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Assessment of Diagnostic Value of 'Human Epididymis Factor 4 (HE4)' in women with adnexal masses

Adneksiyel kitlesi olan kadınlarda 'Human Epididimis Faktör (HE4)'ün diagnostik değerinin belirlenmesi

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Abstract

Purpose: Human epididymis factor 4 (HE4) is a novel biomarker for ovarian cancer. The aim of this study is to assess the usefulness and efficacy of HE4, in comparison with CA-125 in the differential diagnosis of malignant and benign gynecological diseases in women with adnexal masses.

Materials and methods: 85 patients diagnosed with adnexal mass who were operated at a tertiary referral center between the years of October 2012 and February 2013 were included in the study. Demographic data, physical examination, results of the laboratory tests, imaging, and pathology were recorded from all subjects. Blood samples were collected before surgery for the evaluation of HE4 and CA-125 levels. Patients were divided into 4 groups according to their histopathologic diagnosis: benign (n:58), malignant (n:17), borderline (n:5) and metastatic (n:5) and serum CA-125 and HE4 levels were compared considering menopausal status.

Results: Using 35 U/mL as the cut-off value, CA-125 had a sensitivity of 82.4%, spesificity of 67.2%, a negative predictive value of 92.9%, and a positive predictive value of 42.4%. For the same sensitivity level, specificity was 87.9%, negative predictive value was 94.4%, and the positive predictive value was 66.7% for HE4. Receiver operator characteristic (ROC) area under the curve was higher in both the premenopausal and postmenopausal group for the HE4 curve. Accordingly, sensitivity at set specificity of 90% was 82.4%, and 70.6% for 95% of specificity and 41.1% for 98% for HE4. Same sensitivity values for CA-125 were 47.1%, 35.3% and 23.5%, respectively.

Conclusion: According to our study, especially in the premenopausal period, HE4 shows higher sensitivity and specificity values than CA-125.

Key words: Adnexal mass, ovarian cancer, CA-125, HE4.

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Özet

Amaç: Human epididymis faktör 4 (HE4) over kanserinde kullanılan yeni bir tümör belirtecidir. Bu çalışmanın amacı, adneksiyel kitlesi olan kadınlarda malign ve benign hastalık ayırıcı tanısında HE4'ün CA-125 ile karşılaştırılarak etkinliğinin ve yararlılığının değerlendirilmesidir.

Gereç ve yöntem: Ekim 2012 ve Şubat 2013 tarihleri arasında 3. basamak referans merkezi olan bir Kadın Hastalıkları ve Doğum Kliniği'nde adneksiyel kitle nedeniyle opere edilen 85 hasta çalışmaya dahil edildi. Çalışmaya dahil edilen tüm hastaların demografik verileri, anamnez bilgileri, muayene bulguları, görüntüleme tetkikleri, laboratuar tetkikleri ve patoloji sonuçları kaydedildi. Hastalardan preoperatif CA-125 ve HE4 düzeylerini belirlemek amacı ile venöz kan örneklemesi yapıldı. Çalışma grubu histopatolojik tanılara göre benign (n:58), malign (n:17), borderline (n:5) ve metastatik (n:5) olmak üzere 4 gruba ayrıldı. Serum CA-125 ve HE4 düzeyleri menapozal durum göz önünde bulundurularak karşılaştırıldı.

Bulgular: CA-125 için uluslarası kabul gören 35 U/ml eşik değer olarak alındığında adneksiyel kitlelerde benign malign ayrımında toplam grupta %82,4 sensitivite, %67,2 spesifite, %92,9 negatif prediktif değer, %42,4 pozitif prediktif değer saptandı. Aynı sensitivite değerinde HE4 için %87,9 spesifite, %94,4 negatif prediktif değer, %66,7 pozitif prediktif değer saptandı. ROC analizine göre HE4 eğrisinin altında kalan alan hem premenapozal hem de postmenapozal dönemde daha büyüktü. Buna göre HE4'ün; %90 spesifite değerinde %82,4 sensitif, %95 spesifite değerinde %70,6 sensitif, %98 spesifite değerinde %41,1 sensitif olduğu izlendi. Aynı sensitivite değerleri CA-125 için ise sırasıyla %47,1, %35,3, %23,5 olarak izlendi.

