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Epidemiology

Lead, selenium and nickel concentrations in epithelial ovarian cancer, borderline ovarian tumor and healthy ovarian tissues

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ABSTRACT

Objective: Wide variation exists in ovarian cancer incidence rates suggesting the importance of environmental factors. Due to increasing environmental pollution, trace elements and heavy metals have drawn attention in studies defining the etiology of cancer, but scant data is available for ovarian cancer. Our aim was to compare the tissue concentrations of lead, selenium and nickel in epithelial ovarian cancer, borderline tumor and healthy ovarian tissues.

Methods: The levels of lead, selenium and nickel were estimated using atomic absorption spectrophotometry in formalin-fixed paraffin-embedded tissue samples. Tests were carried out in 20 malignant epithelial ovarian cancer, 15 epithelial borderline tumor and 20 non-neoplastic healthy ovaries. Two samples were collected for borderline tumors, one from papillary projection and one from the smooth surface of cyst wall.

Results: Pb and Ni concentrations were found to be higher both in malignant and borderline tissues than those in healthy ovaries. Concentrations of Pb and Ni in malignant tissues, borderline papillary projections and capsular tissue samples were not different. Comparison of Se concentrations of malignant, borderline and healthy ovarian tissues did not reveal statistical difference. Studied metal levels were not found to be different in either papillary projection or in cyst wall of the borderline tumors.

Conclusions: This study revealed the accumulation of lead and nickel in ovarian tissue is associated with borderline and malignant proliferation of the surface epithelium. Accumulation of these metals in epithelial ovarian cancer and borderline ovarian tumor has not been demonstrated before.

1. Introduction

It is well recognized that carcinogenesis is a multistep process that requires genetic and epigenetic alterations facilitated by multiple environmental effects. Due to the global burden of cancer, several epidemiologic studies have been undertaken to identify the risk factors. Trace elements and heavy metals have drawn attention as a considerable and potentially modifiable environmental factor. WHO described 19 trace elements as being critical for human health. These elements can be found naturally in various sources such as ground water, air and food [1]. Insufficiency of the trace elements adopted as essential for homeostasis such as cofactors, as well as accumulation of some toxic metals may disrupt the host resistance against cancer. For example, selenium (Se) has a role in counteracting oxidative stress due to being a substantial component of selenoproteins and some epidemiologic studies suggest the inverse relation between Se and cancer [2–4]. Arsenic, nickel (Ni), cadmium have been designated as group 1 human carcinogens by International Agency for Research on Cancer (IARC) and identified in several malignancies [5]. Lead (Pb) is also defined as a potentially toxic element for human body. Wastes from domestic, industrial and commercial sources may contain various metals and contaminate the soil and water sources such as ground water and rivers.

Ovarian cancer has the highest mortality rate among gynecological cancers and it is the seventh leading cause of cancer-related deaths in women [6]. Risk factors of ovarian cancer have not been clearly identified but up to five-fold international variation in its incidence suggest the importance of environmental factors. Epidemiological studies suggest that ovarian cancer is primarily a disease of the industrialized world [7,8]. There is a substantial interest in trace elements and heavy metals regarding carcinogenesis, but the data is

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limited for ovarian cancer.

The aim of this study was to compare the tissue concentrations of Pb, Se and Ni in healthy non-neoplastic ovaries, epithelial ovarian cancer and epithelial borderline tumor which carries an intermediate behavior between benign and frankly malignant neoplasms both in clinical and histopathological aspects. For this purpose, concentrations of Pb, Se and Ni were determined by atomic absorption spectrometry in formalin-fixed paraffin-embedded tissue samples. Despite the fresh tissue samples are always preferable, it is known that fresh and formalin-fixed tissues yield the same results for some trace metal analysis [9,10]. For example, Gellein et al. reported that Ni and Pb were leached from tissue to formalin with a negligible degree due to being strongly bounded to sulfhydryl groups [11]. Furthermore, Sato et al. showed no alteration in Se concentration of the tissue with formalin preservation, suggesting the limited diffusion [12]. Se is covalently bound within large molecules such as proteins and selenocysteins and withstands the tissue processing and embedding. To the best of our knowledge, this is the first study comparing metal concentrations in invasive epithelial ovarian cancer, borderline ovarian tumor and healthy ovarian tissues.

