Brazilian Dental Science

unesp
UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Instituto de Ciência e Tecnologia
Campus de São José dos Campos

ORIGINAL ARTICLE

DOI: https://doi.org/10.14295/bds.2022.e2867

A study on the correlation between smoking and non-enzymatic antioxidant factors of the saliva of healthy smokers and non-smokers

Estudo da correlação entre fumo e fatores antioxidantes não-enzimáticos na saliva de pacientes saudáveis fumantes e não fumantes

Mahdieh ZARABADIPOUR[1], **Seyed Amir Hadi HOSSEINI**[2], **Hashem HAGHDOOST-YAZDI**[3], **Ehsan AALI**[4], **Pedram YUSEFI**[2], **Monirsadat MIRZADEH**[3], **Hossein PIRI**[3]

1 - Department of Oral and Maxillofacial Medicine, School of Dentistry, Dental Caries Prevention Research Center, Qazvin University of Medical Sciences, Qazvin, Iran.
2 - Student Research Committee, School of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran.
3 - Cellular and Molecular Research Center, Research Institute for prevention of Non-Communicable Disease, Qazvin University of Medical Sciences, Qazvin, Iran.
4 - Department of Pharmacology, School of Medicine, Qazvin University of Medical Sciences, Qazvin, Iran.

ABSTRACT

Objective: Smoking is among the most destructive habits which have numerous effects on the body.The chemical components of cigarettes destroy the anti-oxidant content of the saliva.In this study, the concentration of albumin and uric acid of healthy non-smokers and smokers was measured based on the frequency of smoking. **Material and Methods:**In this cross-sectional study, 26 heavy smokers, 27 normal smokers, and 29 non-smokers between the ages of 25 to 40 were selected.The subjects did not suffer from any systemic or periodontal conditions. Unstimulated saliva was collected by spitting. The level of salivary albumin was measured by Bromocresol Green, and the level of salivary uric acid was measured by the uricase method.The selected method of analysis, using SPSS software, was One-Way ANOVA. **Results:** Mean albumin content of saliva was 33.52 ± 1.52 mg/dl in non-smokers and 23.88 ± 8.93 mg/dl in heavy smokers.The mean uric acid concentration in non-smokers was 2.98 ± 0.79 μmol/L and in heavy smokers was 2.32 ± 0.77 mg/dL.The differences between levels of both salivary uric acid and salivary albumin were significant in heavy smokers and non-smokers(P=0.001). **Conclusion**: Based on the findings of this study, saliva concentrations of both Albumin and Uric Acid change based on the frequency of smoking.Decreased level of salivary albumin and decreased level of salivary uric acid can be considered as markers of the harmful effects of smoking on oral health.

KEYWORDS

Uric Acid; Albumin; Saliva; Cigarette Smoking; Oxidative stress.

RESUMO

Objetivo: Tabagismo está entre os hábitos mais deletérios, que causam inúmeros efeitos no organismo. Os componentes químicos do cigarro destroem os compostos anti-oxidantes da saliva. Neste estudo, a concentração de albumina e ácido úrico em pacientes saudáveis fumantes e não-fumantes foi mensurada e correlacionada coma frequência de fumo. **Material e Métodos:** Neste estudo transversal, 26 fumantes pesados, 27 fumantes moderados, e 29 não fumantes entre 25 e 40 anos foram incluídos. Os participantes não apresentavam nenhuma condição sistêmica ou periodontal. Saliva não estimulada foi coletada. Os níveis salivares de albumina foram avaliados por Verde de bromocresol, e o nível de ácido úrico foi mensurado pelo método de uricase. Os dados foram analisados utilizando-se One-way ANOVA no software SPSS. **Resultados:** A albumina salivar foi de 33.52 ±1.52 mg/dl nos não-fumantes e 23.88 ± 8.93 mg/dl nos fumantes pesados. A concentração média de

ácido úrico em não-fumantes foi de 2.98 ± 0.79 μmol/L e em pacientes fumantes pesados de 2.32 ± 0.77 mg/dL. As diferenças entre os níveis de ambos, ácido úrico e albumina, foi significante entre fumantes pesados e não-fumantes (p=0.001). **Conclusão:** Baseados nos achados deste estudo, concentrações salivares de albumina e ácido úrico baseados na frequência de fumo. A diminuição dos níveis salivares de albumina e ácido úrico podem ser considerados marcadores dos efeitos nocivos do cigarro na saúde oral.

