Original Article

Serum levels of Copper, Zinc and Copper/Zinc Ratio in Patients with Ovarian Cancer

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ABSTRACT

Objective: To investigate the serum concentrations of copper (Cu), zinc (Zn) and copper/zinc ratio (Cu/Zn) in patients with ovarian cancer and benign ovarian lesions.

Methodology: The study included 50 patients, with ovarian cancer (malignant group) and 50 patients with benign ovarian lesions (benign group). After overnight fasting, blood samples were collected. The concentrations of Cu and Zn were measured by Atomic Absorption Spectrophotometry (AAS).

Results: There was a significant increase in mean serum levels of Cu (p=0.0001) and the ratio of Cu/Zn (p=0.003) in ovarian cancer patients. However, there was no significant difference in the mean serum levels of Zn between two groups (p=0.71).

Conclusions: The study showed that serum copper and copper/zinc ratio were significantly higher in ovarian cancer patients when compared to the patients with benign ovarian lesions. It seems that determination of serum copper and copper/zinc ratio may be used as a test for diagnosing of ovarian cancer.

KEY WORDS: Ovarian cancer; Zinc, Copper.

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INTRODUCTION

Ovarian cancer etiologic factors are not well established and mortality has not significantly decreased during the past two decades causing disappointment.[1,2] Combining the internal and external factors such as trace elements have been suggested to have some effect on induction and progress of cancers.[3] Trace elements are essential components of biological structures, including the oxidative processes. Oxidative processes have been implicated in inducing human cancers.[4] Two of these trace elements are Zinc and Copper. A number of studies[5-19] have been carried out on serum Copper and Zinc levels in human cancer patients and the relationship between their concentration and disease activity were investigated.

Zinc is an essential trace element and is known to play an important role in metabolic processes. For many years, the ability of Zn to induce some antioxi-

dants, such as the metallothioneins, has been recognized.[5-7] Significantly lower Zn levels and higher Cu/Zn ratios were found in both cervical intraepithelial neoplasia and cervical cancer patients compared with the controls.[10]

Copper is another trace element which acts as a vascular endothelial growth factor (VEGF). Copper induces tumor cell growth by angiogenesis. Lowering Copper levels offer a safe and effective way to stabilize the growth of advanced and metastatic cancers.[9] Patients with carcinoma had a significantly higher level of serum Cu and Cu/Zn ratio compared with the controls.[13]

As the role of trace elements in various cancers has been the subject of investigation, and the evidence linking Cu and Zn to cancers is not conclusive, the present study was designed to evaluate serum Cu, Zn concentrations and Cu/Zn ratio in patients with carcinoma of ovary, and to compare these concentrations with those of patients with benign tumors of the ovary.

METHODOLOGY

Sample collection: This cross sectional study was carried out on 100 patients, admitted in Alzahra teaching hospital for operation between October 2006 to September 2009. Fifty patients with ovarian cancer (malignant group) and fifty patients with ovarian benign tumors (benign group) randomly were recruited. The diagnosis of ovarian cancer and benign tumors were confirmed histologically by senior gynecologic pathologist. All patients were informed of the purpose of the study, and written consent was obtained. Ethical approval was obtained from the Research Vice Chancellor Office, Tabriz University of Medical Sciences. An information form was used to obtain all the patients' sociodemographic and obstetrics data. Subjects had complete physical examination to determine existence of other disease that might alter trace elements.

Excluded criteria from eligibility were:

1. Patients who had other cancer history or received previous chemotherapy or radiotherapy.
2. Patients who suffered from diabetes mellitus and hepatic cirrhosis.
3. Patients with poor liver function (bilirubin>1.5mg/dl), poor renal function (creatinine>1.5mg/dl) and pregnant patients.
4. Patients who used oral contraceptive or intrauterine devices within the past 6 months.
5. Patients who had taken a zinc tablet or any complementary medicine with zinc in its content.
6. Patients with low potential malignant tumors.

All patients with ovarian cancer were staged and graded according to the recommendations of International Federation of Gynecology and Obstetrics (FIGO).

