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Correlation between Total Antioxidant Level and Dental Caries in Adults - an In *vivo* Study

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ABSTRACT

The objective of the study is to evaluate the correlations between the physicochemical properties of saliva such as pH, hydration, buffering capacity, total antioxidant level, level of *Streptococci mutans* and dental caries in adult patients with DMFT>5. Study included 100 healthy adult subjects. Saliva was tested for hydration viscosity, pH of resting saliva, quantity, buffering capacity of stimulated saliva. Level of *Streptococcus mutans* in saliva was found. Swab collected from caries lesion of patient was inoculated in S-L agar for 24 hrs. Gram staining method identified *Streptococcus mutans* colonies. Body Mass Index (BMI) of each patient was calculated. Results obtained were statistically analyzed. The total antioxidant level in saliva was significantly higher in the study group than the control group but there was no significant correlation between the number of *Streptococci mutans* and total antioxidant level. The resting saliva pH and the buffering capacity of the stimulated saliva were statistically significant. From the study it is concluded that TAC of saliva increases with caries activity. pH and buffering capacity of saliva decreases with caries activity, suggesting that these factors play an important role in development of caries.

Keywords: DMFT, Body mass index (BMI), Total antioxidant level, Buffering capacity.

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INTRODUCTION

Dental caries is a complex multifunctional disease, as multiple factors influence the initiation and progression of the disease. Among the factors that have been related to greater cariogenic activity are inadequate dental hygiene and care. Changes in salivary components are in connection with caries formation and it may be used for recognizing risk in patients and to maintain prevention [1].

Total or whole saliva refers to a complex mixture of bodily fluid secreted by major and minor salivary glands, gingival folds, oral mucosal transudate, non-adherent oral bacterial, epithelial and blood cells. Saliva is a major determinant of oral environment and serves as an easily available diagnostic tool of systemic conditions since it plays an important role in acquired pellicle formation on tooth surfaces, crystal growth, bacterial adhesion, plaque formation and also functions as a cleansing solution, a lubricant, a buffer and an ion reservoir of calcium and phosphate which are essential for remineralization of initial carious lesions [2,3]. Consequently, more intense saliva research can be observed in recent decades, which leads to a higher amount of scientific data presented by numerous researchers of this far reaching field. Recently it has been claimed that the imbalance in levels of free radicals, reactive oxygen species and antioxidants in saliva play an important role in the onset and development of dental caries [14]. As for the literature available very little has been discussed about dental caries and antioxidants.

Antioxidants are found in all biological species and protect against the potentially harmful effects of processes or reactions that cause excessive oxidations. Therefore, biological antioxidants form an important part of our diet and together with intracellular antioxidants and antioxidant enzyme systems may prevent various pathological diseases [4]. Halliwell (1997) suggested that “an antioxidant is any substance that when present at low concentrations compared to those of an oxidisable substrate significantly delays or prevents oxidation of that substrate [5]. Antioxidants interact with free radicals and terminate the chain reaction. However since free radicals / reactive oxygen species and antioxidant systems appear to act in concert rather than alone, investigations of individual antioxidant activity may be misleading and the measurement of any individual antioxidant may be less representative of the whole antioxidant status. Research is now being directed towards assays that evaluate the so called “Total antioxidant capacity” (TAC) of biological fluids [2,14]. Hence evaluation of TAC in saliva can pave way in understanding the risk of individuals to dental caries.

MATERIALS AND METHODS

This study was conducted in the Central Research Laboratory of Nitte University after the approval from institutional ethical committee.

Subjects

Study group:

75 healthy adult patients coming to the OPD of Department of Conservative Dentistry and Endodontics, A.B.Shetty Memorial Institute of Dental Sciences, Mangalore with DMFT>5 under the age group of 25-50 years were included in the study.

Control group:

25 healthy adults without caries, in the same age group were taken.

Patients fulfilling the inclusion and exclusion criteria were selected for the study. The inclusion and exclusion criteria used are as follows :

Inclusion criteria:

- Free from systemic or local disease which affect salivary secretions.
- Caries status was assessed according to WHO criteria. Caries active adult having atleast 5 decayed tooth surfaces.

