Original Article

Simultaneous Measurement of two Serum Markers (*CA-125* and *HE-4)* while Diagnosing Malignant Ovarian Epithelial Tumors

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ABSTRACT

Objective: To evaluate the benefits of simultaneous measurement of CA-125 and HE-4 markers while diagnosing malignant epithelial tumors in the ovary. By this, the combined measurement of serum markers will possibly add to the accuracy of diagnosing such ovarian tumor.

Methodology: Performing a cross- sectional study on 87 women with ovarian mass, serum levels of CA125 and HE4 markers were measured before surgery or biopsy. In the wake of the surgery or biopsy, the results obtained from these tests were compared and analyzed with pathological report.

Results: The average serum level of CA-125 and HE-4 serum was notably higher in women with ovarian malignancy than in those with benignancy (CA-125: 502 vs. 19.3 v/ml, P < 0.001- HE4: 195 vs. 15.8 P mol/L, P < 0.001). As the disease stage rises, the level of these markers increases significantly. The two markers were also directly proportionate. (r = 0.85 and P < 0.001). There is also a meaningful difference between the levels of markers, specifically HE-4, in epithelial and non-epithelial tumors of ovary (HE-4: 195 vs. 93 P mol/L P <0.001). The simultaneous measurement of CA-125 and HE-4 increases the sensitivity and specificity of diagnosing malignant epithelial tumors in ovary, compared with one- by- one measurement guideline. The sensitivity and specificity of simultaneous measurement of CA125 and HE4 for diagnosing epithelial ovarian cancer were calculated to be 99.5% and 100%, respectively.

Conclusion: Simultaneous measurement of CA-125 and HE-4 increases the sensitivity and keep the specificity still high in diagnosing malignant epithelial tumors in ovary, compared with one-by-one measurement system.

KEY WORDS: CA-125, HE-4, Ovarian Malignant Epithelial Tumors.

How to cite this article:

Modarres-Gilani A, Ghaemmaghami F, Mousavi A, Abbasi F, Abdollahi A, Shoar S. Simultaneous Measurement of two Serum Markers (*CA-125* and *HE-4)* while Diagnosing Malignant Ovarian Epithelial Tumors. Pak J Med Sci 2011;27(4):858-861

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INTRODUCTION

Ovarian carcinoma is the fifth leading cause of cancer-related mortalities in women in United States of America.[1] In 2010, it is estimated that ovarian cancer accounts for approximately 14,000 deaths and 5% of the cancer deaths in women.[1] The stage at which diagnosis is made is an important factor to determine the patient's survival; however, most cases are diagnosed after the tumor metastasizes through their bodies.[1,2] According to multiple previous studies, 62% of women with ovarian cancer were diagnosed when the disease has spread to other organs (Stage

IV) and this was associated with a 27.6% 5– year survival rate.[2-8] Fifteen percent of women diagnosed with localized cancer (Stage 1) had a 93.5% 5– year survival rate[4]; among these, epithelial ovarian tumor accounts for 85–90% of ovarian cancers.[4]

There is no reliable test to differentiate between benign and malignant ovarian tumors. CA-125 marker alone has proven to be incapable of diagnosing a malignant tumor in the ovary or differentiating the malignancy or benignancy of an ovarian tumor.[2,3] Currently, in most clinics in Iran and the Middle East, the CA-125 is the solo lab marker used for diagnosis of ovarian cancer and monitoring its treatment. CA125 level can also be elevated in benign conditions such as endometriosis, congestive heart failure and cirrhosis[4,5] and it tends to be higher in premenopausal women, increasing the likelihood of false positives cases when used in this population.[5,9] Sensitivity and specificity of CA125 does not fully protects all malignant epithelial ovarian carcinoma. Human Epididymis Protein 4 (HE4) is made up of two whey acidic proteins with a four disulfide core domain.[5,6,9] It has been found to be over-expressed by epithelial ovarian cancer tumors and to circulate in the serum of such patients.[6,7] Levels of HE4 are less likely to be elevated in benign conditions as is the case of CA–125 makes it a candidate to replace or at least complement role of CA–125 as a serum marker. In addition, two serum markers will result in more accuracy and sensitivity than only one marker.

Hence, this study aimed to evaluate the simultaneous measurement of CA-125 and HE-4 markers in the view of diagnosing malignant epithelial tumors in the ovary of patients referring to a high referral hospital of Tehran to determine if this combination will serve the accuracy of diagnosis for the better; and if yes, what are the sensitivity and specificity results.

