

Research Article**THE PERFORMANCE OF HE4 BIOMARKER IN PREDICTION OF MALIGNANCY IN OVARIAN MASS****Dr. Sushma Rachel S¹, Dr. Nagarathna Gopala², Dr. Rohanchandra R Gatty³**

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ABSTRACT: In India, ovarian cancer is the third leading cause of cancer deaths in women after cervical and breast cancer. The high mortality of ovarian cancer is attributed mainly to late diagnosis. The poor long term survival rates are mostly due to presentation of patients in advanced stages.

Objectives: 1. To validate the diagnostic performance of serum Human Epididymal Protein 4 (HE4) in prediction of malignancy in ovarian mass. 2. To compare the diagnostic accuracies of serum Carbohydrate Antigen 125 (CA125) and serum HE4. **Methodology:** Women presenting with ovarian mass/masses planned for surgery were included in the study according to the inclusion and exclusion criteria. Preoperative serum samples for estimation CA125 and HE4 levels were obtained. Risk of malignancy was predicted as low-risk or high-risk according to the cut-off values. Post- surgery, the histopathology report was noted and predicted values were compared to the pathologic diagnosis.

Results: Sixty women were included in the study. Out of which 35 were postmenopausal and 25 were premenopausal. In differentiating benign from malignant disease, in postmenopausal group, the sensitivity and specificity of serum HE4 was found to be superior 90.90% and 95% respectively, compared to serum CA125 (sensitivity=63% and specificity=60%). In premenopausal group, the sensitivity was 100% for both the tests, but serum HE4 had a greater specificity (70.60%) compared to CA125 (41.2%). **Conclusion:** Serum HE4 can be widely used as an individual biomarker for detection of ovarian malignancy. **Keywords:** Ovarian cancer, CA125, HE4

INTRODUCTION: The incidence of Ovarian cancer in India is 5.4 to 8.0 per 100000 population in different parts of the country.¹ The high mortality rate associated with this cancer can be attributed to late diagnosis, delayed presentation of the patients mostly in advanced stages and lack of appropriate screening tests. Due to delay in presentation and diagnosis, there is in turn delay in referral to gynaecologic oncologist. And because mortality is closely related to disease stage, the 5-year survival is higher than 70% in stage I or II but decreases to 40 and 20% in stage III or IV, respectively.² The high mortality rates can be reduced by screening the high risk population as well as early diagnosis. Unfortunately, effective screening tools for early detection of ovarian cancer have not been established yet.^{3,4} Currently Carbohydrate antigen 125 (CA125) is a widely used single biomarker for detecting ovarian cancer. Risk of malignancy index (RMI), where M is menopausal status, U is ultrasound findings and C is serum CA125 level, is the most widely used tool for the detection of ovarian cancer. Even though CA125 performs well in early detection of ovarian cancer, it is also elevated in benign conditions like pelvic inflammatory disease (PID), ovarian endometriosis, uterine myomas, acute and chronic salpingitis, and peritonitis. And thus a great decrease in its specificity. Pregnancy, menstruation, age, race, hysterectomy, and other benign conditions are few factors that influence the serum CA-125 levels.⁵ However, serum CA-125 is not very sensitive in the early stages of

ovarian cancer. The sensitivity of CA-125 drops to 50% in the early stages. In a meta-analysis by Ferraro et al,⁶ the specificity of CA125 for detecting ovarian cancer was 78%.

Plenty of new markers have been proposed and investigated for detection of ovarian cancer. One among them is serum Human Epididymal Protein 4 (HE4) which has proven to be a novel marker in detection of ovarian cancer. It is a glycoprotein belonging to the family of whey acidic four-disulfide core proteins, accounting for its alternative name of WAP Four Disulfide Core Domain 2 (WFDC2.)

There have been pilot studies suggesting that HE4 has increased sensitivity to discriminate ovarian cancer from benign ovarian neoplasms compared with CA125, especially in stage I disease.^{7,8}

This biomarker is weakly expressed in the epithelium tissues of respiratory and reproductive organs but is overexpressed in ovarian tumors, especially in endometrioid ovarian cancer. HE4 is less frequently elevated in benign conditions which are common in younger women.