Exclusion Criteria:

Patients with periodontal disease, hypertension, diabetes, radiotherapy, chemotherapy, systemic disease of the vital organs and history of long term medications.

A detailed case history of the patient was taken. A case history format was filled, with an informed consent which was duly signed by each patient.

Calculation of DMFT and BMI:

- Patients Body Mass Index (BMI) was calculated using the formula : Weight (Kg)/Height (m^2).
- The smooth and occlusal surfaces of teeth were cleaned with soft bristle brush, dried and examined. DMFT score calculated.

Salivary Analysis :

Saliva test was done with a GC saliva–check buffer kit (GC Corp, Belgium) for hydration, viscosity and pH of resting saliva and also for the quantity and buffering capacity of stimulated saliva (according to manufacturer's instructions).

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Total antioxidant capacity of saliva:

Total antioxidant capacity was evaluated using a spectrophotometric assay. The optical density wasread at 695nm.

Estimation of *Streptococci mutans* level:

For the estimation of *Streptococci mutans* level in saliva, swab was collected from caries lesion of the patient using a wooden swab stick and inoculated in S-L agar for 24 hrs. *Streptococci mutans* colonies were identified by gram staining method.

Statistical analysis:

Results are presented as mean ± standard deviation value. Student 't' test was used to correlate between total antioxidant level and dental caries in study and control groups. A 'p' value of 0.05 or less was considered significant.

RESULTS

Total antioxidant levels of saliva were higher in study group when compared to that of control group. It was found that total antioxidant levels in study group was 521.20 ±111.75 and that of control group was 286±81.95 and it was statistically significant ($p<0.05$) (Table-1,fig-1).

The correlation between DMFT and saliva properties were evaluated using Spearman's rho. The resting saliva pH and buffering capacity of the stimulated saliva were shown to be statistically significant ($p<0.05$). Whereas resting saliva hydration and viscosity were not significant. The quantity of the stimulated saliva showed no correlation with DMFT (Table 2).

The correlation between *Streptococci mutans* and total antioxidant levels of saliva were evaluated using Pearson correlation and it was not statistically significant ($p>0.05$) (Table 2).

The correlation between DMFT and BMI were evaluated using Pearson correlation and it was not statistically significant ($p>0.05$) (Table 2).

Table 1:Total antioxidant level in the control group and the study group

Group	N	Mean	Std. Deviation	Std. Error Mean	p value
Antioxidant					
Case	75	521.2000	111.74730	12.90347	< 0.001
Control	25	286.0800	81.95218	16.39044	(significant)

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Table 2: Correlation between total antioxidant level and *Streptococcus mutans* level, Body Mass Index and salivary parameters with DMFT

Variables	Correlation coefficient	p value
DMFT and resting saliva – hydration	-0.159	0.172 (not significant)
DMFT and resting saliva – viscosity	-0.096	0.415 (not significant)
DMFT and resting saliva - pH	-0.504	<0.001 (significant)
DMFT and stimulated saliva - quantity	Normal	No correlation
DMFT and stimulated saliva - buffering capacity	-0.471	<0.001 (significant)
S. mutans and total antioxidant	-0.114	0.331 (not significant)
DMFT and BMI	0.126	0.280 (not significant)

DMFT = Decayed Missing Filled Teeth, BMI = Body mass index, *S.mutans* = *Streptococci mutans*

