

INFLUENCE OF THE TOTAL ANTIOXIDANT CONTENT OF SALIVA ON DENTAL CARIES.

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Abstract:

Objective: The aim of the study was to evaluate the Total Antioxidant level in saliva of caries patients with and without smoking habit.

Methods: A total of 80 individuals were included in the study. Unstimulated saliva was collected. Samples of smoking and non smoking caries patients showing only *Streptococcus mutans* growth were analysed for Total Antioxidant level and smokers and nonsmokers without any caries were considered as control groups. Caries isolates were confirmed as *Streptococcus mutans* by Gram staining and biochemical tests. Total Antioxidant level was determined by spectrophotometric method.

Result: Non smokers with caries showed significantly ($P < 0.0001$) higher TAC level to Smokers with caries. Smokers without caries showed significantly ($P = 0.0005$) lower level of antioxidants than nonsmokers without caries.

Conclusion: This invasive study shows that smoke adversely affects the Total Antioxidant Level in caries patients.

Keywords: Dental caries, *Streptococcus mutans*, Total antioxidant level. Smokers and nonsmokers

Introduction

Dental caries is one of the most common, but rarely life threatening disease¹. It is characterized by a localized, transmissible, pathological infectious processes that ends up in the destruction of hard dental tissue². Among the factors that have been related to greater cariogenic activity are inadequate dental hygiene and care, the existence of active caries or a family history of caries, a high concentration in the mouth of bacteria with acidophilic activity (*Streptococcus mutans* or *Lactobacillus*), reduced salivary flow a cariogenic diet or inadequate levels of fluoride in drinking water³.

Streptococcus mutans is considered to be the main cause of dental caries. A study stated that *Mutans Streptococci* participate in the formation of biofilms on tooth surfaces⁴. Antioxidants constitute an important part of our diet and these, together with extra cellular antioxidants and those of the enzymatic systems, can prevent various inflammatory, infectious or tumoural processes⁵. Various

authors^{5,6} have examined the relationship between the composition of the saliva and cariogenic activity. A study showed that the amount of caries in deciduous teeth is in direct proportion to the observed total antioxidant capacity of saliva⁷.

Cigarette or tobacco smoke is involved in the pathogenesis of several diseases including local toxic effects in the oral cavity. The noxious effects of smoke compounds justify the high incidents of periodontal diseases, caries and neoplastic diseases of oral tissues in smokers⁸. Exposure to oxidant chemicals in smoke is associated with depletion of endogenous levels of antioxidants in the systemic compartment. Studies have reported that smoking results in low antioxidant concentrations in plasma⁹.

The aim of the present study is to evaluate the status of Total Antioxidant Capacity (TAC) in caries patients with and without smoking habit.

Materials and Methods

The present study was done at A. B. Shetty Memorial Institute of Dental Sciences after getting the institutional ethical clearance. The study was comprised of 80 participants, belonging to age group 30-40 years, in four groups. A written informed consent was obtained prior to enrollment of participants.

Group I: 20 Smoking individuals with caries.

Group II: 20 Smoking individuals without caries.

Group III: 20 Healthy individuals without any caries or smoking habits.

Group IV: 20 Non-smoking individuals with caries.

Collection and isolation of *Streptococcus mutans*

Unstimulated saliva was collected in sterile sample collection bottles from patients with caries and without caries with and without smoking habit¹⁰. Saliva samples were vortexed and serially diluted to 10 folds in 0.05M phosphate buffer. 100µl of the aliquotes were cultured onto Mutans Sanguis Agar (Himedia, Bombay) plates. The plates were incubated at 37°C for 24-48 hrs. Colonies were identified as *Streptococcus mutans* by doing gram staining and biochemical tests like Inulin and Mannitol fermentation, Esculin hydrolysis in the presence and absence of bile, Urease and resistance to Bacitracin. The *St. mutans* ferments both Inulin and Mannitol. Esculin hydrolysis is negative in presence of bile and positive in absence of bile. Urease is negative and it is resistance to 2U of Bacitracin.

Estimation of total antioxidant capacity (TAC)

Total antioxidant capacity was estimated by phosphomolybdenum method¹¹. This quantitative assay is based on the conversion of Molybdenum (Mo VI) by reducing agents like antioxidants to Molybdenum (Mo V), which further reacts with phosphate under acidic pH resulting in the formation of a green coloured complex. The intensity of which can be read spectrophotometrically at 695 nm.

Results

The data of TAC was analyzed by one way Anova using Prism software. Table 1 is representing the level of TAC as mean \pm standard deviation in all four groups. The data shows significant decrease in the TAC of smokers with caries than non-smokers with caries ($P < 0.0001$) and it is also significant over smokers without caries ($P = 0.0005$). Nonsmokers with caries showed higher level of TAC ($162.0 \pm 6.339 \mu\text{g/ml}$) than healthy individuals without any smoking habit ($136.5 \pm 17.08 \mu\text{g/ml}$) (Table 2 and Fig 1)

Discussion

Dental Caries is a bacterial plaque-dependent disease of the dentition that is characterized by a progressive, intermittent demineralization of enamel, dentin and cement with a characteristic pattern of decay that may lead to total destruction of coronal dental tissues and the formation of pulpal abuses, Oral microorganisms, when organized in voluminous masses as dental plaque on tooth surfaces, hydrolyze starches and metabolize sugars to form acids (mainly lactic acid) that demineralize the hard tissue underneath¹².

