



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

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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  $\beta$ -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.<sup>1</sup> Esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC) are the two



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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.<sup>2,3</sup> Golestan province of Iran has the highest ESCC rate worldwide.<sup>4</sup> The north and south districts of Golestan province can be classified as low and high-risk areas of EC (Figure 1).<sup>5</sup> 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.<sup>6</sup> 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.<sup>7</sup> 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.<sup>8</sup> 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  $\beta$ -carotene are reactive oxygen species (ROS) scavengers that play a key role in cellular responses to oxidative stress.<sup>9,10</sup>

Selenium is an essential trace element for human health, which may play a protective role against some cancers.<sup>11,12</sup> 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.<sup>13</sup> Vitamin E is a hydrophobic lipid-soluble molecule including eight isoforms:  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherols and  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocotrienols. Vitamin E acts as a chain-breaking antioxidant and has been shown to have anti-cancer properties.<sup>14</sup> In many studies, lower vitamin E intake or nutritional status was associated with an increased risk of various types of cancer.<sup>15</sup> A meta-analysis of 12 articles reported that higher dietary vitamin E intake was correlated with a lower risk of EC, especially for ESCC.<sup>16</sup>

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.<sup>17</sup> Vitamin A derivatives play an important role in cell differentiation, proliferation, and apoptosis.<sup>18</sup> A meta-analysis of 14 publications suggested that intake of vitamin A may reduce the EC risk.<sup>19</sup> In the high-risk area of Golestan province, daily vitamin A intake was lower than the lowest threshold intakes in rural women.<sup>20</sup>

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.<sup>4</sup> 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.

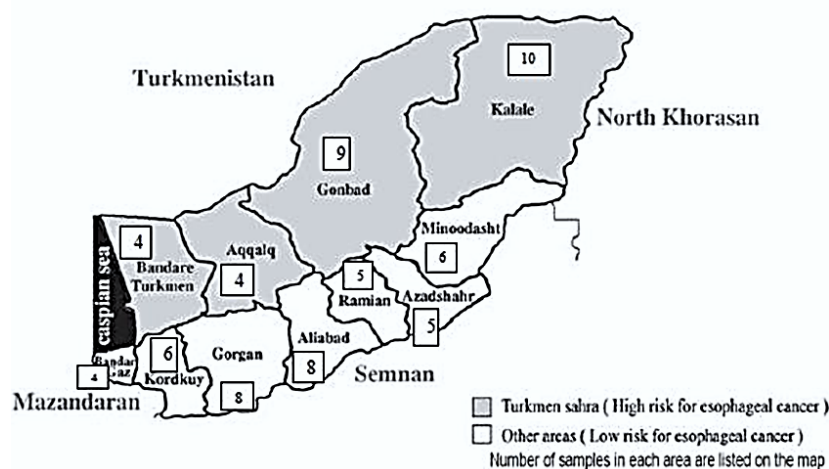


Figure 1. Map of the Golestan province shows the high-and low-risk areas of esophageal cancer

## 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.<sup>21</sup> 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  $51.7 \pm 13.8$  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  $146.8 (\pm 40.9)$  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  $57.5 (\pm 15.2)$  and  $66.1 (\pm 21.3)$ , 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 ( $\pm 14.4$ )	51.6 ( $\pm 13.2$ )
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  $\pm$  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 ( $\mu\text{g/L}$ ), Mean $\pm$ SD			Serum vitamin A ( $\mu\text{g/L}$ ), Mean $\pm$ SD			Serum vitamin E, ( $\mu\text{g/L}$ ), Mean $\pm$ SD		
	High-risk area	Low-risk area	<i>P</i> value	High-risk area	Low-risk area	<i>P</i> value	High-risk area	Low-risk area	<i>P</i> value
Gender									0.01*
Total	146.8 ( $\pm$ 40.9)	158.8 ( $\pm$ 40.2)	0.13	57.5 ( $\pm$ 15.2)	66.1 ( $\pm$ 21.3)	0.07	4.1 ( $\pm$ 1.7)	5.2 ( $\pm$ 2)	
Male	141.7 ( $\pm$ 43.7)	153.2 ( $\pm$ 35.3)		60.3 ( $\pm$ 16.7)	69.4 ( $\pm$ 19.5)		3.9 ( $\pm$ 1.5)	4.7 ( $\pm$ 1.6)	
Female	149.7 ( $\pm$ 43.7)	155.9 ( $\pm$ 43.6)		55.7 ( $\pm$ 14.2)	63.6 ( $\pm$ 22.5)		4.1 ( $\pm$ 1.7)	5.4 ( $\pm$ 2.2)	
<i>P</i> value	0.31	0.71		0.45	0.45		0.77	0.3	
Residence place									
Urban	150.6 ( $\pm$ 50.1)	149.8 ( $\pm$ 32.5)	0.98	59.6 ( $\pm$ 14.5)	69.4 ( $\pm$ 22.3)	0.55	4.6 ( $\pm$ 1.9)	5.2 ( $\pm$ 1.8)	0.71
Rural	144.4 ( $\pm$ 33.8)	162.1 ( $\pm$ 48.3)	0.63	56.2 ( $\pm$ 15.8)	61.4 ( $\pm$ 15.5)	0.70	3.6 ( $\pm$ 1.4)	5.1 ( $\pm$ 2.2)	0.37
<i>P</i> value	0.45	0.09		0.56	0.3		0.13	0.87	

