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Original Article

A comparative evaluation of total antioxidant capacity of saliva in children with and without Gingivitis

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ABSTRACT: Gingivitis is a reversible and non-destructive form of periodontal disease. Oxidative stress contributes in the pathogenesis of periodontal diseases[5]. The oxidative stress has been implicated as one of the important contributory etiologic factors in many of the oral inflammatory pathologies including gingivitis. This research analyzed the ''Total antioxidant capacity'' (TAC) of biological fluids including saliva**.** The present cross-sectional study was conducted to evaluate the total antioxidant capacity (TAC) of saliva in children with/ without gingivitis and its relation with Age and Gender**.** For measuring the TAC of saliva: Cayman's Antioxidant Assay Kit was used and Gingival Index Measured through The Gingival Index (Löe and Silness, 1963). The results were analyzed using descriptive statistics and making comparisons between cases and control by using SPSS software version 20. In this result, mean TAC of saliva in case children group was found lower 0.203 ± 0.053 compared to control children group was higher 0.236 ± 0.048. While, in male and female children of aged 3-5 years were found antioxidant activity (TAC) lower in compared to control groups. But among males aged 6-13 years it was found that the mean antioxidant capacity of saliva in case group was 0.259 ± 0.040 while in control group it was 0.295 ± 0.026. The TAC of saliva in males was found high compared to female. A weak negative correlation was found between the TAC and gingival index. In conclusion TAC decreases in children with gingivitis compared to healthy children. The gingivitis was more observed in female leading to lower TAC value.

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INTRODUCTION

Gingivitis is a reversible and non-destructive form of periodontal disease [1, 2]. In gingivitis, marginal gingiva becomes inflamed and it may progress to include free and attached gingiva but loss of attachment does not occur [3, 4]. Untreated gingivitis may result to periodontitis. Hence, if gingivitis and periodontitis are assessed in early stages it will minimize the chance of tooth loss. The gingivitis occurring in primary dentition tends to remain confined to marginal gingiva for the reason yet unexplained. Several studies also indicate that the prevalence of destructive form of periodontal disease is lower in young individuals than in adults.

According to Chapple IL and Matthews JB oxidative stress contributes in the pathogenesis of periodontal diseases [5]. A harmful increase in the production of reactive oxygen species (ROS) leads to oxidative stress [6]. ROS are important signaling molecules in the regulation of several cellular processes [7]. Sequelae of the oxidative stress include adaptation, damage or cell death [8] through a variety of mechanisms, such as DNA, lipid and protein damage [9].

The oxidative stress has been implicated as one of the important contributory etiologic factors in many of the oral inflammatory pathologies including gingivitis [10].

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. Moreover, the number of different antioxidants makes it difficult and expensive, to measure each antioxidant separately, especially during daily clinical treatments. So, the present study is now being directed towards assays that evaluate the ‘‘Total antioxidant capacity’’ (TAC) of biological fluids including saliva [11].

MATERIALS AND METHODS

The aims of this study were to estimate the total antioxidant capacity (TAC) of saliva in pediatric dental patients with / without gingivitis, and relation of TAC in respect to age and gender. Study was carried out in the Department of Pediatric and Preventive Dentistry, Faculty of Dental Sciences, King George’s Medical University, Lucknow, in collaboration with the Department of Biochemistry, King George’s Medical University, Lucknow (U.P.).

Study Design: The present cross-sectional study was conducted to evaluate the TAC of saliva in patients with/ without gingivitis and its relation with Age and Gender. Ethical clearance **(Ref-code-76 *ECM IIB-Thesis/P*49)** was obtained from the Institutional Ethics Committee, King George’s Medical University, Lucknow, UP.

Methodology: The present study was planned as a cross sectional study, conducted among 72 patients. A brief medical and dental history of patient was recorded and a thorough oral health examination was done. After taking written informed consent from their parents, patients were screened for gingivitis by the help of gingival index and saliva collection was done.

Criteria for Group Division: The subjects selected for the study were divided into two groups on the basis of their age, (Group A and Group B).

- Patients within 3-5 years of age were assigned to Group A.
- Patients within 6-13 years of age were assigned to Group B.

Each group comprised of 36 subjects who were further divided into two (each 18 subjects) sub groups on the basis of gingival index score, the patients with gingival index score >1 were included in Study group (Subgroup AI) and those with score<1 were included in Control group (Subgroup AII). Similarly, Group B was categorized into Subgroup BI (study group) and Subgroup BII (Control group). Among these subgroups data for males and females were recorded separately.

Materials Used:

- For oral examination: Mouth mirror (API), Explorer (API), Tweezers (API), Sterilized cotton, Autoclave, UNC-15 Periodontal Probe (PCP UNC 15; Hu-Friedy Manufacturing Co., Chicago, IL, USA).
- For collecting saliva: Sterile saliva collecting vials (Specican), Ice-Box, - 20 Refrigerator.
- For measuring the TAC of saliva: Cayman's Antioxidant Assay Kit was used to measure the TAC of saliva **(Item: 709001, Batch: 0495414).**
- Gingival Index Measured By**:** The Gingival Index (Löe and Silness, 1963) [12] was used for the assessment of the gingival condition and to record qualitative changes in the gingiva. It scores the marginal and interproximal tissues separately on the basis of score 0 to 3.

