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HE4: really a novel biomarker for ovarian cancer?

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Summary

Ovarian cancer forms 4% of all cancers and approximately 23% of all gynecological cancers in women and is responsible for the 47% of deaths related to cancers of the genital tract of women. Tumor markers are the biochemical substances which can be detected in the presence of tumors. Generally they are either the products of tumoral tissues or secreted from the normal cells which are in the interaction with tumoral ones. The present authors attempted to determine the efficacy of the tumor marker CA-125 and HE4 to differentiate the malign cases from the benign adnexal masses. A total of 76 patients with the appropriate criteria were included in the study. They were divided into three groups; healthy control group (n=31), ones with benign masses (n=23), and ones with malign ovarian masses (n=22). In the study, when the cut-off values were accepted as 55I U/ml for CA-125 and 150 pM for HE4 in differentiation of benign and malign groups, the sensitivity was found as 59.09%, specificity 91.3%, PPV 86.67% and NPV 70% LR = +6.8. This combination gives one false positive result to every five positive cases which were detected as high. With the combination of CA-125 and HE4, the value of sensitivity was found decreased as expected, although the value of the specificity increased.

Key words: Tumor markers; HE4; CA-125; Adnexal mass; Ovarian cancer.

Introduction

Ovarian cancer forms 4% of all cancers and approximately 23% of all gynecological cancers in women. Ovarian cancer is responsible for the 47% of deaths related to cancers of the genital tract of women [1]. The incidence of ovarian cancer increase after the age of 40. The incidence is 60 case per 100,000 women between the ages of 60-80 whereas 0.4-8.9 cases per 100,000 women under the age of 40 [2]. The survival rate after the age of 65 is the almost half of the rate under the age of 65 [1]. More than one-third of the cases are seen after the age of 65. The incidence reaches the highest rate between the age of 75-79 [3, 4]. Because the morphological scoring system alone is incapable of differentiating the malign tumors from the benign ones, explorative laparotomy has to be performed for the definite diagnosis. It is clear that the possibility of determining asymptomatic cases at early levels by routine pelvic examination is very poor.

Tumor markers are the biochemical substances which can be detected in the presence of tumors. Generally they are either the products of tumoral tissues or secreted from the normal cells which are in the interaction with tumoral ones [5]. Optimum tumor marker is the one that is specific to the tumor; however, the marker, that is only occurring in the presence of the tumor, is not yet found. CA-125 is a marker which was first used by Bast *et al.* and has been still used for 30 years in gynecology, especially for the diagnosis and following of the ovarian cancer [6]. CA-125 is a mucin-like glycoprotein which can be detected by a monoclonal antibody in the serum that is prepared from mice and in the form of immunoglobulin G1 [7].

Human epididymis protein 4 (HE-4) is a protein tumor marker and 11kDa in weight. HE-4 is a secretary protein precursor of the epididymis. It is first detected in the male reproductive system (distal epithelium of the epididymis) hence the name and later it was shown that it is expressed in female reproductive system (fallopian tube, endometrial and endocervical glands) and most of normal tissues consisting of epithelium of the respiratory system [8, 9]. It is shown that HE-4 can be determined as a new serological marker for the early diagnosis of the ovarian cancer in the studies of last decade [10-13]. Like CA-125, the physiological functions of HE-4 has not been known yet.

In the present study the authors attempted to determine the efficacy of the tumor markers CA-125 and HE4 to differentiate the malign cases from the benign adnexal masses.

Materials and Methods

Population of the study

The patients operated by laparotomy or laparoscopy with the indication of adnexal mass were included in this study. The study protocol was formed by the approval of the ethics committee of Maltepe University Faculty of Medicine and Research Hospital. All the patients were over the age of 18 and they were informed before their blood samples were taken. Written informed consents

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Table 1. — *The median levels of CA-125 and HE4.*

		Healthy	Benign	Malign	KW	*p*
CA-125	Median (IQR)	13.8 (9.02−20.32)	30.7 (16.3−87.8)	335.5 (64.25−744.8)	37.53	0.0001
HE4	Median (IQR)	27.6 (22.16−36.53)	109.4 (56.47−172.19)	188.8 (142.59−269.26)	46.54	0.0001

Table 2. — *The statistical values of CA-125 measurement at different thresholds.*

Threshold	Sensitivity	95% CI	Specificity	95% CI	PPV	NPV	+LR	-LR
> 33	81.8	59.7−94.7	56.5	34.5−76.8	64.3	76.5	1.88	0.32
> 55.35 *	81.8	59.7−94.7	73.9	51.6−89.7	75.0	81.0	3.14	0.25
> 1258.47	13.6	3.1−34.9	95.6	78.0−99.3	75.0	53.7	3.14	0.90

* Optimal threshold. PPV: positive predictive value, NPV: negative predictive value, +LR: positive likehood ratio, -LR: negative likehood ratio.