2. Materials and methods

2.1. Patient selection and collection of the samples

Paraffin embedded, 5 μm sectioned slides of 20 malignant epithelial ovarian cancer, 15 borderline ovarian tumor and 20 non-neoplastic ovarian tissues collected in between May 2011 and June 2014 were reviewed by the pathologist (author of the study) retrospectively. Eligible slides and corresponding paraffin blocks were retrieved from the archive of Pathology Department of XXX University School of Medicine. Institutional review board approved the study. Two distinct samples were selected for each borderline tumor, if present; one from the nodular or papillary projection (n = 15) and one from the smooth surface of the cyst wall (n = 14). In 1 borderline tumor, cyst wall was uniformly including papillary projections. Normal ovarian tissue samples were collected from the patients underwent hysterectomy and salpingo-oophorectomy for benign gynecological diseases other than any ovarian cysts or pelvic infections. A total of 69 tissue samples were analyzed.

2.2. Digestion and spectrometric analysis of the tissues

The paraffin blocks were deparaffinized in xylene at 55 °C for 1 h in the tissue processor. Following deparaffinization, tissue samples were washed with deionized water and weighed. Then tissues were digested by adding 3 mL mixtures of nitric acid-perchloric acid per 1 g tissue. After subsequent heating on electrical hot board at 120 °C until a dense smoke has evolved and periodic stirring, tissue samples were cooled to room temperature. Water used for the analysis was deionized and double distilled. All reagents used were highly pure grade. The concentrations of the elements were calculated on wet tissue basis and expressed as μg/g per tissue. According to each element measurement their diluents were used as blanks and commercial diluted standards were used for calibration curves and calculation of coefficient of variation (CV) and limit of detection (LOD). LOD and CV for each element are provided in Table 1 [13].

2.3. Lead (Pb) measurement

For Pb analysis all samples were diluted 400 μL of “Diluent” containing 0.25% Triton X-100, 2 g/L ammonium dihydrogen phosphate [NH4H2PO4], and 0.75 g/L magnesium nitrate [Mg(NO3)2]. The Pb contents of the samples were analyzed by Perkin-Elmer Analyst 800 atomic absorption spectrophotometer equipped with a graphite furnace and Zeeman background correction Perkin-Elmer, Norwalk, CT, USA. The calibration curves of standards were done with aqueous lead solutions custom-grade standard; Inorganic Ventures Inc. CN:CGPBI-B1, lot:W-PB02114, 1000 mg/L. The Pb standardization curve was linear in the range between 0.50 and 20.00 μg/dL. Element concentrations of the samples were calculated according to calibration curves.

2.4. Selenium (Se) measurement

Selenium measurement was made in graphite furnace atomic absorption spectrophotometer using Zeeman background correction. We used palladium (4 μg in 20 μL sample) and magnesium sulfate (3 μg in 20 μL sample) as matrix modifiers. Samples and calibration standards were diluted 1:3 (1 + 2) with 0.05% Triton X-100 to improve sample viscosity and reproducibility of the results. Selenium calibration standards were prepared from the commercial Se standard (1000 mg/L) by serial dilutions [14].

2.5. Nickel (Ni) measurement

All digested samples were diluted as 1:4 (1 + 3) with%0.2 HNO3. Commercial Ni standard was used (1000 mg/L) and serial dilutions were prepared. Samples were analyzed to produce a standard curve [15].

2.6. Statistical analysis

We compared the levels of studied elements in malignant, papillary projections and capsular samples of the borderline tumors and healthy ovarian tissues: (1) borderline capsular tissue vs borderline papillary tissue, (2) borderline capsular tissue vs malignant tissue, (3) borderline capsular tissue vs healthy ovarian tissue, (4) borderline papillary tissue vs malignant tissue, (5) borderline papillary tissue vs healthy ovarian tissue, (6) malignant tissue vs healthy ovarian tissue. The mean of the metal levels and standard error of mean (SEM) were calculated and parametric tests were used for group comparisons where possible, otherwise nonparametric test were carried out. P values lower than 0.05 were considered as statistically significant. Finally, we performed post hoc comparison tests and power calculations for the 3 groups as a whole and one-to-one basis.

