

## ORIGINAL ARTICLE

# A correlative study of the levels of salivary *Streptococcus mutans*, lactobacilli and *Actinomyces* with dental caries experience in subjects with mixed and permanent dentition

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Received: 18-09-2015

Accepted: 10-02-2016

## ABSTRACT

**Purpose:** The aim of the study was to estimate the salivary levels of *Streptococcus mutans*, *Lactobacilli* and *Actinomyces* and to correlate it with dental caries experience in mixed and permanent dentition. **Materials and Methods:** The sample size comprised 110 subjects. The decayed, missing and filled teeth (DMFT) index of all the individuals participating in the study was calculated. Saliva samples were collected from patients and samples were inoculated on specific culture media and incubated for a period of 48 h. Based on colony characteristics, *S. mutans*, *Lactobacilli* and *Actinomyces* were identified. **Results:** A positive correlation exists between DMFT and *S. mutans*, *Lactobacilli* and *Actinomyces* in mixed dentition and permanent dentition group samples ( $P < 0.001$ ). **Conclusion:** The conclusion from the results obtained was that *S. Mutans*, *lactobacilli* and *Actinomyces* which are the components of the normal microbial flora of the oral cavity play an important role in the pathogenesis of dental caries and increased number of microorganisms is associated with an increased caries frequency.

**Key words:** *Actinomyces*, decayed, missing and filled teeth, dental caries, *lactobacilli*, *Streptococcus mutans*

## INTRODUCTION

Oral diseases qualify as major health problems owing to their high prevalence and incidence in all regions of the world, the greatest burden of oral diseases being the disadvantaged and socially marginalized populations.<sup>[1]</sup> Dental caries continues to plague most of the world's population despite overly optimistic claims of success in the elimination of this disease.<sup>[2]</sup> The disease results due to an imbalance between the demineralization and remineralization process. Thus, an acidic environment is a prerequisite for caries formation and acidogenic microflora play an important role. Oral bacteria

like *Streptococcus mutans* and *Lactobacillus* are the main microorganisms implicated in the initiation and progression of caries, respectively.

*Actinomyces*, *Bacteroides*, *Bifidobacterium*, *Campylobacter*, *Capnocytophaga*, *Cornybacteria*, *Fusobacteria*, *Neisseria*, *Prevotella*, *Selenomonas*, *Veillonella* spp., *Propionibacterium*, *Atopobium* and other low pH non streptococci are the other microorganisms implicated in caries pathogenesis.<sup>[3]</sup> As it is proved that microorganisms play an important role in

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**How to cite this article:** Chokshi A, Mahesh P, Sharada P, Chokshi K, Anupriya S, Ashwini BK. A correlative study of the levels of salivary *Streptococcus mutans*, *lactobacilli* and *Actinomyces* with dental caries experience in subjects with mixed and permanent dentition. J Oral Maxillofac Pathol 2016;20:25-8.

## Access this article online

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### Website:

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### DOI:

10.4103/0973-029X.180916

caries initiation and progression, this study has been taken up to estimate the levels of salivary *S. mutans*, *Lactobacillus* and *Actinomyces* and to correlate the number of individual organisms with the caries incidence.

## MATERIALS AND METHODS

### Source of data

One hundred and ten subjects included in the study were subjected to random sampling method. Ethical clearance was taken from the institution and school authorities before the start of the study. They were further divided into three groups; ten caries-free subjects constituting control group and two study groups each containing fifty subjects. The first group constituted subjects with carious teeth in mixed dentition and the second group constituted subjects with carious teeth in permanent dentition.

### Methods

The oral examination of subjects was carried out under natural light using plane mouth mirrors and World Health Organization (WHO) probes. No radiographs were used. The same examiner examined all subjects. Caries experience was determined by decayed, missing and filled teeth (DMFT), dmft and dmfs-using WHO criteria.

### Salivary analysis

Unstimulated saliva was collected from each subject and microbiological assay and salivary tests commenced within 24 h of saliva collection.

### Method of saliva collection

Saliva collection was scheduled after the clinical examination. The subjects were asked to rinse mouth with normal tap water. They were asked to spit their saliva into the sterile plastic containers (Tarsons). About 3–4 ml of whole unstimulated saliva was collected over 2 min. The saliva samples of all the participants were identified by a code number during the period of sample collection and processing.

### Microbiological procedure

The samples were transported immediately to the laboratory and inoculated on specific culture media (mitis-salivarius agar for *S. mutans*, Rogosa SL agar for *Lactobacillus*, McBeth Scales starch mineral agar for *Actinomyces*). The saliva was taken in a 0.001 µl loop and was inoculated by streaking in all the three media and placed in the incubator at 37°C for 48 h. After 48 h of incubation at 37°C, the growth of the colonies was recorded as positive growth. Based on colony characteristics, *S. Mutans*, *Lactobacilli* and *Actinomyces* were identified. Colony counting was done on the digital colony

counter and the count was expressed as the number of colony forming units per millimeter (CFU/ml) of saliva.