METHODOLOGY

We performed a cross- sectional study on 87 patients from Jan 2010 to Oct 2010 at a referral teaching hospital in Tehran, Iran. In this study, women already diagnosed with adnexal mass were referred to the hospital to undergo surgery or laparoscopy and biopsy for a definite diagnosis or treatment procedure. Based on the pathological reports, the patients were divided into three groups: benign, epithelial malignant and non-epithelial malignant.

The patients with any adnexal tumor whether benign or malignant who needed to undergo a surgery or biopsy were included in the study. The patients with history of previous breast or ovary tumor and pregnancy were excluded. A questionnaire including information about demographic parameters, history of breast or ovarian cancer in immediate family members, etc was also completed for each patient. Before surgery and biopsy all peripheral blood samples were collected in five milliliters Vacutainer tubes. Samples were kept at a room temperature for a maximum of thirty minutes. Separation of the blood sample was accomplished by centrifugation at 3000g for ten minutes.

Serum HE4 was measured by Enzyme immunometric assay (ELISA) method, Can-Ag Company SWEDEN Kit. Expected value of this marker was below 150pM. The functional sensitivity of the HE4 EIA assay was d" 25pM. The cut off for detection of the HE4 EIA assay was d"15pM. The HE4 assay precision was d" 15% of total CV. Serum CA125 was measured by Enzyme immunometric assay (ELISA) method, Can-Ag Company SWEDEN Kit. Expected value of this marker was 5.06- 47.9 U/ml. Measurement range of this kit was between 1.5 and 500 U/ml and detection cut off for this assay was < 1.5.

After surgery or biopsy, results of laboratory tests (CA125 and HE4 serum levels) were compared with pathology report (histopathology) based on the aims of the study. This study was carried out according to the principles of the Declaration of Helsinki. The local ethics review committee of Tehran University of Medical Sciences approved the study protocol. All participants gave written informed consent before participation. Laboratory observer and data analyzer didn't have any idea about the relationship of samples to patients.

After collecting the data, statistical package for the social science, SPSS version 17 for Windows (SPSS Inc., Chicago, IL, USA), was used for statistical analysis. Correlation between serum markers and histopathological data were analyzed by Spearman correlation coefficient and differences were evaluated by chi- square test and considered significant at $p < 0.05$.

RESULTS

Primary characteristics of the patients including demographics and malignancy distribution of the tumors are presented in Table-I. Eighty seven patients, 50 with benign and 37 with malignant tumors, were included in the study; among married patients, 3 cases were reported to be infertile. 43 patients were in pre-menopausal status and the rest were in their post-menopausal.

Table-I: Primary characteristics of the patients
with malignant ovarian cancers compared
with those of benign ovarian cancers.

Tumor Demographics	Benign (n=50)	Malignant (n=37)	Total=87
Age(Yrs)	42.84 ±16.64	45.85 ± 16.20	
Pre-menopause	29 (60%)	14 (40%)	43
Post-Menopause	21 (40%)	23 (60%)	44
			Total=87
Single	14(28%)	4 (11%)	18
Married	36 (72%)	33 (62%)	69
			Total=87

Groups are matched for age and BMI. Quantitative variables are expressed as
Mean ± Standard Deviation otherwise number.

Serum HE- 4 levels were significantly higher among patients with malignant ovarian cancer compared to those with benign tumors (Table-II). This was significant after multiple adjustments for age, BMI, and menopausal status using general linear model. The average serum levels of CA-125 and HE- 4 were notably higher in women with ovarian malignancy than those with benignancy (CA-125: 502 vs. 19.3 U/ml, P < 0.001 HE-4: 195 vs. 15.8 P mol/L, P< 0.001).

As the stage of disease rises, the markers increase significantly. These two markers were also directly proportionate to each other (r=0.85 and P < 0.001). There is also a significant difference between the levels of markers (specifically HE-4) in epithelial and non-epithelial tumors of ovary (HE-4: 195 vs. 93 P mol/L P <0.001).

The sensitivity and specificity of CA- 125 using a cut – off level of 37 U/ml were 85% and 100%, respectively (AUC = 0.93, p < 0.001); while the sensitivity and specificity of HE- 4 using a cut – off level of 25 P mol/l were 99 and 100%, respectively (AUC = 0.1, p < 0.001). Combined together, the sensitivity and specificity of simultaneous measurement of CA125 and HE- 4 were 99.5% and 100 %, respectively (Table-III).