SD, standard deviation. Data were expressed as mean $\pm$ 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  $\mu\text{g/mL}$ ) were significantly lower than in low-risk areas (5.2  $\mu\text{g/mL}$ ), 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  $\mu\text{g/dL}$  and 66.1  $\mu\text{g/}$

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.<sup>20,22</sup>

The results of our study showed that serum selenium levels were high in high-risk areas (146.8  $\mu\text{g/L}$ ) and low-risk areas (158.8  $\mu\text{g/L}$ ) 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.<sup>23</sup> A study conducted in Golestan province, a high-risk area for the incidence of ESCC, showed that the median serum selenium concentration was 155  $\mu\text{g/L}$  (141-173) in this population and above the level required to saturate serum selenoproteins.<sup>24</sup> 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.<sup>25,26,27,28</sup> The case-control study nested within the Golestan Cohort reported that toenail selenium concentrations did not differ significantly between cases of ESCC in high-risk areas and healthy subjects, and there was no association between toenail selenium concentrations and the ESCC risk in this population.<sup>29</sup> Another study in Golestan province showed that in the high-risk areas

of EC, the association between selenium and the risk of developing ESCC was non-linear and U-shaped.<sup>30</sup> 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.<sup>31</sup> 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.<sup>32</sup>

Although selenium is commonly known as an antioxidant, it becomes toxic at a high dose depending on chemical species and may even increase carcinogenesis.<sup>33</sup> 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.<sup>34</sup> Intracellular oxidative stress may promote DNA damage and oncogenic mutations, cytotoxicity, and genotoxicity.<sup>35</sup> High selenium exposure may decrease global DNA methylation through a decreased DNA methyltransferase activity.<sup>36</sup> 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.<sup>37</sup>

The association between cancer risk and vitamin E has been investigated in various epidemiological studies.<sup>38</sup> Oxidative stress has been implied in various cancer pathogenesis, especially gastrointestinal cancers. Vitamin E isoforms sweep ROS due to the presence of a phenyl group in their chromanol ring, hence lowering their radical damaging abilities and inhibiting oxidative DNA damage.<sup>39,40</sup> Transcription factor Nrf2 regulates the induction of antioxidant enzymes. Natural forms of vitamin E, especially  $\gamma$ -tocopherol stimulate Nrf2, which induces gene expression of several antioxidant enzymes like superoxide dismutase, catalase, and glutathione peroxidase.<sup>41</sup> 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.<sup>42</sup> 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.<sup>43</sup>

Vitamin E and vitamin A can inhibit the formation of N-nitroso compounds, which are possible carcinogens in the esophagus.<sup>44</sup> 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.<sup>7</sup> Furthermore, in N-Nitrosomethylbenzylamine-treated rats, vitamin E and selenium supplementation reduced 8-hydroxy-2'-deoxyguanosine in each category of lesions.<sup>45</sup> Vitamin E and selenium may suppress esophageal carcinogenesis of N-Nitrosomethylbenzylamine-treated rats by blocking the activation of the nuclear factor-kappa B pathway.<sup>46</sup> 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.<sup>47</sup>

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 ESCC in this region is a multifactorial disease and requires a combination of exposures for its progression.<sup>7</sup> 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 ESCC 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.

#### Ethical 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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