**Influence of the blood group reactive substances in saliva on the aggregation of *Streptococcus mutans***

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**ABSTRACT**

*Streptococcus mutans* is one of the most dominant species of oral cavity that also initiates the dental plague formation. An attempt has been made to establish the correlation between the secretor status and aggregation of *Streptococcus mutans* with the salivary blood group reactive substances. The level of aggregation of *S. mutans* with salivary mucin was studied spectrophotometrically (630 nm) and has been greatly influenced by the blood group reactive substances. A person can be either a secretor or a non-secretor. This is completely independent of whether one’s blood type is A, B, AB or O. In the present study it was observed that out of the 98 individuals 94 were secretors. The secretor status was determined by absorption elution technique. The presence of *Streptococcus mutans* in the saliva was detected by the colony morphology grown on Nutrient agar followed by Gram’s staining and then the presence of *S. mutans* in the saliva were confirmed by their biochemical test of the same samples. The aggregation in the non-secretors is almost constant whereas the level of aggregation in blood group A, B, AB and O are found to be reducing with respective dilution.

**Key words:** *Streptococcus mutans*, aggregation, Infection immunity, Secretor.

**INTRODUCTION**

Interactions between bacteria and salivary components are of profound interest in oral biology. Salivary components facilitate and influence the adherence of micro-organisms on the teeth and oral epithelia and thus in dental plaque formation (1). Substances in saliva can interact both with mucosal-cell surfaces and with invading bacteria and these interactions may have a profound influence on the fate of bacteria entering the oral cavity. Dental caries is a complex infectious and transmissible disease (2), resulting from the interaction of several factors including the host, agent, substrate and time. Modern concepts consider dental caries as an interaction between genetic and environmental factors, in which social, biological, psychological and behavioral factors are expressed in a complex interactive manner (3).  

*D. mutans* was isolated for the first time from the dental plaque by Clarke in 1924. The name ‘mutans’ was chosen because of its tendency to exhibit both cocal and rod shaped (mutant) cell morphology. It is a primary aetiological agent in dental caries and is reported to colonize orthodontic patients in significant numbers (4). *Streptococcus mutans* is a Gram-positive, facultatively anaerobic bacterium commonly found in the human oral cavity and is a significant contributor to tooth decay (5, 6). *S. mutans* is one of a few specialized organisms equipped with receptors that improve adhesion to the surface of teeth. Sucrose is used by *S. mutans* to produce a sticky, extracellular, dextran-based polysaccharide that allows them to cohere to each other, forming plaque. *S. mutans* produces dextran via the enzyme dextranusecra (hexosyltransferase) using sucrose as a substrate in the following reaction:

\[ \text{n sucrose} \rightarrow (\text{glucose})^n + n \text{fructose} \]

Sucose is the only sugar *S. mutans* can use to form this sticky polysaccharide (5). Conversely, many other sugars—glucose, fructose, lactose can be digested by *S. mutans*, but they produce lactic acid as an end product. It is the combination of plaque and acid that leads to dental decay (7). In saliva there are various substances able to aggregate bacteria like α-amylase (8), lysozyme (9), secretory IgA (10) and blood group reactive glycoproteins (11). Blood group antigens are oligosaccharides found on the surface of erythrocytes and can be detected in other tissue cells and in body fluids like saliva, sweat, semen, milk etc. It has been established that, secretion of group specific substances in body fluids is controlled by a pair of alleles Se and se. Thus, a person can be homozygous [SeSe], heterozygous [SeSe] or homozygous [se]. The first 2 classes are called secretors and third class, non-secretor (12).

The aim of the research work is to determine the connection between secretor status in saliva and aggregation of *S. mutans* with salivary blood-group reactive substances.

**MATERIALS AND METHODS**

1. **Collection of saliva sample:** The saliva samples were collected randomly after taking consent from 98 subjects (irrespective of gender, caste, diet) for blood group A (23), B (18), O (13) and AB (9). (13)

2. **Bacterial strain:** The standard strain of *Streptococcus mutans* MTCC 890 was procured from IMTECH, Chandigarh, India.

3. **Spectrophotometric Analysis of Bacterial aggregation:** 0.1 ml saliva was taken in appendorf (collection vials, 1.5ml.) and serially diluted in 10mM potassium phosphate buffer (pH-7.0) with 0.5 mM calcium chloride. Subsequently, 0.2 ml of bacterial cell suspension (fresh bacterial culture of *Streptococcus mutans* grown in nutrient broth) was added to it. By aggregation the absorbance of a cell suspension decreases, which can be measured spectrophotometrically (630nm) (14). A non-aggregating cell suspension with buffer instead of saliva was used as a control. Two parameters were chosen to quantify the aggregation process: - the decrease in absorbance after one hour (6Abs), which was a measure for the rapidity of the aggregation process, and - the bacterial aggregating titer, which was a measure for the minimum salivary concentration inducing aggregation. For aggregation inhibition studies the bacterial cell suspensions were pre-incubated at 7°C for 10 to 30 min and were observed at 1000 x magnification.

4. **Determination of secretor status**

The secretor status of an individual was determined using Absorption Elution technique and was found to yield the most accurate results as compared to Absorption Inhibition technique and Mixed Agglutination technique. In this technique strong antiserum is used directly instead of its titre.

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because it takes less time to determine the secretor status of the individual.

