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Evaluation of the sensitivity and specificity of serum level of prostasin, CA125, LDH, AFP, and hCG+β in epithelial ovarian cancer patients

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Summary

Objectives: The aim of this work was to compare and analyze the diagnostic value of serum prostasin, cancer antigen 125 (CA125), lactate dehydrogenase (LDH), alpha-fetoprotein (AFP), and human chorionic gonadotropin (hCG+β) in epithelial ovarian cancer (EOC) and evaluate if their serum levels could be used as a potential diagnostic markers of EOC from benign tumors and healthy women. *Materials and Methods:* Preoperative serum samples of 110 women (24 healthy controls, 66 ovarian benign tumors, and 20 EOC) were tested for prostasin, CA125, AFP, and hCG+β. The level of CA125, AFP, and hCG+β serum tumor markers were determined by electrochemiluminescence immunoassay (ECLIA) and the serum level of prostasin was measured using enzyme-linked immunosorbent assay (ELISA) and LDH activity was measured by spectrophotometer and analyzed using SPSS version. *Results:* The Area Under the Curve (AUC) values of prostasin, CA125, LDH, AFP, and hCG+β for the discrimination of EOC from benign and healthy controls were, respectively, 0.89, 0.91, 0.77, 0.54, and 0.65, and significant increase in serum levels of prostasin, CA125, and LDH were observed for EOC compared with benign and control groups. *Conclusion:* The present study showed that CA125 and LDH levels of serum increased in high stages, while prostasin level was decreased in high stages. The present results indicate that prostasin, CA125, and LDH are differentially expressed in EOC than in benign and healthy control population, that may be an indicative of a better diagnostic value, with higher sensitivity and specificity. Here the authors used a multimarker approach, consisting of CA125, AFP, beta hCG, prostasin, and LDH that could provide a more accurate tool for a differential diagnosis of patients with EOC.

Key words: Epithelial ovarian cancer; Prostasin; Cancer antigen 125; Lactate dehydrogenase; Alpha-fetoprotein; Human chorionic gonadotropin.

Introduction

Ovarian cancer is one of the common gynecologic malignancies and the fifth most common cause of cancer death in women. Epithelial ovarian neoplasms derived from tissues that arise from the coelomic epithelium or mesothelium have the highest incidence rate among ovarian cancer patients [1]. In developing countries, ovarian cancer is the seventh most common neoplasm among women, with an estimated 239,000 new cases diagnosed in 2012 worldwide [2]. In Iran, ovarian cancer is the eighth most frequent type of cancer affecting women with a five-year survival rate of 61% [3, 4].

Despite advances in anti-tumor therapy, the survival rate of the disease has not improved during recent decades [5]. Progress in the treatment of ovarian cancer over the past 20 years has resulted in prolongation of patients' survival without an equal improvement in the cure rate [6].

The dismal prognosis of ovarian cancer is mainly caused by its aggressive biological characteristics and lack of specific symptoms. Approximately 70% of patients are diagnosed with advanced Stage III or IV ovarian cancer in which tumor cells have already spread beyond the ovaries and pelvic organs [7-9].

Only 15% of ovarian cancer is diagnosed at early stages and if diagnosed the survival rates are more than 90% [10]. As the stages increase, the survival significantly decreases to as low as 18% for Stage IV ovarian cancer [11]. The significant discrepancy between the early- and late-stage survival rate requires an urgent need to develop highly specific and sensitive screening tests for ovarian cancer at an early stage [12]. Currently, there are no US Food and Drug Administration–approved specific biomarkers for ovarian cancer–targeted therapy [13].

Despite intensive efforts to find and develop new effective population-based screening test, no biomarkers have been identified yet. There are, however, a number of potential candidate diagnostic biomarkers of ovarian cancer under intense investigation that include prostasin, cancer antigen 125 (CA125), alpha-fetoprotein (AFP), lactate dehydrogenase (LDH), and human chorionic gonadotropin

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(hCG).

