**Pesquisa Brasileira em Odontopediatria e Clínica Integrada 2019, 19(1):e4897**
**DOI: http://doi.org/10.4034/PBOCI.2019.191.91**
**ISSN 1519-0501**

**ORIGINAL ARTICLE**

# Analysis of the Total Antioxidant Capacity of Saliva in Smokers

Ratna Kumala Indrastiti[1], Febrina Rahmayanti[2], Anandina I. Soegyanto[3]

[1]Oral Medicine Residency Program, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia. 0000-0001-6817-1410
[2]Department of Oral Medicine, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia. 0000-0002-9918-1571
[3]Department of Oral Medicine, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia. 0000-0001-9253-2810

Author to whom correspondence should be addressed: Febrina Rahmayanti, Department of Oral Medicine, Faculty of Dentistry, Universitas Indonesia, Jakarta, 10430. Phone: +62 212303257. E-mail: febrina_r@ui.ac.id.

Academic Editors: Alessandro Leite Cavalcanti and Wilton Wilney Nascimento Padilha

Received: 27 January 2019 / Accepted: 30 April 2019 / Published: 14 May 2019

**Abstract**

**Objective:** To analyze the total antioxidant capacity (TAoC) of saliva in smokers based on type of cigarette, duration and frequency of smoking. **Material and Methods:** 51 male smokers, aged 20-55 years were enrolled. Information regarding cigarette type and smoking duration and frequency was collected using a questionnaire. Unstimulated saliva samples were collected in the morning following fasting for 2 h, and TAoC was measured using a commercial kit. The data were evaluated through the independent t-test and Kruskal-Wallis test. **Results:** Mean TAoC for the consumption of Kretek cigarettes was 0.29 (±0.15) and for that of non-Kretek cigarettes was 0.36 (±0.10). Mean TAoC based on smoking duration was 0.31 (±0.14) for 5-10 years and 0.27 (±0.15) for >10 years. Median TAoC based on smoking frequency was 0.23 (0.11-0.44), 0.31 (0.06-0.64), and 0.27 (0.06-0.68) for 1-5, 6-10, and 11-20 cigarettes/day. Mean TAoC of the saliva from participants who consumed Kretek cigarettes was lower than that of the saliva from those who consumed non-Kretek cigarettes (p=0.3). Mean TAoC for a duration >10 years was lower than that for a duration of 5-10 years, although the difference between these two groups was not significant (p=0.4). **Conclusion:** There were tendencies of lower total antioxidant capacity on smokers with kretek type cigarettes, smoking duration >10 years and frequency of 1-5 cigarettes/day. This study indicates that the type, duration, and frequency of smoking may affect the salivary total antioxidant capacity.

**Keywords:** Tobacco Smoking; Cigarette Smoking; Antioxidants; Free Radicals.

**Introduction**

Smoking can result in the absorption of various harmful compounds, such as nitrous oxide, carbon monoxide, nicotine, tar, cadmium, methanol, and polycyclic aromatic hydrocarbons (PAH) in the body. Such compounds or their metabolites may react with biological macromolecules, resulting in oxidative stress and the formation of reactive species or the initiation of a radical chain reaction. Smokers are more susceptible to tissue damage due to the free radicals generated against cigarette smoke. Free radicals are highly reactive molecules produced during biochemical reactions that constitute normal processes of cell metabolism [1].

An imbalance between the reactive oxygen species (ROS) and reactive nitrogen species load and the biological antioxidant system that counteracts these causes oxidative stress [2]. There are several types of antioxidants in the body: (1) enzymes such as, superoxide dismutase, glutathione peroxidase, and catalase; (2) large molecules (albumin, ceruloplasmin, ferritin, and other proteins); (3) small molecules (ascorbic acid, glutathione, uric acid, tocopherol, carotenoids, and (poly) phenols); and (4) certain hormones (estrogen, angiotensin, and melatonin) [3]. There have been several studies that observed antioxidants level on human body, including in smokers. The total antioxidant capacity (TAoC) in smokers and non-smokers shower that TAoC in non-smokers was higher than that in smokers [4].

