# Serum Selenium, Vitamin A, and Vitamin E Levels of Healthy Individuals in High- and Low-Risk Areas of Esophageal Cancer

Mehdi Darbani Torshizi[1], Ommolbanin Younesian[2], Maryam Aboomardani[3], Gholamreza Roshandel[1], Sara Hosseinzadeh[4], Seyedeh Somayeh Hosseini Alarzi[5], Hamidreza Joshaghani[4*]

[1]Golestan Research Center of Gastroenterology and Hepatology, Golestan University of Medical Sciences, Gorgan, Iran
[2]School of Medicine, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran
[3]Department of Nutrition, Golestan University of Medical Sciences, Gorgan, Iran
[4]Laboratory Sciences Research Center, Golestan University of Medical Sciences, Gorgan, Iran
[5]Department of Medical Biotechnology, School of Allied Medical Sciences, Iran University of Medical Sciences, Tehran, Iran

[*]**Corresponding Author:**
Hamidreza Joshaghani, MD
Laboratory Science Research Center, Golestan University of Medical Sciences, 60 Kola Road, Falsafi Building.
Telefax: +98 17 32450093
Email: joshaghani@goums.ac.ir

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## Abstract

**Background:**
Esophageal cancer is one of the main causes of cancer mortality in the world. Golestan province, in the northern part of Iran, has the highest esophageal cancer rate in the world. The north and south districts of Golestan province can be classified as low and high-risk areas for esophageal cancer. One of the potential risk factors for esophageal cancer in this population is a nutrient-deficient diet. Dietary antioxidant compounds such as selenium, vitamin E, vitamin A, and β-carotene are reactive oxygen species (ROC) scavengers that play a key role in cellular responses to oxidative stress and preventing DNA damage. This study aims to compare the serum levels of selenium, vitamin E, and vitamin A in healthy individuals in high and low-risk areas of esophageal cancer.

**Methods:**
This study is a population of 242 healthy individuals. Serum selenium levels were assessed by atomic absorption spectroscopy. Vitamin E and A were assessed by reversed-phase high-performance liquid chromatography.

**Results**:
Vitamin E levels of healthy individuals in high-risk areas were significantly lower than in low-risk areas, while there was no significant difference between the selenium and vitamin A levels of healthy individuals in high-risk areas and low-risk areas. Also, there was no significant difference between selenium, vitamin E, and vitamin A levels in urban and rural areas and men and women in Golestan province.

**Conclusion:**
High levels of selenium with lower levels of vitamin E, along with other risk factors, may be associated with esophageal squamous cell carcinoma in high-risk areas of Golestan province.

**Keywords:**
Esophageal cancer, Trace element, Vitamin, Antioxidant

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## Introduction

Esophageal cancer (EC) is one of the main causes of cancer mortality in the world. In 2018, there were 572 000 (seventh rank) new cases of EC and 509 000 (sixth rank) deaths due to EC. The incidence and mortality rate of EC is higher in men than in women.[1] Esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) are the two

main histological types of EC. In the so-called Asian EC belt from northern China through central Asia to northern Iran, approximately 90% of all incident EC cases are ESCC.[2,3] Golestan province of Iran has the highest ESCC rate worldwide.[4] The north and south districts of Golestan province can be classified as low and high-risk areas of EC (Figure 1).[5] In Gonbad and Kalaleh counties, located in the eastern area of the Golestan province, the incidence rate of ESCC is higher than in the other counties.[6] Potential risk factors for ESCC in this population are nutrient deficient diet, consumption of hot tea, poor oral health, indoor air pollution, exposure to polycyclic aromatic hydrocarbons, and lack of access to piped water.[7] The human dietary regime is a combination of oxidants and antioxidants, and the gastrointestinal tract is thought to be the main site of antioxidant action.[8] Oxidative stress, by stimulating gene mutation and pro-oncogenic signaling pathways, could play an important role in the initiation and promotion of cancer. Dietary antioxidant compounds such as selenium, vitamin E, and β-carotene are reactive oxygen species (ROS) scavengers that play a key role in cellular responses to oxidative stress.[9,10]

