Evaluation of plasma reactive oxygen metabolites levels in obese subjects with periodontal disease

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ABSTRACT

Background: Obesity represents the systemic condition capable of influencing the onset and progression of periodontal disease. Obesity is associated with oxidative stress. Plasma level of reactive oxidative metabolites (ROMs) is measured as an indicator of oxidative stress in the body. The aim of this study is to assess and compare the plasma ROM levels in obese subjects with healthy and inflammatory periodontal status.

Materials and Methods: Sixty subjects selected were grouped as 15 obese or overweight subjects with generalized chronic periodontitis, 15 obese or overweight subjects with generalized chronic gingivitis, 15 obese or overweight subjects with healthy periodontium, and 15 nonobese and healthy periodontium. The clinical periodontal parameters such as plaque index, gingival index, probing pocket depth, and clinical attachment level were measured. Blood samples were obtained to measure the plasma levels of ROM.

Result and Conclusion: In this study, obese subjects with chronic periodontitis (Group I) had mean plasma ROM levels (442.3 ± 15.65 Carratelli unit [CARR U]) showing 100% subjects with high oxidative stress. Obese subjects with chronic gingivitis (Group II) had mean plasma ROM levels (358.7 ± 20.61 CARR U) indicating 86.7% subjects with oxidative stress. Obese subjects with healthy periodontium (Group III) had 46.7% subjects with slight oxidative stress, and the mean ROM level was 320.2 ± 17.57. Nonobese subjects with healthy periodontium (Group IV) had 80% of subjects with normal oxidative stress and the mean plasma ROM level was 296.9 ± 20.35 CARR U. The intra- and inter-group comparison showed significant difference (P < 0.001). From our study, we report that obese subjects with periodontitis have more oxidative stress compared to obese subjects with healthy periodontium.

Key words: Obesity, oxidative stress, periodontal disease, reactive oxygen metabolites

Obesity is one of the most serious metabolic disorders, affecting 5% of India’s population in this 21st century. Obesity is considered to be a worldwide epidemic, which is related to secondary morbidities such as diabetes, cardiovascular disease, and cancer. Obesity is the condition with excess amount of body fat in proportion to lean body mass to the extent that health is impaired.1 The World Health Organization defines obesity as a body mass index (BMI) more than 30 and defines overweight as with a BMI of more than 25.2 Periodontitis has been described as an irreversible, cumulative condition, initiated by bacteria but propagated by host factors.3 Susceptibility to development of periodontitis may increase owing to the interaction of environmental, acquired, and genetic risk factors that modify the host response to the putative pathogenic microbes.4 The majority of tissue destruction in periodontitis is considered to be the result of an aberrant inflammatory/immune response to microbial plaque and to prolonged release of neutrophil enzymes and reactive oxygen species. Reactive oxygen species released from

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neutrophils and other immune cells lead to oxidative damage in biomolecules which triggers morphofunctional changes in the endothelium of the vessels and therefore in the periodontal tissues that nourish them.\textsuperscript{[5]} Reactive oxygen species are oxygen-derived free radicals that can disrupt cellular proteins, nucleic acids and membrane lipids and also cause depolymerization of matrix components such as collagen, hyaluronan, and proteoglycans.\textsuperscript{[6]}

Obesity represents the systemic condition capable of influencing the onset and progression of periodontal disease. Obesity also induces oxidative stress due to the presence of excessive adipose tissue. The adipocytes and preadipocytes have been considered as a source of proinflammatory cytokines such as tumor necrosis factor-\(\alpha\), interleukins-1 and interleukin-6. These cytokines are potent stimulators for the production of reactive oxygen and nitrogen by monocytes and macrophages. Adipose tissue also secretes angiotensin II, which stimulates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity. NADPH oxidase comprises the major route for reactive oxygen species production in adipocytes, leading to systemic increase in reactive oxygen species and this may lead to progression of periodontal inflammation.\textsuperscript{[7]} Reactive oxygen species when chronically produced can cause oxidative stress within tissues and result in direct damage to cells and the extracellular matrix. A method of measuring plasma level of reactive oxidative metabolites (ROMs) has been developed for evaluation of oxidative stress in the body.\textsuperscript{[8]} ROM can help to determine the total oxidative status in serum. This analysis measures the whole oxidant capacity of blood against \(N, N\)-diethyl-para-phenylenediamine in acidic buffer. The main components of ROM are hydroperoxides, which are intermediate oxidative products of lipids, peptides, and amino acids. These hydroperoxides in the plasma are relatively stable compared to their parent free radicals; therefore, their levels can be detected. This test is based on the reaction of plasma samples with transition metal ions to form alkoxy and peroxy radicals.