When antioxidant defenses are weakened, body cells and tissues become more prone to develop dysfunction and/or disease. Total antioxidant capacity evaluation is the first step in the search of diseases in biochemistry, medicine, food and nutritional sciences¹³.

Saliva is considered as mirror of the body is readily available and collection process is fairly straight forward¹⁴. Saliva being the diagnostic tool for detection of dental caries shows lower flow rate, viscosity, pH and buffering capacity¹⁵. In this study unstimulated saliva was collected for the assessment of *Streptococcus mutans* and total antioxidant level.

In the present study smokers with and without caries (group 1 and 2) shows decrease in Total Antioxidant level compared to the nonsmokers with and without caries

(group 3 and 4). This may be due to the presence of high amounts of free radicals in cigarette smoke that generate an oxidative stress in the smokers body that may cause exhaustion of antioxidants of the body¹⁶.

In our study we found significant increase (P=0.0142) of Total Antioxidant level in nonsmokers with caries (group 4) than healthy individuals (group3) without any habit of smoking. Similar studies done on children reveals the same results^{17,18,19}. The increase may due to an increase in the suspension of proteins and of cariogenic activity⁶ and it has been suggested that the levels of antioxidants could be altered in response to an infection or disease²⁰.

There is significant decrease (P=0.0005) in total antioxidant level of Smokers without any caries when compared to nonsmokers without any dental caries. This finding was also reported in few previous studies^{7,16}.

The Total antioxidant level in smokers with *St.mutans* caries (Group 1) decreased significantly (P<0.001) than in

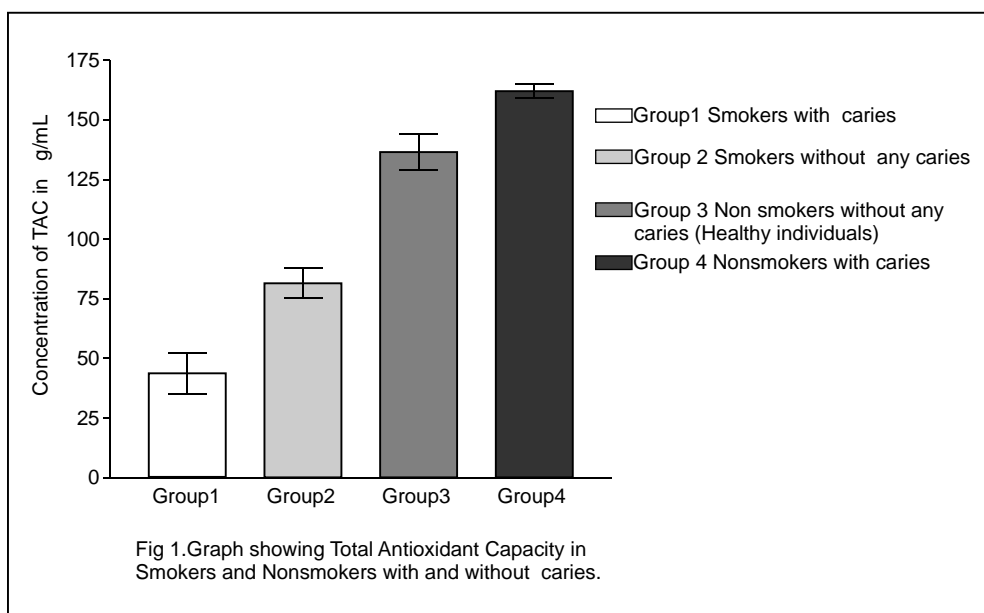
nonsmokers with *St.mutans* caries (Group 4). Cigarette smoking was shown to be associated with the prevalence of caries²¹ and free radicals in cigarette smoke^{22,23} deplete the antioxidant level.

Conclusion

In this noninvasive study we considered the patients with *St.mutans* caries and smoking habit. We have found that the Total antioxidant level does not decrease in individuals with *St.mutans caries* without any smoking habit, but it decreases in individuals with smoking habit. So it may be suggesting that smoking may increase the prevalence of dental caries or it may worsen the conditions in dental caries as the free radical produced by the smoke reported to deplete the total antioxidant level.

