Comparative analysis of subgingival red complex bacteria in obese and normal weight subjects with and without chronic periodontitis

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INTRODUCTION

Obesity and overweight are considered as abnormal or excessive fat accumulation that may impair health and are associated as major risk factors for a number of chronic diseases, including diabetes, cardiovascular diseases, and other life-threatening diseases. The National Family Health Survey-3 stated that, the number of obese participants has doubled in India in the past 10 years. As per this survey, 28.2% males and 30.9% females are obese or overweight in Tamil Nadu. This survey also highlights that urban population is more prone to overweight or obesity than their rural counterparts and also three in ten women were obese in Tamil Nadu.

One of the most common chronic diseases in the world is periodontal disease and is the main cause for extraction of permanent teeth. Periodontitis is associated with inflammation of periodontal apparatus, leading to the destruction of connective tissue attachment and loss of teeth. The bacterial challenge in periodontitis leads to low-grade chronic infection and exacerbate the ongoing inflammation in distant organs. Obesity is one of the systemic conditions which influences the onset and progression of periodontal disease. Several systematic reviews showed the association of periodontal disease with obesity.

Adipose tissue is considered as an endocrine organ which is metabolically active and secretes numerous immunomodulatory factors, so-called adipokines. Adipose tissue during obesity also contains increased number of macrophages, which in turn may further lead to periodontal inflammation.
leading to production of inflammatory mediators by adipose tissue. The pro-inflammatory and immune responses modulation associated with obesity may contribute to periodontal disease. Red complex bacteria such as Porphyromonas gingivalis (Pg), Tannerella forsythia (Tf), and Treponema denticola (Td) were commonly detected from the oral cavity of patients with periodontitis. Recent studies carried out in Japanese population showed the association of the proportion and levels of certain subgingival species and periodontal status in obese participants. Modulation of metabolic changes associated with obesity, alter subgingival microbial colonization, leading to progression of periodontal disease. The microbiota in the gut and oral cavity is affected by the pro-inflammatory effect of circulating adipokines, thereby altering microbial colonization. Although studies in the past have shown significant association between diabetes and periodontal disease; however, limited studies have reported the presence of red complex bacteria in obese population with periodontal disease. Due to lack of studies in the quantification of red complex bacteria in obese participants with periodontitis, especially in the South Indian Population. Our study was aimed to quantify and compare the red complex microorganisms in obese or overweight and normal weight participants with and without chronic periodontitis to identify obesity as a risk for the presence of red complex bacteria.

**MATERIALS AND METHODS**

One hundred and twenty participants of both the sexes with age between 20 and 45 years were selected. The written informed consent was obtained and the protocol was explained to all the participants. The study period was from June 2016 to March 2017. The study was approved by University ethical committee, in accordance with the Helsinki Declaration of 1975 as revised in 2013 (Dr.MGRDU/TMDCH/RES/2015-2016/0302582).

According to periodontal status, the participants were categorized into four groups as, thirty overweight or obese individuals with generalized chronic periodontitis (Group I), thirty normal weight individuals with chronic periodontitis (Group II), thirty overweight or obese individuals with healthy periodontium (Group III), and thirty normal weight individuals with healthy periodontium (Group IV). Inclusion criteria included individuals who were within 20–45 years of age having minimum number of twenty natural teeth and with clinical signs for their respective groups, and Individuals with pregnancy, previous or current smokers, menopause, cardiovascular disorders, thyroid disorders, diabetes mellitus, use of antioxidant supplements, long-term steroid medications, patient who had taken anti-inflammatory or antibiotics within previous 3 months or underwent periodontal treatment in the past 6 months were excluded from the present investigation similar to our previous study.

Groups I and III (overweight or obese individuals) were selected by assessing waist circumference (WC) and body mass index (BMI). The calculation of BMI was done by dividing weight of an individual in kilograms by height of an individual in meters squared. Individuals were considered as overweight when the BMI was >25 kg/m², WC of >90 cm (men) and >80 cm (women). The healthy periodontium was defined by having "0" gingival index (GI) score. Generalized chronic periodontitis is defined by individuals having 3–5 mm clinical attachment loss in more than 30% of sites. The periodontal parameters such as GI, plaque index (PLI), probing pocket depth (PPD), and clinical attachment level (CAL) were recorded similar to our previous study. PLI was recorded at midbuccal, mesiobuccal, distobuccal, and mid-palatal sites in each tooth. The facial, mesial, distal, and lingual gingival areas were examined for GI. PPD was measured in millimeters and were recorded at six sites per tooth and CAL was recorded for all teeth, which is measured from cementoenamel junction to the base of the periodontal pocket.