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Sonuç: Çalışma sonuçlarımıza göre özellikle premenapozal dönemde HE4, CA-125'e göre daha yüksek sensitivite ve spesifite değerleri göstermektedir.

Anahtar kelimeler: Adneksiyel kitle, over kanseri, CA-125, HE4.

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Introduction

Approximately 5-10% of women need surgery because of an adnexal mass, and 13-21% of these masses were subsequently diagnosed as malignant [1]. Early diagnosis and proper treatment of ovarian cancers are key to good prognosis. But there has been no gold effective screening test available for ovarian cancer [2]. Therefore, triage is very important in cases presenting with adnexal mass in order to prevent unnecessary or inadequate treatment. For determining the appropriate approach, detailed anamnesis information, physical examination, use of ultrasonography (USG) and other radiological examinations, detection of tumor marker levels and combination of all these with other predictors are required [3].

Many tumor markers are being investigated for screening, diagnosis, and determining prognosis and the response to treatment of ovarian cancer [4]. The best known and most commonly used of these is the Carcinoembryonic antigen (CA-125). However, existing tumor markers, including CA-125, do not have sufficient sensitivity and specificity in the differential diagnosis of ovarian cancer, and the search on this issue continues [5]. With recent current studies, a new tumor marker, Human Epididymis Factor 4 (HE4), stands out in this issue.

The aim of this study was to determine the predictive value of HE4, a new tumor marker in the evaluation of adnexal masses and benign-malignant differential diagnosis, and to compare its efficacy with CA-125.

Materials and methods

Prior to the study, the institutional approval was obtained from the Ethics Committee. Patients between the ages of 18-80 who applied to Obstetrics and Gynecology Clinic, Izmir Katip Celebi University Atatürk Education and Research Hospital, and decided to be operated due to adnexal mass were prospectively included in the study. Informed consent was obtained from all the participants before being included in the study. Patients with renal failure or with serum creatinine levels >1.2 were excluded from the study. Detailed anamnesis was taken from the patients, demographic data, additional diseases, gynecologic and abdominal examinations were recorded. Patients were evaluated with the ultrasonography (USG), while patients deemed to need for further examination were evaluated with Magnetic Resonance Imaging (MRI) or Computed Tomography (CT). All collected blood samples were analyzed in the biochemistry laboratory of the same center.

Preoperative venous blood sampling was performed to examine CA-125 and HE4 levels. After 10 minutes of centrifugation of the blood samples at 4000 rpm, the serum was separated. Serum samples were not dull, being without fibrin and hemolysis. CA-125 levels were researched with Siemens CA-125 II Readypack kit using the chemiluminescence method in Siemens Advia Centaur device during the preoperative period. The serums separated for HE4 examination were placed in clean and dry godets; their caps were sealed with parafilm and stored in deep freezers at -40°C for being analyzed together. After the patient's consent was obtained, laparoscopy or laparotomy was performed taking into account demographic data, examination findings, imaging methods, and tumor marker levels. Based on the results, patients underwent standard or radical surgery. Surgeons and pathologists were unaware of the results of HE4 levels.

HE4 levels were analyzed from serum samples in Biotech semiautomatic Elisa device by Solid Phase Sandwich Enzyme-Linked ImmunoSorbent Assay (ELISA) method using Human HE4/WFDC2 Quantikine ELISA Kit from R&D SYSTEMS, INC Human Epididymis Protein 4 (HE4) EIA.

The patients were divided into 4 groups: benign, malignant, borderline malignant

and metastatic malignant according to the histopathological diagnoses and compared with each other based on serum CA-125 and HE4 levels.

Statistical methods: The SPSS (for Windows, Version 20.0) package software was used in statistical analysis. The normal distribution of numerical variables was checked with the Shapiro-Wilk Test. Since there is usually a lack of normal distribution, non-parametric methods were preferred in the analysis. Chi-Square or Fisher's exact probability test was used to compare categorical variables. The presence of a linear relationship between numerical variables was examined by Spearman Correlation analysis. In addition, Kruskal-Wallis and Mann-Whitney U Tests were preferred for comparing numerical variables between groups. ROC analysis was performed to determine the threshold (cut-off) to differentiate the two groups of paraffin. All hypothesis controls were performed at α=0.05 significance level, namely, $p<0.05$ was considered significant.

