Study of Osteopontin as the "CA125" Adjunct for Detection of Ovarian Carcinoma in Patients with Pelvic Mass

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Abstract

Introduction: Ovarian cancer is the most lethal gynecologic cancer, whose high mortality rate is partly due to late diagnosis. Tumor-specific markers that can be detected in early stages of the disease could probably improve treatment results. In this study, we evaluated the diagnostic value of serum levels of osteopontin in patients with epithelial ovarian tumors.

Patients and methods: Twenty five patients with ovarian cancer and 25 patients with pelvic masses and 25 healthy women were enrolled in this study. Blood samples were taken from the patients from the first two groups before surgery and the healthy controls donated blood at the same time. Osteopontin serum level was measured by Enzyme-Linked Immunosorbent Assay method using human osteopontin enzyme immunoassay kits. Finally osteopontin levels before surgery were compared between groups.

Results: The mean age in patients with ovarian cancer, benign tumor and normal group were 35.1, 32.4 and 31.6 years respectively. The most prevalent clinical symptom in almost half of patients with either benign or malignant pathology was abdominal pain. The average level of CA125 and osteopontin in ovarian cancer patients was 1818.4 and 9.6, respectively. The average level of CA125 and osteopontin in epithelial ovarian tumors was significantly higher than other groups. Significant linear relationship was detected between CA125 and osteopontin.

Conclusion: Our study results support the fact that commitment measurement of osteopontin and CA125- due to their significant increase among the ovarian cancer patients- could be helpful in early detection of ovarian cancer.

Keywords: Ovarian cancer; tumor marker; diagnosis; osteopontin; CA125.

Introduction

In the United States, 21,550 new cases of epithelial ovarian cancer were diagnosed and 14600 patients died of this disease in 2009 [1,2]. Ovarian carcinomas and surface epithelial tumors are the most common forms of ovarian cancer [3]. Surgical intervention for an ovarian cyst or a pelvic mass is the appropriate choice for the majority of patients, and this treatment is better to be performed in a tertiary care center with multidisciplinary specialist teams [4-6].

Different screening strategies have been used for early detection of the disease including ultrasonography [7–9], serum markers such as CA125 [10–12], or a combination of these two modalities, where a rising serum marker would prompt the physician to do a transvaginal sonography [10-13]. Currently, CA125 is the only tumor marker which has a well-defined and validated role in monitoring ovarian cancer [14]. Decreasing levels of CA125 have been shown to be associated with response to the treatment, while its increasing levels are usually a sign of tumor progression [13, 15]. However, most of the patients with normal levels of CA125 (<35 units/ml) and no clinical symptoms, still have microscopic residual cancer at the time of completion the chemotherapy [16].

Recently, it has been found that osteopontin (OPN) (secreted as glycoprotein in body fluids) in ovarian cancer cell lines shows a 184-fold over-expression compared to healthy women ovarian surface epithelium [17]. Plasma levels of this protein have been shown to correlate with disease status in several types of carcinomas [18, 19]. The average levels of OPN are significantly higher in epithelial ovarian cancer patients compared with healthy controls [20]. The efficacy of this putative biomarker has not been directly compared with CA125 in monitoring patients with ovarian cancer and pelvic masses. The purpose of this study was to test the hypothesis whether OPN is a clinically useful adjunct to CA125 in detecting ovarian cancer.

Patients and Methods
Study Participants
The patients were randomly selected for this study according to the inclusion criteria from November 2011 to May 2012. Serum samples from 25 patients diagnosed with ovarian cancer as well as 25 women with pelvic masses (including benign cyst, leiomyoma and eccysis) and 25 healthy women (referred due to vaginal discharge) was provided by department of gynecology/oncology, Ghaem Hospital, Mashhad University of Medical Sciences, Mashhad, Iran. The inclusion criteria for the cancer group included age between 15-83 years old, epithelial ovarian cancer, and not having renal failure. The patients with non-epithelial ovarian cancers, renal failure and other age ranges were excluded. Written informed consent was obtained from each patient. The study procedure was approved by the ethics committee of Mashhad University of Medical Sciences. All patients with cancer underwent cytoreductive surgery for ovarian cancer based on the International Federation of Obstetricians and Gynaecologists (FIGO) staging system. All plasma samples were centrifuged at 2000g at 4°C for 15 minutes. The separated plasma was removed, aliquoted, and frozen at - 80°C for future analysis. Serum OPN level was measured by Enzyme-Linked Immunosorbent Assay (ELISA) technique in the studied groups.

A partial response was defined by a decrease

Table1: Pregnancy status in three studied groups.

Pregnancy Status	Studied groups (Mean ± SD)			P-value
	Epithelial ovarian cancer	Benign ovarian tumor	Healthy women	
Parity	3.04±2.2	2.19±1.8	1.8±1.5	0.51
Gravidity	3.04±2.6	3.18±2.6	2.3±1.77	0.16
Abortion	0.82±0.52	0.91±0.4	0.65±0.48	0.53

Table2: Evaluation of clinical manifestations and treatment methods in the three studied groups.