Serum HE4 levels in patients with endometrioma were comparable to levels in patients with other benign ovarian cysts (53.0pmol/L vs. 52.8pmol/L)⁹, quite understandable since the gene coding for HE4 is not overexpressed in endometriotic lesions.¹⁰

HE4 has been found in more than half of ovarian tumors without CA125 expression.¹¹ Serum HE4 levels vary in smokers and hormonal contraceptive users.¹² Yanaranop et al.¹³ reported a specificity of 86% for HE4, and the AUC was higher than CA125 alone, with values of 0.893 and 0.865, respectively. These data, following those reported in a recent Italian multicenter study, included 387 patients, showed that HE4 for diagnosing ovarian epithelial cancer appeared more reliable than CA125.¹⁴

OBJECTIVES:

1. To validate the diagnostic performance of serum HE4 as an individual biomarker.
2. To compare the diagnostic accuracies of serum CA125 and serum HE4 using specificity and sensitivity values in prediction of malignancy.

METHODOLOGY:

Source of data: It is a validation study conducted from November 2017 to June 2019 in Father Muller Medical College, Mangalore, India. Women who presented with ovarian mass/masses to Gynecology and Surgical Oncology OPD who were planned to undergo surgery were enrolled in the study.

Inclusion criteria: Women >35 years of age with ovarian mass/masses.

Exclusion criteria: 1. Women diagnosed with ovarian cancer 2. Women with ongoing chemotherapy for ovarian cancer 3. Women who underwent bilateral salpingo-oophorectomy 4. Women not consenting for study.

The women who presented with ovarian mass/masses undergoing surgery were selected according to the inclusion and exclusion criteria. Upon obtaining consent for the study, they were enrolled into study. Preoperatively serum samples were collected for estimation of CA125 and HE4 levels. The risk of malignancy was predicted as low-risk and high-risk according to the cut off values. Post-surgery, the tissue specimens were subjected to histopathologic study. The predicted values were compared with pathologic diagnosis. The cut off values for serum CA125 and serum HE4 are shown in table 1.

Table 1. SERUM CA125 AND HE4 ASSAYS:

Tumor marker	Cut off values
CA125	35 (U/mL)
HE4	<70 (pmol/L)
Premenopausal	<140 (pmol/L)
Postmenopausal	

STATISTICAL ANALYSIS: According to the pathology patients were categorized into; Benign, Borderline neoplasia, Malignant Epithelial ovarian cancer (EOC) and Malignant Non-EOC. Two types of comparisons are presented for premenopausal and postmenopausal separately: Benign vs Malignant + Borderline ovarian tumors and Benign vs Malignant EOC. Cross tabulations were applied to assess the differences in the distribution of serum CA125 and serum HE4 levels among two groups respectively. The following test indicators of diagnostic performance were used in the study; sensitivity (SN), specificity (SP), positive predictive value (PPV) and negative predictive value (NPV). The diagnostic performance of serum CA125 and serum HE4 was evaluated using receiver operating characteristics (ROC) curves and area under the curve (AUC). All tests were considered statistically significant for a p -value <0.05 .

RESULTS: Sixty women were enrolled into the study. Out of which 25 were premenopausal and 35 were postmenopausal. The age distribution is shown in table 2.

TABLE 2. AGE DISTRIBUTION

	Age	Valid Percent
<40	12	20
41-50	16	26.7
51-60	17	28.3
61-70	13	21.7
>70	2	3.3
Total	60	100

Histopathology: Out of 60 women, 60% (n=36) were reported to have benign ovarian tumors. Ten percent (n=6) were reported as borderline ovarian tumors. Thirty percent (n=18) women were found to have malignant ovarian mass i.e. 26.7% (n=16) were malignant epithelial ovarian tumors and 3.3% (n=2) were non-epithelial ovarian tumors.