PALAVRAS-CHAVE

Ácido úrico; Albumina; Saliva; Fumantes; Estresse oxidativo.

INTRODUCTION

Smoking is one of the modifiable risk factors of cancer, by smoking a wide range of harmful chemicals and reactive oxygen species enter the oral cavity which can cause tissue damage and cytotoxic effects inside and outside of cells and result in cells losing their function [1]. Oxidative stress means the imbalance between free radicals and the antioxidant defense system, it happens when the body cannot detoxify the mentioned free radicals [2]. There is a direct correlation between oxidative stress and certain diseases and the evaluation of the factors influencing the oxidative stress in body fluids, including saliva, can be used to monitor and treat these conditions [3]. The smoke inhaled from cigarettes contains toxic compounds including carbon monoxide, nicotine, and benzopyrenes, and these compounds along with the aforementioned reactive oxygen species comprise most of the cigarette smoke [4]. These compounds have significant correlations with inflammatory diseases related to the respiratory system, cardiovascular diseases, and a broad range of cancers [5,6]. Generally, Gingivitis is one of the periodontal diseases and it affects about 50% of different populations [7]. Periodontal diseases usually begin by the formation of bacterial colonies such as Porphyromonas gingivalis. These bacteria activate the immune system of the host, and by the accumulation of neutrophils in the infected region and the production of reactive oxygen species by neutrophils, tissue damage also occurs.These bacteria also employ special antioxidant systems which protect them from reactive oxygen species which are produced by the neutrophils [8]. It appears that the progression of periodontal diseases is caused by the inability of antioxidant systems to remove free radicals and reactive oxygen species [9].Cigarette smoke is one of the major factors which contribute to the decrease in the ability of antioxidant systems, speeding up the periodontal tissue damage [10].Generally, Saliva is a mixture of different compounds, secreted by salivary glands, and it covers the internal area of the mouth and teeth.The antioxidant system of saliva plays an important role in its anticancer capacity, and it contains a variety of enzymes and molecules such as Uric acid and the peroxidase system [11]. Saliva has two antioxidant components:enzymatic (glutathione peroxidase, superoxide dismutase, etc.) and non-enzymatic (albumin, uric acid, glutathione, etc.), and saliva is the first biologic fluid that contacts cigarette smoke [12]. Uric acid is the most important non-enzymatic antioxidant component of saliva, and the level of uric acid in the saliva is related to the level of uric acid in serum directly. It appears that uric acid enters from plasma into saliva, and it seems to be the most important ingredient of salivary antioxidant defense system in the epithelium of nasal and oral cavities to combat ROSs and cigarette smoke.Another function of salivary uric acid is the ability to break water-soluble chains to destroy free radicals [7]. One of the salivary proteins is albumin which is filtered from serum into saliva and the oral cavity. Albumin and other proteins found in saliva act as reservoirs for antioxidants, and for this reason the protective role of these proteins should be studied [13]. By Considering the prevalence of smoking in the past few decades, cigarette smoking remains the leading cause of preventable disease.Tobacco smoking provides sufficient evidence to establish a causal association between smoking and different cancers [14]. Due to the lack of sufficient information on the relationship between smoking and non-enzymatic salivary antioxidants such as albumin and uric acid this study was conducted so that we can use the gathered information of this study to investigate the effect of smoking on levels of salivary uric acid and albumin.The aim of this study is to compare salivary uric acid and albumin of non-smokers, normal smokers and heavy smokers.

MATERIAL AND METHODS

The present research was approved by the Ethics Committee of the Qazvin University of Medical Science and health services, marked as Project No:IR.QUMS.REC.1396.449.This cross-sectional study was performed at Qazvin University of Medical Sciences and health services.The 82 male smokers ages 25 to 40 were selected and signed an Informed Consent Form. The subjects did not suffer from diseases such as diabetes, hypertension, thyroid disease or Sjögren's syndrome and without moderate to severe periodontal disease based on the Ramford index. It should be noted that subjects with poor oral health (With plaque index of 25% or higher) and history of medication and dietary supplement intake including hypertension medication, anti-allergy medication, multivitamin supplements, and passive smokers were dropped out [15].

