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# **ORIGINAL ARTICLE**

# ASSESSMENT OF SALIVARY STREPTOCOCCUS MUTANS LEVELS IN PREGNANT WOMEN: A COMPARATIVE STUDY

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#### **ABSTRACT:**

**Background:** One of the major etiologic factor in the occurrence of dental caries is Streptococcus mutans. In pregnancy, it is said that oral microbial flora shows a considerable alteration. Hence; we planned the present study to assess and compare the salivary streptococcus mutans levels in pregnant and non-pregnant subjects. **Materials & methods:** The present study included evaluation and comparison of salivary streptococcus mutans levels in pregnant and non- pregnant subjects. A total of 50 subjects were included, out of which, 25 were pregnant and 25 non- pregnant. Due to smaller sample size, we didn't sub- classified the pregnant subjects based on their trimester of pregnancy. A caries-associated bacteria testing kit with a culture method was used in the present study. Each participant was given a paraffin pellet (provided in the kit), to be chewed upon for 1 min, and was instructed to swallow the stimulated saliva after the removal of the pellet. Sterile Strip was rotated ten times on the participant's tongue and was withdrawn gently with the teeth apart and lips closed to obtain a thin layer of saliva on the strip. After doing the incubation procedure, all the strips were carefully removed from the incubator and were assessed for colony forming units as criteria given previously in literature. All the values were recorded, summarized and analyzed by SPSS software. **Results:** In pregnant and non- pregnant subjects, the mean salivary streptococcus mutans levels in the two study groups. **Conclusion:** Special attention should be given to the oral health status of the pregnant subjects as hormonal changes can alter the oral environment in them. **Key words:** Salivary, Streptococcus mutans, Pregnant

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#### NTRODUCTION

One of the major etiologic factor in the occurrence of dental caries is Streptococcus mutans.<sup>1, 2</sup> Tooth surfaces colonized with S. mutans are at a higher risk for developing caries. In populations with a relatively high caries experience, a positive association between salivary levels of S. mutans and dental caries experience have been reported.<sup>3-6</sup> Individuals with high levels of S. mutans also develop more coronal and root caries in temporary and permanent restorations than do individuals in the same population with lower concentration of S. mutans.<sup>7</sup> In pregnancy, it is said that oral microbial flora shows a considerable alteration.<sup>8</sup> Hence; we planned the present study to assess and compare the salivary streptococcus mutans levels in pregnant and non-pregnant subjects.

#### **MATERIALS & METHODS**

The present study was conducted in a private medical hospital and included evaluation and comparison of salivary streptococcus mutans levels in pregnant and nonpregnant subjects. After taking the ethical clearance from the hospital's ethical committee, we obtained written consent from all the patients, we took the salivary samples. A total of 50 subjects were included, out of which, 25 were pregnant and 25 non- pregnant. Due to smaller sample size, we didn't sub- classified the pregnant subjects based on their trimester of pregnancy. A caries-associated bacteria testing kit with a culture method was used in the present study. Each participant was given a paraffin pellet (provided in the kit), to be chewed upon for 1 min, and was instructed to swallow the stimulated saliva after the removal of the pellet. Sterile Strip was rotated ten times on the participant's tongue and was withdrawn gently with the teeth apart and lips closed to obtain a thin layer of saliva on the strip. This act was rehearsed in each individual before the actual procedure. The vials containing strips were coded and were transported within an hour of collection to the laboratory in an upright position, by placing them in their original boxes. In the laboratory the vials were given another code by one of the authors, in order to make it a blind study. The screw cap of the vials was opened by quarter of a turn so as to release any gas which could

form during incubation. The vials were incubated at 37° centigrade for 48 h. After doing the incubation procedure, all the strips were carefully removed from the incubator and were assessed for colony forming units as criteria given previously in literature.<sup>9</sup> All the values were recorded, summarized and analyzed by SPSS software. Chi- square test and independent t test were used for the assessment of level of significance. P- value of less than was taken as significant.

## RESULTS

**Table 1** shows the comparison of salivary streptococcus mutans levels in pregnant and non- pregnant subjects. In pregnant and non- pregnant subjects, the mean salivary streptococcus mutans levels were found to be 3.56 and 3.02,  $x10^5$ cfu/ml respectively. Non- significant results were obtained while comparing the mean salivary S. mutans levels in the two study groups.

 Table 1: Mean salivary streptococcus cfu according to pregnancy status

| S. mutans score | Mean CFU<br>(x 10 <sup>5</sup> cfu/ml) | p-value |
|-----------------|--|---------|
| Pregnant        | 3.56                                   | 0.89    |
| Non pregnant    | 3.02                                   |         |

NS: Non- significant

## DISCUSSION

Systemic condition of the body does affect the oral health status of the individual. The composition of human saliva altered during pregnancy, menstruation, is and menopause due to changes in steroid hormone levels. Many human studies indicate that during ovulation time, hormonal change influences the composition of human saliva. During pregnancy, there are profound physiologic changes due to complex hormonal interactions. Pregnancy brings changes in the salivary flow rate, pH of saliva. and biochemical composition. Salivary composition is of great importance for preventing caries incidence.<sup>10-12</sup> Hence; we planned the present study to assess and compare the salivary streptococcus mutans levels in pregnant and non-pregnant subjects.