Studied variable		Frequency (Percentages)			P-value
		Group I	Group II	Group III	
Complaint and clinical symptoms	Painful Masses	12 (48)	12 (48)	5 (20)	0.031
	abdominal distention	7 (28)	2 (8)	2 (8)	
	vaginal secretions	-----	7 (28)	13 (52)	
	Refer for Surgery	6 (24)	4 (16)	1 (4)	
	Nulliparity	-----	----	4 (16)	
Oral Contraceptive Pills	Negative	15(60)	23 (92)	25 (100)	0.001
	Positive	10 (40)	2 (8)	----	
Hormone replacement therapy	Negative	21 (84)	23 (92)	25 (100)	0.083
	Positive	4 (16)	2 (8)	-----	

Group I; Epithelial ovarian cancer, Group II; Benign ovarian tumor, Group III; Benign ovarian tumor

of at least 50% in the sum of the largest tumor dimensions as measured by computed tomographic scanning. Any tumor with smaller decrease or increase in size during primary chemotherapy was defined as a nonresponder. The duration of overall survival was defined as the interval between initiation of treatment (surgery or chemotherapy) and death.

Enzyme-Linked Immunosorbent Assay

Levels of OPN were measured in plasma samples with a solid-phase sandwich ELISA using a commercially available kit (Code17158, Immuno-Biological Laboratories, Quantikine ELISA Kits, USA). Briefly, microplateshave was first precoated with anti-human OPN rabbit IgG [100 µl of 20 µg/ml in 0.1 M carbonate buffer (pH: 9.5)] and blocked with 1% BSA and 0.05% Tween 20. Plasma and standard OPN samples were diluted with 1% BSA and 0.05% Tween 20 in PBS and incubated for 1 hour at 37°C. After seven washes with 0.05% Tween 20 in phosphate buffer, horseradish peroxidase-labeled conjugated antihuman OPN (10A16) mouse monoclonal antibody (100µl of 2ng/ml) was added and incubated for 30 minutes at 4°C. After nine washes, 100µl of tetramethyl benzidine buffer was added, and the signal was allowed to develop for 30 minutes at room temperature. The reaction was stopped with 100µl of 1N sulfuric acid. The absorbance at 450nm was measured by an automatic ELISA reader (Bio-Rad, Hercules, CA). The applied cutoff value for CA125 was 35units/ml, as recommended by Bast et al. [21].

Statistical Analysis

The basic descriptive statistical analysis was represented using mean± standard deviation. SPSS version 16 (SPSS Inc., Chicago, IL) was used to perform statistical analysis. Nonparametric test, Wilcoxon signed rank test and rock curve were used to perform paired comparisons between pretreatment and postoperative OPN and CA125 measurements. Also $\chi2$ or the Fisher's exact test was applied for dichotomous variables between studied groups. P values less than 0.05 were considered as significant for all statistical analysis.

Results

The study participants were divided into three groups with 25 individuals in each group; patients with ovarian epithelial cancer (Group I), pelvic masses or benign ovarian pathology (Group II) and healthy women (Group III). The mean age of studied individuals was not significantly different between the studied groups (P=0.17). The mean age of participants in groups I, II and III was 35.1±14.48, 32.4±11.27 and 31.6±7.85 years respectively. History of pregnancy was evaluated in all three studied groups (Table 1). Table 2 shows that the frequency of patients complaints differs significantly between the groups (P=0.031). Complaint about painful masses in the healthy group was lower than groups I and II, as they were referred to our clinic due to vaginal discharge. There was also a significant relation between the consumption of oral contraceptive pills and ovarian cancer (P<0.001), as patients in Group I had used oral contraceptive pills more than two other groups. The three groups did not differ in using Hormone Replacement Therapy (HRT). Pathological evaluation of epithelial tumors in patients with malignant pathology revealed that 15 cases (60%) had serous adenocarcinoma and 10 cases (40%) had mucinous adenocarcinoma. The tumor stage of patients in group I (patients with ovarian epithelial cancer) is presented in Figure 1. Most of the studied patients were in the stage I according to FIGO staging system.

OPN and CA125 levels in the three studied groups are presented in table 3. This table shows that there was a significant difference between CA125 levels among the three studied groups (P=0.001). There was also a meaningful difference in OPN levels between groups (P=0.03).Considering the cutoff value of 35units/ml for CA125 as a positive result,

Table3: Osteopontin and CA125 levels in the three studied groups.

