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Salivary Levels of Cariogenic Streptococcus and Lactobacillus among Tobacco Abusers in Andhra Pradesh, India.

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ABSTRACT

Saliva is the first biological fluid that is exposed to tobacco which is responsible for the changes in salivary pH. The aim of this study was to examine the relationship of caries risk, salivary pH, and levels of cariogenic Streptococcus and Lactobacillus in relation to tobacco abuse. A total of 96 patients aged (20-80) years, chosen from the Department of Oral Medicine and Radiology of Sri Sai College of Dental Surgery, Andhra Pradesh, India. They were divided equally into tobacco smokers, chewers and smokers/chewers with caries and without caries and interviewed about tobacco abusing behavior. Non-stimulated salivary sample was analyzed for cariogenic Streptococcus, Lactobacilli count and salivary pH. Present study indicates that the level of Streptococcus is appreciably higher among smokeless tobacco abusers. Whereas, a non-significant relation was obtained among the groups presented with Lactobacilli. A lower salivary pH was observed in tobacco smokers as compared with chewers. There was a significant relation between Streptococcus and salivary pH in caries-free chewers and Lactobacilli and salivary pH in subjects (consume smoke and smokeless tobacco) with dental caries. These alterations in bacterial count and salivary pH due to long-term effect of tobacco usage can render oral mucosa vulnerable to various dental diseases.

Keywords: Tobacco abusers, Effects of Tobacco on dental caries, Mutans Streptococcus, Lactobacillus spp.

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INTRODUCTION

Tobacco abuse has become a global epidemic and also a serious worldwide health concern. It is estimated that by 2025 the number of smokers would rise by 1.6 billion and the number of deaths due to this habit is expected to reach 6.4 million in 2015 and 8.3 million in 2030 [1].

In India, a survey carried out in 2009-2010 reported that more than one-third of the adults are addicted to tobacco in some form which included 48% males and 20% females. Among them 29% of adults used tobacco on a daily basis [2].

Tobacco in its many forms is a risk factor for various systemic diseases, periodontal disease, and gingivitis. Although, several studies indicate an association with tobacco abuse and caries, its relation to dental caries is still a subject of controversy [3].

The cariogenic capacity of oral microorganisms is necessary to be facilitated by appropriate substrates and host-related physiological conditions to enable microbial colonization and survival facilitating the development of caries lesions [5]. Saliva possesses an important impact through functions relying on its physicochemical characteristics such as flow rate, pH and buffering capacity; so variations under threshold levels are considered risk factors for the development of dental caries [6] and a shift of the bacterial population towards higher number of Lactobacilli and cariogenic streptococci [7]. Although, saliva is the first biological fluid to expose and be affected by the numerous toxic compositions of tobacco (smoked/smokeless form), yet the means by which tobacco modifies the caries process and its relationship with availability of saliva in the mouth is still unclear [8].

The purpose of this study was to investigate the effect of tobacco on count of cariogenic Streptococcus, Lactobacilli, salivary pH and finally on dental caries.

MATERIAL AND METHODS

Samples

A total of 96 individuals aged 20 – 80 years were selected and classified into 6 groups of 16 each as follows:

Group I: Tobacco chewers with dental caries.
Group II: Tobacco chewers without dental caries (caries free).
Group III: Smokers individuals with dental caries.
Group IV: Smokers individuals without dental caries.
Group V: Smokers and Tobacco Chewers with dental caries.
Group VI: Smokers and Tobacco Chewers without dental caries.

The source of material for the isolation of the caries pathogens was saliva. The specimens were received after obtaining the informed consent from the patients. A proforma was recorded for each study case to analyze the age, sex, marital status, risk factors such as smoking, tobacco chewing and detailed clinical examination. Tobacco users were further enquired regarding the type (cigarettes or beedis or any other forms; pan masala or ghutka or other chewing tobacco; snuff), smoked duration and frequency and the placement of the quid in the oral cavity was elicited from the users of smokeless tobacco. All selected individuals were required to have no history of focal infection in the three months prior to the study or prior to the dental treatment at the time of examination, the absence of dental abscesses and of any medication therapy. Each person was instructed not to eat or drink anything for two hours before the appointment. The dental examination was performed by trained dentist in a dental chair, using a dental mirror and an explorer.

All patients with history of tobacco consumption were made aware of the harmful effects of tobacco and were motivated to quit the habit.

The one month investigation was conducted in the Department of Microbiology among the patients visiting the Department of Oral Medicine and Radiology of Sri Sai College of Dental Surgery, Andhra Pradesh, India and was approved by the ethics committee of the college.

