

## Comparison of Total Antioxidant Capacity and Vitamin C in Smokers and Non-smokers

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### ABSTRACT

The aim of this study was to evaluate salivary antioxidant's levels in smokers in comparison to non-smokers. Few studies have shown the role of oxidative stresses in smokers. Decreased antioxidant's level can be one of the etiologic factors of oral Mucosal lesions. With consideration of the importance of saliva and its defense system such as antioxidants and minerals in creation of different disease and destructive effects of cigarette smoking and lack of researches about this topic, we have assessed the effect of cigarette's smoking on saliva's antioxidants. In this descriptive analytical study that was done in Tabriz, 5 ml unstimulated saliva by spitting method from 50 smokers and 50 healthy non smokers was collected. Then the levels of salivary antioxidants in smokers and non-smokers were assessed separately. These antioxidants are as follows: total antioxidant, Vit.C. Data was analyzed using descriptive and analytical statistical methods and SPSS.16 software. Average total antioxidant capacity in smokers' saliva was  $1.87 \pm 0.49$  mol/dL and the mean vitamin C was  $1.60 \pm 0.30$  mg/dl. Average total antioxidant capacity in smokers' saliva was  $4.10 \pm 0.73$  mo/dL and the mean vitamin was  $1.22 \pm 0.22$  mg/dl. T-test showed that this difference was statistically significant. Total antioxidant capacity and vitamin C in smokers' saliva was less than non- smokers' saliva.

**Key words:** Antioxidant, saliva, smoke.

### INTRODUCTION

Saliva is complex liquid containing water, organic and inorganic components, which is produced by the major and minor salivary glands. It also contain buffers, enzymes, growth factors, cytokines, immunoglobulins, Mosin, and other host glycoproteins as well as defense systems such as antioxidant defense system that serves as the first line of defense against oxidative stress<sup>1, 2</sup>.

Defense systems of the saliva antioxidants contains the enzymes and different molecules while the most important of them is uric acid that compose almost 70% of the total antioxidant capacity of saliva as well as peroxidase enzyme, that both are soluble in water. Other antioxidants include superoxide dismutase, glutathione peroxidase, catalase, and low molecular weight molecules like vitamin E, thymol, glutathione, and vitamin C<sup>3-5</sup>. In addition to measuring the concentrations of specific

antioxidants, total antioxidant capacity is also widely used<sup>6</sup>.

Oxidative stresses caused by imbalance between production of reactive oxygen species and antioxidant defenses. Oxidants prevent excessive production of reactive oxygen species which are the causes of tissue damage, under the normal conditions<sup>6, 4</sup>.

Smoking is an important source of free radicals that has a direct connection with damage DNA. Damage to and other macromolecules have been proposed as pathogenesis of various diseases<sup>2</sup>.

Cigarette contains 4,000 different chemicals that some of these compounds are known including: nicotine, ammonia, acrolein, phenols, acetaldehyde, Benzopyrene, nitrogen oxides, carbon monoxide, polonium, radium and thorium<sup>7-9</sup>.

Two main phases have been identified in cigarette smoke: tar phase and gas phase. Both phases are rich in oxygen, carbon, nitrogen and free radicals. Analysis has shown that a puff of cigarette contains about 1014 free radicals in the tar phase and 1015 free radicals in the gas phase<sup>10, 11</sup>.

Therefore smoking has free radicals that can cause cell damage, and studies have shown that smoking reduces antioxidant levels and increases oxidative stress play an important role in the development and pathogenesis of a wide range of degenerative disease such as cancer, heart disease and, periodontal disease<sup>12, 13</sup>.

The total capacity of antioxidants, which represents oxygen radical absorbance capacity, is highly important. Among the factors that affect the overall capacity of antioxidants, vitamins E and C and also uric acid can be noted. Among these, uric acid is one of the most important determinants of overall capacity of antioxidants<sup>6</sup>.

In this regard, in studies conducted by Kanehira and colleagues<sup>14</sup> and Reddy and colleagues<sup>15</sup>, it was aimed to compare the

antioxidant enzymes in the saliva of smokers and non-smokers, and reduced levels of these antioxidants were seen in smokers. However, the study of Buduneli *et al.*,<sup>16</sup> and Zappacosta and colleagues<sup>17</sup> didn't demonstrate significant difference in overall antioxidant capacity between smokers and non-smokers. It seems that the antioxidant power of saliva may be influenced by susceptibility to stress of smoking, feeding of smokers and non-smokers, their systemic disease, the number of smoked cigarettes, and etc.

Chavez *et al.*,<sup>18</sup> and Budunelli and colleagues<sup>16</sup> observed significant differences between smokers and nonsmokers in the levels of ascorbic acid.

