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HE4 -Are We Looking At A Better Marker? A Prospective Study In Comparison With CA125

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ABSTRACT

Aim: *Human epididymis protein 4 (HE4) has been suggested to be a new novel biomarker of epithelial ovarian cancer. The aim of this study is to evaluate HE4 and the combination of HE4 and CA125 in diagnosis of ovarian cancer.* ***Material & methods:*** *CA125 and HE4 serum levels were determined in thirty six patients presenting with pelvic mass or suspected to have ovarian mass at our institute. Ten women including five premenopausal & five postmenopausal were taken as healthy controls. CA125 and HE4 were analyzed by Electro chemiluminescent immunoassay on Roche Cobas e411.* ***Results:*** *HE4 and CA125 concentrations were significantly higher in ovarian cancer patients compared with benign disease and non ovarian malignancies ($p < 0.001$). Area under curve (AUC) for HE4 in differentiating ovarian cancer from benign and non-ovarian malignancies was 0.84 [95% Confidence interval (CI), 0.71-0.98] and that for CA125 was 0.71 (95% CI, 0.54-0.89). Compared to CA125, HE4 had higher specificity (64.29% vs. 50%) and Positive predictive value (PPV) (76.19% vs. 75.86%). CA125 is 100% sensitive and has 100% Negative predictive value (NPV) compared to HE4 (72.73% & 60%). By combining HE4 and CA125, the sensitivity and specificity reached 100% and 58.38% respectively.* ***Conclusion:*** *The finding of AUC values for HE4 (0.84) being significantly higher than CA125 (0.71), suggests a better performance of HE4 in differentiating ovarian cancer from benign and non ovarian malignancies. Hence HE4 is a good single marker than CA125. Further studies are needed to explore in more detail.*

KEY WORDS

CA125, HE4, Ovarian malignancy, pelvic mass.

INTRODUCTION

Ovarian cancer (OC) remains the most lethal among gynecologic malignancies. Screening can improve survival, but the impact of screening on mortality from OC is unclear [1].Carbohydrate antigen 125 (CA125) is elevated only in 50% of women with early stage disease [2]. The sensitivity and specificity of CA125 are not high enough for population screening, since it is elevated in many benign conditions [3, 4]. HE4 (Human epidydimis protein 4) was found to complement CA125 and improve its sensitivity for early detection [3, 5]. HE4 is found in reproductive and respiratory tracts [6] and over expressed in ovarian cancer cells, especially in serous or endo metroid carcinoma [7] and suggested to be a marker of OC [8]. Presently, we aimed to compare HE4 and CA125 in epithelial OC, benign gynecological diseases, and other malignancies.

PATIENTS AND METHODS

In this study thirty six female patients were included who presented to the O.P with a pelvic mass and those suspected to have an ovarian mass. The inclusion criteria were – patients may be premenopausal or postmenopausal, above 18yrs of

age, presenting with pelvic mass (? Suspected ovarian mass). The Exclusion criteria were - patients <18yrs old, pregnant women, those with history of smoking, patients previously treated for malignancy or currently under treatment, women presenting with abnormal renal conditions like Chronic kidney disease.

The second group included 10 healthy controls –five premenopausal and five postmenopausal women.

Method:

Venous samples were collected from the patients and healthy controls. Serum creatinine was analyzed on Vitros 350 analyzer to check the renal status. CA125 and HE4 were analyzed by Electrochemiluminescent immunoassay (ECLIA) on Roche Cobas e411. Both assays are non-competitive immunoassays, based on sandwich technique, and were run according to manufacturer's instructions. The appropriate controls were within the ranges provided by the manufacturer for all runs. For CA125, the normal upper limit is 35 U/ mL, whereas that for HE4 is 70 pmol/L and 140 pmol/L for premenopausal and postmenopausal women respectively.