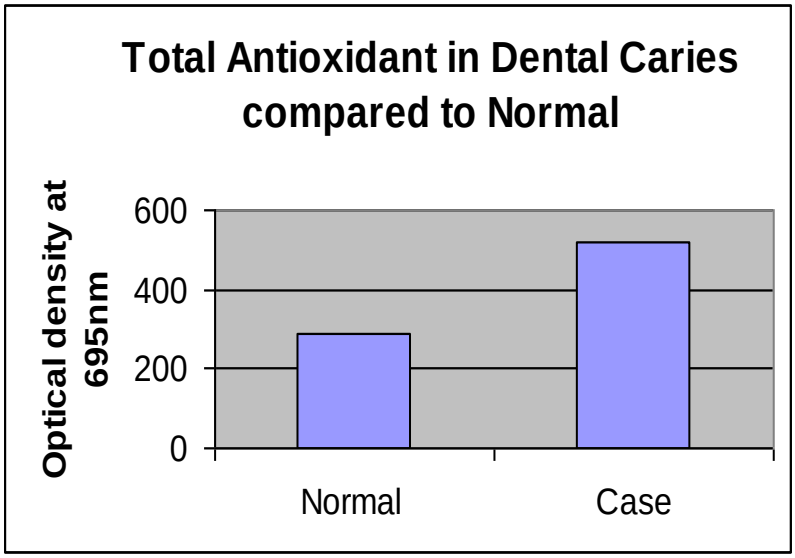

Figure1: Bar diagram showing an increase in total antioxidant level in study group with respect to the control group

DISCUSSION

Cells and biological fluids have an array of protective antioxidant mechanism both for preventing the production of free radicals and for repairing oxidative damage. Free radicals are produced during dental decay. The number appeared to vary directly with caries activity [6]. A free radical is any atom with atleast one unpaired electron in the outermost shell, and is capable of independent existence. Free radicals are very unstable and react quickly with other compounds, trying to capture the needed electron to gain stability. Normally the body can handle the free radicals, but if the antioxidants are unavailable, or if the free radical production becomes excessive, damage can occur. Antioxidants can neutralize free radicals by donating one of their own electrons. Studies have been done to correlate the total antioxidant level of saliva in children related to caries. The authors reported that total antioxidant capacity increased with increase in caries activity [4,7].

In this clinical study correlation between total antioxidant level in saliva and dental caries have been assessed. Total antioxidant capacity is evaluated because free radical and TAC appear to act together rather than alone and measurement of an individual antioxidant may be less representative of whole antioxidant status [5,14].

In the present study it was seen that total antioxidant level in saliva increases with caries activity. It has been suggested that levels of antioxidants could be altered in response to an infection or disease [5] . The presence of caries in our study group could be one of the factors for increased levels of total antioxidant capacity of saliva .An important factor could also be triggered function of salivary peroxidase system which constitutes one of the major salivary antioxidant systems [8].

At rest, without exogenous or pharmacological stimulation there is small continuous salivary flow, that moisturizes and lubricates the oral tissues. Small amount of salivary flow can influence caries formation by decreasing rinsing of the dentition [9]. But in the present study salivary flow did not differ between the control and study groups.

Testing the pH of the unstimulated saliva indicates the environment of mouth. In our study resting saliva pH showed significant results and this was in accordance with the previous study [10].

Saliva behaves as a buffering system to protect the mouth from colonizing microorganisms [8]. In the present study buffering capacity showed significant results [10]. Salivary parameters (hydration, viscosity, and quantity) were tested and showed no correlation with caries prevalence scored using DMFT index and supports the findings of previous studies [10,11]. This may be due to lack of sensitivity of DMFT index.

Researchers have observed an association between prevalence of *Streptococci mutans* in plaque and dental caries. Many dietary factors and some substances of well-known antioxidant effect can influence the adherence of *Streptococci mutans* [11,12]. Our study showed no correlation between *Streptococci mutans* and total antioxidant capacity in saliva.

The present study also investigated the correlation between DMFT and BMI which did not reveal any correlation and is in accordance with previous studies done [13].

CONCLUSION

Dental caries is one of the common diseases in children as well as in adults. Saliva is one of the important factors that influence the development of caries. Oxidative stress which occurs as a result of an imbalance between free radical/reactive oxygen species and antioxidant system has been implicated as one of the important contributory etiologic factors in many of oral inflammatory pathologies and dental caries is no exception.

From our results, it can be concluded that total antioxidant capacity of saliva has a linear relation with caries i.e., as the severity of caries increases, the TAC level also increases. There also appeared to be a correlation between the resting saliva pH and prevalence of early lesions as well as saliva buffering capacity and potential lesion activity of dental caries.

More clinical and laboratory tests are needed to determine the exact relationship between salivary parameters, BMI and *Streptococci mutans* level.

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