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Original Article

The risk of gastrointestinal cancer and nitrate intake due to vegetable consumption: A case-control study in Minab, Iran

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Abstract

Background: Nitrate (NO_3) is a necessary element for plant growth, but its excessive use in agricultural products causes different health problems. This study aimed to investigate the relationship between NO_3 concentrations in vegetables and the prevalence of gastrointestinal cancers in Minab city, Iran.

Methods: This case-control study was conducted on 60 people with cancer as the case group and 120 healthy people as the control group in Minab city. Data were collected through a questionnaire and measuring NO_3 concentration levels in vegetables. All samples were examined for NO_3 by reverse-phase HPLC (RP-HPLC) method.

Results: The concentration levels of NO_3 in all vegetables ranged from 15.08 (onion) to 1143.55 mg/kg (spinach). There was no significant difference between the concentrations of NO_3 in all vegetables among the different regions. The most common cancer in the case group was stomach cancer (61.7%). There was a significant difference between the amount of daily intake of NO_3, through different vegetables, and the prevalence of gastrointestinal cancer between the case and control groups ($P < 0.05$). The results showed that increasing the consumption of vegetables increases the chance of getting gastrointestinal cancer (OR: 5.72; $P < 0.001$).

Conclusion: According to the results, there is a significant relationship between the NO_3 concentration in vegetables and the prevalence of gastrointestinal cancers in the studied areas. It is highly recommended to closely monitor the cultivation, fertilization, and spray process of agricultural products, and frequent monitoring of NO_3 levels in fruits and vegetables.

Keywords: Nitrates, Risk assessment, Vegetables, Gastrointestinal neoplasms, Risk cancer

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Introduction

Recently, human activities have caused a fundamental change in the global nitrogen cycle and an increase in the amount of nitrates in most parts of the earth (1,2). Environmental pollution in the air (3-6), pathogenic bacteria in meat (7), glufosinate-ammonium residue in wheat (8), palmitic acid (9), acrylamide (10) and eugenol (11) is increasing over recent decades. One of the main factors of nitrate (NO_3) increase in the environment is the use of fertilizers in agricultural activities (12). Today, NO_3 concentration is an important issue, because of short- and long-term adverse effects on humans, which can be referred to as methemoglobinemia, effects on fetuses, and different health effects (13-15). Among the health effects of nitrates and nitrites, cancer is one of the most important diseases that require high attention (15-17). Cancer is the third cause of death after accidents and cardiovascular diseases in Iran, and the second cause of death in the world (18,19). In Iran, about 70 000 people get cancer every year, which is twice the average in developed countries, and cancer leads to the death of 30 000 Iranians annually (20,21). Among the most common types of cancer, gastrointestinal cancer is one of the most well-known cancers in the world, and among gastrointestinal cancers, the most common and deadly cancer is related to stomach cancer (22-24). Several risk factors, including improper nutrition, inactivity, aging, carcinogens produced in the digestive system (nitrosamines), toxins, and nitrogen fertilizers (nitrate and nitrite) in agriculture play a role in the occurrence of stomach cancer (19,22). One of the main environmental factors is the amount of nitrates and nitrites in food and drinking water. Today, unfortunately, excessive use of chemical fertilizers, including nitrogen fertilizers (nitrate and nitrite) in agriculture and their entry into water and food sources has caused the spread of stomach cancer. The prevalence of stomach cancer in the northern part of the country including Golestan (25), Mazandaran (19), Gilan (26), and Ardabil (21), which is the agricultural pole of Iran is high (19,25). According to the studies in Golestan (19) and Isfahan (27) provinces, there is a direct relationship between the use of nitrogen fertilizers and stomach cancer. As well as in a study in Denmark, there was a positive relationship between nitrates in drinking water and stomach cancer (28). Several epidemiological studies have been conducted to determine the relationship between nitrates in drinking water and the risk of cancer, and most of these studies focused on stomach cancer and showed different results (29-35). In a study by Zarei et al, the results demonstrated that the NO_3 levels had the greatest effect in adults and infants compared to other subgroups (36). In a study by Samadzadeh et al, the mean levels of nitrate in soil were 353 mg/kg and higher than the recommended standard (37). Based on the reports of the Hormozgan Province Cancer Center and Minab city Health Center, the prevalence of gastrointestinal cancers, including stomach cancer, is high in the east of Minab city (Tokhor-Hashtabandi), which is the agricultural pole of Iran in autumn and winter seasons. This study aimed to investigate the association between the NO_3 concentrations in the vegetables and the prevalence of gastrointestinal cancers in Minab, Iran.

