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A survey of micro flora present in dental caries and it's relation to enviornmental factors

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Abstract
Periodontal disease (PD) is a chronic inflammatory process that occurs in response to infection from bacteria in dental plaque. This may lead to dental caries that destroys the periodontal tissues causing teeth loss. It is also associated to systemic diseases. In the present study an attempt has been made to find out the general bacterial fauna present in the dental caries. We also tried to find out the relationship between dental caries and age, sex, food habits, C-reactive protein, salivary pH, blood groups. Hundred dental carried subjects and 50 non carried subjects (aged between 23 to 50) volunteered for the study. The result shows a clear association of caries and salivary pH. Also a significant relation was found between the levels of C-reactive protein in saliva and dental caries. The results of study of bacterial fauna show that out of 80 different types of bacteria isolated 85% were gram positive and had a tendency to produce acid.

Keywords: Dental caries, Bacteria, C-reactive protein, salivary pH.

INTRODUCTION

Periodontal disease constitutes an oral physical, physiological disorder contributing to the evolution of systemic diseases (Fowler, 2001). Causative agents assumed to be include specific bacteria, smoke, systemic diseases, genetic factors age etc., however the main reason of PD is the individual's chronic exposition to a pathogenic oral microflora existing in bacterial biofilm (Darveau *et. al*., 1997; Socransky *et. al*., 1998) Among the PD, dental caries is an irreversible microbial disease of calcified tissues of the teeth characterized by demineralization of the inorganic portion and destruction of organic substances of the tooth which often leads to cavitations. It is a complex and dynamic process where a multitude of factors influence and initiate the progression of disease. Two stages of dental caries are decalcification and dissolution of softened residue. It is a major health problem affecting mankind and its manifestation persists throughout the life despite treatment. It is a multi factorial disease which is caused by host agents and environmental factors.

It affects persons of both genders in all races, all socioeconomic strata and every age group. The caries experience varies greatly among countries and even within countries. The difference in caries rates noted in different parts of the world are extreme from rates fewer than one decayed, missing and filled (DMF) tooth per person at all ages unto 34 years in Ethopia (Littleton, 1963) to 60 times greater in Alaska-Aleuts (Russel, et al 1961). Numerous epidemiological studies have been carried out to establish base lines of the caries experience. They show that there are no areas in the world which have been investigated where the people are totally free of caries. There are several studies in which an attempt has been made to study the prevalence of dental caries with increase in age (Weddell & Klein, 1981; Tang, 1997). Regarding gender, studies indicate that the total caries experience in permanent teeth is greater in females than in males of the same age. Siblings of individuals with high caries susceptibility are also generally caries active, where as siblings of caries immune individual generally exhibit low caries rate. Children of parents with a low caries; the converse is true for children whose parents have a high caries rate (Gran, 1976).

Till date there is no universally accepted opinion of the etiologic of dental caries. Numerous theories have evolved through years of investigation and observation which have stood the test of time. Robertson (1835) proposed that dental decay was caused by acid formed by fermentation of food particles around the teeth. Later several physicians related microorganisms to caries Underwood and Miller (1881) postulated a septic theory with a belief that acids capable of causing decalcification was actually produced by bacteria feeding on the organic fibrils of dentine. They reported sections of decayed dentine having micro cocci as well as oval and rod shaped forms. Goadby (1990) isolated a gram positive bacillus from various dentines and concluded that they played important role in decalcification of both enamel and dentine. Clark (1924) described new *streptococcus* species *streptococcus mutants*, which was invariably isolated from various lesions in the teeth of British patients. A wide group of microorganisms are identified from various lesions of which *S. mutants, Lactobacillus acidophilus* and *Actinomyces viscosus* are the main pathogenic species involved in the initiation and development of dental caries. (Shivkumar *et. al*. 2009)

Some other factors which cause dental caries were studied by certain workers. Sellman (1949) studied the buffer capacity of saliva and its relation to dental caries and found that total amount of acid needed to reduce the salivary pH to given level was always greater

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for saliva from caries resistant persons. It was pointed out that regardless of the quality of the saliva, including the relative presence or absence of inhibitory principles, saliva always appears to contain bacteria capable producing caries if carbohydrate is present. Slephal and Miller (1943) provided evidence that decrease in plague pH after exposure to fermentable carbohydrate is the causative agent of caries. It was found that the critical value for enamel dissolution is 5-6 and average pH of 5.5 is generally acceptable value.

A dynamic relation exists between pH and oral health. Diet affects the integrity of the teeth quality. Compositions of the saliva and its pH, plague pH sugar and other fermentable carbohydrates after being hydrolyzed by salivary amylase provide substrate for the action of oral bacteria which in turn lower plaque and salivary pH. The resultant action is the beginning of teeth demineralization (Offenbacher S, Weathers DR 1978 American Dietetic Association, 2003).

