Original Article

TIPS Trends in Pharmaceutical Sciences 2021: 7(3): 161-168.

# Does vitamin E improve the smokers' salivary antioxidant status?

Mostafa Rezaei[1], seyed mohammad Mazloomi[2], Fatemeh Mohammadi[4], Maryam Shahrokhi[4*]

[1]Department of Oral and Maxillofacial Medicine, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran.
[2]Research Center and School of Nutrition and Food Sciences, Shiraz University of Medical Sciences, Shiraz, Iran.
[3]School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran.
[4]Students' Research Committee, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran.

## Abstract

Vitamin E is known as a preventive or therapeutic antioxidant that improves the total antioxidant capacity (TAC) of saliva in individuals at risk of different oral cavity oxidative stresses. This study aimed to compare the effect of this vitamin on the salivary TAC between smokers and non-smokers. In this single-blind crossover clinical trial, non-stimulated saliva samples were collected from smoker and nonsmoker participants (n=60 per group) at three stages (baseline and after the two interventional phases). They were divided into subgroups to receive daily vitamin E (200 IU) and placebo for three weeks alternatively in the first or second phase. The salivary TAC was measured via fluorescence recovery after photobleaching method. The TAC changes were calculated in each phase. Data were analyzed by using SPSS software through repeated measures ANOVA, independent sample t-test, and covariate test. The mean changes of TAC of smokers first receiving vitamin E and then placebo were 0.06±0.091 (IU/mL) and 0.25±0.089 (IU/mL), respectively(P=0.017). The mean TAC changes in non-smokers after taking vitamin E and placebo were 0.059±0.13 versus 0.053±0.129 (IU/mL), respectively,; being statistically insignificant (P=0.791). Accordingly, vitamin E improved the salivary TAC in both non-smokers and smokers.

*Keywords*: saliva, smoking, total antioxidant capacity, vitamin E

## 1. Introduction

Among the numerous negative effects of smoking on the oral health are increased risk of periodontal disease, tooth loss,pre-cancerous oral lesions and oral cancers,exacerbation of the alveolar bone resorption, reduced periodontal treatment efficacy and delayed healing of oral ulcers.(1-6). Cigarette smoke is responsible for 30-90% of oral cancers (6), asitweakensthe antioxidant properties of the defense system and triggers inflammatory diseases and oraldisorders (7-8). The oral squamous cell carcinoma is much more prevalent in smokers than non-smokers (9). According to the field cancerization concept, gradual accumulation of the free radicals and reactive species of oxygen and nitrogen in the inhaled cigarette smoke causes malignant transformation (10).

The antioxidant compounds of saliva form the first line of defense against oxidative stress caused by free radicals (11). Total antioxidant capacity (TAC) of saliva depends on enzymatic(e.g. superoxide dismutase, catalase and glutathione peroxidase) and non-enzymatic agents (e.g. uric acid, vitamin C, glutathione and oxidized glutathione, vitamin E, carotenoids, and bilirubin) (12). The salivary TAC isreported to be lower in patients with systemic diseases such as Crohn's

*Corresponding Author*: Maryam Shahrokhi, Students' Research Committee, School of Dentistry, Shiraz University of Medical Sciences, Shiraz, Iran.
Email: mshahrokhisa@sums.ac.ir


Recieved: 08/03/2021; Accepted: 01/05/2021

disease (13), asthma (14), multiple sclerosis (15), type 2 diabetes mellitus (16), Sjögren syndrome (17), and some oral diseases such as tooth decay (18), periodontal disease (19), oral lichen planus (20), pre-cancerous lesions (21), and squamous cell carcinoma (22). This condition is alsoassociated withsmoking (23-25).

Vitamin E is one of the essential antioxidants that helps removing free radicals (26). It is said to improve the salivary antioxidant capacity in individuals at risk ofdifferent oral cavity oxidative stressesas preventive or therapeutic agents (27). Vitamin E functions primarily as a chain-breaking antioxidant that causes the propagation of lipid peroxidation (28). Cigarette smoke can affect the γ-tocopherol (γ-T) nitration and the changes in the synthesis, plasma concentrations, and urinary excretion of the vitamin E metabolite (29). This study aimed to evaluateand compare the effect of vitamin E on total antioxidant capacity of saliva in smokers and non-smokers population.