Statistical Analysis: The results were analyzed using descriptive statistics and making comparisons between two groups- cases and control, with respect to various parameters. Discrete (categorical) data was summarized as in proportions and percentages (%) and Mean ± SD (standard deviation), Chi-square test, Unpaired Student’s 't’ test and Pearson’s Correlation. All the statistical analysis was done by using SPSS software version 20.0 (Armonk, NY, USA). A p-value < 0.05 was considered as statistically significant for all the data.

RESULTS

Comparison of TAC of Saliva between Case & Control Groups (Group AI, BI & Group AII, BII):

On comparing the TAC of saliva between case & control groups, it was found that the mean antioxidant capacity of saliva in case group was 0.203 ± 0.053 while in control group it was 0.236 ± 0.048 as tabulated in table 1. Therefore, according to unpaired student’s t-test the mean antioxidant capacity of saliva in cases was found to be significantly less than the control group (p<0.01).

Table 1: Comparison of TCA of Saliva between Case & Control Groups (Group AI, BI & Group AII, BII)

Group	Mean	SD	t-value	p-value
Case	0.203	0.053	2.808	0.006**
Control	0.236	0.048		

* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

Comparison of TAC of Saliva between Case & Control Groups according to gender and aged (Group AI- M & AII-M):

On comparing the TAC of saliva between case & control group among males and female aged 3-5 years, in the male group it was found that the mean antioxidant capacity of saliva in case group was 0.226 ± 0.020 while in control group it was 0.255 ± 0.011 and in the female group it was found that the mean antioxidant capacity of saliva in case group was 0.149 ± 0.041 while in control group it was 0.188 ± 0.029 as tabulated in table no.2 Therefore, according to unpaired student’s t-test the mean antioxidant capacity of saliva in cases was found to be significantly less than the control group in both male and female. On comparing the TAC of saliva between case & control group among males aged 6-13 years it was found that the mean antioxidant capacity of saliva in case group was 0.259 ± 0.040 while in control group it was 0.295 ± 0.026 as tabulated in table 2. Therefore, according to unpaired student’s t-test the mean antioxidant capacity of saliva in cases was found to be significantly less than the control group (p<0.05).

Table 2: Comparison of TAC of Saliva between Case & Control Groups according to gender and aged (Group AI- M & AII-M)

Gender/ Age	Group	TAC value of saliva (mM)		t-value	p-value
		Mean	SD		
Male 3-5 yrs	Case	0.226	0.020	3.834	0.001***
	Control	0.255	0.011		
Female 3-5 yrs	Case	0.149	0.041	2.329	0.033*
	Control	0.188	0.029		
Male 6-13 yrs	Case	0.259	0.040	2.286	0.036*
	Control	0.295	0.026		
Female 6-13 yrs	Case	0.177	0.022	2.752	0.014*
	Control	0.206	0.024		

* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

Comparison of TAC of Saliva between the Two Age Groups (Group A & B):

On comparing the TAC of saliva between age Groups 3-5 years and 6-13 years, it was found that the mean antioxidant capacity of saliva in age group 3-5 years was 0.205 ± 0.048 while in age group 6-13 years it was 0.234 ± 0.054 as tabulated in table 3. Therefore, according to unpaired student's t-test the difference in mean antioxidant capacity of saliva between the two age groups was found to be significant (p=0.017).

Table 3: Comparison of TAC of Saliva between the Two Age Groups (Group A & B)

Age Group	TAC value of saliva(mM)		t-value	p-value
	Mean	SD		
3-5 yrs	0.205	0.048	2.456	0.017*
6-13 yrs	0.234	0.054		

* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

Comparison of TAC of Saliva between Male & Female (AB-M & AB-F):

On comparing the TAC of saliva between males and females, it was found that the mean antioxidant capacity of saliva in males was 0.259 ± 0.036 while in females it was 0.180 ± 0.035 as tabulated in table 4. Therefore, according to unpaired student's t-test the difference in mean antioxidant capacity of saliva between males & females was found to be significant (p<0.001).

Table 4: Comparison of TAC of Saliva between Male & Female (AB-M & AB-F)

Gender	TAC value of saliva (mM)		t-value	p-value
	Mean	SD		
Male	0.259	0.036	9.390	<0.001*
Female	0.180	0.035		

* = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$

Correlation between TAC of Saliva and Gingival Index:

A weak negative correlation was found between the TAC and gingival index as tabulated in table 5 and graphically represented in figure 1.