Table 3. — *The statistical values of CA-125 measurement at different thresholds.*

Threshold	Sensitivity	95% CI	Specificity	95% CI	PPV	NPV	+LR	-LR
> 147.56 *	77.2	54.6−92.1	73.9	51.6−89.7	73.9	77.3	2.96	0.31
> 355.66	4.5	0.8−22.9	94.3	78.0−99.3	33.3	50.0	0.52	1.05

* Optimal threshold.

were taken after having read the form.

The exclusion criteria were: pregnancy, previous history of ovarian malignancy or surgery for benign adnexal masses, and usage of oral contraceptive pills. Comprehensive history evaluation of the patients were done. All patients had radiologic imaging either by pelvic ultrasound (US), computed tomography scanning (CT), and/or magnetic resonance imaging (MRI) prior to surgery to document the presence of an ovarian cyst or pelvic mass.

A total of 76 patients with the appropriate criteria were included in the study. They were divided into three groups; healthy control group (n=31), ones with benign masses (n=23), and ones with malign ovarian masses (n=22). Immediately prior to surgery, blood samples were obtained. Histopathological diagnosis was regarded as gold standard for the evaluation of the results.

The authors aimed to determine the efficacy and the role of these two serum markers in preoperative evaluation and management of adnexal masses by detecting the result's correlation with the histopathological diagnosis.

Sampling and laboratory analyses

Approximately five ml blood samples taken from all patients were saved in non-heparinized tubes. They were kept in -40°C until the time of assesment. The measurement of HE4 was done according to the manufacturer's instructions through the enzyme immunometric assay (EIA). Unit of measurement was used as picomoles/liter (pmol/L). Though the threshold value changes by the techniques (colorimetric or chemiluminescence) used, the value was excepted as 150 pmol/L by following the manufacturer's instructions (EIA).

The level of CA-125 was detected by an autoanalyser with the method of immuno-chemiluminescence on the same day and processing after the same control and calibration. The threshold value was accepted as 35 IU/ml.

Statistical analyses were done by using the SPSS-16.0. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likehood ratio (+LR), and negative likehood ratio (-LR) values were calculated for the tumor markers.

Results

A total of 71 patients were enrolled in the study. The mean ages were 45.19 ± 12.72, 35.7 ± 9.87 and 57.64 ± 9.94 for healthy, benign and malign groups, respectively. All the malign cases (n=22) were epithelial ovarian cancer, serous=16 (72.72%), mucinous = 3 (13.63%), endometroid = 2 (9.1%), and clear cell = 1 (4.55%). The mean ages of control and benign groups were statistically lower then the malign group ($p = 0.0001$).

The mean number of pregnancies of patients with epithelial ovarian cancer was significantly higher compared to other groups (4.64 ± 2.44 versus 3.16 ± 2.42 for healthy and 1.91 ± 1.95 for benign group $p = 0.001$). Also there was a statistically significant difference in the distribution of the diabetes mellitus among the groups ($p = 0.028$). (The patients with diabetes mellitus were detected in higher rates in malign group). Among the complaints, abdominal distension, pain, and irregular vaginal bleeding, were the mostly seen symptoms for malignant cases. Benign masses were diagnosed with abdominal and pelvic pain.

The serum levels of HE4 and CA-125 were evaluated among the groups. The median serum levels of CA-125 were 335.5 U/ml (interquartile range IQR, 64-744) in the malign cohort, 30.7 U/ml (IQR, 16-87) in the benign cohort, and 13.8 U/ml (IQR, 9-20) in the healthy cohort. The serum CA-125 levels were significantly different between the groups ($p < 0.001$ between malign-healthy, benign-healthy, and $p = 0.001$ between malign-benign).The median serum levels of HE4 were 188.8 pM ([IQR], 142-269) in the malign group, 109.4 pM (IQR, 56-172) in the benign group, and 27.6 pM (IQR, 22-36) in the healthy group with statistically significant differences ($p < 0.001$ between malign-healthy, benign-healthy, and $p = 0.023$ between ma-

Table 4. — *The ROC curve analysis for CA-125 and HE4 for optimal thresholds.*

Benign / malign	AUC	95% CI
CA-125	0.78	0.63−0.89
HE4	0.69	0.543−0.82

AUC: area under curve.

