

# Relation of caries status on the salivary total antioxidant levels in asthmatic children

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## Abstract

**Aim:** To study the correlation between the caries status and the salivary antioxidant levels among asthmatic children. **Settings and Design:** One hundred children within the age group of 6-12 years were selected and equally subdivided into children having a history of asthma and their healthy siblings. **Materials and Methods:** Salivary antioxidant levels were assessed using spectrophotometric method, and the caries status was recorded using DMFT (Decayed, missing and Filled Teeth Index) and dft (decayed, filled teeth index) index. **Result:** The salivary antioxidant levels were reduced among the asthmatic children which was statistically highly significant, whereas the caries prevalence was reduced, but not statistically significant.

**Keywords:** Antioxidants, asthma, dental caries

## Introduction

Bronchial asthma is a chronic inflammatory disorder of airways characterized by variable air flow obstruction and airway hyper-responsiveness.<sup>[1]</sup> It is a serious global health problem affecting more than 100 million people worldwide and its prevalence is rising in most countries of the world, including India.<sup>[2]</sup> In asthma, the inflammatory cells infiltrating the airways produce several mediators that modulate the inflammatory response. In recent years, oxidative stress has been increasingly recognized as one of the major factors contributing to the chronic inflammatory process.<sup>[3]</sup> Children with asthma show evidence of oxidant-antioxidant imbalances in their peripheral blood such as increased superoxide generation from leukocytes, increased total nitrates and nitrites, increased protein carbonyl, increased lipid peroxidation products and decreased protein sulfhydryl in plasma, indicating an increased oxidative stress.<sup>[4]</sup> At the same time, as asthmatic children are

on long term inhaler and liquid medicaments, it leads to a higher caries prevalence and this is found to increase with the severity of the bronchial asthma.<sup>[5,6]</sup>

Saliva, which serves as a mirror of the body's health is described as a heterogeneous fluid composed of proteins, glycoproteins, electrolytes, and small organic molecules, as well as compounds transported from blood. Saliva may constitute a first line of defense against free radical (FR) mediated oxidative stress, since the process of mastication promotes a variety of such reactions, including lipid peroxidation. Human whole saliva contains a complex peroxidase system; which controls the oral bacteria that form dental plaque, leading to dental caries.<sup>[7]</sup> Saliva has been used in various studies as an important lab adjunct for diagnosis.<sup>[7,8]</sup>

As for the current literature available, little has been discussed about salivary antioxidants in children with asthma and their dental caries status. Hence, this study was undertaken to evaluate the same.

## Materials and Methods

The study was carried out among 100 children, aged 6-12 years who reported to the Department of Pedodontics and Preventive Children Dentistry, A.B. Shetty Memorial Institute of Dental Sciences, Mangalore and Department of Paediatrics, Justice K S Hegde Charitable Hospital, Derlakatte, Mangalore. One hundred selected children were divided into two groups of 50 each, group 1:50 children with the history of asthma (study group) and group 2:50 children who were healthy siblings of group 1 children (control group). The total antioxidant capacity (TAC) of saliva was evaluated among all the selected children using the spectrophotometric assay.<sup>[9]</sup> The dental caries status was assessed using the modified WHO oral assessment form.<sup>[10]</sup> The statistical analysis was done using Student's unpaired *t*-test.

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### Method of collection of saliva

Patients were seated with their head held slightly down and were asked not to swallow or move their tongue or lips during the period of collection. The saliva samples were collected in the mornings before the consumption of food. The saliva was allowed to accumulate in their mouth for 2 min followed by asking them to spit the accumulated saliva into the receiving vessel. Similar method of collection of saliva was used in study and control group. Two milliliter of unstimulated saliva was collected and stored at a temperature of 4°C in plastic or glass vials. Then, the collected saliva was subjected to TAC analysis using the spectrophotometer.<sup>[9]</sup>

### Method of estimating total antioxidant capacity

An aliquot of 0.1 ml of sample solution containing a reducing agent (ethanol) was combined in an Eppendorf tube with 1 ml of a reagent solution, which contained 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The tube was capped and incubated in thermal block at 95°C for 90 min. A typical blank solution containing 1 ml of reagent solution and the appropriate volume of the same solvent used for the sample was prepared and it was incubated under the same conditions as the rest of the samples. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank.

Quantitation of total antioxidant level was based on the molar absorption coefficient of the phosphomolybdenum complex. Linearity was evaluated by obtaining calibration curves with multiple standards of the appropriate reducing species in parallel with the sample.<sup>[9]</sup>

## Results

In a sample size of 100 children, the mean TAC level among the control groups were 1.0296 mmol/l, which when compared to the study group was 0.9196 mmol/l, suggesting a decrease in TAC in the study group, which was statistically highly significant [Table 1].

The dental caries status using the WHO modified oral assessment form revealed mean DMFT scores of 0.81 and 0.76 in the control and study group and mean dft score of 2.04 and 1.98 respectively [Table 2].

The antioxidant level and caries experience values were both low in the study group as compared to control, but these combined factors were not statistically significant [Table 3].

## Discussion

A FR may be defined as an atomic or molecular species with one or more unpaired electrons in its structure. FR can be positively ( $\text{NAD}^+$ ) or negatively charged ( $\text{O}_2^-$ ) or electrically neutral ( $\text{OH}^\cdot$ ).