Several studies have shown that an increased smoking duration and frequency leads to an increased risk of diseases, like oral cancers and oral precancerous lesions like leukoplakia, erythroplakia and oral submucous fibrosis [5-7]. Smoking can affect different parts of the oral cavity, such as the gingiva, mucosa, teeth, and periodontal tissue [8]. In smokers, free radicals are generated via various sources that can increase the occurrence of oxidative stress and ultimately decrease the antioxidant capacity in the body. The consumption of even a single cigarette can significantly reduce the concentration and disrupt glutathione (GSH) action in protecting the body from harmful chemical components of cigarettes. A decrease in GSH action is associated with an unsaturated aldehyde component of cigarettes that reacts with the cysteine in GSH [2].

The saliva is the most appropriate bodily fluid that can be used to determine an individual's antioxidant capacity status related to smoking, since it contains gingival crevicular fluid, immune cells, and metabolic tissue, predominantly reflecting intraoral circumstances. Reportedly, the antioxidant contents of the saliva and blood correlate well; however, due to excessive free-radical production in the oral cavity, the saliva can exhibit increased enzyme effects [9]. The saliva has buffering, antibacterial, lubricating, and demineralizing abilities but is also a source of enzymatic and non-enzymatic antioxidants, maintains the redox balance, and prevents disturbances in redox homeostasis in the oral cavity and the body as a whole [10].

In recent years, saliva has often been used for diagnostic and research purposes due to its easy, non-invasive retrieval, relatively lower protein content, less complexity, and varying composition than serum [11]. It has been suggested that saliva could constitute a first line defense

in contrast to free radical mediated oxidative stress, so the salivary parameter could showed an antioxidant defense mechanism to scavenged the free radical activity [12].

The present study investigated the effects of cigarette type and smoking duration and frequency on total antioxidant capacity (TAoC) of the saliva. This salivary parameter is crucial, since a decrease in its antioxidant levels can cause abnormalities in bodily functions, such as cancer, lung disease, heart disease, kidney disease, diabetes, and some neurodegenerative diseases and also diseases in the oral cavity [10].

## Material and Methods

### Study Design and Sample

In this cross-sectional analytical study, 51 male smokers were selected using a convenience sampling method; the study population consisted of employees of the University of Indonesia.

The inclusion criteria were: male; aged 20-55 years old; had smoked cigarettes for a minimum period of 5 years, whether kretek cigarettes or non-kretek cigarettes type; consumption of at least 5 packs/year; and signed informed consent. Kretek is a type of cigarette made from tobacco, cloves, and other addictive substances, whereas non-kretek cigarettes was made only from tobacco, and less addictive substances than kretek cigarettes. The exclusion criteria were: a history of systemic disease or any medication taken in the previous 3 months; or a disability affecting involvement in the study. Following informed consent, a questionnaire regarding smoking behavior was completed, and subjects who fulfilled the study requirements were enrolled.

### Collection of Saliva Samples

The participants were requested not to eat or drink two hours prior to saliva collection. The smokers were also prohibited from smoking for one hour prior to saliva collection [4]. To gather whole unstimulated saliva, the participants were asked to spit their saliva into 15 mL centrifuge tubes. Collection of saliva samples was performed in an upright position, at 09.00-12.00 AM. The samples were preserved at -80$^{0}$C immediately until assessment.

### Assay Procedures

Samples were centrifuged at 1000 x g for 25 minutes at 4$^{0}$C. The supernatant was used for the assay, and TAoC was evaluated using the QuantiChrom Antioxidant Assay Kit (DTAC-100; BioAssay Systems, Hayward, CA, USA). Each sample was assessed in triplicate in three independent experiments, and the results were read using an Enzyme Linked Immunosorbent Assay (ELISA) Reader. The mean value from three readings were use for the statistical analysis.

### Data Analysis

Data was analyzed using the Statistical Package for Social Sciences software version 20.0. Statistical significance was set at $p<0.05$. The normality test was used to measure the data

distribution. The data distribution of TAoC based on cigarette type and smoking duration was normal so an independent t-test was applied. Analysis using the Kruskal-Wallis test was applied to assess TAoC based on smoking frequency.

