

Estimation of total antioxidant capacity levels in saliva of caries-free and caries-active children

RESHMA DODWAD, ANUPAMA V. BETIGERI¹, PREETI B. P.²

Abstract

Background and Objectives: The purpose of this study was to evaluate total antioxidant levels in caries-free and caries-active children. **Materials and Methods:** The present study included one hundred and twenty healthy children who were divided into two groups; Group I and Group II, comprising of age groups 7 to 10 years and 11 to 14 years, respectively. Both the groups were then sub-divided equally according to gender. They were further divided into caries-free and caries-active children with 15 children in each group. Unstimulated saliva was collected by suction method. The samples were then analyzed for total antioxidant capacity. The data was then statistically analyzed using Student's t-test (unpaired). **Results:** The results revealed that the total antioxidant capacity of saliva increased significantly in caries-active children when compared to caries-free children. **Conclusion:** Within the limitation of this study, we can conclude that the physicochemical properties of saliva in particular total antioxidant capacity play a role in the development of caries.

Keywords: Dental caries, saliva, total antioxidant capacity

Introduction

Among oral diseases, dental caries is the most common chronic disease of mankind.^[1] It affects all people regardless of their sex, socioeconomic strata, race, and age. It is also profoundly affected by other factors like oral hygiene and saliva.^[2]

The saliva circulating in the mouth at any given time is termed as whole saliva and it comprises of a mixture of secretions from the major and minor salivary glands and traces from the gingival crevicular fluid. Saliva definitely promotes oral health, and hence, lack of its secretion contributes to the disease process.^[2,3] The saliva by constantly bathing the teeth and oral mucosa, 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.

It has been long 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. However, unlike wholeblood, saliva is easy to collect, less painful to the patient, and is less infectious for the healthcare provider. Healthy individuals produce about a litre and a quarter of saliva per day. Nearly all analytes that are in blood are also present in saliva. Although saliva has not been used till date as a sampling media, it does have a strong potential to do the same types of tests that are done currently using blood.^[4] As for the literature available, little has been discussed about dental caries and antioxidants.

Recently, it has been claimed that the imbalances in levels of free radicals, reactive oxygen species, and antioxidants in saliva may play an important role in the onset and development of dental caries.^[5] Hence, evaluation of those factors in saliva that may increase the risk of individuals to dental caries, can pave way to make recommendations that will cater specifically to needs of an individual.^[6]

Departments of Pedodontics and Preventive Dentistry, College of Dental Sciences, Davangere, Karnataka, ¹Department of Physiology, VMMC and Safdarjung, New Delhi, ²Department of Biochemistry, JJM Medical College, Davangere, Karnataka, India

Correspondence: Dr. Reshma Dodwad, Department of Pedodontics and Preventive Dentistry, College of Dental Sciences, Davangere, Karnataka - 577004, India.
E-mail: reshma.dodwadpedo@gmail.com

Materials and Methods

Subjects

One hundred and twenty children aged between 7 and 14 years reporting to the Department of Pedodontics and Preventive Dentistry, Bapuji Dental College and Hospital, Davangere, were included as subjects of this study. They were divided into two groups; Group I and Group II, comprising of age groups 7 to 10 years and 11 to 14 years, respectively.

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Both the groups were then sub-divided according to gender with 30 children in each group. They were then divided into caries-active and caries-free groups, with 15 children in each group. Salivary analysis was carried out in the Biochemistry department of J.J.M. Medical College, Davangere. The study protocol was approved by the Ethics Committee of J.J.M. Medical College, Davangere. Informed consent was obtained from parent/guardian of each child.

The inclusion and exclusion criteria used are as follows:

Inclusion criteria

- Free from systemic or local diseases, which affect salivary secretions.
- Children should be permanent residents of Davangere city and should be consuming only municipal water.
- Caries status was assessed according to the WHO criteria
 - Caries-active children having at least five decayed tooth surfaces.
 - Caries-free children having no caries, DMFS = 0.

Exclusion criteria

- Patients who are physically and medically compromised.
- Patients who are on medications.
- Patients who have arrested carious lesions

Stratified randomized sampling procedure was employed for statistical analysis.

Unstimulated saliva was collected for the study. The saliva was allowed to accumulate in patient's mouth for 2 minutes and was aspirated directly from the floor of the mouth. Each sample was estimated for total antioxidant capacity.

Salivary analysis

Estimation of total antioxidant capacity of saliva

Total salivary antioxidant levels were estimated by using a spectrophotometer.^[7]

Principle: A standardized solution of Fe-EDTA complex reacts with hydrogen peroxide by a Fenton-type reaction leading to the formation of hydroxyl radicals. These reactive oxygen species degrade benzoate, resulting in release of thiobarbituric acid reactive substances (TBARS). Antioxidants from the added human sample (saliva) cause suppression of production of TBARS. This reaction can be measured spectrophotometrically and inhibition of color development as Total antioxidant capacity.

Statistical analysis

Results are presented as mean \pm standard deviation values. Student t-test was used to compare the mean values between caries-free and caries-active groups.

A *P* value of 0.05 or less was considered for standard significance.

Results

Results of this study revealed that total antioxidant capacity levels were increased in caries-active children when compared to caries-free children. It was found that as age increased total antioxidant capacity levels also increased [Table 1].