The participants of this study include 26 heavy smokers, 27 normal smokers, and 29 non-smokers.Heavy smokers are defined as subjects who smoked more than 1 packet of cigarettes per day in the past 5 years.While normal smokers are comprised of subjects who smoked less than a packet per day during the aforementioned period [15]. Saliva samples were collected from 11:00 to 15:00 during the day.The subjects did not smoke nor ate or drank 90 minutes prior to saliva sampling, and they were seated during the sampling. After rinsing physiologic serum, they were provided with special sampling containers and they were asked to collect their non-stimulated saliva for 5 minutes.All of the samples were moved to a refrigerator (-20 degrees celsius) in a short time after sampling.Samples were centrifuged for 10 minutes with 400 RPM after thawing.The centrifuging process homogenized the samples and isolated different cellular components. It should be noted that prior to sampling, subjects were interviewed and examined clinically (Oral, non-oral, periodontal) to check for study criteria and remove them if needed.None of the subjects had active periodontal disease and they had acceptable oral hygiene. Also, no interventions were made.

In this study, the concentrations of salivary uric acid and albumin were assessed.The level of Uric acid was measured by using the Uricase method (With high specificity).This enzymatic method is appropriate since there is no need for protein Isolation because Uricase does not interfere with the protein components of saliva.The only substances that caused minor interference were guanine, xanthine, and other structural analogs of uric acid. In the enzymatic reaction, uric acid is converted to Allantoin by uricase.The reduction in absorption of light (related to the usage of Urate) is measured at the wavelength of 282-292 nanometer with a spectrophotometer [16]. Also, measuring uric acid was made possible by the Uric Acid Measurement Kit of Pars Azmon company, using the colorimetric method.To measure the level of salivary albumin, the Bromocresol green (BCG) method was used which works based on Colorimetry (Using end-point method). In this method, the albumin present in saliva creates an acidic PH with BCG (which creates a green-blue complex) which can be detected at the wavelength of 546 nanometers using Spectrophotometry.The intensity of the created color is related to the albumin content of the sample [17]. Also, measuring Albumin was made possible by Albumin Measurement Kit of Pars Azmon company, using colorimetric method. As for the spectrophotometer, HALO-XB 10 (Manufactured by Dynamica) was used.

It should be noted that the data regarding the variables were reported based on Mean ± SD and were analyzed by the SPSS version 19.The Kolmogorov-Smirnov normality test was first used, and since the data followed normal distribution, parametric tests were also used.One-way ANOVA test was then used to analyze the data and the P-value for all cases was considered at $P<0.05$.

RESULTS

Laboratory findings based on saliva samples show that the mean uric acid concentration in non-smoking men was 2.98 ± 0.79 μmol/L and the level of salivary uric acid in male normal smokers was 2.65 ± 0.89 μmol/L (Figure 1). Mean uric acid concentration in heavy smokers was 2.32 ± 0.77 mg/dL. Also, using one-way ANOVA and $P<0.05$ showed that there is a significant relationship between uric acid and different quantities of smoking (Figure 1). Also, after performing statistical tests we found that there is a significant difference in levels of salivary uric acid between non-smokers and heavy smokers ($p = 0.034$), but no such significant difference was found between normal smokers and non-smokers ($p=0.52$).

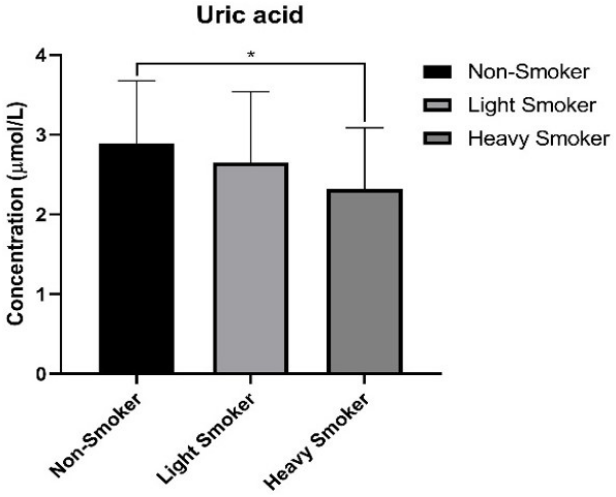

Figure 1 - Salivary Uric acid mean concentration in groups. *P< 0.05.

