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Original article

Determination of nitrate concentration in consumed vegetables and estimation of that's dietary intake in Shabestar and Khameneh City, northwest of Iran: Azar Cohort study

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Summary. Nitrate is one of the risk factors of stomach cancer due to the formation of carcinogenic chemicals known as N-nitroso compounds. Vegetables are natural sources of dietary nitrate. In this study, nitrate concentration in consumed vegetables and that's intake in two cities in northwest of Iran were assessed. About 72 leafy and root vegetables were purchased from local supermarkets or wholesale in cities of Shabestar and Khamneh and analyzed using Cataldo method. The highest concentration among leafy vegetables was determined in Coriander (814.31±52 mg $NO_3^-kg^{-1}$) and between root vegetables in Garlic (4739±89 mg $NO_3^-kg^{-1}$). The results showed that nitrate intake viavegetables in Khamneh and in Shabestar 164.14 and 149.27 $mgday^{-1}$ per person, respectively, and the difference between concentration of nitrate in vegetables was not significant (p = 0.72) *Conclusion:* The results of this study showed that the daily intake of nitrate is higher than recommended by the World Health Organization. Therefore, the need to plan for reducing the amount of food nitrate (more monitoring on the use of chemical fertilizers and agricultural water abstraction) seems necessary.

Key words: stomach cancer, nitrate, vegetable, Iran

Background

In the past several decades, there have been many studies on diet-cancer associations which have produced inconsistent results (1). Among different cancers, the highest concern of the public health has focused on the relationship between nitrates and stomach cancer (2). High dietary intakes of nitrate have been implicated in the etiology of human gastric cancer (3). New information has clearly established that nitrite and nitrate are important biological compounds and nitrosation is an important feature of NO metabolism in human physiology (4). Nitrates form a part of essential chemistry of soils and plants. Thus, vegetables' roots are able to absorb nitrate directly from soil. Vegetables play an important role in human nutrition since they are an outstanding source for vitamins, minerals and biologically active compounds (5).

Although nitrate is apparently non-toxic at below maximum residue levels (MRLs), it may be endogenously transformed into nitrite which can react in stomach with amines and amides to produce N-nitroso compounds (NOC), which are mainly carcinogens (3, 6-8)

Dietary source of NOCs is among the main environmental sources of NOCs in human body (9). From dietary sources, vegetables contribute the largest amount of nitrates (10).

Nitrates are inherently present in all plant materials, especially vegetables accumulated when the plant matures in a nitrate rich environment. Dietary intake of nitrates in vegetables can vary greatly from region to region depending on some factors such as farming practices, climate, soil quality, manufacturing processes and legislation (3).

According to the Iranian Ministry of Health and Medical Education, gastric cancer is one of the most common cancers in Iran. Therefore, in this study, nitrate levels as one of the important risk factors for gastric cancer were measured in leafy and root vegetables consumed in mentioned cities and the daily intake of nitrate was estimated and compared in them.

Material and methods

All the reagents used in this study were of analytical grade. De-ionized water was used for preparing all the solutions. Potassium nitrate (Merck), activated carbon (Merck), aluminum sulphate (Merck), sodium hydroxide (Merck) and acid salicylic (Sigma) were also used.

Standards

As mentioned before, all the reagents used in this study were of analytical grade. Potassium nitrate (KNO_3) was mixed with distilled water in volumetric flasks to give 0.2- 20 milligram per liter NO_3-N.

Vegetable samples

All the vegetables including cabbage, red cabbage, lettuce, spinach, parsley, coriander, leek, fenugreek, fumitory and mint as leafy vegetable and onion, potato, eggplant, radish, tomato, carrots, zucchini and garlic as root vegetable were purchased from 3-5 supermarkets and wholesalers in each city and then were analyzed. By considering 18 different vegetable samples (leafy and root) from 3-5 supermarkets (averagely 4 sellers) and also considering sampling in tow cities about 72 samples were taken during study.

After analyzing nitrate content in all of the studied vegetables, the amount of consumed vegetables (leafy and root) was determined according to a national comprehensive study and was used in calculation of daily nitrate intake (11).

Extracting nitrate from the samples

In the laboratory, the vegetables were washed with tap water, rinsed with distilled water and dried up at laboratory temperature for 2 h. Then, each type of vegetable samples was mixed and dried at 70° C in an oven. The dried samples were ground and then sieved with mesh no. 20. and, 0.4 g of the sieved sample plus 0.05 gram of activated carbon and 40 ml of 0.025 M aluminum sulphate were poured to a flask and were shaken for 30 min. After shacking, the samples were filtered with Whatman no. 100 filter paper and again 0.05 g of activated carbon was added to the extraction solution and shaken and filtered like before (12). Finally, the extracted nitrate was measured using Cataldo method (13). In this method, 0.2 ml was extracted from each extracted sample in a suitable test tube, then 0.8 ml of salicylic acid was added to each test tube and immediately stirred well and was given 30 minutes, and then 19 ml of sodium hydroxide solution was added to each sample and was well stirred, and finally, the absorbance of the samples at 410 wavelengths was read in a spectrophotometer. The amount of nitrate intake was estimated using findings of nitrate extraction as well as food consumption in Shabestar and Khameneh cities. Amounts of examined food group intake were determined using food frequency questionnaire (FFQ). We used data of completed FFQ in Azar cohort study. Finally the amount of nitrate intake (per kg body weight / day) was calculated by the following formula:

$$I=(F.C1)+(W.C2)/M$$

I=Nitrate intake (per kg body weight/day)
F= amount of food consumption (kg/day)
C1=nitrate concentration in food sample (mg/kg)
W=amount of water consumption (L/day)
C2= nitrate concentration in water sample (mg/L)
M=Average body weight(70kg)

Results and Discussion

Vegetables are recognized to provide a significant portion of nitrates in nutritional regime. There are numerous factors affecting NO_3 uptake and accumulation in vegetable tissues, e.g., (day) light, type of soil, temperature, humidity, frequency of plants in field,

plant maturity, genetic, harvesting time, size of vegetable unit, storage time and source of nitrogen. Nitrate can be unstable and different sampling methods and extraction procedures can affect its concentration level in analysed vegetables(14). The method used in current study was chosen because of its applicability, availability, partial simplicity, etc. The results of the quantification of nitrate contents in the selected fresh leafy vegetable samples (mean values) in the studied cites (Shabestsr and Khamneh, Iran) are presented in Table 1. The result of determined nitrate in the root vegetable samples in the mentioned cites are given in Table 2.

Table 1. Mean and SD nitrate concentration in leafy vegetables ($mgNO_3^-kg^{-1}$ wet weight)

	Shabestar		Khamneh	
Vegetables	Sample 1	Sample 2	Sample1	Sample2
Cabbage	100.00±11	81.52±19	269.18±25	231.29±51
Red cabbage	26.23±39	139.21±42	297.07±31	228.26±16
Lettuce	43.80±51	132.07±22	27.86±21	26.47±41
Coriander	814.31±52	744.80±15	796.74±14	640.06±61
Parsley	48.77±29	113.05±32	280.45±62	310.71±58
Fumitory	94.40±11	64.66±21	234.48±13	31.83±46
Mint	756.33±62	26.15±31	309.67±77	87.64±22
Fenugreek	151.07±78	83.93±26	140.94±66	240.94±64
Leek	128.78±47	21.46±11	61.74±23	102.49±55
Spinach	55.04±27	44.39±10	77.59±18	42.32±20

Values are means of at least two replicate determinations from three sources.

Table 2. Mean and SD nitrate concentration in root vegetables (mg $NO_3^-kg^{-1}$ wet weight)

Vegetables	Shabestar		Khamneh	
	Sample 1	Sample 2	Sample 1	Sample 2
Onion	148.72±71	235.86±83	388.00±81	64.90±13
Potato	124.14±65	182.76±80	190.60±56	86.64±19
Eggplant	277.83±69	388.33±62	322.21±54	142.83±75
Radish	25.29±11	51.51±20	91.76±56	135.76±69
Tomato	619.24±81	213.97±55	238.38±44	233.03±66
Carrots	73.61±23	91.38±36	329.12±72	289.07±48
Zucchini	69.21±15	34.24±14	51.29±32	48.13±21
Garlic	3315.96±83	1986±85	2188.28±91	4739.34±89

Values are means of at least two replicate determinations from three sources.

As indicated in tables 1 and 2 the highest concentration among leafy vegetables was determined in Coriander (814.31±52 mg $NO_3^-kg^{-1}$) in the Shabestar and between root vegetables in Garlic (4739.34±89 mg $NO_3^-kg^{-1}$) in the Khamneh. Many studies have been conducted to measure nitrate content in vegetables. There is considerable variation in nitrate contents within the same vegetable species reported in the previous researches. As mentioned, nitrate content in vegetables depends on many factors. In the current study, for leafy vegetables in both cities, the highest nitrate value was obtained for Coriander. Of course, in Khamneh, nitrate content in this vegetable was higher than Shabestar. Therefore, it can be concluded that Coriander contributed the most dietary intake of nitrate from leafy fresh vegetables.

The lowest nitrate in leafy vegetables in Khamneh belonged to Lettuce while in Shabestar the lowest nitrate level was determined for Red cabbage and for Leek. It was lower in comparison with the other study which was carried out in Varzeghan (spiring 972.19-autumn1701 $mgkg^{-1}$) and parsabad(spring 2232- autumn 1952 $mgkg^{-1}$) by taghipoure and et al. (15, 16).

The mean nitrate concentration obtained for the onion samples in Khamneh was 388.00 and 64.90 mg per kg, respectively, which showed considerable difference in samples. And, it was lower in comparison with the other studies in Varzeghan (spiring 220.88-autumn 240.44 $mgkg^{-1}$) and parsabad(spring 1030- autumn 708.64 $mgkg^{-1}$).and it was higher with the other studies in Ardabil, Iran (165 $mgkg^{-1}$), Korea (23 $mgkg^{-1}$) and most European countries (164 $mgkg^{-1}$) (15-17). In addition, mean nitrate concentration in Shabestar samples for onion was 148.72 and 235.86 $mgkg^{-1}$, respectively.