Results

The mean age of the patients involved in the study was 48.1±14.9 (18-80) years and the median value was 46 years. The study group was divided into two groups as premenopausal and postmenopausal group. Of the 85 patients, 49 were in the premenopausal period and 36 were in the postmenopausal period.

The 85 patients involved in the study were divided into 4 different groups as benign, malignant, borderline malignant and metastatic malignant according to the final pathological results. Of the 85 patients, 58 (68.2%) had benign, 17 (20%) had malignant, 5 (5.9%) had borderline malignant, and 5 (5.9%) had metastatic malignant mass. Of the 49 premenopausal patients, 37 (75.5%) had benign, 7 (14.3%) had malignant, 2 (4.1%) had borderline malignant and 3 (6.1%) had metastatic malignant mass. Of the 36 postmenopausal patients, 21 (58.3%) had benign, 10 (27.8%) had malignant, 3 (8.3%) had borderline malignant, and 2 had metastatic malignant mass. Of the 17 patients in the malignant group, 5 (29.4%) were at stage-1A, two (11.76%) were at stage-1B, one (5.9%) was at stage 2C, and 9 (52.9%) were at stage-4, according to FIGO. Histopathological subtypes of benign, malignant, borderline malignant and metastatic malignant groups and their percentages in groups are shown in Table 1.

Four groups were compared with each other in terms of age by the Kruskal-Wallis test. No significant difference was observed for age distribution between the groups ($p>0.05$).

Table 1. Histological subsets of the study group.

Benign (n:58)	Serous (n:17, 29.3%) Mucinous (n:4, 6.9%) Endometrioma (n:10, 17.2%) Mature cystic teratoma (n:9, 15.5%) Fibroma (n:12, 20.7%) Tubaovarian abscess (n:1, 1.7%) Endometrioma + mature cystic teratoma (n:1, 1.7%) Intraligamental leiomyoma (n:1, 1.7%) Paraovarian cyst (n:1, 1.7%) Leiomyoma + Paraovarian cyst (n:1, 1.7%) Benign steroid cell tumor (n:1, 1.7%)
Malignant (n:17)	Serous (n:9, 52.9%) Mucinous (n:3, 17.7%) Endometrioid (n:4, 23.5%) Malign mixt (serous + endometrioid) (n:1, 5.9%)
Borderline malignant (n:5)	Serous borderline (n:1, 20%) Mucinous borderline (n:4, 80%)
Metastatic malignant (n:5)	Colon cancer (n:3, 60%) Gastric cancer (n:1, 20%) Diffuse B cell lymphoma (n:1, 20%)

(n: number)

In the entire study group, the median value for CA-125 was 27 U/ml; the median value for HE4 was 354 pmol/l. The median value for CA-125 in the premenopausal group was 19 U/ml, the median value for HE4 in the premenopausal group was 272 pmol/l, the median value for CA-125 in the postmenopausal group was 41 U/ml, and the median value for HE4 in the postmenopausal group was 374 pmol/l. Serum HE4 levels according to histological subtypes are schematized in Figure 1. Age, CA-125 and HE4 mean rank values according to histopathological subtypes are shown in Table 2.

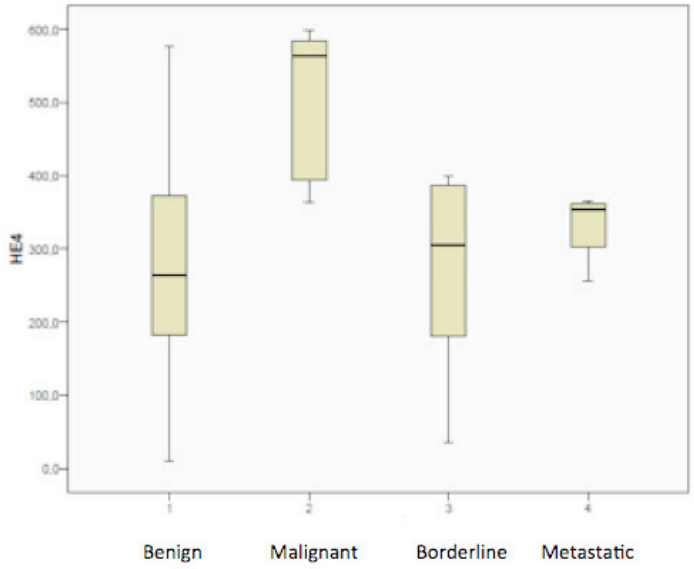

Figure 1. Serum HE4 levels according to histopathological subtypes.

