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Study of Cariogenic Bacteria and Its Anti-Microbial Susceptibility in Systemic Condition like Diabetes Mellitus with Hypertension

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Abstract: Dental caries is a concern of every person regardless of race, age, gender, or socioeconomic level. The ability to determine a person's susceptibility to dental caries is a prominent area of research to identify both cause and effect. It is estimated that more than 51 million school hours are lost each year to dental-related illness. Department of Health and Human Services, In addition, The Academy of Pediatrics reported that human dental flora is site specific, and an infant is not colonized with normal dental flora until the eruption of the primary dentition. It has been well documented that mothers who have a high caries risk can pass the cariogenic bacteria *Streptococcus mutans* to their infants. In other words, this automatically predisposes the infant to a high caries risk. On the other hand, employed adults lose approximately 164 million hours of work each year due to dental disease. It is apparent that the ability to assess caries susceptibility is advantageous for all populations and groups. Looking at the specificity of the *Streptococcus mutans* test, we recognize that "specificity of a diagnostic procedure is the percentage of disease-free individuals who are diagnosed correctly". The data collected concerning specificity showed that the test did identify the proportion of patients who were correctly identified by the test. The test is an accurate test to use as a chair side tool. In addition, we have concluded that are important tools as well in the diagnosis of dental caries. It is important to recognize that a false positive test result could eventually result with a positive DMFT and x-rays. With this in mind, the dental professional could educate the patient and start preventive measures to decrease the microbial load in the oral cavity. Although this test was performed on an 18 and above population, it can be a useful tool on patients of all ages in the quest to detect caries susceptibility.

Keywords: dental caries, *Streptococcus mutans*, cariogenic bacteria, pathogenic microbes, data collected, acid-tolerating

I INTRODUCTION

Dental caries is formed when the balance inside mouth is disturbed. If the proportion of acidogenic and aciduric (acid-tolerating) bacteria increases it leads to demineralization of

enamel by rapid metabolism of dietary sugars to acid, resulting into a low pH. Thus, favourable micro environment is created for these organisms enhancing their growth and multiplication whereas most species associated with enamel health are sensitive to acidic environmental conditions [1]. Thus, oral bio film can

be studied as an ecological plaque signifying that disease can be prevented not only by targeting the pathogenic microbes directly by antimicrobial or anti-adhesive strategies, but also by regulating the selection pressures responsible for their growth and multiplication. Under prehistoric evolutionary conditions, the presence of *Streptococcus mutans* would have no adverse affects, and might even have benefited humans by preventing the colonization of harmful bacteria. Yet the taste for sugar that once ensured that our ancestors consumed foods with the maximum amount of calories now keeps us snacking in between meals and has created a multibillion dollar industry for what Loesche has called "the slow-release device for sucrose known as a candy" [2,3].

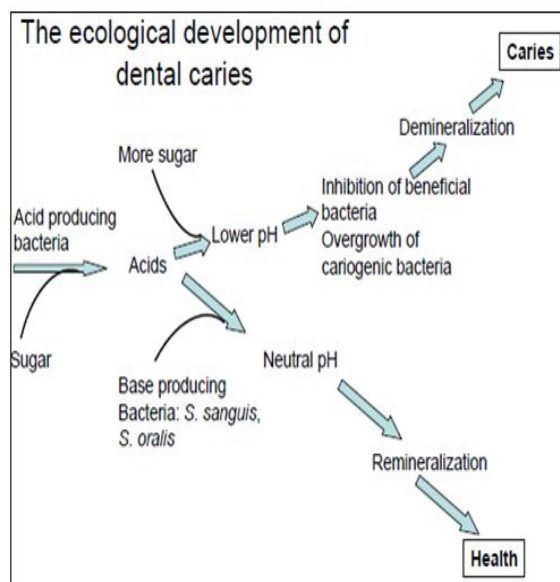


Fig 1. Schematic representation of ecological development of dental caries

Studies of societies in which access to sugar is drastically altered show the expected matching change in caries rates. For example, Inuit families following culturally traditional (low-sucrose) diets had many fewer cavities than wealthier Inuit families who could afford more Western foods (Mayhall, Dahlberg et al. 1970). Similarly, Norwegian children who grew up during World War Two, when sugar was severely rationed, experienced fewer caries than children growing up shortly after, when sugary foods were again common [4]. Humans' relationship with both food and with *Streptococcus mutans* evolved in very different conditions than those of today. In effect, the disease initiated by *S. mutans* in our mouths is a case of evolutionary incongruence with the lifestyle enjoyed by those in developed countries. Early Childhood Caries One of the most striking examples of the problems caused by the conflict between the evolutionary past

and the modern lifestyle is the pediatric dental disease known as Early Childhood Caries, also called Nursing-Bottle Caries. Early Childhood Caries is associated with the availability of a sugar-rich drink (often in a bottle or sippy cup) to infant or young child during periods of sleep [5].