The role of extracellular proteolysis in cancer progression has been a subject of study for many years with a large focus on secreted proteases [14-15]. Prostasin is a trypsin-like serine protease, and its expression was found in a variety of human tissues with the highest expression in the prostate and semen [16]. In prostate epithelial cells, prostasin regulates inflammatory gene expression and is known to be able to suppress tumor growth and invasion of prostate and breast cancer cells [17]. In contrast, prostasin has been demonstrated to be overexpressed in ovarian cancer cell lines and has been detected at an elevated level in the serum of patients with ovarian cancer [18].

CA125, also known as mucin 16, is an antigenic determinant of a high molecular weight glycoprotein recognized by the murine monoclonal antibody OC-125 and/or M-11 as performed by a routine blood test [19]. It has an established role in monitoring treatment and detecting recurrence of ovarian cancer and has been advocated as a prognostic marker for advanced ovarian cancer [20, 22]. Serum levels of CA125 are believed to correlate with the intensity of disease [23].

Mammalian AFP is classified as a member of the albuminoid gene superfamily. AFP, a tumor-associated fetal protein, has long been employed as a serum fetal defect/tumor marker to monitor distress/disease progression [24, 25].

The serum enzymes might be efficient tumor markers was shown by Awais in 1973 when he first reported considerably high level of LDH in the serum of patients with ovarian cancer [26]. Peritoneal fluid and serum LDH levels in ovarian cancer patients were significantly higher than those in patients with benign ovarian tumor or other gynecological malignancies [27].

HCG is the first hormone message that is produced during pregnancy and is essential for establishing and maintaining early pregnancy [28]. Due to structural heterogeneity, hCG exists in biological fluids as a mixture of different isoforms, i.e., intact active hormone (hCG), nicked hCG (hCGn) which is enzymatically cleaved, free β subunits (hCGβ), free α subunit (hCGα), without biological activity, β-core fragment, and nicked free β-subunit (hCGβn). In a recent investigation, Whittington *et al.* also confirmed that hCG immunoassays might vary remarkably in detecting different hCG variants and hCG+β assay can detect intact hCG, free β-hCG, and other variants, tend to be more sensitive [29]. HCG has also been found to be secreted by some tumors in both men and women such as malignant tumor in the reproductive system of male or ovarian cancer in female where its expression could be an indicator of differentiating between some ovarian tumors [30, 31]. In many ways, detecting the free-subunit in the blood of a person with cancer is like finding an indicator of poor prognosis [32].

The aim of this study was to analyze the levels of four potential biomarkers of ovarian cancer and present results that support a higher specificity and sensitivity of them in epithelial ovarian cancer (EOC) than benign tumor and healthy control population.

Materials and Methods

This study was performed in 2012 at Motahari Hospital in Urmia in 110 cases, including 20 patients with EOC, 66 patients with benign tumor, and 24 healthy women. The control group was selected from medical students and staff of the hospital who had no history of benign and malignant ovarian tumors or irregular menstruation. This study was approved by the ethics committee of Urmia Medical University. Phlebotomy was conducted in all subjects and the serum samples were stored in the freezer at -40°C.

The serum level of prostasin was measured using enzyme-linked immunosorbent assay (ELISA). Briefly, standards or samples were added to the appropriate micro ELISA plate wells and combined with the pre-coated anti-prostasin immunoglobulin G. Afterwards, a biotinylated detection antibody specific for the antigen and avidin-horseradish peroxidase (HRP) conjugate was added to each micro plate well and incubated at 37°C for 60 minutes. Free components were washed and the substrate solution was added to each well. The wells containing the antigen appeared blue as the results of the biotinylated detection antibody and avidin-HRP conjugate. The enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution. Absorbance was measured spectrophotometrically at 450 nm. The absorbance value is proportional to the concentration of the antigen.

The level of CA125, AFP, and hCG+β serum tumor markers were determined by electrochemiluminescence immunoassay (ECLIA) using immunoassay analyzers. Briefly, for the detection of CA125, samples were incubated with biotinylated monoclonal CA125-specific antibody and a second ruthenium labelled monoclonal CA125-specific antibody acting as a capture antibody to form a sandwich complex. After addition of streptavidin-coated microparticles, the complex bounds to the solid phase via interaction of biotin and streptavidin. The reaction mixture was then aspirated into the measuring cell where the microparticles were magnetically captured onto the surface of the electrode. After removing the unbound substances with ProCell/ProCell M, chemiluminescent emission was measured by a photomultiplier and analysed according to the manufacturer recommendations. Similar procedure was used to quantitate the levels of hCG+ free β-subunit, and AFP.

