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Association between selenium, cadmium, and arsenic levels and genetic polymorphisms in DNA repair genes (XRCC5, XRCC6) in gastric cancerous and non-cancerous tissue

Mahdieh Safarzad[a], Sima Besharat[b,**], Saeedeh Salimi[c], Ramin Azarhoush[b], Naser Behnampour[d], Hamid Reza Joshaghani[e,b,*]

[a] Metabolic disorders research center, Golestan University of Medical Sciences, Gorgan, Iran
[b] Golestan Research Center of Gastroenterology and Hepatology, Golestan University of Medical Sciences, Gorgan, Iran
[c] Cellular and Molecular Research Center, Zahedan University of Medical Sciences, Zahedan, Iran and Department of Clinical Biochemistry, School of Medicine, ZahedanUniversity of Medical Sciences, Zahedan, Iran
[d] Biostatistics Department, Faculty of Health, Golestan University of Medical Sciences, Gorgan, Iran
[e] Laboratory sciences research center, Golestan University of Medical Sciences, Gorgan, Iran

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ABSTRACT

Gastric cancer is one of the most prevalent cancers in northern Iran. The DNA repair genes X-ray repair cross-complementing (XRCC) group 5, XRCC6, which are important members of non-homologous end-joining repair system, play an important role in repairing the DNA double-strand breaks. Chronic exposure to heavy metals has long been recognized as being capable of augmenting gastric cancer incidence among exposed human populations. Since trace elements could directly or indirectly damage DNA, and polymorphism in DNA DSBs-repair genes can alter the capacity of system repair, we assumed that XRCC5 VNTR and XRCC6-61C > G polymorphism also impress the DSBs-repair system ability and contribute to gastric cancer. Therefore, the objective of this research was to evaluate the tissue accumulation of Selenium (Se), Cadmium (Cd) and Arsenic (As), and XRCC5 VNTR, XRCC6-61C > G polymorphisms in cancerous and non-cancerous tissues in Golestan province. The study population included 46 gastric cancer patients and 43 cancer-free controls. Two polymorphisms of XRCC5, XRCC6 were genotyped using polymerase chain reaction (PCR) or polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Further employed was atomic absorption spectroscopy so as to determine the levels of Se, Cd and As. Finally, the data were analyzed by SPSS (version 16) statistical software. The Se level was significantly higher in tumors as compared to non-tumor tissues, but there was no significant correlation between As and Cd in cancerous and noncancerous tissues. Allele frequencies of the selected genes were not statistically different between groups regarding XRCC6 (-61C > G). XRCC5 0R/0R, 0R/1R, 1R/1R, and 0R/2R genotypes were more common in cancerous group. High levels of Se in cancerous tissues vs. non-cancerous tissues may be one of the carcinogenic factors; in Golestan province, unlike other regions of Iran and the world, the level of Se is high, hence the higher risks of gastric cancer.

1. Introduction

Gastric cancer is one of the most prevalent cancers worldwide, and the third major cause of cancer mortality, especially in developed countries [1]. Regions in the north and northwest of Iran are at a high risk of developing gastric cancer; Ardabil has the highest incidence of gastric cancer in Iran. Semnan, Golestan and East Azerbaijan provinces, as well as Tehran's urban areas, have high rates of gastric cancer in both men and women [2–7]. Several factors play roles in gastric cancer, among which, genetic factors are considered as very important agents through the modulation of DNA repair [8–10]. Helicobacter pylori infection, dietary factors, smoking and alcohol, occupational exposure

* Corresponding author at: Laboratory Science Research Center, Golestan University of Medical Sciences, 60 Kola Road, Falsafi Building, P.O:49165-513, Gorgan, Iran.
** Corresponding author at: Golestan Research Center of Gastroentrology and Hepatology, 3rd floor, Salim heart complex, Sayyad-e-Shirazi hospital, Sayyad-e-Shirazi Boulevard, Gorgan city, Golestan province, Iran.
E-mail addresses: S_besharat_gp@yahoo.com (S. Besharat), joshaghani@goums.ac.ir (H.R. Joshaghani).

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such as cadmium and lead are other leading factors in gastric cancer incidence [11–13]. Trace elements such as Selenium, Cadmium and Arsenic are among cancer risk factors.

Cadmium is a ubiquitous, toxic and non-essential heavy metal [14–16]. Approximately 3–10% (average 5%) of the ingested cadmium is absorbed from the gastrointestinal tract [17]. Following absorption, Cadmium is transported into different organs by certain proteins, especially divalent metal transporter 1 (DMT1) and albumin [18,19].