Table 2. Age, serum CA-125 and HE4 mean rank values according to histopathological subtypes.

		mean rank	*p* value
Age (years)	Benign	38.8	0.106
	Malignant	52.5	
	Borderline	58.3	
	Metastatic	44.2	
CA-125 (U/ml)	Benign	34.9	**<0.001**
	Malignant	**67.0**	
	Borderline	47.8	
	Metastatic	50.8	
HE4 (pmol/l)	Benign	35.1	**<0.001**
	Malignant	**72.5**	
	Borderline	37.2	
	Metastatic	40.7	

Overall, Kruskal Wallis analysis revealed a significant difference between CA-125 and HE4 levels among the groups and therefore, two-group comparisons were subsequently performed. In the two-group comparison by the Mann-Whitney U test, all groups were compared to each other with Bonferroni correction. When benign and malignant groups were compared with each other, both HE4 and CA-125 were found to be significantly higher in the malignant group (p<0.01 for both markers). It was observed that both HE4 and CA-125 levels did not differ significantly between groups when benign and borderline groups were compared with each other (p>0.05). When the benign and metastatic malignant groups were compared, it was observed that both HE4 and CA-125 levels did not significantly differ between the groups (p>0.05). When malignant and borderline malignant groups were compared, HE4 levels were found to be significantly higher in the malignant group (p<0.05), and CA-125 levels did not significantly differ between the groups (p>0.05). When malignant and metastatic malignant groups were compared, HE4 levels were found to be significantly higher in malignant groups (p<0.01) and CA-125 levels did not significantly differ between the groups (p>0.05). Finally, when borderline malignant and metastatic malignant groups were compared with each other, it was observed that both HE4 and CA-125 levels did not differ significantly between the groups (p>0.05 for both). CA-125 was not found to be distinctive for the malignant and metastatic malignant group, while HE4 was found to be distinctive for the malignant and metastatic malignant group.

ROC analysis was performed to determine HE4 and CA-125 cut-points for distinguishing between the groups whose histopathological results were benign and malignant. According to these results, the area under the curve for HE4 is larger than the area under the curve (AUC) for CA-125 (Figure 2, Figure 3) and more distinctive in the benign-malignant diagnosis of adnexal masses than CA-125 (AUC: 0.932, p<0.01; AUC: 0.871, p<0.01, respectively). The groups were divided into two as premenopausal and postmenopausal, and ROC analysis was performed again for each 2 groups (Figure 4). According to these results, HE4 in the premenopausal period is more distinctive than CA-125 in the premenopausal period (AUC: 0.967, p<0.01; AUC: 0.896, p<0.01, respectively for premenopausal period and AUC: 0.881, p<0.01; AUC: 0.833, p<0.01, respectively for postmenopausal period).

