Introduction

According to WHO Report 2008, In India, 53% of the deaths occurs due to Non Communicable Diseases (NCD). These NCDs are the result of lifestyle related behavioral risk factors such as use of tobacco, physical inactivity, unhealthy diet and harmful use of alcohol leading to metabolic/physiological changes like increased in blood pressure (BP), overweight/obesity, raised blood glucose and raised cholesterol levels. The clinical cluster of hypertension, cardiovascular disease, hyperlipemia, hyperuricemia and type 2 Diabetes had been recognized as syndrome X which is finally named as metabolic syndrome (Met.S) which was the subjects of this study.

There is a known association between periodontitis and Met.S. The microorganisms associated with periodontitis are diverse. The association between Porphyromonas gingivalis and periodontitis is well established but the association between Porphyromonas gingivalis and Met.S is not so evident. Studies have also reported presence of Porphyromonas gingivalis in periodontitis, but microbiological status in Met.S patients is not yet evaluated. Hence, with this aim in mind, we conducted the present study to evaluate the incidence of Porphyromonas gingivalis in periodontitis patients with Met.S & patients with periodontitis without Met.S.

Materials and Method

The present study was carried out in the Department of Oral Pathology and Microbiology of Sharad Pawar Dental College. The patients were selected from the Outpatient Department (OPD) of the department of Periodontics, Sharad Pawar Dental College and department of General Medicine, Achaya Vinoba Bhave Rural Hospital Sawangi, Meghe Wardha. Before the start of the study, an informed consent was obtained from all the study subjects. The study was

Evaluation of Porphyromonas Gingivalis in Periodontitis Patients of Met.S and Periodontitis Patients without Met.S

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Abstract

Oral cavity is said to be the mirror of systemic health. Many systemic diseases first manifest in the oral cavity. Also an oral disease has a potential to act as an independent risk factor for causing systemic disease. This bidirectional view is gaining acceptance currently, due to findings that, association between periodontal disease and systemic conditions such as Metabolic Syndrome. Effort has brought advances in revealing the etiological and pathological links between the chronic inflammatory dental disease, periodontitis and systemic condition, Metabolic Syndrome. The role of microorganisms in periodontal disease is well documented. In the last 10 years, studies have been published indicating a positive or negative relationship between periodontitis and various systemic diseases, including Met.S. The most frequently isolated microaerophilic pathogens are (A. actinomyctemcomitans, Campylobacter rectus, and Eikenellacorrodens) and anaerobic pathogens are (P. gingivalis, Bacteroidesforsythus, T. denticola, Prevotella intermedia, Fusobacterium nucleatum, Eubacterium, and spirochetes). In the present study P. gingivalis periodontal pathogen confirmed increased colonization of periodontal pathogens, are cultured in both periodontitis patients without Met.S and Periodontitis patients with Met.S.

Keywords: Metabolic Syndrome, Periodontitis, Porphyromonas gingivalis
Evaluation of Porphyromonas Gingivalis in Periodontitis Patients of Met.S and Periodontitis Patients without Met.S

approved by the Institutional Ethics Committee, Datta Meghe Institute of Medical Sciences, Wardha (Deemed University) Ref. No. DMIMS(DU)/ IEC/ 2013-14/ 127 date-30.09.2013. The study was performed on subjects, which were divided into three groups according to their periodontal status and presence or absence of Met.S.

2. Group II: Chronic Periodontitis patients without Met.S (n=50).
3. Group III: Chronic Periodontitis patients with Met.S (n=50)

The Criteria for Periodontal Disease Sites were as follows
1. Probing pocket depth (PPD) > 3mm, with the help of Williams graduated periodontal probe.
2. Presence of bleeding on probing (BOP).
4. Gingival Index score (GI ≥ 2)

The Criteria for Diagnosis of Met.S were as follows
1. Body Mass Index (BMI), Obesity > 30(mass/height²)
2. Pressure (BP) > 140/90 mmHg
3. Triglycerides (TG) > 160mg%
4. Fasting Blood Sugar (FBS) >125mg/dl
5. High-Density Lipoprotein (HDL) > 75mg%

Detailed clinical history was recorded and clinical assessment was carried out in all the three groups. Subgingival plaque samples were collected and subjected to conventional microbial culture method.

For Group I- Subgingival plaque sample was collected from molar area of periodontally healthy individuals. The sampling site was isolated using cotton rolls and supragingival plaque was removed with the help of sterile cotton. The subgingival plaque sample was then collected using sterile Gracey curettes.