Ethical Aspects

The present study was approved by the Ethics Committee of the Faculty of Dentistry, University of Indonesia. All subjects signed the free and informed consent form.

## Results

A total of 51 smokers participants were included in the present study; all were males ranging from 20 to 55 with the mean age 27.0 ± 6.4. Majority of the participants with a smoking habit consumed Kretek cigarettes (88.2%) and had a smoking duration of 5-10 years (72.5%) at a frequency of 5-10 cigarettes/day (47.1%). Mean TAoC of the saliva from participants who consumed Kretek cigarettes (mean = 0.29) was lower than that of the saliva from those who consumed non-Kretek cigarettes (mean = 0.36); however, the difference was not significant ($p>0.05$).

Mean TAoC for a duration >10 years was lower (mean = 0.27) than that for a duration of 5-10 years (mean = 0.31), although the difference between these two groups was not significant ($p>0.05$).

Median TAoC of the saliva from participants who smoked at a frequency of 1-5 cigarettes/day was lower (median = 0.23) than that of the saliva from the participants who smoked 6-10 cigarettes/day or 11-20 cigarettes/day; however, the differences among these three groups were not significant ($p>0.05$) (Figure 1).

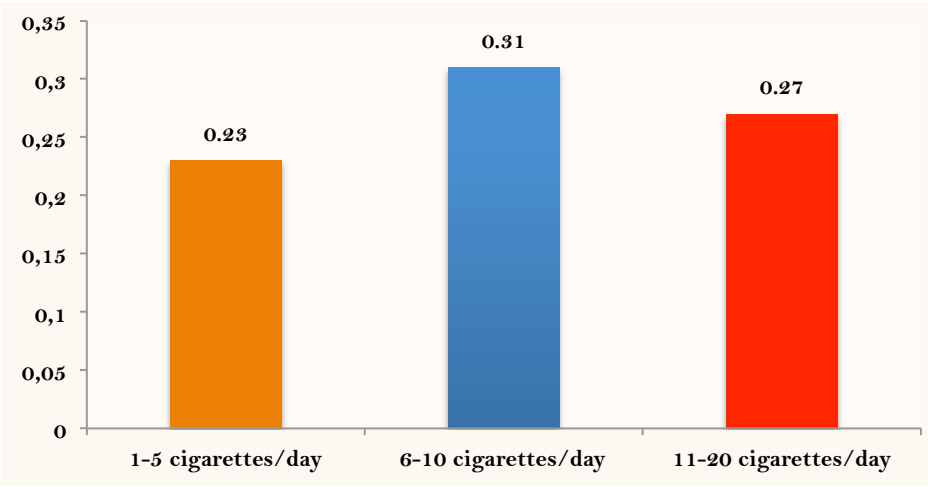


**Figure 1. Total Antioxidant Capacity (TAoC) of saliva in smokers based on frequency of smoking.**

## Discussion

Cigarette smoke contains gas and particle components that can potentially generate free radicals [13,14], which are highly and easily reactive with other compounds such as lipids, DNA,

and proteins [15]. Free radicals and ROS are toxic substances that can cause oxidative damage to biological targets. Oxidative damage to DNA and other macromolecules is thought to play a role in the pathogenesis of various diseases [16]. Tobacco can cause various changes in the mucosa, including the development of cancerous and pre-cancerous lesions. The type and location of such lesions vary and are influenced by the type of tobacco, way tobacco is used, and frequency and duration of tobacco use [17].

In the present study, the majority of participants had been smoking for 5-10 years at a frequency of 5-10 cigarettes/day. The distribution of the smoking duration and frequency in the present study was slightly different from the findings of an Indian study, in which the majority of subjects had been smoking for 11-20 years at a frequency of 1-5 cigarettes/day [6].

Kretek is a type of cigarette made from tobacco, cloves, and other addictive substances. It contains higher levels of carbon monoxide, nicotine, and tar than regular cigarettes, leading to a greater risk of disease [18]. Such compounds or their metabolites may react with biological macromolecules and cause oxidative stress via the formation of reactive species or the initiation of a radical chain reaction [1]. The effects of free-radical deletion can be contained when a balance between the body's antioxidant defense system and the generated free radicals exists. The antioxidant methods that detoxify excessive free radicals cause severe antioxidant deficiency [19, 20]. The present study indicated that the TAoC of the saliva in smokers who consume Kretek cigarettes was lower than that of the saliva in smokers who consume non-Kretek type cigarettes.

