Total Antioxidant Capacity of Saliva and its Relation with Early Childhood Caries and Rampant Caries

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Dental caries is an infectious and communicable disease and multiple factors influence the initiation and progression of the disease. Recently it has been claimed that oxidative stress may play an important role in the onset and the development of several inflammatory oral pathologies and dental caries may also be included. Saliva could constitute a first line of defense against free radical- mediated oxidative stress. This is the reason why antioxidant capacity of saliva has led to increasing interest and hence the need for this study.

The antioxidant capacity of saliva was investigated in 100 children who were divided into four groups. Two of which comprised the study and control groups of children with ECC (below 71months of age) and the other two groups comprised of the study and control groups of the children with rampant caries [(RC) (6-12yrs)]. Unstimulated saliva samples were collected from all the groups. Total antioxidant capacity of saliva was evaluated by spectrophotometric assay.

The results indicated that the total antioxidant capacity (TAC) of saliva increased in children with caries. TAC also increased with the age of the children.

Keywords: *Dental caries; free radicals (FR); total antioxidant capacity (TAC).*

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INTRODUCTION

The science of dentistry has existed for long, ever since there has been theorizing about the cause for dental caries. Today, all experts on dental caries generally agree that it is an infectious and communicable disease and that multiple factors influence the initiation and progression of the disease.[1]

Many of the details of the dental caries process have been known for a long time. Research findings are continually being published on various aspects of dental caries such as the microbiology of caries, the biofilm, demineralization and remineralization, fluoride applications, dietary components, saliva, and fluoride- containing dental materials.[2] Apparently it is still not possible to embrace the science and fully implement it to reduce the level of dental caries in the population or even try to implicate a particular cause for the same.[2]

The reduction of molecular oxygen to water is accompanied by a large free energy release that can give rise to Free Radicals (FR) and/or Reactive Oxygen Species (ROS).[3] The most important FR in biological systems are radical derivatives of oxygen. Other highly reactive compounds are known as ROS. ROS include not only oxygen FR but also non-radical oxygen derivatives involved in oxygen radical production. The reactivity and associated toxicity of these may be major contributors to the pathogenesis of several chronic degenerative diseases including dental caries.[3, 4]

It has been recognized that saliva serves as a mirror of the body's health, as it contains proteins, hormones, antibodies and other molecules that are frequently measured in standard blood tests to monitor health and disease.[5] However, unlike whole blood, saliva is easy to collect, less painful to the patient and is less infectious for the health care provider.[5]

Very little has been discussed about total antioxidant capacity of saliva with Early Childhood Caries and Rampant Caries.

MATERIALS AND METHOD

100 children who reported to the Department of Pedodontics and Preventive Children Dentistry, A.B. Shetty Memorial Institute of Dental Sciences, Mangalore were included in the study. These children were divided into two main groups,

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Early Childhood Caries (<71months of age) and Rampant Caries (6-12yrs of age) as *per* their definitions.[23, 1] Each of these main groups was further subdivided into two subgroups, study and controls each having 25 children. The dental caries status was assessed using the WHO Oral Assessment Form.[6] The total antioxidant capacity was evaluated using spectrophotometric assay.[7] Informed consent from the parents and all the selected children were taken. Patients who were physically and medically compromised and who had arrested carious lesions were excluded. For the collection of saliva the child was seated in the coachman's position, head slightly down and was asked not to swallow or move his tongue or lips during the period of collection. The saliva was allowed to accumulate in the mouth for 2 minutes and he or she was asked to spit the accumulated saliva into the receiving vessel.[8] 2ml of unstimulated saliva was collected and stored at a temperature of 4ºC in plastic or glass vials. The collected saliva was subjected to analysis using Spectrophotometer.[9]

The total antioxidant capacity of saliva was evaluated using the spectrophotometric assay.[9] The method is based on the principle that, when a standardized solution of Fe–EDTA complex reacts with hydrogen peroxide by a Fenton-type reaction, it leads to the formation of hydroxyl radicals (•OH). These reactive oxygen species degrade benzoate, resulting in the release of thiobarbituric acid reactive substances (TBARS). Antioxidants from the added sample of human fluid cause suppression of the production of TBARS. This reaction can be measured spectrophotometrically and the inhibition of color development defined as the antioxidant capacity. [8, 10, 11]

All the data obtained were subjected to statistical evaluation using student's unpaired *t* test.

RESULTS

The children of the ECC study and control groups were compared for the TAC of saliva. It was found that the study group generally had higher levels of antioxidants than the controls. When the mean TAC of saliva was compared, it was found that the children belonging to the study group had higher values and the results were found to be statistically significant (Table 1)

Table 1. Comparison of mean scores of TAC of saliva among study and control groups

	Group	N	Mean TAC (µmol/l)	Std. Deviation	Sig. P value
TAC (µmol/l)	Control	25	14.152	4.246	.000 (vhs)
	ECC	25	25.584	12.123	
	Control	25	22.96	4.76	.000 (vhs)
	RC	25	46.12	.99	

P > .05 NOT SIGNIFICANT (ns)
P < .05 SIGNIFICANT (s)
P < .01 HIGHLY SIGNIFICANT (hs)
P < .001 VERY HIGHLY SIGNIFICANT (vhs)

Similarly when the children belonging to the RC study and control groups were compared, the results showed that the study groups had higher levels of antioxidants and when the mean TAC of saliva was compared it was found that the study group had higher values than the controls and the results were statistically significant. (Table 1)