For Group II and III- The sampling sites were isolated with cotton roles and subgingival plaque was removed using sterile cotton. Subgingival plaque samples were obtained using sterile gracy curettes from deepest periodontal pocket. Plaque was subsequently transferred into a sterile container (Himedia) with 0.85% of sterile saline that was immediately processed. Serial Dilution was done. A **serial dilution** is the stepwise dilution of a substance in solution. Usually the dilution factor at each step is constant, resulting in a geometric progression of the concentration in a logarithmic fashion. In 100 ml distilled water, 0.85gms Sodium chloride (NaCl - SD fine company) was added. The prepared 9ml of 0.85% NaCl was dispensed into 3 test-tubes (Borosil) each and autoclaved. After autoclaving 5-fold serial dilutions of the sample was made in the test-tube containing 0.85% NaCl. The serial dilution of samples were done immediately and cultured on agar plates. Brucella Agar Plate with Hemin and Vitamin K1. Hi MP1039 is recommended for the isolation of Porphyromonas gingivalis. The prepared culture plate were used for inoculation. The plaque sample which was already serially diluted was added to each medium using streak culture method. Streak culture method is a routinely employed method for bacterial isolation in pure culture. A platinum or nichrome wire loop of 2-4 nm in internal diameter is used. A loopful of specimen is smeared onto the surface of dried plate near the peripheral area. This known as primary inoculum. From the primary inoculum, it is spread thinly over the plate by streaking with loop in parallel lines. The streak plate technique is essentially a method to dilute the number of organisms, decreasing the density. This allows for individual colonies to be isolated from other colonies. Each colony is considered "pure," since theoretically, the colony began with an individual cell. Each inoculated petri-plate was sealed with Parafilm (Himedia) to prevent any contamination while incubation, and then placed in an anaerobic jar (Himedia) with AnaeroGas Pack system (Himedia) and incubated at 37°C for 48-72 hours. All the plates are then placed in the anaerogas jar and anaerogas pack is placed inside the jar to maintain the anaerobic condition. The anaerobic gas jar is then placed inside the incubator at 37°C for 72 hours. After 72 hours the colonies cultured on Petri plates are subjected to biochemical identification test for confirmation of bacteria intended to be cultured. The identification of a bacterial species is based on factors, including colony morphology, chemical composition of cell walls, biochemical activities, and nutritional requirements. 3 points should be considering during microorganism identification. (Table 1) *Porphyromonas gingivalis* is gram negative and *Indole Test* was positive.
In this study, a quantitative analysis of *P. gingivalis* in periodontitis patients with Met.S and periodontitis patients without Met.S done. The results of the present study were subjected to statistical analysis. The comparison of the *P. gingivalis* counts in periodontitis patients with Met.S (Group III), periodontitis patients without Met.S (Group II) and normal control (Group I) was carried out to test whether there is significant difference between those values. The statistical tests used for the analysis of the results were: Chi-square Test, One way ANOVA (F-Test), Tukey HSD and ‘Descriptive statistical analysis’ (i.e. mean, standard deviation and standard error) was carried out for all the groups in this study.

In Normal controls (Group I), mean total count for *P. gingivalis* was 0.04±0.19x10^5 with range of 0.00-1.00. In Periodontitis patients without Met.S (Group II), mean total count for *P. gingivalis* was 4.68±3.93x10^5 with range of 0.00-11.00. In Periodontitis patients with Met.S (Group III), mean total count for *P. gingivalis* was 10.94±5.95x10^5 with range 0.00-20.00. Thus, the sample suggested that *P. gingivalis* count in periodontitis patients with Met.S was six times more than periodontitis patients without Met.S. In periodontitis patients without Met.S the count was about four times more than normal control. In periodontitis patients with Met.S the count was about eight times more than normal control. (Table 3) The statistically significant variations of mean of total count of *P. gingivalis* count were found among all groups (p<0.05). The total counts of *P. gingivalis* were observed in decreasing order in periodontitis with Met.S (Group III) 10.94x10^5±5.95x10^5, periodontitis without Met.S (Group II) 4.68x10^5±3.93x10^5, normal control (Group I) 0.04x10^5±0.19x10^5. (Table 4)

