Comparative Evaluation of Quantitative Changes in Microbiota around Mobile and Non-mobile Teeth in Chronic Periodontitis

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ABSTRACT

Background: Various periodontopathic micro-organisms and their products are thought to play a major role in periodontal disease progression. This causes persistent inflammation, tissue destruction further leading to tooth mobility. The present study aimed at quantitative assessment of specific pathogenic micro-organisms around mobile and non-mobile teeth.

Materials and Methods: The study included 24 chronic periodontitis subjects aged between 30 and 50 years with the presence of minimum 20 teeth. Subgingival microbial samples were collected from mobile and non-mobile teeth from each patient with a probing pocket depth of 4 to 12 mm. The collected samples were cultured for identification and quantification of specific periodontal pathogens.

Results: The quantity of subgingival micro-organisms expressed in CFUs was compared between mobile and non-mobile teeth using paired and unpaired t-test. There was statistically significant increase in Platsysaurus intermedius (p = 0.021), Actinobacillus actinomycetemcomitans (p = 0.002), Campylobacter rectus (p = 0.000) and P. micros (p = 0.000) around mobile teeth.

Conclusion: There was a statistical increase in pathogenic micro-organisms around mobile teeth especially C. rectus and P. micros.

INTRODUCTION

Periodontal disease is initiated by extension of supragingival plaque to the subgingival space, leading to migration of the junctional epithelium in an apical direction to form a periodontal “pocket” thus resulting into chronic periodontitis.1,2 Periodontitis in its early stage is relatively asymptomatic. Thus, individuals with early chronic periodontitis do not report for treatment. This results in steady proliferation of subgingival microbiota and paves the way for their systemic influences.1,4 The interactions between micro-organisms and human hosts have been emphasised, debated and extensively studied to characterise the composition of the human microbiome at different body sites.5,6 The oral cavity primarily consisting of teeth and mucosa provides a nutrient surface for microbial colonization, offering diverse habitats wherein different species of micro-organisms can prosper.5,6

The mobility of teeth puts dentist in dilemma whether to save the tooth or not. Single rooted teeth develop mobility commonly than multi-rooted teeth. Primary trauma from occlusion and secondary trauma from occlusion increases tooth mobility.9 Similarly, hormonal changes in women during pregnancy may also increase the tooth mobility.10 Thus, tooth mobility is a factor which has multiple dimensions for its etiological variation.

The periodontal prognosis of teeth is decided most of the time by mobility, plaque index, and alveolar bone support, in descending order, which shows that mobility is the important predictor of tooth survival.11 Mobility is mainly caused due to various micro-organisms colonizing around the teeth and destructing the periodontal structures indicating the prime role played by the micro-organisms.

As the periodontal ligament space is widened around mobile teeth there is increased blood vasculature in dentogingival connective tissue.12 Hence this ecological niche (gingival sulcus) is slightly different from that present around non-mobile teeth. In view of ecological plaque hypothesis whether this change in biological niche around mobile teeth and non-mobile teeth leads to the variation of microbiota around them needs to be verified.

The understanding of microbiome of periodontal disease is continuously evolving. We have identified many micro-organisms involved in periodontal disease progression; still the question remains unanswered, whether there is any difference between microbiota around mobile and non-mobile teeth.

The studies with regards to microbiome around the mobile tooth and non-mobile tooth are very few. So, in the present study, the subgingival microbiota of mobile and non-mobile teeth was examined for major suspected periodontopathic organisms using selective culture methods.

KEYWORDS: microbiota, mobility, periodontitis, plaque, subgingival

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MATERIALS AND METHODS

The present study was conducted in the Department of Periodontology, School of Dental Sciences, KIMS University, Maharashtra, India. Ethical clearance was obtained before commencement of the study [KIMSDU/IEC/01/2015]. This study was approved by the ethics board of Krishna Institute of Medical Sciences, Maharashtra, India and was conducted in accordance with the Helsinki Declaration of 1975 as revised in 2013.

Thirty (n=30) chronic periodontitis patients aged between 30 and 45 years, with at least one multirooted mobile and non-mobile teeth with a probing pocket depth of 4 to 12 mm were selected for the study. To avoid bias in the sample collection only multirooted teeth with pockets were considered ignoring single rooted teeth. Patients with systemic diseases, receiving systemic antibiotics for at least 3 months, teeth with class II restorations, teeth with prosthetic restorations, supra-erupted teeth and root caries were excluded.

Clinical Examination

A mobile and non-mobile tooth, each exhibiting probing pocket depths ranging from 4 to 12 mm, were identified in each study patient for clinical and microbiological examination.

