Detection of S. mutans and S. sobrinus from Dental Plaques Adhered on Zirconia, PFM and Ni-Cr Crown by RT-PCR

Shikha Gupta¹, Om Prakash², Dr. Raj Kumar Kalyan³, Dr. Dev Kumar Verma⁴, Dr. Jitendra Rao⁵, Dr. Chandkiram Gautam⁶

Abstract: The purposes of this study was to detect S. mutans and S. sobrinus from Dental Plaques adhered on Zirconia, PFM and Ni-Cr crown by RT-PCR amplification. Supragingival and the subgingival plaque was taken from the crown site of the 118 participants from all the three groups who had crown restoration and were categorized as Group I (Zirconia) (27 cases), Group II (PFM) (49 cases) and Group III (Ni-Cr) (42 cases), respectively. In the present study, the purposes of the study was to detect S. mutans and S. sobrinus from Dental Plaques adhered on Zirconia, PFM and Ni-Cr crown by RT-PCR amplification.

Keywords: Dental Plaques; Streptococcus Mutans; Streptococcus Sobrinus; Polymerase Chain Reaction; Crown

1. Introduction

Dental caries (tooth decay) is a chronic disease, caused by acidogenic bacteria, may lead to dissolution of enamel and dentin, (coronal caries) and cementum and dentin (root caries) (1). Streptococcus mutans and Streptococcus sobrinus are powerfully compromised in the onset of human dental caries. Many studies have shown the relationship of S. mutans and S. sobrinus with higher caries levels (2-4) and many studies have found that S. sobrinus occurrence is more strongly related with high caries activity than that of S. mutans (5-6). It is considered that determination of these two species in early childhood could be important for diagnosing and preventing dental caries.

Various authors (7-8) have developed PCR methods to detect S. mutans in samples of dental plaque. To facilitate biological sample collection for epidemiological studies, it has been developed a PCR method to detect S. mutans and S. sobrinus in saliva, using specific primers for the genes that encode glucosyltransferase (gtfB in S. mutans and gtfI in S. sobrinus), which can be used to assess the prevalence of these organisms in epidemiological studies (9).

Based on a strong relationship between microorganism counts in saliva and plaque, S. mutans determination in saliva has been suggested as a suitable method for identifying patients at high risk of dental caries (10). As saliva is continuously in contact with all the teeth, it provides a better reflection of the colonization of mutans streptococci on all dentition (11).

The purposes of this study was to detect S. mutans and S. sobrinus from Dental Plaques adhered on Zirconia, PFM and Ni-Cr crown by RT-PCR amplification.

2. Material and Methods

Plaque Sampling

Supragingival and the subgingival plaque was taken from the crown site of the 118 participants from all the three groups who had crown restoration and were categorized as...
Group I (Zirconia) (27 cases), Group II (PFM) (49 cases) and Group III (Ni-Cr) (42 case), respectively by using sterile curettes (Gracey Curettes). Dental examinations were performed under natural light, using a plane dental mirror and explorer. The plaque samples were suspended in 1 ml of sterile Phosphate buffer saline PBS (0.12 M NaCl, 0.01 M Na₂HPO₄; 5 mM KH₂PO₄; pH 7.5). The samples were transported on icebox to the laboratory until processed within 24 hours.

Genomic DNA Extraction from Plaque Samples

Phenol: chloroform method was used for extraction of genomic DNA according to Paster et. al. (16) with few modifications as 100 μL of plaque sample transferred to a new sterile micro centrifuge tube where 100 μL of the lysis buffer was added (50 mM tris buffer, 1 mM EDTA, 0.5% Tween 20, and proteinase K (200μg/ml) pH 8), then incubation at 55-60 °C was done for 1.5 hour. Inactivation of proteinase K was done at 90 °C for 5 minutes. After that, samples were cooled on ice for few minutes and the DNA was precipitated by the addition of an equal volume of ice-cold isopropanol and incubated at refrigerator overnight. The DNA pellet was washed with 70% ethanol, and then rehydrated in 20-30 μl TE buffer (10 mM Tris-HCl, and 1 mM EDTA) (pH 8) (16).

RT-PCR

Oligonucleotide primers F5'-ACTACAATTTGATGGGCTTGG and R5'-CAGTATAAAGCGCCAGTTTCATC were designated to amplify a 517 bp-DNA fragment of the gtf B sequence of S. mutans, and primers F5'-GATAACTACCTGACAGCTGACT and R5'-AAGCTGCTTAAAGGTAATCCT were designated to amplify a 712 bp-DNA fragment of the gtf B sequence of S. sobrinus. The PCR mixture was the same used by Oho et al. (16). The PCR conditions were denaturation at 95 °C for 1 min. This amplification was repeated for 30 cycles. The final cycle was run at 72 °C followed by annealing at 59 °C (7). The PCR products were separated through the gel at an electric current of 80 V for 1 hour by using horizontal gel electrophoresis apparatus. Gels were stained with etidium bromide (0.5μg/ml) and visualized on a UV transilluminator.

Statistical Analysis

Categorical variables will be presented in number and percentage (%) and continuous variables will be presented as mean and SD. Quantitative variables will be compared using one-way ANOVA between three groups. A p value of <0.05 will be considered statistically significant. The data will be entered in MS EXCEL spreadsheet and analysis will be done using Statistical Package for Social Sciences (SPSS) version 21.0.