LDH activity of serum samples measured by the optimized standard method recommended by the German Society for Clinical Chemistry (DGKC) using the commercial kit and measured on an autoanalyser. Decrease of the absorbance value at 340 nm, due to the NADH oxidation in NAD+, is directly proportional to the enzyme activity.

The data was analyzed using SPSS version 18, and Receiver Operator Characteristic (ROC) curve and ANOVA test were used for data analysis, meanwhile the level of statistical significance was considered to be $p < 0.05$.

Results

Demographic and clinical features of the study group are summarized in Table 1. The mean age of the patients with EOC of 49.8 years, was significantly higher ($p < 0.05$) than patients with benign ovarian tumor with a mean of 39.5 years. Fifty-five percent of patients with EOC were in post-

Table 1. — *The demographic and clinical characteristics of the study groups.*

	Malignant	Benign	Healthy control
Case number	20	66	24
Age (years)	49.8	39.5	40.4
Premenopausal	9(45%)	51(77.2%)	20(83.3%)
Postmenopausal	11(55%)	15(22.8%)	4(16.7%)
AUB	8(40%)	17(25.7%)	0
Familial history of gynecologic malignancy	0	2(3%)	0
OCP consumption	10(50%)	32(48.5%)	7(29.2%)

EOC: epithelial ovarian cancer, AUB: abnormal uterine bleeding, OCP: oral contraceptive.

Table 3. — *Receiver Operating Characteristics (ROC) curve analysis for the prostasin, CA-125, AFP, hCG+β and LDH.*

Test	AUC	Error	Sig.	Asymptotic 95% Confidence interval	
				Lower bound	Upper bound
CA125	0.91	0.048	0.000	0.819	0.997
Prostasin	0.89	0.042	0.000	0.806	0.972
LDH	0.77	0.060	0.001	0.639	0.874
hCG+β	0.65	0.073	0.069	0.496	0.781
AFP	0.54	0.075	0.509	0.404	0.696

AUC: Area Under Curve.

menopausal stage and 40% had abnormal uterine bleeding (AUB). Fifty-five percent of ovarian cancer cases had previous history of oral contraceptive consumption (Table 1).

A significant increase in serum prostasin level was observed for EOC (mean: 12.36 μg/ml) comparing with the benign (mean: 8.36 μg/ml) and control groups (mean: 7.21 μg/ml) ($p < 0.05$) [33]. No significant increases were observed in serum prostasin level of patients with benign tumors in comparison with the control group (Table 2). For evaluation of prostasin sensitivity and specificity in diagnosis of EOC, ROC curve was constructed and Area Under the Curve (AUC) was calculated. The AUC of prostasin for diagnosis of EOC from benign ovarian tumors and healthy women was 0.89, suggesting a cut-off point, and sensitivity and specificity of prostasin in diagnosis of EOC from ovarian benign tumors and healthy women had a suggested cut-off point of 9.3 μg/ml, and sensitivity and specificity of prostasin in diagnosis of EOC from ovarian benign tumors and healthy women were 89% and 75 %, respectively (Table 3, Figure 1). Determination of prostasin in different stages of EOC showed that prostasin serum level was decreased in high stages (Figure 2a). Otherwise prostasin can be used as a good marker of EOC in early stages.

The mean serum level of CA125 for patients with EOC was 621 IU/ml, for group with benign tumors it was 44.2 IU/ml, and for control groups it was 15.3 IU/ml (Table 2). A significant increase in serum level of CA125 was observed for malignant group compared with benign and control group ($p < 0.05$). Serum CA125 level also was slightly higher in patients with benign tumor compared with control group, although this increase was not significant. The AUC of CA125 for diagnosis of EOC from benign ovarian tumors and healthy women was 0.91. Sensitivity and specificity of CA125 in diagnosis of EOC from benign ovarian tumors and healthy women were 85% and 76%, respectively with routinely used cut-off (35 IU/ml) (Figure 1, Table 3). CA125 serum levels were increased in high stages of EOC in comparison with low stages (Figure 2b).