An important biochemical consequence of exposure to cadmium is oxidative stress, where reactive oxygen species (ROS) and lipid peroxidation are generated [20–22]. Cadmium reduces the antioxidant activity of various enzymes such as glutathione peroxidase [23–25]. The main carcinogenesis mechanism of cadmium may be the suppression of gene expression, inhibition of DNA damage repair, and consequently, increased genomic instability, apoptosis inhibition, and oxidative stress induction [26,27].Certain studies have shown that cadmium, in addition to testicular and breast cancer, cause stomach, kidney and liver cancer, because liver, kidney and stomach are target sites for cadmium carcinogenesis in humans [28].

Cd^{2+} can affect E-cadherin function by replacing Cd^{2+}; as a result, the cell junctions are disrupted, affecting cell-cell junctions which may indicate a key step in both cancer incidence and tumor-promoting properties of Cd [29]. Cd disrupts tight cell junctions by affecting the function of E-cadherin, consequently triggering ß-catenin-mediated activation of oncogenes in epithelial cells. The affected function of E-cadherin by Cd reduces mucus thickness, mucous content, and basal acid output, and increases lipid peroxidation, disrupting the gastric mucosal barrier [29–31].

Cd affects DNA repair and acts as an antagonist to alter the Cd/Zn ratio in protein p53 (a tumor suppressor). Zn can be replaced by Cd, thereby disrupting p53 binding activity and repair-related processes, which in turn, induces high error rates and ineffective DNA repair [32].

Cd can replace iron and copper in different cytoplasmic and membrane proteins, thereby increasing the amount of unbound, free, or chelated copper and iron ions, which then take part in oxidative stress via Fenton reactions. Also, Cd acts as a mitogen, inhibiting DNA methylation, stimulating cell proliferation, inhibiting apoptosis and DNA repair, and promoting cancer in a number of tissues [33].

Selenium (Se) protects cells against the toxic effects of ROS via its antioxidant properties [34]. Se compounds may have anti-cancer effects at low concentrations, while at higher concentrations, they can be genotoxic and possibly carcinogenic. Se compounds' toxicity is attributed to the production of ROS inducing damage of DNA Single-Stranded Binding-proteins (SSBs) and Double-Stranded Binding-proteins (DSBs) [35].

Inorganic form of Arsenic is highly toxic and considered as Group 1 Carcinogenic to humans [36,37]. The precise mechanism of the relationship between iAs and cancer is still unknown, but it has been shown that iAs can interfere with a number of biological processes, such as chromosomal damage, DNA methylation and DNA repair, further producing ROS [38–40].

Heavy metals, such as Cd and As, are common exogenous sources of ROS, which generation causes gene abnormalities, gastric mucosal and DNA lesions, all of which lead to various GI diseases, including GI cancers [41,42]. Studies have also shown that chronic As exposure via the generation of oxidative stress causes genomic instability, such as DNA lesions, defects in DNA repair, and dysfunction in telomere and mitosis [42].

As can lead to the survival of damaged cells by inducing proapoptotic gene silencing or mutation [43]. In addition, heavy metals such as As can induce the expression of microRNAs, promoting tumorigenesis [44].

DNA repair mechanisms protect the genome from environmental and endogenous agents that attack DNA integrity [45]. Deleterious DSBs breaks may be repaired through non-homologous end joining (NHEJ) or homologous recombination repair (HRR) mechanisms [46]. In humans, NHEJ have predominant roles in DSBs repairing [47].

Several proteins are involved in the NHEJ pathway encoded by certain members of X-ray repair cross-complementing (XRCC) gene family such as XRCC5 (KU80), XRCC6 (KU70), and XRCC7 (DNA dependent protein kinase (DNA-PK)) [48–50]. One of the key components of NHEJ repair machine is DNA-PK, which includes a DNA binding subunit called the KU complex and a DNA-PKcs subunit. KU70 and KU80 form a KU complex that is a DNA-linked heterodimer complex acting as a vital member of the NHEJ restorative pathway. KU80 and KU70 are expressed by XRCC5 and XRCC6 genes, respectively. DNA-PK subunit is also expressed by the XRCC7 gene [51,52]. Four genetic variations in NHEJ genes, such as single nucleotide polymorphism (SNP), may prevent the cell from monitoring the checklist and entail inadequate DNA repair, causing DNA damage accumulation, and ultimately form a tumor.