Given that the collection of saliva is a non-invasive, simple and affordable method in order to assess the antioxidants in different diseases<sup>19</sup> and given the importance of saliva and its defense system including anti-oxidants and due to decrease in antioxidants in the saliva of smokers and increased prevalence of oral lesions, malignancies, dental and periodontal problems of these people, as well as given the limited and contradictory studies in this field, in the present research it was aimed to investigate the effect of smoking on salivary antioxidants including vitamin c and the total antioxidant, so that in the future these antioxidants can be used as additives in foods, toothpaste or mouthwash in order to prevent the harmful effects of tobacco.

## MATERIALS AND METHODS

The design and method of this study were approved Tabriz Dental and Periodontal Research Center of the Tabriz University of Medical Sciences' Investigation Committee (TBZMED.REC.1394.234) In this cross - sectional study, statistical population consisted of 50 subjects in the control group and 50 subjects in the exposed group 20 to 50-year-old who referred to the Department of Oral Medicine, School of Dentistry Tabriz. The collection method of saliva samples was performed using Spitting method. In this method, the patient was allowed to collect saliva in the mouth and then put it into a special sterile plastic tube (falcon). This is usually done every 60 seconds for 15-5 minutes. In order

to achieve unstimulated samples, patients were asked to refuse eating or drinking or having any oral stimulation for 90 minutes before collecting. About 5 ml of saliva was collected using this method<sup>3, 7</sup>.

The collected saliva was immediately placed on ice and was transported to the laboratory at 4 ° C. It was centrifuged for 10 minutes at 800 g in order to squamous cells and cellular debris be removed. The samples were frozen at -80 ° C until preparation of all samples<sup>4, 7</sup>. When all samples were collected using the prepared kits, then the necessary tests (TAC and vitamin C kits) and atomic absorption using spectrophotometry were conducted.

**RESULTS**

As it can be seen in the followed table, the average total antioxidant capacity in saliva of smokers was 1.87± 0.49 mol/dL and the mean vitamin C was 1.06± 0.03mg/dl.

As it can be seen in the followed table, the average total antioxidant capacity in saliva of non-smokers was 4.10±0.73 mol/dL and the average vitamin C is 1.22 ±0.22 mg dl.

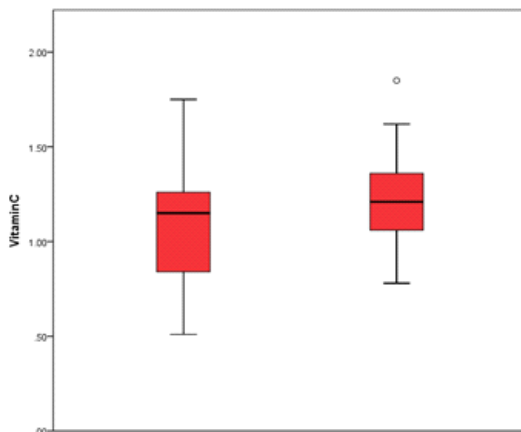
Kolmogorov- Smirnov test showed normal distribution of data, therefore the comparison of total antioxidant capacity and vitamin C between both

**Table 2: total antioxidant capacity and vitamin C in the saliva of non-smokers**

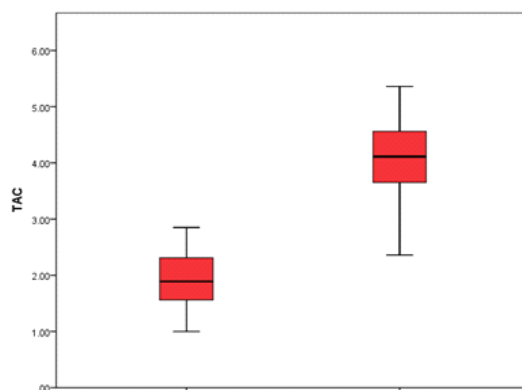
	Number	Average	Standard Deviation
Total antioxidant capacity	50	1.87	0.49
Vitamin C	50	1.06	0.03

**Table 1: The total antioxidant capacity and vitamin C in smokers**

	Number	Average	Standard Deviation
Total antioxidant capacity	50	4.10	0.73
Vitamin C	50	1.22	0.22



**Fig. 1: Comparison of vitamin C in smokers and non-smokers**



**Fig. 2: Comparison of total antioxidant capacity in smokers and non-smokers**

groups was conducted by t-test for independent groups. The results showed that there are significant differences between the studied groups so that the total antioxidant capacity ( $P = 0.0001$ ) and vitamin C ( $P = 0.005$ ) in smokers were lower than non-smokers.

## DISCUSSION

Cigarette smoke is a major source of free radicals that cause harmful effects on oral health and cancer and precancerous lesions and plays most important role in the pathogenesis of periodontal diseases<sup>20</sup>. Antioxidants counter the damaging effects of free radicals and maintain the integrity of the tissue. An imbalance between the levels of free radicals and antioxidants play an important role in the onset and evolution of the mouth disease<sup>1, 21, 22</sup>.