Statistical analysis

The data was presented as mean ± standard deviation (SD) or median (range) and number (n). Linear relationships between variables were determined using Spearman's rank correlation test. Sensitivity, specificity, Positive predictive value (PPV) and Negative predictive value (NPV) were calculated using Medcalc software. Non-parametric receiver operating characteristic (ROC) analyses were performed to evaluate diagnostic efficacy of individual parameters generated by graphically plotting sensitivity versus specificity using 95% confidence intervals (CI). The diagnostic accuracy of the test is measured by the area under the curve (AUC). Statistical significance is considered a value of P <0.05. All statistical analyses were performed using SPSS software, version 20.0.

RESULTS

Clinical characteristics and laboratory variables of the studied groups were demonstrated in Table 1. The median CA125 and HE4 levels in the healthy pre menopausal controls were 16.8 U/mL and 39.9 pmol/L respectively & in healthy postmenopausal controls were 13.1U/mL & 51.8pmol/L. There was significant difference in HE4 & CA125 values between the ovarian cancer group (median 573.1 U/mL in premenopausal, 1657.pmol/L in postmenopausal for CA125, and median 157.9 U/mL in premenopausal, 368pmol/L in postmenopausal for HE4) and the control group. Table 2 showed FIGO stage of the studied woman. 54% of the patients had stage 3 and the serum levels of HE4 and CA125 in relation to histological types showed higher significant level in serous ovarian cancer (p <0.01 and p <0.05 respectively). In 36 women studied, HE4 was showing higher specificity than CA125 (64.29% vs. 50%, respectively) and lower sensitivity (72.73% vs. 100% respectively). Also, the PPV and NPV of CA125 and HE4 were 75.86% vs. 76.19% and 100% vs. 60%, respectively. Sensitivity and PPV were increased reaching 100% and 76.19% respectively when the two markers were combined (Table 3). Tables 4-5 show Spearman's correlation of CA125 and HE4 in the studied population. Figure 1 shows the Spearman's correlation graph between CA125 and HE4 (P<0.001). ROC plot was shown in Figure 2 for HE4 and in Figure 3 for CA125. AUC for CA125 was 0.71 (95% CI, 0.54-0.89) and for HE4 was 0.84 (95% CI, 0.71-0.98) (p <0.01) for distinguishing between EOC from benign & non ovarian malignancy. ROC –AUC values for HE4 is 0.97 (95% CI 0.91-1.0) when only ovarian cancers were taken into account, which is shown in Figure 4.

Table 1. Clinical and laboratory variables of studied groups

	Group I-Ovarian cancer (n = 22)		Group II(n = 14)		Controls (n = 10)	
Age (range)	premenopausal	postmenopausal	premenopausal	postmenopausal	premenopausal	postmenopausal
Samples	7	15	8	6	5	5
S.creatinine (mg/dL)	0.7 ± 0.08	0.7 ± 0.28	0.7 ± 0.19	0.8 ± 0.24	0.6 ± 0.11	0.8 ± 0.24
CA125 U/mL	573.1 (0-2133)	1657.5 (0-7073)	130 (0-655.8)	105.5 (0-423.9)	16.83 (2.47-31.19)	13.14 (0-28.72)
HE4 pmol/L	157.9 (0-643.9)	368 (0-1574)	68.4 (0-166.8)	110.4(0-222)	39.93 (22.29-57.57)	51.85 (18.75-84.95)

Table 2. HE4 & CA125 serum levels in patients with EOC according to tumour stage and histological type

	n	CA125 median (range)	HE4median (range)
Stage I-II	1	89.1	86.49
Stage III	12	1921.28	425.74
Stage IV	5	863.59	595.95
Serous	17	989.15	230.47
Sero-Mucinous	3	3700.58	265.89
Stromal	1	825	37.28
Germ cell	1	133.1	85.05

