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Streptococcus mutans and Lactobacillus Proportion in the Saliva of the patients Undergoing Extraction Procedure

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ABSTRACT

To assess the influence of salivary bacterial flora in the complete loss of tooth structure leading to extraction. The objective of this study is to evaluate the influence of salivary levels of Streptococcus mutans and Lactobacillus that leads to tooth extraction. If the etilogy is known prophylactic measures or the steps to retain the tooth can be initiated at the early stage of the lesion. Dental extraction is the process of removal of tooth from the dental socket in the alveolar bone. It is performed for wide variety of reasons the most common reason is tooth that has become unrestorable through tooth decay and the surrounding tissues. This is caused by both facultative anaerobes and obligate anaerobes. This study is aimed to assess the contribution of two major populations of bacterial species in saliva that contributes to tooth loss. The study comprised of 60 subjects divided into three groups, 20 healthy individuals, 20 individuals both male and female reporting for restoration due to dental caries and 20 subjects both male and female reported for tooth removal were randomly selected. The saliva samples were collected in sterile container and subjected for microbiological analysis. The study was conducted in the year 2019. There was a significant increase in the mutans and lactobacillus in the patients coming for dental extraction. This study concludes that there is a many fold increase in the total bacterial count of the saliva and there is also a significant proportionate increase in Streptococcus mutans and Lactobacilli. Further study can be conducted to demonstrate that penetration into deeper layers and bone structures.

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INTRODUCTION

The mouth is a complex structure it consist of both smooth and rough structures that are coated with saliva and numerous microbes. The oral cavity houses more than 700 microbial species. Only few among those are predominantly found in the oral cavity and some of these belong to the normal microbial flora. [1]. The normal microbial flora can be further divided into resident flora and transient flora , which is further divided into supplemental flora those that are present constantly but in lower numbers and indigenous flora these are organisms that are present constantly in high numbers.[2] The most predominate among them are the resident bacterial flora, whenever there is shift in the normal flora the transient bacterial flora proliferates to cause disease.[3] There is constant change in the oral microbial flora as it ages, during the first few months of life before the eruption of the tooth the commonly seen organisms are Lactobacillus, S.salivarius and Fusobacterium are commonly seen, as it ages following tooth eruption the commonly seen organism are S.mutans, S.sanguis and Spirochetes. As age progresses there is tooth eruption, tooth loss, insertion of artificial teeth or prosthesis, tooth is subjected to scaling and

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restoration this affects the ecological system within the mouth.[4] There is a transient change in the stability oral ecosystem which is caused due to type of food ingested, periods of antibiotic therapy, hormonal changes of the host, any alterations in the salivary flow caused by any medications which results in decreased salivary flow predisposing to caries. In old age the host immunity reduces and this explains the increased isolation of Staphylococci and enterococci in the elderly individuals. [5][6] The oral cavity houses various bacterial species which includes both gram positive and gram negative organisms, the commonest of which being Streptococcus mutans, Streptococcus mitis, Streptococcus sanguis, Staphylococcus, Fusobacterium, Enterococcus, ,Peptostreptococcus, Lactobacillus, Actinomyces bifidus, Actinomyces israeli, Actinomyces naeslundi, Nocardia, Cornybacterium, Neisseria, and small amount of Treponemas.[7] There exists microbial homeostasis i.e., the composition of micro flora at a specific site in the oral cavity remains constant over time despite regular minor alteration.[8]. The oral microbes face multiple changes that are not faced by any other microbes, the host has the ability to maintain good oral hygiene, also in response to eating, salivating, tooth brushing , flossing , the oral microbes have evolved the skills to survive and tolerate the changing oral environment due to these inhibitory practices.[9] These microbes living in a biofilm community increases its tolerance to antimicrobial agent and host defence mechanism and thereby enhancing the bacterial virulence. The resident microbial flora that are non-pathogenic play an important role in contributing to host physiology by preventing colonisation by potentially pathogenic microorganisms. [10] Studies have shown there is a significant difference in the bacterial flora among healthy and diseased oral cavity. [11] Dental plaque, a biofilm containing multiple organisms that binds onto the tooth surface should be periodically detached from the tooth surface. Failure to detach from the tooth surface leads to growth of pathogenic microorganism. [12] This results in pathology such as dental caries and periodontal disease [13]