**Collection of plaque sample**

The plaque samples were collected subgingivally by curette and transported using phosphate buffered saline and stored at −80°C for further analysis. The red complex bacteria were quantified by real-time polymerase chain reaction (RT-PCR).

**Statistical analysis**

The SPSS SPSS (IBM Corporation, Chicago IL USA) software program version 16 was used to perform statistical analysis. Thirty individuals per group sample size were needed to get 80% power of the study, hence 30 individuals were included one-way ANOVA was used to compare the mean values of demographic, clinical parameters, and red complex bacteria levels between the groups. Multiple comparison was done between Groups I and II, Groups I and III, Groups I and IV, Groups II and III, Groups II and IV, and Groups III and IV for demographic, periodontal, and microbiologic variables. Tukey’s honest significant difference post hoc tests were carried for multiple comparisons. Pearson’s correlation analysis was carried out to correlate WC, BMI, PL index, GI, PPD, and CAL with red complex bacteria.

**Table 1: Primers**

| Description Sequence (5’-3’) | Forward | Reverse |
|-----------------------------|---------|---------|
| Pg                          | AGG CAG CTT GCC ATA CTG CG | ACT GGT AGC AAC TAC CGA TGT |
| Td                          | CCG AAT GTG CTC ATT TAC ATA AAG GT | GAT ACC CAT CGT TGC CTT GGT |
| Tf                          | AGC GAT GGT AGC AAT ACC TGT CC | TTC GCC GGG TTA TCC CTC |

Pg – *Porphyromonas gingivalis*; Tf – *Tannerella forsythia*; Td – *Treponema denticola*; A – Adenine; C – Cytosine; T – Thiamine; G – Guanine

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RESULTS

The variables such as demographic, periodontal parameters, and red complex bacteria were compared among the four groups [Table 2]. On comparing the mean age between the groups, no statistically significant difference was shown \((P = 0.332)\). Comparison of BMI, WC, PLI, GI, PPD, CAL, and red complex bacteria between the groups showed a significant difference \((P = 0.000)\) [Table 2]. The multiple comparison of various groups also showed a significant difference among demographic variables, periodontal parameters, and red complex bacteria. The significant mean difference of BMI and WC was found between Group I and II. Multiple comparison of clinical parameters such as PLI, GI between Groups I and III were found to be significant. Comparison of the mean PPD and CAL between Group I and II was also significant \((P = 0.008 \text{ and } 0.000)\). Multiple comparison of red complex bacteria \((Pg, Tf, \text{ and } Td)\) showed a significant difference among all the groups [Table 3 and Figure 1].

The demographic variables and periodontal parameters were correlated with red complex bacteria among the various groups [Tables 4-6]. The positive but weak correlation was observed between \(Pg, Tf,\) and \(Td\) with BMI and WC in Groups I and III. A similar correlation was also found between \(Pg, Tf,\) and \(Td\) with PLI and GI scores in Groups I and II.

DISCUSSION

Obesity is a commonly emerging public health problem. Increased fat accumulation related to overweight and obesity causes a high risk to general health. Chronic diseases have shown to result from systemic inflammation related to obesity.[23] The white adipose tissue secretes numerous immunomodulatory factors named as adipokines, which play a role in regulating vascular and metabolic biology. The adipokines include highly active and novel molecules released by adipocytes such as leptin, adiponectin, resistin, visfatin, or chemerin and also classical cytokines released by inflammatory cells infiltrating adipose tissue. These adipokines are soluble proteins that bind receptors on target cells and initiate intercellular signaling cascades resulting in phenotypic changes to the cell through altered gene expression and regulation. Abnormal levels of metabolites from adipose tissue also activate monocytes which increases the production of inflammatory cytokines. These adipokytotkines play an important role in the initiation of periodontal disease.[4]

The human oral microbiome is composed of diverse community of microorganisms, consisting of more than 700 species of microorganisms.[23] These microorganisms live in harmony with the host and cause disease when there is increased mass/pathogenicity of a microorganism or reduced host response.[24] Periodontal diseases are caused by a group of organisms and five microbial complexes are commonly found in the subgingival biofilm. The red complex consists of three species \(Pg, Tf,\) and \(Td\) which have been considered as the most pathogenic microbial complex.[25] In humans, obesity might increase the risk of periodontitis, by increasing the amount of pathogenic species. Many studies have focused on the ratio of \(Bacteroides\) to \(Firmicutes\) in obese and lean individuals displaying higher levels of \(Firmicutes\) and lower level of \(Bacteroides\) in the gut.[26,27] Amar et al. reported that obesity interferes with the immune system response against \(Pg\) in an animal model.[28] Furthermore, Haffajee and Socransky reported enhanced colonization of \(Tf\) in subgingival biofilm of obese individuals in Japanese Population, whereas Goodson et al. detected \(Selenomonas\) \(noxia\) in saliva from overweight women.[11,29] To the best of our knowledge, this is the first study to compare the red complex bacteria in obese or overweight and normal weight individuals with and without periodontal disease in South Indian population. We hypothesized that in obese individuals, there is an alteration in red complex microflora, increase the risk for the periodontal disease.