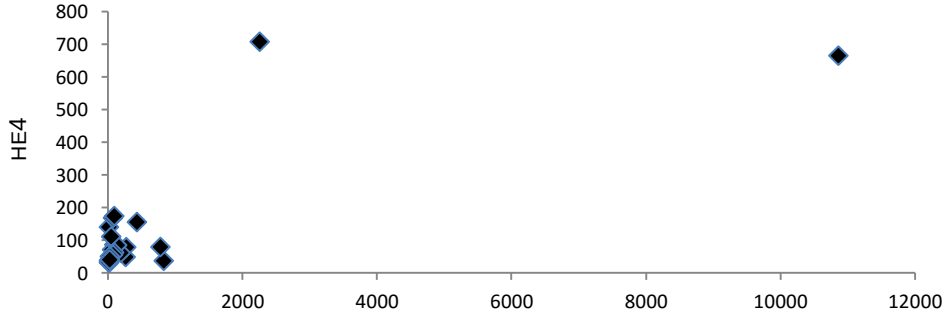
Table 3: Sensitivity, specificity, positive and negative predictive values of CA125, HE4 and in combination

	Sensitivity	Specificity	PPV	NPV
HE4	72.73%	64.29%	76.19%	60%
CA125	100%	50%	75.86%	100%
HE4 + CA125	100%	58.38%	76.19%	100%

Table 4: Spearman's rank correlation of HE4 and CA125 individually with diagnosis

			Diagnosis	HE4			Diagnosis	CA125
Spearman's rho	Diagnosis	Correlation Coefficient	1.000	.908**	Diagnosis	Correlation Coefficient	1.000	.907**
		Sig. (2-tailed)	.	.000		Sig. (2-tailed)	.	.000
		N	36	36		N	36	36
	HE4	Correlation Coefficient	.908**	1.000	CA125	Correlation Coefficient	.907**	1.000
		Sig. (2-tailed)	.000	.		Sig. (2-tailed)	.000	.
		N	36	36		N	36	36

Figure 1- Spearman's correlation graph between CA125 and HE4

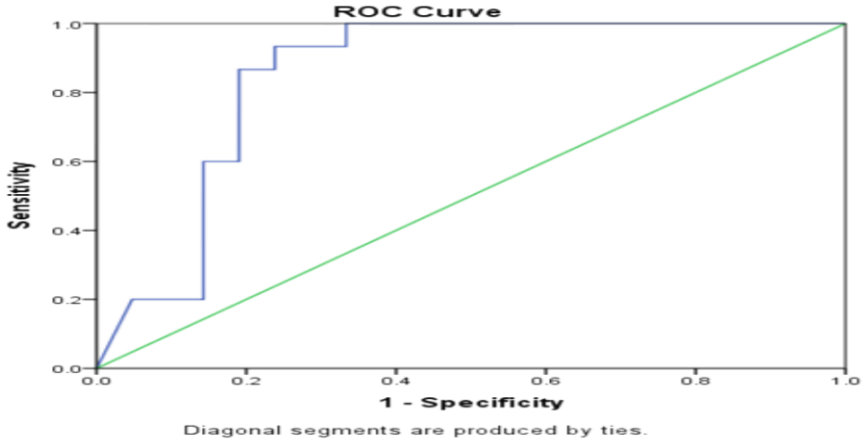


The value of R is 0.59.

Table 5. Spearman's rank correlation between HE4 and CA125 in post and premenopausal women

			CA125 post	HE4 post	CA125 pre	HE4 pre
Spearman's rho	CA125	Correlation Coefficient	1.000	.996**	1.000	.994**
		Sig. (2-tailed)	.	.000	.	.000
		N	21	21	15	15
	HE4	Correlation Coefficient	.996**	1.000	.994**	1.000
		Sig. (2-tailed)	.000	.	.000	.
		N	21	21	15	15