Nitrate concentration of potatoes in the studied cities was close to the other studies such as Varzeghan (spiring 160-autumn 141 $mgkg^{-1}$) and parsabad(spring 102-autumn162$mgkg^{-1}$)and 97 $mgkg^{-1}$ for Ardebil, Iran and 168 $mgkg^{-1}$ for European countries (15, 16).

In the study which was carried out in Ardabil by Shahbazzadghan and et al. on radish nitrate, 428 $mgkg^{-1}$was obtained and,Varzeghan (spiring 316.91-autumn 620.21 $mgkg^{-1}$) and parsabad(spring 976.68-autumn1048 $mgkg^{-1}$) in another study in Europe, it was 967 $mgkg^{-1}$(15, 16). In this study, mean nitrate in

radish samples of Shabestar and Khamneh was 38.40 $mgkg^{-1}$and 113.76 $mgkg^{-1}$, respectively. Thus, mean nitrate concentration in Shabestar was less than that of Ardabil, Khamneh and European countries.

Mean nitrate in tomatoes samples of Shabestar was 416.60 $mgkg^{-1}$ that was higher in comparison with mean of other studies such as,Varzeghan (spiring 256.03-autumn 176.1 $mgkg^{-1}$) and parsabad(spring 273.78-autumn233.88 $mgkg^{-1}$) European countries (43 $mgkg^{-1}$), Hong Kong, China (57 $mgkg^{-1}$) and Beijing, China (35 $mgkg^{-1}$) (16, 18, 19) as well as Khamneh (235.71 $mgkg^{-1}$) [16].

The results of measurements of carrots' nitrate from Shabestar samples showed that nitrate obtained was 73.61 $mgkg^{-1}$. Mean nitrate of carrots in Shabestar (82.50 $mgkg^{-1}$) was lower than that of Khamneh city (309.09 $mgkg^{-1}$) and also that of other studies including Varzeghan (spiring 632.83-autumn 820.27 $mgkg^{-1}$) and parsabad(spring 514.07-autumn901.55 $mgkg^{-1}$), Ardebil, Iran (104 $mgkg^{-1}$), New Zealand (98 $mgkg^{-1}$), Korea (316 $mgkg^{-1}$), Beijing, China (457 $mgkg^{-1}$) (15, 16).

The mean nitrate concentration in total (leafy and root) vegetables and the amount of daily intake via vegetables in the studied cites are presented in Table 3.

Levels of nitrate obtained in this study from vegetables were compared with those of previous studies. There was considerable variation in nitrate contents within the same vegetable species reported in the previous researches. As described previously, nitrate content in vegetables depended on many factors.

Gangolli et al. (1994) estimated that mean daily intake of nitrate in the US was 106 and, in the UK, it was 104 (20). In 1994, van Vliet, Vaessen, van de Burg and Schothorst (1997) estimated mean intake of nitrate for Dutch people as 80 mg/day per person. Moreover, Italy had mean daily intake of nitrate of 245 mg/day whereas Poland and Switzerland recorded mean daily nitrate intakes of 178 and 125 mg/day, respectively (21). France's mean daily intake of nitrate was reported as 150.7 followed by Netherlands, Germany and Norway where mean daily nitrate intakes were 71, 68 and 43 mg/day, respectively (20). According to James Hsu et al. (2009) based on mean consumptionof vegetables and cured meat in Australia, dietary nitrate intake was estimated as 267 mg/adult/day for Australians (3).

Based on the finding of this study, mean consumption of vegetables in Shabestar and Khamneh cities is shown in Table 3. Daily intake of nitrate in Khamneh was 164.14 and, in Shabestar, it was 149.27 mg/adult/day. But, the difference of daily intake of nitrate from vegetables between the above cities were not statistically significant (P=0.72).

Table 3. Intake of nitrate and amount of vegetable usage in Shabestar and Khamneh

City	Use of vegetable kg day^{-1}	Intake of nitrate mg day^{-1} per person
Shabestar	0.316	149.27
Khamneh	0.242	164.14

Conclusion

Different studies on nitrate toxicity and gastric cancer have been done based on the daily intake of nitrate from major food groups, most of which have reported that vegetables contribute to the majority of dietary nitrate intake. In this study, leafy and root vegetables' nitrate concentration level in Shabestar and in Khamneh indicted that concentration of nitrate in the studied cities was almost in the same range of other studies. The difference between the concentration of nitrate in consumed vegetables in the studied cities was not significant (p = 0.72. According to our results, the daily intake of nitrate is higher than recommended by the World Health Organization. Therefore, the need to plan for reducing the amount of food nitrate (more monitoring on the use of chemical fertilizers and agricultural water abstraction) seems necessary.

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