In our study, individuals between the age of 25–45 years were included. The age was found to be similar in all the groups [Table 2]. This age range was selected since women more than 45 years of age are expected to attain menopause which is the confounding factor for obesity and

Table 2: Comparison of demographic, periodontal parameters and red complex bacteria among the groups

| Variables | Group I | Group II | Group III | Group IV | F   | P    |
|-----------|---------|----------|-----------|----------|-----|------|
| Age       | 35.30±.13 | 37.17±.476 | 35.40±.555 | 36.52±.59 | 1.151 | 0.332 |
| BMI       | 31.50±.205 | 22.60±.77 | 30.93±.42 | 22.37±.09 | 258.177 | 0.000* |
| WC        | 101.00±.349 | 78.13±.399 | 102.37±.68 | 78.20±.13 | 294.606 | 0.000* |
| PLI       | 2.60±.31 | 2.78±.191 | 0.93±.58 | 0.36±.20 | 339.168 | 0.000* |
| GI        | 2.78±.27 | 2.85±.14 | 0.00 | 0.00 | 3.317 | 0.000* |
| PPD       | 6.27±.38 | 5.87±.41 | 1.90±.48 | 1.77±.57 | 805.725 | 0.000* |
| CAL       | 6.43±.41 | 6.04±.53 | 0.00 | 0.00 | 345.03 | 0.000* |
| Pg        | 16.76±.179 | 22.13±.62 | 26.51±.20 | 30.81±.42 | 353.426 | 0.000* |
| Tf        | 17.88±.54 | 19.91±.38 | 23.83±.09 | 25.99±.13 | 117.433 | 0.000* |
| Td        | 16.71±.039 | 19.76±.47 | 22.47±.89 | 25.73±.43 | 551.481 | 0.000* |

*Level of significance: \(P<0.05\) significant. BMI – Body mass index; WC – Waist circumference; PLI –Plaque index; GI – Gingival index; PPD – Probing pocket depth; CAL – Clinical attachment level; Pg – Porphyromonas gingivalis; Tf – Tannerella forsythia; Td – Treponema denticola; F – Ratio of mean squares
periodontal disease.\[30\] The National Health and Nutrition Examination Survey (NHANES III) reported that 18–34 years aged individuals showed association between obesity and periodontal disease with odds ratio (OR) of 1.76 and between increased WC and periodontal disease with OR of 2.27.\[31\] This study did not show any gender difference in obese individuals with respect to the prevalence of periodontal disease. However, few studies in the past have shown that the association of obesity and periodontitis was stronger in female younger adults.\[10,32\] BMI and WC levels were higher in Groups I and III as they were obese or overweight compared to Groups II and IV and the mean PLI, GI, PPD, and clinical attachment loss scores were significantly higher in Groups I and II as compared to Groups III and IV [Tables 2 and 3] which was similar to study done by Maciel et al.\[33\] Although Group I and II were chronic periodontitis individuals, the mean values of clinical parameters such as PPD and CAL were higher in Group I, when compared to Group II and the difference was significant [Table 3]. Hence, obese individuals with periodontitis had a highest score of periodontal parameters as compared to other groups. This was in accordance with the study by Suvan et al. who stated that obesity was associated with PPD.\[14,35\] Similarly, Chaffee and Weston also found greater mean clinical attachment loss in obese individuals with periodontal disease.\[5\]