3. Results

In cancer group, histological diagnosis was serous cystadenocarcinoma in 17 patients, mucinous cystadenocarcinoma in 1 patient, and clear cell carcinoma in 2 patients. In borderline tumor group, histological diagnosis was serous borderline in 6 patients, mucinous borderline in 8 patients and seromucinous tumor in 1 patient. Distribution of the element concentrations according to the tissue is presented in Table 1.

Tissue concentration of Se was below the LOD in 1 borderline tumor-capsular sample. Pb levels were below the LOD in 1 borderline tumor-papillary sample and 1 malignant tissue sample. These measurements were excluded from the statistical analysis. In 1 malignant tissue, Ni concentration was extremely high (30 μg/g) in comparison with distribution of the group (0.22–3.78 μg/g) and excluded from the analysis.

3.1. Pb

Pb concentrations were found to be significantly higher in malignant tissues (3.809 μg/g; range, 0.65–19.43) *than normal ovaries* (0.62 μg/g; range, 0.06–1.67) ($p < 0.001$). *Pb concentrations of both papillary and capsular samples of the borderline tumors were also higher than normal ovarian tissue* (Borderline capsule, mean 4.93 μg/g, range 0.34-20.99, $p < 0.001$; Borderline papillary projection, mean 1.96 μg/g, range 0.18–-6.65, $p < 0.001$). *No difference was observed in between papillary projections and capsular samples of the borderline tumors* ($p = 0.192$).

Table 1
Distribution of metal concentrations in the tissues; mean values + SEM (range).

	Borderline tumor- capsular tissue	Borderline tumor-papillary tissue	Malignant tumor	Healthy ovary	[a]LOD	[b]CV	p
Pb, μg/g	4.93 ± 1.76 (0.34–20.9)	1.96 ± 0.54 (0.18–6.65)	3.809 ± 1.22 (0.654–19.43)	0.62 ± 0.11 (0.06–1.67)	3.05	1.01%	p < 0.001
Ni, μg/g	3.11 ± 0.92 (0.24–9.68)	2.169 ± 0.53 (0.49–6.81)	2.704 ± 1.65 (0.22–3.78)	0.49 ± 0.07 (0.104–1.487)	4.25	1.42%	p < 0.001
Se, μg/g	0.74 ± 0.35 (0.005–3.305)	0.267 ± 0.07 (0.03–1.13)	0.477 ± 0.21 (0.04–3.396)	0.17 ± 0.05 (0.009–1.126)	5.86	7.82%	p = 0.231

[a] LOD: Limit of detection.
[b] CV: Coefficient of variation.

