



Research Article

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Association Between the Salivary *Streptococcus Mutans* Levels and Dental Caries Experience in Adult Females

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Abstract

Objectives: The aim of this study was to look at the possible relationship between heavy colonization of salivary *Streptococcus mutans* and caries occurrence and other factors affect the colonization among adult mothers in Sana'a city, Yemen.

Study design: A total of 261; 19-55 year old mothers were selected. Clinical examination of mothers were conducted to estimate dental caries experience with the Silness-Loe index, as well as stimulated saliva were collected to assess *S.mutans* levels by colony forming units (CFU). Difference in proportions calculated the differences between the groups and the associated OR of colonization with tested factors were estimated and significance was determined by chi square and P value.

Results: Out of 261 mothers, 72 (27.6%) showed heavy colonization of *S.mutans*. Overall 12 (4.6%) mothers were caries free (Score 0) and 249 (95.4%) presented with caries (Score 1-3). There was significant growth in the rate of *S.mutans* heavy colonization with growing caries score (score 1=20.8%, 32.2% for score 2 and 46.2% for score 3). There was an increase in the rate of *S.mutans* heavy colonization with growing age. Regarding oral hygiene practices, there was significant growth in the rate of *S.mutans* heavy colonization with using mouthwashes while significant drop in the rate with using flossing and regular visit of dentist.

Conclusion: A high rate of heavy colonization of *S.mutans* occurred, with significant growth in the rate with growing caries score, there is a negative effect of using regular mouthwashes while a positive one with using flossing and regular visit of dentist.

Keywords: Colony forming units (CFU); Saliva; Dental caries; *Streptococcus mutans*; Colonization; Adults

Introduction

Streptococcus mutans has been concerned like one of the main etiology of dental caries [1,2]. It is known that tooth surfaces inhabited with *S. mutans* are on a high risk of developing caries [1-3]. Appearing in people with a relatively high caries experience, a positive relationship between high salivary levels of *Streptococcus mutans* and dental caries experience have been reported [4,5]. Those with high levels of *Streptococcus mutans* as well acquire more coronal and root caries in permanent restorations and temporary restorations than persons appearing in the same population with lower intensity of *Streptococcus mutans* [5-7]. *S. mutans* salivary levels are straight linked to the number of tooth sites colonized [8] and to their amount in dental plaque [9].

Greater part of the studies on frequency distribution of *Streptococcus mutans* and its correlation with dental caries have been carried out on children [3,10] and a few on adolescents [11-13] while the data on adults are uncommon [13-17]. Saliva has been used to screen the oral load of *Streptococcus mutans* and other micro-organisms [10]. In adults the mechanisms of pathogenesis of dental caries are to a great extent more complex than in children. Pay no attention to the levels (concentration) of *S. mutans*; data from previous researches prove that the numbers of carious lesions per tooth in the adult population are to a certain extent higher [13-17,18]. Therefore, more information is required regarding the spreading of *Streptococcus mutans* and correlation

of levels of *Streptococcus mutans* and caries in adults. The current study was planned in mother's population of Sana'a city, Yemen (i) to determine the *S. mutans* levels in their stimulated saliva, (ii) to correlate the dental caries in these individuals with their *S. mutans* titers (iii) to correlate the heavy *S. mutans* colonization with host and oral hygiene practices, and (iv) to determine antibiotic sensitivity of *S. mutans* isolates.

Material and Methods

The present study was conducted in the Department of Conservative Dentistry and Oral Health, Faculty of Dentistry, Sana'a University, Republic of Yemen. The study protocol was approved by the ethics committee of Sana'a University. A written informed consent was obtained from the selected participants.

Study participants

The study included 261 mothers attending dental clinics in Sana'a city. The selected mothers were selected randomly from adult mothers attending dental clinics of public hospitals and private dental clinics in Sana'a city during a period of three months, starting in June and ending in August 2015.

Recording of dental caries

All of the study mothers were examined by the same examiner. The intra-examiner calibration was performed with respect to the diagnostic criteria of caries. The Silness-Loe plaque index for mothers was done. This index is based on the field clinical examination of the study participants using a probe, mirror and cotton rolls, in addition to simply counting the number of decayed, missing (due to caries only) and restored teeth.

Salivary analysis

Method of saliva collection: Saliva collection was scheduled after the clinical examination. Participants were made to swallow their preexisting saliva, in order to clear the mouth of any residual un-stimulated saliva. After this each participant was asked to chew a standard piece of paraffin wax, for 5 min to induce the stimulated saliva needed for collection. The saliva samples of all the

participants were labeled using a code number during the period of sample collection and processing.