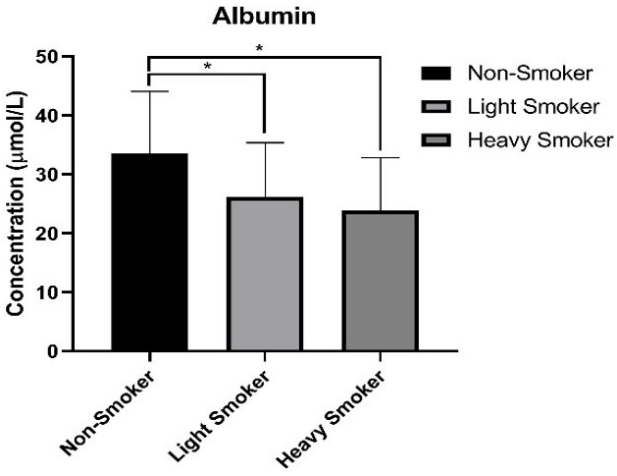

Figure 2 - Salivary Albumin mean concentration in groups. *P< 0.05.

In addition, there was no significant difference between normal and heavy smokers (p=0.32). The mean albumin concentration of non-smokers was 33.52 ± 1.52 μmol/l, and this number was 26.16 ± 9.19 μmol/l in normal smokers and 23.86 ± 8.93 μmol/l in heavy smokers (Figure 2).

One-way ANOVA and P<0.05 showed that there is a significant difference between normal smokers and non-smokers (p=0.01), and also between non-smokers and heavy smokers (p=0.001). It should be mentioned, however, that no significant difference was observed between normal and heavy smokers.

DISCUSSION

Antioxidants combat the destructive effects of free radicals and conserve the structure and integrity of the tissues. It has been reported that the imbalance between free radicals and antioxidant levels plays an important role in the onset and progression of oral inflammatory diseases [18]. As mentioned in the findings section, we found that due to the significant difference in levels of salivary uric acid between non-smokers and heavy smokers and another significant difference between non-smokers and heavy smokers in levels of salivary albumin we can investigate the relationship between decreased salivary albumin and decreased salivary uric acid with smoking-induced diseases and smoking-induced oxidative stress in oral cavity and Periodontal tissues, Greabu et al, showed that total antioxidant capacity of saliva in smokers is lower than non-smokers, findings which conform to our study [9,19]. Also, Sediqh Bakhtiari et al conducted a study in 2015 on healthy male smokers and non-smokers and found that the total antioxidant capacity of healthy non-smokers was higher than smokers.These findings also validate our study [1]. The antioxidant system of saliva includes both enzymatic and non-enzymatic components, and considering that numerous studies were performed on the relation between smoking and total antioxidant capacity, Taniguci et al study was also reviewed, which showed that saliva as a multi molecular complex of proteins, changes after cigarette use.In this study which was performed on the premenopausal smoker and non-smoker women, it was shown that the number of sulfhydryl bonds of Proteins in saliva, in smokers was 20 to 25-kilo dalton less than non-smokers, and they discussed that the saliva of smokers contains oxidized proteins which can reduce the conservation of epithelium.In another study [20], Esculley et al showed that patients with poor periodontal status also had higher levels of oxidative stress while also having significantly lower urate and other antioxidants (except albumin with a significant increase) in their saliva. They also pointed out that saliva contains markers that indicate oxidative stress in the oral cavity which can be used to monitor oxidative damage [7]. In another study by Meurman et al in 2002, it was showed that salivary albumin in hospitalized patients with generally poor physical health was higher than outpatients [13]. It is worth mentioning that in most studies performed on salivary albumin in different groups, the target population consisted of mostly people with poor periodontal health, and no study was done solely on the direct effect of smoking on salivary albumin of otherwise healthy people.In other studies, smokers with

unhealthy periodontal status were observed, and in these cases, the periodontal status of the subjects can be considered as a strong confounder agent and interfere with the clear understanding of the role of smoking.Our study tried to overcome this issue by studying the salivary albumin smoker subjects who were otherwise healthy, to remove the confounding effect of periodontal disease on salivary albumin, so that we can observe the direct effect of smoking on salivary albumin.

The other important antioxidant component of saliva, however, is uric acid.In a study performed by Novakovic et al it was shown that the concentration of salivary uric acid and also albumin can be increased by non-surgical Periodontitis treatment and thus discussed that after non-surgical treatment of Periodontitis, a significant increase in salivary albumin and uric acid activity is observed, which are indicative of the antioxidant capacity of saliva [12]. In Pullichery's study in 2015, however, it was seen that the uric acid levels were lower in non-tobacco users than tobacco users, although this difference was not significant [21]. Young et al. [22] showed in 2017 that hyperuricemia in male smokers compared to non-smokers was lower (22.8% vs 26.5%) which indicates a negative relationship between smoking and hyperuricemia, although this study did not show any relation between hyperuricemia and smoking in females. Roohi et al. [23] showed in 2017 that smoking can significantly affect serum protein profile of male smokers, and concluded that serum albumin level lowers if smoking is increased.