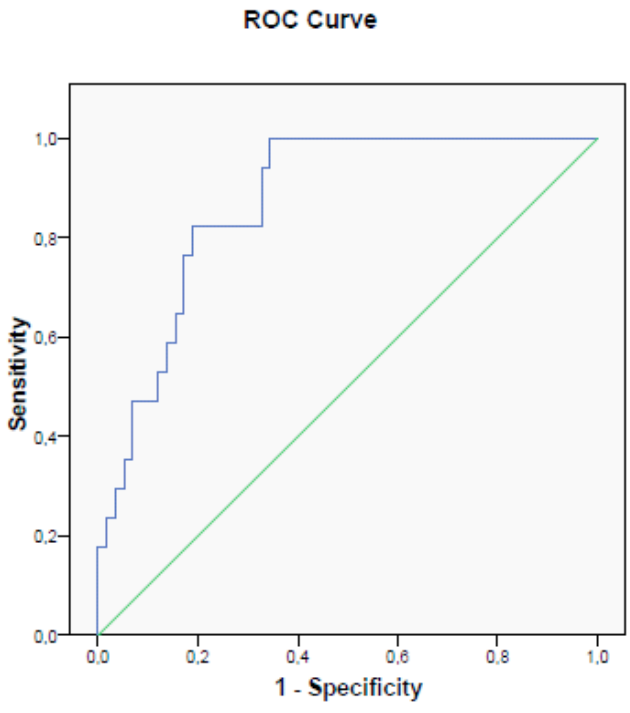

Figure 2. ROC curve analysis of CA-125 in diferentiating benign and malignant cases.

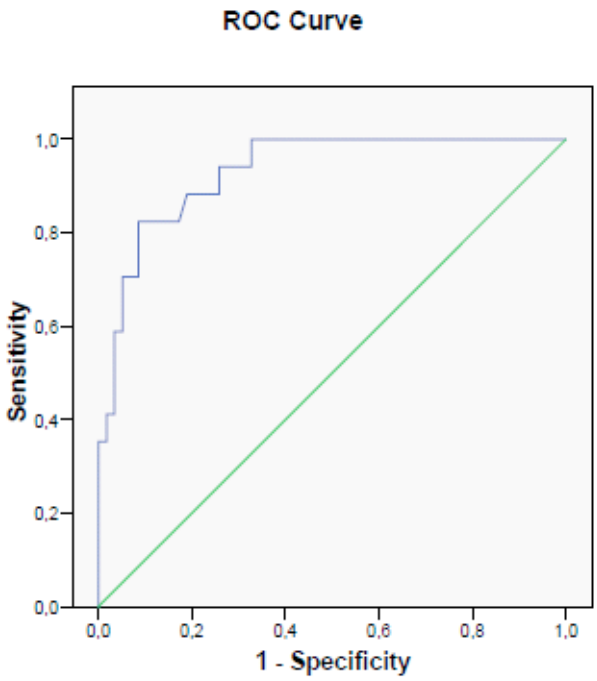

Figure 3. ROC curve analysis of HE4 in diferentiating benign and malignant cases.

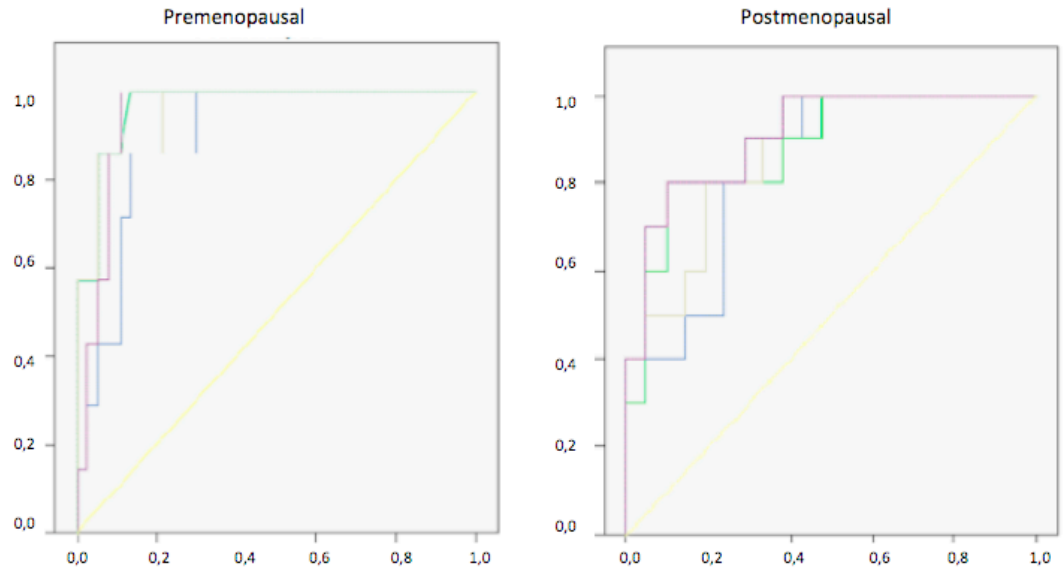

Figure 4. ROC analysis of both CA-125 and HE4 with respect to menopausal status in diferentiating benign and malignant cases.