## 2. Materials and Method

### *2.1. Design, participants, and ethical consideration*

This single-blind randomized cross-over clinical trial was approved by the Ethics Committee of Shiraz University of Medical Sciences. A total of 120 participants were selected through restricted randomization method of block randomization sampling method out of those referring to the clinic of School of Dentistry, Shiraz University of Medical Sciences. The enrolled smokers had a history of smoking five or more pack/year (number of daily cigarettes divided by 20 and multiplied by the years of smoking). Healthy individuals were considered as the non-smokers group. Those with systemic diseases, dry mouth, history of oral ulcer or any inflammatory oral disease, alcohol consumption, drug and medication use, or supplemental vitamin consumption within the preceding three months were excluded from the study.

### *2.2. Randomization method*

In this study, we used the restricted randomization method of block randomization. Blockage is usually used to balance the number of samples allocated to each of the studied groups. This feature helps researchers to equalize the number of samples allocated to each of the studied groups in cases where intermediate analyzes are required during the sampling process. All blocks are the same size, and in this two-group experiment we will have 6 blocks (including 3 participants in the intervention group and 3 participants in the control group). Random allocation software (StataCorp, College Station, TX, USA) was also used to randomize random sequence production software (Random allocation software). To conceal, we used Allocation concealment, which refers to the method used to perform a random sequence on study participants, so that the assigned group was not identified before the individual was assigned. Using non-transparent envelopes sealed with random sequences (Sequentially numbered, sealed, opaque envelopes). They are placed in order. In order to maintain the random sequence, numbering is done on the outer surface of the envelopes in the same way. Finally, the lids of the letter envelopes are glued and placed inside a box, respectively. At the beginning of the registration of participants, based on the order of entry of eligible participants in the study, one of the envelopes of the letter will be opened in order and the assigned group of the participant will be revealed.

Each group contain 60 participants which were randomly divided into two subgroups (n=30) to receive vitamin E 200 IU (Dana Pharmaceutical Company, Tabriz, Iran) and placebo (DanaPharmaceutical Company, Tabriz, Iran) alternatively in the first (vitamin-placebo) or second phase (placebo-vitamin) of intervention for three weeks. Accordingly, the groups were defined as smokers vitamin E-placebo, smokers placebo-vitamin E, non-smokers vitamin E-placebo, and non-smokers placebo-vitamin E.

### *2.3. Saliva sample collection and preparation*

The saliva samples were collected in three phases at the baseline, and after each washing out of interventional phases (phase I and II). In all the three phases, the participants were asked to stop eating, drinking and smoking one hour before collecting saliva. To collect 20 mL of non-stimulated saliva during the period of 9 to 12 A.M., the participants were in seated position, spitted in a 50-mL Falcon tube (Isan Med, Tehran, Iran) every 60

seconds. There was 5 to 15-minutes break between each spit depending on the participant's status. The samples were stored at 0 to 5 °C, and centrifuged (4000 rpm;Sigma, Germany) at 4 °C for 10 minutes. By using Pasteur Pipette (ISO-Lab, Germany), the supernatant was collected in 2-mL tubes (IsanMed, Tehran, Iran) and froze at -70 °C till used.

### *2.4. Measurement method*

The experiments were carried out by a blind skilled technician. The TAC was measured through fluorescence recovery after photobleaching technique. This method is based on the ability of saliva to recover ferric ions to the ferrite in the presence of a substance called 2,4,6-Tri (2-pyridyl)-s-triazine (TPTZ), which is used as a reagent, resulting in a blue complex of TPTZ-Fe with a maximum absorption of 593 nm. The amount of salivary oxidation/reduction capacity was measured through increasing the concentration of TPTZ-Fe blue complex up to 593 nm by spectrophotometer.

### *2.5. Statistical analysis*

Data were analyzed by using SPSS software (version 18, SPSS Inc., Chicago, USA) through repeated measures ANOVA, independent sample t-test, and covariate test (α=0.05).

## 3. Results

The participants' mean (±standard deviation) age was 38.46 (±13.68) in the non-smoker group and 44.63 (±15.98) years in the smoker group, with no significant difference between the two groups (P=0.214).