II RELATED WORK

Many systemic diseases are believed to be affected by periodontal disease and the association may be bidirectional in some. Management of these entities may need to target periodontal disease [6]. Thorough patient education and improved motivation for following guidelines and follow-up and regular assessment by clinicians working as a team (physicians/specialists/dentists etc) will ensure improved health of various systems as a whole. The inter-link between periodontitis and various diseases discussed above has not been unanimously and universally established in all the studies. An older study established no significant radiographic evidence of periodontitis in a study on diabetics with considerable dysglycemia of long duration Likewise another study on diabetics with significant periodontal disease found no demonstrable improvement in glycemic control up on reception of nonsurgical periodontal therapy including root planning. A similar lack of improvement in glycemic control was found in another study done on diabetics who underwent periodontal therapy for 4 months.

A large prospective cohort study did not find convincing evidence of a causal association between periodontal disease and CHD risk Pregnant subjects with periodontal disease were assessed for the risk of adverse pregnancy outcomes such as preterm birth, preeclampsia, fetal growth restriction, or perinatal death and no association was found with periodontal disease. A multicenter, randomized clinical trial on pregnant subjects with periodontal disease compared scaling and root planning to tooth polishing and found no association with the occurrence of spontaneous preterm birth at <35 weeks of gestation. A clear relationship between periodontitis and respiratory infectious diseases such as bacterial pneumonia and bronchitis has not been established. An epidemiologic study found no association between periodontal state or poor oral hygiene and acute respiratory disease in the community-dwelling population. Most systemic conditions like diabetes, CHD, CKD, etc., may stem from multi factorial etiologies. Periodontal disease may act as a modifying factor in conjunction with the variety of factors influencing the outcome of systemic diseases. The degree and extent of periodontal disease may be different in the subsets of patients with these systemic diseases and may help explain the non-universality of such an inter-link[7,8].

As depicted by a few studies that questioned the association, we need to keep in mind that those with adequately managed systemic diseases do not guarantee a healthy periodontium and vice versa. Treating periodontal infection may have promising practical advantages that translate into better management of systemic diseases. In specifically those diseases that demonstrate bidirectional association, adequately treated systemic diseases mirror improved oral hygiene and minimize periodontal disease morbidity. Universal management protocol and guidelines may necessitate further exploratory studies into this interlink but it is evident that in battling systemic disease and periodontal disease, taking into account the contribution of each to one another, shall bolster our approach in bettering the all-round health [9].

III. PURPOSE of RESEARCH

The objectives of this study is to perform a detailed microbiological investigation of some potential pathogens associated with caries, to characterize the isolates, and the epidemiological factors related to this disease in diabetic and hypertensive patients .

1. Does the presence of Streptococcus mutans relate to caries susceptibility as indicated by the RESEARCHRapid Detection Streptococcus mutans saliva test using the 500,00 cfu/ml biomarker?
- 2.To detect and identify cariogenic bacterial pathogens in samples from diabetic and hypertensive patients.
- 3.To assess the antimicrobial susceptibility in isolated bacteria in systemic conditions like hypertension and diabetes mellitus.
- 4.To investigate is there any other possible factors (opportunistic bacteria) present in the sample.

IV DESIGN AND METHODOLOGY

The oral cavity is home to many different types of bacteria. However, Streptococcus mutans is considered to be a major human cariogen (Dasanayake et al., 1995). Therefore, having an increase in the number of Streptococcus mutans in the oral cavity is “considered as a risk factor for the onset of caries”[10]. Moreover, given the uncertainty about the association between Streptococcus mutans and the entire caries experience in adults, Giacaman et al. decided to research if high colonies of Streptococcus mutans and the presence of biofilm could predict caries susceptibility in adults. It was concluded that “while the relation between MS and dental caries remains debateable, the cariogenic potential of MS is indisputable”[11]. Further research

should “consider differences in the number of colonies with and without biofilm in order to determine if a quantitative relation also exists” (Giacaman et al.). Many methods have been developed to identify Streptococcus mutans and predict caries susceptibility. Earlier detection methods of Streptococcus mutans used several different media to grow colonies. These media were mitis-salivarius-bacitracin agar (MSB), trypticase yeastextract cysteine-sucrose-bacitracin agar (TYCSB), and glucose-sucrose-potassium telluritebacitracin (GSTB) agar (Dasanayake et al., 1995). With this in mind, the newest technology suggests an immunochromatography process that uses two monoclonal antibodies for rapid detection and accurate results. This test is currently available from RESEARCH(Saliva-Check Mutans) and will deliver results in 15 minutes. It is well noted that within the last 10-15 years there has been a dedicated interest to the concept of caries predictability. It has been well established that Streptococcus mutans is a transferable bacteria from mother to infant and from caregiver to infant (Caufield et al., 1993). However, the ability to develop an accurate test is still in progress. RESEARCH has developed a rapid detection Streptococcus mutans saliva test utilizing monoclonal antibodies with immunoassay. This study determined the specificity of the RESEARCH rapid detection test.