Figure 2 - ROC-AUC for HE4

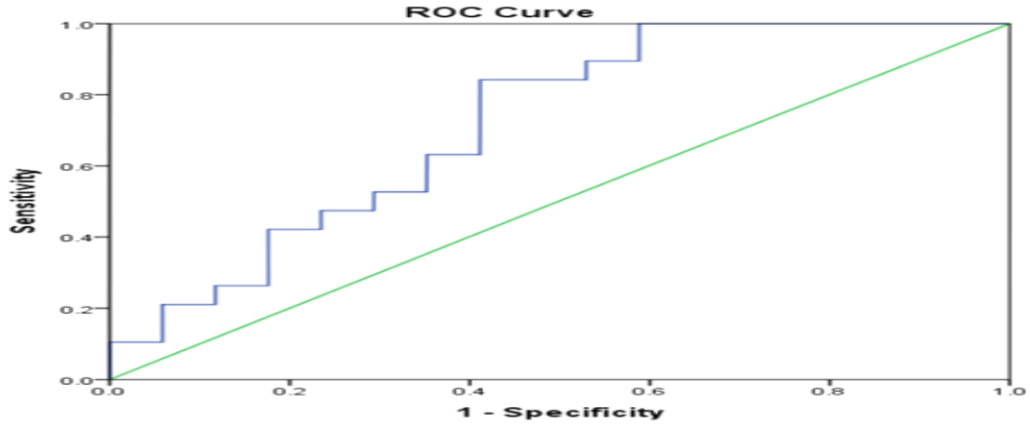


Area Under the Curve

Test Result Variable(s): HE4

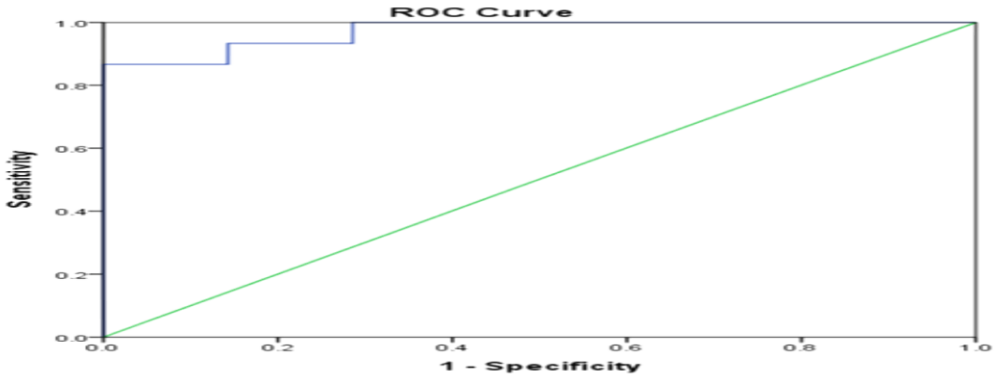
Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.849	.068	.000	.716	.983

Figure 3 - ROC-AUC for CA125



Area Under the Curve				
Test Result Variable(s): CA125				
Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.718	.088	.025	.546	.891

Figure 4 - ROC-AUC for HE4 in only ovarian cancer group



Area Under the Curve				
Test Result Variable(s): HE4				
Area	Std. Error[a]	Asymptotic Sig.[b]	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.971	.030	.000	.912	1.000

DISCUSSION

Worldwide, ovarian cancer is the second leading cancer in women and the fifth common cause of death from cancer. It is a gynecological disease with one of the highest mortality rates. The more the disease has progressed, the lower the survival rate is and unfortunately most of the ovarian cancer cases are detected in later stages where the chances for a cure are rather low. In the early stages of ovarian cancer, symptoms are nonspecific and cause little, if any, discomfort. Later, women may suffer from so-called pelvic or adnexal masses which can result in abdominal pain. It is estimated that 5 to 10 percent of women will present with a pelvic mass to their physician during their lifetime and undergo a surgical procedure for a suspected ovarian malignancy. In approx. 13 to 21 percent of these women, ovarian malignancies will be found [9]. Therefore, new methods and biomarkers which can help in diagnosing this disease at an earlier stage are highly desirable.