There are numerous genetic polymorphisms in XRCC5 and XRCC6 genes that influence DNA repair capacity and ensue predisposition to several cancers such as gastric, head and neck and skin cancers [53–55].

Since trace elements are capable of directly or indirectly damaging DNA [56], and polymorphism in DNA DSBs-repair genes can alter the capacity of system repair [57], we assumed that XRCC5 VNTR and XRCC6-61C > G polymorphism may also impress the DSBs-repair system ability and conduce to gastric cancer. Therefore, in this paper, we evaluated the tissue accumulation of Se, Cd and As, and XRCC5 VNTR, XRCC6-61C > G polymorphisms in cancerous and non-cancerous gastric tissues in Golestan province.

2. Material and method

2.1. Ethical considerations

This study was approved by the local ethical committee of Golestan University of Medical Sciences (IR.GOUMS.REC.1395.261). All patients admitted due to gastrointestinal complains at academic hospital in Gorgan (5th Azar Hospital), Golestan University of medical sciences between 2014–2018. Those candidates suspicious to gastric cancer (N = 89) have been included. Informed consents have been taken before endoscopy.

2.2. Sample collection

Eighty-nine archived formalin fixed paraffin embedded (FFPE) tissue were selected among samples that had been taken by endoscopy from patients admitted due to gastrointestinal complains between 2014–2018 in our academic hospital in Gorgan (5th Azar Hospital), Golestan University of Medical Sciences.

In department of Pathology, 46 gastric cancers were confirmed by expert pathologist and 43 other samples were gastritis that has been selected as control group.

The specimens included 46 gastric cancer FFPE blocks (33 male, 13 female) mean age 56.27 ± 15.5 years and 43 free gastric cancers FFPE block(27 male, 16 female), mean age 51.7 ± 17.2, that were age/sex-matched with gastric cancer patient selected as control group.

2.3. Preparation of samples

For each tissue block, 10-μm sections were cut from each specimen; a new sterile blade was used for each one (outer sections were discarded). Four or five scrolls from serial sections were placed in 2 different Eppendorf tubes. All specimens were cut to very small pieces by a bistoury blade.

2.3.1. Deparaffinization and digestion of FFPE Tissue Cores had been done for trace element assay

(1) Deparaffinization has been carrying out in 1.5 ml tubes, and then 1 ml n-octan was added to the tissue core, vortexing vigorously for 10 s and heated for 3 min at 50 ˚C.
(2) Centrifuging tubes for 2 min (at RT and 11,000 g) then placed on ice for 5 min (allows the waxy residue to solidify on the top).
(3) Collected paraffin was removed by a pipette tip and n-octan treatment had been repeated (steps 2.2.1.2-2.2.1.3).
(4) 1 ml ethanol (96%) has been added, vortex vigorously for 10 s, centrifuged for 2 min at RT (maximum speed), and ethanol has been carefully discarded. This step has been repeated once again.

After deparaffinization, each tissue has been weighted and placed in the test tube. Then100 µl concentrated nitric acid and 100 µl hydrogen peroxide (35%) was added to a known mass of tissue (0.1-0.5 mg) in the test tube. After 1 min vortex vigorously tubes have been placed in heater for 24 h at 50 ˚C. After 24 h, if there were cell debris the samples centrifuged for 2 min. Upper phase (containing tissue digestion components) has been transferred to a new tube. These were subsequently diluted with distilled deionized (DD) water as needed.

2.3.2. Reagents and glassware

Ultra-pure water was used throughout the work. Concentrated nitric acid (65%), hydrogen peroxide (30%) and other chemicals were used as analytical reagent-grade obtained from E. MERCK.co. Standard solutions for the calibration curve made from the standard solution of Se, As (Chem Lab) and Cd (MERCK) have been prepared immediately before their use; by stepwise dilution of the certified standard solution (1000 ppm) with HNO3. The modifier used for stabilization was palladium (II) chloride. All glassware were soaked for 24 h in 10% nitric acid, washed with distilled water and finally rinsed with ultra-pure water, then dried and stored in laminar hoods.

2.3.3. Determination of Se, Cd, As

Se, Cd and As concentrations have been assayed by Agilent SpectrAA-240 Z atomic absorption spectrometer equipped with Zeeman background correction and fitted with a PSD-120 sampler by graphite furnace and graphite tubes with integrated L'vov platform. Se, Cd, and As were measured at 196.0 nm, 228.8 and 193.7 nm wavelengths, respectively. The calibration graph for Se, Cd and As was prepared from standards 0–40 µg/L, 0–4 µg/L and 0–50 µg/L, respectively.