Materials and Methods

Study area

This case-control study was conducted on patients with cancer of the digestive system (stomach and intestine) as the case group and healthy people as the control group from Minab city, Tokhor-Hashtbandi region. Based on the list of patients with gastrointestinal cancer (stomach and intestine) at Hormozgan Cancer Center, 60 patients were included in the study. Also, 120 people were selected as a control group.

To determine the consumption pattern of the group of leafy, bushy, and tuberous vegetables in three regions with high, medium, and low risk of cancer prevalence, the data of the food frequency questionnaire (FFQ) of the subjects participating in the Persian cohort were used (27). The questionnaire was completed by referring to patients and their families in both case and control groups. In the second stage, the samples of vegetables and herbs consumed by both control and case groups were collected and transferred to the laboratory. To track and separate nitrate and nitrite concentration in vegetables and green plants, the selected species included leafy (lettuce and cabbage), tuberous (potato and onion), and bush (tomato and cucumber) products.

Study design and sample collection

Three samples of 20 vegetables including green cucumber, onion, tomato, pumpkin, eggplant, potato, hot pepper, bell pepper, green bean, cabbage, lettuce, local vegetable (turnip greens), cilantro, celery, spinach, dill, basil, parsley, and leeks were obtained from agricultural lands, markets, and peddlers selling in three steps. Sampling was done in three different periods and a total of 18 samples were analyzed (9 samples from each vegetable). The samples were selected from three regions as follows: The first region with a high risk of cancer and with a high cultivation of agricultural products, the second region with an average cultivation of crops, and the third region with no cultivation of agricultural products (Figure 1). In each sampling, 10-15 of each type of vegetable were collected, and all samples were ground using a Moulinex machine (shredder). The samples were placed in sterile plastic bags inside the ice box and were transferred to the laboratory. The sample preparation and extraction methods were according to studies by Hongsibsong et al and Ghaffari et al (38,39). Nitrate was measured using an HPLC device (KNAUER) with a UV detector (UV-Detector-K2500) with three repetitions, and their mean

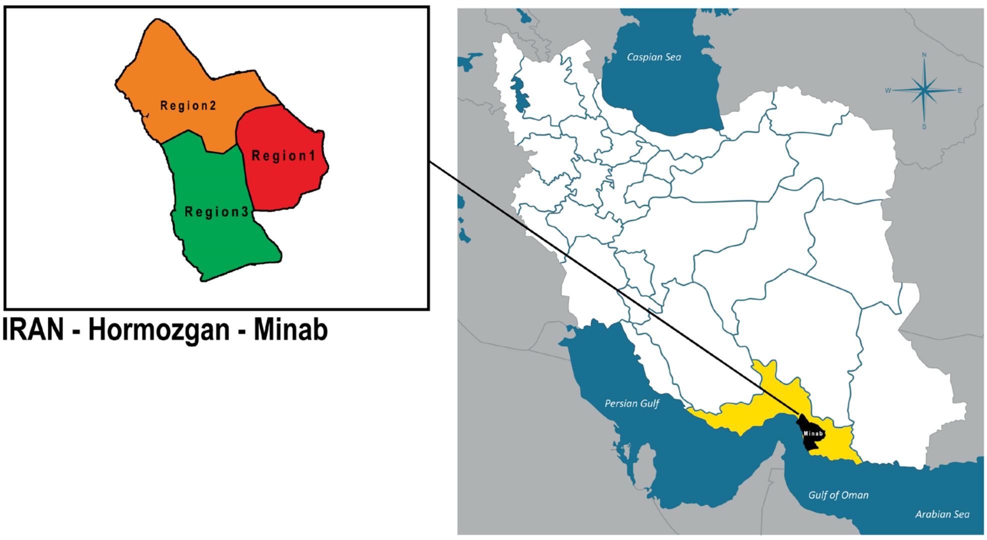

Figure 1. Map of studied areas (27)

values were considered as the level of nitrite and nitrate in each sample.

Sample analysis

To extract the nitrate, first, 50 mL of distilled water was added to 1 g of prepared fruits and vegetables, and then, mixed at 70-80 °C for 15 minutes. Then, the samples were cooled at room temperature and their volume reached 100 ml using distilled water, 10 mL of each sample was taken and filtered using a 0.45-micron syringe head filter before injecting into the HPLC device (KNAUER: at a wavelength of 213 nm). The first three ml of the filtered sample was discarded and the rest was kept for injection into the HPLC device. The injection was done immediately after extraction. Nitrate measurement was done using an HPLC device with a detector (UV) and reverse phase column (C18). The mobile phase consisted of methanol solution (50%), distilled water (50%) and 0.01 M octylamine, and pH was adjusted to 7 using phosphoric acid.