According to Shafer Dental caries is an irreversible microbial disease of the calcified tissues of the teeth characterised by degeneration of inorganic substance and destruction of organic substance of the teeth. Miller found that the bacteria produced acids that dissolved the tooth structure in the presence of fermentable carbohydrates. The three primary factors in the etiology of dental caries is host, microbial flora and substrate. Unlike the oral epithelium the epithelium of the tooth do not shed and the tooth has many inaccessible areas that cannot be accessed by mechanical cleansing. The tooth surfaces are unique as they are the only body part that is not subject to metabolic turnover. In living individuals the tooth surface is subjected to bacterial challenge that results in dental infection called dental caries. Caries initially are painless as it progresses to the tooth pulp it manifests as pain. The initial treatment includes restoration of the tooth, if not treated at the right time it results in complete loss of tooth structure and the final option of treatment is extraction of the tooth. The most common cause for tooth extraction is known to be dental caries [14] [15] [16] [17] [18] . A decrease in the number of teeth may affect the nutrition intake thereby affecting the quality of life. [19] The destruction of the tooth structure to a non-restorable extent is mainly by facultative anaerobes and obligate anaerobes. Various studies have shown Streptococcus mutans as the main cause of dental decay various lactobacillus are associated with the progression of dental caries. [21] [22] The present study aims at accessing the bacterial proportion of Streptococcus mutans and Lactobacillus in patients coming for extraction due to severe carious tooth, and healthy individual.

MATERIALS AND METHODS

Study location

The study was conducted among patients reporting to the out-patient department of oral and maxillofacial surgery for tooth extraction in a private dental college, located in the suburban area of Chennai, Tamil Nadu, and India. The study was conducted in the year 2019.

Study Groups

The study was conducted among 60 subjects who were made into three groups- Control group (A), Control group (B) and Study group (C).

The Control group (A) comprised of 20 healthy individuals both male and female selected after careful examination of the oral cavity for any active caries.

The Control group (B) comprises of 20 individuals including male and female with carious tooth that can be restored.

The Study group (C) comprised of 20 subjects randomly selected, including male and female who were approved for tooth removal.

All the subjects were explained about this study in detail and the patients have given their consent in the written format. The saliva samples were collected in a labelled sterile disposable container for microbiological analysis.

Inclusion criteria

1. Healthy subjects without any systemic disease were included in this study.
2. Subjects not under any antibiotics therapy at present.
3. Subjects not using any mouth wash

4. The control Group A are the subjects without any caries or periodontal disease.
5. The control Group B are the subjects reporting for restoration due to dental caries.
6. The study Group C includes patients reporting for extraction due to complete loss of tooth structure due to dental caries.

Exclusion criteria

1. Subjects with systemic disease.
2. Subjects reported for extraction of periodontally compromised teeth, prophylactic removal for orthodontic treatment, supernumerary tooth removal.
3. Subjects under any antibiotics and mouth wash.

Resting Saliva sample collection and microbial analysis

The participants rinsed their mouth with water prior to collection, and waited 10 minutes before commencing with the collection. Resting drooling (minimal oral movements) was used to collect whole mouth saliva from the oral cavity Participants were asked to sit comfortably in an upright position and tilt their heads down slightly to pool saliva in the mouth. The first expectoration was discarded to eliminate food debris and unwanted substance contaminating the sample that may cause analytical inaccuracy. The subsequent sample was then expectorated into a pre-labelled sterile disposable plastic container and was immediately transferred to the microbiology lab. The saliva samples were diluted in the ratio of 1:40 using sterile normal saline. The sample was mixed thoroughly. Media used were Mutans sanguis agar (Himedia code no: M977 -500G), MacConkey agar (Himedia code no: M008- 500G) and Nutrient agar (Himedia code no: MV001-500G). 10 µl of the diluted sample was transferred to the indicative media. The plates were incubated aerobically at 37°C for 24 hours. After incubation the growth were examined and each colonies were counted and tabulated.