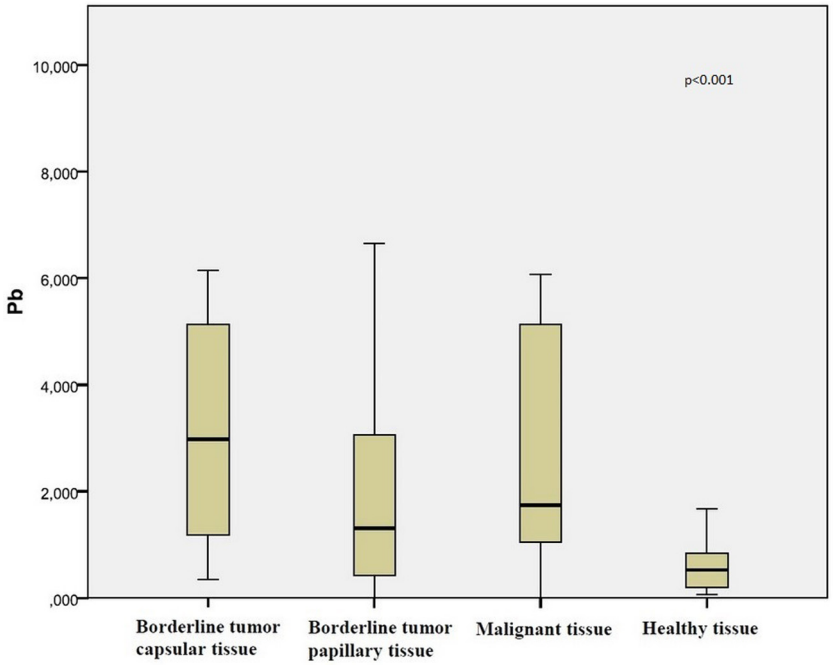

Fig. 1. Histogram representing Pb concentrations of the tissues. Pb concentrations were higher in malignant, borderline capsular and papillary tissue samples than healthy ovaries. No statistical difference among malignant and borderline tissue samples was observed.

Comparison of Pb concentrations of malignant and borderline tumors did not reveal any difference (malignant tissue vs borderline tumor capsule, p = 0.351; malignant tissue vs borderline tumor papillary projection, p = 0.25). Fig. 1 demonstrates the histogram representing Pb concentrations of the tissues.

3.2. Ni

Ni concentrations were higher in malignant tissue (2.704 μg/g; range, 0.22–3.78), *than healthy ovaries* (0.49 μg/g; range, 0.104–1.487) (p = 0.012). *No statistical difference was observed in between papillary and capsular samples of the borderline tumors* (papillary projection 2.169 μg/g, range 0.49–6.81; capsule 3.11 μg/g, range 0.24–9.68) (p = 0.471). *Ni concentrations of both capsular and papillary samples were higher than those of normal ovarian tissues* (p < 0.001). *Ni concentrations of malignant and borderline tumors were not statistically different* (malignant tissue vs borderline tumor capsule, p = 0.155; malignant tissue vs borderline tumor papillary projection, p = 0.154). Fig. 2 demonstrates the histogram representing Ni concentrations of the tissues (one too high outlier is not depicted).

3.3. Se

Se concentrations were not found to be different among malignant tissue (0.477 μg/g; range, 0.04–3.396), *normal ovaries* (0.17 μg/g; range, 0.009–1.126) *and borderline tissues* (papillary projection 0.267 μg/g, range 0.03–1.13; capsule 0.740 μg/g, range 0.005–3.305) (p = 0.231). *No difference was observed in Se concentrations between papillary and capsular samples of the borderline tumors* (p = 0.476). Fig. 3 demonstrates the histogram representing Se concentrations of the tissues.

We performed post hoc comparison tests and power calculations for the 3 groups as a whole and one-to-one basis. Table 2 is a summary of the ***P*** values for the comparison of the 3 groups (K-W test) and one-to-one comparisons (following ANOVA for transformed rank values) made in our study for lead and nickel. The mean ± SEM values for each group represent transformed rank values. Despite the number of subjects being limited, the comparisons are statistically distinctive enough for the annotated parameters.

4. Discussion

Metal genotoxicity can be related with several mechanisms. Firstly, they can interfere with cellular redox regulation and cause an oxidative stress that could damage DNA [16]. Secondly, they can inhibit DNA repair mechanisms that will result in genomic instability and accumulation of critical mutations. Another mechanism is the inhibiting cell control by inactivating tumor suppressor genes or activating oncogenes in the cell cycle. Recent developments suggest that epigenetic changes may play important role in metal-induced carcinogenicity [17]. Epigenetic mechanisms such as DNA methylation and histone modification

