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An estimation and evaluation of total antioxidant capacity of saliva in children with severe early childhood caries

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Background. The available evidence implicating the involvement of oxidative stress in the caries process suggests that local antioxidant status may be of importance in determining the susceptibility to the caries process.

Aim. The aim of this study was to estimate the total antioxidant capacity (TAC) in unstimulated saliva of healthy children with and without severe early childhood caries (S-ECC) and to correlate the individual TAC level with dmft (d = decayed, m = missing, f = filled, t = teeth) score and age.

Material and methods. The TAC of saliva was investigated in 100 healthy children in the age range of 3–5 years divided in two groups, control and study group based on the absence or presence of caries, respectively. The antioxidant capacity of saliva was estimated by an adaptation of ABTS [2, 2′-Azino-di-(3-ethylbenzthiazoline sulphonate)] assay.

Results. The mean TAC level in the saliva of the children in study group was found to be significantly increased ($P < 0.001$), and a significantly linear regression was seen between the TAC and dmft score ($P < 0.001$) whereas it was insignificant between the TAC and age ($P = 0.078$).

Conclusion. The results indicated that TAC of saliva increased significantly in children with S-ECC and increasing prevalence of dental caries predisposes to the increase in TAC of saliva.

Introduction

Early childhood caries (ECC) is one of the most severe and pandemic form of dental caries affecting almost all sections of the modern world with a global incidence ranging from 3% to 45%.[1] The disease of ECC is defined as 'the presence of one or more decayed (noncavitated or cavitated lesions), missing (due to caries), or filled tooth surfaces' in any primary tooth in a child 71 months of age or younger.[2] In children younger than 3 years of age, any sign of smooth-surface caries is indicative of severe early childhood caries (S-ECC). From ages 3 through 5, 1 or more cavitated, missing (because of caries), or filled smooth surfaces in primary maxillary anterior teeth, or a decayed, missing, or filled score of ≥4 (age 3), ≥5 (age 4), or ≥6(age 5) surfaces constitutes S-ECC.[3] Biologically, ECC is an infectious process catalysed by the frequent and prolonged exposure of sugars, such as those present in milk, formula, and fruit juice, to the teeth's surface with the *Streptococcus mutans* being the primary microbiological agent.[4] The children with S-ECC have a higher susceptibility to dental caries in permanent dentition and are likely to be underweight with slower growth.[5]

The caries process is controlled to a large extent by a natural protective mechanism inherent within the saliva. The role of saliva is so unique and special that further discussion is warranted regarding its influence on several aspects of caries process that may help to produce favourable environments to combat the disease process.[6] Saliva serves as a mirror of the body's health and contains protein, hormones, antibodies, and other molecules that are frequently measured in standard blood tests to monitor health and disease.[7]

Antioxidants are present in all body fluids and tissues which protect against endogenously formed free radicals (FR). The reduction of

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molecular oxygen to water is accompanied by large free energy release that can give rise to FR and/or reactive oxygen species (ROS). Oxidative stress which occurs as a result of an imbalance between the FR/ROS and the antioxidants system has been implicated as one of the important contributory etiologic factors in many of the oral inflammatory pathologies[8] in which dental caries may also be included. Recently, it has been claimed that saliva could constitute a first line of defence against free radical–mediated oxidative stress.[8] A protective potential role of saliva in the pathogenesis of caries process has been implicated in various studies, but the possible role of endogenous host-associated attributes of saliva in this disease process has so far received little attention.

The aim of this study was to estimate the total antioxidant capacity (TAC) in unstimulated saliva of healthy children with and without S-ECC and to correlate the individual TAC level with dmft (d = decayed, m = missing, f = filled, t = teeth) score and age.

Material and methods

This study was carried out in the Department of Pedodontics with Preventive Dentistry, Faculty of Dental Sciences (FODS), CSM Medical University, Lucknow in collaboration with Cardiovascular Toxicology Division, Indian Institute of Toxicology Research (IITR), Lucknow. The study was approved by the Institutional Ethics Committee, CSM Medical University, Lucknow. Consent forms were signed by parents or legal guardians, prior to patient enrolment in the study.