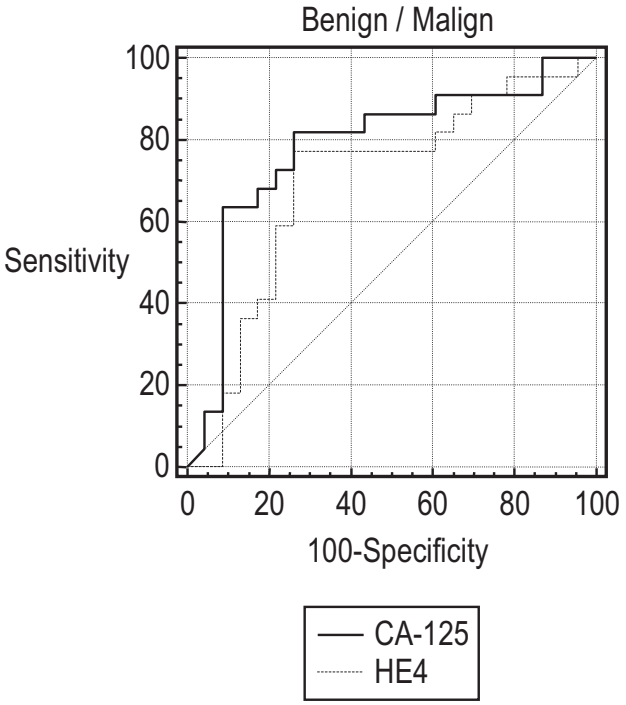

Figure 1. — Receiver-operating characteristics (ROC) curves for Ca-125 (AUC = 0.78, 95% CI: 0.63–0.89) and HE4 (AUC = 0.69, 95% CI: 0.54–0.82) with optimal thresholds.

lign-benign) (Table 1).

The sensitivity of CA-125 for detecting malign ovarian cancer were found to be 81.8% (95% CI: 59.7% to 94.7%) at 35 IU/ml (standard threshold) with 56.5% (95% CI: 34.5% to 76.8%) specificity compared with benign population. The optimal threshold value for the assay was different from the upper normal values indicated by the manufacturers, as it was 55 IU/ml with the same sensitivity but an increased specificity of 73.9% (95% CI: 51.6% to 89.7%). Setting the specificity of the assay at 95% (95% CI: 78% to 99.3%), the sensitivity of CA-125 was unacceptable, 13.6% (95% CI: 3.1% to 34.9%) (Table 2).

According to the clinical data and using as cut-off the reference value suggested by the assay manufacturers (150 pmol/L), the sensitivity of HE4 assay was 77.2% (95% CI: 54.6% to 92.1%) and the specificity was 73.9% (95% CI: 51.6% to 89.7%). Setting the specificity of the assay at 95% (95% CI: 78% to 99.3%), the sensitivity of HE4 was incredibly low as 4.5% (95% CI: 0.8% to 22.9%). The optimal threshold seemed to be 150 pmol/L as mentioned by the manufacturing company (Table 3).

The ROC curve analysis (Table 4 and Figure 1) showed a very similar accuracy of the CA-125 (AUC = 0.78, 95% CI: 0.63–0.89) and HE4 (AUC = 0.69, 95% CI. 0.54 to 0.82); none of the differences was significant by Z test.

Discussion

Because ovarian masses are commonly seen pathologies in gynecology, the differentiation of malign from benign ones is very important in distinguishing patients that require to be followed-up and have a surgical treatment. It is also important to decrease the number of unnecessary surgical procedures and related morbidity and mortality [14].

Ovarian cancer not only has the highest rate of mortality in gynecological malignancies but is also the fifth common cancer among cancer-related deaths [15]. Because of the incidence increase, especially in developed countries, the mortality rate is higher in late levels of the disease, the symptoms are non-specific in early levels, therefore improving imaging techniques or assessing new biochemical markers become an important necessity.

CA-125

CA-125, since its discovery 25 years ago has shown to be a good marker in diagnosis, follow up, and recurrence of ovarian cancer in most of the studies. In the present study the median values of CA-125 were found as 13.82, 30.72, and 335 IU/ml in healthy, benign, and malign groups of patients, respectively. The values of CA-125 were found significantly higher in both the benign and malign groups compared to heathy ones. Furthermore the value of CA-125 was found higher in malign group than benign one and it was statistically significant ($p = 0.001$). Paek *et al.* also found similar median values of CA-125 for healthy, benign, and malign groups (11.6I, 16.1I, and 370 IU/ml) [16].