Given the importance of saliva and its defense system, due to the limited and contradictory studies in this field, in the present study it was aimed to investigate the effect of smoking on salivary antioxidants such as total antioxidants and vitamin c.

The results showed that total antioxidant capacity and vitamin C in saliva of smokers were lower than than non-smokers. In the study conducted by Arbabi and colleagues in 2014 on the 50 smokers and 50 non-smokers, total antioxidant capacity in saliva was evaluated in two groups using FRAP. The results also showed that the levels of antioxidant in smokers were lower than non-smokers which were consistent with the results of this study<sup>23</sup>. Greabu and colleagues showed that total antioxidant capacity of saliva was lower in smokers than non-smokers<sup>24</sup>.

Preston and colleagues in 2003 studied 512 children aged 2 to 12 years old, and have concluded that exposure to even small amounts of smoke caused a significant reduction in the plasma levels of vitamin C<sup>25</sup>.

However Chavez and colleagues in 2007 studied 15 smokers and 15 non-smokers and did not find significant differences in salivary levels of vitamin C between two groups<sup>18</sup>.

Fujinami *et al.* in a study on rats found that salivary peroxidase activity and total protein in saliva of rats that were exposed to smoke was less than other rats<sup>26</sup>.

Baharvand and colleagues evaluated the activities of superoxide dismutase, which is a component of antioxidant system, in smokers and non-smokers and showed that the enzyme activity is higher in smokers, but given that the total antioxidant capacity of saliva was not examined, then their results are not comparable with the present study<sup>27</sup>.

On the other hand, Zappacosta and colleagues demonstrated that total antioxidant capacity of saliva was similar in smokers and nonsmokers, but the glutathione content in smokers was higher than non-smokers<sup>17</sup>.

Charalabopoulos and colleagues examined saliva and plasma antioxidant capacity of 25 smokers and 25 non-smokers without oral lesions and showed that despite an increase in plasma antioxidant levels in smokers, total antioxidant capacity of saliva had no difference in the two groups<sup>28</sup>.

Buduneli and colleagues studied 15 healthy male volunteers and 15 healthy smokers and revealed that total antioxidant capacity of saliva was not different between the two groups<sup>16</sup>.

The present study had several limitations. First, in this the total antioxidant content was measured and not enzyme activity. The amount of protein in the saliva has always a direct association with its activity or biological role in the mouth. The second limitation of this study was that it was a cross-sectional type and may indicate the temporary relationship between smoking and antioxidant capacity of saliva. Given the importance of saliva and its defense system including antioxidants and according to a decrease in antioxidants including total antioxidant capacity and vitamin C in the saliva of smokers, and the increased prevalence of oral lesions, malignancies, dental, and periodontal problems in these people, in the future these antioxidants can be used in food supplements, toothpaste or mouthwash in order to prevent the harmful effects of tobacco.

**CONCLUSION**

With consideration of the importance of saliva and its defense system such as antioxidants,

Total antioxidant capacity and vitamin C in smokers' saliva was less than non-smokers' saliva. Decreased antioxidant's level can be one of the etiologic factors of oral Mucosal lesions.