Selenium is an essential trace element for human health, which may play a protective role against some cancers.[11,12] Selenium, as part of the amino acid selenocysteine, is required for the production of selenoproteins, such as antioxidant enzymes. Under oxidative stress, antioxidant enzymes protect cells from the toxic effects of free radicals.[13] Vitamin E is a hydrophobic lipid-soluble molecule including eight isoforms: α-, β-, γ-, and δ-tocopherols and α-, β-, γ-, and δ-tocotrienols. Vitamin E acts as a chain-breaking antioxidant and has been shown to have anti-cancer properties.[14] In many studies, lower vitamin E intake or nutritional status was associated with an increased risk of various types of cancer.[15] A meta-analysis of 12 articles reported that higher dietary vitamin E intake was correlated with a lower risk of EC, especially for ESCC.[16]

Vitamin A, a fat-soluble micronutrient, cannot be produced by the human body and must be provided from the diet in the form of preformed vitamin A and provitamin A carotenoids.[17] Vitamin A derivatives play an important role in cell differentiation, proliferation, and apoptosis.[18] A meta-analysis of 14 publications suggested that intake of vitamin A may reduce the EC risk.[19] In the high-risk area of Golestan province, daily vitamin A intake was lower than the lowest threshold intakes in rural women.[20]

Although in the past three decades, age-standardized incidence and mortality rates of EC have decreased globally, absolute numbers of new cases and deaths of EC have increased with the population growth and aging.[4] In this study, we evaluated serum selenium, vitamin A, and vitamin E levels of healthy individuals in two populations living in Golestan province, Kalaleh from high-risk areas and Kordkuy from low-risk areas.

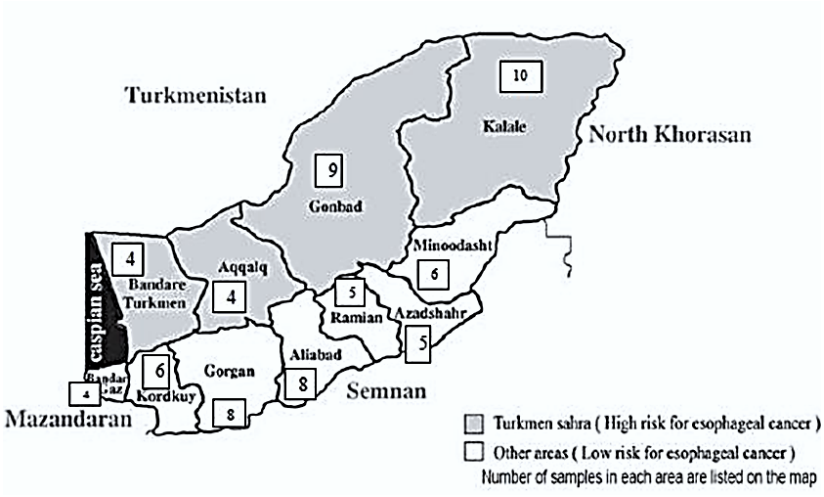


**Figure 1.** Map of the Golestan province shows the high-and low-risk areas of esophageal cancer

## Material and Methods

### Study design

In this cross-sectional study, 242 serum samples were selected randomly from urban and rural individuals who had been recruited for the research project on non-communicable diseases in the Golestan province. Demographic information from the samples was extracted using questionnaires available at Golestan Gastroenterology and Liver Research Center. After being matched in terms of age and sex, the samples were divided into two groups: high-risk area (Kalaleh) and low-risk area (Kordkuy). Exclusion criteria in this study were people with various types of cancer, heart disease, kidney and gastrointestinal diseases, and people taking dietary supplements.

### Serum selenium, vitamin E, and vitamin A analysis

To measure serum selenium, the atomic absorption spectroscopy method was used by graphite furnace using YOUNG LIN AAS 8020 model equipped with graphite furnace, along with deuterium lamp modifier, to eliminate background absorption. According to the method described by Jacobson and Lockitch, serum samples were diluted with reducing agents, including ascorbic acid, Triton X-100, and Antifoam B emulsion. Palladium chloride was also added as a matrix modifier.[21] Concentrations of vitamin E and vitamin A were analyzed by Reversed-Phase HPLC model KNAUER V7057-3 10/2003, Smartline Pump 1000 V7603 10/2005, Smartline UV Detector 2500 V7604 10/2003, Smartline manager 5000 V7602 10/2003, Diaphragm Vacuum Pump Model: GM-0.50 6/2008. SN: 0126, and Column chromatography C18. The mobile phase consisted of methanol-water (95:5, v/v). The flow rate was set at 1.5 mL/min. The wavelength range scanned was 292–325 nm. The total run time was 20 min.