Saliva Collection

From each subject, fresh whole non-stimulated saliva samples were collected from the oral cavity where it was allowed to accumulate at the floor of the mouth and was transferred in sterile plastic sample vials (HiMedia, India), discharging the first portion.

Isolation of Bacterial Strains

The freshly collected saliva samples were cultured on plates of selective media: Mitis Salivarius Bacitracin Agar [3, 9] and Rogosa SL agar [4, 10] (HiMedia, India) for the primary isolation of Streptococcus spp. and Lactobacilli respectively. The cultured mitis salivarius agar plates were incubated aerobically in a candle jar (5% to 10% CO_2), whereas the cultured Rogosa agar plates were incubated anaerobically. All plates were kept at 37°C for 48h. The levels of microorganisms were expressed as LogCFU/ml.

The Streptococcus isolates were identified by gram's staining (Figure 1), colony morphology (Figure 2) and was characterized biochemically (Figure 3) as described elsewhere [3, 9, 11]. Gram's staining (Figure 4), colony morphology (Figure 5), catalase test (not illustrated) and Hugh-Leifsons fermentation test (Figure 6) confirmed the identity of Lactobacilli [10, 12].

Saliva pH Measurement

The pH of the samples was measured in a digital pH meter (pHep, Hanna, Italy) according to the manufacturer's instructions. Briefly, 1 mL of saliva was mixed with 3 mL of distilled water, subject to bubbling.

Statistical analysis

The Statistical Package for Social Science (SPSS INC Chicago link), was used for analysis. Difference in microbial counts was tested using ANOVA followed by Turkey's test for intragroup comparison. ANOVA followed by Turkey's post hoc was used for the analysis of difference in means of salivary pH. Levels of caries associated flora were correlated with salivary pH using Pearson correlation coefficient. Level of statistical significance was assumed at $p < 0.05$.

RESULTS

Relation between Dental Caries, Streptococcus, Lactobacillus Counts and Tobacco Abuse

Association between tobacco consuming, dental caries and numbers of Streptococcus is well documented in (Table 1). Increased numbers of Streptococcus have been associated with tobacco chewing in groups (I and V). Smokers reported more frequent healthy oral health behavior than did non-smoke tobacco abusers (Table 2). Table 3 shows the identified species of Streptococcus.

The count of lactobacilli LogCFU/ml saliva was statistically insignificant between the groups evaluated (P=0.374) as represented in Table 1.

Relation between Dental Caries, Salivary pH and Tobacco Abuse

Salivary pH was statistically significant (P<0.001) among the 6 groups (Table 4).

Salivary pH of tobacco chewers with caries was significantly higher than that of individuals practicing both smoke and smokeless tobacco having dental caries; similarly pH of chewers with no caries was significantly higher than that of caries-free smokers and tobacco smokers and chewers with caries. The salivary pH of the individuals (with caries) practicing both the forms of tobacco is further lesser than that of the caries-free group. No other significant differences were seen.

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Correlation between Salivary pH, Cariogenic Streptococcus and Lactobacillus Count

Table 5 demonstrates the correlation of tobacco abusing behaviors, dental caries experience with salivary variables which include pH and bacterial load; there was a highly significant relation between salivary pH and Streptococcus (LogCFU/ml) in caries-free chewers, also with the level of Lactobacillus in chewing/smoking abusers with caries.

Oral Hygiene Practice by the Study Groups

About 80% of all subjects in each study group used toothpaste, a toothbrush (except for Group III) and more than 90% of them brushed once a day to maintain oral hygiene as indicated in Table 6.