(15, 16)

5). Biochemical test
Biochemical test for identification of unknown bacterial samples was done including Arginine Hydrolysis, 6.5% NaCl Tolerance Test, Starch Hydrolysis Test, Urea Hydrolysis test, Voges-Proskauer Test, (17), Manitol test, MR-VP (18)

RESULTS
It was observed that out of 98 individuals participated in the study for determining the secretor status, 4 were found to be non-secretor and remaining were secretors. The secretors showed good level of aggregation (Figure-1) as compared to non-secretors in which there was no aggregation (Figure-2) or less aggregation (Figure-3) seen of S. mutans with saliva sample. The absorption (630nm) was calculated for 70 individuals as shown in Table-1 and Table-2 interprets that the level of aggregation in the non-secretors remains nearly constant whereas the level of aggregation in secretors were found to be reducing up to 32-fold dilution. It is seen that at all the dilutions the bacterial aggregation is maximum in saliva samples that secrete Antigen O, followed by AB, B and the minimum in A. The aggregation in saliva samples of the non-secretors is negligible (Figure-4). Out of 98 saliva samples when 40 were employed for the biochemical characterization test, almost all the samples were found to be showing positive result for the presence of S. mutans (Table 3). All the samples were found to be Gram positive, and showing alike colony morphology when grown on the nutrient agar media. They were absent when grown in 6.5% NaCl and not utilizing Arginine but there is a fluctuation in the utilization of Mannitol. 15 samples out of 40 exhibited the utilization of Mannitol whereas 25 did not exhibit its utilization. They are also found to be utilizing starch.

DISCUSSION
Presence of microorganisms in saliva is based on the findings that there is an association between the type and number of bacteria in plaque and saliva. The total counts and the Actinomyces viscous/naeslundii and Streptococcus sanguis counts in dental plaque had increased by approximately two log units, while the Streptococcus mutans counts had increased by more than one log unit.(19). Evaluation of patients for Sjogren’s syndrome and radiation-induced xerostomia had the lowest salivary gland performance but displayed a mucosal status similar to denture-wearing healthy patients with normal salivary flow who had idiopathic xerostomia. However, those patients with a total lack of salivary flow rarely had normal-appearing oral mucosa. These results confirm a role for saliva in oral mucosal preservation and also suggest that other factors may act to maintain oral mucosal integrity (20). There is a strong correlation between salivary blood group antigen or secretor status and caries susceptibility. Authors have studied lower caries prevalence in secretors than in non-secretors (21) and among blood

Figure 1. The aggregation of Streptococcus mutans mutans with saliva sample

Figure 2. No aggregation of S. mutans with saliva sample

Fig: 1 Aggregation of S. mutans with saliva sample

Fig: 3 Less level of aggregation of Streptococcus mutans with saliva sample.

Fig: 4 Variation of optical density (cell concentration) with dilution

Reading interpretation for Biochemical Test:
1. Arginine Hydrolysis- Out of 40 samples only 3 samples showed positive result as the broth shows a deep purple colour indicating an alkaline reaction due to release of ammonia.
2. 6.5% NaCl Tolerance Test- All samples showed negative results as no turbidity appeared.
3. Starch Hydrolysis Test- A clear zone surrounding the growth is observed in all samples showing positive test
4. Urea Hydrolysis test- No colour change in the slants indicates a negative test in urea hydrolysis test for all samples.
5. Voges-Proskauer Test (VP)- Out of 40 samples, red colouration was observed only in 4 samples showing positive result.
6. Mannitol Test- The positive results for Mannitol Test were seen for 36 samples out of 40 samples.
<table>
<thead>
<tr>
<th>S.No</th>
<th>Sample No</th>
<th>Sex</th>
<th>Diet</th>
<th>Group tested from saliva</th>
<th>Reaction with Anti-A, B, D, H</th>
<th>Blood group from 4-fold</th>
<th>8-fold</th>
<th>16-fold</th>
<th>32-fold</th>
<th>Dilution Factor</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>29</td>
<td>F</td>
<td>Veg</td>
<td>B+</td>
<td>+ + + + B+</td>
<td>-0.03</td>
<td>-0.081</td>
<td>-0.09</td>
<td>-0.09</td>
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<tr>
<td>2</td>
<td>30</td>
<td>F</td>
<td>Veg</td>
<td>B+</td>
<td>+ + + + + A+</td>
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<td>-0.084</td>
<td>-0.08</td>
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<tr>
<td>3</td>
<td>31</td>
<td>M</td>
<td>Veg</td>
<td>+ + + + + A+</td>
<td>-0.05</td>
<td>-0.09</td>
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<tr>
<td>4</td>
<td>32</td>
<td>F</td>
<td>Veg</td>
<td>+ + + + + A+</td>
<td>-0.02</td>
<td>-0.01</td>
<td>-0.01</td>
<td>-0.02</td>
<td>-0.02</td>
<td>-0.02</td>
</tr>
<tr>
<td>5</td>
<td>33</td>
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<td>Veg</td>
<td>+ + + + + A+</td>
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<td>-0.076</td>
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<td>Veg</td>
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<td>-0.073</td>
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<tr>
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<td>Veg</td>
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<td>0.17</td>
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<tr>
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<td>Veg</td>
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</tr>
<tr>
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<td>37</td>
<td>M</td>
<td>Veg</td>
<td>+ + + + + A+</td>
<td>-0.215</td>
<td>0.084</td>
<td>0.08</td>
<td>0.045</td>
<td>0.084</td>
<td>0.084</td>
</tr>
</tbody>
</table>

Out of 98 samples 4 found to be non secretor and The O.D was taken at 630 nm interprets that the level of aggregation in the non-secretors remains nearly constant whereas the level of aggregation in secretors were found to be reducing up to 32-fold dilution.
The Optical density of \( S. mutans \) in Saliva samples of secretors and non-secretors interprets that the level of aggregation in the non-secretors remains nearly constant whereas the level of aggregation in secretors were found to be reducing up to 32-fold dilution. From the table it is seen that at all the dilutions the bacterial aggregation is maximum in saliva samples that secrete Antigen O, followed by AB, B and the minimum in A.

### REFERENCES


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