Daily intake of nitrates

To calculate the daily intake of nitrates per person through vegetables, the mean levels of nitrate in each sample were multiplied by its per capita daily consumption, and the total amount for each person was calculated by adding the levels received from all vegetables and according to Eq. (1) (40,41):

$$ADI = \frac{\sum_{i=1}^{n} C_i \times IR_i}{BW} \quad (1)$$

Where C_i is the nitrate concentration (mg/g) in each vegetable, IR_i is the amount of consumption (g/day) of each vegetable, and *BW* is the body weight.

Statistical analysis

Statistical analyses were performed using SPSS version 16 and Excel version 2016. The Shapiro test was used to determine the normality of the data. The independent student *t* test was used to compare the values between the two groups. The ANOVA and post-hoc tests were used to analyze the mean comparison for variables that were more than two groups. Logistic regression was used to determine the effective and predictor factors in both control and case groups. The statistically significant level was considered at $P<0.05$.

Results

Out of 60 people in the case group, 24 (40%) were female and 36 (60%) were male. In the case of control group, 46 (38.3%) were female and 74 (61.7%) were male. The mean ages of the case and control groups were 56.22 ± 13.88 and 55.15 ± 8.58 years, respectively. Based on the analysis, no significant difference was observed between age, gender, and place of residence in the two case and control groups, but there was a significant difference between marital status and education level in the two groups (Table 1). In the case group, 37 people (61.7%) had stomach cancer, 16 people (26.6%) had colon cancer, 6 people (10%) had liver cancer, and 1 people (1.7%) had esophageal cancer (Table 2).

The mean concentration levels of nitrates in all examined vegetables and herbs were 347.44 mg/kg. The highest concentration was related to spinach (1143.55 mg/kg) and the lowest one was related to onion (15.08 mg/kg). The nitrate concentration levels in different vegetables and herbs are shown in Figure 2. The mean concentration levels of leafy, bushy, and tuberous vegetable groups were 685.87 ± 52.84, 153.48 ± 20.15, and 63.58 ± 27.32 mg/kg, respectively (Figure 3).

The comparison of the mean concentration levels of nitrate (mg/kg) in vegetables in the regions (three regions) shows that there was no significant difference in the concentration levels of nitrate among the different regions ($P>0.05$). Also, there was no significant difference in the concentration levels of nitrate among different types of vegetables such as bushy, tuberous, and leafy vegetables ($P>0.05$; Table 3).

Based on the comparison of the average daily intake of nitrates and using the permissible daily intake amount of 3.7 mg/kg of body weight per day, which was determined by the World Health Organization (WHO), people were divided into two groups with permissible intake and illegal intake (42). The daily intake of nitrate through the consumption of vegetables and summer fruits was associated with gastrointestinal cancers in the case group (OR: 5.72; $P>0.001$) (Table 4).

As shown in Table 5, all the people whose daily intake of nitrates through the consumption of vegetables and herbs was above 3.7 mg/kg were patients who had agricultural jobs and lived in Region 1. In the other two regions, the

Table 1. Frequency distribution of demographic variables

Variable	Groups	Case group No. (%)	Control group No. (%)
Gender	Female	24(40)	46 (38.3)
	Male	36(60)	74 (61.7)
Age (year)	<50	37 (62)	72 (60)
	≤50	23 (38)	48 (40)
Marital status	Single	2 (3.3)	0 (0)
	Married	58 (96.7)	120 (100)
Education level	Illiterate	32 (53.3)	6 (5)
	High school	23 (38.3)	77 (64.2)
	Diploma and above	5 (8.3)	37 (30.8)
Regions	The first region (with high agriculture)	36 (60)	72 (60)
	The second region (with medium agriculture)	7 (11.7)	14 (11.7)
	The third region (no agriculture)	17 (28.3)	34 (28.3)