### Statistical analysis

In this study, statistical analysis was performed using SPSS software 16. Data on sex and residence place were expressed as numbers and percentages. Age, the value of serum selenium, vitamin E, and vitamin A levels were expressed as means and the respective standard deviations (SD). T test was used to compare the two groups. Spearman's correlation test was used for the correlation assessment. $P$ values $<0.05$ were considered statistically significant.

## Results

### Baseline characteristics

A total of 242 healthy subjects with a mean age of 51.7±13.8 years were included in this study. 47.5% of the subjects (a total of 115) were from the high-risk area (Kalaleh), and 52.5% of the subjects (a total of 127) were from the low-risk area (Kordkuy). The baseline characteristics of subjects are presented in Table 1.

### Selenium analysis

The mean serum selenium level in high and low-risk areas was 146.8 (±40.9) and 158.8 (40.2), respectively. The difference in selenium levels between the two groups was not significant ($P=0.13$). In both areas, selenium levels were lower in men than in women, but this difference was not significant ($P>0.05$). No statistically significant correlation was found between age and serum selenium level in high and low-risk areas ($r=0.268$; $P>0.05$). Also, there was no significant difference between the mean serum selenium levels in urban and rural areas ($P=0.70$) (Table 2).

### Vitamin A analysis

The mean serum vitamin A level in the high and low-risk areas was 57.5 (±15.2) and 66.1 (±21.3), respectively. The difference in vitamin A levels between the two groups was not significant ($P=0.07$). In both areas, vitamin A levels were higher in men than in women, but this difference was not significant

**Table 1.** Baseline characteristics of the subject in high- and low-risk areas in Golestan province

| Variables | High-risk area (n=115) | Low-risk area (n=127) |
|---|---|---|
| Age (years) | 49.7 (±14.4) | 51.6 (±13.2) |
| Gender | | |
| Male | 42 (36.5) | 54 (42.5) |
| Female | 73 (63.5) | 73 (57.5) |
| Residence place | | |
| Urban | 43 (37.4) | 74 (58.3) |
| Rural | 72 (62.6) | 53 (41.7) |

SD: standard deviation. Data were expressed as mean±SD, or numbers and percentages.

**Table 2.** Comparison of serum selenium, vitamin A, and vitamin E levels in high- and low-risk areas in relation to sex and residence

| | Serum Se (µg/L), Mean±SD | | | Serum vitamin A (µg/L), Mean±SD | | | Serum vitamin E, (µg/L), Mean±SD | | |
|---|---|---|---|---|---|---|---|---|---|
| | High-risk area | Low-risk area | *P* value | High-risk area | Low-risk area | *P* value | High-risk area | Low-risk area | *P* value |
| Gender | | | | | | | | | 0.01* |
| Total | 146.8 (±40.9) | 158.8 (±40.2) | 0.13 | 57.5 (±15.2) | 66.1 (±21.3) | 0.07 | 4.1 (±1.7) | 5.2 (±2) | |
| Male | 141.7 (±43.7) | 153.2 (±35.3) | | 60.3 (±16.7) | 69.4 (±19.5) | | 3.9 (±1.5) | 4.7 (±1.6) | |
| Female | 149.7 (±43.7) | 155.9 (±43.6) | | 55.7 (±14.2) | 63.6 (±22.5) | | 4.1 (±1.7) | 5.4 (±2.2) | |
| *P* value | 0.31 | 0.71 | | 0.45 | 0.45 | | 0.77 | 0.3 | |
| Residence place | | | | | | | | | |
| Urban | 150.6 (±50.1) | 149.8 (±32.5) | 0.98 | 59.6 (±14.5) | 69.4 (±22.3) | 0.55 | 4.6 (±1.9) | 5.2 (±1.8) | 0.71 |
| Rural | 144.4 (±33.8) | 162.1 (±48.3) | 0.63 | 56.2 (±15.8) | 61.4 (±15.5) | 0.70 | 3.6 (±1.4) | 5.1 (±2.2) | 0.37 |
| *P* value | 0.45 | 0.09 | | 0.56 | 0.3 | | 0.13 | 0.87 | |

SD, standard deviation. Data were expressed as mean±SD.
*Significant group difference at $P<0.05$.

