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ESTIMATION OF VARIATION IN THE PROPORTION OF VIRIDANS POPULATION IN SALIVA OF HEALTHY AND CARIOUS INDIVIDUALS

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ABSTRACT

Background: Oral cavity is colonized by many bacterial species, fungi and parasites. Though all these organisms are present in the oral cavity and are considered to be commensals, most of them are opportunistic. Some of these species in the oral cavity dominates in the number or proportion with others. Dominant members are usually harmless and the rest acts during pathological conditions. Maintaining bacterial proportion is directly proportional to proper maintenance of oral health. Any alteration in the respective proportion disturbs the normal flora which ultimately drives into pathology. **Aim:** Any pathology in oral cavity is a result of existence of one or more predisposing factors. The environment that is conductive to increase the bacterial proportion has to be identified. Diet, Host-Organisms relationship, salivary pH, oral hygiene maintenance are some of the factors responsible for the change in this proportion. In oral lesion etiological factors are multiple and the organisms involved are numerous and complex. This study is aimed at exploring the involvement of five common habitats of oral cavity in the caries process. **Objective:** Though there are many organisms associated in caries development, mutans are considered to be solely responsible for caries initiation. Studies report that voluntary colonization of oral cavity with probiotics has an effect in reducing the caries production. Hence this study is done to explore the possibility of other organisms involved in cariogenic activity.

KEYWORDS: Commensals, Bacterial proportion, Cariogenic activity, Oral hygiene maintenance.

INTRODUCTION

Oral cavity is a complex structure which is comprised of several smooth and rough structures that are coated with plethora of microorganisms, the predominant one being the bacterial species forming biofilm. More than 700 bacterial species have been detected so far in oral cavity. Only few among those are predominantly found in the oral cavity and some of these belong to the normal flora.[1] The normal flora can be divided into resident flora and transient flora, which is further divided into indigenous and supplemental flora. Organisms that are present constantly in high numbers in a particular site are indigenous and those that are present but at lower numbers are supplemental species.[2] Resident flora is more predominant and whenever there is a disturbance in the normal flora, the transient flora proliferates to cause disease.[3] Oral cavity harbors various bacterial species including both gram positive and gram negative organisms, the commonest of which being *Streptococcus mutans, Streptococcus sanguis, Streptococcus mitis, Staphylococcus, Enterococcus, Fusobacterium, Peptostreptococcus, lactobacillus, Actinomyces bifidus, Actinomyces israelii, Actinomyces naeslundii, Neisseria, Nocardia, Corynebacterium* and few *Treponems*.[4]

Oral flora changes with age. For example, during the first few months of life oral cavity harbors less organisms as it consists only of mucosal surface. During this period lactobacillus, S. salivarius and Fusobacterium are commonly seen. As the tooth erupts, the complexity of the oral flora increases due to the availability of additional nutrition from the gingival crevicular fluid and at this stage S. mutans, S. sanguis and spirochetes dominates. With the progression of age there is teeth loss which brings about disappearance of many aerobes and anaerobes. Insertion of prosthesis such as dentures may facilitate the growth of some organisms in old age.[5]

Oral microorganisms have the ability to tolerate the changing oral environment.[6] These microbial colonies have its significance in maintaining oral health.[7] Saliva contains most of these organisms and plays its role in maintaining homeostasis and defense mechanism.[8] The bacterial flora in healthy oral cavity and diseased oral cavity are reported to be distinctly different.[9] The need for this oral biodiversity in maintaining oral hygiene suggests that every species has its own significance in maintaining equilibrium and homeostasis. They act by Preventing the adherence of pathogens over the surface,

thus preventing them from occupying and multiplying. They also act by degrading the virulence of those organisms.[10]

Any change in the oral environment predisposes pathological changes causing the microorganisms to initiate disease. Alterations in proportion of organisms, diet, poor oral hygiene, change in relationship between the host and organisms, increased virulent factor, immunodeficiency state and genetics are the common factors responsible for the ecological shift, thus causing pathological changes.[11]

