Original Research Article

Association of Heavy Metals and Selenium Content in Rice with Incidence of Esophageal Cancer in Golestan Province, Iran

Hadi Rahimzadeh[1], *Mahdi Sadeghi[1,2], Somayeh Beirami[1], Abotaleb Bay[1], Morteza Mansurian[3], Gholamreza Roshandel[4]

[1]Environmental Health Research Center, School of Health, Golstan University of Medical Sciences, Gorgan, Iran, [2] Cereal Health Research Center, Golestan University of Medical Sciences, Gorgan, Iran,[3] Faculty of Health, Iran University of Medical Sciences, Iran,[4] Golestan Research Center of Gastroenterology and Hepatology, Golestan University of Medical Sciences, Gorgan, Iran

ABSTRACT

Introduction: Heavy metals have an important role in human health because of their nutritive value and possible adverse effects. The objective of this study was to assess level of heavy metals and selenium in rice harvested in the Golestan Province, Iran. **Materials and Methods:** This study was conducted in the Golestan Province, northern Iran. The area under rice cultivation is 45714 acres. Overall, 69 rice samples were collected from rice farms in different areas of the province including high-risk (42 samples) and low-risk areas (27 samples) for esophagus cancer (EC). Concentrations of heavy metals were measured by polarograph. Voltammetric analysis was carried out using 797 VA computrace (Metrohm). **Results:** Maximum concentrations of zinc, cadmium, lead, copper, nickel, cobalt and selenium in high-risk areas for EC were 70.6, 0.41, 5.05, 14.4, 8.8, 0.88 and 0.48 mg/kg as dry weight, respectively. Maximum concentrations of these elements in the low-risk area for EC were 88.4, 0.37, 3.9, 17.2, 8.1, 0.27 and 0.25 mg/kg as dry weight, respectively. The concentration of Se in high-risk areas were significantly higher than that in low-risk areas ($P<0.05$). **Conclusions:** Heavy metals and Se may act as possible risk factors for EC in the Golestan Province, which should be considered when designing cancer control programs in this area.

KEYWORDS: Heavy metal, Rice, Golestan province, Esophageal Cancer

[1]**Correspondence:** Mahdi Sadeghi, Address: Golstan University of Medical Sciences, Gorgan, Iran, Telephone: +98-1732436102, Fax: +98-1732436107, Email: dr-sadeghi@goums.ac.ir

INTRODUCTION

Dietary intake of heavy metals from crops and the potential public health risk associated with heavy metals have become an increasing concern. The main pathway of human exposure to heavy metals is intake via the soil-crop system [1]. Among heavy metals, cadmium (Cd), lead (Pb), arsenic and mercury are considered toxic to plants, humans and soil microbial population [1, 2]. Heavy metals have an important role in human health because of their nutritive value and possible harmful effects [3,4]. Heavy metals are non-biodegradable and persistent elements in the ecosystem. Therefore, they can accumulate in plant tissues and may be released under certain physicochemical conditions, consequently entering the food chain and accumulate in vital human organs over a long period of time [5-7]. The adverse effects of heavy metals on human life are exerted via bioaccumulation and bio-augmentation in the food chain [8]. Main sources of heavy metals in plants are soil, growth media, pesticides, fertilizers and nutrient solutions [9]. Rice is the staple food for many people in the world. Heavy metal concentrations in rice have been studied in rice-producing countries such as China and Thailand [1,10]. Rice is grown in different areas in Iran, mainly in Northern provinces near the Caspian Sea such as Golestan Province. Golestan Province is an important area for cultivation of rice in Iran [11].

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Esophageal cancer (EC) is among the ten most common diseases in the world [12]. Golestan Province has been known as a high risk area for EC [13,14]. Se concentration in soil has been suggested as a possible risk factor for EC in the Golestan Province [15]. Se is a metalloid, present in nature and in organisms as organic and/or inorganic forms [16]. Considering the high rate of rice consumption and the high risk of EC in the Golestan Province, this study aimed to assess the relationship of concentration of heavy metals including Pb, Cd, copper (Cu), zinc (Zn), nickel (Ni), cobalt (Co) and Se in rice with incidence of EC in this area.

MATERIALS AND METHODS

This study was conducted in the Golestan Province, northern Iran. The area under rice cultivation is 45714 acres. Overall, 69 rice samples were collected from farms in different areas of the province (Figure 1). Samples were taken from rice harvested in autumn. Of 69 samples, 42 were collected from low-risk areas for EC and 27 samples from high-risk areas for EC. Samples were collected in impermeable plastic bags and kept in refrigerator until analysis. They were washed with deionizer water, and then over-dried at 60°C for 24 hr. The dried samples were milled to fine powder. The samples were weighted (1g) and digested in a high-walled beaker with HNO_3-H_2O_2 mixture solution. Rice sample (1g) and 5 ml of HNO_3 (65%) were transferred into a 100 ml beaker. The mixture was evaporated gently using a hotplate until its volume reached about one-third of the initial volume. Then, 2 ml of H_2O_2 were added before the mixture was heated again. The solution was cooled at room temperature. After filtration, the mixture reached the volume of 100 ml with triply distilled water. The solution was transferred to a PET bottle and kept in refrigerator until analysis.