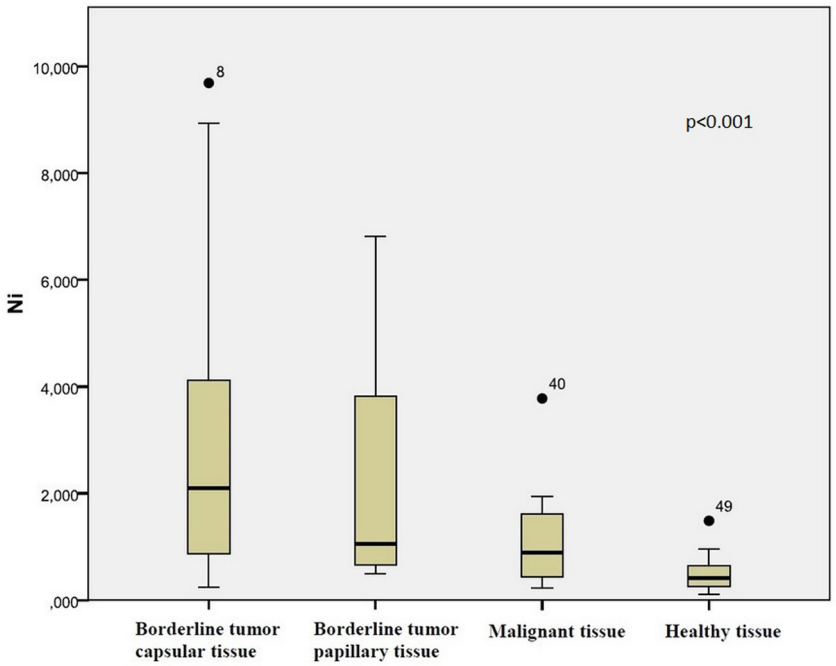

Fig. 2. Histogram representing Ni concentrations of the tissues. Ni concentrations were higher in malignant, borderline capsular and papillary tissue samples than healthy ovaries. Ni concentrations of malignant and borderline tumors were not different.

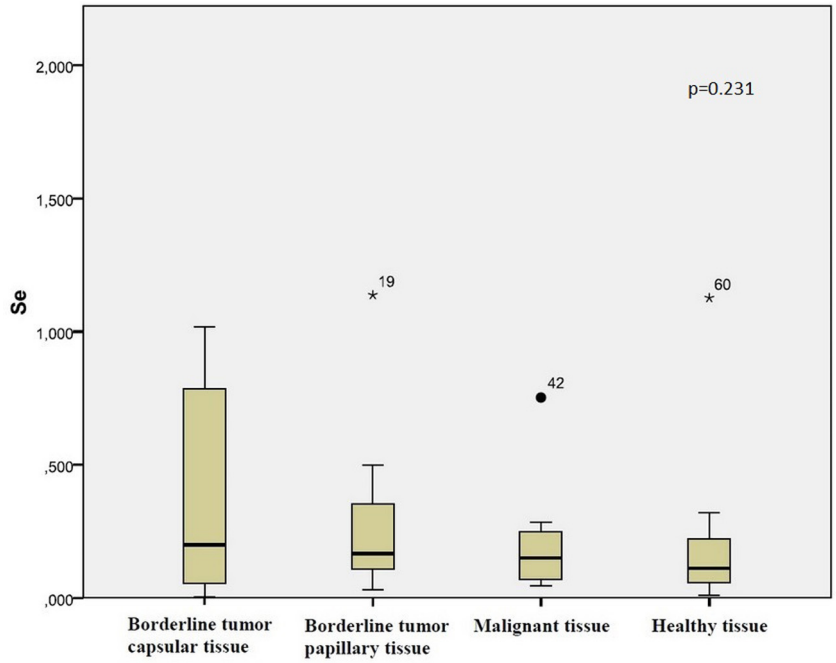

Fig. 3. Histogram representing Se concentrations of the tissues. No statistical difference was observed among the tissues.

are essential in controlling gene expression. Hypermethylation in the promoter region of tumor suppressor genes represses the transcription, while decreasing in DNA methylation has been shown to be associated with activation of the oncogenes [18–20]. Cumulative exposure of these metals is particularly of interest rather than short time exposure regarding the etiology of cancer.

In this study, we observed an increased tissue concentration of lead and nickel both in malignant and borderline ovarian tumors than healthy non-neoplastic ovaries. In 1996, WHO classified lead as a toxic element. Toxic effects of lead may involve several organs and may range from evident clinical manifestations to obscure biochemical effects. High lead consumption may cause peripheral neuropathy, nephropathy and hypertension in adults [21]. Moreover, given its proven carcinogenicity in animals, lead is classified as category 2B carcinogen by IARC [22]. Lead is a mitogen in vivo and has been shown to have proliferative effects on liver cells [23]. This proliferation was showed to be direct hyperplasia and associated with TNF-a [24]. A similar proliferative effect on renal tubular epithelial cells with occasional atypia has also been demonstrated in rats with lead exposure in a dose dependent way; but with the lower doses that induce the nephrotoxic effects [25,26]. To our knowledge, lead and nickel accumulation in ovarian epithelial cancer and borderline ovarian tissue has not by now, been reported in literature. According to the current study, accumulation of these metals in ovarian tissue is associated with borderline or malignant proliferation of the surface epithelium.