Subjects

One hundred children within the age range of 3–5 years, comprising of 51 boys and 49 girls who attended the paediatric dental clinic at FODS, CSM Medical University, Lucknow, were included in the study. The children who were physically and mentally compromised, were on medications, had dental fluorosis, and arrested carious lesions were excluded from the study. The children included were divided into two equal groups: control group (n = 50) with no dental caries (dmft = 0) and study group (n = 50) who had dmft score of ≥4 (age 3), ≥5 (age 4), or ≥6 (age 5). The control group included 26 boys and 24 girls, whereas the study group included 25 boys and 25 girls. Caries status was recorded using the World Health Organization recommendations.[9]

Saliva

Special diet was given to the subjects 24 h before saliva collection by a dietician. Unstimulated saliva samples were collected in the morning 1 h after routine dental brushing and a fasting period of at least 2 h to minimize diurnal variation. The subjects were asked to seat comfortably with eyes open, head tilted slightly forward with restricted orofacial movements and were instructed to rest for 5 min. Saliva was allowed to accumulate in the floor of the mouth, and the subjects were asked to spit into a sterile saliva collecting vial. The collected samples were stored in a hermetically sealed case containing ice and transported to laboratory within 1 h of collection and were stored at −80°C, until analysis.

Antioxidant assay analysis

Total antioxidant capacity of saliva was measured using antioxidant assay kit. The antioxidant assay kit was provided by Cayman Chemical Company, Ann Arbor, USA. The kit is based on the principle of inhibition of the oxidation of ABTS [2, 2′-Azino-di-(3-ethylbenzthiazoline sulphonate)] to radical cation $ABTS^{\cdot+}$ by the antioxidants present in the sample. $ABTS^{\cdot+}$ is formed by the interaction of ABTS with the ferrylmyoglobin radical species, generated by the activation of metmyoglobin with H_2O_2 and this reaction produces a blue/green colour. The antioxidants in the sample causes suppression of the absorbance of $ABTS^{\cdot+}$ at 750 or 405 nm to a degree which is proportional to their concentration. The capacity of the antioxidants to prevent ABTS oxidation is compared with that of Trolox, a water soluble tocopherol analogue. This reaction was measured spectrophotometrically, and the inhibition of blue/green colour development depicted the TAC.

Statistical analysis

The statistical analysis was performed using SPSS 15.0 software (Chicago, IL, USA). Kolmogorov–Smirnov was used to test the normality prior to analysis. Data were expressed as mean ± standard deviation (X ± SD). The children in control and study group were compared for TAC of saliva using independent Student's *t*-test. Simple linear regression analysis was performed to assess the association of TAC with age and dmft score, considering age and dmft score as independent variable and TAC the dependent variable. Only differences with *P*-value <0.05 were considered as statistically significant.

Results

TAC levels in S-ECC

The children in the study and control group were compared for the TAC of saliva. The mean TAC level in the saliva of the children in control group was found to be 0.568 ± 0.169 mM, whereas in the study group, it was 1.729 ± 0.297 mM ($t = 24.03$, $P < 0.001$). The mean TAC level of saliva is significantly increased in study group when compared to the control group.

TAC and dmft score

The association between mean TAC and mean dmft score was studied using linear regression analysis as shown in Fig. 1. A statistically significant linear regression was seen between the TAC and the dmft score ($R^2 = 0.93$, $F = 128.92$, $P < 0.001$). TAC increase is in direct proportion with number of teeth affected by caries.

TAC and age

The association between mean TAC and mean age was studied using linear regression analysis as shown in Fig. 2. A statistically insignificant linear regression was evident between TAC and age ($R^2 = 0.30$, $F = 3.94$; $P = 0.078$).

TAC and gender

The results were found to be statistically not significant when the mean TAC level of saliva was compared for gender. In the control group, mean TAC was 0.551 ± 0.175 mM and 0.586 ± 0.164 mM in boys ($n = 26$) and girls ($n = 24$) ($P = 0.619$), respectively, whereas in the study group, mean TAC was 1.726 ± 0.298 mM and 1.731 ± 0.302 mM in boys ($n = 25$) and girls ($n = 25$) ($P = 0.934$), respectively, as shown in Fig. 3.

Discussion

A paradox in metabolism is the majority of multicellular organisms require oxygen for its existence; however, oxygen is highly reactive molecule that damages living organisms by producing FR/ROS.[10] The reactivity and associated toxicity of FR/ROS may be the major contributors of the pathogenesis of several chronic degenerative diseases. Antioxidants are present in all biological species and protect against the potentially harmful effects of FR. Therefore, biological antioxidants form an important part of our diet as together with

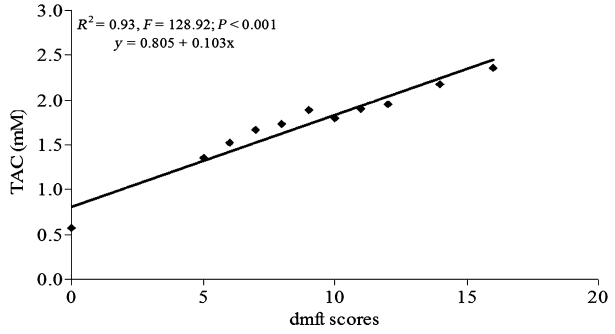

Fig. 1. Linear regression analysis of mean total antioxidant capacity with mean dmft scores.