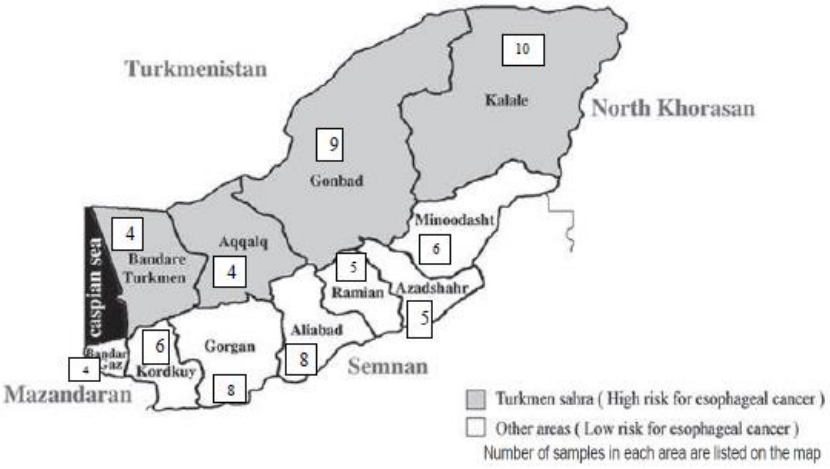

Figure 1: Map of the Golestan Province shows the number of samples collected from each area [11]

The concentrations of heavy metals and Se were determinate by polarograph. Voltammetric analysis was carried out with a 797 VA Computrace (Metrohm). A classic three-electrode cell consisting of a mercury electrode, a Pt electrode and an Ag/AgCl reference electrode was used with a hanging mercury drop electrode. Only high purity grade reagents (Merck and Chemlab Suprapur) and de-ionized water were used. For calibration, the standard addition method was used. Calibration standards were prepared using Chem-Lab (Belgium) at 1000 ppm of heavy metals and Se. Heavy

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metals were quantified using linear regression based on height of the peaks in the voltammograms. Limit of detection (LOD) for Pb, Cd, Cu, Zn, Ni and Co was 0.1, 0.1, 1, 1, 0.1and 0.1µgL^{-1}, respectively. The maximum LOD was 50 mgl^{-1} for Pb, Cd, Cu and Zn, and 10 µgL^{-1} for Ni and Co. Data was analyzed using SPSS software (version 16). P-values less than 0.05 were considered as statistically significant.

RESULTS

Results of the heavy metals measurement in samples from various areas of the Golestan Province are presented in Tables 1 and 2. The mean concentration of heavy metals varied widely in different areas (Table 1). The maximum concentrations of Zn, Cd, Pb, Cu, Ni, Co and Se for high-risk areas for EC were 70.6, 0.41, 5.05, 14.4, 8.8, 0.88 and 0.48 mg/kg, respectively. The maximum concentrations of these heavy metals in low risk area for EC were 88.4, 0.37, 3.9, 17.2, 8.1, 0.27 and 0.25 mg/kg, respectively. All the obtained values were higher than the permissible limit. Normality of the data in low- and high-risk areas was analyzed. Since data related to Cu and Se was normal, independent t-test was used for statistical analysis. For other heavy metals (non-normal data), Mann-Whitney test was used (Table 1).

Table 1: Mean (SD) level (mg/kg) of heavy metals and Se in rice samples from different areas of the Golestan Province.

Elements	High risk area for EC (ASR* of EC=44 per 10^5 person years)		Low risk area for EC (ASR of EC=19.62 per 10^5 person years)		Permissible limit (mg/Kg)	p-value
	Mean	SD	Mean	SD		
Cd	0.043	0.085	0.029	0.06	0.06	0.415
Pb	0.68	1.04	1.1	0.54	0.15	0.167
Ni	1.5	1.9	2.4	1.05	-	0.57
Co	0.05	0.06	0.18	0.037	-	0.786
Zn	29.2	6.35	38	14.2	50	0.372
Cu	3.3	2.57	4.28	3.5	10	0.815+
Se	0.35	0.02	0.16	0.02	-	0.002+

*Age Standardized Rate + Independent Sample T Test

Concentrations of Se in high-risk and low-risk areas differed significantly (P< 0.002). The maximum values of Zn and Cu were observed in Azadshahr and maximum values of Cd, Pb, Ni and Co were observed in Gonbad (Figure 1). In addition, concentration of Se in all high-risk areas for EC was significantly higher than in the low-risk areas. The permissible limit of Zn, Cd, Pb and Cu in rice is 50, 0.06, 0.15 and 10 mg/kg, respectively. Ni and Co have no guideline value. Generally, most heavy metals are present in the lowland and flat areas (high-risk areas) because of high application of chemical fertilizers and pesticides.

Table 2: Mean concentration of heavy metal in rice samples from different farms in the province.