Table 2
Post hoc comparison tests and power calculations for the groups.
[b]Post hoc tests used for intergroup comparisons following ANOVA for transformed rank values.

Nickel (Ni)	Group Mean ± SEM	
Borderline Tumors	42.8 ± 3.2	
Malignant Tumors	32.9 ± 4.3	
Control Group	18.7 ± 2.9	
KW[a] ***P*** value < 0.001	**Power** %	***P***[b]
Borderline Tumors-Control Group	100	< 0.001
Malignant Tumors-Control Group	100	0.026
Lead (Pb)	**Group Mean ± SEM**	
Borderline Tumors	38 ± 3.6	
Malignant Tumors	40.7 ± 3.8	
Control Group	18 ± 2.6	
KW[a] ***P*** value < 0.001	**Power** %	***P***[b]
Borderline Tumors-Control Group	100	< 0.001
Malignant Tumors-Control Group	100	< 0.001

[a]Kruskall-Wallis test.
[b]Post hoc tests used for intergroup comparisons following ANOVA for transformed rank values.

Even though the mechanism under lead-associated carcinogenicity remains obscure, studies showed that lead destabilizes the DNA structure, induces chromatin aggregation through histone-DNA cross-links [27]. Lead is also known to induce oxidative stress and lipid peroxidation. However, lead is a redox-inactive metal and can not readily undergo valance changes. The major effect of lead is depletion of the cellular antioxidant pool by binding sulfhydryl groups of the proteins and decrease in the level of glutathione [28]. Lead inhibits delta-aminolevulinic acid dehydratase and as a consequence, the substrate delta-aminolevulinic acid which is known to stimulate the ROS production increases. These independent however related mechanisms cause the free-radical induced damage by lead. In a prospective study of 4740 lead and zinc smelter workers, authors found for a long time and low level exposure of these metals was a risk factor for lung cancer. Furthermore, they reported nine-fold increased risk in peritoneal and retroperitoneal cancers [29]. Tissue level of lead was also found to be higher in patients with gallbladder carcinoma [30]. Moreover, lead exposure was reported to be associated with breast cancer due to acting as a metalloestrogen and activating the estrogen receptor-α [31]. Concentrations of Pb, Cd and Mn in the nails and scalp hair of the patients with lung and prostate cancer were also found to be higher than those of healthy controls [32,33]. On the other hand, in addition to previous studies comparing various cancerous and healthy human tissues, the current study revealed that Pb and Ni accumulation in ovarian surface epithelium is not only associated with malignant transformation, but also present in borderline tumors. These results indicate the possible relevance of additional risk factors for frankly malignant transformation.

Nickel is a known carcinogenic metal and commonly used in modern industry, producing stainless steel, batteries, coins and jewelry [34]. Ni-induced carcinogenesis has been linked to inhibition of DNA repair enzymes, thus increase in genotoxicity of DNA-damaging agents [35,36]. Epigenetic changes and alterations in chromatin structure by histone modifications are suggested as the primary mechanisms underlying the Ni carcinogenesis. Silencing tumor suppressor gene p16 by hypermethylation of the promoter region and activation of MAP kinase pathway has been demonstrated in mice with tumor induced by Ni sulfide [37]. It is conceivable that co-exposure of the genotoxic agents can increase the possibility of Ni carcinogenesis. An increased risk of lung and nasal cancers have been reported with the occupational exposure in refining industries [38]. Ni levels were showed to be higher in p53 mutant lung cancer tissues than those with p53-wild type and this was attributed to increased occurrence of p53 mutation by high Ni levels [39]. Furthermore, a positive but weak correlation was reported with Ni plating and stomach cancer [40]. In an ecological study from China showed soil Ni exposure in general population was associated with increased risk of liver and lung cancer [41]. The current study showed Ni levels were higher both in invasive ovarian cancer and borderline tumors than those of healthy ovarian tissues. These findings suggest Ni accumulation is associated with cancerous changes of ovarian surface epithelium but underscore the necessity of co-exposure to different genotoxic agents or genetic predisposition for frankly malignant transformation.