The premenopausal and postmenopausal groups are examined separately at internationally accepted 35 U/ml threshold for CA-125. 85.7% sensitivity, 70.3% specificity, 96.3% negative predictive value, 35.3% positive predictive value was obtained in the premenopausal group and 80% sensitivity, 61.9% specificity, 86.7% negative predictive value, 50% positive predictive value was obtained in the postmenopausal group. False positivity rates were 29.7% and 38.1% and false negativity rates were 14.3% and 20% in the premenopausal and postmenopausal group respectively. Table 3 shows the results if 80 U/ml is accepted as a threshold for CA-125 in the premenopausal group and 30 U/ml is accepted as a threshold for CA-125 in the postmenopausal group.

Table 3. The evaluation of CA-125 cut-off values.

	Premenopausal group >80 U/ml	**Postmenopausal group >30 U/ml**	**Total group >30 U/ml**
Sensitivity	85.7%	90%	94.1%
Specificity	86.5%	61.9%	67.2%
Negative predictive value	97%	92.9%	97.5%
Positive predictive value	54.5%	52.9%	45.7%
False positivity	13.5%	38.1%	32.8%
False negativity	14.3%	10%	5.9%

According to the ROC analysis for HE4 in our study group, the values of 370 pmol/l and 390 pmol/l are taken as threshold values and the sensitivity and specificity values for the benign malignant distinction of adnexal masses were evaluated. When premenopausal and postmenopausal groups are examined separately; the sensitivity and specificity values for the same factors are shown in Table 4.

The sensitivity and specificity values of CA-125 and HE4 tumor markers according to the selected threshold levels in total patient groups are summarized in Table 5.

Histological subsets in the malignant group were examined according to HE4 levels, and serum HE4 levels were observed as higher than mucinous groups in serous and endometrioid subtypes. In the benign group, histological subtypes were examined according to CA-125 and HE4 levels, and CA-125 levels were high in 7 of the 11 patients with endometrioma, and serum HE4 levels were high in only 3 of these 7 patients.

Accordingly, HE4 levels were found in patients with endometrioma at similar levels with other benign groups, and HE4 levels in the malignant group were higher than in all benign groups. The benign group was compared to the metastatic malignant group, HE4 levels were not different, and HE4 levels were higher than metastatic malignant groups as mentioned earlier.

Of the 17 patients in the malignant group, 5 were in the stage-1A, 2 were in the stage-1B, 1 was in the stage 2C and 9 were in the stage-4. The CA-125 levels did not elevate in 3 patients, and stage-1 ovarian cancer was observed in these 3 patients. A fairly wide distribution range (minimum value: 30 U/ml, maximum value: 5881 U/ml, median value: 90 U/ml, standard deviation: 2659 U/ml) was detected in stage-1 patients with high CA-125 levels. CA-125 levels were found to be high in all stage-4 patients and a similarly wide distribution range (minimum value: 66 U/ml, maximum value: 4467 U/ml, median value: 294 U/ml standard deviation: 1437 U/ml) was detected.

HE4 levels elevated in all groups with early and advanced malignant tumors, and HE4 levels were high in 3 patients in the early stages where CA-125 did not rise. HE4 levels were found to have a narrower dispersion range in the early stages (minimum value: 371, maximum value: 591 pmol/l, median value: 563 pmol/l, standard deviation: 112 pmol/l) and later stages (minimum value: 363 pmol/l, maximum value: 598 pmol/l, median value: 575 pmol/l, standard deviation: 95 pmol/l). Although mean

Table 4. The evaluation of HE4 cut-off values in premenopausal and postmenopausal groups.

	>370 pmol/l cut-off value		**>390 pmol/l cut-off value**	
	Premenopausal	**Postmenopausal**	**Premenopausal**	**Postmenopausal**
Sensitivity	100%	90%	85.7%	80%
Specificity	78.4%	61.9%	91.9%	81%
Negative predictive value	100%	92.9%	97.1%	89.5%
Positive predictive value	46.7%	52.9%	66.7%	66.7%
False positivity	21.6%	38.1%	8.1%	19%
False negativity	0%	10%	14.3%	20%

Table 5. Sensivitiy and specifity of CA-125 and HE4 for the prediction of malignancy at the total group.