2.4. Genomic DNA extraction and genotyping

2.4.1. DNA isolation

Genomic DNA was extracted by the MACHEREY-NAGEL (tissue DNA EXT NucleoSpin® DNA FFPE XS) kit from the FFPE samples of patients and controls as per the manufacturer's protocol. Extracted DNA was stored at -20∘c.

2.4.2. PCR and PCR-RFLP procedure

The XRCC5 VNTR polymorphisms (0R, 1R, 2R, 3R) and XRCC6-61C > G were genotyped by using PCR and PCR-RFLP(Polymerase chain reaction- restriction fragment length polymorphism) techniques with XRCC5-f/-r and XRCC6-f/-r primers, which were designed by Wang et al [46]. PCR was performed in a total volume of 20 µl containing 12 µl Taq DNA Polymerase Master Mix- Ampliqon, 1 µl (10 pM) of each forward and reverse primers, 2 µl gDNA (˜100 ng) and the total reaction volume was raised to 20 µl with DD water. PCR products were electrophoresed on the 2.5% agarose gel. XRCC5 VNTR polymorphism contains four different alleles; zero repeat (0R), one 21 bp repeat (1R), two 21 bp repeats (2R) and three 21 bp repeats (3R); the size of fragments was 224, 245, 266 and 287 bp for, respectively. DNA segment of the XRCC6 -61C > G gene was applied in PCR-RFLP technique according to Jahantigh [58]. The PCR product of XRCC6-61C > G polymorphism size was 320 bp digested to 240 and 80 bp fragments for G allele by BanI (Fermentas Co.). There was no BanI cleavage site for C allele and we had a single 320 bp band.

Table 1
Mean and standard deviation of Se, Cd, As, frequency of XRCC5 VNTR polymorphisms and comparison of the frequency distribution of XRCC6 polymorphism between cancerous and non-cancerous tissue.

	case	control	p.value
element			
Se[1] (µg/g), Mean ± SD*	1.175 ± 0.657	0.696 ± 0.700	0.0001
Cd[2] (µg/g), Mean ± SD	0.500 ± 0.401	0.518 ± 0.425	0.663
As[3] (µg/g), Mean ± SD	0.096 ± 0.13	0.145 ± 0.243	0.587
Frequency of XRCC 5 Genotype			
0R/0R	4.3%(2)	%(0)0	
0R/1R	6.5 %(3)	2.3 %(1)	
0R/2R	4.2%(2)	2.3%(1)	
1R/1R	19.6%(9)	9.3%(4)	
1R/2R	45.7%(21)	58.1%(25)	
1R/3R	4.3%(2)	4.7%(2)	
2R/2R	13.0%(6)	20.9%(9)	
2R/3R	2.2%(1)	2.3%(1)	
Frequency of XRCC 6 Genotype			
CC	52.2%	42.9%	
CG	43.5%	42.9%	
GG	4.3%	14.3%	
			0.374

1.selenium, 2.cadmium, 3.arsenic / * standard deviation.

2.5. Statistical methods

Statistical evaluation was conducted using descriptive analysis, normality test (Shapiro–Wilk), and Mann-Whitney test. The data analysis was performed using SPSS version 16 (SPSS Inc., IL, USA). The significance level was less than 0.05.

3. Results

The study samples included 46 gastric cancer and 43 (age/sex-matched) free gastric cancers FFPE blocks (described in Section 2.1).

Results showed a significantly higher mean (SD) concentration of Se in cancerous tissues compared to the non-cancerous tissues ($p = 0.001$), but no significant differences concerning Cadmium and Arsenic concentrations in cancerous and non-cancerous tissues ($p > 0.05$) (Table 1).

There was no significant difference regarding Selenium, Cadmium and Arsenic concentrations between males and females in both case and control groups. Spearman's correlation coefficient showed a significantly direct relationship between age and cadmium concentration in the control group (P-Value = 0.031) (Fig. 1).

The allelic and genotypic distributions of XRCC5 VNTR polymorphism and XRCC6 SNPs in the studied population are listed in Table 1. All loci were in the Hardy–Weinberg equilibrium.