Detection of anaerobic bacteria by culture methods are challenging because of their specific growth requirements such as anaerobic environment and certain technical barriers.\[16,37\] Advances in molecular biology such as PCR have enabled the identification of specific bacteria in large number of periodontitis cases. The PCR is a relatively simple and rapid test for successful detection of oral anaerobic bacterial pathogens. The quantity of red complex microorganisms identified was expressed in CT units. CT value is inversely proportional to bacterial counts. Multiple comparison of mean difference in red complex bacteria levels among the four groups was statistically significant with $P = 0.000$. The mean $Pg$, $Tf$, and $Td$ levels in obese individuals with chronic periodontitis (Group I) were higher when compared to the other groups, which was also statistically significant (0.000) [Tables 2 and 3]. This suggests that obesity is related to increase in proportion of red complex bacteria as reported by Haffajee and Socransky and Matsushita et al.\[11,12\] The values of red complex bacterial count of Group II was higher as compared to Group III and IV signifies the presence of increased levels of red complex bacteria compared to Groups III and IV [Table 3]. The results of our study are comparable to Mahalakshmi et al., who reported the higher odds of detecting $Pg$, $Tf$, and $Td$ in individuals with periodontitis as compared to those in

Table 3: Multiple comparison of demographic, clinical, and microbiological variables

| Variable | Group I and II ($P$) | Group I and III ($P$) | Group I and IV ($P$) | Group II and III ($P$) | Group II and IV ($P$) | Group III and IV ($P$) |
|----------|---------------------|----------------------|---------------------|-----------------------|----------------------|-----------------------|
| Age      | 0.397               | 1.00                 | 0.749               | 0.446                 | 0.951                | 0.795                |
| BMI      | 0.000*              | 0.580                | 0.000*              | 0.000*                | 0.953                | 0.000*                |
| WC       | 0.000*              | 0.615                | 0.000*              | 0.000*                | 1.00                 | 0.000*                |
| PLI      | 0.190               | 0.000*               | 0.000*              | 0.000*                | 0.000*               | 1.00                  |
| GI       | 0.261               | 0.000*               | 0.000*              | 0.000*                | 0.000*               | 0.000*                |
| PPD      | 0.008*              | 0.020*               | 0.000*              | 0.000*                | 0.000*               | 0.000*                |
| CAL      | 0.000*              | 0.000*               | 0.000*              | 0.000*                | 0.000*               | 0.000*                |
| Pg       | 0.000*              | 0.000*               | 0.000*              | 0.000*                | 0.000*               | 0.000*                |
| Tf       | 0.000*              | 0.000*               | 0.000*              | 0.000*                | 0.000*               | 0.000*                |
| Td       | 0.000*              | 0.000*               | 0.000*              | 0.000*                | 0.000*               | 0.000*                |

*Level of significance: $P<0.05$ significant. BMI – Body mass index; WC – Waist circumference; PLI – Plaque index; GI – Gingival index; PPD – Probing pocket depth; CAL – Clinical attachment level; $Pg$ – Porphyromonas gingivalis; $Tf$ – Tannerella forsythia; $Td$ – Treponema denticola

Table 4: Pearson correlation among variables and Porphyromonas gingivalis in four groups

| Variables | Group I | Group II | Group III | Group IV |
|-----------|---------|----------|-----------|----------|
| $Pg$ ($r$) | $P$     | $P$      | $P$       | $P$      |
| Age       | 0.128   | 0.502    | 0.126     | 0.506    |
| BMI       | 0.293   | 0.030    | 0.107     | 0.573    |
| WC        | 0.386   | 0.035*   | 0.264     | 0.020*   |
| PLI       | 0.324   | 0.041*   | 0.282     | 0.040*   |
| GI        | 0.015   | 0.940    | 0.007     | 0.971    |
| PPD       | 0.088   | 0.190    | 0.146     | 0.374    |
| CAL       | 0.008   | 0.968    | 0.120     | 0.527    |

*Level of significance: $P<0.05$ significant. BMI – Body mass index; WC – Waist circumference; PLI – Plaque index; GI – Gingival index; PPD – Probing pocket depth; $Pg$ – Porphyromonas gingivalis; $r$ – Correlation

Table 5: Pearson correlation among variables and Tannerella forsythia in four groups

| Variables | Group I | Group II | Group III | Group IV |
|-----------|---------|----------|-----------|----------|
| $Tf$ ($r$) | $P$     | $P$      | $P$       | $P$      |
| Age       | 0.003   | 0.987    | 0.031     | 0.872    |
| BMI       | 0.374   | 0.042*   | 0.098     | 0.605    |
| WC (cm)   | 0.276   | 0.041*   | 0.029     | 0.224    |
| PLI       | 0.235   | 0.036*   | 0.351     | 0.047*   |
| GI        | 0.199   | 0.041*   | 0.287     | 0.023*   |
| PPD (mm)  | 0.053   | 0.786    | 0.141     | 0.277    |
| CAL (mm)  | 0.061   | 0.747    | 0.166     | 0.380    |