A cross sectional quantitative research design was used in this study to determine the

specificity of the RESEARCHRapid Detection Saliva Streptococcus mutans test. Giacaman et al.[12] performed a cross-sectional study using 96 randomly selected patients ranging in age 15- 17 years to determine whether biofilm formation by S. mutans was associated with high caries susceptibility. “Patients were randomly chosen for the study amongst those in treatment by the students” (Giacaman et al., 2010 p.551). Informed consent was requested and age and gender were collected. Patients were examined clinically. Bitewing radiographs and a DMFT were used to supplement the clinical examination. Stimulated saliva was collected using paraffin wax and seeded on TYSCB agar plates to culture MS counts. The collection of data allowed for anonymity and confidentiality. “Anonymity exists when there is no link between personal information and the research participant” (Cottrell & McKenzie[13]. “Confidentiality exists when there is a link between personal information and the research participant’s identity but that information is protected from others”. Age and gender were collected from the patient’s dental chart and the patient was assigned a confidential number. All data were stored on a password protected laptop that remained in the researcher’s possession at all times. Using these techniques protects the

privacy of the research participants. Demographic data was collected including age and gender to determine if differences among various population demographics exist. Other data collected included, two horizontal bitewing radiographs, a DMFT index, and the administration of the RESEARCHRapid Detection Streptococcus mutans Saliva test. The researcher was trained to administer the saliva test by a representative from INDIA[14].

V PERFORMANCE EVALUATIONS AND RESULTS

A total of 100 samples will be collected from patients with diabetes mellitus and hypertension. Fresh samples without any preservatives will be collected in containers for culture. Sample will be completely labeled by the necessary data (date, time of collection, sample type etc.....).

- Detection of cariogenic bacteria in sample by routine culture.
- Antimicrobial surveillance for the bacterial isolates
- Isolation Procedures of cariogenic bacteria.
- Identification of cariogenic bacteria by Gram’s stains.

Gram's stain reagents:

Crystal violet, Grams Iodine, Ethyl alcohol 95% v/v, Safranin and Distilled water .

Enumeration of Total viable cell count [15]

Total viable count was determined from selected plates having 30 to 300 colonies (Table

1).

Total viable count was calculated from the formula

THB = No. of colonies × Dilution factor / Inoculum size
CFU/ml

Table 1. Viable cell count

S.NO	Number of bacterial colonies	Dilution factor	TBH(CFU/ml)
1	290	10 ⁻²	2.8×10 ⁵
2	210	10 ⁻³	2.0×10 ⁵
3	170	10 ⁻⁴	1.6×10 ⁵

10 bacterial strains with observable difference in colony morphology were randomly selected from initial spread plate and restreaked

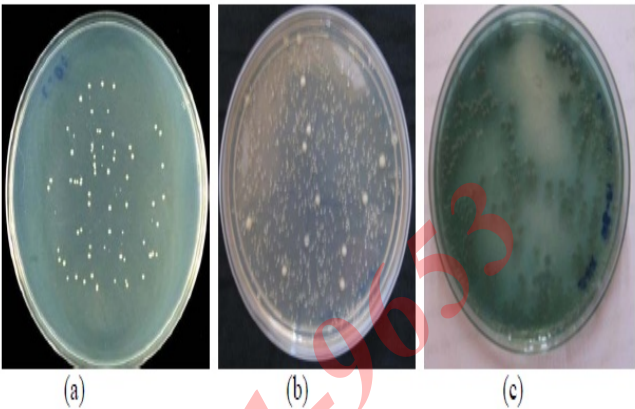


Fig.2(a)-(c) Spread plate culture of dental plaque bacteria.

Isolation of strains:

The 10 strains selected with observable difference in colony morphology were

pure cultured by quadrant streaking.

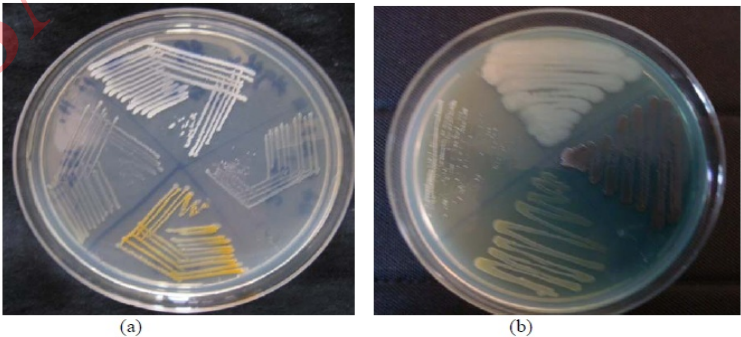


Fig.2 (a),(b) Pure cultures of the isolated strains.