Microbiological procedure: The sample was transported to the laboratory immediately after collection using Thioglycollate broth and processed on same day. The sample was vortexed (15 sec) and diluted 1:1000 in isotonic saline solution prior to inoculation. One loop (1/1000th ml of sample) was inoculated on the Mitis Salivarius agar with potassium tellurite medium, bacitracin and 20% sucrose. The plates were incubated at 37°C anaerobically. After 72 hour, colony characteristics were studied and the number of colony-forming units of *S. mutans* (CFU/ml) in saliva was determined using a colony counter in which ≥ 105 CFU/ml indicated heavy colonization.

Statistical analysis: Epi Info version 7 was used for analysis data. Difference in proportions and associated odds ratio and test of significance were calculated using 2X2 tables and selected uncorrected statistical test for chi square and 2 tailed p values for significance. Level of statistical significance was assumed at $p < 0.05$.

Results

The detailed results of this study are presented in 3 tables. Out of 261 mothers, 72 (27.6%) showed heavy colonization of *S. mutans*. There was ascending in the rate of *S. mutans* heavy colonization with growing age but not significant (Table 1). Overall 12 (4.6%) mothers were caries free (Score 0) and 249 (95.4%) mothers presented with caries (Score 1-3). In regards to the study mothers whom have presented with caries; 120 (45.9%) had score 1, 90 (34.5%) had score 2 and only 39(14.9%) had score 3, moreover, there was significant grow in the rate of *S. mutans* heavy colonization with growing caries score (score 1=20.8%, 32.2% for score 2 and 46.2% for score 3) (Table 2). Regarding oral Hygiene practices and oral conditions of the mothers, there was significant grow in the rate of *S. mutans* heavy colonization with using mouthwashes while significant drop in the rate with using flossing and regular visit of dentist (positive effect) (Table 3).

Table 1: The association between heavy colonization of *S. mutans* and different age groups of mothers under study.

| Age groups (years) | Positive <i>S. mutans</i> heavy colonization $\geq 10^5$ n=72 | | OR | CI 95% | χ^2 | p |
|--------------------|---|------|-----|---------|----------|------|
| | Number | % | | | | |
| ≤ 20 n=63 | 14 | 22.2 | 0.7 | 0.3-13 | 1.9 | 0.27 |
| 20-29 n=81 | 17 | 21 | 0.6 | 0.3-11 | 2.5 | 0.1 |
| 30-39 n=105 | 36 | 34.3 | 2.7 | 1.0-3 | 3.9 | 0.04 |
| 40+ n=12 | 5 | 41.6 | 1.9 | 0.5-6.3 | 1.2 | 0.2 |
| Total n=261 | 72 | 27.5 | | | | |

Table 2: The association between heavy colonization of *S. mutans* with the Silness-Löe Index for mothers.

| Index | Positive <i>S. mutans</i> heavy colonization $\geq 10^5$ n=72 | | | | CI | χ^2 | p |
|---------|---|----|------|-----|-----------|----------|-------|
| | Total No (%) | NO | % | OR | | | |
| Score 0 | 12 (4.6%) | 0 | 0 | 0 | undefined | 4.7 | 0.02 |
| Score 1 | 120 (45.9%) | 25 | 20.8 | 0.5 | 0.2-0.9 | 5.1 | 0.02 |
| Score 2 | 90 (34.5%) | 29 | 32.2 | 1.4 | 0.8-2.4 | 1.4 | 0.2 |
| Score 3 | 39(14.9%) | 18 | 46.2 | 2.7 | 1.3-5.3 | 7.9 | 0.004 |

Silness-Löe Index: Score 0 = The tooth surface is clean; score 1 = The tooth surface appears clean; but dental plaque can be removed from the gingival third with a sharp explorer; Score 2=Plaque is visible along the gingival margin; Score 3=The tooth surface is covered with abundant plaque

Table 3: The association of heavy *S. mutans* oral colonization and oral hygiene practices and oral conditions for the adult mothers.

| OR | Positive <i>S. mutans</i> heavy colonization $\geq 10^5$ n=72 (27.6%) | | | | CI | χ^2 | p |
|----------------------|---|----|------|------|-----------|----------|--------|
| | Frequent use of | NO | % | OR | | | |
| Mouth wash | yes, n=120 | 42 | 35 | 1.9 | 1.1-3.4 | 6.1 | 0.01 |
| | No, n=141 | 30 | 21.3 | 0.5 | 0.2-0.6 | 6.1 | 0.01 |
| Tooth brushing | Yes, n=186 | 51 | 27.4 | 1 | 0.5-1.6 | 0.09 | 0.92 |
| | No, n=75 | 21 | 28 | 0.9 | 0.1-1.7 | 0.09 | 0.92 |
| Flossing | Yes, n=36 | 4 | 11.1 | 0.26 | 0.09-0.6 | 5.6 | 0.01 |
| | No, n=225 | 68 | 30.2 | 3.4 | 1.3-10.1 | 5.6 | 0.01 |
| picks | yes, n=84 | 24 | 28.6 | 1.1 | 0.6-1.9 | 0.06 | 0.8 |
| | No, n=187 | 48 | 27.1 | 0.9 | 0.5-1.6 | 0.06 | 0.8 |
| Gum bleed | Yes, n=111 | 27 | 24.3 | 0.7 | 0.4-1.3 | 1.02 | 0.31 |
| Dry mouth | yes, n=87 | 21 | 24.1 | 0.76 | 0.4-1.3 | 0.7 | 0.37 |
| Bad odor | yes, n=135 | 24 | 9.2 | 0.35 | 0.19--.6 | 13.4 | <0.001 |
| regular dental visit | yes, n=183 | 18 | 33.3 | 0.04 | 0.02-0.09 | 96 | <0.001 |
| | No, n=78 | 54 | 26.1 | 20 | Oct-40 | 96 | <0.001 |