As displayed in Table 1, the mean salivary TAC of smokers vitamin E-placebo group (first vitamin E and then placebo) increased after using both vitamin (0.06±0.91U/mL) and placebo (0.025±0.089U/mL). The difference was statistically significant between the two phases (P=0.017). In smokers placebo-vitamin E group, the mean salivary TAC decreased in the placebo phase (-0.021±0.103 U/mL) and increased after using vitamin E (0.039±0.107 U/mL). The difference between the two phases was statistically significant (P<0.001) (Figure 1).

The mean salivary TAC of non-smokers vitamin E-placebo group increased after using both vitamin E (0.059±0.131U/mL) and placebo (0.053±0.129U/mL). However, the difference was not statistically significant between the two phases (P=0.791). The salivary TAC in non-smokers placebo-vitamin Egroup decreased after the placebo phase (-0.015±0.121 U/mL) and increased by using vitamin E (0.055±0.120 U/mL). The difference was statistically significant between the two phases (P<0.001) (Figure 2). Table 2 compares the TAC changes in each phase with the baseline (Δvitamin E and Δplacebo). Vitamin E increased the salivary TAC level in both groups.

## 4. Discussion

Findings of the present study approved the effect of vitamin E on the total antioxidant capacity of saliva in smokers and non-smokers. Vitamin E is a lipid-soluble antioxidant vitamin that prevents the tissue damage and pre-cancerous oral lesions by neutralizing free radicals (30), improves the function of salivary glands against ionizing radiation (26), increases the salivation and amelioratesxerostomia in patients with Sjögren syndrome (31). Saliva creates a defensive barrier against free radicals and oxidative stress (24). Cigarette smoke elevates the salivary superoxide dismutase activ-

Table 1. Mean total antioxidant capacity in smokers and non-smokers in the three phases.

| Groups | Subgroups | Baseline | Phase I | Phase II | Δvita | Δplaa | *P |
|---|---|---|---|---|---|---|---|
| Smokers | Vitamin-placebo | 0.084±0.073 | 0.144±0.089 | 0.109±0.083 | 0.060±0.091 | 0.025±0.089 | 0.017 |
| | Placebo-vitamin | 0.150±0.078 | 0.128±0.089 | 0.189±0.097 | 0.039±0.107 | -0.021±0.103 | <0.001 |
| **P value | | 0.001 | | | 0.419 | 0.064 | |
| Non-smokers | Vitamin-placebo | 0.136±0.104 | 0.199±0.119 | 0.186±0.125 | 0.509±0.131 | 0.053±0.129 | 0.791 |
| | Placebo-vitamin | 0.110±0.075 | 0.094±0.091 | 0.165±0.092 | 0.055±0.120 | -0.015±0.121 | <0.001 |
| **P value | | 0.259 | | | 0.905 | 0.038 | |

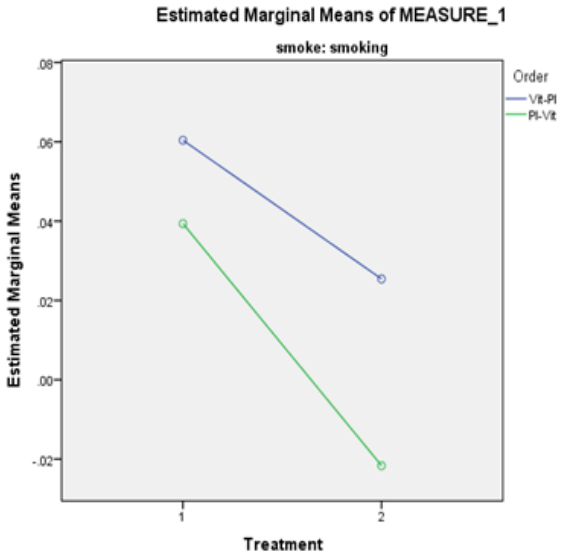


Figure 1. The Total antioxidant capacity changes of treatment phases in smokers.

ity. Maras *et al*. (32) encouraged everyone to take at least the vitamin E required for nonsmokers (15 mg α-T/d); although, smokers need even higher doses.