Another important factor present in saliva is CRP indicator (C-reactive protein indicator).CRP is a protein produced by liver and released into the blood during the acute phase of inflammation. It is regarded as a golden marker for inflammation. It is a pentameric non immunoglobulin protein having five identical subunits. It is a member of pentraxin family proteins. It plays important role in innate immunity. It assists in compliment binding to foreign and damaged cells and enhances phagocytosis. Its level rises with the rise in inflammation. During an inflammatory response or infection the levels of CRP may be detected as early as 5-10 hours after the tissue damage. In the last few decades saliva has been advocated as a non invasive alternative to blood as a diagnostic fluid. Studies on dental caries suggest that it may include a systemic immune response. Numerous clinical studies have established elevated serum CRP as a strong, independent risk factor for the development of cardiovascular disease (CVD). CVD has also been associated with oral infections oral infections and there is evidence that systemic CRP may be a link between the two (Lagrand *et. al.,* 1999; Slade et. al., 2000; Lloyd-Jones DM, 2006). Studies also suggests that raised levels of CRP may be indicative of diabetes (Pradhan 2001; Dehghan 2007)

Regarding Blood groups and susceptibility to dental caries, reports are there that individuals of blood group O have greater severity of periodontal disease but individuals of blood group have greater resistance to periodontal disease (Gawrzewska, 1975). Several other authors have found a significant relationship between the ABO blood groups and oral disease (Pradhan, 1971; Suk, 1971).

MATERIAL AND METHODS

Two types sample were collected and processed

1. Caries sample for bacterial isolation

Caries sample were collected from the caried persons ⟶ diluted with N saline ⟶ plated in nutrient agar medium ⟶ incubated and cultured ⟶ used for standard morphological and biochemical studies**.**

2. Saliva sample for pH measurement and CRP test

Saliva samples were collected in sterilized vials for detecting the pH and CRP levels pH was measured by lab pH meter CRP was measured with the help of CRP measurement kit (CRP Latex Agglutination KIT)

RESULTS

A. Morphological Analysis

Total 80 different types of bacteria were isolated from the caries. Out of which 45% were Rod shaped and 55% were Coccus shaped.Results of gram staining show that 85% of bacteria were gram +ve and 11% were gram –ve Only 50% of the bacterial colonies showed endospore formation.

B. Biochemical Test Results:

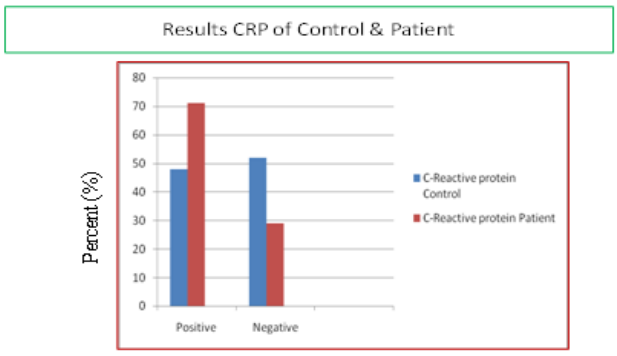
The Results are presented as Table-1

Table 1. Results of biochemical analysis

Sl. no	Name of Biochemical Test	Positive (%)	Negative (%)	No growth (%)
1.	Gelatin Hydrolysis	46	16	38
2.	Starch Hydrolysis	30	54	16
3.	Methyl Red	48	52	0
4.	Voges Proskauer	65	35	0
5.	Nitrate Reduction	60	40	0
6.	Indole Production	1	99	0
7.	DNase Utilization	0	35	65
8.	Citrate Utilization	19	29	52
9.	Urease Test	91	9	0
10.	Hydrogen Sulphide Production	45	55	0
11.	Casein Hydrolysis	54	46	0
12.	Arginine Utilization Test	91	9	0
12.	Arginine Utilization Test	91	9	0
13.	Lysine Utilization	86	14	0
14.	Ornithine Utilization	64	36	0
15.	Tween 20	5	41	54
16.	Tween 60	16	56	28
17.	Tween 80	28	55	18
18.	Litmus Utilization	67	3	30
19.	[illegible]	85	15	0
20.	Fermentation	85	15	0

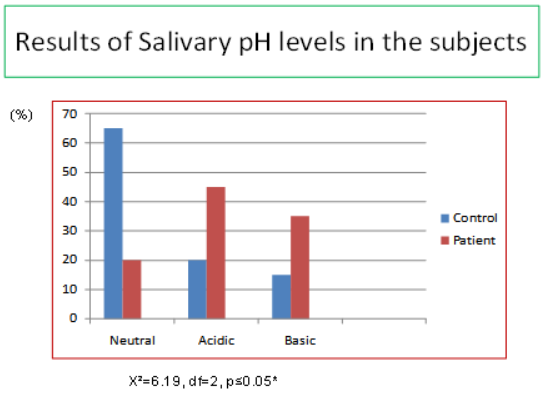
Results of Environmental factors:

1. **C-reactive protein**- The results of C-reactive protein show a significant relationship between CRP and dental caries. X^2 =4.569, df=1, P≤0.032. (Fig-1)