Blood collection: After an overnight fasting, preoperatively a 5-ml blood sample was collected from the arm vein of each patient. The blood was then allowed to clot and centrifuged for 10 minutes at 3000 rpm to extract the serum at 4°c. The serum was aliquoted into eppendorf tubes and stored at -70°c until analyses were performed at the laboratory of Drug Applied Research Center, Tabriz University of Medical Sciences. Atomic absorption spectrophotometry (AAS) was used to determine Zn and Cu levels. Serum Zn and Cu contents were determined by direct aspiration of 1:5 dilution of serum in deionized water. Extensive precautions were taken for both collection and subsequent handling of serum in order to avoid or minimize trace elements contamination.

Statistical analysis: Descriptive statistics were used for all variables. Values were expressed as percentage, mean, and standard deviation. The independent sample t-test was used to compare elements in serum between the two groups. The association of numerical data in tumor stages and grades was analyzed by one-way Anova test.

Diagnostic value was determined by sensitivity, specifity, positive predictive value (PPV) and negative predictive value (NPV). Roc curve was used for cut off estimation. Sociodemographic and obstetrics data between the groups were tested using the Chi-square method and independent samples t-test. Odds ratio (OR) and its 95% confidence interval (CI) were calculated to estimate the associations between various factors and ovarian cancer by the $\times^2$ test and Fisher's exact test.

The statistical analysis was carried out by SPSS.15 software and Pvalues of < 0.05 were considered significant.

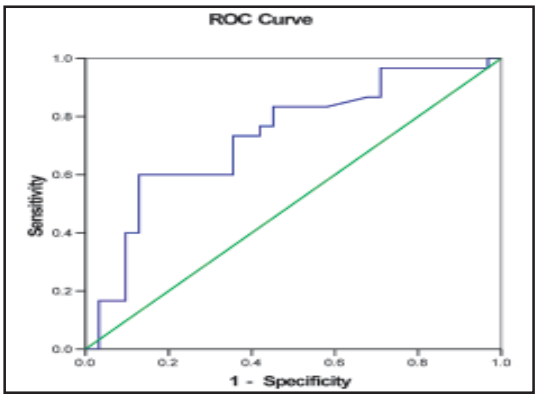

Fig-1: Roc curve for diagnostic value of serum Copper level

RESULTS

The mean age of patients in malignant and benign groups was 48.69±12.90 and 38.95±13.25 respectively, p=0.42. Table-I shows the sociodemographic and obstetrics characteristics of the two groups. In the malignant group, 28% were over 50 yr of age. The corresponding figure in the benign group was 18% (p=0.34). In the benign group, 78% of patients and in the malignant group 50% of patients had breast-fed their children. Most patients with malignant tumors were illiterate. Frequency of blood RH positive was high in the malignant group.

The most common histologic type in the malignant and benign groups was serous cystadenocarcinoma (50%) and serous cystadenoma (22%) respectively. In the ovarian cancer group 18(36%) were stage I, 7(14%) were stage II, 22(45%) were stage III and 3(5%) were stage IV. In this group 14(28%) were grade 1, 12(14%) were grade2 and 24(48%) were grade 3.

Serum copper, zinc and copper/zinc ratio are presented in Table-III. The concentrations of Zn and Cu were 0.68±0.15 mg/l and 1.65±0.32 mg/l, respectively in the malignant group, and 0.69±0.12 mg/l and 1.25±0.31 mg/l, respectively, in the benign group. Trace elements analysis indicated that the concentrations of serum Cu were increased significantly ($p<0.05$) in the malignant group compared with the benign group, while the concentrations of serum Zn were not changed significantly ($p>0.05$). The concentrations of serum Cu/Zn ratio in the malignant group were increased significantly (p=0.003) compared with the benign group.

We also evaluated the correlation between serum trace elements levels of the malignant patients and tumor stage and grade, No significant correlations were found among the concentrations of trace elements and various FIGO stages and grades (Table-IV).

To evaluate the utility of preoperative trace elements in predicting malignancy, sensitivity and specificity calculations were performed for various cut-off values of trace elements in all patients. Using the Receiver Operator Characteristic (ROC) curve, 1.18 mg/l was determined as the cut- off value for serum Cu in differentiating malignant from benign lesions. At this cut-off concentration the sensitivity, specificity, PPV and NPV of the test were 69%, 62%, 58% and 73% respectively, Fig 1. At a cut-off serum Zn level of 0.7mg/l, the sensitivity was 51%, the specificity was 50%, the PPV was 67%, and the NPV was 33% for predicting malignancy. For serum Cu/Zn ratio, the cut-off value was calculated as 2.04 mg/l. At this cut-off, the sensitivity, specificity, PPV and NPV were 73%, 70%, 70%, and 73% respectively, Fig 2.