The results of the present study showed that consumption of vitamin E for three weeks increased the level of salivary TAC in both non-smokers and more significantly in smokers. Some studies investigated the effects of vitamin E supplements on the antioxidant-oxidative system balance; yet, the changes of the salivary TAC have not been studied so far. Naziroğlu and Cay (33) observed that intraperitoneal injection of vitamin E and selenium significantly protected the blood, liver and musclesagainst oxidative damages in rats. A similar study on rats by Dilsiz *et al*. (34) reported that combination of vitamin E and selenium moderated the oxidative stress caused by cigarette smoke. Schneider *et al*. (35) found that the free radical formation in plasma following the short-term supplementation with vitamins C and E decreased the number of micronuclei in blood lymphocytes, and thus, the DNA damage in smokers.

The current study used a crossover method to reduce or restrict the confounding effects of individual differences such as psychological factors. In this study, the baseline salivary TAC was not significantly different between the smokers and non-smokers. However, some contrasting studies reported lower salivary TAC in non-smokers than that in smokers with/without periodontal diseases (7, 36). In line with the present study, Azimi *et al*. (37) detected similar salivary TAC in smokers and non-smokers; although, glutathione level was higher in smokers than non-smokers.

While some studies used Cayman ELISA kit to measure the total antioxidant capacity of saliva (9, 24, 38) the present evaluated was done through the fluorescence recovery after photobleaching method. Bakhtiari *et al.* (9) detected that anti-oxidant supplementation (vitamin C) did not

Table 1. Changes of mean total antioxidant capacity compared with the baseline.

| Groups | Subgroups | Δvitamin a | Δplacebo a | *P |
|---|---|---|---|---|
| Smokers | Vitamin-placebo | 0.060±0.091 | 0.025±0.089 | 0.017 |
| | Placebo-vitamin | 0.039±0.107 | -0.021±0.103 | <0.001 |
| **P value | | 0.419 | 0.064 | |
| Non-smokers | Vitamin-placebo | 0.509±0.131 | 0.053±0.129 | 0.791 |
| | Placebo-vitamin | 0.055±0.120 | -0.015±0.121 | <0.001 |
| **P value | | 0.905 | 0.038 | |

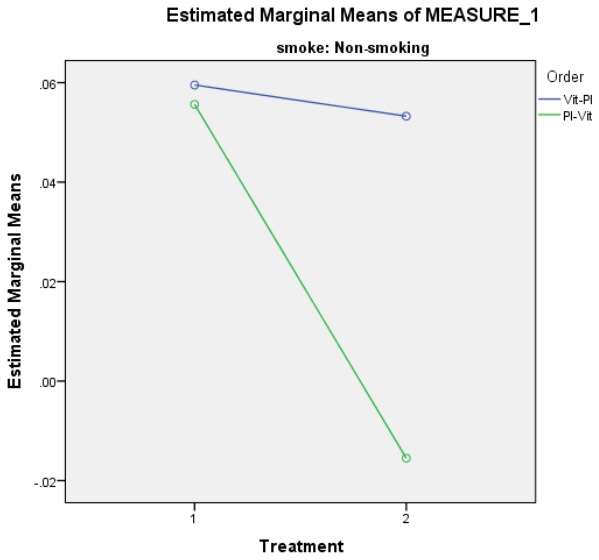


Figure 2. The Total antioxidant capacity changes of treatment phases in non-smokers.

significantly altered the salivary TAC in smokers, compared with the non-vitamin supplementation (placebo phase). They concluded that vitamin C supplementation reduced the stress, and that the smoke would not trigger oxidative salivation.

In the present study, the enrolled smokers had a history of five or more pack/year of smoking according to Mahmood *et al.* and Møller *et al*. (38-39). The effect of cigarette smoke on oral peroxidase activity in saliva causes a 70% loss of enzyme activity (40).

## 5. Conclusion

The findings of the present study revealed that vitamin E can increase the salivary total antioxidant capacity. However, it should be considered that these results were derived from a pilot study with a limited sample size. Hence, further studies with larger sample sizes are recommended.