O'Connel *et al.*, in their 56 case of study with 26 of them with ovarian cancer, found 100% sensitivity, 43% specificity, 60% PPV, and 75% NPV in detecting primary ovarian cancer, while accepting the threshold value of 35 U/ml for the serum level of CA-125 [17]. Also Milojkovic *et al.*, in their retrospective study including 121 malign and 91 benign cases, to evaluate the efficacy of preoperative serum CA-125 level in differentiating the benign and malign tumors, and accepting a cut-off value of 35 U/ml, found the 80.2% sensitivity, 76.1% specificity, 81.5% PPV, and 74.5% NPV. They concluded that in differentiating the malign and benign adnexal masses, the measurement of serum CA-125 level was helpful [18]. Moore *et al.* evaluated 531 patients with pelvic masses and 352 of them were benign. When they accepted a cut-off value of 35 U/ml, they found a sensitivity of 78.3% and specificity of 82%. When they

accepted a value of 65 U/ml, they found that the sensitivity decreased to 71.72%, whereas the specificity increased to 92.5% [19]. In the present study, the authors found a sensitivity of CA-125 for detecting malign ovarian cancer as 81.8% (95% CI: 59.7% to 94.7%) at 56.5% (95% CI: 34.5% to 76.8%) specificity compared with benign population. The cut-off for CA-125 was 35 IU/ml at this specificity. When setting the cut-off value to 55 IU/ml, they obtained the same sensitivity with a high specificity (73.9%, 95% CI: 51.6% to 89.7%).

HE4

In recent studies, it was shown that the tumor marker CA-125 alone, was unsatisfactory in differential diagnosis and in screening of ovarian malignancies [20-23]. A number of new novel markers for ovarian cancer have been identified in recent years, including HE4, which is one of the most intensively studied [24-31]. HE4 was first described as an epididymis-specific gene using northern blot analysis and in situ transcript hybridization [32, 33]. Recently, HE4 is regarded as a new biomarker for epithelial ovarian cancer in the serological study. Moore *et al.* analyzed the serum samples for levels of CA-125, HE4, and many markers and as a single tumor marker, HE4 had the highest sensitivity in detecting ovarian cancer and combined CA-125 and HE4 were a more accurate predictor of malignancy than either alone [11]. Also, it was found that HE4 is less frequently elevated in patients with benign gynecologic disease compared with CA-125 [10].

In the present study, the median serum levels of HE4 were 27.6 pM in the healthy, 109.4 pM in the benign, and 188.8 pM in the malign group, with statistically significant differences ($p < 0.001$ between malign-healthy, benign-healthy, and $p = 0.023$ between malign-benign). Paek *et al.* also found similar median values of HE4 in healthy, benign, and malign groups (7.5, 12, and 155 pM) with statistically differences ($p < 0.001$) [16]. Wang *et al.* [34], when they compared 56 patients with benign pelvic mass and 30 patients with malign pelvic mass to 46 healthy people in their study, they found mean serum values of HE4 as 34.1 pM in healthy control group, 39.1 pM in patients with benign pelvic mass, and 248.7 pM in patients with malign ovarian mass. The cut-off value in this patient population was 51.6 pM and they found the sensitivity and specificity as 86.7% and 98,0% respectively. Huhtinen *et al.* [35] in their study with a population of 12 control, 21 patients with benign mass and 45 patients with malign mass, compared patients with ovarian cancer with control group accepting the specificity as 95% and found the sensitivity of CA-125 as 78.6%, the sensitivity of HE4 as 78.6%, and 92.9% together. They detected that HE4 was less likely to rise in benign gynecological conditions compared to CA-125. Andersen *et al.* compared 137 healthy women to 74 patients with ovarian cancer and found the sensitivity and specificity of HE4 as 71.4% and 94.6% respectively [13].

In the present study, the sensitivity of HE4 for detecting malign ovarian cancer was found to be 95.4% (95% CI: 77.1% to 96.7%) at 95% (95% CI: 83.2% to 99.5%) specificity compared with healthy population. The cut-off for HE4 was 48.8 pM at this specificity. According to the clinical data and using as cut-off the reference value suggested by the assay manufacturers (150 pmol/L), the sensitivity of HE4 assay was 77.2% (95% CI: 54.6% to 92.1%) and the specificity was 73.9% (95% CI: 51.6% to 89.7%) when comparing the malign and benign cases.