Figure 1: Gram's stained Streptococcus mutans

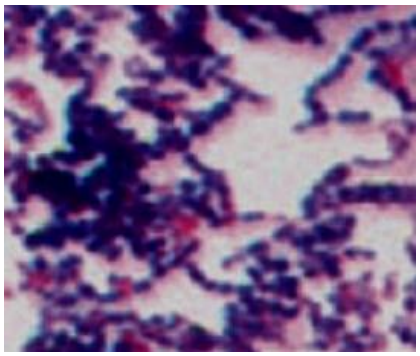


Figure 2: Streptococcus mutans on Mitis Salivarius Bacitracin Agar

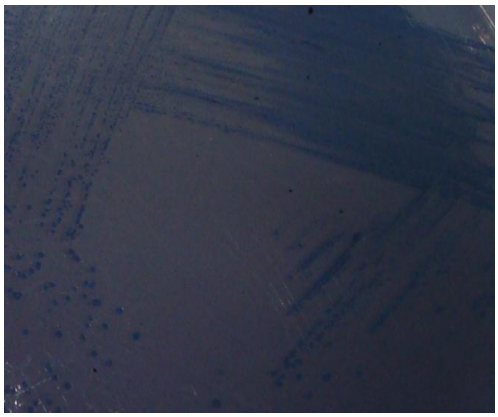
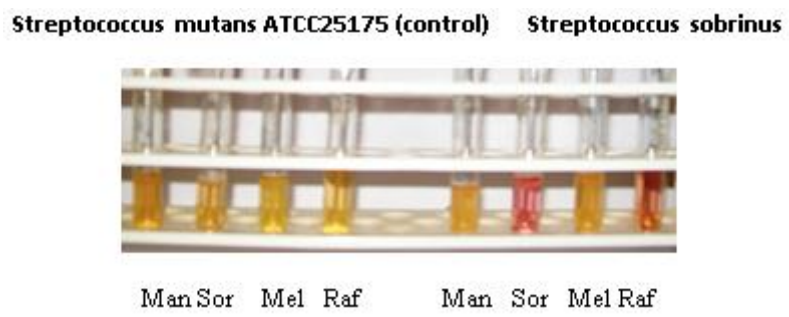


Figure 3: Carbohydrate utilization tests by Streptococcus sobrinus isolated from tobacco abusers



Man = Mannitol; Raf = Raffinose; Sor = Sorbitol; Mel = Mellibiose. Yellow = Positive reaction, Red = Negative reaction.

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Figure 4: Gram's stained Lactobacillus

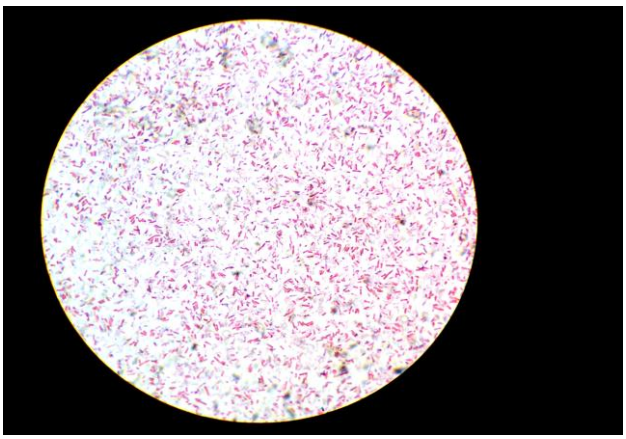

Figure 5: Rogosa SL Agar with Lactobacillus growth

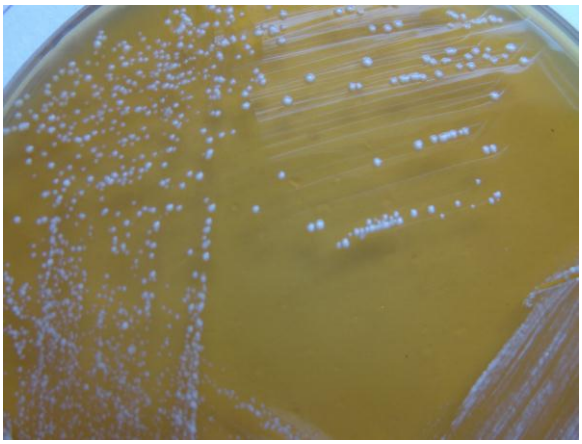

Figure 6: Hugh-Leifsons fermentation test of Lactobacillus

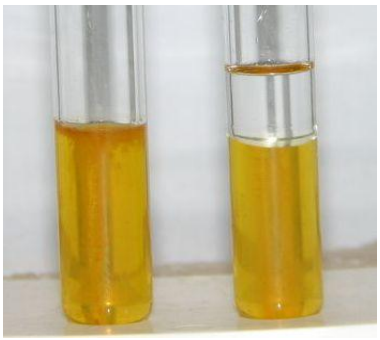

Table 1: Mean AND Standard deviation (SD) of count(LogCFU/ml) of specific bacteria in each studied group

	GROUP												P-value
	I		II		III		IV		V		VI		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Log Streptococcus	4.70	.12	4.71	.08	4.03	.20	4.20	.48	4.01	.49	4.52	.41	<0.001
Log Lactobacilli	4.51	.42	4.45	.47	4.50	.27	4.52	.17	4.15	.42	4.40	.08	0.374
ANOVA with turkey's test ($p < 0.05$; significant)													

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Table 2: Intergroup comparison of count of specific bacteria