*Level of significance: $P<0.05$ significant. BMI – Body mass index; WC – Waist circumference; PLI – Plaque index; GI – Gingival index; PPD – Probing pocket depth; $Tf$ – Tannerella forsythia; $r$ – Correlation

Table 6: Pearson correlation among variables and Treponema denticola in four groups

| Variables | Group I | Group II | Group III | Group IV |
|-----------|---------|----------|-----------|----------|
| $Td$ ($r$) | $P$     | $P$      | $P$       | $P$      |
| Age       | 0.157   | 0.409    | 0.179     | 0.344    |
| BMI       | 0.267   | 0.041*   | 0.475     | 0.008*   |
| WC        | 0.423   | 0.020*   | 0.242     | 0.082*   |
| PLI       | 0.203   | 0.048*   | 0.292     | 0.018*   |
| GI        | 0.249   | 0.016*   | 0.230     | 0.020*   |
| PPD       | 0.109   | 0.573    | 0.144     | 0.464    |
| CAL       | 0.121   | 0.523    | 0.040     | 0.464    |

*Level of significance: $P<0.05$ significant. BMI – Body mass index; WC – Waist circumference; PLI – Plaque index; GI – Gingival index; PPD – Probing pocket depth; $Td$ – Treponema denticola
The present study showed higher subgingival red complex bacterial levels in obese individuals with chronic periodontitis than normal weight individuals with chronic periodontitis (Group II). Few studies were conducted in different populations linking periodontal disease and obesity. The results of the present study were comparable with findings of these previous studies. A systematic Review by Amelie Keller suggested that obesity, overweight, and increased WC may be risk factors for periodontitis or worsening the periodontal measures. Chaffee et al. found an increase in the prevalence of obesity in general adult population with and without periodontitis.

Our study showed the prevalence of more subgingival red complex bacteria in obese individuals with healthy periodontium than normal weight individuals with healthy periodontium. This finding was comparable to studies done by Matsushita et al. and Maciel et al. They reported that the number of red complex bacteria were high in individuals with high WC or BMI independent of periodontitis. The inflammatory cytokines were also released at periodontal tissues by red complex bacteria. More inflammatory cytokines are also released due to insulin resistance. Our study demonstrated the presence of increased red complex microorganism in obese individuals with high BMI and WC [Tables 2 and 3]. Goodson et al. proposed three pathways that are hypothesized to link periodontal bacteria to obesity. The first hypothesized mechanism suggests that certain oral bacteria may cause increased fat storage. The second hypothesized mechanism is through the control of appetite through controlling, leptin and ghrelin, which in turn control food intake. A third hypothesized mechanism is through the upregulation of systemic inflammatory markers such as tumor necrosis factor-α and downregulation of adiponectin, which leads to increased insulin resistance. Pg can influence immune system and when in a virulent state, may produce a large amount of pro-inflammatory mediators, which may affect the pathological mechanisms involved in the development of obesity.

We observed positive correlation between Pg, Tf, and Td levels and BMI and WC in Group I and III, which implies the prevalence of increased proportions of red complex bacteria in the periodontally healthy/diseased sites of obese individuals [Tables 4-6]. This finding is in accordance with Haffajee et al., who suggested that the periodontal pathogen Tf overgrow in healthy sulcus or shallow pocket of periodontally healthy or gingivitis sites of female obese or overweight individuals. The metabolic changes associated with obesity affect the subgingival microbial colonization or compromised periodontal tissue host defenses, thereby altering the progression of periodontal disease. Increased proportions of periodontal pathogens in periodontally healthy sites of obese individuals increase the risk of initiation of periodontal disease.

Positive correlation was also found between Pg, Tf, and Td levels with PLI and GI scores in Group I and II [Tables 4-6]. The results showed the presence of red complex bacteria in chronic periodontitis individuals with higher plaque scores. This study also showed the association of red complex bacteria with increased GI which accepts the fact that these bacteria are associated with the bleeding on probing sites.

The limitation of this study was being a cross-sectional study. Future interventional studies in a similar population are needed to more strongly elucidate the association between the red complex microorganism and obesity.

**CONCLUSION**

The oral microbiota has significant impact on oral and general health. In our study, obese individuals with periodontal disease harbor increased red complex bacteria. These microorganisms were also found to be higher in obese individuals with healthy periodontium. This states the strong association of obesity and red complex bacteria. Hence, reduction of bacterial burden by periodontal therapy may show a greater impact in the prevention of periodontal disease progression, especially in obese or overweight individuals. It is suggested that periodontal therapy should be included as a part of prevention program in obesity-related diseases.

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**Conflicts of interest**

There are no conflicts of interest.

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