AUC- ROC ANALYSIS

ROC for Serum CA125 and Serum HE4 for Benign Vs Borderline + Malignant Masses

nopausal status	Test Result Variable(s)	Area	Std. Error	c 95% Confidence Interval	
				Lower Bound	Upper Bound

Postmenopausal	CA125	.683	.092	.503	.864
	HE4	.970	.024	.924	1.000
Premenopausal	CA125	.801	.105	.596	1.000
	HE4	.831	.092	.651	1.000

ROC for Serum CA125, Serum HE4, AND ROMA for Benign Vs Malignant EOC

Menopausal status	Test Result Variable(s)	Area	Std. Error	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
Postmenopausal	CA125	.736	.093	.555	.918
	HE4	.995	.008	.981	1.000
Premenopausal	CA125	.918	.063	.794	1.000
	HE4	.965	.036	.894	1.000

As shown in table 3.1, in differentiating benign from malignant and borderline tumors, in premenopausal group, the AUC for both tests was comparable, but HE4 (AUC 0.831) had better value indicating better performance. In postmenopausal group, the AUC-ROC for HE4 (AUC 0.970) was clearly larger than that for CA125 (AUC 0.683) indicating a superior performance of HE4.

Comparing the ROC-AUC in table 3.2, for differentiating benign from malignant EOC, in postmenopausal women, HE4 had the larger AUC of 0.995, compared to CA125 (AUC of 0.736). In the premenopausal group, the AUC for both tests were comparable, but HE4 (0.965) had better value compared to CA125 (AUC 0.918) indicating the superior performance of HE4 over CA125 in the study.

COMPARISON OF PERFORMANCE OF SERUM CA125 AND SERUM HE4 IN PREMENOPAUSAL GROUP

PATHOLOGY	PARAMETER	SN	SP	PPV	NPV	DIAGNOSTIC ACCURACY	P VALUE
Benign Vs Malignant + Borderline	CAs125	87.50%	41.20%	41.20%	87.50%	56.00%	0.2050
Benign Vs Malignant + Borderline	HE4	75.00%	70.60%	54.50%	85.70%	72.00%	0.0810
Benign Vs Eoc	CA125	100.00%	41.20%	33.30%	100.00%	54.55%	0.1350
Benign Vs Eoc	HE4	100.00%	70.60%	50.00%	100.00%	77.27%	0.0100

In table 4.1, the overall comparison of the performance of serum CA-125 and serum HE4 was made in the premenopausal group. In differentiating benign from malignant tumors (malignant + borderline), serum HE4 had a sensitivity of 75%, but serum CA125 had a better sensitivity of 87.50%. The diagnostic accuracy of HE4 was 72% which was better than serum CA125 (56%). None had a significant p-value. In differentiating benign from malignant EOC, both biomarkers had sensitivity and negative predictive value of 100%, but only serum HE4 had p-values 0.0100, which were statistically significant. And serum

HE4 had a better specificity of 70.6% compared to CA125 (41.20%). The diagnostic accuracy of HE4 (77.27%) was better than that of CA125 (54.55%).

4.2 COMPARISON OF PERFORMANCE OF SERUM CA125, SERUM HE4 AND ROMA IN POSTMENOPAUSAL GROUP

PATHOLOGY	PARAMETER	SN	SP	PPV	NPV	DIAGNOSTIC ACCURACY	P VALUE
BENIGN VS MALIGNANT +BORDERLINE	CA125	60.00%	60.00%	52.90%	66.70%	60.00%	0.3150
BENIGN VS MALIGNANT +BORDERLINE	HE4	66.70%	95.00%	90.90%	79.20%	82.86%	<0.001
BENIGN VS EOC	CA125	63.60%	60.00%	46.70%	75.00%	61.29%	0.2730
BENIGN VS EOC	HE4	90.90%	95.00%	90.90%	95.00%	93.55%	<0.001

In table 4.2, the overall comparison of the performance of serum CA-125 and serum HE4 was made in the postmenopausal group. In differentiating benign from malignant tumors (malignant + borderline), serum HE4 had better specificity of 95% compared to serum CA-125 (60%). Serum HE4 also had a better sensitivity of 66.70% compared to serum CA125 (60%). Diagnostic accuracy of serum HE4 was 82.86% and with p -value of <0.001 which were significant compared to serum CA125 (60%, p -value of 0.3150).