($P>0.05$). No statistically significant correlation was found between age and serum vitamin A levels in high and low-risk areas ($r=0.128$; $P>0.05$). Also, there was no significant difference between the mean serum vitamin A levels in urban and rural areas ($P>0.05$, Table 2).

### Vitamin E analysis

Mean serum levels of vitamin E in high-risk areas were significantly lower than in low-risk areas ($P=0.01$). In both areas, vitamin E levels were lower in men than in women, but this difference was not significant ($P>0.05$). No statistically significant correlation was found between age and serum vitamin E levels in high and low-risk areas ($r=0.178$; $P>0.05$). Also, there was no significant difference between the mean serum vitamin E levels in urban and rural areas ($P>0.05$, Table 2).

## Discussion

The present study showed that vitamin E levels of healthy subjects in high-risk areas (4.1 µg/mL) were significantly lower than in low-risk areas (5.2 µg/mL), while there was no significant difference between the selenium and vitamin A levels of healthy subjects in high-risk areas and low-risk areas. Also, there was no significant difference between selenium, vitamin E, and vitamin A levels in urban and rural areas and men and women in Golestan province. In this study, serum vitamin A levels were 57.5 µg/dL and 66.1 µg/dL in high and low-risk areas, respectively, and serum vitamin A levels in both areas were not significantly different between men and women, as well as in urban and rural areas, while the results of other studies in healthy participants in the high-risk areas of Golestan showed severe vitamin A deficiency intake in women and rural dwellers.[20,22]

The results of our study showed that serum selenium levels were high in high-risk areas (146.8 µg/L) and low-risk areas (158.8 µg/L) and did not differ significantly. According to the selenium content in the soil and drinking water of each area, selenium status is different in various countries of the world and corresponds to its intake.[23] A study conducted in Golestan province, a high-risk area for the incidence of ESCC, showed that the median serum selenium concentration was 155 µg/L (141-173) in this population and above the level required to saturate serum selenoproteins.[24] Also, total selenium in soil, grain, loess, sediments, and rice seeds was higher in high-risk areas of Golestan province than in low-risk areas of Golestan province. These studies suggest that high levels of selenium in this area may play a possible role in EC pathogenesis.[25,26,27,28] The case-control study nested within the Golestan Cohort reported that toenail selenium concentrations did not differ significantly between cases of ESSC in high-risk areas and healthy subjects, and there was no association between toenail selenium concentrations and the ESCC risk in this population.[29] Another study in Golestan province showed that in the high-risk areas

of EC, the association between selenium and the risk of developing ESSC was non-linear and U-shaped.[30] In East Africa, a high-risk area for the incidence of ESCC, there was also a significant positive association between serum selenium level and the occurrence of esophageal squamous dysplasia.[31] Also, studies show that long-term consumption of inorganic selenium in drinking water with a concentration range of 8-10 µg/L, increases the risk of cancer, especially cancers of the melanoma, pharynx, urinary tract, and lymphoid tissue.[32]

Although selenium is commonly known as an antioxidant, it becomes toxic at a high dose depending on chemical species and may even increase carcinogenesis.[33] Some chemical species of selenium may react with thiols in glutathione to form disulfide bonds, thus indirectly increasing the production of superoxide and hydrogen peroxide.[34] Intracellular oxidative stress may promote DNA damage and oncogenic mutations, cytotoxicity, and genotoxicity.[35] High selenium exposure may decrease global DNA methylation through a decreased DNA methyltransferase activity.[36] Genome-wide hypomethylation plays a key role in the instability of the genome and carcinogenesis. Hypomethylation of long interspersed nucleotide element-1 (LINE-1), a good indicator of genome-wide hypomethylation, may be an early event during the carcinogenesis of ESCC.[37]

