Qualitative Analysis Of Salivary pH In Dental Caries Using Snyder Test: Original Research

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Abstract: Background : Caries activity can generally be defined as the occurrence and the rate at which, teeth are destroyed by acid production by the plaque bacteria.
Aims and objectives : To test the salivary pH in dental caries patients using Snyder test.

Materials and methods : DMFT index was calculated and patients were categorized according to the score. Saliva samples were collected from patients. Prepared Snyder's test medium was melted in the micro centrifuge tubes, which were then kept in hot water bath at 50°C. The fluid thiglycolate medium was prepared and poured in test tubes and kept ready. 0.2 ml of saliva was pipetted into the melted Snyder's medium at 50°C. After the addition of saliva into the medium, tubes were rotated for proper mixing of saliva and the medium. The micro centrifuge tube was incubated at 37°C for up to 72 h. The observations for colour change and pH were made every 24 h for a period of 72 h.

Results : A statistically significant difference was seen for the values between the groups for DMFT index, pH values at 24 hrs, 48 hrs and 72 hrs (p<0.01). There was a statistically highly significant difference seen for the frequencies of various types of colours at 48 and 72 hrs between the sub groups 1 & 2 (p<0.01) with lower frequency for yellowish green in subgroup 1 while higher of yellowish green in subgroup 2 at 48 hrs. Gender and age did not have an influence on the outcome of variable.

Conclusion : Snyder test was performed and it was found to be a simple and an inexpensive method for assessment of caries activity. We observed a close relationship between DMFT index and salivary pH value which could be suggestive of increased risk of dental caries within an acidic environment.

Keywords : Dental caries, DMFT, Snyder test.

INTRODUCTION

Dental caries, a multi-factorial disease is considered to be the most prevalent oral disease worldwide and also the main cause of tooth loss among the population of all ages.¹ Dental caries can be defined as a localized post eruptive, pathological process of external origin involving softening of the hard tooth & proceeding to the formation of a cavity.² This multi-factorial disorders, dental caries can affect the human in various ways i.e. presence of tooth pain, infection or dysfunction of the stomatognathic system can limit the necessary ingestion of energetic foods, affecting the growth in children and adults as well as their learning communication skills and recreational activities.³

The microbial community of caries is diverse and contains many facultatively and obligately-anerobic bacteria belonging to genera Actinomycyes, Bifidobacterium, Eubacterium, Lactobacillus and Parvimonas. It can also be caused by other bacteria Enterococcus fecalini, Prevotella Fusobacterium.⁴ However, the most common microorganisms related to caries are Streptococcus and Lactobacilli, present in the dental plaque formed along the tooth. Worldwide, the main species associated with caries in humans is Streptococcus mutans.⁵

Dental caries gets established in the mouth long before it manifests clinically in the form of visible lesions. This makes it possible to assess the caries activity in a patient or in a population. Caries activity can be defined as the occurrence and the rate at which, teeth are destroyed by acid production by the plaque bacteria.⁶ Currently there are many tests available which check for caries activity and caries susceptibility. One such test is Snyder Test used to estimate the relative number of lactobacilli in saliva. This test is a colorimetric analysis which measures the rapidity of acid formation when a sample of stimulated saliva is inoculated into glucose agar adjusted to pH 4.7 to 5 and with bromocresol green as colour indicator. Indirectly the test is also a measure of acidogenic and acidicuric bacteria.⁷⁻⁸ Hence the purpose of this study was to test the salivary pH in dental caries patients using Snyder test.
MATERIALS AND METHODS

The present study was carried out in a private dental college in Patna, Bihar. A total of 100 randomly selected individuals of either sex and any age group who gave their consent formed the study population. Individuals who had brushed their teeth at least 2 hours prior formed the study population. Subjects taking antibiotics for the last one month, additional fluoride use or the ones wearing any orthodontic or prosthodontic appliance were excluded from the study. Moreover, individuals with reduced salivary flow were also not included in the study.

Oral examination

All the participating subjects received a thorough oral examination using a mouth mirror and World Health Organization (WHO) probe in adequate illumination. The decayed, missing and filled teeth (dmft/DMFT) was recorded as per WHO criteria. The caries experience was calculated as the dmft + DMFT. The mean index value of the sample was 6.30 ± 1.85. The subjects were divided into two groups according to their dmft/DMFT scores.

Group I: with score between 1-5.
Group II: with score between 6-10.

All the subjects were taken for the study only after a gap of 90 min after the last intake of food or drink.

Collection of salivary sample

Subjects were asked to rinse their mouth with water and then instructed to chew paraffin for 25 chewing strokes on the same side and spit. This process was continued. The first two saliva samples were discarded, the third sample collected in a sterile vial. The entire collection procedure was repeated until 10ml of saliva had been collected.