Among 10 probable genotypes of XRCC5 VNTR polymorphism (rs6147172), 8 genotypes were observed in this study. For VNTR XRCC5 polymorphism, in comparison with the 0R/0R, 0R/1R, 1R/1R, 0R/2R and 1R/3R, 2R/2R, 2R/3R (OR = 3.556; 95% CI = 0.993–12.733; P-value = 0.051) genotypes, the 1R/2R (OR = 3.175; 95% CI = 1.053–9.567; P-value = 0.04) genotype more significantly increased the risk of gastric cancer.

Based on the number of replicates, the VNTR polymorphisms of the XRCC5 gene were divided into three groups; Group 1: repeat number less than 1R/2R (including 0R/0R, 0R/1R, 1R/1R, and 0R/2R), Group 2: 1R/2R, Group 3: replicate number of more than 1R/2R (including1R/3R, 2R/2R, and 2R/3R).

For XRCC6 polymorphism, by considering CC as reference genotype,

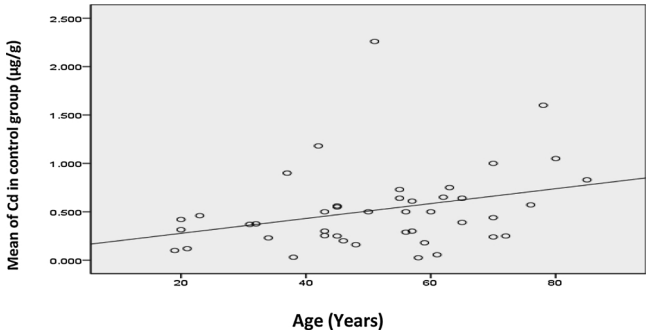

Fig. 1. The relationship between age and Cd level (µg /g) in control group; Spearman's correlation coefficient showed a significantly direct relationship between age and cadmium concentration in the control group (P-Value = 0.031).

CG (OR = 0.83; 95% CI = 0.278–2.499; P-value = 0.745) and GG (OR = 0.25; 95% CI = 0.036–1.751; P-value = 0.163) genotypes were less observed in gastric cancer.

There was no significant relationship concerning XRCC6 (-61C > G), XRCC5 VNTR polymorphisms and Cadmium, Arsenic levels between the case and control groups (P-Value > 0.05). However, there was a significant relationship between Selenium levels and three groups of XRCC5 genotypes (Fig. 2) and between CG genotype and Selenium levels (Table 2).

As shown in Fig. 2, in the patients group, the subject with 0R/0R-0R/2R genotype had the highest Selenium levels, while in the control group, the subject with 1R/2R had the highest Selenium levels. Also, the high levels of Selenium in the individual with CG (xrcc6) genotype is more likely to lead to gastric cancer.

4. Discussion

The present study showed that Selenium levels in gastric cancerous tissues were significantly higher than the control group (1.75 vs 0.69 µg/g), while Arsenic and Cadmium levels were higher in healthy tissues compared with gastric cancerous tissues, which was not statistically significant.

Extensive studies have shown that Selenium is an anti-cancer agent whose deficiency entails the onset and development of cancer [59].

In a cohort study, Steevens J et al. observed a significant reverse relationship between the level of nail Se and incidence of gastric and esophageal cancers [60]. In a prospective cohort study in Finland, Knekt et al. observed that gastric cancer was reduced in subjects with high serum Se concentrations, prior to the administration of selenium in

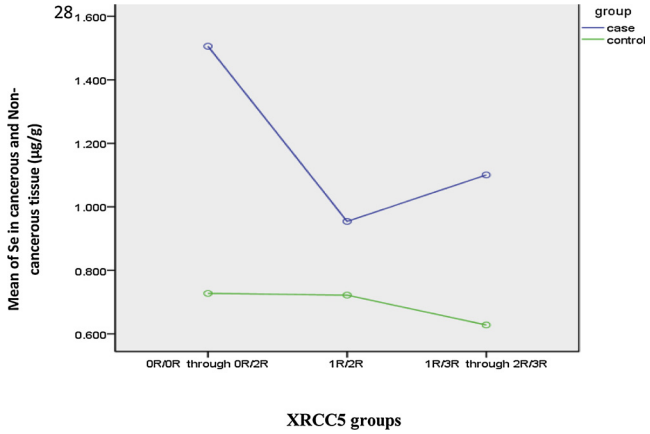

Fig. 2. Relationship between selenium levels and three groups of XRCC5 genotypes in the case and control group.

Table 2
Mean and SD of selenium according to XRCC5 and XRCC6 CG genotype.

