The Lancet, 384(9945), 766-781.

Ochanji, A., Matu, N., & Mulli, T. (2017). Association of salivary RANKL and osteoprotegerin levels with periodontal health. Clinical and Experimental Dental Research, 3(2), 45-50.

Organization, W. H. (2000). *Obesity: preventing and managing the global epidemic*. World Health Organization.

Östberg, A.-L., Nyholm, M., Gullberg, B., Råstam, L., & Lindblad, U. (2009). Tooth loss and obesity in a defined Swedish population. Scandinavian Journal of Public Health, 37(4), 427-433.

Page, R. C. (1991). The role of inflammatory mediators in the pathogenesis of periodontal disease. Journal of Periodontal Research, 26(3), 230-242.

Pfeilschifter, J., Köditz, R., Pfohl, M., & Schatz, H. (2002). Changes in proinflammatory cytokine activity after menopause. Endocrine Reviews, 23(1), 90-119.

Pischon, N., Heng, N., Bernimoulin, J.-P., Kleber, B.-M., Willich, S., & Pischon, T. (2007). Obesity, inflammation, and periodontal disease. Journal of Dental Research, 86(5), 400-409.

Prakash, S., Dhingra, K., & Priya, S. (2012). Similar hematological and biochemical parameters among periodontitis and control group subjects. European Journal of Dentistry, 6(3), 287.

Queiroz, A. C. d., Taba Jr, M., O'Connell, P. A., Nóbrega, P. B. d., Costa, P. P., Kawata, V. K. d. S., Trevisan, G. L., Novaes Jr, A. B., Souza, S. L. S. d., & Palioto, D. B. (2008). Inflammation markers in healthy and periodontitis patients: a preliminary data screening. Brazilian Dental Journal, 19(1), 3-8.

Saito, T., & Shimazaki, Y. (2007). Metabolic disorders related to obesity and periodontal disease. Periodontology 2000, 43(1), 254-266.

Saloom, H. F., Carpenter, G. H., & Cobourne, M. T. (2019). A cross-sectional cohort study of gingival crevicular fluid biomarkers in normal-weight and obese subjects during orthodontic treatment with fixed appliances. The Angle Orthodontist, 89(6), 930-935.

Sarlati, F., Akhondi, N., Ettehad, T., Neyestani, T., & Kamali, Z. (2008). Relationship between obesity and periodontal status in a sample of young Iranian adults. International Dental Journal, 58(1), 36-40.

Schätzle, M., Löe, H., Bürgin, W., Ånerud, Å., Boysen, H., & Lang, N. P. (2003). Clinical course of chronic periodontitis: I. Role of gingivitis. Journal of Clinical Periodontology, 30(10), 887-901.

Scorzetti, L., Marcattili, D., Pasini, M., Mattei, A., Marchetti, E., & Marzo, G. (2013). Association between obesity and periodontal disease in children. European Journal of Paediatric Dentistry, 14(3), 181-184.

Seneviratne, C. J., Zhang, C. F., & Samaranayake, L. P. (2011). Dental plaque biofilm in oral health and disease. Chinese Journal of Dental Research, 14(2), 87.

Simonet, W., Lacey, D., Dunstan, C., Kelley, M., Chang, M.-S., Lüthy, R., Nguyen, H., Wooden, S., Bennett, L., & Boone, T. (1997). Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. Cell, 89(2), 309-319.

Sufaru, I.-G., Solomon, S.-M., Pasarin, L., Martu-Stefanache, M. A., Oanta, A. C., Martu, I., Ciocan-Pendefunda, A., & Martu, S. (2016). Study regarding the quantification of RANKL levels in patients with chronic periodontitis and osteoporosis. Romanian Journal of Oral Rehabilitation, 8(4), 42-46.

Suvan, J. E., Petrie, A., Nibali, L., Darbar, U., Rakmanee, T., Donos, N., & D'Aiuto, F. (2015). Association between overweight/obesity and increased risk of periodontitis. Journal of Clinical Periodontology, 42(8), 733-739.

Swee-Hock, S. (2015). *The population of Malaysia* (Vol. 514). Institute of Southeast Asian Studies.

Teles, R., Likhari, V., Socransky, S., & Haffajee, A. (2009). Salivary cytokine levels in subjects with chronic periodontitis and in periodontally healthy individuals: a cross-sectional study. Journal of Periodontal Research, 44(3), 411-417.

Tobón-Arroyave, S. I., Isaza-Guzmán, D. M., Restrepo-Cadavid, E. M., Zapata-Molina, S. M., & Martínez-Pabón, M. C. (2012). Association of salivary levels of the bone remodelling regulators sRANKL and OPG with periodontal clinical status. Journal of Clinical Periodontology, 39(12), 1132-1140.

Weingarten, H. P., & Elston, D. (1991). Food cravings in a college population. Appetite, 17(3), 167-175.

Yasin, M. M., Daher, A. M., Nasir, N. M., Ramli, A. S., Miskan, M., Keat, N. K., Razak, S. A., Krishnapillai, A. D. S., Ariffin, F., & Hamid, H. A. (2012). 220 Overweight And Obesity In Malaysia: An Epidemiology Survey. Journal of Hypertension, 30, e67.

Yasin, M. M., Daher, A. M., Nasir, N. M., Ramli, A. S., Miskan, M., Keat, N. K., Razak, S. A., Krishnapillai, A. D. S., Ariffin, F., Hamid, H. A., Majid, F. A.,

Bakar, N. A., Khan, N. A. M. N., Ismail, T. S. T., Nawawi, H. M., Yusoff, K., & Investigators, f. t. C. (2012). 220 overweight and obesity in malaysia: an epidemiology survey. Journal of Hypertension, 30, e67.

Ylöstalo, P., Suominen-Taipale, L., Reunanen, A., & Knuuttila, M. (2008). Association between body weight and periodontal infection. Journal of Clinical Periodontology, 35(4), 297-304.

Zellner, D., Garriga-Trillo, A., Rohm, E., Centeno, S., & Parker, S. (1999). Food liking and craving: A cross-cultural approach. Appetite, 33(1), 61-70.

Zuza, E. P., Barroso, E. M., Fabricio, M., Carrareto, A. L. V., Toledo, B. E., & Pires, J. R. (2016). Lipid profile and high-sensitivity C-reactive protein levels in obese and non-obese subjects undergoing non-surgical periodontal therapy. Journal of Oral Science, 58(3), 423-430.