Clinical examination was carried out by single calibrated examiner to assess mobility according to Miller’s criteria. Probing pocket depth was measured using UNC-15 probe (HU- Friedy, Chicago, IL, USA) and Gingival index (Loe and Silness in 1963) was used to assess the severity of gingival inflammation.

Sample Collection

Following clinical examination, prior to sample collection, oral prophylaxis was performed to eliminate supragingival deposits. The patients were recalled after 1 week in interval for collection of plaque samples from subgingival areas of mobile and non-mobile teeth. A time interval of 1 week between supragingival scaling and subgingival plaque collection was maintained in order to avoid contamination of subgingival plaque with supragingival plaque and debris. The samples were collected at the sites where the study probing measurements were made. At 1 week interval, the representative sites were isolated with cotton rolls and acurette was inserted to the bottom of the pocket and moved coronally in contact with the root surface to remove the most apical plaque. Each plaque sample was immediately suspended in a sterile tube containing 2 ml of reduced transport fluid (RTF). The maximum time between sample collection and laboratory processing was 24 hours. The samples were selectively cultured for Porphyromonas gingivalis (P.g), Prevotella intermedia (P.i), Aggregatibacter actinomycetemcomitans (A.a), Campylobacter rectus (C.r) and Peptostreptococcus micros (P.m). These micro-organisms were assessed and quantified in terms of colony forming units (CFUs).

Transport Media

RTF was used as transport media to collect plaque samples. The RTF fluid was a mixture of stock solution 1 and stock solution 2. Stock solution 1 consisted of diabasic potassium phosphate and distilled water. Whereas stock solution 2 consisted of potassium chloride, ammonium sulphate, potassium phosphate, magnesium sulphate and distilled water.

Later sodium carbonate 8%, L-cystine, sodium thioglycollate were filtered, sterilised and added to the stock solution land 2 and stored separately under sterile conditions.

The prepared, stock 1 and stock 2 solutions were mixed prior to sample collection resulting into final transport medium, which was stored at 4°C.

Microbiological Analysis

Plaque samples received in the transport media were first vortexed and then inoculated in the culture medium according to the requirement of the selected micro-organisms in enriched and selective medium.

For P. gingivalis and P. intermedia, blood agar was used as an enriched medium. Brucella agar with hemin, vitamin K and blood agar was incubated at 37°C for 3–4 days in anaerobic jar. The blood agar medium was mixed with kanamycin which makes the medium selective for P. gingivalis and P. intermedia.

A. actinomycetemcomitans were incubated in Actino Agar for 3–4 days in anaerobic jar at 37°C. Further dental agar which was used as a selective medium for A.a was incubated at 37°C in 5–10% CO₂ jar for 48–72 hrs.

C. rectus, is strictly an anaerobe, the selective media used was Columbia blood agar supplemented with campylobacter solution. CAMPY-WOI (enriched medium) was inoculated directly with clinical material. Inoculated plates were streaked to obtain isolated colonies and incubated at 35–37°C for 18–48 hours.

P. micros were cultured using Columbia CNA agar, a selective medium that was supplemented with glutathione and lead acetate.

After completion of incubation of all respective cultured micro-organisms, the plates were removed and noted for the colony characters of the required organism and also the colony count was done for quantification. The micro-organisms were confirmed by gram staining and key biochemicals.

Statistical Analysis

The collected data was analysed statistically using SPSS software version 16.0 (statistical package for the social sciences 16, IBM corporation, United States). The
and *P. micros* showed near about three-fold increase in their quantity around mobile teeth as compared around non-mobile teeth.

Distribution of subgingival micro-organisms with mobile and non-mobile teeth when compared between male and female patients showed no statistical significant difference in CFUs of both the groups (Table 4).

### RESULTS

A total of 24 (n=24) patients (10 females, 14 males) with a mean age of 39 years, diagnosed with chronic generalised periodontitis were enrolled in this study. A total of 48 (n = 48) multi-rooted teeth (24 mobile and 24 non-mobile) affected by moderate-to-severe chronic periodontitis were included in the study.

Table 1 shows the demographic and clinical variation in study population. Out of 24 mobile teeth 4 exhibited Grade I mobility, 11 exhibited Grade II mobility and 9 exhibited Grade III mobility. The mean probing depths when evaluated around mobile and non-mobile teeth were almost similar (Table 2).