Urban soil is a very important pathway of exposure to heavy metals. Due to the increasing environmental pollution, traffic loads, industrial emissions and residential blocks, heavy metal contents in urban area are found much higher than those in rural soil. Lead is the element most enriched in the city [42,43]. Incidental ingestion of soil particles is possible through hand contact [44]. Moreover, lead and the other heavy metals such as Cd, Co and Cu could pose health threat through their introduction into the food chain by the water irrigating the vegetable farms as previously showed in Ethiopia [45].

The relationship between heavy metals and ovarian cancer may not be restricted with oral consumption alone. When we take into consideration of seminal compounds regardless of their size may enter the oviducts; a plausible way of potential toxic elements to reach the ovarian surface epithelium may be the retrograde transmission by seminal plasma. Talc and asbestos exposure are previously described factors that stimulate local inflammation on the ovary by retrograde transmission [46,47]. Human seminal plasma contains several trace elements that have essential functions in sperm function and quality. Several studies showed exposure in toxic work environment results in trace element alteration in human seminal plasma. Decreased levels of the elements that can oppose the oxidation and elevated concentrations of toxic metals are associated with poor sperm quality and infertility [48,49]. Toxic element accumulation in semen was also showed in occupationally unexposed infertile men [50–52]. Infertility in male partner has been reported in association with epithelial ovarian cancer in some previous studies [53]. Despite the increase in epithelial ovarian cancer risk was not confirmed in recent studies, an unexpected 3.15-fold increase in uterine and 2.85-fold increase in colon cancers was reported in women with male factor-related infertility [54]. Ascendant transmission of toxic elements by seminal fluids should be investigated in future studies regarding the local inflammatory reactions and

oxidative stress on ovarian surface epithelium. Positive correlation of infertility with ovarian cancer and a protective role of tubal ligation might support this standpoint.

In normal conditions, harmful effects of free radicals and ROS on cells are neutralized by antioxidant defense of the body. Se is primary component of selenoproteins and glutathione peroxidase is the best selenoprotein capable to detoxify hydrogen and lipid peroxidation. While there is growing body of evidence supporting an inverse association between Se and some cancers, the evidence is not consistent to draw a firm conclusion [55,2]. Epidemiological data support people living in geographic areas with low Se, have higher rate of malignancy [56]. Significantly lower levels of Se were found in sera of the patients developing esophagus, breast, liver and colorectal cancers [57–60]. Similarly, reduced serum, bile and tissue levels of Se and increased levels of Pb were demonstrated in gallbladder cancer [30]. In contrast, larger prospective studies did not support protective role of Se against cancer in women [61]. Current study did not show a difference in Se concentrations among the malignant, borderline and non-neoplastic ovarian tissue samples. These findings indicate that further investigations are needed before reaching a definite conclusion.

The limitations of this study are its retrospective design, analyzing only three elements in a limited number of patients and comparison of the elements in paraffin-embedded tissue samples not in the fresh tissues. However, this study demonstrated the relative concentrations of Pb, Ni and Se in the malignant, borderline and healthy ovarian tissues processed and preserved in the same manner. Moreover these elements were previously showed as being strongly bounded to tissue proteins and sulfhydryl groups and able to withstand the tissue processing [11,12]. Still with the limitations, this is the only study revealing that nickel and lead accumulation in ovarian tissue is associated with borderline and malignant proliferation of the surface epithelium. A prospective analysis of fresh tissue samples in conjunction with the scalp hair or nail samples of the cancer patients and controls are warranted to reveal a more consistent conclusion regarding the long term metal exposure and ovarian cancer.

Conflict of interest

We declare that we have no conflict of interest.

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