RESULTS

Table 1: Showing mean value of colony forming units in 1ml of saliva

ORGANISM ISOLATED	CONTROL GROUP A	CONTROL GROUP B	STUDY GROUP
Total CFU	9,97,050	23,02,000	48,57,412
Streptococcus mutans	3,08,200	9,92,000	44,08,333
Lactobacilli	0	0	4,22,000

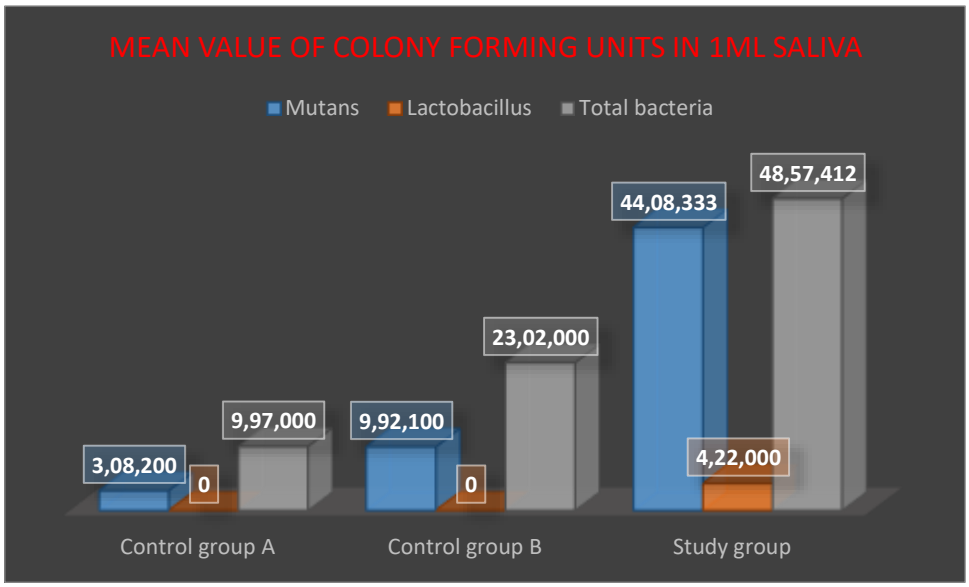

Figure 1: Graphical representation of mean colony forming units in 1ml of saliva

Table 2: Showing proportion of each organism in the total CFU

ORGANISM	CONTROL GROUP A	CONTROL GROUP B	STUDY GROUP
Mutans	30.91%	43.09%	90.75%
Lactobacilli	0.00%	0.00%	9%

The percentage of each of the organism was calculated separately the predominant organism in the study group was found to be streptococcus mutans which constituted 90.7% of the total bacteria. The lactobacillus constituted 9% of the total bacteria.

DISCUSSION

The saliva is a fluid, over 99% of it is made of water. The saliva collected from the mouth is a complex mixture that comprises of secretions of all the glands, desquamated epithelial cells , microorganism and their products, fluids from gingival crevice , food remnants and leucocytes.[23] The normal pH of saliva ranges from 6.2 -7.0 and pH 6.7 being the average. The resting pH of the saliva does not fall below 6.3. There is maintenance of the salivary pH by two possible mechanisms. Firstly because of the saliva there is flushing of the carbohydrates if they are not flushed these carbohydrates are metabolised by the bacteria and there is acid production thereby decreasing the pH. The saliva has a buffering activity that neutralises the acidity from the drinks and foods as well as from the bacterial activity thereby maintaining the salivary pH within limit.