**Table 1: Comparison of mean scores of total antioxidant capacity of saliva among asthma and control groups**

Group	N	Total antioxidant levels (mmol/l)		
		Mean	SD	P value
Control group	50	1.0296	0.2203	0.001
Asthma group	50	0.9196	0.0571	

Control: Siblings of asthmatic children (not having asthma). SD: Standard deviation

**Table 2: Correlation of DMFT, difference between asthma and control group**

Group	N	Mean	SD	T	P value
DMFT					
Control group	50	0.86	0.81	1.912	0.059
Asthma group	50	0.56	0.76		
dft					
Control group	50	2.04	1.35	0.204	0.839
Asthma group	50	1.98	1.58		

SD: Standard deviation, DMFT: Decayed, missing and filled teeth index, dft: decayed, filled teeth index

**Table 3: Correlation of DMFT and dft with antioxidant levels**

Caries	Group	TAC	P value
Mean DMFT (0.86)	Control	0.137	0.347
Mean DMFT (0.56)	Asthma	0.097	0.535
Mean dft (2.04)	Control	0.135	0.350
Mean dft (1.98)	Asthma	0.133	0.357

TAC: Total antioxidant capacity, DMFT: Decayed, missing and filled teeth index, dft: decayed, filled teeth index

FR/reactive oxygen species (ROS) production and actions are rather complex and their interaction is frequent.<sup>[8]</sup> The antioxidant defense systems are also highly complex,<sup>[11]</sup> constituting an effective network capable of counteracting FR/ROS effects. It is essential to evaluate the amounts and/or the activities of the different antioxidants when assessing antioxidant status *in vivo*. However, since FR/ROS 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 various assays that evaluate the so-called "TAC" of biological fluids, including saliva,<sup>[11]</sup> similar to our study.

Asthma and other pulmonary diseases have long been associated with inflammation and oxidative stress, which is the final result of numerous molecular pathways involving ROS and reactive nitrogen species (RNS), leading to an increase in airway reactivity and secretions, increased production of chemo attractants and increased vascular permeability.<sup>[12]</sup> Oxidative-antioxidative balance also changes

in the plasma and blood cells apart from its main effect on the respiratory system.<sup>[13]</sup>

Even though the blood is the gold standard for doing many medical tests it is not particularly convenient. Expecterated saliva serves as an alternative. Healthy individuals produce about a liter and a quarter of saliva per day. Nearly all the analytes that are in blood are also present within saliva. Although saliva has not been used extensively as a sampling media, it does have a strong potential for the same.<sup>[14]</sup>

The unstimulated saliva samples have been preferred to determine antioxidant defense parameters to stimulated saliva as TAC is higher in unstimulated saliva.<sup>[14,15]</sup> Even in the present study unstimulated saliva was found to be a good sampling media to estimate the TAC of children.

The TAC values of the children from the control group ranged between 0.81 and 1.25 mmol/l with a mean value of 1.0296 mmol/l in our study, which is in accordance with the study done by Hegde *et al.*, where the mean value was 1.41 mmol/l among healthy children. It has been suggested that the levels of antioxidants could be altered in response to an infection or disease.<sup>[16]</sup> The absence of an infectious challenge in these children with good immunity may be a factor for the comparatively increased levels of TAC in saliva among them.

The study groups had significantly lower levels of TAC when compared to the control groups, which is in accordance with the decreased antioxidant level activity in asthmatic patients found by Smith *et al.*<sup>[17]</sup>

During the process of repair and reconstitution of the damaged bronchioalveolar-lavage cells and membranes with enzymes among the asthmatic children, the antioxidants in the body may be used, thereby reducing its level in the saliva. The superoxide dismutase activity which produces FR, has been found to be increased in asthmatic children, thereby decreasing their TAC value.<sup>[18,19]</sup> But the tissue used in these studies was erythrocytes in contrast to saliva used in this study.

Antioxidant function in the oxidative process shows conflicting results: On one hand, it aids in the scavenging of potent superoxide radical while on the other, it progrades for the ROS and RNS creation causing an increased FR level and reduced antioxidant levels in blood and saliva in inflammatory conditions like asthma.

The caries experience in the study group was found to be decreased when compared to the control group, though not statistically significant. This is in contradiction to an earlier study by Hegde *et al.*, where there was an increased caries experience in asthmatic children compared to healthy control.<sup>[5]</sup> This decreased caries experience in the present study group may be due to their better oral hygiene practices and reduced use of liquid medicaments.

Tulonoglu *et al.*,<sup>[20]</sup> studied the relation between caries and TAC, and showed that there was an opposite linear relation between the two, i.e., an increase in TAC is associated with an increased caries experience among the healthy children. In our study, contradicting the above, even though the TAC value increased in healthy children, dental caries did not increase in these children. In fact, there was a minimal decrease in caries experience among these children. These observations clearly shows that dental caries prevalence is not directly related to only one factor like TAC but can be modified by various other factors such as good oral hygiene practice and good diet.

Thus as caries with its multifactorial etiology can be controlled by modifying the various etiological factors, the TAC of the child can also be modulated by a diet rich in antioxidants to improve the immune system, irrespective of the medically compromising condition. Hence, further research should be directed towards the TAC of children with various medically compromising conditions and their interplay with the various predisposing and health promoting factors.

## Conclusion

The TAC of saliva decreased in children with asthma. Prevalence of dental caries did not increase in children with asthma.

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