Considering the fact that exposure to cigarette smoke can cause a variety of oxidative damages in different tissues, we expected that the albumin level of smokers would increase similar to non-smokers with poor periodontal status, but in practice, this was not observed. Our observation showed that smoking causes salivary albumin to decrease, and the same was observed with regards to uric acid level.In this study, a narrow age group of single-sex subjects was chosen to remove their potential confounding effects. A limited number of studies, however, were performed on smokers without periodontal diseases, and the results of some of those studies conform to ours.

CONCLUSION

The results found in this study show that salivary albumin and salivary uric acid concentration are significantly lowered by smoking, and this decrease can be an indication of biochemical changes that can be markers of early periodontal disorders in smokers.To have a broad conclusion, years of smoking and other antioxidants of saliva can be observed.Also, to achieve better results, large samples can be selected, and also the expression of inflammatory genes and proteins in periodontal tissues can be studied.

Acknowledgments

The authors of the present research project would like to appreciate the Deputy for the Research and Technology Division of Qazvin University of Medical Sciences for supporting the project.

Author Contributions

HP: contributing to the study conception and design, Writing the first draft of the manuscript, Approving the final manuscript. MZ: contributing to the study conception and design. Approving the final manuscript. AHH: writing the first draft of the manuscript. Data collection and analysis. HH-Y: approving the final manuscript. EA: Writing the first draft of the manuscript. Approving the final manuscript. PY: data collection and analysis. Approving the final manuscript. MM: Data collection and analysis. Approving the final manuscript.

Conflict of Interest

The authors declare no competing interests.

Funding

This research project was financially supported by Deputy for the Research and Technology Division and the Cellular and Molecular Research Center of Qazvin University of Medical Sciences.

Regulatory Statement

The present research was approved by the Ethics Committee of the Qazvin University of Medical Science and health services, marked as Project No:IR.QUMS.REC.1396.449.