Dental plaque is known to be a biofilm containing multiple organisms that binds onto the tooth surface. It is necessary that this biofilm should get detached from the tooth surface for obtaining proper ecological balance, as it can manifest the pathogens within itself and cause disease.[12] Failure of the accumulated plaque to detach from the surface leads to overgrowth of microorganisms that in coarse of time becomes pathogenic. The progression may ultimately cause pathology such as dental caries and periodontal diseases.[13]

Recent studies have demonstrated the relationship between the biofilm induced periodontitis and systemic diseases including diabetes mellitus, cardiovascular diseases, respiratory diseases, Alzheimer's disease and others due to the ability of bacterial products to gain access to the systemic circulation through the ulcerated epithelium of the periodontal pocket.[14,15,16] Dental caries is a multifactorial chronic bacterial disease that causes demineralization and destruction of the dental hard tissues. Primary colonizers are *Streptococcus, Actinomyces, Neisseria and Veillonella* and secondary colonizers includes *Fusobacterium nucleatum, Prevotella intermedia and capnocytophaga*. It is believed that *S. mutans* and *Lactobacillus* from the biofilm produces weak acids as a by-product of metabolism of fermentable dietary carbohydrates, which causes fall in the pH below a critical level, thus resulting in the demineralization of the tooth.[17] There are several studies which reports that *S. mutans* initiates caries and *Lactobacillus* is responsible for caries progression.[18] This study aims at accessing the bacterial proportion of three other organisms (*S. sitis, S. salivalius, and S. sanguis)* including *S. mutans and lactobacillus* in carious and healthy individual.

MATERIALS AND METHODS

Study location

The study was conducted among the patients reported to the outpatient clinics of Saveetha Dental College and Hospitals, Chennai, Tamilnadu, India.

Study groups

The study was conducted among 50 subjects who were grouped into two - control group and study group. The study group comprised of 25 patients who reported to the outpatient clinic and the control group comprised of 25 healthy individuals selected after careful examination of oral cavity for any active lesion. The subjects were informed about the study and a verbal informed consent was obtained. The saliva samples were collected in a sterile container for microbiological analysis.

Inclusion criteria

- Subjects with no systemic illness
- Subjects not under any antibiotics in the recent past
- Subjects who do not use any mouthwash
- Subjects who have not undergone scaling for the past one month
- Subjects with no plaque or calculus accumulation and caries (for group A) and with local factors accumulation and caries with no periodontitis (for group B).

Exclusion criteria

- Subjects under antibiotics
- Subjects with systemic illness
- Subjects with any other oral lesions other than caries and calculus accumulation
- Subjects not willing for participating in the study

Sample collection and study methodology

The saliva samples of both the control and study group was collected in a sterile disposable plastic container and was immediately transferred to the microbiology lab and stored at 8°C until it was processed. Saliva samples were diluted in the ratio of 1:40 using sterile normal saline. Samples were mixed thoroughly. Media used were lactobacillus MRS agar (Himedia-M641), mutans sanguis agar (Himedia-M977) and mitis salivarius agar (Himedia-259). These indicative media were used to identify and differentiate the five organisms included in the study. 10 µL was transferred to each of the indicative media used. Plates were incubated aerobically at 37°C overnight. After incubation the growth were examined and each colonies were counted and tabulated. The colonies were identified based on the manuals provided by Himedia laboratories.

RESULTS

The total number of Colony Forming Units (CFU) in both the control and study group were analyzed which showed a two fold increase in number of CFU of the study group when compared to the control group.

