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VISUALIZATION OF ORAL BACTERIAL LOAD USING ACRIDINE ORANGE DYE AND ITS APPLICATION FOR ORAL HEALTH ASSESSMENT

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Oral health; Bacterial Load; Fluorescent dye; Acridine Orange

Abstract

Background: Assessment of oral health is essential as oral health may impact overall health condition. Traditionally used culture medium to assess bacterial load is costly and time consuming. Hence, use of fluorescent dye to assess the estimation of bacterial load. Materials and Method: Swab of 100 subjects was taken and stained the section with Acridine Orange fluorescent dye and number of bacteria in unit area was measured. Result: At all points, the number of oral bacteria in the latter was higher than in the former. It was significantly higher in latter than former ($p<0.05$). Conclusion: Fluorescent dyes can be used to assess bacterial load in oral cavity and thus oral health status can be evaluated.

Introduction

Oral health management is important for all age group persons to prevent aspiration pneumonia in children and elderly persons, patients with consciousness disorder, and severe-status patients under respiratory control[1,2]. These microbes including bacteria, fungi, which can cause chronic respiratory infectious diseases, are present in oral cavity due to insufficient oral care[3]. In debilitated patients, aspiration of these bacteria is easy and may cause pneumonia related to the reduction of the oral self-cleaning function[4].

Oral commensal microorganisms, which do not usually show pathogenicity, cause respiratory infectious diseases in infection-prone persons such as the elderly patients. In order to reduce this oral bacterial load proper oral care is mandatory[1] Therefore, bacteriological examination of the oral cavity is essential to evaluate the efficacy of oral care. Bacterial medium culture and identification of the bacteria is complex and take for a long time. In order to clarify these issues, the presence and change of number of oral bacteria must be evaluated using a simple method. In the current study we are visualizing the number of oral bacteria using a fluorescent dye Acridine Orange for staining of bacterial DNA, and to ascertain whether this method can be applied for the assessment of oral care.

Material and methods

The current study was a prospective cohort study done between the year 2016-17 Informed consent was obtained from all subjects. Aim of the current study was to visualize oral bacteria and its counts with a fluorescent dye so that it can be applied for the assessment of oral care. Also, we investigated changes in the number of oral bacteria in the oral cavity of two group of population.

The study consisted of 100 subjects divided into two groups; one group consisted of 50 healthy adults of age less than 40 and 50 persons of age above 40 on random bases. The study included all the patients attending the dental

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OPD for minor dental problems. Persons suffering from any oral mucosal lesion, chronic systemic diseases, infection and those who were not willing to be part of the study were excluded from the study.

Sample Collection:
Sample was collected from three sites (tongue, buccal mucosa, and labial sulcus) in each subject's oral cavity with clean wooden spatula. It is then stored in a bottle containing a fixative (isopropyl alcohol). The presence or absence of dental disease or denture was noted in the subjects.

Calculation of the number of bacteria and visualization:
Samples were collected on a glass slide with the help of wooden spatuala. Each sample was immediately fixed in isopropyl alcohol. Each sample was stained with Acridine-orange stain (phosphate-buffered saline, pH 7.4,Alfa Aesar) for 2 minutes, washed in distilled water, covered with a slide cover, mounted in Phosphate buffer solution and photographed under a fluorescent microscope. Bacteria stain orange against a green to yellow background of human cells and debris. In addition, the photographed images were then input to a computer, and bacteria will be counted. The number of all bacteria in unit area was measured. The total number of bacteria in 5 different fields per sample was calculated.

Analytical method:
The data will be expressed as the mean±standard deviation (S.D.). For statistical analysis, the Mann-Whitney U test was applied using SPSS version 17 IBM software

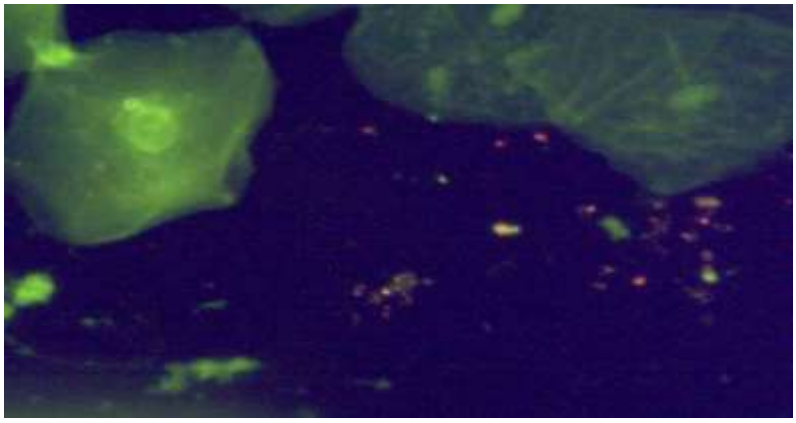

Figure-1: Staining of Fluorescent Acridine Orange stain

Result

The study comprised of 68 % females and 32% males. 43% of all subjects were denture wearers. Dental disease such as dental caries, periodontitis was present in 63% of subjects. The bacteria measured approximately 2 to 3 µm, being globular or in chains. Bacteria were observed singly or in colonies. Figure-1 shows fluorescence microscopy photographs of oral bacteria in subjects. Thus, oral bacteria were fluorescently visualized on Acridine Orange stain in all subjects.