	Comparison	P-value
Streptococcus	I vs II	1.000
	I vs III	**<0.001**
	I vs IV	**.007**
	I vs V	**<0.001**
	I vs VI	.870
	II vs III	.123
	II vs IV	.415
	II vs V	.104
	II vs VI	.983
	III vs IV	.842
	III vs V	1.000
	III vs VI	**.039**
	IV vs V	.770
	IV vs VI	.423
	V vs VI	**.029**

	Comparison	P-value
Lactobacillus	I vs II	1.000
	I vs III	1.000
	I vs IV	1.000
	I vs V	.415
	I vs VI	.991
	II vs III	1.000
	II vs IV	1.000
	II vs V	.719
	II vs VI	1.000
	III vs IV	1.000
	III vs V	.302
	III vs VI	.988
	IV vs V	.613
	IV vs VI	.995
	V vs VI	.774

Table 3: Distribution of cariogenic Streptococcus (n/N) %

SPECIES	GROUP-I	GROUPII	GROUPIII	GROUP-IV	GROUP-V	GROUP-VI
Streptococcus mitis	(5/16)31.3	(2/16)12.5	(4/16)25	0	(8/16)50	(2/16)12.5
Streptococcus sanguis	(9/16)56.3	(1/16)6.3	(8/16)50	(6/16)37.5	(6/16)37.5	(4/16)25
Streptococcus mutans	(2/16)12.5	(1/16)6.3	(2/16)12.5	(5/16)31.3	(1/16)6.3	0
Streptococcus sobrinus	0	0	(2/16)12.5	0	(1/16)6.3	(1/16)6.3

Table 4: Mean and standard deviation (SD) of salivary pH in each studied group

	GROUP												P-value	Post-hoc test
	I		II		III		IV		V		VI			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
pH	8.09	.78	8.58	.65	7.82	.77	7.60	.73	7.15	.56	8.09	.95	<0.001, significant	I>V II>IV,V VI>V
ANOVA with post-hoc turkey's test														

Table 5: Correlation between salivary pH, cariogenic Streptococcus and Lactobacillus count

GROUP			Log Streptococcus	Log Lactobacilli
I	**pH**	Pearson Correlation	-.062	-.447
		p-value	.819	.374
		N	16	6

II	**pH**	Pearson Correlation	-1.000**	-.695
		p-value	.	.305
		N	2	4
III	**pH**	Pearson Correlation	-.068	.110
		p-value	.803	.747
		N	16	11
IV	**pH**	Pearson Correlation	.131	-.146
		p-value	.702	.907
		N	11	3
V	**pH**	Pearson Correlation	-.326	-.814*
		p-value	.218	.049
		N	16	6
VI	**pH**	Pearson Correlation	.123	-.441
		p-value	.792	.381
		N	7	6
**. Correlation is significant at the 0.01 level (2-tailed). *. Correlation is significant at the 0.05 level (2-tailed).				

Table 6:Oral hygiene measures in study populations

	GROUP-I n (%)	GROUP-II n (%)	GROUP-III n (%)	GROUP-IV n (%)	GROUP-V n (%)	GROUP-VI n (%)
Cleaning material						
Toothpaste	14 (87.5)	13 (81.25)	10 (62.5)	15 (93.75)	14 (87.5)	14 (87.5)
Toothpowder	0	0	01 (6.25)	0	0	0
Others	02 (12.5)	03 (18.75)	05 (31.25)	01 (6.25)	02 (12.5)	02 (12.5)
Frequency						
Once	16 (100)	16 (100)	15 (93.75)	16 (100)	16 (100)	16 (100)
More than once	0	0	01 (6.25)	0	0	0
Cleaning method						
Toothbrush	14 (87.5)	13 (81.25)	11 (68.75)	14 (87.5)	14 (87.5)	14 (87.5)
Fingers	0	0	03 (18.75)	02 (12.5)	01 (6.25)	0
Others	02 (12.5)	03 (18.75)	02 (12.5)	0	01 (6.25)	02 (12.5)

DISCUSSION

Use of tobacco in various forms [13,14] and its interaction is known to cause abnormality in salivary pH , flow rate as well as the oral micro-flora thereby influencing the initiation and progression of dental caries [15,16]. Bartoloni examined dental caries in Air Force personnel and reported that the tobacco use had an elevated risk of developing caries [17]. Aguilar-Zinser examined the relationship of smoking of professional truck drivers and reported that the number of cigarettes is positively correlated to the number of large caries. [18, 19] Collectively, the evidence suggests smoking is a possible risk factor for caries and thereby responsible for tooth loss, probing attachment loss and dental caries [20]. Offenbacher and Weathers [21]

reported that the presence of gingivitis was an indicator of oral hygiene and that poor oral hygiene was a cofactor with smokeless tobacco use in the development of dental caries. Liede et al indicated that tobacco smokers implicated in dental/oral conditions, such as increased Lactobacilli [18, 22, 23] and Streptococcus mutans [18, 24, 25] demonstrated reduced buffering capacity, thereby, an increased susceptibility to caries.