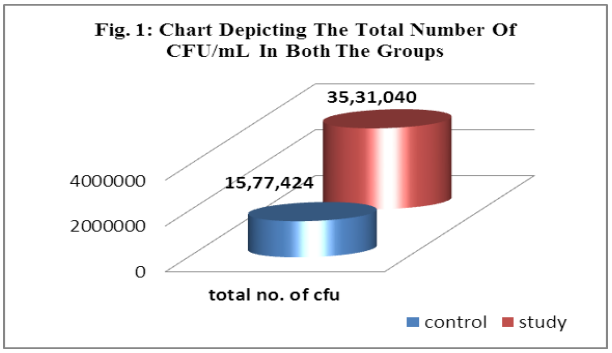


Fig. 1: Chart Depicting The Total Number Of CFU/mL In Both The Groups

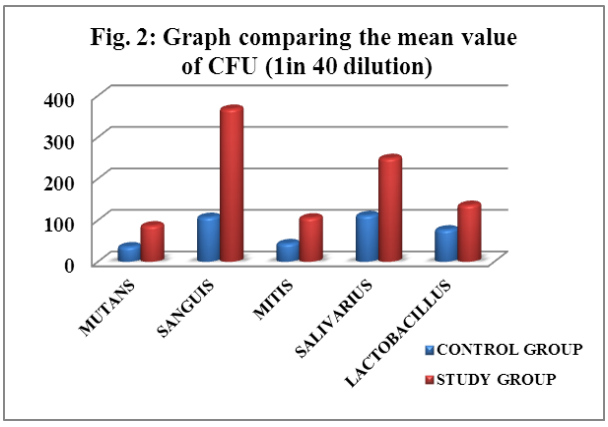
When individual species were considered, the mean values of each showed a marked difference between the two groups which is tabulated below (Table 1). Graphical representation of it is respresented in fig. 2.

Table 1: Table Showing the Mean Value of no. of colonies (1 in 40 dilution).

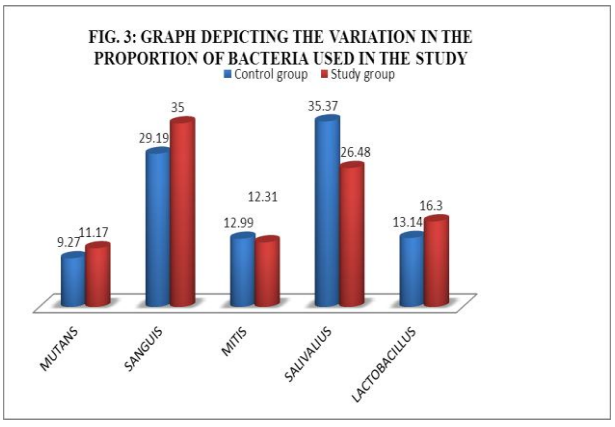
Microbe	Control Group	Study Group
Mutans	36	87
Sanguis	107	340
Mitis	44	106
Salivarius	111	249
Lactobacillus	77	136

Table 2: Table Showing the Mean value of CFU in /mL of saliva.

Microbe	Control Group	Study Group
Mutans	134,464	356,000
Sanguis	420,160	1,251,520
Mitis	201,760	424,160
Salivarius	552,240	997,120
Lactobacillus	268,800	545,913



The percentage of each organism was calculated individually. The mean % value of each organism was calculated and depicted in Fig.3.there is no significant alteration in the proportion of the bacteria included in the study except for *S. salivarius and S. sanguis*.