When the whole population in the study was divided into a range of age groups, it was found that there was a gradual increase in the Total Antioxidant Capacity of saliva with age and the results were found to be statistically significant. (Table 3)

The intergroup comparison was done and it was found that children belonging to the RC group (6-12yrs) had higher levels of antioxidants than children belonging to the ECC group (below 71months of age) which was statistically significant. (Table 2)

When the gender was compared with the levels of antioxidants, though minor differences were found the results were not statistically significant (27.19 $\pm$ 17.85µmol/l for males and 27.22 $\pm$ 16.05 µmol/l for females). (Table 4)

DISCUSSION

For a clinician, saliva means ' whole saliva', which is the fluid present in the mouth and comprises not only pure

Table 2. Table for Correlation Comparison of Mean Age Between Study and Control Group & Its Correlation With TAC of Saliva

Group	N	Mean TAC (µmol/l)	Mean Age (yr)	Correlation between TAC and Age
Control	25	14.152	4.44 SD = 0.58	
ECC	25	25.584	4.36 SD =0.70	
Control	25	22.96	7.56 SD = 1.04	
RC	25	46.12	7.80 SD = 0.82	
Total	100	27.816	6.16 SD = 0.78	r = .428, p = .000 (vhs)

Table 3. Comparison of mean TAC of saliva between different age groups

Age Groups	Mean TAC (µmol/l)	N	Std. Deviation	F	Sig. p value
< 4yrs	20.0462	26	9.7939	7.843	**.000 (vhs)**
4-6yrs	19.6750	24	11.61376		
6-8yrs	32.7269	26	18.54567		
>8yrs	36.5083	24	19.23457		

Table 4. Comparison of means between the genders

Sex	Mean	N	Std. Deviation
males	27.1900	50	17.85767
females	27.2200	50	16.05929

secretions from the major and minor salivary glands but also gingival exudates, microorganisms, and their products, epithelial cells, food remnants, and also to some extent nasal exudates.[12]

Saliva may constitute a first line of defense against FR mediated oxidative stress, since the process of mastication promotes a variety of such reactions including lipid peroxidation.[15]

Unstimulated saliva samples was used as it is preferred in determination of antioxidant defense parameters to stimulated saliva and moreover it is claimed that Total Antioxidant Capacity is higher in unstimulated saliva.[13]

The total antioxidant capacity of saliva was evaluated as it is suggested that FR/ROS and antioxidant system 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. Moreover, the number of different antioxidants makes it difficult, and also expensive, to measure each of them separately.[16]

In all the previously reported literatures where individual antioxidants have been evaluated in caries free and caries active individuals, no statistically significant results were ever reported and they suggested that total antioxidant capacity be evaluated rather than individual antioxidants.[15]

Oxidative stress which occurs as a result of an imbalance between the FR/ROS and antioxidants (AO) system has been implicated as one of the important contributory etiologic factors in many of the oral inflammatory pathologies and supposedly dental caries is no exception.[14]

When we compared the TAC of saliva in children with ECC and rampant caries (RC), the results suggest an increase in the TAC of saliva in children with caries be it ECC or RC indicating a linear relation between TAC of saliva and caries.

It has been suggested that the levels of antioxidants could be altered in response to an infection or disease.[19] The presence of an infectious challenge in the form of caries or poor oral hygiene as observed in our study groups could be one of the factors for the comparatively increased levels of total antioxidant capacity of saliva.

An important factor could also, be the triggered function of the salivary peroxidase system which constitutes one of the major salivary antioxidant systems. Salivary peroxidase brings about the control of the oral bacteria that form dental plaque, to imbalances in the ecology and which lead to dental caries. Salivary peroxidase catalyses the peroxidation of the thiocyanate ion (SCN^-) to generate oxidation products (more stable $OSCN^-$) that inhibit the growth and metabolism of many microorganisms thereby inhibiting caries or at least slowing down the progress of caries.[18]

Previously reported quantitative studies of salivary composition and caries activity, though have been inconclusive, there is evidence that similar proteins in saliva from caries active and caries free persons may have different levels of biological activity and it was reported that salivary proteins were increased in individuals with dental caries.[20] The higher TAC levels in caries active children may also be attributed to elevated protein levels.

We also observed that the total antioxidant capacity of saliva increases with age showing increased levels in the older age groups when compared to the younger age groups.

The differences in the nutritional requirements and also the change of food pattern from softer and semisolid foods to harder and more solid foods which may contain larger volumes of antioxidants in the form of micronutrients can also be a reason for the increased levels of antioxidants in the older age group.

The immune status improves with age and at an older age owing to the optimal immunity; the corresponding total antioxidant capacity levels of saliva could also be increased.[22]

The relationship between total antioxidant capacity and gender of the children could not be established in this study though we feel that the levels of antioxidants can get altered with age owing to the hormonal fluctuations encountered during the pubertal ages.

Recent clinical trials have found that antioxidant supplementation can significantly improve certain immune responses.[21] Supplementation with the antioxidant vitamins also protected immune responses in individuals exposed to certain environmental sources of free radicals. [21] Therefore it also needs to be evaluated in the future if the levels of antioxidants differ after the supplementation with antioxidants.

We are yet far away from establishing the role of antioxidants in oral health nevertheless this study hopefully shall begin the journey.

CONCLUSIONS

From the study we derived the following:

1. Total antioxidant capacity of saliva increases with ECC and RC.
2. Total antioxidant capacity of saliva has a linear relation with age.
3. Total antioxidant capacity of saliva has no statistically significant relation with respect to gender.

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