**REFERENCES**

- Battino M, Ferreiro MS, Gallardo I, Newman HN, Bullon P. The antioxidant capacity of saliva. *Journal of clinical periodontology*. **29**(3):189-94 (2002).
- Little J, Falace D, Miller C, Rhodus N. Dental management of the Medically Compromised Patient. 8 th ed. p. 119 (2013).
- Garg N, Singh R, Rixit J, Jain A, Tewari V. Level of lipid peroxides and antioxidants in smoker and non smoker. *J Periodont Res*. **41**(5):405-10 (2006).
- Saggu TK, Masthan KMK, Dudanakar MP, Nisa SUI, Patil S. Evaluation of Salivary Antioxidant Enzymes among Smokers and Non smokers. *World J Dent*, **3**(1):18-21 (2012).
- Casanueva E, Viteri FE. Iron and oxidative stress in pregnancy. *The Journal of nutrition*. **133**(5 Suppl 2):1700S-8S (2003).
- Chen X, Scholl TO. Oxidative stress change in pregnancy and with gestational diabetes mellitus. *Current Diabetes Report* **5**(4):282-8 (2005).
- Bakhtiari S, Bigom Taheri J, Bakhshi M, Mortazavi H, Shah Hoseini A, Vahid Dastjerdi E, et al. Effect of vitamin C on salivary total antioxidant capacity in smokers. *Iranian journal of pharmaceutical research : IJPR*. **11**(4):1045-9 (2012).
- Pryor WA, Stone K. Oxidants in cigarette smoke. Radicals, hydrogen peroxide, peroxyxynitrate, and peroxyxynitrite. *Annals of the New York Academy of Sciences*. **686**:12-27; discussion -8 (1993).
- Tsuchiya M, Asada A, Kasahara E, Sato EF, Shindo M, Inoue M. Smoking a single cigarette rapidly reduces combined concentrations of nitrate and nitrite and concentrations of antioxidants in plasma. *Circulation*. **105**(10):1155-7 (2002).
- Abdolsamadi HR, Goodarzi MT, Mortazavi H, Robati M, Ahmadi-Motemaye F. Comparison of salivary antioxidants in healthy smoking and non-smoking men. *Chang Gung medical journal*. **34**(6):607-11 (2011).
- Kosecik M, Erel O, Sevinc E, Selek S. Increased oxidative stress in children exposed to passive smoking. *International journal of cardiology*. **100**(1) :61-4 (2005).
- Morrissey PA, O'Brien NM. Dietary antioxidants in health and disease. *International Dairy Journal* **8**: 463-72 (1998).
- Güven M, Öztürk B, Sayal A, Özet A. Lipid peroxidation and antioxidant system in the blood of patients with Hodgkin's disease. *Clinical biochemistry*. **33**(3):209-12 (2000).
- Kanehira T, Shibata K, Kashiwazaki H, Inoue N, Morita M. Comparison of antioxidant enzymes in saliva of elderly smokers and non-smokers. *Gerodontology*. **23**(1): 38-42 (2006).
- Reddy S, Swapna LA, Ramesh T, Singh TR, Pradeep K. Influence of cigarette smoking on blood and salivary super oxide dismutase levels among smokers and non-smokers. *Journal of investigative and clinical dentistry*. **3**(4): 298-303 (2012).
- Buduneli N, Kardesler L, Isik H, Willis CS, 3rd, Hawkins SI, Kinane DF, et al. Effects of smoking and gingival inflammation on salivary antioxidant capacity. *Journal of clinical periodontology*. **33**(3): 159-64 (2006).
- Zappacosta B, Persichilli S, De Sole P, Mordente A, Giardina B. Effect of smoking one cigarette on antioxidant metabolites in the saliva of healthy smokers. *Archives of oral biology*. **44**(6):485-8 (1999).
- Chavez J, Cano C, Souki A, Bermudez V, Medina M, Ciszek A, et al. Effect of cigarette smoking on the oxidant/antioxidant balance in healthy subjects. *American journal of*

- therapeutics*. **14**(2):189-93 (2007).
19. Kaufman E, Lamster IB. The diagnostic applications of saliva—a review. *Critical reviews in oral biology and medicine* : an official publication of the American Association of Oral Biologists. **13**(2):197-212 (2002).
  20. Yildiz L, Kayaoglu N, Aksoy H. The changes of superoxide dismutase, catalase and glutathione peroxidase activities in erythrocytes of active and passive smokers. *Clinical chemistry and laboratory medicine : CCLM / FESCC*. **40**(6):612-5 (2002).
  21. Khalili J. Oral cancer: risk factors, prevention and diagnostic. *Experimental oncology*. **30**(4):259-64 (2008).
  22. Preston AM. Cigarette smoking-nutritional implications. *Progress in food & nutrition science*. **15**(4):183-217 (1991).
  23. Arbabi-Kalati F, Nosratzahi T, Salimi S, Sadeghi R, Arbabi-Kalati P. Comparison of Total Antioxidant Capacity of Saliva in Smokers and Non-Smokers. *J Mash Dent Sch*. **38**(2): 93-8 (2014).
  24. Greabu M, Totan A, Battino M, Mohora M, Didilescu A, Totan C, et al. Cigarette smoke effect on total salivary antioxidant capacity, salivary glutathione peroxidase and gamma-glutamyltransferase activity. *BioFactors*. **33**(2):129-36 (2008).
  25. Preston AM, Rodriguez C, Rivera CE, Sahai H. Influence of environmental tobacco smoke on vitamin C status in children. *The American journal of clinical nutrition*. **77**(1):167-72 (2003).
  26. Fujinami Y, Fukui T, Nakano K, Ara T, Fujigaki Y, Imamura Y, et al. The effects of cigarette exposure on rat salivary proteins and salivary glands. *Oral diseases*. **15**(7):466-71 (2009).
  27. Baharvand M, Maghami AG, Azimi S, Bastani H, Ahmadih A, Taghibakhsh M. Comparison of superoxide dismutase activity in saliva of smokers and non-smokers. *South Med J* **103**(5): 425-7 (2010).
  28. Charalabopoulos K, Assimakopoulos D, Karkabounas S, Danielidis V, Kiortsis D, Evangelou A. Effects of cigarette smoking on the antioxidant defence in young healthy male volunteers. *International journal of clinical practice*. **59**(1):25-30 (2005).