In the present study there was a considerable increase in the level of Streptococcus mutans and the Lactobacillus and there was a significant reduction in the harmless commensal bacteria in the study group. According to Marsh et al., the frequent exposure to low pH leads to inhibition of acid sensitive species and there is growth of organism with an aciduric physiology such as mutans streptococci and lactobacilli.[24] Similar to that, there is a corresponding reduction of the harmless bacteria and increase in the streptococcus and lactobacillus count in this study. Thus the parameters of the oral environment determine which microbe can occupy the site, the metabolic activities of those microbes alter the environment facilitating the growth of unfavourable organisms. It is evident from the study that in patients with advanced carious lesion there is a considerable increase in the Streptococcus mutans and the Lactobacillus level due to probable decrease in the salivary pH, causing an acidogenic environment favouring their growth. So these patients with an increased susceptibility of dental caries have a high relative proportion of Streptococcus mutans and lactobacillus in their saliva. According to data obtained in the present study in the patients reporting for extraction there is an increase in the total bacterial load when compared to healthy volunteers and patients reporting for restoration, there is a fivefold increase in bacterial load among the patients reporting for extraction when compared to healthy individuals. When compared to patients reporting for restoration of carious tooth, there is a two fold increase in the bacterial load in patients reporting for extraction of carious tooth. According to Meenakshi et al., there was a similar increase in the bacterial load where they compared healthy individuals and caries susceptible individuals.[25] The mean value of Streptococcus mutans in the control group A and control B is 3, 08,200 and 9, 92,000 respectively where as in the study group is 44, 08,333.There is a 15 fold increase in the mutans count in the study group when compared to the control group A without any caries, 5 fold increase when compared to control group B with caries that were restorable. Whereas the lactobacillus count was 0 among both the control group A and B and it was 4, 22,000 among the study group. The mean percentage of Streptococcus mutans in the control group A and control group B was 30.91% and 43.09% respectively, where as in the study group there was predominance of Streptococcus mutans about 90.75% of mutans and 9% of lactobacillus was observed[26]. These two microorganism decreased the proportion of other non- pathogenic bacteria.

CONCLUSION

This study is done to associate the increase in the bacterial population in the saliva to cause tooth decay. It is found from the study that there is a many fold increase in the total bacterial count of the saliva and there is also a significant proportionate increase in Streptococcus mutans and Lactobacilli. The percentage increase of these two bacteria in the extraction cases are found to be significantly high when compared with the corresponding count in the caries individual. With the data obtained with this study the mutans and Lactobacilli, which is found to be exceedingly increased among the other facultative anaerobes found in the saliva of the study group. It is possible to be a contributing factor in the tooth decay and tooth loss. There are many anaerobic bacteria involved in caries development and tooth decay but according to the study the volume of increase of these two bacteria will have a definite influence in tooth decay and loss. Further study can be conducted with more sample size and to demonstrate that penetration into deeper layers and bone structures.

REFERENCES

1. Jørn A. Aas, Bruce J. Paster, Lauren N. Stokes and Floyd E. Dewhirst. Defining the Normal Bacterial Flora of the Oral Cavity. JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2005; 43(1): 5721–5732. 10095-1137/05/$08.00.
2. Ahrne S, Nobaek S, Jeppsson B, Adlerberth I, Wold AE, Molin G. The normal Lactobacillus