	Studied groups (Mean ± SD)			
	Epithelial ovarian cancer	Benign ovarian tumor	Healthy women	P-value
Osteopontin	9.60±9.6	5.80±3.86	5.2±2.94	0.032
CA125	1818.4±5019.94	51.71±85.96	0	0.001

the ROC curve (Figure 2) shows that the relation between CA125 and OPN is significant. The analysis of multiple biomarkers in this study demonstrated that the addition of CA125 significantly increases both sensitivity and specificity.

Discussion

The levels of several tumor markers including OPN, CA72-4, EGFR, ERBB2 (Her-2), activin, and inhibin are elevated in patients with ovarian cancer [22–24]. Increased expression of OPN along with elevated plasma levels of this marker have been reported in patients with ovarian cancer. However, to obtain a detection sensitivity of 80%, the specificity declined to 80% [20].

OPN might be a clinically useful adjunct to CA125 for detection of ovarian cancer. After primary surgery and platinum-based chemotherapy, most patients with advanced disease would achieve a complete clinical remission. However, the majority of these women (75–80%) will relapse and neither consolidation therapy nor maintenance therapy has been shown to improve their survival.

CA125 is the most widely used serum biomarker in patients with ovarian cancer. Its usefulness in determining response to treatment or as a marker for the detection of recurrent disease is well established. The mean age of our studied individuals with ovarian cancer and benign tumors were 35.1 and 31.6 years respectively, and most of the tumors were in stage I. In a similar study by More et al. [25] the mean age of studied patients with ovarian cancer and benign tumors were 65 and 50 years respectively and most of the studied cases (38 cases from 48 individuals) were in the stage III. The reason of lower mean age and lower tumor stage in our study compared to their study remains elusive [26].

In the present study the CA125 and OPN levels in patients with ovarian cancer and then in patients with pelvic masses or benign tumors were significantly higher than healthy individuals. These findings are in line with previous studies [27, 28]. Although we did not evaluate the expression of tumor markers (CA125 and OPN) in the tissue, we found a significant direct relation between the OPN, and CA125 serum levels and the presence of tumor.

Conclusion

Our study results support the fact that concomitant use of osteopontin with CA125, due to their significant increase among the ovarian cancer patients, might be useful for early detection of ovarian cancer.
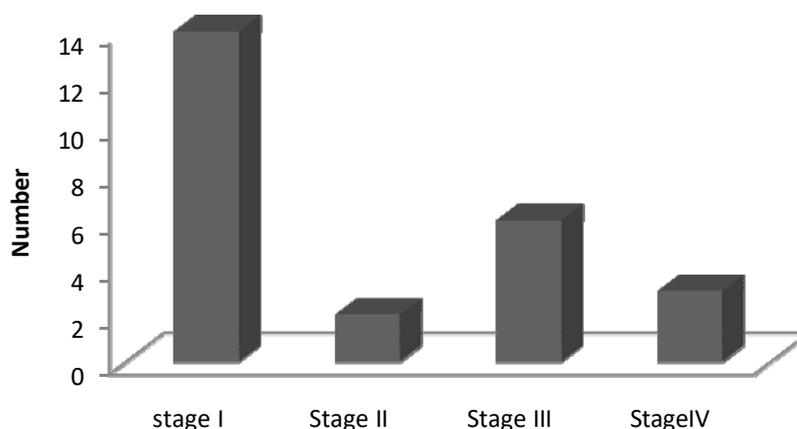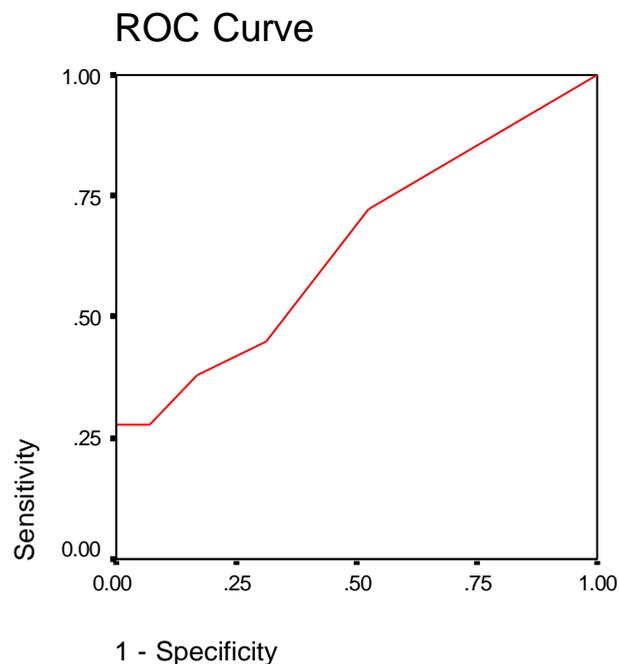
Acknowledgment

Figure 1: The number of studied patients with epithelial ovarian cancer in different stages.

ROC Curve

Diagonal segments are produced by ties.

Figure 2: The comparison of OPN level with CA 125 in all studied patients.

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