DISCUSSION

The main finding of this study was that serum HE- 4 is significantly higher in patients with malignant epithelial ovary tumors among Iranian patients. In our study, the simultaneous measurement of CA-125 and HE- 4 increased the sensitivity while keeping specificity in diagnosing malignant epithelial tumors in the ovary, compared with one- by- one measurement system. Our results may have been similar to some others' from previous studies[10,12-20]; but they did not necessarily endorse them. The differences seem to have stemmed from the samples, the

Table-II: Serum level of biomarkers in patients
with malignant ovarian cancers compared
with those of benign ovarian cancers.

Tumor Marker	Benign	Malignant	P value
CA-125 (U/ml)	502	19.3	<0.0001
HE-4 (P mol/L)	195	15.8	<0.0001

short duration of the study as well as the sensitivity and specificity of ELISA kits.

Ovarian cancer is diagnosed annually in more than 200,000 women worldwide, with the greatest incidence in the US and Northern Europe, and lowest incidence in Africa and Asia.[3] Approximately 1 in every 57 women in the US will die of this disease.[9] Fewer than 30% of all ovarian cancer are diagnosed in stages I/II.[10]

Using precise lab methods with high sensitivity and specificity can contribute to more precise and accurate diagnosis while avoiding unnecessary costs and surgeries. Highest sensitivity of biomarker measuring tests is achieved when the cut off is determined in diagnostic stage I/II of epithelial ovarian cancer; however, this costs the system at the price of raising false positives in benign tumors cases or healthy women leading to an undesirable specificity.[11] That explains such high sensitivity and specificity of biomarkers especially CA- 125 in our study.

Hellstrom et al showed that there is 67% sensitivity and 96% specificity for HE4 in the detection of ovarian cancer.[6] Moore et al in a subsequent study evaluating numerous known biomarkers for ovarian cancer showed that HE- 4 has the highest sensitivity for the detection of ovarian cancer, particularly in early stage disease. In this study, the combination of HE- 4 was a more accurate predictor of malignancy than each marker alone, with a sensitivity of 76% and a specificity of 95%.[5]

The serum level of CA-125 may rise in many other clinical stages in different circumstance rather than ovarian malignancies and therefore it cannot serve as an effective indicator when used alone.[12,13] The serum CA- 125 blood test is effective for monitoring women with ovarian cancer for progression or recurrence. The serum measurement of HE- 4 may have an advantage over CA- 125, because the former is

Table- III: Sensitivity and specificity of
each biomarker alone and in combination.

Serum marker	Sensitivity %	Specificity %
CA-125	85	100
HE-4	99	100
Combination measurement	99.5	100

less frequently raised in patients with non malignant ovary disease.[8]

HE4 (WFDC2) is made up of two whey acidic protein (WAP) domains and a 4 disulfide core and has been shown to be over expressed by epithelial ovarian cancer tumors. Similarly HE4 is not elevated in many common benign gynecologic and medical conditions where CA- 125 is elevated.[10]

In premenopausal women, CA- 125 suffers from a lack of specificity secondary to its tendency to be elevated in many common benign gynecologic and non gynecologic conditions. Because HE4 is not falsely elevated in many of these conditions it may complement CA- 125.[14,15]

Thus, measuring both HE- 4 and CA- 125 together, rather than relying on either of them alone, provides a more accurate tool for differential diagnosis of patients with ovarian cancer. It may also help clinicians in the follow – up of patients suffering from advanced epithelial ovarian cancer.

The limitation of our study is mainly its small size of investigated population. Those inherent limitations in cross- sectional analysis which precludes the determination of the direction of causality may not be desirable in performing such a study which aims to determine benefits and accuracy of a group of biomarkers, especially in comparison to already introduced tests; however, we took advantage of a close similarity between groups in most of the confounding variables.

Our findings are confirmatory to previous studies somehow as we showed that serum HE- 4 levels is significantly higher among patients with ovarian cancer. Whether these findings are confounded by other factors, has to be studied in future. In the light of the limitations of this study, investigations with much more significant samples is strongly recommended.

In conclusion, simultaneous measurement of CA- 125 and HE-4 seems to increase the sensitivity, while keeping its 100% specificity, in diagnosing malignant epithelial tumors in ovary, compared with one-by-one measurement system.

Conflict of interest statement: The authors declare hereby that there is no conflict of interest.

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