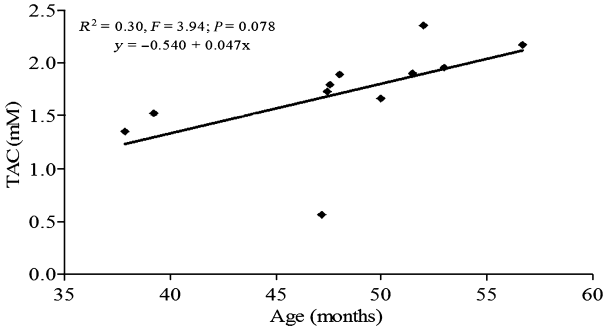

Fig. 2. Linear regression analysis of mean total antioxidant capacity with mean age.

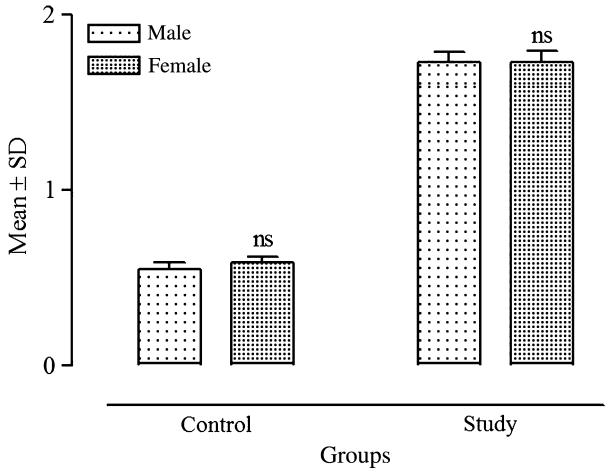

Fig. 3. Bar graph showing mean (±SD) saliva total antioxidant capacity level (mM) of boys and girls in control and study group.

intracellular antioxidants, and antioxidant enzyme systems may prevent various pathological processes.[8,11] Moreover, the available evidence implicating the involvement of oxidative stress in the caries process suggests that local antioxidant status may be of importance in determining the susceptibility to the caries process.

Saliva is a complex biological fluid composed of enzymes, hormones, antibacterial constituents, electrolytes, as well as the compounds transported from blood.[8,12] Thus, it is functionally equivalent to serum in reflecting the physiological state of body including hormonal, nutritional, and metabolic disturbances.[12] Unstimulated saliva was favoured in the determination of antioxidant defence parameters to stimulated saliva as it is reported that TAC is higher in unstimulated saliva.[13]

As for the literature available, very little has been discussed about the association between TAC of saliva and ECC; moreover, the study regarding the estimation of TAC of saliva in S-ECC has still not been reported. In previous studies where individual antioxidants have been evaluated in caries-free and caries-active individuals, no statistically significant results were ever reported. Investigations of individual antioxidant activity may be expensive as well as misleading and less representative of the whole antioxidant status;[14] therefore; in this study, the TAC of saliva was evaluated as it has been suggested that FR/ROS and antioxidant system appear to act in concert rather than alone.[14] Tulunoglu *et al.*[15] evaluated the TAC of saliva in 7–10 and 11–15 years of children and reported that the TAC were higher in caries active children, but the differences were not statistically significant ($P > 0.05$). This might be due to the relatively small sample size ($n = 10$) in each group of their study. Preethi *et al.*[16] also estimated the TAC of saliva in 7–10 and 11–14 years of children, but with relatively larger sample size ($n = 30$) and reported that the mean level of TAC is increased in caries active children when compared to caries-free children and is statistically significant. Uberos *et al.*[17] evaluated the relationship between TAC of saliva and the presence of caries in primary and permanent dentition and reported statistically insignificant results ($P = 0.06$). They, however, observed a statistically significant linear regression between the number of primary teeth with caries and the salivary TAC ($P = 0.004$). Hegde *et al.*,[18] in their study, estimated TAC of saliva in children with ECC and rampant caries (RC) and reported that the TAC of saliva increases with ECC and RC and had a linear relation with the age. In this study, we found an increase in mean TAC level in saliva in children with S-ECC ($P < 0.001$) and a statistically significant linear regression between the number of primary teeth with caries and TAC of saliva: $y = 0.0805 + 0.103x$ ($R^2 = 0.93$, $F = 128.92$, $P < 0.001$). Insignificant linear regression, however, was seen between the age and the TAC of saliva: $y = -0.540 + 0.047x$ ($R^2 = 0.30$, $F = 3.94$, $P = 0.078$), which might be due to narrow age range included in this study (3–5 years) when compared to Hegde *et al.*[18] who had compared the TAC levels in a much wider age group (<71 months and 6–12 years).