The diagnostic performance of CA-125 and HE4 in discriminating ovarian cancer from healthy and benign gynecologic conditions was also verified using ROC analysis. The resultant AUC values were 0.97 for HE4 and 0.94 for CA-125 (healthy versus malign) and 0.69 for HE4 and 0.78 for CA-125 (benign versus malign), which would make them feasible for use as tumor markers to differentiate ovarian cancers from especially healthy and benign gynecologic conditions. In a few previous studies which utilized preliminary HE4 assays developed by the respective authors, the AUC values of HE4 and CA-125 for differentiating ovarian cancer were 0.85 to 0.96 and 0.81 to 0.95, respectively [10, 36, 37], which were similar to the present findings. Slight differences of the results between the studies might be caused by differences in the number of patients and the histologic types or disease stages of ovarian cancer enrolled in each study.

CA-125 and HE4

In a study of which the nine different markers, like CA-125, HE4, CA72, osteopontin, inhibin, activin, EGFR, were investigated alone or in several combinations, it was found that the sensitivity (76.4%) of the combination of HE4 and CA-125 was higher than other markers and combinations compared [11].

In the present study, when the cut-off values were accepted as 55 IU/ml for CA-125 and 150 pM for HE4 in differentiation of benign and malign groups, the sensitivity was found as 59.09%, specificity 91.3%, PPV 86.67% and NPV 70%, and LR= +6.8. In the combination of CA-125 and HE4 when the cut-off value of CA-125 was accepted as 35 IU/ml, LR was detected as +5,2 and this indicated that the ratio of patients with a diagnosis that was confirmed versus not confirmed, was 5.2 times greater. This combination gives one false positive result to every five positive cases which were detected as high. With the combination of CA-125 and HE4, the value of sensitivity decreased as expected, although the value of the specificity increased.

Moore *et al.* [11] decided that the dual marker combination improved upon CA-125 alone without using predefined cut-offs for either serum tumor markers. Notably, HE4 levels were elevated in greater than 50% of tumors that did not express CA-125. Therefore, the addition of HE4 to CA-125 enabled the detection of malignancies in patients with tumors that did not express CA-125 and will be missed by algorithms that employ CA-125 alone. In ad-

dition, HE4 and CA-125 levels increased in some benign conditions or other gynecologic and non-gynecologic malignancies; for example, breast, pancreatic, and endometrial cancers. These results imply that HE4 and CA-125 are not especially specific to ovarian cancer, although increases in both markers were more evident in certain histologies of ovarian cancer than in others. Galgano *et al.* [38] researched expression patterns of HE4 in various benign and malignant diseases, and reported that HE4 proteins or genes were expressed strongly in serous papillary, clear cell, and endometrioid carcinoma of the ovaries. However, other histologies of ovarian cancer or non-gynecologic malignancies including invasive ductal carcinoma of breast, endometrial, pancreaticobiliary, and renal cell carcinoma also exhibited strong or weak expressions of HE4 proteins or genes. Another study demonstrated that HE4 might be associated with the innate immune defences of the lung, nasal and oral cavities, and the authors suggested that re-expression of HE4 in lung was related to the carcinogenesis [39]. Several researchers have also investigated the usefulness of HE4 in other malignancies, including transitional cell carcinoma of the urinary tract and endometrial cancer [40, 41]. Thus, a single marker is not enough for an accurate diagnosis, especially in certain histologies, and it would be necessary to complement one marker with another. In a recent study, as stated earlier in this article, a combination of CA-125 and HE4 was demonstrated to be the most sensitive marker for the detection of ovarian cancer in patients with a pelvic mass, with the AUC value of 0.91, which was superior to other markers including CA-125 and soluble mesothelin-related peptide (SMRP), or other marker combinations [11].

As a conclusion, in the differentiation of benign and malign adnexal masses preoperatively, despite extensive studies, an exact way of diagnosis has not yet been found. Although the most common ways of preoperative diagnosis are pelvic examination, ultrasonography, tumor marker CA-125, in the new studies, the combination of HE4 and CA-125 is encouraging especially in epithelial ovarian cancers. The optimal value for CA-125 was found to be 55 IU/ml with sensitivity of 81.82%, specificity of 73.91%, PPV 75%, and NPV 81%, and the optimal value for HE4 was 150 pM with sensitivity of 77.2%, specificity of 73.9%, PPV 73.9%, and NPV 77.3%. In the combination of CA-125 and HE4, LR was detected as +5.2: this indicated that the ratio of patients with a diagnosis that was confirmed versus not confirmed, was 5.2 times greater. It has however been reported in the recent literature that dual or more combinations of the tumor markers did not always confirm the certain diagnosis. Studies with large population of patients are in limited numbers in the literature except for a few number of multicentric public studies with larger population. In this case, larger numbers of studies are needed to formulate a certain conclusion.

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