Antioxidants are molecules that scavenge free radicals and consist of enzymatic and non-enzymatic molecules. Antioxidants in the saliva comprise various molecules and enzymes, primarily uric acid molecules and peroxidase enzymes, which are water soluble [21]. Smoking attacks the antioxidant defense system in the saliva, since it is the first fluid to come in contact with the free radicals produced by cigarettes. Antioxidants in the saliva cannot protect the oral cavity from continuous attack, causing cellular and extracellular damage [4].

The present study indicated that the TAoC of the saliva in participants who had smoked for a duration of >10 years was lower than that of the saliva in participants who had smoked for 5-10 years. A decrease in the antioxidant capacity in heavy smokers with a long smoking duration as compared with that in those with a short smoking duration was observed [22]. This may be due to the fact that participants with shorter smoking duration have more active body defense mechanisms that partially counteracts the influence of free-radical invasion from cigarette smoke than those with longer smoking duration [22].

It was observed that free radicals decreased in participants who smoked continuously compared with those who smoked less frequently [22]. This was an opposite with the present study that showed a lower TAoC of the saliva in participants who smoked less frequently, at a frequency of 1-5 cigarettes/day. A previous study showed a significant decrease in total antioxidant capacity in smokers at a rate of 10 cigarettes per day and also there was no significant relationship between total antioxidant capacity and duration of smoking [23]. The result of this study can be affected by

several confounding factors such as air pollutant, food consumption, and other confounding factors that has not been controlled [19].

## Conclusion

Cigarette type, smoking duration and frequency led to differences, albeit non-significant, in TAoC of the saliva of smokers. The participants who consumed kretek cigarettes, with the duration of smoking >10 years and frequency of smoking 1-5 cigarettes/day has a lower TAoC than other groups. These results may be used as guidelines for further research regarding the antioxidant capacity of smokers, for inhibiting the negative effects of smoking, particularly those associated with oral cancer. Future investigation approaches with more research variables and restricted confounding factors should be designed in order to gain more detailed information.

**Acknowledgements:** We would like to thank all the staff and students of the Oral Medicine Department, all the staff of the Oral Biology Laboratory, and all participants for their cooperation throughout this study.

**Authors' Contributions:** All authors contributed individually to the development of this article. RKI contributed with the project design, data analysis and interpretation, critical content review, and approval of the final version of the manuscript. FR and AIS contributed to the intellectual conceptualization of the study, data analysis and reviewed the manuscript.

**Financial Support:** This study was supported by a grant from the Directorate of Research, Universitas Indonesia (Hibah PITTA 2017).

**Conflict of Interest:** The authors declare no conflicts of interest.

## References

[1] Pasupathi P, Saravanan G, Farook J. Oxidative stress bio markers and antioxidant status in cigarette smokers compared to nonsmokers. J Pharm Sci Res 2009; 1(2):55-62.

[2] Avezov K, Reznick AZ, Aizenbud D. Oxidative stress in the oral cavity: Sources and pathological outcomes. Respir Physiol Neurobiol 2015; 2018(209):91-4. https://doi.org/10.1016/j.resp.2014.10.007

[3] Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J Agric Food Chem 2005; 53(10):4290-302. https://doi.org/10.1021/jf0502698

[4] Bakhtiari S, Azimi S, Mehdipour M, Amini S, Elmi Z, Namazi Z. Effect of cigarette smoke on salivary total antioxidant capacity. J Dent Res Dent Clin Dent Prospects 2015; 9(4):281-4. https://doi.org/10.15171/joddd.2015.049

[5] Garg KN, Raj V, Chandra S. Trends in frequency and duration of tobacco habit in relation to potentially malignant lesion: A 3 years retrospective study. J Oral Maxillofac Pathol 2013; 17(2):201-6. https://doi.org/10.4103/0973-029X.119760

[6] Naveen-kumar B, Tatapudi R, Sudhakara-reddy R, Alapati S, Pavani K, Sai-Praveen KN. Various forms of tobacco usage and its associated oral mucosal lesions. J Clin Exp Dent 2016; 8(2):e172-7. https://doi.org/10.4317/jced.52654

[7] Keluskar V, Kale A. An epidemiological study for evaluation of oral precancerous lesions, conditions and oral cancer among Belgaum population with tobacco habits. Biosci Biotechnol Res Commun 2010; 3(1):50-4.