Table-I: Comparison of sociodemographic and obstetric characteristics in the two groups.

Demographic & obstetrics characteristics	*Benign (n=50) n (%)*	*malignant (n=50) n (%)*	*p value*
Age (years)			0.34
>50	9 (18)	14 (28)	
Gravid (n)			0.18
0	18 (36)	11 (22)	
ed 1	32 (64)	39 (78)	
Parity (n)			1
0	32 (64)	31 (62)	
> 1	18 (36)	19 (38)	
Breast feeding	39 (78)	25 (50)	0.006
Illiteracy	14 (28)	28 (56)	0.008
Blood RH type			0.002
Positive	36 (72)	48 (96)	
Negative	14 (28)	2 (4)	

DISCUSSION

Trace elements are essential components of biological structures, but their role in the development and inhibition of cancer is complex, and have raised many questions because of their essential and toxic effects at concentrations beyond those necessary for their biological functions on human health. Literature on this subject demonstrates conflicting results.[11,17-19] A major reason for these different results is the difficulty in evaluating trace elements and the interaction between the elements. Another reason is the fluctuability of the test levels and interpretation of elements in the human body.

Lower serum Zn concentrations in patients with ovarian and cervical cancers have been reported.[15,16] Our study demonstrated lower Zn concentrations in the patients with ovarian cancer than those with benign lesions, but the differences were not statistically significant, which is in agreement with the findings of Piccinini et al.[11] It seems that determination of zinc levels in ovarian cancer patients and comparison with a normal controls without ovarian pathology is

Table-II: Serum Cu, Zn levels and Cu/Zn ratio in the two groups.

Variables	*Mean ± SD (mg/l)*		*P value*
	Benign (n=50)	*malignant (n=50)*	
Zn	0.69±0.12	0.68±0.15	0.71
Cu	1.25±0.31	1.65±0.32	0.0001
Cu/Zn	1.93±0.72	2.39±0.8	0.003

Table-III: Serum Cu, Zn levels and Cu/Zn ratio according to different FIGO stages and grades in the malignant group (n=50).

	Cu (mg/l) Mean ± SD	*Zn (mg/l) Mean ± SD*	*Cu/Zn (mg/l) Mean ± SD*
Stage			
I	1.68±0.41	0.63±0.09	2.08±0.43
II	1.08±0.31	0.69±0.34	1.69±0.37
III	1.34±0.31	0.49±0.18	2.29±0.58
IV	1.18±0.27	0.47±0.08	2.39±0.28
P value	0.18	0.44	0.37
Grade			
1	1.43±0.24	0.48±0.13	2.54±0.28
2	1.35±0.29	0.67±0.18	1.59±0.36
3	1.35±0.31	0.61±0.09	2.21±0.53
P value	0.07	0.75	0.38

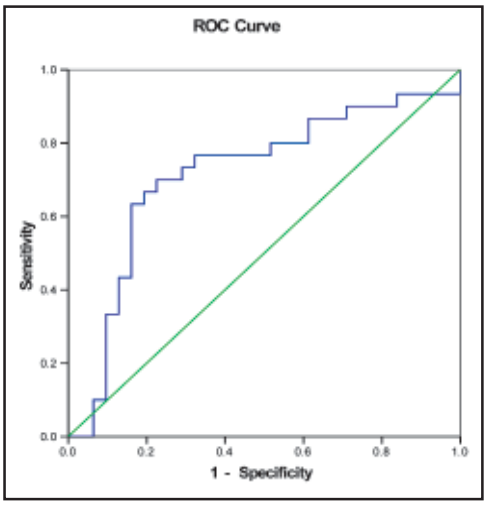

Fig-2: Roc curve for diagnostic value of serum Cu/Zn ratio

necessary to fully elucidate the relationship between the zn level and ovarian cancer.