Table 5: Correlation between TAC of Saliva and Gingival Index

Variable		Gingival Index
TAC value of saliva (mM)	Pearson Correlation (r)	-0.136
	p-value	0.254[ns]

ns- non-significant

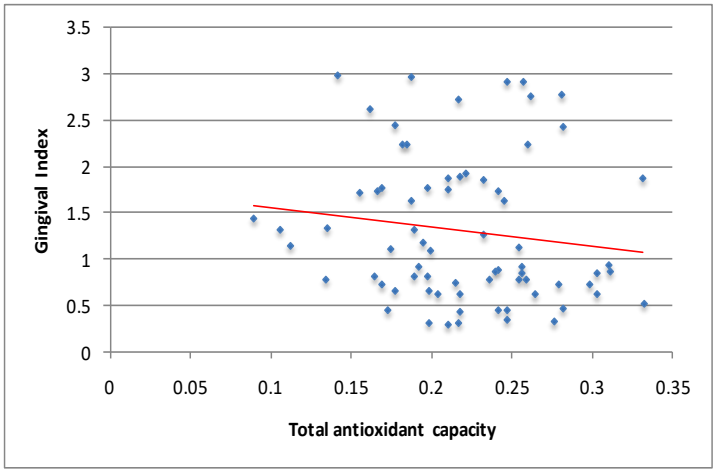

Figure 1: Correlation between TAC of Saliva and Gingival Index

DISCUSSION

In the present study, when mean antioxidant capacity of saliva within control group (patients without gingivitis) was compared with that of case group (patients with gingivitis) of all the age groups, we observed that antioxidant level of saliva in control group was significantly higher than case (p=0.006), concluding that salivary total antioxidant levels were less in patients with gingivitis than the control group showing that the levels of antioxidant could be altered in response to an infection or disease [13].

These findings were in accordance with those reported by Taowen Zhang *et al*. (2016) [14] and Fatemeh Ahmadi-Motamayel *et al*. (2017) [15] similar results were also observed by Baser *et al*., (2015) [16] and Tamaki *et al*. (2015) [17].

In the present study, the mean antioxidant content of saliva in male among control group (3-5years) was 0.255±0.011mM as compared 0.226±.020 mM in case group (3-5 years) and a statistically significant difference was found between case and control groups (p=0.001). Similarly, on comparing TAC value within females it was found that for control group TAC value was 0.188±0.029 mM as compared to 0.149±0.041 mM for case group. This difference was also statistically significant (p=0.033). In the present study the mean antioxidant content of saliva in males in control group (6-13 years) was 0.295±0.026mM as compared to 0.259±0.040mM in case group (6-13 years), which was statistically significant (p=0.036). For females it was 0.206±0.024mM as compared to 0.177±0.022mM for study group, that is also statistically significant (p=0.014).

When the TAC value of males was compared to that of females, it was observed that the value was significantly less in females showing that, females have higher prevalence of gingivitis. This could be related to the hormonal changes during puberty, moreover the fact that the girl child in rural areas is being provided with less oral hygiene facilities and priority compared to boys. These results were in agreement with Furuta M *et al.* (2011) [18]. On contrary it was shown by Pepe H *et al*. (2009) [19] that antioxidant protection in females was higher than that of males due to increased activity of antioxidant enzymes in females.

In the present study on comparing the TAC value between 3-5 years age group (Group A) and 6-13 years age group (Group B), it was significantly less for Group A, in comparison to group B showing that Group A has higher degree of gingivitis when compared to Group B. These results were in agreement with Matsson *et al.* (1986) [20]. So, the finding of present study suggested that TAC value of saliva in patients with gingivitis significantly decreases in both age groups. This occurs due to increase in the oxidative stress in response to excessive ROS release. Various reasons have been suggested for this increase in oxidative stress. Firstly, it may reflect an underlying genetic predisposition of an individual, which allows for disease initiation and its progression. Secondly, it may be because of a consequence of disease process where the ROS consumes radical quenching species faster than they can be replenished. A third reason suggested was that there might be defects in the protein antioxidant response.

In present study on comparing the relationship of TAC value with genders of both groups, it is found that mean TAC value of saliva was 0.180 ±0.03 mM for females compared to 0.259±0.036mM in males which was statistically significant (p<0.001).When the gingival index score of the patients was correlated with the TAC of saliva it was found that *Pearson correlation* value was 0.136 with the p value (0.254) exhibiting the negative correlation, confirming that there is an inverse relation between gingivitis and TAC value of saliva i.e. if gingival index score is more than TAC value will be less. This result was in accordance with the study conducted by Zhang *et al*. (2016) [14].

Furthermore, studies with more sample sizes are necessary to evaluate the more significant relationship of TAC of saliva with age and gender and also its exact relationship with gingivitis in terms of severity.

In view of the findings of the present study it can be concluded that TAC decreases in children with gingivitis compared to healthy children. The gingivitis was more observed in female leading to lower TAC value.

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Conflict of Interest: The authors declare that they have no conflict of interests.

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