DISCUSSION

The resident oral micro flora plays an important role in the normal maintenance of homeostasis and in the maintenance of oral health. Clinicians should beaware of the beneficial properties of the resident microbial flora, and their treatment strategies should be focused on the controlling their progression to pathology rather than the elimination.[19] This study is done to know about the alteration in the proportion of the bacteria that are considered to be the risk factors in cariogenic activity. This study is done by compiling the normal controls to obtain a baseline titer value and to demonstrate the alternative. According to the data obtained in this study in caries individual, there is an overall increase in the bacterial load in the saliva, it is more than double when compared with healthy oral cavity. *S. mutans* count is also doubled in the carious individual. The mean value of *S. mutans* in the control group is 134,464 whereas in the carious group is 356,000. The value of S. sanguis when compared to the control group shows a threefold increase in the carious group. The *S. mitis* proportion also doubled in the carious group. The mean value of *S. salivarius* and *Lactobacillus* has also increased near to double (Table 2). When the mean% of individual organism was recorded for the analysis of variation in the proportion of the bacteria between the control and carious group, no significant alteration in proportion is noticed except for *S. salivarius and S. sanguis*. There is an increase in proportion of *S.mutans, S. sanguis and Lactobacillus*, whereas a decrease in the proportion of *S. mitis anf S. salivarius* (fig. 3).

CONCLUSION

Cariogenesis is a cyclic event that involves many contributing factors. These factors are mostly dependent on each other. When all these contributing factors are favorable for the progression of microorganism, the risk of caries development increases. This is due to the overall increase in the bacterial load. The caries activity can be kept under control by revising the dietary habits and by maintaining proper oral hygiene. Usage of Probiotics (to restore colonization resistance) can alter the relative proportion of the bacterial flora (20).

REFERANCES

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. doi:10.1128/JCM.43.11.5721–5732.2005.
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 Appl Microbiol, 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. Nolte WA. Oral microbiology with basic microbiology and immunology. C.V. Mosby, St. Louis, 2nd edition, 1973; chapter-3: 193-200.
5. Biswajit Batabyal, Sukanta Chakraborty and Shibendu Biswas. role of the oral micro flora in human population: A brief review.CODON(USA):IJPLCP. ISSN: 0976-7126.
6. Avila M, Ojcius DM, Yilmaz O. The oral microbiota: living with a permanent guest. DNA Cell Biol, 2009; 28: 7.
7. Flemmig TF, Beikler T (2011). Control of oral biofilms. Periodontol, 2000; 55: 9–15.
8. Nieuw Amerongen AV, Veeman ECI Saliva – the defender of the oral cavity. Oral Dis, 2002; 8: 12–22.
9. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE. Defining the normal bacterial flora of the oral cavity. J Clin Microbiol, 2005; 43: 12.
10. Socransky SS, Haffajee AD. The bacterial etiology of destructive periodontal disease: current concepts. J Periodontol, 1992; 63: 322–331.
11. Parahitiyawa NB, Scully C, Leung WK, Jin LJ, Samaranayake LP. Exploring the oral bacterial flora: current status and future directions. Oral Dis, 2010; 16: 10.
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. Costerton JW, Lewandowski Z, Deebeer D, et al.. Biofilms, the customized microniche. J Bacteriol, 1994; 176: 2137-2142.
15. Wood SR, Kirkham J, Marsh PD, et al. Architecture of intact natural human plaque biofilms studied by confocal laser scanning microscopy. J Dent Res, 2000; 79: 21-27.
16. Levine MJ, Reddy MS, Tabak LA, et al. Structural aspects of salivary glycoproteins. J Dent Res, 1987; 66: 436-441.
17. Caufi eld PW, Griff en AL. Dental caries. An infectious and transmissible disease. *Pediatr Clin North Am*, 2000; 47: 1001–19.
18. Byun R, Nadkarni MA, Chhour KL, et al. quantitative analysis of diverse lactobacillus species present in advanced dental caries. J Clin Microbiol, 2004; 42: 3128-3136.
19. Marsh PD. The control of oral biofilms: new approaches for the future. In: Guggenheim B, Shapiro S, eds. Oral Biology at the Turn of the Century. Misconceptions, Truths, Challenges and Prospects. Basel: Karger, 1998; 22–31.
20. Hillman JD. Replacement therapy for dental caries. In: Newman HN, Wilson M, eds. Dental Plaque Revisited: Oral Biofilms in Health and Disease, 1999; 587–599.