Discussion

Oral diseases are major public health problems as a result of the high prevalence in all countries of the world and the greatest impact on the socially marginalized populations. Therefore, the assessments of carries risks are very important. The researches in this topic give an opportunity to improve oral health, oral hygiene practices, and implement preventive measures in representation populations [19].

Overall 12 (4.6%) mothers were caries free (Score 0) and 249 (95.4%) mothers presented with caries (Score 1-3) and there was significant grow in the rate of *S. mutans* heavy colonization with growing caries score with odds ratio of 2.7 for score 3 (Table 2). The results are in accordance with the studies conducted by some other researchers who reported a positive correlation between the concentration of heavy level *mutans streptococci* in saliva and dental caries [9,14]. Individuals with lower concentrations showed a significantly lower number of decayed surfaces compared with the individuals with higher concentrations of *mutans streptococci* in their saliva [20]. Result of a study conducted by Ito et al. in Japan showed that *S. mutans* heavy colonization was correlated with the onset of primary and secondary caries, with odds ratios of 2.34 and 2.22, respectively [21]. Giacaman et al. and van Palenstein et al. in their studies reported that *S. mutans* heavy colonization was not associated with high caries experience which is different from the results of the present study [22,23].

There was ascending in the rate of *S. mutans* heavy colonization with growing age in the present study. When *S. mutans* heavy colonization were reported by growing age in the dental literature, older ages were found to exhibit typically higher prevalence rates of *S. mutans* heavy colonization than younger [24] and this finding is similar to our results. A high *S. mutans* heavy colonization rate in older ages can be attributed to fact that *Streptococcus mutans* older patients affected with more periodontitis leads to elevated counts of *S. mutans* than younger patients [24].

In the present study, using antiseptic mouthwashes contain fluoride / chlorhexidine etc caused a significant increase of

salivary *S. mutans* colonization (OR= 1.9, p=0.01) (Table 3). This finding is at variance with several previous reports on the effect of mouth washes contain chlorhexidine and fluoride in which they confirmed that mouthwashes kill and prevent *S. mutans* and *Lactobacillus* to colonize on teeth surfaces and prevent forming bacterial plaque [25,26]. This result is in agreement with findings of Gunsolly [27]; and Tal and Rosenberg [28] in which they affirmed that the use of mouthwashes alone does not eliminate bacterial colonization (biofilm formation) and there is need beside that for both brushing and flossing to control *S. mutans* colonization (kill the bacterial plaque).

Regarding brushing, there was no significant effect of brushing in the rate of *S. mutans* heavy colonization (Table 3). This result is in contrast with previous studies in which they confirmed that regular proper brushing eliminated bacterial colonization (biofilm formation) [27,28]. Our non-significant result of brushing might be due to irregularity improper technique of brushing practices in our study group.

One of the limitations of this study is that a cross-sectional study design was used to determine the correlation between *S. mutans* and dental caries experience like several other studies. However a single saliva sample would record the microbial counts at one particular point of time and its well understood that dental caries develops over a considerable period of time, during which bacterial counts would fluctuate in response to the changing oral environments [29].

Conclusion

Longitudinal studies, where microbial samples are taken at regular intervals would help to study the variation in counts of microorganisms. However, the results of this study will add to the existing data on the host and hygiene factors correlated with *S. mutans* heavy colonization and in caries, which can be used for planning preventive programs in adults, to reduce the factors of *S. mutans* heavy colonization hence prevent them.

Conflict of Interest

No conflict of interest associated with this work.

Author's Contribution

This research work is part of A MSc thesis. The candidate is the third author (ALAAM) who conducted field and laboratory works and wrote up the thesis. The corresponding author (HAA) help in laboratory works, supervised the works, revised and edited the thesis draft and the manuscript. (IZA) was co-advisor of the work. KMA also helped in the field works.

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