The association between cancer risk and vitamin E has been investigated in various epidemiological studies.[38] Oxidative stress has been implied in various cancer pathogenesis, especially gastrointestinal cancers. Vitamin E isoforms sweep ROC due to the presence of a phenyl group in their chromanol ring, hence lowering their radical damaging abilities and inhibiting oxidative DNA damage.[39,40] Transcription factor Nrf2 regulates the induction of antioxidant enzymes. Natural forms of vitamin E, especially γ-tocopherol stimulate Nrf2, which induces gene expression of several antioxidant enzymes like superoxide dismutase, catalase, and glutathione peroxidase.[41] Vitamin E isoforms inhibit cyclooxygenase-2, which is necessary for prostaglandin synthesis. Many investigations have reported the relationship between overexpressed cyclooxygenase-2 in gastrointestinal cancers, such as EC.[42] The relationship between vitamin E and cancer can be significantly modified by selenium, and vitamin E has a strong synergism with selenium on cancer risk. A Nested case control study reported that high selenium (median > 83.7 mg/L) levels with higher levels of vitamin E were significantly related to a lower risk of total cancer, gastrointestinal cancer, and EC, while low selenium (< 83.7 mg/L) levels with higher vitamin E levels were significantly associated with a higher risk of total cancer and non-gastrointestinal cancer.[43]

Vitamin E and vitamin A can inhibit the formation of N-nitroso compounds, which are possible carcinogens in the esophagus.[44] The production of N-nitroso compounds is the result of the reaction of nitrates with amides and amines. Nitrates in un-piped water are converted to nitrites by oral bacteria. In Golestan province, poor oral health and drinking un-piped water increase exposure to N-nitroso compounds.[7] Furthermore, in N-Nitrosomethylbenzylamine-treated rats, vitamin E and selenium supplementation reduced 8-hydroxy-2′-deoxyguanosine in each category of lesions.[45] Vitamin E and selenium may suppress esophageal carcinogenesis of N-Nitrosomethylbenzylamine-treated rats by blocking the activation of the nuclear factor-kappa B pathway.[46] A study in esophagoduodenal anastomosis rats showed that selenium supplementation with a dose of 1.7 mg/kg promoted EAC, while selenium supplementation along with vitamin E supplementation inhibited carcinogenesis.[47]

In summary, we found low serum vitamin E levels in high-risk areas. Serum selenium levels were high in both areas and above the level required to saturate serum selenoproteins. Vitamin E has a strong synergism with selenium on cancer risk. The results of the Golestan cohort study suggest that ESSC in this region is a multifactorial disease and requires a combination of exposures for its progression.[7] As a result, this study hypothesizes that high levels of selenium with lower levels of vitamin E, along with other risk factors, probably may be associated with ESSC in high-risk areas of Golestan province. Further large-scale cross-sectional, case-control, and cohort studies are necessary to confirm this hypothesis.

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## Author Contributions

MDT, SSHA and SH collected the data. OY wrote the paper. MA conceived and designed the analysis. GR performed the analysis. HJ wrote the paper, conceived and designed the analysis

## Availability of Data and Materials

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

## Conflict of Interest

The authors declare no conflict of interest related to this work.

## Ethics Approval

This study was approved by grant number 921120187 from Golestan Research Center of Gastroenterology and Hepatology at Golestan University of Medical Sciences. All procedures performed were in agreement with the principles of the Declaration of Helsinki (1964) and later amendments. Informed consent was obtained from all individual participants included in the study.