### Observations and Results

<table>
<thead>
<tr>
<th>Groups</th>
<th>Present</th>
<th>Absent</th>
<th>Total</th>
<th>N2-value±SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>50</td>
<td>02(4%)</td>
<td>48(96%)</td>
<td>50(100%)</td>
<td>p&lt;0.01</td>
</tr>
<tr>
<td>Periodontitis without Met.S (Group II)</td>
<td>50</td>
<td>31(62%)</td>
<td>19(38%)</td>
<td>50(100%)</td>
<td>7.55±1.69</td>
</tr>
<tr>
<td>Periodontitis with Met.S (Group III)</td>
<td>50</td>
<td>40(80%)</td>
<td>10(20%)</td>
<td>50(100%)</td>
<td>13.68±2.48</td>
</tr>
<tr>
<td>Total</td>
<td>73</td>
<td>77</td>
<td>150</td>
<td></td>
<td>p&lt;0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>50</td>
<td>0.40</td>
<td>0.19</td>
<td>0.027</td>
<td>0.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Periodontitis without Met.S (Group II)</td>
<td>50</td>
<td>4.68</td>
<td>3.93</td>
<td>0.55</td>
<td>0.00</td>
<td>11.00</td>
</tr>
<tr>
<td>Periodontitis with Met.S (Group III)</td>
<td>50</td>
<td>10.94</td>
<td>5.95</td>
<td>0.84</td>
<td>0.00</td>
<td>20.00</td>
</tr>
</tbody>
</table>

### One way ANOVA for *P. gingivalis* count in all three groups (All the values in 10^5)

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Sum of Squares</th>
<th>Df</th>
<th>Mean Square</th>
<th>F</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>2992.12</td>
<td>2</td>
<td>1496.06</td>
<td>88.123</td>
<td>0.000 S,p&lt;0.05</td>
</tr>
<tr>
<td>Within Groups</td>
<td>2495.620</td>
<td>147</td>
<td>16.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>5487.74</td>
<td>149</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Evaluation of Porphyromonas Gingivalis in Periodontitis Patients of Met.S and Periodontitis Patients without Met.S**

The statistical significant difference in total count of *P. gingivalis* was noted between all groups *(p=0.000)*. Thus, statistical analysis revealed that *P. gingivalis* count was highest in periodontitis patients with Met.S (Group III) than periodontitis patients without Met.S (Group II) and Normal control (Group I). *(Table 5)*

**Table 5. Multiple Comparisons: Tukey Test for *P. gingivalis* count in all three groups (All the values in 10^5)**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean Difference (I-J)</th>
<th>p-value</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (GroupI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Periodontitis without Met.S (GroupII)</td>
<td>-4.64</td>
<td>0.000 *&lt;p&lt;0.05</td>
<td>-6.59</td>
</tr>
<tr>
<td>Periodontitis with Met.S (GroupIII)</td>
<td>-10.90</td>
<td>0.000 *&lt;p&lt;0.05</td>
<td>-12.85</td>
</tr>
<tr>
<td>Periodontitis patients with Met.S (GroupIII)</td>
<td>6.26</td>
<td>0.000 *&lt;p&lt;0.05</td>
<td>4.30</td>
</tr>
</tbody>
</table>

**Discussion**

Oral cavity is said to be an open system which is exposed to the environment. So, the possibilities of foreign material entering the system are heightened because of constant intake of food and liquids through the mouth. Large numbers of bacteria in oral cavity can start tissue destruction by activating on host defense cells indirectly, which in turn, produce as well as release mediators which will stimulate the effectors of connective tissue breakdown.5-6-7

Met.S refers to a constellation of disturbances including glucose intolerance, central obesity, dyslipidemia (hypertriglyceridemia, elevated nonesterified fatty acids (NEFAs), and decreased high-density lipoprotein (HDL) cholesterol, and hypertension.4

The microbiological analysis revealed that the mean count for *P. gingivalis* in periodontitis patients was six times more than control. The results of our study were in an agreement with the study of Ann L. Griffenetal, which showed that *P. gingivalis* were present in low levels in healthy subjects and increased 11.2-fold in individuals with periodontitis.8-9-10

The reasons for increased colonies of *P. gingivalis* in periodontitis patients could be that, *P. gingivalis* is best characterized as an opportunistic oral pathogen that inhibits the oral biofilm. Many virulence mechanisms have been identified for *P. gingivalis*. *P. gingivalis* has a carbohydrate capsule on its outer surface which prevents opsonization by complement and inhibits phagocytosis and killing by neutrophils. The lipopolysaccharide which is produced by it is not very strong, but it could inhibit chemotaxis and killing by leucocytes. This organism possesses several putative virulence factors (including proteases which degrade immunoglobulin, complement, collagen fibres, hyaluronic acid; adhesins, endotoxins, and cytotoxins) that can directly affect the periodontium or elicit host functions that result in the periodontal disease.11-12-13-14-15-16-17-18