A few studies show a decreased activity of Streptococci and other oral commensals in smokers [15] and other studies failed to show any differences [26]. In another study, dental caries were significantly related to the presence of Streptococcus mutans as well as tobacco smoking [3]. Results of this study indicate a significant positive association between the presence of caries and associated Streptococcus in tobacco chewers (Group I and V). This could be explained by two factors: first, smokeless tobacco in India [27] contained varying amount of sugars which could be responsible for root caries as well as an increased amount of gingival recession in smokeless tobacco users [28]. Second, studies showed that smoking is associated with lower salivary cystatin activity and output of cystatin C during gingival inflammation, are thought to contribute to maintain oral health in smokers by inhibiting certain proteolytic enzymes [3].

Nagarajappa et al. showed that use of chewing tobacco decreased the colony-forming units' count of Lactobacillus [29]. Al-Weheb et al. found lactobacilli count to be higher in smokers group than non-smokers which were statistically significant [4]. In the present study, no statistically significant difference was recorded between Lactobacilli in smokers, chewers and chewers/smokers, indicating that the Lactobacilli count did not vary with respect to the forms of tobacco being practiced. This was in contradiction to the above studies.

Alterations in salivary pH have a significant impact on oral and dental health and can be used for the diagnosis of a wide range of diseases [30, 31]. Saliva pH changes have been cited as variables for modifying caries risk [18]. A report suggested that over longer time periods smokers had a lower pH in stimulated whole saliva however another report showed no difference [32, 33]. Khan et al observed a lower salivary pH in smokers than in non-smokers [34]. Rooban et al observed a mean pH of 6.77 in non-chewers and the mean pH turns acidic in raw form of areca nut chewers [30]. In contrast, Reddy et al observed no difference in salivary pH between the chewers and non-chewer [35]. In another study, salivary pH was found to be lower in tobacco smokers and tobacco chewers than in subjects with no such habits, but the difference was statistically insignificant [36]. In the present study, salivary pH was significantly different across groups with lower pH in smokers than in chewers. Moreover, this study revealed a highly significant relation between salivary pH and Streptococcus in caries-free chewers; Lactobacillus in subjects (having smoking and chewing habit) with caries.

The method of oral hygiene care used by the subjects in the present study did not differ among study groups indicating that the tobacco abuse is an important factor that differed in the study population.

Several limitations of the study design have to be considered when interpreting the findings from this present study. Data on tobacco use are based on the survey participants' self-reported information which carries an inherent potential for bias and have relatively low rates of misreporting. Non-use of radiographic diagnostic aids would have understated the actual incidence of dental caries. Also, as one cannot assume a casual association between tobacco abuse and tooth loss, DMF, DMFS and/or DMFT because the point of time when tooth loss or decay occurred cannot be established; these variables were not included in this study. Finally, comparison of all results with other studies was not possible, as disparity between results exist which could be attributed to difference in dietary pattern, oral hygiene practice, genetic and several other factors peculiar to the study population and distinctness in the technique of sampling saliva and cultivation of bacteria [12].

CONCLUSIONS

The present study, to the best of our knowledge, is the first study to document and compare till date the risk of dental caries and compare it across various commonly abused tobacco, viz., chewing, smoking or both forms in relation to salivary pH.

These findings reiterate the followings:

- A relationship exists between tobacco chewing and Streptococcus.
- There is not a relationship between tobacco abusing status and Lactobacilli.

- A relationship exists between non-stimulated salivary pH and Streptococcus in caries-free chewers; Lactobacilli in individuals (with caries) practicing both smoke and smokeless tobacco.

From the present study, we can conclude that the long-term use of tobacco especially the smokeless form can cause significant alteration in salivary pH. The alterations in bacterial count and salivary pH due to long-term effect of tobacco usage can render oral mucosa vulnerable to various oral and dental diseases. Therefore, tobacco chewing and smoking cessation should be considered in the treatment of caries and be a part of health prevention in dentistry. Finally, prospective studies as well as genetic microbiological analyses are required to elucidate controversial influence of tobacco on caries associated bacteria and salivary pH. Also, future studies with a larger sample size should account for protective and contributing factors such as oral hygiene regimens and dietary pattern.

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