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## References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;68(6):394-424. doi: 10.3322/caac.21492
2. Abnet CC, Arnold M, Wei WQ. Epidemiology of esophageal squamous cell carcinoma. *Gastroenterology* 2018;154(2):360-73. doi: 10.1053/j.gastro.2017.08.023
3. Smyth EC, Lagergren J, Fitzgerald RC, Lordick F, Shah MA, Lagergren P, et al. Oesophageal cancer. *Nat Rev Dis Primers* 2017;3:17048. doi: 10.1038/nrdp.2017.48
4. GBD 2017 Oesophageal Cancer Collaborators. The global, regional, and national burden of oesophageal cancer and its attributable risk factors in 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Gastroenterol Hepatol* 2020;5(6):582-97. doi: 10.1016/s2468-1253(20)30007-8
5. Roshandel G, Sadjadi A, Aarabi M, Keshtkar A, Sedaghat SM, Nouraie SM, et al. Cancer incidence in Golestan province: report of an ongoing population-based cancer registry in Iran between 2004 and 2008. *Arch Iran Med* 2012;15(4):196-200.
6. Pourshams A, Khademi H, Fazeltabar Malekshah A, Islami F, Nouraei M, Sadjadi AR, et al. Cohort profile: the Golestan Cohort Study--a prospective study of oesophageal cancer in northern Iran. *Int J Epidemiol* 2010;39(1):52-9. doi: 10.1093/ije/dyp161
7. Sheikh M, Poustchi H, Pourshams A, Etemadi A, Islami F, Khoshnia M, et al. Individual and combined effects of environmental risk factors for esophageal cancer based on results from the Golestan Cohort Study. *Gastroenterology* 2019;156(5):1416-27. doi: 10.1053/j.gastro.2018.12.024
8. Halliwell B, Zhao K, Whiteman M. The gastrointestinal tract: a major site of antioxidant action? *Free Radic Res* 2000;33(6):819-30. doi: 10.1080/10715760000301341
9. Gorrini C, Harris IS, Mak TW. Modulation of oxidative stress as an anticancer strategy. *Nat Rev Drug Discov* 2013;12(12):931-47. doi: 10.1038/nrd4002
10. Noaman E, Zahran AM, Kamal AM, Omran MF. Vitamin E and selenium administration as a modulator of antioxidant defense system: biochemical assessment and modification. *Biol Trace Elem Res* 2002;86(1):55-64. doi: 10.1385/bter:86:1:55
11. Kuria A, Fang X, Li M, Han H, He J, Aaseth JO, et al. Does dietary intake of selenium protect against cancer? A systematic review and meta-analysis of population-based prospective studies. *Crit Rev Food Sci Nutr* 2020;60(4):684-94. doi: 10.1080/10408398.2018.1548427
12. Abedi J, Vakili Saatloo M, Nejati V, Hobbenaghi R, Tukmechi A, Nami Y, et al. Selenium-enriched *Saccharomyces cerevisiae* reduces the progression of colorectal cancer. *Biol Trace Elem Res* 2018;185(2):424-32. doi: 10.1007/s12011-018-1270-9
13. Adeniran SO, Zheng P, Feng R, Adegoke EO, Huang F, Ma M, et al. The antioxidant role of selenium via GPx1 and GPx4 in LPS-induced oxidative stress in bovine endometrial cells. *Biol Trace Elem Res* 2022;200(3):1140-55. doi: 10.1007/s12011-021-02731-0
14. Ghosh N, Das A, Khanna S. Vitamin E: tocopherols and tocotrienol and their role in health and disease. In: Prasad AS, Brewer GJ, eds. *Essential and Toxic Trace Elements and Vitamins in Human Health*. Academic Press; 2020. p. 283-93. doi: 10.1016/b978-0-12-805378-2.00020-6
15. Yang CS, Luo P, Zeng Z, Wang H, Malafa M, Suh N. Vitamin E and cancer prevention: studies with different forms of tocopherols and tocotrienols. *Mol Carcinog* 2020;59(4):365-89. doi: 10.1002/mc.23160
16. Cui L, Li L, Tian Y, Xu F, Qiao T. Association between dietary vitamin E intake and esophageal cancer risk: an updated meta-analysis. *Nutrients* 2018;10(7):801. doi: 10.3390/nu10070801
17. Tanumihardjo SA, Russell RM, Stephensen CB, Gannon BM, Craft NE, Haskell MJ, et al. Biomarkers of nutrition for development (BOND)-vitamin A