TOTAL	HE4 >370 mol/L	CA-125 >30 U/ml	HE4 >390pmol/L	CA-125 >35 U/ml
Sensitivity	94.1%	94.1%	82.4%	82.4%
Specificity	72.4%	67.2%	87.9%	67.2%
Negative predictive value	97.7%	97.5%	94.4%	92.9%
Positive predictive value	50%	45.7%	66.7%	42.4%
False positivity	27.6%	32.8%	12.1%	32.8%
False negativity	5.9%	5.9%	17.6%	17.6%

rank values were highest in stage-4 disease for both HE4 and CA-125 levels, there was no statistically significant relationship between the serum levels of both markers and stage of the disease (p>0.05).

Discussion

In this study we evaluated 85 patients with their preoperative CA125 and HE4 levels and compared the histopathological results. We have documented that HE4 was 82.4% sensitive at 90% specificity, 70.6% sensitive at 95% specificity, and 41.1% sensitive at 98% specificity in discriminating benign and malignant adnexal masses. However, CA-125 was found to be 47.1% sensitive at 90% specificity, 35.3% sensitive at 95% specificity, and 23.5% sensitive at 98% specificity for the same purpose. When the groups were evaluated according to menopausal status, and ROC analysis was performed again for each 2 groups, HE4 is found to be more distinctive than CA-125 in the premenopausal period. In the premenopausal period; the area below the curve for CA-125 and HE4 were 0.896 and 0.967, respectively. In the postmenopausal period; the area below the curve for CA-125 and HE4 were 0.833 and 0.073, respectively.

CA-125 is a frequently used tumor marker for ovarian cancer. However, its major limitation is the low sensitivity and specificity values especially in the premenopausal period and early stages [5]. Our results indicate that, HE4 has an additional clinical value in differential diagnoses of these patients. We determined that the false positivity rate of CA-125 as 29.7% in the premenopausal period and the

CA-125 increase was not detected in 3 of the 7 stage 1 patients. Although the combination of CA-125 and USG may increase sensitivity and specificity rates of CA-125, the researchs resulted in low positive predictive values of 10-21% [6-8]. The main problem in these studies is that the experience of the clinician in the use of USG leads to huge differences between the results [9]. In addition, CA-125 is not expressed in approximately 20% of ovarian cancers [8]. On the other hand, HE4 levels elevated in all groups with early and advanced malignant tumors, and HE4 levels were high in 3 patients in the early stages where CA-125 did not rise in our study. HE4 levels were found to have a narrower dispersion range in the early stages (minimum value: 371, maximum value: 591, median value: 563, standard deviation: 112) and later stages (minimum value: 363, maximum value: 598, median value: 575, standard deviation: 95).

Moore et al. [9] studied with a variety of biomarkers including CA-125, mesothelin, HER2 oncogene, HE4, CA72-4, activin, inhibin, osteopontin, EGF receptor in 259 patients with adnexal masses. Two hundred thirty three of whom were eligible for analysis, 67 with invasive epithelial ovarian carcinoma and 166 with benign ovarian neoplasm. Sensitivity values of these markers were calculated at 90-95-98% specificity. HE4 alone had the highest sensitivity as 95%, with a sensitivity value of 72.9%. Similarly, according to ROC analysis, we found that HE4 with 95% specificity alone had a sensitivity of 70%. CA-125 with 95% specificity was found to have 35% sensitivity. Our findings also support that HE4 is a more specific marker than CA-125. Moore et al. [9] found that the combination of CA-125 and HE4 had the highest sensitivity and specificity values among all other single and combined markers as 76.4% sensitivity and 95% specificity. Moreover, the addition of other markers did not raise sensitivity values [9].

Afterwards, the authors created a model called 'Risk of Ovarian Malignancy Algorithm (ROMA)'. This algorithm, which included a combination of HE4 and CA125, classified patients in the high-risk group with a 93.8% accuracy rate [10]. The ROMA combines CA125 and HE4 using two formulas, taking into account the menopausal status of each patient. Following reports after Moore et al. [9] have been consistently documented that a combination of HE4 and CA125 as in the ROMA compared to either HE4 or CA125 alone improves the specificity [11-13]. The CA125 and HE4 combination has proved to be highly efficient with an area under the curve (AUC) of up to 0.96 and to date, the most efficient biochemical diagnostic tool for the differential diagnoses of adnexal masses seems to be these combination [13].