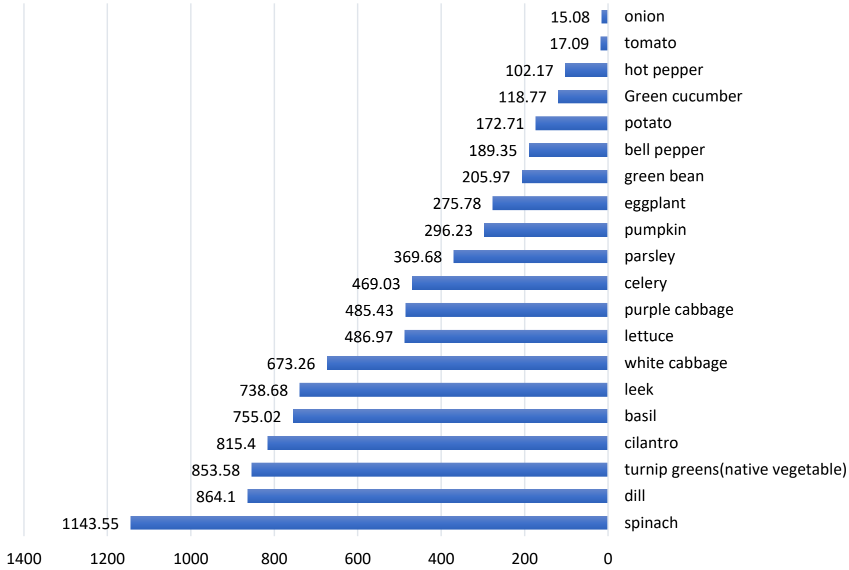

Figure 2. The mean concentration levels (mg/kg) of nitrate in different vegetables and herbs

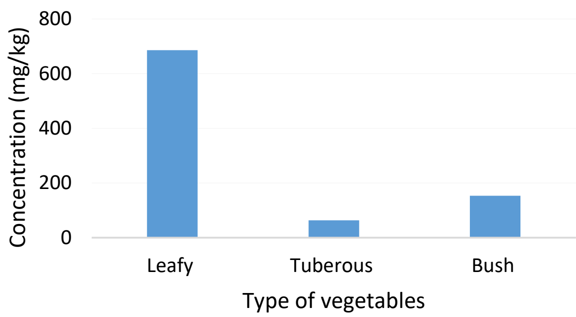

Figure 3. The mean concentration levels of nitrate (mg/kg) in different types of vegetables

Table 2. The distribution of different cancers in the case group

Type of cancer	No. (%)
Stomach	37 (61.7)
Intestine	16 (26.6)
Liver	6 (10)
Esophageal	1 (1.7)
Total	60 (100)

daily intake of nitrate was less than 3.7 mg/kg.

Discussion

In the present study, the mean concentration levels of nitrate in vegetable groups follow the following order: leafy > bushy > tuberous. Therefore, higher levels of nitrates tend to accumulate in leaves, and lower levels are observed in tubers. Consequently, the leafy vegetables group are considered the main nitrate-enriching vegetables (43). Similarly, it was confirmed that nitrate levels in leafy vegetables were higher than those in root and fruiting vegetables (43-45).

The results of the present study show a significant association between the mean level of daily intake of nitrates through vegetable consumption and the prevalence of gastrointestinal cancer (OR: 5.72; $P < 0.001$). The relationship between dietary nitrate intake and gastrointestinal cancer risk is controversial. Some studies have reported an increased risk of gastric cancer with increased nitrate intake (46-49) while others reported no association (50-52). In contrast, in some studies, dietary nitrite intake was inversely associated with gastric cancer risk (53-55). Different biological parameters/mechanisms may affect the association between nitrate intake and gastric cancer risk (53,56). For example, these inconsistent results may be related to diet because different food sources may contain nitrite/nitrate (53). Animal products (especially processed meats) are the main sources of dietary nitrite (57). Also, heterogeneity in the method and type of study may explain the different results between studies. Other causes of the heterogeneity in observed results may be due to different geographical conditions and population groups, nitrate levels, methods of nitrate measurement, as well as the difficulty of estimating intake in the population. Environmental pollutants including microbial (58-63), mycotoxins (64,65), hormones (66,67) and heavy metals (68-71) can endanger human health. Gastric cancer is the third most common cause of cancer-related deaths (72-74) and vegetables rich in nitrate are one of the risk factors for the occurrence of this type of cancer (75).

Excessive use of nitrate fertilizers in agricultural fields has caused different problems such as increasing nitrate concentration in crops, agricultural soils, and environmental pollution (69,76). Food products contaminated with nitrates and nitrites easily enter the human and animal bodies. Nitrates enter the human body through vegetables and drinking water, a significant part of which is removed from the body as a result of daily activities. Residual nitrates are converted to nitrites in the digestive tract, which are carcinogenic compounds (22).