The aim of this study is to assess the plasma ROM levels in obese subjects with healthy and inflammatory periodontal status and to compare plasma ROM levels in obese or overweight subjects with periodontal inflammatory status.

MATERIALS AND METHODS

The present case–control study was carried out at the Department of Periodontics, Thaimoogambigai Dental College and Hospital, Chennai, Tamil Nadu, India, which took place from July 2014 to November 2014. The study protocol was approved by the ethical committee of Dr. MGR Educational and Research University, Chennai, India. Before enrollment, a written informed consent was obtained from the patients. Sixty subjects both males and females aged 20–45 years, out of which 15 obese or overweight subjects with Generalized chronic periodontitis, 15 obese or overweight subjects with generalized chronic gingivitis, 15 obese or overweight subjects with healthy periodontium, and 15 nonobese and healthy periodontium were enrolled in the study. Overweight and obesity was defined by subjects having BMI in a range of >25 kg/m\(^2\) and waist circumference of >90 cm (men) and >80 cm (women) and categorized into healthy periodontium, chronic gingivitis, and chronic periodontitis, based on gingival index and clinical attachment level (CAL). Subjects with healthy periodontium had gingival index score of 0. Generalized chronic gingivitis was defined by having the gingival index score of <1 and without clinical attachment loss. Generalized chronic periodontitis was defined by having clinical attachment loss of 3–5 mm in more than 30% of sites. Exclusion criteria included pregnancy, previous or current smokers, menopause, cardiovascular disorders, thyroid disorders, diabetes mellitus patients, use of antioxidant supplements, patients on long-term steroid medications, anti-inflammatory or antibiotics within previous 3 months and underwent periodontal treatment in the past 6 months. A null hypothesis of no difference between control group and test group was set at the beginning of the study.

The clinical periodontal parameters such as plaque index, gingival index, probing pocket depth (PPD), and CAL were measured. Plaque index was recorded at four sites (mesiobuccal, midbuccal, distobuccal, and midpalatal sites) around each tooth.\textsuperscript{[9]} Four gingival areas of the tooth (facial, mesial, distal, and lingual) were assessed for gingival index. PPD and CAL were recorded at six sites per tooth and measured in millimeters. CAL was measured as the distance in millimeters from the cementoenamel junction to the bottom of the periodontal pocket. BMI was calculated by dividing the weight of an individual in kilograms by height of an individual in meters squared.\textsuperscript{[2]} Blood samples were obtained to measure the plasma levels of ROM.

Reactive oxidative metabolite detection from blood sample

About 2 ml of blood was drawn from the lateral aspect of the cubital vein with the needle and syringe. Transfer the blood to a heparinized test tube and then centrifuge at 3000 \(\times\) g for 5 min. Transfer plasma into Eppendorf tube. The measurements of plasma ROM level were performed using a spectrophotometer, according to previously reported methods.\textsuperscript{[10,11]} Twenty microliters of the plasma sample was mixed with 1 ml of the prepared acetate buffer and 20 \(\mu\)l of the chromogenic substrate and incubated for 5 min at 37°C. The density of the magenta color reflects the concentration of hydroperoxides in the blood, which is proportional to the quantity of ROMs. The measurements were expressed in Carratelli unit (CARR U). It has been established that 1 CARR U corresponds to 0.08 mg/dl hydrogen peroxide. According to the manufacturer’s instructions, a normal value for ROMs is between 250 and 300 CARR U in healthy
individuals.\textsuperscript{[12]} A value between 301 and 320 CARR U indicates a borderline condition of oxidative stress status, whereas a value above 320 CARR U indicates light oxidative stress. A value between 341 and 400 CARR U indicates oxidative stress and when the value is above 401 CARR U indicates high oxidative stress.

Statistical analysis

All statistical analyses were performed using SPSS software program. The normality tests Kolmogorov–Smirnov and Shapiro–Wilks test results showed that the variables follow normal distribution. To compare mean values between groups, one-way ANOVA was applied followed by Tukey’s honest significant difference post hoc tests for pairwise comparisons. Chi-square test is applied to compare proportions between groups. Significance was set at $P < 0.05$.

RESULTS

A total of sixty subjects (28 males and 32 females) were included in this study. The mean scores of plasma ROM levels in Groups I, II, III, and IV were 442.3 ± 15.6 CARR U, 358.7 ± 20.6 CARR U, 320.2 ± 17.2 CARR U, and 296.9 ± 20.3 CARR U, respectively. The difference in mean plasma ROM levels was statistically significant with the $P < 0.001$ [Table 1]. There were significant differences in mean plaque index, gingival index scores and CAL between 4 groups. The mean plasma ROM levels of obese subjects with chronic periodontitis (Group I) was higher compared to obese subjects with chronic gingivitis (Group II). The mean plasma ROM of obese subjects with healthy periodontium (Group III) was higher compared to nonobese subjects with healthy periodontium (Group IV) and the difference in mean plasma ROM levels were statistically significant [Table 2]. Table 3 and Figure 1 shows the percentage of subjects with different levels of oxidative stress in all the groups.