### Statistical analysis

Correlation between DMFT and the levels of salivary *S. mutans*, *Lactobacilli* and *Actinomyces* in both mixed and permanent dentitions were analyzed using Spearman's rank correlation. Mean and standard deviation were calculated for all the variables using *t*-test and Mann–Whitney Test.

## RESULTS

The results of the study showed that a strong positive correlation existed between DMFT and the levels of salivary *S. mutans*, *Lactobacilli* and *Actinomyces* ( $P < 0.001$ ) in both mixed [Table 1] and permanent dentitions [Table 2]. The difference in mean *S. mutans* between mixed dentition and permanent dentition was found to be statistically significant [Table 3], but a difference in mean *Lactobacilli* [Table 4] and *Actinomyces* [Table 5] between mixed dentition and permanent dentition was not found to be statistically significant.

## DISCUSSION

Dental caries ranks among the most common of human diseases simply because of its frequency of occurrence. Dental caries is often seen as a multifactorial disease with the interplay of

**Table 1: Correlation between decayed, missing, filled teeth and microorganisms in mixed dentition with study group and mixed dentition with control group samples**

Organism	Mixed dentition (study group)		Mixed dentition (control group)	
	$\rho$	<i>P</i>	$\rho$	<i>P</i>
<i>Streptococcus mutans</i>	0.957	<0.001*	0.968	<0.001*
<i>Lactobacillus</i>	0.838	<0.001*	0.874	<0.001*
<i>Actinomyces</i>	0.734	<0.001*	0.768	<0.001*

Spearman's rank correlation test done for DMFT and the salivary microorganism in mixed dentition of study and control group showed a statistically significant correlation with  $P < 0.001$  ( $*P < 0.005$  is considered as statistically significant). DMFT: Decayed, missing and filled teeth

**Table 2: Correlation between decayed, missing, filled teeth and microorganisms in permanent dentition (study group) and permanent dentition (control group) samples**

Organism	Permanent dentition (study group)		Permanent dentition (control group)	
	$\rho$	<i>P</i>	$\rho$	<i>P</i>
<i>Streptococcus mutans</i>	0.866	<0.001*	0.896	<0.001*
<i>Lactobacillus</i>	0.733	<0.001*	0.784	<0.001*
<i>Actinomyces</i>	0.646	<0.001*	0.693	<0.001*

Spearman's rank correlation test done for DMFT and the salivary microorganism in permanent dentition of study and control group showed a statistically significant correlation with  $P < 0.001$  ( $*P < 0.005$  is considered as statistically significant). DMFT: Decayed, missing and filled teeth

**Table 3: Comparison of *Streptococcus mutans* (t-test)**

Group	Mean ( $\times 10^3$ CFU/ml)	SD	SEM	MD	P
Mixed dentition	27.58	6.05	0.86	3.680	0.003*
Permanent dentition	23.90	6.07	0.86		

\* $P < 0.005$  is considered as statistically significant. The difference in mean *Streptococcus mutans* between mixed dentition and permanent dentition was found to be statistically significant ( $P < 0.01$ ). SD: Standard deviation, SEM: Standard error of mean, MD: Mean difference, CFU: Colony forming units

**Table 4: Comparison of *Lactobacillus* (t-test)**

Group	Mean ( $\times 10^3$ CFU/ml)	SD	SEM	MD	P
Mixed dentition	17.94	3.45	0.49	1.400	0.062
Permanent dentition	16.54	3.93	0.56		

\* $P < 0.005$  is considered as statistically significant. The difference in mean *Lactobacillus* between mixed dentition and permanent dentition was not found to be statistically significant ( $P > 0.05$ ). SD: Standard deviation, SEM: Standard error of mean, MD: Mean difference, CFU: Colony forming units

**Table 5: Comparison of *Actinomyces* (Mann-Whitney U-test)**

Group	Mean ( $\times 10^3$ CFU/ml)	SD	SEM	MD	P
Mixed dentition	4.08	2.52	0.36	0.540	0.321
Permanent dentition	3.54	2.04	0.29		

The difference in mean *Actinomyces* between mixed dentition and permanent dentition was not found to be statistically significant ( $P > 0.05$ ). SD: Standard deviation, SEM: Standard error of mean, MD: Mean difference, CFU: Colony forming units