	Se (µg/g) Mean ± Sd*		
	Case	control	p-Value
Genotypes			
XRCC5			
0R/0R-0R/2R	1.505 ± 0. 838	0.727 ± 0.575	0.040
1R/2R	0.954 ± 0.431	0.721 ± 0.842	0.010
1R/3R-2R/3R	1.100 ± 0.539	0.627 ± 0.405	0.015
XRCC6			
CG	1.270 ± 0.821	0.618 ± 0.558	0.030

* Standard Deviation.

a low-selenium area [61]. Hashemi and his colleagues in Zahedan (a county in Sistan and Baluchestan Province in Iran) evaluated the serum Se levels and Zinc in patients with esophageal and gastric cancer; they observed that selenium levels in patients were significantly ($P < 0.05$) lower than control groups, concluding that lower levels of Se can affect the incidence of cancer. Hosseini Nezhad et al., in a similar study with Hashemi, found that the low levels of serum Se in Kerman province (in the southeast of Iran) can be related to gastric cancer.

However, studies focusing on the Se level in Golestan province, have proposed that serum Se levels in the soil of this region are higher than other regions of Iran [62,63]; certain ecological studies have also shown that the mean of Se in areas with a high risk of esophageal cancer is significantly higher than low risk areas, and the amount of Se in Golestan province soil is more than allowed [62]. Furthermore, Nouarie et al. showed that Se median (IQR) in Golestan province(155 µg / l) was higher than Ardebil, Kerman and Mazandaran provinces, concluding that the high levels of Se may be a risk factor for gastric cancer in Golestan province [63].

Despite the fact that the tissue levels of trace elements is a more accurate index for chronic contact with these elements, few studies have reported Se levels in human gastric tissues, most of which have assessed the serum levels of Se in gastric cancer patients; in some of these studies, serum Se levels were significantly lower compared to the healthy controls [64,65]. Charalabopoulos et al. reported the serum Se level in gastric cancer patients to be lower than the control group (43µg/l vs 68.7 µg/l), but Se in gastric cancerous tissues was much higher than healthy tissue (2640 mg/g vs 685 mg/g) [66]; some studies have shown that in a variety of cancers, there is an inverse relationship between serum Selenium levels and neoplastic growth. It has further been suggested that the process of tumor development can lead to selenium absorption by malignant cells [67–69]. Higher concentrations of Se in tumor compared to healthy tissues in the present research and Charalabopoulos study may be due to these findings.

High levels of Se in gastric cancer tissue may be due to the high levels of Se in soil and serum province's inhabitants, causing the accumulation of this element in the tissues.

Our study revealed that Se levels in cancerous tissue were higher than non-cancerous tissues; it seems that the deficiency of this element in Golestan province cannot be a cause of cancer, rather it can be hypothesized that high levels of selenium not only inhibit cancer, but may also be its facilitator (especially GI cancer) in this province.

In this study, Cd concentrations in non-cancerous gastric tissues were higher than cancerous tissues (0.51 vs 0.5µg/g), a difference not much significant. Yaman et al. showed that the mean of Cd concentration in non-cancerous gastric tissues was higher than the cancerous tissues (65 vs 51 ng/g respectively), which was not statistically significant[70].

One reason for the slight increase in Cd level in control group may be the occupational poisoning of these individuals; we did not investigate the occupation of the subjects, or the consumption of high cadmium concentration products. However, previous studies have

found positive associations between Cd and risk of various types of cancer. Ostadrahimi et al. examined the association between serum and urinary concentrations of cadmium and the risk of gastrointestinal cancer. Cd levels in the cancer group were significantly higher than the control group (P = 0.037). However, the results of the multivariate regression model showed no significant relationship between the concentration of cadmium in the blood and the risk of gastrointestinal cancers, while there was a significant correlation between urinary cadmium concentrations and the risk of gastrointestinal cancers [71]. Unlike tissue, blood and urine concentrations of Cadmium cannot indicate chronic contact with cadmium.

Our results revealed that Cd and As levels were higher in the control group compared with the cases. On the contrary, RimKhlifi et al. showed that the blood levels of As and Cd in head and neck cancer cases were respectively 3.5 and 4.7 times higher than those of controls; like our finding (Fig. 1), they further showed that Cd levels in controls increased significantly with age (P-Value = 0.031). Elevated As and Cd levels in human blood might be attributed to the occupational exposure, but Cd and As are rapidly and extensively cleared from the blood via the kidney. Cd and As concentrations in blood have been considered to reflect only recent exposures, and the tissue levels of these elements is a better and more accurate indicator of chronic exposure.