The results of our study revealed that mean count for *P. gingivalis* in periodontitis patients with Met.S was four times more than periodontitis patients without Met.S and 8 times more than controls. Our results are in accordance with the study results by Takahashi etal, 2001 who states that levels of *P. gingivalis* was increased in periodontitis patients with Met.S as compared to normal patients.19-20

The reasons for increased in *P. gingivalis* levels in periodontitis patients with Met.S could be explained as, *P. gingivalis*, have the known ability to invade deep vascular endothelium of the periodontium of Met.S patients comprising of T2DM. Once periodontal pathogens were entered and established in the
diabetic host, periodontal infection may aggravate microvascular complications. In periodontitis patients, chronic low-level systemic exposure to periodontal microorganisms exists, that will lead to significant changes in plasma levels of cytokines and hormones. Due to the dynamic nature of the inflamed periodontium, the tissue may act as an endocrine-like source of inflammatory mediators. Among the inflammatory biomarkers, TNF-alpha, CRP, IL-1β, IL-6 and IL-8 and IL-6 are liberated. Excessive concentrations of TNF-alpha negatively regulate insulin signaling and glucose uptake. Additionally, the elevated levels of soluble TNF receptor 1 and 2 (sTNFalphaRI and sTNF-alphaRII) shown in obese patients may lead to a hyperinflammatory state, thereby increasing the risk for periodontal disease. The hyperinflammatory state may be caused by adipocytes, which secrete proinflammatory cytokines, and providing the link between the pathogenesis of type 2 diabetes, obesity, and periodontal disease. The highest levels of TNF-alpha and sTNFalpha receptors were found in those individuals in the highest quartile for BMI. These observations may suggest, an increased acute phase response is associated with insulin resistance contributing to periodontitis.5-21-22-23

One of the complications of diabetes is elevation of blood glucose concentrations (hyperglycemia). Hyperglycemia leads to the formation of AGEs. These AGEs make endothelial cells and monocytes more susceptible to stimuli that will then induce the cells to produce inflammatory mediators. Some author suggested that AGE accumulations in the gingival tissue will leads to increased vascular permeability, and increased breakdown of collagen fibers, and more destruction of both nonmineralized connective tissue and mineralized bone. As diabetes is a metabolic disorder and periodontal disease is an infectious disease, the pathophysiological relationship between these two entities is, through the ability of both conditions to induce an inflammatory response, because of AGE or bacterial accumulation, respectively, which further leads to the production of inflammatory mediators.5-24-25-26-27

The following conditions in Met.S favor the growth of Porphyromonas Gingivalis attached to the components of extracellular matrix and to the Type I collagen. This helps in colonization of Porphyromonas Gingivalis to gingival margin. The arg-gingipain protease of Porphyromonas Gingivalis helps either directly or indirectly in this attachment. Directly by acting as an adhesion and indirectly by affecting the expression of an adhesion. A strong association between gingival protease activity and periodontal infection were found in studies. In Met.S serum levels of Cystatin C (cysteine protease) are synthesized at constant rate.28 Therefore it could be hypothesized that constant increased in protease in Met.S patients favours the colonization of Porphyromonas Gingivalis. Other reasons for increase colonization of Porphyromonas Gingivalis could be that Porphyromonas Gingivalis has the ability to identify receptors on gingival epithelial cell receptors even in low chronic inflammatory condition, Porphyromonas Gingivalis also has the property to respond and adopt to oxidative stress as they are oxidative stress resistance. Porphyromonas Gingivalis growth requirements include iron, which is provided by elevated iron stores in GCF of Met.S patients.29 Therefore it could be hypothesized that constant increased in protease in Met.S patients favours the colonization of Porphyromonas Gingivalis. Other reasons for increase colonization of Porphyromonas Gingivalis could be that Porphyromonas Gingivalis has the ability to identify receptors on gingival epithelial cell receptors even in low chronic inflammatory condition, Porphyromonas Gingivalis also has the property to respond and adopt to oxidative stress as they are oxidative stress resistance. Porphyromonas Gingivalis growth requirements include iron, which is provided by elevated iron stores in GCF of Met.S patients.29

Thus, Patients diagnosed with Met.S are thought to be at higher risk due to a compromised immune system. Infectious and opportunistic microbes responsible for periodontal infection may thus bring a burden onto the rest of the body and release products that elicit an inflammatory response.

**Conclusion**

The fact that increases in colonization of periodontal pathogens may be attributed to the assumption that, Periodontitis releases proinflammatory cytokines and ROS at the site of inflammation, leading to oxidative stress situation.30 This will contribute to aggravate existing Met.S.

**References**


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