CA-125 may increase in relation to many different benign conditions. This reduces the specificity of this marker especially during the premenopausal period, and causes the cut-off values to change with age [14]. The best example is endometriosis. CA-125 can be used in ovarian cancer as well as in the evaluation of the treatment effectiveness of endometriosis and in the follow-up of recurrence. In addition, neoplastic ovary mass may be similar to endometrioma in the USG [15]. Therefore, these cases should be evaluated with different diagnostic methods.

One of the major advantages of HE4 is that its level is not affected by endometriosis cysts [16, 17]. Huhtinen et al. [18] studied serum concentrations of HE4 and CA-125 in a total of 225 women, including 14 with ovarian cancer, 16 with endometrial cancer, 129 with endometriosis, and 66 healthy. The combination of these two markers showed higher sensitivity (92.9%) and higher specificity (95%) in ovarian and endometrial cancer compared to only HE4 (78.6% sensitivity) and only CA-125 (78.6% sensitivity). The most important result of this study was that the mean serum HE4 levels increased in both endometrial and ovarian cancer patients but did not increase in ovarian endometrioma or different types of endometriosis. As a result, the authors suggested that the combination of HE4 and CA-125 can be used by clinicians to follow up patients with advanced endometriosis and to follow up malignancy transformation. In our study, 7 of the 11 patients with endometrioma were found to have high levels of CA-125, while serum HE4 levels were found to be high in only 3 of these 7 patients. In our study, compared to CA-125, HE4 in the premenopausal period was found to be more diagnostic than in the postmenopausal period. At the same time, HE4 levels were found in patients with endometrioma similar to other benign groups, and HE4 levels

in the malignant group were higher than in all benign groups. This result suggested that the higher specificity of HE4 than CA-125 in the premenopausal period where endometriosis is more common may be related to this finding. Our finding is in accordance with current literature [17].

HE4 shows higher sensitivity and specificity than CA-125 and other complementary markers. However, it is not yet known how HE4 concentrations have changed in other diseases. To avoid misinterpretation, HE4 levels should be studied with extended research in other benign and malignant conditions. Interestingly, unlike CA-125, HE4 concentrations increase with age in healthy individuals. However, CA-125 is higher in healthy individuals during the premenopausal period [19]. The upper limit of the normal clinical level is considered to be 35 U/ml of CA-125. However, it is recommended in the literature that the upper limit of CA-125 should be 20-26 U/ml in the group of patients in the postmenopausal period and with hysterectomy [20]. In early-stage disease, about half of cases have CA-125 levels above 35 U/ml, which leads to questioning of this threshold in ovarian cancer, where the main purpose is to diagnose at an early stage [20, 21]. In our study, compared to 35 U/ml, which is the accepted cut-off value of CA-125, higher sensitivity and specificity values were obtained with 30 U/ml cut-off values in the postmenopausal period and 80 U/ml in premenopausal period.

In our study, low HE4 levels in the borderline malignant group may be associated with that 80% of the group is in the mucinous subtype. Studies with more patients are needed to determine the diagnostic value of HE4 in borderline malignant ovarian tumors.

The major limitation of this study is the limited number of the patients with early stage ovarian cancer and borderline malignant ovarian cancer. However, this can be attributed to the biology of ovarian cancer. Our results are sufficient to give an idea regarding the clinical value of HE4 in the differential diagnoses of adnexal masses. Larger studies are needed to be implemented in screening and diagnostic algorthims.

In summary, HE4, a new tumor marker in the differential diagnosis of adnexal masses, shows higher sensitivity and specificity values than CA-125 in accordance with the results of our study and literature knowledge. CA-125 shows high sensitivity in the late stages (stage III-IV) and in the postmenopausal period, whereas HE4 is more sensitive in the early stages (stage I-II) than CA-125. These two tumor markers are complementary and the combination of these two markers increases sensitivity rates compared to the use alone in ovarian cancers.

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