## Acknowledgments

This article was based on the thesis by Dr Fatemeh Mohamadi. The authors would like to thank the Vice-Chancellery of Research of Shiraz University of Medical Sciences for financially supporting this research (Grant#8895175). This research also has an ethics code (IR.SUMS.REC.1395.209) and a clinical evaluation code (IRCT20110428006322N3). Appreciations are also expressed to Dr. Mehrdad Vossough of the Dental Research Development Center of the school of Dentistry for the statistical analysis, and Ms. Farzaneh Rasouli for proofreading, editing and improving the English structure of this manuscript.

## Conflict of Interest

None declared.

## References

1. Anand PS, Kamath KP, Shekar BR, Anil S. Relationship of smoking and smokeless tobacco use to tooth loss in a central Indian population. *Oral Health Prev Dent.* 2012;10(3):243-52. PMID: 23094267.

2. Balaji SM. Tobacco smoking and surgical healing of oral tissues: a review. *Indian J Dent Res.* 2008 Oct-Dec;19(4):344-8. doi: 10.4103/0970-9290.44540. PMID: 19075440.

3. Fresquez MR, Pappas RS, Watson CH. Establishment of toxic metal reference range in tobacco from US cigarettes. *J Anal Toxicol.* 2013 Jun;37(5):298-304. doi: 10.1093/jat/bkt021. Epub 2013 Apr 2. PMID: 23548667; PMCID: PMC4547355.

4. Haitami S, El Mokhlis K, Hamza M, Ben Yahya I. Detection of potentially malignant lesions and squamous cell carcinomas in consultation of surgical denstitry. *Tunis Med.* 2016 Jan;94(1):29-

33. PMID: 27525602.
5. Juntanong N, Siewchaisakul P, Bradshaw P, Vatanasapt P, Chen SL, Yen AM, et al. Prevalence and Factors Associated with Oral Pre-Malignant Lesions in Northeast Thailand. *Asian Pac J Cancer Prev.* 2016;17(8):4175-9. PMID: 27644680.
6. Kumar M, Nanavati R, Modi TG, Dobariya C. Oral cancer: Etiology and risk factors: A review. *J Cancer Res Ther.* 2016 Apr-Jun;12(2):458-63. doi: 10.4103/0973-1482.186696. PMID: 27461593.
7. 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. doi: 10.1002/biof.5520330205. PMID: 19346588.
8. Wagenknecht DR, BalHaddad AA, Gregory RL. Effects of Nicotine on Oral Microorganisms, Human Tissues, and the Interactions between Them. *Curr Oral Health Rep*. 2018;5(1):78-87. doi:10.1007/s40496-018-0173-3.
9. Bakhtiari S, Bigom Taheri J, Bakhshi M, Mortazavi H, Shah Hoseini A, Vahid Dastjerdi E, Azimi S. Effect of vitamin C on salivary total antioxidant capacity in smokers. *Iran J Pharm Res.* 2012 Fall;11(4):1045-9. PMID: 24250535; PMCID: PMC3813165.
10. Nagler RM, Reznick AZ. Cigarette smoke effects on salivary antioxidants and oral cancer--novel concepts. *Isr Med Assoc J*. 2004 Nov;6(11):691-4. PMID: 15562808.
11. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev*. 2010;4(8):118-126. doi:10.4103/0973-7847.70902