Table 2. — *Comparison of the serum prostasin, CA125, AFP, hCG+β, and LDH levels among patients with EOC versus other benign ovarian tumors and healthy control women.*

		Mean	95% confidence interval		S.E.M	Tukey HSD multiple comparisons	*p* value
			Lower limit	Upper limit			
Prostasin (μg/ml)	Ovarian cancer	12.4	11.3	13.5	0.5	Cancer vs Benign	< 0.001
	Benign tumor	8.3	7.5	9.0	0.4	Cancer vs Control	< 0.001
	Healthy control	7.2	6.5	8.0	0.4	Control vs Benign	0.169
CA125 (IU/ml)	Ovarian cancer	621.3	381.8	860.7	114	Cancer vs. Benign	< 0.001
	Benign tumor	44.2	30.4	58.0	6.9	Cancer vs Control	< 0.001
	Healthy control	15.3	12.3	18.1	1.3	Control vs Benign	0.855
AFP (IU/ml)	Ovarian cancer	3.4	0.5	6.6	1.50	Cancer vs Benign	0.988
	Benign tumor	1.6	1.3	1.9	0.15	Cancer vs Control	0.953
	Healthy control	2.5	2.0	3.3	0.30	Control vs Benign	0.855
hCG+β (IU/L)	Ovarian cancer	1.5	0.8	2.3	0.3	Cancer vs Benign	0.653
	Benign tumor	1.2	0.7	2.15	0.3	Cancer vs Control	1.000
	Healthy control	0.3	-0.1	0.7	0.2	Control vs Benign	0.612
LDH (IU/L)	Ovarian cancer	480	411	549	33	Cancer vs. Benign	0.002
	Benign tumor	378	337	418	20	Cancer vs. control	< 0.001
	Healthy control	243	210	280	17	Control vs Benign	< 0.001

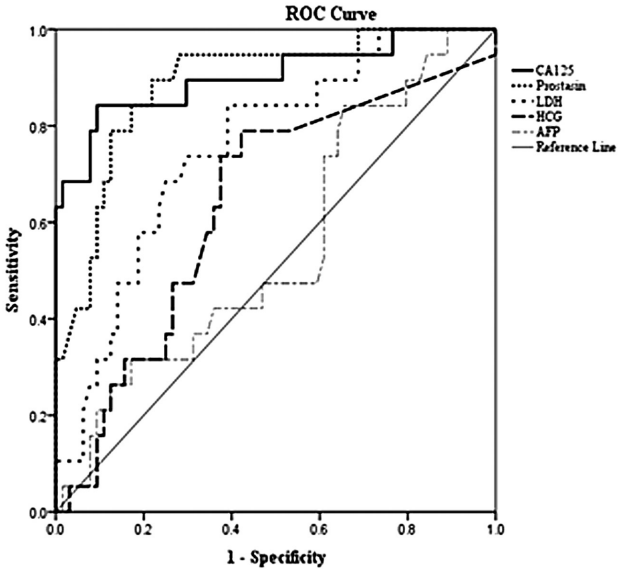

Figure 1. — Comparison of the Area Under the Curve (AUC) from the Receiver Operating Characteristics (ROC) curve analysis for serum prostasin, CA-125, AFP, hCG+β, and LDH for diagnosis of EOC from benign tumor and control group.

Table 4. — *Pearson correlations between prostasin, CA125, AFP, hCG+β, and LDH.*

	Prostasin	CA125	hCG+β	AFP	LDH
Prostasin					
Pearson correlation	1	0.412**	-0.048	0.085	0.401**
Sig. (2-tailed)		0.000	0.665	0.446	0.000
CA125					
Pearson correlation	0.412**	1	0.618	0.629	0.370**
Sig. (two-tailed)	0.000		-0.048	-0.047	0.000
hCG+β					
Pearson correlation	-0.048	-0.048	1	0.330**	0.032
Sig. (two-tailed)	0.665	0.618		0.000	0.744
AFP					
Pearson correlation	0.085	-0.047	0.330**	1	0.015
Sig. (two-tailed)	0.446	0.629	0.000		0.877
LDH					
Pearson correlation	0.401**	0.370**	0.032	0.015	1
Sig. (two-tailed)	0.000	0.000	0.744	0.877	