3. Results

Table 1: Percentage distribution of patients according to their gender in all three groups

<table>
<thead>
<tr>
<th>Gender</th>
<th>Group I (Zirconia) (N=27)</th>
<th>Group II (PFM) (N=49)</th>
<th>Group III (Ni-Cr crown) (N=42)</th>
<th>Total (N=118)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>11(40.7)</td>
<td>29(59.2)</td>
<td>25(59.5)</td>
<td>55(45.1)</td>
</tr>
<tr>
<td>Female</td>
<td>16(59.3)</td>
<td>20(40.8)</td>
<td>17(40.5)</td>
<td>33(44.9)</td>
</tr>
</tbody>
</table>

Table 1 shows Percentage distribution of patients according to their gender in samples of all three groups.

Table 2: Age distribution: (18 to 40 years)

<table>
<thead>
<tr>
<th>Group</th>
<th>Age Mean±SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Zirconia) (N=27)</td>
<td>25.0±2.63</td>
<td>1.0000</td>
</tr>
<tr>
<td>Group II (PFM) (N=49)</td>
<td>35.2±3.12</td>
<td>0.8326</td>
</tr>
<tr>
<td>Group III (Ni-Cr crown) (N=42)</td>
<td>38.3±3.45</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Distribution of S. mutans and S. sobrinus in different base metal alloy groups. (N=118)

<table>
<thead>
<tr>
<th>Organism Present</th>
<th>S. mutans</th>
<th>S. sobrinus</th>
<th>Group I (Zirconia) (N=27)</th>
<th>Group II (PFM) (N=49)</th>
<th>Group III (Ni-Cr crown) (N=42)</th>
<th>Total (N=118)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td></td>
<td></td>
<td>10(37.0)</td>
<td>17(34.7)</td>
<td>15(35.7)</td>
<td>42(35.6)</td>
</tr>
<tr>
<td>-</td>
<td></td>
<td></td>
<td>5(18.5)</td>
<td>13(26.5)</td>
<td>11(26.2)</td>
<td>29(24.6)</td>
</tr>
<tr>
<td>+</td>
<td></td>
<td></td>
<td>3(11.1)</td>
<td>11(22.4)</td>
<td>10(23.8)</td>
<td>24(20.3)</td>
</tr>
<tr>
<td>-</td>
<td></td>
<td></td>
<td>9(33.4)</td>
<td>8(16.4)</td>
<td>6(14.3)</td>
<td>23(19.5)</td>
</tr>
</tbody>
</table>

The S. mutans and S. sobrinus was found to be present in 60.2% and 44.9% of cases respectively. In the present study, S. mutans alone was present only in 35.6% of cases where as S. sobrinus alone was present only in 20.3% of cases A significant finding of the present study was presence of both S. mutans and S. sobrinus in 24.6% of cases and 19.5% were negative for both (Table-3).

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Genomic DNA (gDNA) extracted from *S. mutans* was analyzed on 1% agarose gel electrophoresis and found to be good quality and quantity off or further use. Total gDNA isolated from dental plaque underwent PCR amplification using primer pair specific to 16S rDNA of the genus Streptococci [Figure 1]. One percent agarose gel electrophoresis depicts the positive PCR amplification at 712 bp when compared with standard 1kbp. Another PCR reaction was set using gtfB primers specific for *S. mutans* for functional gene identification. 1.2% agarose gel electrophoresis was done and a positive ampiclulation was observed at 517 bp when compared to 1000 bp standards, depicting the presence of functional genes (gtf) in *S. mutans*.

In the present study, dental plaque adhered on Zirconia, PFM and Ni-Cr crown was used for the detection of cariogenic micro-organisms rather than saliva. Various previous studies have used saliva for detecting cariogenic bacteria; however, the tendency in recent studies has been towards the use of dental plaque. As the intention was to relate the presence of cariogenic bacteria and dental caries, it was found that using saliva as a source of cariogenic bacteria does not permit establishing an effective association. Although the presence of *S. mutans* is high in saliva, it is lower on the surface of enamel, where this bacterium actually manifests its capacity to produce acids and result in subsequent demineralization.

In present study, gtfB was targeted as a functional gene, PCR amplification was done both with positive and negative controls with species-specific gtf primers. The amplification observed in 1.2% agarose gel electrophoresis depicts the presence of gtfB genes, hence confirming the presence of *S. mutans* in the dental plaque samples. Moreover, the amount of bacterial load can also be determined using real time PCR.

### References

1. Loeches WJ. Microbiology of dental decay and periodontal disease.

### Table 4: Oligonucleotidesquences, nucleotide (bp), used in this study for *S. mutans* and *S. sobrinus*

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Targeted gene</th>
<th>Oligonucleotide sequence (5'–3')</th>
<th>Nucleotide (bp)</th>
<th>Location</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. mutans</em></td>
<td>GTFB</td>
<td>(F) ACTACACTTTTCGGGTGGCTTGG</td>
<td>517</td>
<td>793–814</td>
<td></td>
</tr>
<tr>
<td><em>S. mutans</em></td>
<td>GTFB</td>
<td>(R) ICAGTAFAGCGCCAGTTTCACT</td>
<td>517</td>
<td>1288–1309</td>
<td></td>
</tr>
<tr>
<td><em>S. sobrinus</em></td>
<td>GTFI</td>
<td>(F) GATAACTACCTGACAGCTGACT</td>
<td>712</td>
<td>871–892</td>
<td></td>
</tr>
<tr>
<td><em>S. sobrinus</em></td>
<td>GTFI</td>
<td>(R) AAGCTGCCTTAAGGTAACACT</td>
<td>712</td>
<td>1561–1582</td>
<td><em>Oho T et al.</em> 2000</td>
</tr>
</tbody>
</table>

### Figure 1: Amplification of 16S gene using *S. mutans* DNA (gel picture)


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