Arsenic is a known carcinogen and is classified as carcinogenic in group 1 [72]. Gastric is more exposed to Arsenic in the diet, hence absorbed in the gastric and affected by its presence.

Kohzadiet al. measured the tissue levels of certain elements (Ca, Cu, Fe, As, Mg, Ni, Cd, Cr) in three different types of gastric tissues; cancerous, non-cancerous gastric tissues of gastric cancer patients and healthy gastric tissues of the control group. Concentrations of Cd and As in cancer patients were higher than healthy subjects; 0.22, 0.21, and 0.2 mg/kg were the mean of Cd concentrations and 1.76, 0.709, and 0.24 mg/kg were the mean of As concentrations in the cancerous tissues from cancer patient, normal tissues from cancer patient, and normal tissues from the normal patients, respectively; but there was no significant difference between the levels of Cd and As in the three tissue groups [73], findings slightly different from the present research where the mean of Cd and As in the control group was slightly higher than patients. Also, in the study conducted by Reddy et al. As was higher in cancerous tissues [74]. According to the findings of Ponomarenko et al. [75], it can be concluded that high concentrations of selenium can play an important role in discharging Arsenic from various organs of the body; since in our study, Selenium and Arsenic concentrations were respectively higher and lower in the case group compared with the control group, it can be concluded that low Arsenic concentrations in the case group may be due to the high concentrations of Selenium in these individuals.

We have no absolute knowledge regarding the patients and controls exposed to Cadmium and Arsenic. However, we know various sources of heavy metals including soil erosion, natural weathering of the earth's crust, mining, industrial effluents, urban runoff, sewage discharge, insects or disease control agents applied to crops, and many others [76]. Since Golestan province has a moderate and humid climate, agriculture is the main occupation; it seems that a possible way for these elements to enter is agriculture and contaminated agricultural products [77].

Regarding XRCC5 VNTR polymorphism, the present research showed that 1R / 2R genotype was the common genotype in both patient and control groups. It should be noted that there was no association between the genotypes of VNTR polymorphism and gastric cancer (see Table 3). 0R/0R, 0R/1R, 1R/1R, and 0R/2R genotypes compared to 1R/2R, 1R/3R, 2R/2R, and 2R/ 3R genotypes were more observed in gastric cancer group. In other words, gastric cancer patients had lower repeats.

Saadat et al. investigated the associations between the polymorphisms of VNTR in the promoter region of XRCC5 and the risk of gastric cancer in Shiraz (Fars province, south of Iran). In their study, 1R / 2R genotype was the common genotype in both patients (41.5%) and control group (41.7%). They divided the alleles of the VNTR XRCC5 polymorphisms into two groups of L (0 and 1 repeat) and H (2 and 3 replicates), where they found that the gastric cancer risk was more in individuals with LH + HH genotype with a positive family history of gastric cancer, compared to the reference group (those without a family history of gastric cancer or genotype LL) [78]. The results of Saadat et al. are to some extent different from the current research as it did not consider the family history of gastric cancer; moreover, we found that gastric cancer group had fewer repetitions (0 R / 0R, 0R / 1R, 1R / 1R, 0R / 2R) compared to the control group (Table3).

Table 3
Comparison of the frequency distribution of XRCC5 gene polymorphisms in both case and control groups based on the number of replications.

Genotype			
	0R/0R-0R/2R	1R/2R	1R/3R - 2R/3R
Group			
Case(n = 46)	34.8%	45.7%	19.6%
Control(n = 43)	14.0%	51.7%	27.9%
p-Value		0.073	

XRCC5 VNTR polymorphisms have been examined in other cancers; Rajaei et al. determined XRCC5 VNTR polymorphisms in women with breast cancer and a healthy group in Shiraz. Their findings showed that 0R / 0R, compared with 1 R / 1R genotype, increased the risk of breast cancer (OR 9.55, 95% CI 1.19–76.64, P = 0.034), while 1R / 3R, compared with 1R / 1R genotype, reduced the risk of breast cancer; they concluded that VNTR polymorphism in the XRCC5 promoter region is associated with the risk of breast cancer. They further observed that 1R/2R genotype was the most abundant genotype in the case and control groups [79]. In other words, Rajaei's and our findings indicate that the frequency of lower repeat genotypes is higher in the cancer group. Regardless of the chance of contracting cancer, according to previous studies on XRCC5 VNTR polymorphisms in Iran, 1R / 2R genotype is a common genotype in Iranian population.