In the present study, we observed a non significant difference in the salivary mutans levels of pregnant and non- pregnant subjects. In pregnancy, there is occurrence of decreased gastroesophageal sphincter tone and prolonged gastric emptying times. These changes along with decreased esophageal tone lead to ptyalism. Further, decreased large bowel motility leads to increased water absorption and constipation. These factors have been hypothesized to the increase in the saliva flow. Also, increase in salivary flow rate results in decreased microbial attack.<sup>13, 14</sup>

evaluated the salivary flow rate and pH in pregnant and non-pregnant Indian women and, consequently, to compare and correlate the salivary flow rate, pH, and prevalence of dental caries in both groups. A crosssectional study was conducted in dental institute on a sample of 30 pregnant and 30 non-pregnant women. The clinical findings for Decayed-Missing-Filled Teeth (DMFT) index were recorded. Unstimulated whole saliva was collected to determine the salivary flow rate and pH. Data were statistically analyzed using Student's t-test. Results: Salivary flow rate was lower in pregnant women as compared to that in non-pregnant women and the pH was also lesser in pregnant women than in non-pregnant women. DMFT index showed a strong negative correlation with pH in pregnant women and non-pregnant women. A difference was observed between the salivary parameters of pregnant and non-pregnant women in this sample. However, all the values were within the normal range. A significant inverse relation was found between salivary pH and dental caries for both the groups.<sup>15</sup>

Pannu P et al determine the level of Streptococcus mutans (S. mutans) in adults of Chandigarh and to correlate the dental caries in these individuals with their S. mutans titers. Salivary levels of Streptococcus mutans, using Dentocult SM commercial kits were estimated in 200, 25-35 year old adults (males and females). Streptococcus mutans were detected in 87% of the study sample. Score 2, representing 105-106 CFU/ml (Colony Forming Unit) of saliva was found to be most prevalent, i.e. in 80 of 200 adults, followed by score 1, depicting S mutans with <105 CFU/ml, in 56 of 200 adults and score 3, with bacterial titer >106 CFU/ml in 38 of 200 adults. Dental caries, recorded using Møller's index, was found to be maximum in individuals with score 3, followed by score 2,1 and 0, thereby showing a positive correlation of dental caries with increasing titers of S. mutans. This correlation was statistically highly significant in males with figures as 8.73 decayed surfaces at score 2 rising to 17.38 at score 3. The mean of DMFT was higher among females than in the males in their study. The split up data in males and females, showed a positive association between caries experience and salivary S. mutans scores. The results of the study will serve as a baseline data for future planning of preventive programs in adults.<sup>16</sup>

Seibert W et al showed an association between S. mutans levels and caries prevalence in a sample of elementary school children. The study group consisted of 242 school children, ages 5-13 years. The subjects were divided into two age groups, 5-8 years and 9-13 years. Approximately 59 percent were African Americans. The sample of 242 children were equally females and males, 50 percent in each group. The Dentocult SM Test was used to make S. mutans determinations. The df-t index was used to determine the number of decayed and filled teeth of children ages 5-8 years; the DMF-T Index estimated the number of decayed, missing or filled teeth of children ages 9-13 years. Dental caries were found in 58 percent of the children (mean = 2.67, and range of 1-11). Approximately 47 percent of the children with caries had high S. mutans levels (100K-1M). Females had higher S. mutans levels than males in the 9-13 age group, p < .05. Analysis of Variance Test indicated that S. mutans levels for older females (ages 9-13) were significantly higher than those observed in males the same age (p < .01). This trend was not observed in younger children, ages 5-8 years. In addition, no significant difference or interaction was noted by sex for S. mutans levels and decayed or

filled teeth (df-t) for younger children. We conclude that high levels of Streptococcus mutans are related to increased number of decayed teeth and conversely, low Streptococcus mutans levels are related to fewer dental caries.<sup>17</sup>

Kishi M et al assessed the relationships of quantitative salivary levels of Streptococcus mutans and S. sobrinus in mothers with the colonization of mutans streptococci (MS) in plaque and caries status in their 2.5-year-old children. Furthermore, the dynamics of caries status in the children was evaluated in a 2-year follow-up survey. After oral examination of 54 mother-and-child pairs, the saliva samples from the mothers and the plaque samples from the children were collected. The levels (log DNA copies/ml saliva) of S. mutans and S. sobrinus were quantified using real-time polymerase chain reaction (PCR) assays, while MS in the plaque samples were detected using a cultivation method. In addition, 50 of the 54 children participated in a 2-year follow-up survey of caries prevalence. In the 2.5-year-old children, the percentage of dft-positive subjects and mean number of dft were significantly higher in the MS(+) group when compared with the MS(-) group. Findings from the 2-year follow-up survey indicated that MS(+) subjects had a persistently higher mean number of dft at 4.5 years. The 2.5-year-old children were divided into three groups based on the quantitative levels of salivary S. mutans and S. sobrinus in their mothers: those whose mothers had low levels of S. mutans (<4 log DNA copies/ml) and S. sobrinus  $(\leq 2)$  (group 1); those whose mothers had a high level of S. mutans (> or = 4) and low level of S. sobrinus  $(\leq 2)$  (group 2); and those whose mothers had high levels of both (> or = 4 and > or = 2, respectively) (group 3). Among the three groups, the percentages of MS(+) and dft-positive children were highest in group 3 and lowest in group 1. Furthermore, multiple logistic regression analyses revealed that grouping the mothers based on salivary level of S. mutans and S. sobrinus was an efficient means to predict both MS colonization (OR = 2.96) and prevalence of dental caries (OR = 9.39) in children at 2.5 years of age. In the 54 mother-and-child pairs tested, the maternal salivary levels of S. mutans and S. sobrinus determined by real-time PCR were significantly related to MS colonization in plaque as well as dental caries in their children at 2.5 years of age. Thus, determination of maternal levels of both organisms using the present cut-off values is proposed as an efficient method to indicate the risks of maternal transmission of MS and childhood dental caries.<sup>18</sup>

## CONCLUSION

Special attention should be given to the oral health status of the pregnant subjects as hormonal changes can alter the oral environment in them. However, future studies are recommended.

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