A tumor cell can multiply to a small cell mass of about 2mm with cells in the mass able to get nutrients by diffusion. However, to grow beyond this, process of angiogenesis is required. For this process, growth factors such as vascular endothelial growth factor (VEGF) are needed. Higher levels of Cu induces neovascularization, tumor growth promotion and metastases.[9] Our data revealed that serum concentrations of Cu and Cu/Zn ratio in ovarian cancer were significantly higher than those in benign lesion. These results are consistent with Zowczak et al's findings.[17]

Oyama et al found that Cu and Cu/Zn ratio were higher and Zn was lower in the advanced stage tumors than early ones.[18] However, the findings of the current study do not support their research. As one could argue that the association between zinc deficiency and advanced disease stage was the systemic tumor effects on dietary intake and increased catabolism. But this was not demonstrated in Prasad et al's study.[5] They showed no significant differences between zinc deficiency and protein status. However, with small sample size, in the current study, caution must be applied, and further studies with larger sample sizes of advanced disease stages are suggested to elucidate the relationship between trace elements and advanced disease.

Gupta et al[19] in their study found a decreased serum Zn level and increased Cu level in breast cancer patients compared to controls. Conversely, Moyad et al[20] and Costello et al[21] found a higher concentrations of Zn in the prostate gland in patients with benign prostatic hyperplasia (BPH) which has the potential to increase the growth of prostate conditions from BPH to cancer. These findings are not consistent with those of Yaman et al[16] who found a lower zinc levels in cancerous endometrium than in noncancerous samples, and not different Zn levels in cancerous ovary samples than those in noncancerous tissues.

It has been reported the average level of Zn was significantly higher in the hair of patients with breast cancer compared to the healthy women.[22] Their findings are not in agreement with the current study findings which was carried out on the patient's serum. This inconsistency may be due to the two different organs (breast and ovary) and samples (hair and serum). Mazdak et al[23] found a significant decrease in the serum Zn and increased in the serum Cu concentrations and Cu/Zn ratio in bladder cancer patients compared with those of the control subjects, suggesting that an increase in the serum level of Cu and a decrease in the level of Zn might be cause-and-effect of bladder cancer occurrence. The current study supports Mazadak et al's findings. However, higher serum levels of Cu and Cu/Zn ratio could not be considered as the ovarian cancer risk factor due to cross sectional study. A possible explanation for the high serum concentrations of Cu and Cu/Zn ratio in ovarian cancer patients might rest in the effect of Cu on cell promoter of cellular growth, which would make tumor cells develop. Increased serum levels of Cu may be the result of the release of Cu in the circulation following destruction and necrosis of the tissues involved.

Kuo et al[24] found a highest level of Cu and Cu/Zn ratio in both serum and tissue of patients with breast cancer. In their study serum level of Zn was low but tissue level of Zn was high. For serum cu/Zn ratio, at a cut-off value of 1/2 μg/l; sensitivity and speci-

ficity were both 100%. They suggested that change of trace elements in serum and tissue might be useful and significant as biological tumor marker. Our data indicate that the ability of serum Cu/Zn ratio to differentiate malignancy from benign lesions at a cut-off value of 2.04 mg/l gave a sensitivity of 73%, a specificity of 70%, a positive predictive value of 70%, and a negative predictive value of 73%. The receiver operating characteristic curve was 0.715 (0.57-0.85) mg/l. This is consistent with the cut-off point in Gupta et al's study[19] which was 0.99 (sensitivity=86%, specificity=87%).

In Gupta et al's study, the average Cn/Zn ratio in breast cancer patients and healthy subjects were, 1.91 and 0.86 respectively. The ratios for the current study were 2.39±0.8 (mg/l) and 1.93±0.72 (mg/l), respectively. Although, the absorption of Zn may be reduced as a result of the presence of high levels of Cu with which it competes, but in the current study this was not demonstrated. However further research is needed to clarify the underlying mechanisms about the serum levels differences of Cu and Zn.

In conclusion, increased serum Cu level and Cu/Zn ratio were found in malignant patients. The cut-off value of serum Cu/Zn was 2.04, giving 73% sensitivity and 70% specificity. If these results are confirmed in larger studies, these elements might be considered as the risk factors for ovarian cancer occurrence, and determination of serum Cu level and Cu/Zn ratio may be used as a tool for diagnosing.

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