flora of healthy human rectal and oral mucosa. J ApplMicrobiol, 1998; 85: 88-94.
3. Ahumada M del C, Bru E, Colloca ME, Lopez ME, Nader- MaciasME. Characterization of lactobacilli isolated from the tongue and gum. Anaerobe, 1999; 5: 129-135.
4. BiswajitBatabyal, Sukanta Chakraborty and Shibendu Biswas. role of the oral micro flora in human population: A brief review.CODON(USA):IJPLCP. ISSN: 0976-7126.
5. Percival RS, Challacombe SJ, Marsh PD (1991). Age-related microbiological changes in the salivary and plaque microflora of healthy adults. J Med Microbiol; 35: 5–11.11.
6. Marsh PD, Percival RS, Challacombe SJ. (1992) The influence of denture wearing and age on the oral microflora. J Dent Res; 71: 1374–1381
7. Nolte WA. Oral microbiology with basic microbiology and immunology. C.V. Mosby, St. Louis, 2nd edition, 1973; chapter-3: 193-200.
8. Marsh PD. (1989). Host defenses and microbial homeostasis: role of microbial interactions. J Dent Res; 68: 1567–1575.
9. Avila M, Ojcius DM, Yilmaz O. The oral microbiota: living with a permanent guest. DNA Cell Biol, 2009; 28: 7.
10. Flemmig TF, Beikler T (2011). Control of oral biofilms. Periodontol, 2000; 55: 9–15.
11. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. J ClinMicrobiol, 2005; 43: 12.
12. Filoche S, Wong L, Sissons CH. Oral biofilms: emerging concepts in microbial ecology. J Dent Res, 2010; 89: 8–18.
13. Zaura E, Keijser BJ, Huse SM, Crielaard W. Defining the healthy ''core microbiome'' of oral microbial communities. BMC Microbiol, 2009; 259: 12.
14. Reasons for permanent tooth extractions in Taiwan. Lee CY, Chang YY, Shieh TY, Chang CS Asia Pac J Public Health. 2015 Mar; 27(2):NP2350-7
15. Reasons for permanent tooth extractions in Japan. Aida J, Ando Y, Akhter R, Aoyama H, Masui M, Morita M J Epidemiol. 2006 Sep; 16(5):214-9.
16. Agerholm DM, Sidi AD. Reasons given for extraction of permanent teeth by general dental practitioners in England and Wales. Br Dent J. 1988;164:345–348.
17. Al-Shammari KF, Al-Ansari JM, Al-Melh MA, Al-Khabbaz AK. Reasons for tooth extraction in Kuwait. Med PrincPract. 2006;15:417–422.
18. Chen SC, Chueh LH, Hsiao CK, Wu HP, Chiang CP. First untoward events and reasons for tooth extraction after nonsurgical endodontic treatment in Taiwan. J Endod. 2008;34:671–674.
19. Snyder ML. Colorimetric method for estimation of the relative number of lactobacilli in saliva. J Dent Res. 1940;19:349–55.
20. Shimono T, Mjzuno J, Nonomura E, Morisaki I, Msuoto N, Matsumura S, et al. Studies on a caries activity test (Cariostat comparison with Snyder test) Jpn J Ped Dent. 1980;18:612–7.
21. Loesche WJ et al. Microbiology of Dental decay and periodontal disease Medical microbiology 4th edition
22. Byun R, Nadkarni MA, Chhour KL, et al. quantitative analysis of diverse lactobacillus species present in advanced dental caries. J ClinMicrobiol, 2004; 42: 3128-3136.
23. Baliga S et al. Salivary pH a diagnostic bio marker J Indian Soc Periodontal. 2013 Jul; 17 (4) : 461-5
24. Marsh PD Microbial Ecology of dental plaque and its significance in health and disease Adv Dent Res. 1994 Jul; 8(2):263-71
25. Meenakshi et al Estimation of variations in the proportion of viridans population in saliva of healthy and carious individuals WJPLS, 2017, Vol.3, issue 4, 150 – 153.
26. Nivedha Srinivasan, N. P. Muralidharan1, S. Niveda ,Alteration in the proportion of bacterial flora in the oral cavity after consuming probiotics, Drug Invention Today , 2018 Vol 10, issue 4, 3676-78.