microorganisms, dietary factors and salivary composition.<sup>[4]</sup> There are large numbers of cariogenic microorganisms that can be defined by their ability to induce caries. *S. mutans*, *Streptococcus sanguis*, *Lactobacillus casei* and *Actinomyces viscosus* fulfill most of these criteria.<sup>[5]</sup> The mutans group of streptococci play a central role in the initiation of caries on the smooth surfaces and fissures of the crowns of the teeth and suggest that they have a potent etiologic role in the induction of root surface caries also.<sup>[6]</sup> *Lactobacillus* is involved in the progression of carious lesions and carious dentin is the main ecological site of lactobacilli.<sup>[7]</sup> The *Actinomyces* are carbohydrate users but are not powerfully acidogenic or acid tolerant.<sup>[3]</sup> *Actinomyces naeslundii* and *A. viscosus* are known to contribute to the initiation and progression of human dental caries, especially root caries. The DMFT index is one of the most widely used indices for presenting epidemiological data about the caries experience of a population. However, this index relates to past signs of the disease, since it allows verifying the incidence or prevalence of decayed, missing and filled teeth but does not reveal if the carious lesion is active or not.<sup>[8]</sup> The present study was carried out to assess the correlation of salivary *S. mutans*, *Lactobacilli* and *Actinomyces* levels with dental caries experience in subjects with mixed and permanent dentition. The salivary

samples were collected to assess the *S. mutans*, *Lactobacillus* and *Actinomyces* and then the CFUs were calculated and tabulated. In the present study, the difference in mean *S. mutans* between mixed dentition and permanent dentition was found to be statistically significant ( $P < 0.01$ ), whereas the difference in mean lactobacilli and *Actinomyces* between mixed dentition and permanent dentition was not found to be statistically significant ( $P > 0.05$ ). In the present study, a positive correlation was found between DMFT and *S. mutans* in mixed dentition and permanent dentition group samples, with a  $P < 0.001$  which denotes significant correlation. These results are in agreement with studies by Pradopo, Hong and Hu, Hebbal *et al.*, Sakeenabi and Hiremath, Ravindran *et al.* and Pannu *et al.* All of them concluded that the levels of *S. mutans* were positively correlated to the caries incidence.<sup>[1,4,9-12]</sup> In the present study, a positive correlation was found between DMFT and *Lactobacillus* in mixed dentition and permanent dentition group samples, with a  $P < 0.001$  which denotes significant correlation. These results were consistent with Pradopo, Farsi, Hebbal *et al.*, Sakeenabi and Hiremath and Bhayat *et al.* These authors stated that the levels of *Lactobacilli* can be an indicator of caries experience.<sup>[4,9,10,13,14]</sup> In the present study, a positive correlation was found between DMFT and *Actinomyces* spp. in mixed dentition and permanent dentition group samples with a  $P < 0.001$  which denotes significant correlation. These results were in accordance with studies by Toi *et al.* and Marchant *et al.* who found out that the proportion of *A. naeslundii* and *Actinomyces odontolyticus* were significantly greater in the plaque samples than in the lesion samples. *Actinomyces israelii* formed 18.2% of the flora from the lesions but was not isolated from the plaque samples. Aparna *et al.* stated that *Actinomyces* occurred most frequently in root carious lesions in older adults as compared to the middle-aged population. Aas *et al.* stated that *Actinomyces* spp. were likely to play an important role in caries progression.<sup>[15-18]</sup>

Microbiologic diagnosis is advantageous in the management of the patients whenever an expensive treatment is planned, such as orthodontic treatment or the placement of dental crowns and bridges to replace missing teeth and also to prevent or minimize future decay. Microbiologic examination is also useful at the end of any restorative treatment to determine the residual levels of microbial colonization on the teeth.<sup>[19]</sup>

Even though many authors have stated that a positive correlation exists between the salivary microorganisms and caries experience of an individual, there are other authors who stated that the correlation is not positive. Fredy Gamboa *et al.* concluded that no statistically significant difference exists in *S. mutans* counts between the group with dental caries and the caries free group and not all the children hosting this microorganism had caries. Hegde *et al.* concluded that a highly significant relation exists between *S. mutans* and DMFT categories, while *Lactobacilli* were not statistically related to DMFT categories.<sup>[20]</sup>

However, the results of the present study reinforce most of the previous scientific literature, which state that the oral microbial flora play an important role in cariogenicity as a result of its ability to colonize teeth causing a marked reduction in pH in the presence of a sugar substrate and consequently inducing caries.

Research in cariology is sky-rocketing, bringing out the hidden facts of this age-old disease, as a result of which caries vaccine is emerging but the education and clinical practice is adopting them in a snail pace.<sup>[21]</sup>

*S. mutans* play a key role for the development of dental caries and that a vaccine directed against this microorganism could be a valuable adjunct to existing preventive measures in some countries. Only a few studies, however, have examined the efficacy of dental caries vaccines in humans. Although several years have passed, active immunization against caries remains a goal yet to be achieved. The successful development of vaccines against oral diseases requires a concerted effort by industry, government and academia and also it is a matter of great importance to ensure safety along with effective protection.<sup>[22]</sup>

## CONCLUSION

The present results conclude that *S. mutans*, lactobacilli and Actinomyces play an important role in the pathogenesis of dental caries. An increase in numbers of these microorganisms is associated with an increase in caries frequency. Further, the detection of salivary levels of microorganisms is important in order to be able to evaluate the occurrence of dental caries and also play a role in its prevention.

## Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.

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