**Correlation is significant at the 0.01 level (two-tailed).
*Correlation is significant at the 0.05 level (two-tailed).

The mean serum concentration of LDH in patients with EOC was 480 IU/L, for groups with benign tumor and in healthy subjects, they were 378 IU/L and 243 IU/L, respectively. Significant increase in serum levels of LDH was observed for EOC compared with benign tumors and control group ($p < 0.05$). Serum levels of LDH also were higher in patients with benign tumors compared with control group ($p < 0.05$) (Table 2). The AUC of LDH for diagnosis of EOC from benign tumors and healthy subjects were 0.77 (Figure 1, Table 3). Serum levels of LDH increased in high stages of EOCs (Figure 2c). Cut-off point, sensitivity, and specificity of LDH in diagnosis of EOC from benign ovarian tumors and healthy control were 360 IU/L, 75%, and 68%, respectively.

The mean serum level of AFP for patients with EOC was 3.4 IU/ml, for group with benign tumor it was 1.6 IU/ml, and for control groups it was 2.5 IU/ml. No significant increase in serum levels of AFP was observed for EOC compared with benign and control group ($p > 0.05$) (Table 2). The AUC of AFP for diagnosis of EOC from benign and healthy controls was 0.54; due to its low value, it failed in diagnosing EOC (Figure 1, Table 3).

The mean serum level of hCG+β for patients with EOC was 1.5 IU/L, for group with benign tumor it was 1.2 IU/L, and for control groups was it 0.3 IU/L. No significant increase in serum level of AFP was observed for EOC compared with benign and control group ($p > 0.05$) (Table 2). The AUC of hCG+β for diagnosis of EOC from benign and healthy controls was 0.65, hence it had a poor value in EOC diagnosis (Figure 1, Table 3).

For determining of correlation between prostasin,

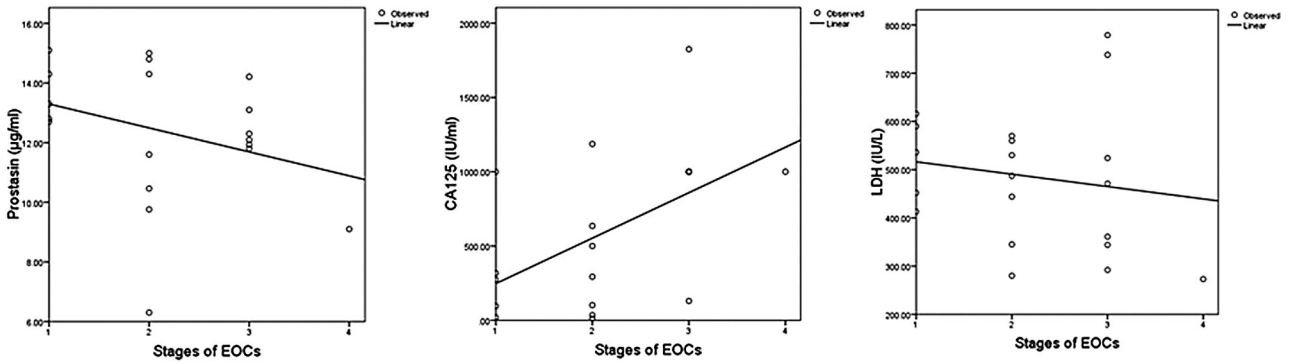

Figure 2. — Estimated regression between prostasin, CA125, and LDH with different stages of EOCs.

CA125, AFP, hCG+β, and LDH, Pearson test was used. The results from this test showed that a weak positive correlation (+0.41) exists between CA125 and prostasin (Table 4).