REFERENCES

1. Bakhtiari S, Azimi S, Mehdipour M, Amini S, Elmi Z, Namazi Z. Effect of cigarette smoke on salivary total antioxidant capacity. 2015;9(4):281-4. https://doi.org 10.15171/joddd.2015.049.
2. Birnboim HC. DNA strand breaks in human leukocytes induced by superoxide anion, hydrogen peroxide and tumor promoters are repaired slowly compared to breaks induced by ionizing radiation. Carcinogenesis. 1986;7(9):1511-7. https://doi.org/10.1093/carcin/7.9.1511.
3. Golusińska-Kardach E, Napierała M, Sokalski J, Kardachi H, Florek E. Periodonta disease in smokers, and the parameters of oxidative stress. Przegl Lek. 2015;72(10):584-7. PMID: 26946573.
4. Kanehira T, Shibata K, Kashiwazaki H, Inoue N, Morita M. Comparison of antioxidant enzymes in saliva of elderly smokers and non-smokers. Gerodontology. 2006;23(1):38-42. https://doi.org/10.1111/j.1741-2358.2006.00077.x.
5. Dwyer TM. Cigarette smoke-induced airway inflammation as sampled by the expired breath condensate. Am J Med Sci. 2003;326(4):174-8. https://doi.org/10.1097/00000441-200310000-00004.
6. Phillips DH. Smoking-related DNA and protein adducts in human tissues. Carcinogenesis. 2002;23(12):1979-2004.
7. Sculley DV, Langley-Evans SC. Periodontal disease is associated with lower antioxidant capacity in whole saliva and evidence of increased protein oxidation. Clin Sci (Lond). 2003;105(2):167-72. https://doi.org/10.1042/CS20030031.
8. Fredriksson M, Gustafsson A, Åsman B, Bergström K. Hyper-reactive peripheral neutrophils in adult periodontitis: generation of chemiluminescence and intracellular hydrogen peroxide after in vitro priming and FcγR-stimulation. J Clin Periodontol. 1998;25(5):394-8. http://dx.doi.org/10.1111/j.1600-051x.1998.tb02461.x.
9. Chapple ILC, Brock G, Eftimiadi C, Matthews JB. Glutathione in gingival crevicular fluid and its relation to local antioxidant capacity in periodontal health and disease. Mol Pathol. 2002;55(6):367-73. http://dx.doi.org/10.1136/mp.55.6.367.
10. Chapple I, Mason G, Garner I, Matthews J, Thorpe G, Maxwell S, et al. Enhanced chemiluminescent assay for measuring the total antioxidant capacity of serum, saliva and crevicular fluid. Ann Clin Biochem. 1997;34(Pt 4):412-21. http://dx.doi.org/10.1177/000456329703400413.
11. Maciejczyk M, Szulimowska J, Skutnik A, Taranta-Janusz K, Wasilewska A, Wiśniewska N, et al. Salivary biomarkers of oxidative stress in children with chronic kidney disease. J Clin Med. 2018;7(8):209. http://dx.doi.org/10.3390/jcm7080209.
12. Novaković N, Čakić S, Todorović T, Anđelski-Radičević B, Dožić I, Petrović V, et al. Antioxidative status of saliva before and after non-surgical periodontal treatment. Srp Arh Celok Lek. 2013;141(3-4):163-8. http://dx.doi.org/10.2298/sarh1304163n.
13. Meurman JH, Rantonen P, Pajukoski H, Sulkava R. Salivary albumin and other constituents and their relation to oral and general health in the elderly. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2002;94(4):432-8. http://dx.doi.org/10.1067/moe.2002.122345.
14. Sasco A, Secretan M, Straif K. Tobacco smoking and cancer: a brief review of recent epidemiological evidence. Lung Cancer. 2004;45 Suppl 2:S3-9. http://dx.doi.org/10.1016/j.lungcan.2004.07.998.
15. Pashaei P, Alizadeh Z, Piri H, Mirzadeh M, Kazemi F. Comparison of total antioxidant capacity of saliva in men based on daily cigarette consumption. Braz Dent Sci. 2021;24(1):1-6.
16. Miller CS, King Jr CP, Langub MC, Kryscio RJ, Thomas MV. Salivary biomarkers of existing periodontal disease: a cross-sectional study. J Am Dent Assoc. 2006;137(3):322-9. http://dx.doi.org/10.14219/jada.archive.2006.0181.
17. Tobón-Arroyave S, Jaramillo-Gonzalez P, Isaza-Guzman DM. Correlation between salivary IL-1β levels and periodontal clinical status. Arch Oral Biol. 2008;53(4):346-52. http://dx.doi.org/10.1016/j.archoralbio.2007.11.005.
18. Arbabi-Kalati F, Nosratzehi T, Salimi S, Sadeghi Sabzevari R, Arbabi-Kalati P. Comparison of total antioxidant capacity of saliva in smokers and non-smokers. J Mashhad Dent Sch. 2014;38(2):93-8.
19. 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. 2008;33(2):129-36.
20. Taniguchi M, Iizuka J, Murata Y, Ito Y, Iwamiya M, Mori H, et al. Multimolecular salivary mucin complex is altered in saliva of cigarette smokers: detection of disulfide bridges by Raman spectroscopy. Biomed Res Int. 2013;;2013:168765. http://dx.doi.org/10.1155/2013/168765.
21. Pullishery F, Panchmal GS, Siddique S. Salivary thiocyanate, uric acid and pH as biomarkers of periodontal disease in tobacco users and non-users-an in-vitro study. J Clin Diagn Res. 2015;9(7):ZC47-ZC50. http://dx.doi.org/10.7860/JCDR/2015/12783.6203.
22. Yang T, Zhang Y, Wei J, Zeng C, Li L-J, Xie X, et al. Relationship between cigarette smoking and hyperuricemia in middle-aged and elderly population: a cross-sectional study. Rheumatol Int. 2017;37(1):131-6. http://dx.doi.org/10.1007/s00296-016-3574-4.
23. Roohi N, Mehjabeen, Ashraf S. Effects of cigarette smoking on serum proteins profile in male active and passive smokers. Punjab Univ J Zool. 2017;32(2):209-15.

Hossein Piri
(Corresponding address)
Cellular and Molecular Research Center, Research Institute for prevention of Non-Communicable Disease, Qazvin University of Medical Sciences, Qazvin, Iran.
Email: hosseinpiry@gmail.com

Date submitted: 2021 Mar 12
Accept submission: 2021 Jun 08