Procedure for Snyder's caries activity test

Prepared Snyder's test medium was melted in the micro centrifuge tubes, which were then kept in hot water bath at 50°C. The fluid thiglycollate medium was prepared and poured in test tubes and kept ready. 0.2 ml of saliva was pipetted into the melted Snyder's medium at 50°C. After the addition of saliva into the medium, tube was rotated for proper mixing of saliva and the medium. The micro centrifuge tube incubated at 37°C for up to 72 h. An uninoculated tube was used as a negative control. The observations for colour change and pH were made every 24 h for a period of 72 h. The rate of colour change of Snyder's medium from green to yellow is indicative of caries activity (Figure 1).

Statistical analysis

Data obtained was subjected to statistical analysis using statistical package for social sciences (SPSS V21, IBM) software. Appropriate statistical tests were used. For all the statistical tests, P<0.05 was considered to be statically significant, keeping α error at 5% and β error at 20%, thus giving a powers to the study as 80%.

RESULTS

Age wise distribution of the sample was done. The minimum age recorded was 6 years and maximum was 65 years. The mean age of the subjects was 34.47 ± 12.00. Gender wise distribution of the subjects revealed that out of 100 samples, 41% were females and 59% males. The mean (dmft+DMFT) index of the sample was 6.30 ± 1.850. Out of 100 cases, the minimum index value recorded was 3 while the maximum was 10.

Inter group comparison of variables as per (dmft + DMFT) subgroups

On the basis of index scores, samples were classified into two subgroups. Subgroup I consisted of 35 individuals with index score ranging between 1-5 while subgroup II had 65 individuals with scores between 6 and 10. Table 1 shows the intergroup comparison in terms of pH value. Mean pH value in group A after 24 hours was 4.60 and in group B was 4.68. After 48 hours, mean pH values were 4.50 and 4.58 in groups A and B respectively. After 72 hours, the pH values had dropped to 4.40 in group A and 4.48 in group B. The difference in pH values of both the groups was found to be statistically significant at all the three time intervals which reduced gradually (Table 1). There was a statistically highly significant difference seen for the values between the groups for DMFT index, pH values at 24 hrs, 48 hrs and 72 hrs (p<0.01).
### Table 1: Intergroup comparison in terms of pH value.

Changes in the colour of the Snyder solution was recorded and was subjected to statistical analysis (Table 2, Figure 1). The colour of the solution at time of inoculation was blue, which changed to bluish green after 24 hours in all the 100 samples. The change in the colour of the solution at 48 and 72 hours was recorded as greenish yellow, pale yellow and yellowish green. After 48 hours, greenish yellow change was found in all the 35 samples of subgroup A and 49 of subgroup B. Pale yellow colour was noted in 2 samples of subgroup B while 14 samples of subgroup B showed yellowish green colour change. Colour change after 72 hours, was recorded as greenish yellow, pale yellow, yellow and yellowish green. After 72 hours, all 35 samples of subgroup A showed greenish yellow change, while 6, 20, 1 and 38 samples of subgroup B showed greenish yellow, pale yellow, yellow and yellowish green colour changes respectively. There was a statistically highly significant difference seen for the frequencies of various types of colours at 48 & 72 hrs between the sub groups 1 & 2 (p<0.01) with lower frequency for yellowish green in subgroup 1 while higher of yellowish green in subgroup 2 at 48 hrs.

*Significant; **Highly significant

<table>
<thead>
<tr>
<th>Time</th>
<th>Colours</th>
<th>DMFT subgroups</th>
<th>Chi square value</th>
<th>p value of chi square test</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 hrs</td>
<td>Subgroup-A</td>
<td>35</td>
<td>4.34, .725, .123</td>
<td>-12.340, 0.000**</td>
</tr>
<tr>
<td></td>
<td>Subgroup-B</td>
<td>65</td>
<td>7.35, 1.340, .166</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Subgroup-B</td>
<td>65</td>
<td>5.00, .000, .000</td>
<td></td>
</tr>
<tr>
<td>48 hrs</td>
<td>Subgroup-A</td>
<td>35</td>
<td>4.60, .071, .012</td>
<td>-2.771, 0.007**</td>
</tr>
<tr>
<td></td>
<td>Subgroup-B</td>
<td>65</td>
<td>4.68, .172, .021</td>
<td></td>
</tr>
<tr>
<td>72 hrs</td>
<td>Subgroup-A</td>
<td>35</td>
<td>4.50, .071, .012</td>
<td>-2.691, 0.008**</td>
</tr>
<tr>
<td></td>
<td>Subgroup-B</td>
<td>65</td>
<td>4.58, .174, .022</td>
<td></td>
</tr>
</tbody>
</table>

The colour of the solution at time of inoculation was blue, which changed to bluish green after 24 hours in all the 100 samples. The change in the colour of the solution at 48 and 72 hours was recorded as greenish yellow, pale yellow and yellowish green. After 48 hours, greenish yellow change was found in all the 35 samples of subgroup A and 49 of subgroup B. Pale yellow colour was noted in 2 samples of subgroup B while 14 samples of subgroup B showed yellowish green colour change. Colour change after 72 hours, was recorded as greenish yellow, pale yellow, yellow and yellowish green. After 72 hours, all 35 samples of subgroup A showed greenish yellow change, while 6, 20, 1 and 38 samples of subgroup B showed greenish yellow, pale yellow, yellow and yellowish green colour changes respectively. There was a statistically highly significant difference seen for the frequencies of various types of colours at 48 & 72 hrs between the sub groups 1 & 2 (p<0.01) with lower frequency for yellowish green in subgroup 1 while higher of yellowish green in subgroup 2 at 48 hrs.
### Table 2: Inter group comparison according to colour change.