DISCUSSION

To the best of our knowledge, this is the first study performed to estimate and compare the levels of ROM in obese subjects with periodontally healthy and inflammatory periodontal status. Systemic oxidative stress, defined as a persistent imbalance between the production of highly reactive molecular species chiefly oxygen and nitrogen and antioxidant defenses, correlates with fat accumulation in humans and mice.\textsuperscript{[13,14]} Obesity-related elevations of free fatty acids cause oxidative stress by increasing mitochondrial uncoupling and beta oxidation, leading to the increased production of reactive oxygen species.\textsuperscript{[15]} The changes in the proinflammatory, immune responses, and oxidative stress associated with obesity may also contribute to their increased susceptibility to periodontal disease. Oxidative stress has been assessed by direct measurements of reactive oxygen species and enzymes which are involved in reactive oxygen species production or indirect measurements of oxidized products of lipids, proteins, or DNA. Reactive oxygen species and free radicals have a very short life making their direct measurement difficult. In this study, we used a simple method of evaluating the systemic oxidative stress that has been developed in order to measure the concentration of hydroperoxide induced by free radicals.\textsuperscript{[16]} It has been considered to be directly proportional to the quantity of ROMs indicating the level of oxidative stress throughout the body. Plasma ROM is considered to be a reliable indicator of oxidative status.

In our study, we found an increase in plasma ROM levels in obese subjects than nonobese subjects and also the plasma ROM levels increase in obese subjects with periodontal inflammatory status. We also observed that obese subjects with chronic periodontitis (Group I) had mean plasma ROM levels (442.3 ± 15.65 CARR U) showing 100% subjects with high oxidative stress. Obese subjects with chronic gingivitis (Group II) had mean plasma ROM levels (358.7 ± 20.61 CARR U) indicating 86.7% subjects with oxidative stress. Obese subjects with healthy periodontium (Group III)
had 46.7% subjects with slight oxidative stress, and the mean ROM level was $320.2 \pm 17.57$ CARR U. Nonobese subjects with healthy periodontium (Group IV) had 80% of subjects with normal oxidative stress and the mean plasma ROM level was $296.9 \pm 20.35$. The intra- and inter-group comparison showed statistically significant difference with a highly significant $P < 0.001$. The findings of our study are similar to Tomofuji et al., who reported that obesity-induced gingival oxidative stress with increasing serum reactive oxygen metabolites in rats and Terumi Kogawa in his study also reported that obese female students showed significantly higher oxidative stress.

Increased oxidative stress in obese subjects is due to increased production of adipokines in white adipose tissue, which induces the production of reactive oxygen species. It has also been proposed that oxidative stress in obesity is a response to hypoxia because of increased distance of the enlarged adipocytes from the vasculature. Furikawa et al. 2004 also suggested that increased oxidative stress in accumulated fat is an early indicator of metabolic syndrome and that redox state in adipose tissue is potential target for obesity-associated metabolic syndrome.

In obese subjects, periodontal inflammatory status also plays a role in increasing the oxidative stress. Obese subjects with generalized chronic periodontitis had increased mean plasma ROM levels compared to obese subjects with generalized chronic gingivitis which is in association with studies by Tamaki et al., where he has shown association of plasma oxidative status and CAL in patients in the maintenance phase of periodontal therapy. In another study, Tamaki et al. reported the effectiveness of nonsurgical periodontal treatment in improving the clinical parameters and reducing plasma ROM levels. There is evidence suggesting that changes in the proinflammatory and immune responses associated with obesity may contribute to their increased susceptibility to periodontal disease. From our study, we report that obese subjects with periodontitis have more oxidative stress compared to obese subjects with healthy periodontium. Excessive reactive oxygen species oxidizes nucleic acids, lipids, and proteins thereby contributing to tissue damage. Animal studies revealed that periodontal inflammation induces oxidative tissue damage in the aorta and liver with increasing serum reactive oxygen species. Periodontitis in association with obesity may injure various organs through increased reactive oxygen species in the blood.

**CONCLUSION**

Both periodontitis and obesity induce chronic inflammation and oxidative stress, thereby putting the individual at risk for systemic diseases. Therefore, it is possible that the reduction of circulating reactive oxygen species by the intervention of obesity and periodontitis offer clinical benefits in terms of reducing the risk for future systemic complications.

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**Conflicts of interest**

There are no conflicts of interest.

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