[8] Rad M, Kakoie S, Brojeni FN, Pourdamghan N. Effect of long-term smoking on whole-mouth salivary flow rate and oral health. J Dent Res Dent Clin Dent Prospects 2010; 4(4):110-4. https://doi.org/10.5681/joddd.2010.028

[9] Diana H, Bhola S, Kalburgi V, Warad S, Kokatnur VM. The influence of cigarette smoking on blood and salivary super oxide dismutase enzyme levels among smokers and nonsmokers - A cross sectional study. J Tradit Complement Med 2015; 5(2):100-5. https://doi.org/10.1016/j.jtcme.2014.11.003

[10] Żukowski P, Maciejczyk M, Waszkiel D. Sources of free radicals and oxidative stress in the oral cavity. Arch Oral Biol 2018; 92(1):8-17. https://doi.org/10.1016/j.archoralbio.2018.04.018

[11] Nunes LA, Mussavira S, Bindhu OS. Clinical and diagnostic utility of saliva as a non-invasive diagnostic fluid: A systematic review. Biochem Med 2015; 25(2):177-92. https://doi.org/10.11613/BM.2015.018

[12] Peluso I, Raguzzini A. Salivary and urinary total antioxidant capacity as biomarkers of oxidative stress in humans. Patholog Res Int 2016; 2016:5480267. https://doi.org/10.1155/2016/5480267

[13] Susanna D, Hartono B, Fauzan H. Penentuan kadar nikotin dalam rokok. Makara Kesehat [ASAP] 2003; 7(2):38-41. [In Indonesian]

[14] Tirtosastro S, Murdiyati AS, Kimia K. Tembakau dan rokok. Bul Tanam Tembakau 2010; 2(1):33-43. [In Indonesian]

[15] Albasayam S, Safyudin S, Arifin M, Oktalisa W. Kadar superoksida dismutase mahasiswa perokok di program studi pendidikan dokter Universitas Sriwijaya, Kedokt Yars J 2016; 3(2):76-82. [In Indonesian]

[16] Battino M, Ferreiro MS, Gallardo I, Newman HN, Bullon P. The antioxidant capacity of saliva. J Clin Periodontol 2002; 29(3):189-94. https://doi.org/10.1034/j.1600-051X.2002.290301x.x

[17] Behura SS, Masthan MK, Narayanasamy AB. Oral mucosal lesions associated with smokers and chewers – A case-control Study in Chennai Population. J Clin Diagn Res 2015; 9(7):ZC17-22. https://doi.org/10.7860/JCDR/2015/14008.6169

[18] Gondodiputro S. Bahaya tembakau dan bentuk-bentuk tembakau. Padjajaran 2007. [In Indonesian]

[19] Pham-huy LA, He H, Pham-huy C. Free radicals, antioxidants in disease and health. Int J Biomed Sci 2008; 4(2):89-96.

[20] Rahman K. Studies on free radicals, antioxidants, and co-factors. Clin Interv Aging 2007; 2(2):219-36.

[21] Lee J, Taneja V, Vassallo R. Cigarette smoking and inflammation: Cellular and molecular mechanisms. J Dent Res 2012; 91(2):142-9. https://doi.org/10.1177/0022034511421200

[22] Kurku H, Kacmaz M, Kisa U, Dogan O, Caglayan O. Acute and chronic impact of smoking on salivary and serum total antioxidant capacity. J Pak Med Assoc 2015; 65(2):164-9.

[23] Shirzaiy M, Ladiz MA, Dalirsani Z, Haghighi JD, Nakhaii A. Evaluation of salivary total antioxidant capacity on smoker with severe chronic periodontitis. Int J High Risk Behav Addict 2017; 6(3):1-7. https://doi.org/10.5812/ijhrba.59486