Table 3. The comparison of the average nitrate concentration (mg/kg) of vegetables and summer herbs according to the regions

Types of vegetables	Region 1	Region 2	Region 3	Test statistics	*P* value
Bush vegetables	1.5	1.3	1.2	0.222	0.801
Tuberous vegetables	1.08	1.01	1.05	0.006	0.994
Leafy vegetables	7.05	6.9	6.8	0.029	0.971
All of vegetables	4.35	4.31	4.12	0.055	0.945

Table 4. The comparison of average daily intake of nitrates (mg/kg) through the consumption of vegetables and summer herbs in both case and control groups

Factor	Case No. (%)	Control No. (%)	*P* value	OR	95% CI	
					Lower Limit	Upper Limit
Illegal (above 3.7 mg/kg)	27 (64.3)	15 (35.7)	0.0001	5.72	2.72	12.03
Allowed (below 3.7 mg/kg)	33 (23.9)	105 (76.1)	0.0001	1	1	1

OR, Odds ratio.

Table 5. The distribution of daily nitrate intake (mg/kg) in different regions

Group	Regions	Number of people with nitrate intake below 3.7 (%)	The number of people with nitrate intake above 3.7 (%)
Case	The first region (with high agriculture)	9 (25)	27 (75)
	The second region (with medium agriculture)	7 (100)	0 (0)
	The third region (no agriculture)	17 (100)	0 (0)
Control	The first region (with high agriculture)	57 (79.2)	15 (20.8)
	The second region (with medium agriculture)	14 (100)	0 (0)
	The third region (no agriculture)	34 (100)	0 (0)

The results of the present study showed that the most common cancer in the studied area was stomach cancer. In this regard, the results of a study by Taghian et al (77) also indicated the prevalence of stomach cancer and the nitrate concentration of water samples were higher in this city. Recent ecological studies in Spain and Hungary have shown a positive relationship between stomach cancer and high nitrate concentration in drinking water (78,79). In an epidemiological study in an area with a high prevalence of stomach cancer in northeastern China, a significant relationship was found between the high nitrate levels in drinking water supplies and neoplastic changes in the stomach (80).

In the present study, the highest nitrate concentration was observed in region 1 and in leafy vegetables. In this region, the occupation of most of the people was agriculture and the people who lived in this region had a daily intake of nitrates of 3.7 mg/kg through the consumption of vegetables and fruits. The daily absorption of nitrates through consumed vegetables was higher than the amount recommended by the WHO (3.7 mg NO_3/kg of human body weight). In a study, it was reported that only with daily consumption of 76 g of lettuce or 94 g of spinach by an 80 kg person, the maximum permissible nitrate enters the consumer's body, and consuming more than this amount increases the risk of health risks (81). It seems that in regions 2 and 3, where the amount of nitrate consumption is less than 3.7 mg/kg, the management of fertilizer consumption, especially nitrogen fertilizers, has been done in a better way, and the result has been shown by the reduction of nitrate accumulation in the agricultural products of the region. On the other hand, the results of a study have shown that the cooking process reduces the amount of nitrates in vegetables (44) because nitrates have a great tendency to dissolve in water, and increasing the temperature and time helps the diffusion process and movement of nitrates from vegetable to the water media, and eventually, reducing the level of nitrate in the vegetables (82,83). Therefore, it is recommended to consume cooked vegetables to reduce the health risks of consuming raw vegetables.

Conclusion

The findings of this study provided evidence that dietary nitrate and nitrite intake were potentially associated with gastrointestinal cancer risk. Recently, due to industrial growth and an increase in population, the production and intake of nitrate, especially in the agricultural sector, are increasing. High consumption of agricultural products with high levels of nitrates can be one of the reasons for increasing the prevalence of gastrointestinal cancers. Probably, with this result, the consumption of agricultural products with high nitrate and nitrite can increase the basis for the prevalence of gastrointestinal cancers in people. Although diet is a very complex and variable factor, due to these limitations and confounding factors, the generalization of the results should be done with caution. To further clarify the relationship between nitrate/nitrite and cancers, comprehensive observational studies considering diet and other factors are highly recommended.

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Authors' contributions
Conceptualization: Mahdi Khaksar.
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Formal analysis: Naeme Soltani.
Funding acquisition: Vali Alipour.
Investigation: Omid Rahmanian.
Methodology: Mahdi Khaksar.
Project administration: Vali Alipour.
Resources: Mahdi Khaksar.
Software: Omid Rahmanian.
Supervision: Vali Alipour.
Validation: Vali Alipour.
Visualization: Omid Rahmanian.
Writing – original draft: Naeme Soltani.
Writing – review & editing: Omid Rahmanian.

Competing interests
The authors declare that there is no conflict of interests.

Ethical issues
The study protocols were approved by the Ethics Committee of Hormozgan University of Medical Sciences, Bandar Abbass, Iran (Ethical code: IR.HUMS.REC.1397.171).

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