Discussion

Saliva may contribute a first line of defense against free radical-mediated oxidative stress, since the process of mastication promotes a variety of such reactions including lipid peroxidation.^[8] In our study, we have evaluated total antioxidant capacity in contrast to other reported literature available where individual antioxidant have been evaluated. We took total antioxidant capacity in our study as it is suggested that free radical/reactive oxygen species and antioxidant system appear to act in concert rather than alone, and measurement of any individual antioxidant may be less representative of whole antioxidant status. Moreover, the number of different antioxidants makes it difficult and also expensive to measure each of them separately.^[9]

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 (TAC) be evaluated rather than individual antioxidants. Oxidative stress, which occurs as a result of an imbalance between free radicals/reactive oxygen species and antioxidant 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.^[10]

Total antioxidant capacity levels in caries-free group

As observed in our study, caries-free children had significantly lower levels of TAC when compared to caries-active children.

Table 1: Total antioxidant capacity levels of saliva in caries-free and caries-active children

Gender	Age	Caries activity	Total antioxidant capacity levels
Girls	7-10	CF	0.16 \pm 0.03
Girls		CA	0.23 \pm 0.05
CF v/s CA			t = 4.18; <i>P</i> < 0.001
Boys	7-10	CF	0.16 \pm 0.05
Boys		CA	0.20 \pm 0.04
CF v/s CA			t = 2.91; <i>P</i> < 0.05
Girls	11-14	CF	0.19 \pm 0.04
Girls		CA	0.23 \pm 0.04
CF v/s CA			t = 2.90; <i>P</i> < 0.01
Boys	11-14	CF	0.19 \pm 0.04
Boys		CA	0.22 \pm 0.04
CF v/s CA			t = 2.21; <i>P</i> < 0.05

Unpaired t-test; *P* < 0.05 – Significant; *P* > 0.05 – Not significant

The results are suggestive of the fact that TAC is lower in children who have good oral hygiene status and also that TAC levels increase with age. The plausible reasons for the levels of TAC in caries-free children could be as follows: It has been suggested that the levels of antioxidants could be altered in response to an infection or disease.^[11] The absence of an infectious challenge in the form of caries or poor oral hygiene in caries-free children could be one of the factors for the comparatively decreased levels of TAC of saliva.

TAC and caries

We observed increase in TAC of saliva in children with caries, the plausible reasons of which could be as follows: Most important would be the function of salivary peroxidase system, which constitutes one of the major salivary antioxidant systems. Salivary peroxidase brings about the control of oral bacteria that form dental plaque, to imbalances in the ecology, and which lead to dental caries. Salivary peroxidase catalyzes the peroxidation of thiocyanate ion (SCN⁻) to generate oxidation products (more stable OSCN⁻); this inhibits the growth and metabolism of many microorganisms thereby inhibiting caries or at least slowing down the progress of caries.^[12] It has been suggested that saliva is also rich in antioxidant mainly uric acid with lesser but definite contributions from albumin, ascorbate, and glutathione and all of these are proteins or have protein in their structure. It has been reported that uric acid is the major antioxidant in saliva accounting for more than 85% of TAC of both unstimulated and stimulated saliva. Parallel results were seen in studies conducted by Moore *et al.*^[13] and Pereslegina *et al.*^[14]

TAC and age

In our study, we also found that TAC of saliva in children increased with age. There was a significant increase of TAC in children belonging to 11–14 years age group when compared to 7–10 years age group. The plausible reasons that can be suggested for an increase in TAC in children with age could be as follows:

A reason that can be suggested is nutrition, which may vary between the two age groups. Children belonging to younger age group may be having nutritious diet but may be consuming lesser volumes of food, and hence lower volumes of micronutrients when compared to the older age group accounting for the higher levels of TAC. This increase in TAC of saliva may be a normal feature with increase in age. Also, immune status of children improves with age suggesting that it is better developed in older age group and could therefore account for the increase in TAC of saliva in children belonging to older age group.^[15] Our results were in sync with a related study conducted by Tulunoglu *et al.* that also reported that TAC of saliva increased with caries activity.^[16]

Another potential factor that may alter the levels of total antioxidant capacity levels with respect to age and gender is that as the child approaches and goes through pubertal

growth spurts, wherein significant hormonal changes occur, there could be change in TAC.

Conclusion

As observed in the present study, TAC of saliva has a linear relation with caries, also with increasing caries indices, i.e., as the severity of caries increases it was observed that TAC levels also increase. We also observed that the TAC levels increased with age. But all these findings need to be confirmed using larger sample sizes. Also, the relation between TAC levels and gender and hormonal differences need to be evaluated and established.

Recommendations

To the parents and children

It has been suggested that dental caries is a worldwide problem and very much prevalent during childhood. It has been found that fruits and vegetables are good sources of antioxidants. We can expect that increased intake of such foods may be important in suppressing the growth of acid-forming bacteria in children with high caries risk or at least these antioxidants may help in preventing the progress of this disease.

To the pedodontist

Antioxidant supplementations can be a possible point of interest. It can be added in very low volumes in dental material like cements, which are commonly used for restorative purposes. Also, antioxidant supplements can be tried in mouthwashes and topical fluorides.

However, before prescribing antioxidant supplements it becomes mandatory to definitely establish its role in the etiopathogenesis of any given oral condition and also its influence on the progress or even its ability to prevent a certain condition. Although there have been numerous reported research papers on the altered antioxidant levels in various conditions, there remains an unanswered query that whether it helps in the prevention or at least halts the progress of the condition once it has set in.

Future studies can be aimed at determining:

1. The relationship between the TAC levels of saliva and gender predilection can be established.
2. The relationship of TAC levels of saliva and serum levels of TAC can be evaluated and compared.
3. The relationship of TAC levels in children before treatment and after treatment of dental caries can be evaluated.
4. The relationship between diet and TAC levels can be investigated inclusive of diet counseling.

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