review. *J Nutr* 2016;146(9):1816s-48s. doi: 10.3945/jn.115.229708
18. Doldo E, Costanza G, Agostinelli S, Tarquini C, Ferlosio A, Arcuri G, et al. Vitamin A, cancer treatment and prevention: the new role of cellular retinol binding proteins. *Biomed Res Int* 2015;2015:624627. doi: 10.1155/2015/624627
19. Li K, Zhang B. The association of dietary β-carotene and vitamin A intake on the risk of esophageal cancer: a meta-analysis. *Rev Esp Enferm Dig* 2020;112(8):620-6. doi: 10.17235/reed.2020.6699/2019
20. Islami F, Fazeltabar Malekshah A, Kimiagar M, Pourshams A, Wakefield J, Goglani G, et al. Patterns of food and nutrient consumption in northern Iran, a high-risk area for esophageal cancer. *Nutr Cancer* 2009;61(4):475-83. doi: 10.1080/01635580902803735
21. Jacobson BE, Lockitch G. Direct determination of selenium in serum by graphite-furnace atomic absorption spectrometry with deuterium background correction and a reduced palladium modifier: age-specific reference ranges. *Clin Chem* 1988;34(4):709-14. doi: 10.1093/clinchem/34.4.709
22. Fazeltabar Malekshah A, Kimiagar M, Pourshams A, Yazdani J, Kaiedi Majd S, Goglani G, et al. Vitamin deficiency in Golestan province, northern Iran: a high-risk area for esophageal cancer. *Arch Iran Med* 2010;13(5):391-4.
23. Stoffaneller R, Morse NL. A review of dietary selenium intake and selenium status in Europe and the Middle East. *Nutrients* 2015;7(3):1494-537. doi: 10.3390/nu7031494
24. Nouarie M, Pourshams A, Kamangar F, Sotoudeh M, Derakhshan MH, Akbari MR, et al. Ecologic study of serum selenium and upper gastrointestinal cancers in Iran. *World J Gastroenterol* 2004;10(17):2544-6. doi: 10.3748/wjg.v10.i17.2544
25. Keshavarzi B, Moore F, Najmeddin A, Rahmani F. The role of selenium and selected trace elements in the etiology of esophageal cancer in high risk Golestan province of Iran. *Sci Total Environ* 2012;433:89-97. doi: 10.1016/j.scitotenv.2012.04.033
26. Semnani S, Roshandel G, Zendehbad A, Keshtkar A, Rahimzadeh H, Abdolahi N, et al. Soils selenium level and esophageal cancer: an ecological study in a high risk area for esophageal cancer. *J Trace Elem Med Biol* 2010;24(3):174-7. doi: 10.1016/j.jtemb.2010.03.002
27. Rahimzadeh-Barzoki H, Joshaghani H, Beirami S, Mansurian M, Semnani S, Roshandel G. Selenium levels in rice samples from high and low risk areas for esophageal cancer. *Saudi Med J* 2014;35(6):617-20.
28. Younesian O, Younesian S, Hosseinzadeh S, Joshaghani HR. Association of selenium and risk of esophageal cancer: a review. *Med Lab J* 2019;14(1):1-9. doi: 10.29252/mlj.14.1.1
29. Hashemian M, Murphy G, Etemadi A, Poustchi H, Brockman JD, Kamangar F, et al. Toenail mineral concentration and risk of esophageal squamous cell carcinoma, results from the Golestan Cohort Study. *Cancer Med* 2017;6(12):3052-9. doi: 10.1002/cam4.1247
30. Hashemian M, Poustchi H, Abnet CC, Boffetta P, Dawsey SM, Brennan PJ, et al. Dietary intake of minerals and risk of esophageal squamous cell carcinoma: results from the Golestan Cohort Study. *Am J Clin Nutr* 2015;102(1):102-8. doi: 10.3945/ajcn.115.107847
31. Pritchett NR, Burgert SL, Murphy GA, Brockman JD, White RE, Lando J, et al. Cross sectional study of serum selenium concentration and esophageal squamous dysplasia in western Kenya. *BMC Cancer* 2017;17(1):835. doi: 10.1186/s12885-017-3837-9
32. Vinceti M, Vicentini M, Wise LA, Sacchettini C, Malagoli C, Ballotari P, et al. Cancer incidence following long-term consumption of drinking water with high inorganic selenium content. *Sci Total Environ* 2018;635:390-6. doi: 10.1016/j.scitotenv.2018.04.097
33. Vinceti M, Filippini T, Cilloni S, Crespi CM. The epidemiology of selenium and human cancer. *Adv Cancer Res* 2017;136:1-48. doi: 10.1016/bs.acr.2017.07.001
34. Ganther HE. Selenium metabolism, selenoproteins and mechanisms of cancer prevention: complexities with thioredoxin reductase. *Carcinogenesis* 1999;20(9):1657-66. doi: 10.1093/carcin/20.9.1657
35. Kalyanaraman B, Cheng G, Hardy M, Ouari O, Bennett B, Zielonka J. Teaching the basics of reactive oxygen species and their relevance to cancer biology: mitochondrial reactive oxygen species detection, redox signaling, and targeted therapies. *Redox Biol* 2018;15:347-62. doi: 10.1016/j.redox.2017.12.012
36. Jabłońska E, Reszka E. Selenium and epigenetics in cancer: focus on DNA methylation. A*dv Cancer Res* 2017;136:193-234. doi: 10.1016/bs.acr.2017.07.002
37. Hoshimoto S, Takeuchi H, Ono S, Sim MS, Huynh JL, Huang SK, et al. Genome-wide hypomethylation and specific tumor-related gene hypermethylation are associated with esophageal squamous cell carcinoma outcome. *J Thorac Oncol* 2015;10(3):509-17. doi: 10.1097/jto.0000000000000441
38. Jiang Q. Natural forms of vitamin E as effective agents for cancer prevention and therapy. *Adv Nutr* 2017;8(6):850-67. doi: 10.3945/an.117.016329
39. Jiang Q. Natural forms of vitamin E: metabolism, antioxidant, and anti-inflammatory activities and their role in disease prevention and therapy. *Free Radic Biol Med* 2014;72:76-90. doi: 10.1016/j.freeradbiomed.2014.03.035
40. Bartsch H, Nair J. Chronic inflammation and oxidative stress in the genesis and perpetuation of cancer:

role of lipid peroxidation, DNA damage, and repair. *Langenbecks Arch Surg* 2006;391(5):499-510. doi: 10.1007/s00423-006-0073-1

41. Abraham A, Kattoor AJ, Saldeen T, Mehta JL. Vitamin E and its anticancer effects. *Crit Rev Food Sci Nutr* 2019;59(17):2831-8. doi: 10.1080/10408398.2018.1474169
42. Nagaraju GP, El-Rayes BF. Cyclooxygenase-2 in gastrointestinal malignancies. *Cancer* 2019;125(8):1221-7. doi: 10.1002/cncr.32010
43. Wang J, Guo H, Lin T, Song Y, Zhang H, Wang B, et al. A nested case-control study on plasma vitamin E and risk of cancer: evidence of effect modification by selenium. *J Acad Nutr Diet* 2019;119(5):769-81. doi: 10.1016/j.jand.2018.11.017
44. Keszei AP, Goldbohm RA, Schouten LJ, Jakszyn P, van den Brandt PA. Dietary N-nitroso compounds, endogenous nitrosation, and the risk of esophageal and gastric cancer subtypes in the Netherlands Cohort Study. *Am J Clin Nutr* 2013;97(1):135-46. doi: 10.3945/ajcn.112.043885
45. Yang H, Fang J, Jia X, Han C, Chen X, Yang CS, et al. Chemopreventive effects of early-stage and late-stage supplementation of vitamin E and selenium on esophageal carcinogenesis in rats maintained on a low vitamin E/selenium diet. *Carcinogenesis* 2011;32(3):381-8. doi: 10.1093/carcin/bgq279
46. Yang H, Jia X, Chen X, Yang CS, Li N. Time-selective chemoprevention of vitamin E and selenium on esophageal carcinogenesis in rats: the possible role of nuclear factor kappaB signaling pathway. *Int J Cancer* 2012;131(7):1517-27. doi: 10.1002/ijc.27423
47. Chen X, Mikhail SS, Ding YW, Yang G, Bondoc F, Yang CS. Effects of vitamin E and selenium supplementation on esophageal adenocarcinogenesis in a surgical model with rats. *Carcinogenesis* 2000;21(8):1531-6.