In Table 3 the quantity of subgingival micro-organisms expressed in CFUs was compared between mobile and non-mobile teeth using paired t-test. There was statistically significant increase in *P. intermedia*, *A. actinomycescomitans*, *C. rectus* and *P. micros* around mobile teeth. The count of *P. gingivalis* CFUs was more round mobile teeth than non-mobile teeth, but this difference did not reach statistical significance. Out of all the micro-organisms, *C. rectus*

<table>
<thead>
<tr>
<th>Micro-organisms</th>
<th><em>Pg</em></th>
<th><em>Pi</em></th>
<th><em>Aa</em></th>
<th><em>Pm</em></th>
<th><em>Cr</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>NM</td>
<td>M</td>
<td>NM</td>
<td>M</td>
</tr>
<tr>
<td>Male</td>
<td>25.07±25.00</td>
<td>19.78±9.14</td>
<td>28.50±9.14</td>
<td>63.57±50.00</td>
<td>24.92±14.21</td>
</tr>
<tr>
<td></td>
<td>12.71</td>
<td>37.00</td>
<td>12.47</td>
<td>9.705</td>
<td>16.73</td>
</tr>
<tr>
<td>P value</td>
<td>0.603</td>
<td>0.338</td>
<td>0.946</td>
<td>0.369</td>
<td>0.947</td>
</tr>
</tbody>
</table>

*Indicates significance (p < 0.05); M: mobile tooth; NM: non-mobile; SD: standard deviation.
Evaluation of microbiota around mobile and non-mobile teeth in chronic periodontitis

Table 5: Mean and standard deviation of micro-organisms (in CFUs) according to the grade of mobility.

<table>
<thead>
<tr>
<th>Mobility grades</th>
<th>Pg Mean±SD</th>
<th>Pi Mean±SD</th>
<th>A.a Mean±SD</th>
<th>Pm Mean±SD</th>
<th>C.r Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade I</td>
<td>22±18.35</td>
<td>20±17.32</td>
<td>30±26.45</td>
<td>40±64.09</td>
<td>36.66±18.93</td>
</tr>
<tr>
<td>Grade II</td>
<td>27.5±15.50</td>
<td>22.2±14.20</td>
<td>30±18.10</td>
<td>56±39.84</td>
<td>48±30.20</td>
</tr>
<tr>
<td>Grade III</td>
<td>26.77±13.45</td>
<td>16.66±10.22</td>
<td>24.88±15.26</td>
<td>81.11±50.66</td>
<td>42.22±12.77</td>
</tr>
</tbody>
</table>

![Chart Title](image)

**Fig. 1** Distribution of micro-organisms according to the grades of mobility.

Table 5 depicts the distribution of micro-organisms according to the grade of mobility. *P. micros* was the only micro-organism which showed a linear quantitative increase with increasing grade of mobility (Fig. 1).

**DISCUSSION**

The results of our study revealed that mobile teeth harboured significantly elevated proportions of all the studied putative periodontal pathogens, despite the fact that mobile and non-mobile teeth had same probing pocket depth.

Hence, the question arises whether mobility alters ecology in a periodontal pocket around mobile teeth which provides favourable environment for the growth of certain periodontopathic species or presence of certain pathogenic bacteria exhibit virulence features that induces aggressive tissue degradation leading to mobility. But as pointed out in the results of present study, out of the five tested perio-pathogens, four (*Pi, A.a, Cr, P.m*) were increased around mobile teeth, so that the possibility of alteration of ecology around mobile teeth due to mobility is more.

The vascular changes which causes an abundance of blood around mobile teeth may lead to altered ecology in periodontal pocket occur due to mobility can alter the ecology in periodontal pocket. Certain periodontopathic bacteria like *P. gingivalis* requires the blood components for their growth, but the results of the present study showed no significant increase in *P. gingivalis* around mobile tooth. Further studies are required to evaluate the proportions of *P. gingivalis* by splinting the mobile teeth which reduces the tooth mobility and in turn decreases the abundance of blood around mobile teeth. Hence, future studies can evaluate if abundance of blood around mobile teeth leads to an increase in periodontopathic bacteria.

In present study *C. rectus* and *P. micros* showed near about three-fold increase around mobile teeth as compared to non-mobile teeth. The current results are in accordance with the study done by Daniel et al. which revealed that mobile teeth harboured significantly elevated proportions of *C. rectus* and *P. micros*. *A. actinomycetemcomitans* and *P. intermedia* were also found to be statistically more around mobile teeth. A primary thought behind *C. rectus* colonising periodontal pockets is due to positive chemotactic response to formate that is released by *P. micros* and the other subgingival species. Such interactions in all the micro-organisms colonising around mobile teeth need to be probed. By understanding this symbiotic association we can plan for the better combating modalities against this microbial cluster around mobile teeth. To achieve this, adjunctive role of systemic antimicrobial therapy against *C. rectus*, *P. micros*, *A. actinomycetemcomitans* and *P. intermedia* needs to be verified for added advantage while dealing with the mobile teeth by further research.
REFERENCES


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