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Tables

Groups	Group 1	Group 2	Group 3	Group 4
Level of TAC in µg/ml	43.50 ± 19.04	81.50 ± 13.85	136.5 ± 17.08	162.0 ± 6.339

Table 1: Showing the levels of Total Antioxidant Capacity in all the four groups. Data are presented as mean ± standard deviation. P < 0.0001 and level of significant at the level of 0.05

Groups	Group 1	Group 4	P value
Level of TAC	43.50 ± 19.04	162.0 ± 6.339	P<0.0001
Groups	Group 2	Group 3	P value
Level of TAC	81.50 ± 13.85	136.5 ± 17.08	P=0.0005
Groups	Group 1	Group 3	P value
Level of TAC	43.50 ± 19.04	136.5 ± 17.08	P<0.001
Groups	Group 2	Group 4	P value
Level of TAC	81.50 ± 13.85	162.0 ± 6.339	P=0.0005
Groups	Group 3	Group 4	P value
Level of TAC	136.5 ± 17.08	162.0 ± 6.339	P=0.0142

Table 2: Showing significance in the levels of Total antioxidant capacity between Group 1 and 4(P<0.0001), Group 2 and 3(P=0.0005), Group 1 and Group 3 (P<0.001) , Group 2 and Group 3 (P=0.005) and group 3 and Group 4 (P=0.0142). level of significance at P<0.05.

References

- Marsh P D, Are dental diseases examples of ecological catastrophes? *Microbiology-Sgm* 2003; 149: 279-294
- Gamboa F, Estupinan M, Galindo A. Presence of *Streptococcus mutans* in saliva and its relationship with dental caries: antimicrobial susceptibility of the isolates. *Universitas Scientialum* 2004;9:23-27
- Palmer ,Woefe SH. Position of the American Diabetic association: the impact of fluoride on health. *J Am Diet Assoc* 2005;105:1620-1628
- Martin A T and David AN. The molecular pathogenesis of dental caries associated with mutans streptococci. *Nature Reviews immunology* 2006;6:555-563
- Perno Goldie M. Antioxidants in oral health care : making the connection. *Int Dent Hyg* 2005; 3:93-95
- Tulunoglu O, Demitras S, Tulunoglu I. Total antioxidant levels of saliva in children related to caries, age and gender. *Int J Paediatr Dent* 2006; 16:186-191
- Uberos J,Alaecon JA,Denalver MA,Molina-Carballo A,Reriz M,Gonzalez E,Castejons J, Munoz-Hoyos A. Influence of the antioxidant content of saliva on dental caries in an at risk community. *British journal* 2008 ;doi:10.1038/SJ.bdj.2008.520
- Zappacosta B,Persichilli S, Mordente A,Minucci A,Lazzaro D,Meucci E,Giarsina B.Inhibition of Salivary enzymes by cigarette smoke and the protective role of glutathione. *Human & experimental Toxicology* 2002; 21:7-11
- Yanbaeva DG, Dentener MA, Creutzberg EC, Wesseling G, Wouters EFM, Systemic Effects of Smoking. *Chest* 2007;131:1557-1566
- Fure S. Five -year incidence of caries,salivary and microbiol conditions in 60,70 and 80yedr old Swedish individuals. *Caries Research* 1998;32:166-174
- Prieto, Manuel, P. and Miguel, A., Spectrophotometric quantitation of antioxidant capacity through the formation of phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical biochemistry* 1999;259:337-341
- Konig KG, Navia J N. Nutritional role of sugars in oral health. *Am J Clin Nutr* 1995; 62:275-283
- Kusana C, Ferrari B. Total antioxidant capacity: a biomarker in biomedical and nutritional studies. *Journal of Cell and Molecular biology* 2008;7 :1-15
- Lenander-Lumikari, M. and V. Loimaranta, Saliva and dental caries. *Adv Dent Res*, 2000;14: 40-7
- Gopinath V K, Arzreanne A R. Saliva as a diagnostic tool for assessment of dental caries. *Archives of orofacial sciences* 2006;1:57-59
- Mohmood IH, Abdullah KS, Othman SH. The total antioxidant status in cigarette smoking individuals. *The medical Journal of Basrah University(MJBU)* 2007; 25: 46-50
- Kumar D,Pandey RK,Agarwal D, Agarwal D. An estimation and evaluation of total antioxidant capacity of saliva in children with severe early childhood caries. *International journal of paediatric dentistry*.2011; doi:10.1111/j.1365-263X.2011.01154
- Dodwad R, Betigeri AV, Preeti BP. Estimation of total antioxidant capacity levels in saliva of caries-free and caries-active children. *Contemporary Clinical Dentistry* 2011;2(1):17-20
- Shreesahana VM,Kumari S,Shetty V, Gowda SKP. Dental carries in relation to serum nitricoxide andTotal Antioxidant status. *Biomed* 2009;4(2):147-151
- Ferreiro MS, Gallardo I. The Antioxidant capacity of saliva. *J Slin Periodonol* 2002;29:189-94
- Heng C K,Bander VM, Freeman K D. Relationship of cigarette smoking to dental caries in a population of female inmates. *Journal of correctional healthcare* 2006;12:164-174
- Midgette AS, Baron JA, Rohan TE. Do cigarette smokers have diets that increase their risks of coronary heart disease and cancer.*Am J Epidemiol* 1993;137:521-529
- Eiserich JP, Van der vliet A, Handelman GJ, Halliwell B, Vross CE. Dietary antioxidants and cigarette smoke –induced biomolecular damage; a complex interaction. *Am J Clin Nutr* 1995;62:1490-1500.