The Streptococcus mutans study was conducted from April 2014 to May 2014. The

dental hygiene patients in a middle-high socioeconomic single practitioner dental office in Louisville, Kentucky were the target population. Results of the RESEARCHRapid Detection Streptococcus mutans saliva test was used as well as results from radiographs and the DMFT. Age and gender were also collected as descriptive data for the population group. The random sampling consisted of 116 female patients and 84 male patients respectively. There was 100% acceptance into the study. The median age of the patients was 57 indicating that patients were

more in the caregiving roles of children rather than the childbearing years. However, regardless of age, the RESEARCHER Rapid Detection Streptococcus mutans test can be a useful tool in detecting caries susceptibility, increasing education, and decreasing dental caries [1,2]. The chi square tests were inconclusive, but the results of the cross tabulation indicated that 26.5% of the time there was a false positive result (i.e. the Streptococcus mutans test was positive and the DMFT index or radiographs were negative). Examining specificity revealed that high specificity existed with this chair side test. Therefore, it was determined that the portion of patients without caries was correctly identified. In summary, the test would be a useful tool to the dental professional and the patient [4].

Table 1. Antimicrobial susceptibility testing methods used and the respective number of isolates tested by each of the participating medical centers in the SENTRY Antimicrobial Surveillance Program

Medical Center	No. of isolates tested by each methodology						Total	Not available ^b	Total
	Agard diffusion	Disk diffusion	Etest ^a	MicroScan ^a	Pasco ^a	Vitek ^a			
039	151	203				25	379	671	1,050
040		162			49		211	898	1,109
041		17				239	256	248	504
042	01						01	976	977
043	124	39				81	244	887	1,131
044		51		288			339	552	891
045		05		96		142	243	314	557
046		112				443	555	574	1,129
047		87	40				127	58	185
048		1,416					1,416	21	1,437
049		71					71	399	470
056						15	15	21	36
057		242					242	427	669
092	60						60	72	132
Total	336 (3.3%)	2,405 (23.4%)	40 (0.4%)	384 (3.7%)	49 (0.5%)	945 (9.2%)	4,159 (40.5%)	6,118 (59.5%)	10,277

a - Etest® (AB Biodisk, Solna, Sweden); MicroScan® (Dade Behring, West Sacramento, California); Vitek® (bioMérieux, Hazelwood); PASCO (Becton-Dickinson Wheatridge, Colombia). b - Methodology not reported.

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VI CONCLUSION

Dental caries is a concern of every person regardless of race, age, gender, or socioeconomic level. The ability to determine a person's susceptibility to dental caries is a prominent area of research to identify both cause and effect. It is estimated that more than million school hours are lost each year to dental-related illness. The RESEARCHER Rapid Detection Streptococcus mutans test was administered to 200 patients in a single practitioner Louisville, Kentucky dental practice. Patients signed an informed consent. The patients were given a qualifying questionnaire and upon acceptance into the study had two bitewing x-rays and a DMFT index charted. Data were collected and stored in a password protected laptop. All information gathered (age, gender, and Streptococcus mutans test results) were analyzed using the statistical package for the social sciences (SPSS) version The demographic data were collected and the demographic variables included age and gender. Of the 200 participants in the study, women comprised the majority of the participants with 58% participation. Because we know that maternal transmission is possible, 58% was a good representation of women for this study. However when examining the age of the participants, it was revealed that the mean was 54.91. This is more representative of the caregiver age group rather than the childbearing age group. Duchin and van Houte. concluded in their study that "although the formation evidence about the relative susceptibility of infants, children, and adults to infection by S. mutans is lacking, the evidence suggests that this organism will readily colonize newly exposed teeth of children and adults in the presence of suitable salivary levels" Therefore, regardless of age, the RESEARCHER Rapid Detection Streptococcus mutans test can be a useful tool in detecting caries susceptibility.

VII FUTURE WORK

In future scope, improvement may be to modify the Findings of It was concluded through this study that a relationship existed between the RESEARCHER Rapid Detection Streptococcus

mutans test, the DMFT, and the x-rays. In addition, the specificity concluded that the test is a useful tool in the dental profession.

Further research could answer the following questions:

1. How would this study's results compare with a study conducted on a lower socioeconomic level? (public health facility, free clinic)
2. How would this study's results compare with a study using patients within childbearing ages only?

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