In differentiating benign from malignant EOC, serum HE4 had a sensitivity of 90.90%, and specificity of 95%, which are both clearly superior to that of CA125 (sensitivity of 63.90% and specificity of 60%). The diagnostic accuracy of serum HE4 i.e. 93.55% was significantly greater compared serum CA125 (61.29%). The p -value of HE4 being <0.001 was statistically significant.

DISCUSSION: Our study was conducted to validate the use serum HE4 as an individual biomaker in differentiating benign from malignant ovarian masses and compare the same with the widely used biomarker serum CA125. The recommended cut off value for serum HE4, was taken as 70pmol/L in premenopausal women and 140pmol/L in postmenopausal women and that of serum CA125 was taken as 35U/ml.

In our study, when we compared the sensitivity and specificity of serum CA-125 and serum HE4, in discriminating benign from malignant EOC, we observed that in premenopausal women, both had a sensitivity of 100%, but HE4 had a better specificity of 70% than serum CA-125 which had the lowest specificity of 41.20%. We achieved a statistically significant p -value for serum HE4 i.e. 0.001. In the postmenopausal group, serum HE4 had a sensitivity of 90.9%, but had a superior specificity of 95%. The p -value was statistically significant for serum HE4 i.e. <0.001. Serum HE4 as an individual marker performed better compared to serum CA-125.

Zheng et al¹⁵, in their study, reported sensitivity and specificity of 50% and 98.38% respectively for serum HE4 and concluded that serum HE4 testing is a more accurate and powerful tool than serum CA-125 assay in discriminating EOC from benign conditions like endometriosis and PID. Kadija et al¹⁶ (2012) conducted a prospective cross-sectional study and concluded that at the predefined specificity of 95%, HE4 showed a better sensitivity of 65.5% compared to CA125 (sensitivity 58.6%).

In our study among the premenopausal group, in discriminating benign from malignant and borderline masses, serum CA-125 had a superior sensitivity of 87.5% compared to serum HE4 with a sensitivity of 75%, but HE4 had the higher specificity of 70.60%. The p -values of both the tests were not statistically significant for the premenopausal group.

In the postmenopausal group we observed that serum HE4 performed better with specificity 95% compared to serum CA125 (sensitivity and specificity of 60%). Serum HE4 had statistically significant p-value i.e. <0.001. Serum CA-125 had a sensitivity and specificity of 60%, with a p-value of 0.3150 which was not statistically significant. As an individual marker, serum HE4 had the higher diagnostic accuracy of 72% compared to serum CA125 which had a lower diagnostic accuracy of 55% in this group.

Stiekema et al (2014) conducted a retrospective observational study at Amsterdam with results showing the sensitivity in predicting ovarian cancer of CA125 was 91% and of HE4 90%. The specificity was 65% and 97% respectively. HE4 demonstrated the highest discrimination (ROC-AUC = 0.96), compared to CA125 (AUC = 0.90). They concluded that HE4 in combination with CT-scan may be incorporated in the diagnostic work-up in women with a pelvic mass.¹⁷ In another study by Romagnolo et al (2016), a multicentre clinical study between June 2010 and January 2013, with 387 patients: 290 benign diseases; 15 borderline neoplasia and 82 tumors (73 EOC), they observed that among the two markers CA125 and HE4, the latter seemed to be more efficient than CA125 in ruling in EOC patients in the disease group, also in early stages tumors, both in pre and postmenopausal women.¹⁴ Hence, our study results also have been consistent with the superior performance of serum HE4 as an individual biomarker in the prediction of ovarian cancer, similar to the observations being reported by the studies conducted to validate these markers.

CONCLUSION: Serum HE4 can serve as an excellent individual biomarker in predicting the risk of malignancy in ovarian mass/masses compared to the widely used serum CA125 especially in postmenopausal women.

LIMITATIONS:

- Smaller cohort was taken as serum HE4 estimation is an expensive test currently.
- The 100% sensitivity of both the tests observed in our study in premenopausal group can be attributed to the fact that benign ovarian masses are common in this group.
- The performance of these individual biomarkers in predicting malignant disease with respect to the clinical stage of the cancer has not been studied.

FUTURE RESEARCH

- The correlation of serum HE4 biomarker levels with different clinical stages of malignant disease, response to treatment.
- Studies with larger cohorts can be conducted to further validate the usefulness of biomarker.

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