12. Peluso I, Raguzzini A. Salivary and Urinary Total Antioxidant Capacity as Biomarkers of Oxidative Stress in Humans. *Patholog Res Int.* 2016;2016:5480267. doi: 10.1155/2016/5480267. Epub 2016 Feb 7. PMID: 26966611; PMCID: PMC4761395.
13. Rezaie A, Ghorbani F, Eshghtork A, Zamani MJ, Dehghan G, Taghavi B, et al. Alterations in salivary antioxidants, nitric oxide, and transforming growth factor-beta 1 in relation to disease activity in Crohn's disease patients. *Ann N Y Acad Sci.* 2006 Dec;1091:110-22. doi: 10.1196/annals.1378.060. PMID: 17341608.
14. Hegde AM, Raj K, Shetty S. Relation of caries status on the salivary total antioxidant levels in asthmatic children. *Contemp Clin Dent.* 2012;3(4):402-405. doi:10.4103/0976-237X.107426
15. Karlík M, Valkovič P, Hančinová V, Krížová L, Tóthová Ľ, Celec P. Markers of oxidative stress in plasma and saliva in patients with multiple sclerosis. *Clin Biochem.* 2015 Jan;48(1-2):24-8. doi: 10.1016/j.clinbiochem.2014.09.023. Epub 2014 Oct 7. PMID: 25304914.
16. Zargari M, Arab H, Ghafouri Z. Evaluation of the Salivary Total Antioxidant Capacity and Lipid Peroxidation Status in Type 2 Diabetes Mellitus Patients. *Iran J Diabetes Obes.* 2018;10(2).
17. Ron' GI, Shmeleva LT, Klein AV, Iashkova EIu. Perekisnoe okislenie lipidov i sostoianie bazal'noĭ membrany atsinarnykh kletok malykh sliunnykh zhelez u bol'nykh sindromom Shegrena [Lipid peroxidation and the status of the basal membrane of the acinar cells in the minor salivary glands of patients with Sjögren's syndrome]. *Stomatologiia (Mosk)*. 1992 Mar-Apr;(2):23-6. Russian. PMID: 1412534.
18. Pandey P, Reddy NV, Rao VA, Saxena A, Chaudhary CP. Estimation of salivary flow rate, pH, buffer capacity, calcium, total protein content and total antioxidant capacity in relation to dental caries severity, age and gender. *Contemp Clin Dent.* 2015 Mar;6(Suppl 1):S65-71. doi: 10.4103/0976-237X.152943. PMID: 25821379; PMCID: PMC4374323.
19. TTartaglia GM, Gagliano N, Zarbin L, Tolomeo G, Sforza C. Antioxidant capacity of human saliva and periodontal screening assessment in healthy adults. *Arch Oral Biol.* 2017 Jun;78:34-38. doi: 10.1016/j.archoralbio.2017.02.003. Epub 2017 Feb 5. PMID: 28189883.
20. Tvarijonaviciute A, Aznar-Cayuela C, Rubio CP, Tecles F, Ceron JJ, López-Jornet P. Salivary Antioxidant Status in Patients with Oral Lichen Planus: Correlation with Clinical Signs and Evolution during Treatment with Chamaemelum nobile. *Biomed Res Int*. 2018 May 27;2018:5187549. doi: 10.1155/2018/5187549. PMID: 29992150; PMCID: PMC5994308.
21. Miricescu D, Totan A, Radulescu R, Calenic B, Greabu M. Salivary and serum biomarkers of oxidative stress in patients with oral premalignant lesions. *Free Radic Biol Med*. 2016(96):S56.