In addition to Iranian populations, XRCC5 VNTR polymorphism has been studied on other population. Gorre et al. studied the association of XRCC5 VNTR polymorphism with the development of chronic myeloid leukemia in India, where the most frequent genotypes in the case and control groups were 1R/2R (37.96%) and 1R/1R (35.05%), respectively. Based on Gorre, Rajaei, and our results, it can be concluded that the risk of cancer is elevated in individuals carrying lower repeats.

According to the previous studies, most frequent alleles of XRCC5 VNTR in Iran are 1R and 2R, which is closer to that of the Indian populations, but different from the frequencies reported in the Chinese populations [80,81].

In our study, there was no significant difference regarding XRCC6 (-61C > G) polymorphism between the case and control group. Compared with CC as a wild type genotype, CG and GG were more seen in the control group.

In addition to gastric cancer, XRCC6 (-61C > G) polymorphism has been assayed in other cancers, including acute myeloid leukemia, esophageal squamous cell carcinoma and colorectal cancer.

Wang and his colleagues showed that there was a significant decrease in the risk of AML associated with the XRCC6 − 61 CG/GG genotype (OR = 0.55; 95%, CI = 0.34-0.89) compared with the −61CC genotype. Contrary to the present research and Wang's findings, Li and his colleagues showed that CG carriers were at higher risks of esophageal squamous cell carcinoma (ESCC) (p = 0.001, OR = 2.040, 95% CI = 1.323–3.147). G allele carriers were also associated with an increased ESCC risk (p = 0.003, OR = 1.868, 95% CI, 1.230–2.836) [82,83]. Our results are incompatible with the findings of Li, yet consistent with the results obtained by Wang and his colleagues. Moreover, DIMBERG et al. did not find any significant association between XRCC6

polymorphism and colorectal cancer in a Swedish population. In their study, C/G genotype was the most common genotype in both cases (52.9%) and control (46.6%) groups. Certain studies in Iran have further shown that C/G genotype is the most abundant genotype [58]. However, in our population, CC genotype with 50% frequency and CC/CG genotype with 42.9% frequency were the most common genotypes in the case and control groups, respectively. These differences may be due to the variation in environmental exposures or the ethnic origin of the studied populations.

In our study, there was no significant relationship concerning different genotypes of VNTR XRCC5 polymorphism and Cd and As between the case and control groups. But, there was a significant association between Selenium level and all three groups of genotypes, a relationship not found in any previous studies. As shown in Fig. 2, the patients with fewer repetitions $_{(0\ R\ /\ 0R,\ 0R\ /\ 1R,\ 1R\ /\ 1R,\ 0R\ /\ 2R)}$ genotypes had the highest mean of Se, and vice versa. According to these findings, in subjects with 0 R / 0R, 0R / 1R, 1R / 1R, 0R / 2R genotypes, the increase in the levels of Se seem to increase the chances of developing cancer.

5. Conclusions

Due to the mortality, social and health burdens induced by gastric cancer, identifying the risk factors is of high importance. Environmental factors such as heavy metals are the most critical risk factors, particularly because they are preventable. Trace metals have been proposed as gastric cancer risk factors due to their biochemically carcinogenic effects. An ecological study in Golestan province showed that Se content of Golestan province soil was more than allowed; moreover, the serum Se median (IQR) in the residents of this region was higher (155 μg / l) than other parts of Iran [63]. In our study, high levels of Se in gastric cancer tissues may be due to high Se content in both Golestan province's soil and the serum of the province's inhabitants, causing the accumulation of this element in tissues. Unlike other regions of Iran and the world where Se deficiency may be a risk factor for gastric cancer, it seems that the high levels of Se, acting as antioxidant agents, could be a carcinogenic factor in Golestan population. There was an inverse relationship between Se and As levels in the case and control groups. It can be concluded that high concentrations of selenium can play an important role in discharging arsenic from various organs of the body. High levels of Se in subjects with 0R/0R-0R/2R genotype for VNTR XRCC5 and CG (xrcc6) genotype may play an important role in the development of gastric cancer.

Golestan province has high Se levels in soil, agricultural products, and the serum and gastric tissues of its inhabitants; it is therefore recommended that agricultural organizations import agricultural products from provinces with lower levels of selenium, and vice versa. Moreover, physicians and specialists should check the amount of Se in the blood prior to administrating it, and Se is to be supplied in controlled amounts to avoid harmful effects.

Declaration of competing interest

The authors declare that they have no competing interests.

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