The increase in TAC can be explained by the fact that the levels of antioxidants could be altered in response to infection or disease.[8] The increased level of TAC in the study group comprising of patients with active carious lesions could be the direct consequence of infective foci within the caries itself, which is further validated by the fact that a statistically

significant correlation was found between TAC and caries activity. Salivary antioxidant level is dependent on very few components that include mainly the uric acid molecule and the peroxidase enzyme;[19] therefore, it can be hypothesized that increase in their levels in saliva can also increase the TAC level. The increase in TAC in saliva might be due to the activation of salivary peroxidase system to counteract the caries process. It has been reported that oral peroxidase systems in human saliva inhibits the growth of *Strep. mutans*, *Strep. sobrinus*, and *Lactobacillus casei* by catalysing the peroxidation of thiocynate ion (SCN^-) to generate more stable oxidation products ($OSCN^-$) at pH 5, and peroxidase systems have strong antistreptococcal effects.[20]

It also has been suggested that saliva is rich in antioxidants, mainly uric acid with lesser but definite contributions from albumin, ascorbate, and glutathione, and all of these are proteins or have proteins in their structure. Some researchers reported that the similar proteins in saliva from caries active and caries-free persons may have different levels of biologic activity, and salivary proteins were increased in individuals with dental caries.[16,21] The increased TAC levels in saliva in caries active children may be accredited to elevated protein levels, but recently Zehetbauer *et al.*[22] reported that there is uniform expression of salivary protein profiles between children with ECC and caries-free children.

The increase in the TAC level can also be attributed to diet in ECC. The sum of endogenous and food-derived antioxidants represents the total antioxidant activity of the system. Uric acid is the major antioxidant present in human saliva accounting for more than approximately 85% of the TAC of both unstimulated and stimulated saliva[19] and is mainly derived from the diet.[23] Children with ECC have frequent and prolonged consumption of sugars from liquids. Caries-promoting sugars such as sucrose, glucose, and fructose, contained in fruit juices, and many infant formula preparations are readily metabolized by *Strep. mutans* and *Lactobacilli* to organic acids that demineralize enamel and dentin.[24] One of the striking features of fructose is its ability to produce uric acid. Serum uric acid can increase rapidly after ingestion of fructose,[25] and studies in which fructose (or sucrose) diets have been administered have found that even fasting uric acid levels will increase after several weeks.[26] Moreover, recently, it has been reported that intake of added sugar and sugar-sweetened drinks increases the serum uric acid concentration.[27] This might increase the salivary uric acid concentration, as to a large extent serum composition is reflected in saliva composition leading to the increase in TAC of saliva. Thus, the habit of increased sugar intake might be responsible for increase in uric acid level and TAC, at least in part, being confounding factor for both the development of caries and increase in uric acid level. Further studies regarding role of uric acid, its contribution in TAC of saliva and its association with diet are required to better understand this phenomenon.

Conclusions

From our results, it can be concluded that the TAC of saliva increases in children with S-ECC and is in direct proportion with number of primary teeth affected by caries. The increasing prevalence of dental caries predisposes to the increase in TAC of saliva. The TAC of saliva has no significant relation with respect to age and gender. Further longitudinal studies may be carried out in future to establish the temporal relationship between the TAC of the saliva and the S-ECC in children.

What this article adds

- The total antioxidant capacity of saliva is significantly increased in children with severe early childhood caries.
- The increasing prevalence of dental caries predisposes to the increase in total antioxidant capacity of saliva.

Why this article is important for paediatric dentists

- This article estimated the total antioxidant capacity of saliva in children with severe early childhood caries and evaluated the reasons for the increase in the total antioxidant capacity, which can be useful measure of caries activity in children and allow paediatric dentists to implement preventive practices that can decrease a child's risks of developing this devastating disease.

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