Site of Sampling	Concentration of heavy metals in rice (mg/kg as dry weight)											
	Zn		Cd		Pb		Cu		Ni		Co	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Bandar Gaz	32.5	9.0	0	0	1.46	0.5	1.8	3.0	3.9	0.75	0.1	0.13
Kordkuy	25.6	4.8	0.015	0.04	0.14	0.4	1.2	3.5	2.8	1.38	.006	0.02
B.Turkamen	26.3	4.7	0	0	0	0	1.0	0.8	0.4	0.5	0	0
Aq-qala	25.6	4.4	0	0	0.19	0.5	2.4	1.8	0.6	0.13	0	0

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Gorgan	31.3	13.5	0.008	0.02	1.14	1.9	3.0	1.2	1.4	0.99	0.02	0.04
Aliabad	20.3	6.8	0.008	0.02	1.72	1.0	0.8	0.9	3.4	2.6	0	0
Azadshahr	56.1	15.9	0.73	0.19	0.78	0.8	10	3.8	2.6	1.14	0	0
Gonbad	38.7	16.8	0.17	0.09	2.24	2.2	7.0	6.8	4.4	2.2	0.11	0.31
Ramian	45.6	5.46	0	0	1.23	0.7	7.6	4.0	0.9	0.52	0	0
Minoodasht	54.8	6.75	0	0	1.6	1	5.6	3.1	2.3	1.7	0	0
Kalale	26.1	17.2	0	0	0.28	0.6	3.0	4.1	0.6	0.76	0.1	0.2
Total	34.8	9.57	0.08	0.03	0.98	0.9	3.9	3	2.1	1.1	0.03	0.06
Max and Min level of heavy metals	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**	**Max**	**Min**
Bandar Gaz	45.3	24.2	0	0	2.12	0.9	6.3	0	4.9	3.2	0.27	0
Kordkuy	31.6	18.7	0.124	0	1.11	0	9.8	0	5	0.96	0.054	0
B.Turkamen	31.2	20.3	0	0	0	0	2.0	0.1	1	0	0	0
Aq-qala	30.3	18.9	0	0	1.18	0	5.3	0.5	0.8	0.46	0	0
Gorgan	51.1	13.1	0.05	0	3.9	0	5.2	1.2	2.9	0	0.13	0
Aliabad	26.8	11.2	0.05	0	3.1	0	2.5	0	8.1	0.88	0	0
Azadshahr	88.4	40.3	0.52	0	1.96	0	17.2	6.7	4.5	1	0	0
Gonbad	70.6	20.9	0.41	0	5.05	0	14.4	0	8.8	1.4	0.88	0
Ramian	51.7	39.7	0	0	2.2	0.6	12.3	3.2	1.7	0.5	0	0
Minoodasht	69	49.2	0	0	3.7	0.8	12.4	2.8	4.6	0.01	0	0
Kalale	50.2	9.7	0	0	1.1	0	9.2	0.6	1.5	0	0.4	0

DISCUSSION

Zn is a metal with antioxidant properties. In our study, 17% of samples had Zn concentrations higher than the guideline value (50 mg/kg). Cd is a carcinogenic agent that can cause kidney problems. According to the results, 5.7% of samples had Cd concentrations higher than the guideline value (0.06 mg/kg). Pb also has adverse health effect. Based on the results, 49.2% of samples had Pb concentrations higher than the guideline value (0.15 mg/kg). In addition, 14.4% of samples had Cu concentrations higher than the guideline value (10 mg/kg). The concentration of Zn, Cd, Pb, Co and Ni did not show any significant difference in the low- and high-risk areas for EC. Several studies have reported the list of heavy metals in various crops such as rice [1,10,17]. Due to high application of chemical fertilizers and pesticides in rice farms, rice produced in this region can act as a main source of heavy metals [18]. According to the Codex Committee on Food Additives and Contaminants, dietary intake of heavy metals is a public health concern that needs to be monitored in developing countries [19]. Some studies have found that concentration of Se has an inverse association with risk of cancer. In the present study, the level of Se in soil from high-risk areas for EC was significantly higher than in low-risk areas ($P< 0.002$). This suggests that Se level in soil could be a

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possible risk factor for EC in this area [11, 15]. Consistent with previous studies, results of the present study indicated that contamination of the environment (soil, rice, etc.) by xenobiotics including heavy metals and Se might be a possible risk factor for EC in this area [11]. Thus, it should be considered when designing cancer control programs in this area. Moreover, regular monitoring is required in developing countries such as Iran that use traditional farming methods.

CONCLUSION

The present study shows that rice from some areas of the Golestan Province is highly contaminated by heavy metals. Most heavy metals are present in the lowland and flat areas, because of high application of chemical fertilizers and pesticides. Since Golestan Province is a high-risk area for cancer and consumption of heavy metals has several adverse health effects, consumption of rice containing high concentrations of heavy metals could further increase the risk of cancer in this area. Regular monitoring is required to control the level of heavy metals in the environment and crops in this area..

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