Discussion

Among Iranian population, ovarian cancer is the eighth most frequent for incidence, the 12th most frequent for mortality, and ranks 16th for cancer burden in Iran [33]. Various proteins are being evaluated to see if they can be used as the potential diagnostic markers for the screening of ovarian cancer [34, 35]. The most widely used serum biomarker in ovarian cancer screening is CA125 [36]. Previous data indicates a sensitivity of more than 73% and a specificity of 79%, of the serum level which are comparable to other biomarkers in predicting ovarian malignancy [37]. However, on the other hand, reports indicate that the increase is also detected in benign cases [29, 30]. Recent data from the US Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial, however, shows no mortality benefit using a screening strategy incorporating CA125 cut-off [38]. Overall, the present results are in line with results observed in other studies that measured serum level of CA125 that can be used as an efficient biomarker for the diagnosis of ovarian cancer, in terms of sensitivity and specificity [30-32, 34, 39].

Prostasin is another widely evaluated potent biomarkers of ovarian cancer. The present results also confirm a significant higher level of this protein in the malignant tumors than benign ovarian tumors [8, 36-37] . In the study of Chen *et al.* on prostate cancer showed that a negative correlation was obtained between prostasin expression and stage of malignancy, therefore prostasin can be consider as an inhibitor of metastases [40]. The results of the present study showed that serum levels of prostasin decreased in high stages of EOC. Therefore prostasin may be used as a good marker in EOC diagnosis in early stages.

An elevated level of LDH has been reported in the total serum of ovarian adenocarcinomas patients (26, 41-43). Similarly Schneider *et al.* reported a significantly higher level of LDH in patients with ovarian cancers than benign ovarian tumors [27]. Boran *et al.* showed that serum LDH levels in ovarian cancer patients were significantly higher than those in patients with benign ovarian tumor but no significant differences were observed in LDH levels of different histological types of ovarian cancer and different stages of the disease [44]. In the present study the authors found that the level of LDH is significantly higher in patients with EOC than women with benign ovarian tumors and healthy control. The present authors also observed a slight, but not significant, increase in the LDH serum level among higher stages of ovarian cancer. Altogether the present findings provide evidence that serum LDH level has sufficient diagnostic sensitivity to be used as a biochemical marker for EOC.

Both AFP and beta-hCG are used as biomarkers of ovarian germ cell tumors and in evaluating potential origins of poorly differentiated metastatic cancer. Both biomarkers, however, are also used in the screening of other malignancies. AFP, for example, is used as a marker of hepatocellular carcinoma and occasionally in the screening of highly selected populations for assessing hepatic masses and in the risk for developing hepatic malignancy and beta-hCG is considered as an integral part of diagnosis and management of gestational trophoblastic disease [45, 46]. The present screening did not reveal statistically significance differences in the serum levels of AFP and hCG+β in advanced stages of EOC in comparison with benign ovarian tumor and healthy women. In agreement with the present results, Panza *et al.* showed that hCG+β levels of plasma were not significantly increased in EOC and there was no correlation between EOC and hCG+β levels of plasma [47]. Similar results have also been reported by Aggarwal and Kehoe where no significant increase in AFP level was observed in EOCs [48]. AFP increased in ovarian cancers such as dysgerminoma and some rare types of ovarian cancer, such as yolk sac tumors and embryonal carcinoma that were not present in this study [49].

Studies of Muller and Cole showed that serum free β-subunit or its urinary degradation product β-core fragment is produced by 68% of patient with malignant ovarian tumors, 51% of endometrial and 46% of cervical malignancies [50]. However, in contrast to them, the present authors did not observe any elevation in the level of hCG+β in ovarian cancer. Overall, in present study, the authors used a multimarker approach, consisting of CA125, AFP, beta hCG, prostasin, and LDH that could provide a more accurate tool for a differential diagnosis of patients with epithelial ovarian.

Conclusion

No significant differences were observed in this study in the levels of AFP and hCG+β among EOC, benign tumor, or healthy controls. CA125 serum levels were increased in high stages of EOC in comparison with low stages. Determination of prostasin in different stages of EOC showed that prostasin serum level was decreased in high stages. Otherwise prostasin can be used as a good marker of EOC in early stages. The serum levels of LDH was significantly higher in EOC in comparison to benign tumors and control healthy women. Serum levels of LDH were also higher in patients with benign tumors compared with control group. Serum levels of LDH increased in high stages of EOCs. Furthermore, combination of prostasin and LDH, with CA125 will improve the prediction of EOC status.

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