CA125 is still the only tumor marker recommended as a diagnostic or prognostic indicator and for monitoring the disease, recurrence after surgery and adjuvant chemotherapy [10-12]. The major drawback of CA125 is the documented lack of specificity, as this marker may show levels exceeding the 95th percentile of normal values in a significant proportion of women with benign or other malignant diseases [13].The new biomarker, HE4 alone or together with the already established marker CA125 can play a very important role here.

This study aimed to investigate the performance of serum tumour markers CA125 and HE4 individually, and in combination in a prospective collection of serum samples from patients with a pelvic or adnexal mass and or suspected ovarian mass.

Present study showed that both CA125 and HE4 individually were significantly increased in ovarian cancer ($p<0.001$) when compared to controls. In our experience, no false positive results for HE4 or CA125 were recorded in healthy women. Correlation between CA125 & HE4 was significant in both pre and postmenopausal groups ($p<0.001$).

Initial results on HE4 testing of this study confirm the high specificity and low sensitivity of this molecule over CA125 for OC (64.29% vs. 50% and 72.73% vs. 100%, respectively).The results were consistent with Moore (2008b) who showed that specificity was 83.3% and sensitivity 74.5%. HE4 has a clear specificity edge over CA125 which was already proved

in previous studies [14-18]. Compared to study of Moore, the specificity is little lower since HE4 is elevated in endometrial cancer also and there were 4 cases of endometrial cancer in the present study. Among which 3 cases showed significantly high values of HE4, Indicating it to be a marker for endometrial carcinoma also. Similar findings were seen in Brennan *et al* study [19].

When the ROC–AUCs of the two tumour markers were compared, HE4 showed larger area when compared to CA125 (0.84 vs. 0.71) in distinguishing ovarian cancer from benign & other malignancies. The diagnostic performance of HE4 improved when only ovarian cancers were taken into account with a value of 0.97 (95% CI 0.91-1.0), similar to study by Moore [20]. Combining HE4 and CA125 improved the performance in terms of sensitivity of HE4 and specificity of CA125 (100% and 58.38% respectively).

Of particular interest, HE4 seems to have a slightly higher specificity in pre menopausal group than post menopausal group which is in concordance with Moore *et al* [20] study who also showed that HE4 performance was better in premenopausal group. In other words, the performance of HE4 was similar to that of CA125 and more specific.

All malignant tumours expressed high levels of CA125 and HE4, but the highest levels were noted for the serous subtype. Out of 22 cases of ovarian cancer, 17 cases (77%) were serous carcinoma, three were sero-mucinous, and one sexcord stromal and one germcell tumour were seen.

Although CA125 & HE4 are not currently recommended as a screening tool, it is interesting to see how well a tumour marker performs in the early stage of disease. In the present study since the cohort constituted mostly of stage III & stage IV OC ,it is seen that CA125 performed significantly good ($p<0.05$) than HE4 when only ovarian cancer group was considered. But when all cases were included HE4 performed better than CA125 (ROC-AUC – 0.84 vs. 0.71). The results were comparable to a study by Montagnana, who showed that CA125 had good discrimination between controls and cancer patients only in later stages and HE4 showed a good discrimination between controls and cancer patients also in early stages [15].

CONCLUSION

The finding of ROC-AUC values for HE4 (0.84) being significantly higher when compared to CA125 (0.71), demonstrated a better performance of HE4 in differentiating ovarian cancer from benign and other non ovarian malignancies. Hence HE4 appears to be a good single marker than CA125. HE4 probably may be useful in early stages of ovarian cancer and in late stages CA125 is equally a good marker. HE4 can also be used as a marker of endometrial carcinoma. HE4 in combination with CA125 has shown to increase the sensitivity and specificity of individual markers. Further studies are needed to explore in more detail.

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