doi:10.1016/j.freeradbiomed.2016.04.120.
22. Agha-Hosseini F, Mirzaii-Dizgah I, Farmanbar N, Abdollahi M. Oxidative stress status and DNA damage in saliva of human subjects with oral lichen planus and oral squamous cell carcinoma. *J Oral Pathol Med*. 2012 Nov;41(10):736-40. doi: 10.1111/j.1600-0714.2012.01172.x. Epub 2012 May 15. PMID: 22582895.
23. Azimi S, Mansouri Z, Bakhtiari S, Tennant M, Kruger E, Rajabibazl M, et al. Does green tea consumption improve the salivary antioxidant status of smokers? *Arch Oral Biol.* 2017 Jun;78:1-5. doi: 10.1016/j.archoralbio.2017.02.002. Epub 2017 Feb 3. PMID: 28189030.
24. 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 Fall;9(4):281-4. doi: 10.15171/joddd.2015.049. Epub 2015 Dec 30. PMID: 26889367; PMCID: PMC4753039.
25. 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 Feb;65(2):164-9. PMID: 25842552.
26. Chitra S, Balasubramaniam M, Hazra J. Effect of α-tocopherol on salivary reactive oxygen species and trace elements in oral submucous fibrosis. *Ann Clin Biochem.* 2012 May;49(Pt 3):262-5. doi: 10.1258/acb.2011.011050. Epub 2012 Feb 15. PMID: 22337705.
27. Yasueda A, Urushima H, Ito T. Efficacy and Interaction of Antioxidant Supplements as Adjuvant Therapy in Cancer Treatment: A Systematic Review. *Integr Cancer Ther.* 2016 Mar;15(1):17-39. doi: 10.1177/1534735415610427. Epub 2015 Oct 26. PMID: 26503419; PMCID: PMC5736082.
28. Bruno RS, Traber MG. Cigarette smoke alters human vitamin E requirements. *J Nutr*. 2005 Apr;135(4):671-4. doi: 10.1093/jn/135.4.671. PMID: 15795416.
29. Cook-Mills JM. Nutrition and Functional Foods for Healthy Aging. Chicago: Elsevier; 2017. p. 167-88.
30. Chitra S, Shyamala Devi CS. Effects of radiation and alpha-tocopherol on saliva flow rate, amylase activity, total protein and electrolyte levels in oral cavity cancer. *Indian J Dent Res.* 2008 Jul-Sep;19(3):213-8. doi: 10.4103/0970-9290.42953. PMID: 18797097.
31. Upadhyaya A, Zhou P, Meng Z, Wang P, Zhang G, Jia Q, et al. Radioprotective effect of vitamin E on salivary glands after radioiodine therapy for differentiated thyroid cancer: a randomized-controlled trial. Nucl Med Commun. 2017 Nov;38(11):891-903. doi: 10.1097/MNM.0000000000000727. PMID: 28806348.
32. Maras JE, Bermudez OI, Qiao N, Bakun PJ, Boody-Alter EL, Tucker KL. Intake of alpha-tocopherol is limited among US adults. *J Am Diet Assoc*. 2004 Apr;104(4):567-75. doi: 10.1016/j.jada.2004.01.004. PMID: 15054342.
33. Naziroğlu M, Cay M. Protective role of intraperitoneally administered vitamin E and selenium on the antioxidative defense mechanisms in rats with diabetes induced by streptozotocin. *Biol Trace Elem Res*. 2001 Feb;79(2):149-59. doi: 10.1385/BTER:79:2:149. PMID: 11330521.
34. Dilsiz N, Olcucu A, Cay M, Naziroglu M, Cobanoglu D. Protective effects of selenium, vitamin C and vitamin E against oxidative stress of cigarette smoke in rats. *Cell Biochem Funct.* 1999 Mar;17(1):1-7. doi: 10.1002/(SICI)1099-0844(199903)17:1<1::AID-CBF800>3.0.CO;2-5. PMID: 10191503.
35. Schneider M, Diemer K, Engelhart K, Zankl H, Trommer WE, Biesalski HK. Protective effects of vitamins C and E on the number of micronuclei in lymphocytes in smokers and their role in ascorbate free radical formation in plasma. *Free Radic Res.* 2001 Mar;34(3):209-19. doi: 10.1080/10715760100300201. PMID: 11264897.
36. Guentsch A, Preshaw PM, Bremer-Streck S, Klinger G, Glockmann E, Sigusch BW. Lipid peroxidation and antioxidant activity in saliva of periodontitis patients: effect of smoking and periodontal treatment. *Clin Oral Investig*. 2008 Dec;12(4):345-52. doi: 10.1007/s00784-008-0202-z. Epub 2008 May 29. PMID: 18509684.
37. Azimi S MZ, Bakhtiari S, Namdari M,. Different effect of green tea consumption on salivary antioxidant status in light versus heavy smokers. *Journal of Dental School*. 2018;36(3):81-5. Corpus ID: 78088089.
38. Mahmood IH, Abdullah KS, Othman SH. The total antioxidant status in cigarette smoking individuals. *Med J Basrah Univ.* 2007;25(1):46-50.
39. Møller P, Viscovich M, Lykkesfeldt J, Loft S, Jensen A, Poulsen HE. Vitamin C supple-

mentation decreases oxidative DNA damage in mononuclear blood cells of smokers. *Eur J Nutr.* 2004 Oct;43(5):267-74. doi: 10.1007/s00394-004-0470-6. Epub 2004 Jan 6. PMID: 15309445.

40. Klein I, Nagler RM, Toffler R, van Der Vliet A, Reznick AZ. Effect of cigarette smoke on oral peroxidase activity in human saliva: role of hydrogen cyanide. *Free Radic Biol Med.* 2003 Dec 1;35(11):1448-52. doi: 10.1016/j.freeradbiomed.2003.08.001. PMID: 14642392.