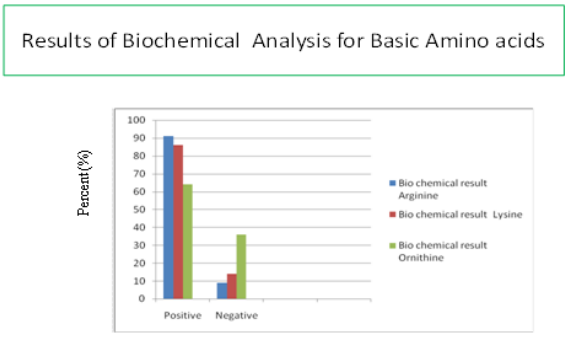
X^2=4.569, and df=1, P≤0.032*

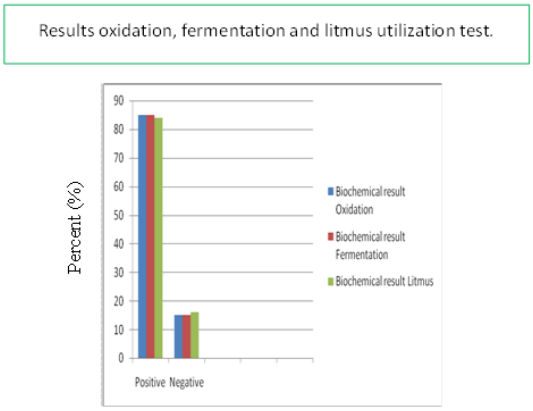
2. **Salivary pH** –Salivary pH has been shown to be a possible indicator for the development of dental caries. Results show significant relationship between dental caries and pH. X^2=6.19, df=2, P≤0.05



X^2=6.19, df=2, p≤0.05*

3. **Gender**- No significant relationship was found gender and dental caries X^2 =6.37, df=1, P≤0.54.
4. **Blood group**- No significant relationship was found gender and dental caries X^2 =7.35, df=3, P≤0.195.





DISCUSSION

Dental caries are initiated due to the decalcification and softening of dental enamel. It is generally believed that it is initiated by lactic acid or other organic acids. The acid production is a result of bacterial fermentation of carbohydrates on the tooth surface. The disease is a result of a universal microbial parasitisation of teeth that occur only in the presence of fermentable carbohydrates. Susceptibility to tooth decay varies among individuals i.e. teeth of certain persons do not decay in the presence of environmental conditions that normally are associated with dental caries. Microorganisms thought to be responsible for dental caries include lactobacillus acidophilus, streptococcus mutans and and actinomyces odontolyticus. These bacteria in the mouth ferment carbohydrates clinging tenaciously to the teeth with the formation of lactic acid and other organic acids, thus reducing the pH of the mouth less than 5 at which decalcification occurs and dental decay begins.

Our results corroborate with the above findings that cariogenic bacteria have a tendency to produce acid as seen in (fig–3). A large number of bacteria isolated showed positive result for oxidation fermentation and litmus milk test by positive result for oxidation and fermentation (fig-4). It can be assumed that they are either facultative aerobes or facultative anaerobes. Litmus milk test also confirms the formation of curd acid and gas. Large number of bacteria showed positive result for Urease and Indole production test indicating a rise in pH, as free ammonia is evolved in both the test. The results of salivary pH supported the above finding. A close relation was found between the salivary pH and dental caries. More than 50% of non carried persons showed neutral ph for saliva where as 72% of the carried persons showed either alkaline or acidic pH. The results of chi-square test were significant. (P≤0.05).

If an organism is able to ferment dextrose acidic byproducts are formed and the media turns yellow. Low pH and the presence of amino acid will cause the organism to begin decarboxylation. Arginene is hydrolyzed to ornithine by an enzyme called dehydrolyase. Ornihine decarboxlation yield puterescine lysine decarboxylation result in cadaverine. These byproducts are sufficient to raise the pH of the media.

None of the bacteria showed positive test for DNase indicating that the bacteria did not produce any extra cellular DNase.

C-reactive protein level rises dramatically during inflammation process occurring in the body. Our results showed a significant

relationship between CRP and dental caries. It can be assumed that high levels of CRP in saliva may be an indicator of high risk of dental caries.

No significant relationship could be found between the blood group, gender and dental caries.

The final conclusions of the study were that out of 80 types of bacteria isolated 85% were gram positive. They were both rod shaped (45%) as well as coccus shaped (55%). From the results of biochemical analysis it can be assumed that most of the bacteria were facultative aerobes and facultative anaerobes. None of the bacteria showed +ve test for DNase indicating that they did not produce any extracellular DNase. A significant relationship was found between dental caries and CRP and it can be assumed that high CRP levels in saliva may be an indicator of high risk of dental caries. Although there are reports which show a relationship of Blood group and dental caries (Blood group A being resistant group and O being least resistant to PD) we could not find so. No significant relation was found between gender and DC. Large % of bacteria showed positive result for oxidation, fermentation, and litmus milk test indicating they are facultative aerobes or anaerobes. These tests also confirm the formation of curd acid and gas.Low pH and presence of basic amino acids will cause organism to begin decarboxylation and the by products are sufficient to raise the pH of the media. Salivary pH either acidic or basic was found important for the formation of dental caries.

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