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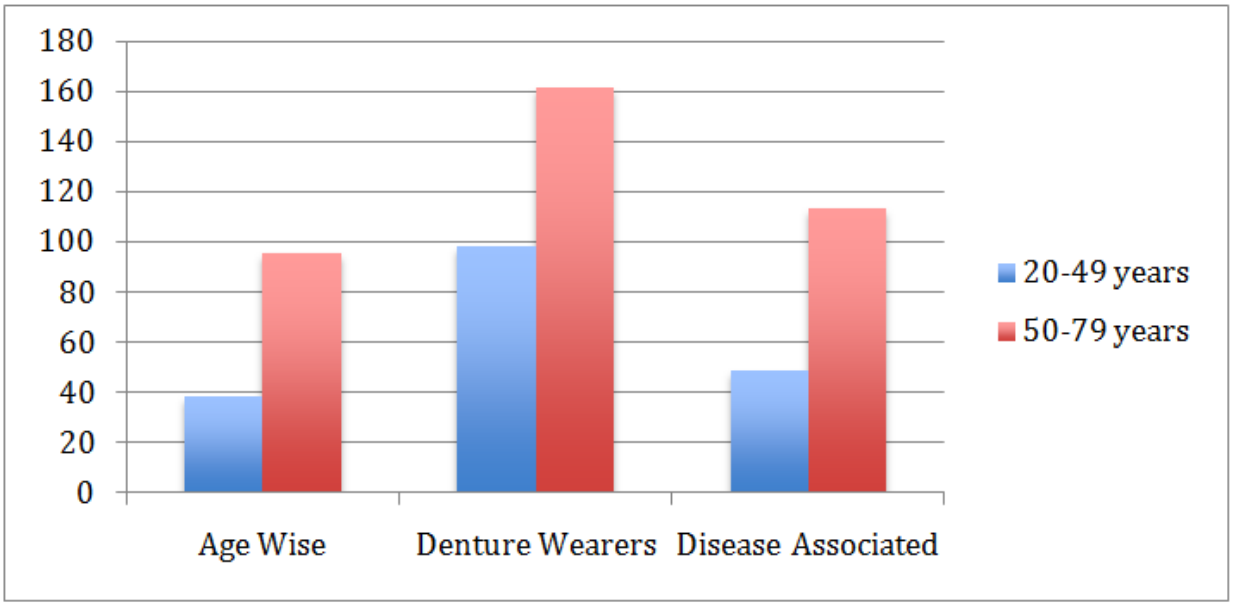

Table-1: Distribution of oral bacterial count in two groups

The mean number of oral bacteria with respect to age is presented in Table-1 In subjects aged 20 to 39 years, the mean number of oral bacteria age wise, in denture wearers and those associated with dental disease (caries, periodontitis) was 38.3±5.1, 98.2±8.4, and 48.6±4.6, respectively. In those aged 70 to 79 years, the values were, 95.3±7.1, 161.6±15.3, and 113.2±18.4, respectively. At all points, the number of oral bacteria in the latter was higher than in the former. It was significantly higher in latter than former ($p<0.05$).

Discussion

Oral health is an essential part of healthy body. Correlation of oral health with aspiration pneumonia is well documented.[5] There is no standardized protocol for oral health assessment.[6] Usually bacterial culture is employed for count of bacteria in oral cavity and is considered as gold standard technique. The drawback with this technique is that it is expensive, tedious and requires longer time duration for result. Hence, we used a fluorescent dye, Acridine Orange for visualization of bacteria for oral care assessment.

We found that number of oral bacterial count increases with age; count increases more in elderly patients. This finding is concomitant with Beighton et al [7] who obtained increase in the count of Lactobacilli and yeast along with root caries in 88% of elderly patient whose age is above 55 years (n=146). The probable reason for increased bacterial load can be reduced efficacy in oral cleanliness and reduction in salivary flow rate. Although, there can be various confounding factors which influences the bacterial count such as dietary intake, medications and other systemic diseases which needs to be taken into consideration. Antibody and cell-mediated responses decline in old age[8,9] and so the increased prevalence of transient organisms and/or opportunistic pathogens in the mouths of the elderly might be due to deficiencies in the integrity of the host defenses leading to the breakdown of microbial homeostasis[10]. Likewise, yeasts, lactobacilli, and mutans streptococci are all aciduric organisms, and their levels might increase due to changes in local environmental conditions.

The number of oral bacterial count was seen increased in denture wearers. Dentures are known to be reservoir of bacteria. A study involving individuals requiring nursing indicated that bacteria that cause aspiration pneumonia, opportunistic infection, or endocarditis were present on dentures.[11] Similar finding was seen by Marsh et. al.[12] Salivary secretion rates[13,14] and sugar clearance times are often reduced in the elderly, especially in hospitalized patients [13]and in subjects with dentures.[12] This would prolong conditions of low pH in plaque and thereby favor the

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selection of aciduric microorganisms.

The number of oral bacteria in both young and elderly subjects with dental diseases was slightly higher than in those without dental diseases. As dental diseases such as caries and periodontal disease are primarily caused by periodontal disease associated bacteria[15], this may have resulted in the finding that the number of bacteria was higher in subjects with dental diseases. Halitosis and dry mouth reflect oral uncleanliness or a reduction in the oral self-cleaning function by saliva; the number of oral bacteria may have been high.

Conclusion

Using a fluorescent dye, Acridine Orange, oral bacteria were investigated. It was possible to visualize the presence of bacteria in a sample in a short period and count them. This method can be applicable for oral-care assessment. Furthur studies with large sample size are needed for validation of Acridine Orange fluorescent dye in bacterial count in oral cavity.

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