<table>
<thead>
<tr>
<th>Colour of solution at I</th>
<th>Blue</th>
<th>35</th>
<th>65</th>
<th>---</th>
<th>----</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour change 24 hrs</td>
<td>Bluish green</td>
<td>35</td>
<td>65</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Colour change 48 hrs</td>
<td>Greenish yellow</td>
<td>35</td>
<td>49</td>
<td>10.256</td>
<td>0.006**</td>
</tr>
<tr>
<td></td>
<td>pale yellow</td>
<td>0</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>yellowish green</td>
<td>0</td>
<td>14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colour change 72 hrs</td>
<td>Greenish yellow</td>
<td>35</td>
<td>6</td>
<td>77.486</td>
<td>0.000**</td>
</tr>
<tr>
<td></td>
<td>Pale yellow</td>
<td>0</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yellow</td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yellowish green</td>
<td>0</td>
<td>38</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Highly significant

A comparison of numerical values between the genders was done. There was a statistically non significant difference seen for the value between the gender (P>0.05) thus indicating that gender did not have an influence on the outcome of variable. Similarly, non significant correlations were seen with age, indicating that age also did not have an influence on the outcome variable.

**DISCUSSION**

Caries activity tests are useful in establishing the risk for incidence of caries and for targeting specific preventive measures to the susceptible groups. They are even more useful in situations of limited resource availability. Saliva serves as a major component in most caries activity tests, and aids in the categorization of patients into high, medium and low caries activity.[9]

Studies carried out by Ferraro et al revealed females were more prone to caries when compared to males. The possible reason for this disparity was postulated to be multi-factorial. It was considered that males express a greater amount of amelogenin which contributes to the strength of the tooth, hence less caries. Salivary IgA, a protective immunoglobulin found in the oral cavity which acts as a protective mechanism against caries was detected in low concentration in women saliva. Moreover, pregnancy also has negative effects on salivary flow, impairing the protective washing and buffering mechanisms of saliva against caries development.[10] The findings of these studies were not in concordance with ours, as we observed a male predilection. On the other hand, Psoter et al suggested that supra and sub gingival biofilms in adolescents and both the gender was in equal proportion, thus showing no difference between the two genders.[11]

In our study, the mean DMFT index was found to be 6.30± 1.850. Based on the DMFT index an intergroup comparison of variables was carried out, which showed statistically significant value. A study carried out by Kunte et al showed similar result, where the DMFT index co-related well with the existing caries status of the individuals.[12] In a study carried out by Ramesh et al, comparison was done between the DMFT indices of the patient with the pH values at 24, 48, and 72 hours which showed statistically significant difference.[13]

In our study, it was found that with increase in the DMFT index, the pH value of saliva decreased. This finding was in accordance with Shetty et al.[14] In our study changes in the colour of the Snyder solution was recorded at 24,48 and 72 hours, and subjected to statistical analysis. There was statistically significant difference seen for the frequencies of various types of colour at 48 and 72 hours between the DMFT sub groups 1 and 2 with lower frequency
for yellowish green in subgroup 1 while higher of yellowish green in subgroup 2 at 48 hours. The change in the colour of the solution was recorded in relation to the change in the pH of the solution.

In our study, DMFT index was co-related with gender and numerical value of pH, but a statistically non significant difference was seen indicating that gender did not have an influence on the pH changes. Similar study was carried out by Lorne et al and they showed similar finding. This finding was in contrary to the results of an Australian study of preschool children that found males to have higher caries rate than females. A bivariate correlation of age with DMFT and pH at various time intervals was done, which also showed statistically non significant correlations, indicating that age did not have an influence on the outcome variables. This finding was in contrary to the findings of Lorne et al, where they stated that caries is age related. They found significantly higher caries rate in 5 year old children than in 3 year olds.

CONCLUSION

Dental caries is a disease with multi-factorial etiology which begins with the loss of ions from the apatite crystals and leads to cavitations. Caries activity tests are valuable adjunct for patient motivation in plaque control program. In our study, Snyder test was performed and it was found to be a simple and an inexpensive method for assessment of caries activity. We observed a close relationship between DMFT index and salivary pH value which could be suggestive of increased risk of dental caries within an acidic environment.

REFERENCES