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Diagnostic Accuracy of CA125 and HE4 in Differentiating Benign and Malignant Ovarian Masses in a Tertiary Care Setting in India: A Prospective Study

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ABSTRACT

Background: Ovarian cancer often follows indolent course resulting in delayed diagnosis and presentation at advanced stage and poor clinical outcomes. Ultrasonography, though widely used has limitations in differentiating benign and malignant lesions. Serum tumor markers such as Cancer Antigen-125 (CA125) and Human Epididymis Protein-4 (HE4) have been investigated for their role in diagnosis of ovarian neoplasms.
Aims: To assess the diagnostic accuracy of serum CA125 and HE4, alone and in combination, in differentiating benign and malignant ovarian neoplasms
Materials and Methods: 186 patients with ultrasound-diagnosed ovarian tumors were included, Detailed demographic and clinicopathological data were recorded after obtaining informed consent. Patients with chronic kidney disease, and pregnancy were excluded. Preoperative serum CA125 and HE4 levels were measured. All patients underwent surgical management, and the final histopathological diagnosis was considered the gold standard. Sensitivity, specificity, and diagnostic accuracy of CA125 and HE4 was assessed individually and in combination.
Results: CA125 demonstrated higher sensitivity (86.1%) compared with HE4 (76.4%), while HE4 showed greater specificity (87.7%) than CA125 (65.8%). The combined use of CA125 and HE4 yielded a sensitivity of 81.3% and specificity of 76.8%, indicating improved diagnostic performance compared with either marker alone.
Conclusion: The Integrated assessment of CA125 and HE4 may improve diagnostic evaluation of ovarian neoplasms and assist in distinguishing benign from malignant lesions, thereby facilitating early clinical decision-making.

Key words: CA125, HE4, epithelial ovarian neoplasm.

INTRODUCTION

Among gynecological malignancies, ovarian cancer has the highest mortality rate and remains a major global health concern**.** Epithelial ovarian carcinoma constitutes the predominant category of ovarian malignancies, with serous carcinoma identified as the most frequent histological subtype. This subtype accounts for approximately 30–70% of ovarian cancer cases and is typically associated with an unfavourable prognosis. While the overall survival rate for ovarian cancer is estimated to be around 47%, outcomes are considerably poorer in patients with high-grade serous ovarian carcinoma (HGSOC), where survival rates have been reported to be as low as 29%.[1]

The development of reliable biomarkers is fundamental to improving the early detection and clinical management of ovarian cancer. Cancer antigen 125 (CA125) is the most extensively used serum biomarker in this context; however, its diagnostic utility is limited, particularly in early-stage disease, owing to suboptimal sensitivity and specificity. Furthermore, CA125 levels may be elevated in a range of benign gynaecological conditions, such as endometriosis, pelvic inflammatory disease, and benign ovarian cysts, thereby diminishing its specificity for malignancy[2]

Human epididymis protein 4 (HE4) has emerged as a valuable biomarker for the detection of ovarian cancer. Compared to CA125, HE4 exhibits higher specificity in distinguishing malignant ovarian tumors from benign ovarian masses.[3] Consequently, the combined assessment of HE4 and CA125 has been proposed to improve diagnostic accuracy and address the limitations associated with the use of either marker alone Considering these limitations, this study was done to assess the diagnostic performance of serum CA125 and HE4, both individually and integrated together, in distinguishing benign from malignant ovarian neoplasms.

MATERIALS AND METHODS

Study design and setting:

Present prospective cohort study was carried out over a duration of two years in the Department of Obstetrics and Gynaecology. Study approval was obtained from the Institutional Ethics Committee (ECR/262/Inst/UP/2013/RR-19; approval no. XIV-PGTSC-IIA/P35, dated 27 February 2023).

Study Population

A total of 186 patients with ovarian tumors detected on ultrasonography were prospectively enrolled after obtaining written informed consent. Detailed demographic and clinicopathological data was collected for all participants.

Inclusion criteria: Patients with ovarian tumors diagnosed on ultrasonography and planned for surgical intervention were included in the study

Exclusion criteria: Patients with chronic kidney disease and pregnancy

Sample Size Determination

Since it was a prospective study all consecutive patients attending OPD and meeting the inclusion criteria were enrolled during the two-year study period. A total of 186 cases of ovarian tumors were identified and included in the final analysis

Specimen Collection and Biomarker Assays

Peripheral venous blood samples were obtained from all cases prior to any surgical intervention. Following clot formation at room temperature, samples were centrifuged at 3000 rpm for 5 minutes. The separated serum was aliquoted and stored at −20°C until analysis. Serum CA125 levels were quantified using an automated chemiluminescent microparticle immunoassay (CMIA). Serum HE4 concentrations were measured using an electrochemiluminescence immunoassay on the Roche Cobas e411 platform, employing a sandwich assay format with monoclonal antibodies (2H5 and 3D8) in department of pathology.

All patients underwent surgical management, and histopathological examination of resected specimens served as the reference standard. Of the 186 patients, 72 were diagnosed with malignant epithelial ovarian neoplasms.

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics (version 23). Continuous variables were expressed as mean ± standard deviation or median (range), while categorical variables were expressed as frequencies and percentages.

Diagnostic performance of CA125 and HE4 was evaluated by calculating sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy. Receiver operating characteristic (ROC) curve analysis was conducted to assess discriminative ability.

Associations between biomarker levels and clinicopathological variables, including tumor stage and histological subtype, were analyzed using appropriate parametric or non-parametric tests. Correlation analyses were performed using Spearman's rank and Kendall's tau-b coefficients where applicable.

Study Outcomes

The primary outcome was to determine the diagnostic accuracy of serum CA125 and HE4, individually and in combination, for identifying Benign and malignant epithelial ovarian tumors. The secondary outcome was to evaluate the correlation between biomarker levels and disease stage.

RESULTS AND OBSERVATIONS

Among the 186 patients included in the study, histopathological examination revealed that the majority of cases were benign ovarian tumors 102 (54.8%), followed by malignant ovarian tumors 79 (42.5%), while borderline tumors constituted the smallest proportion 5 (2.7%).

Table 1-The mean age of patients with benign ovarian neoplasms (n = 102) was 35.9 ± 10.1 years, whereas the mean age of patients with malignant ovarian neoplasms was 48.5±6.92 years. Patients with borderline tumors had a mean age of 38.40 ± 14.08 years.

Most participants were residents of rural areas and belonged predominantly to lower socioeconomic status.

Among patients with malignant epithelial ovarian neoplasms, 50% were multiparous (para 3–4), followed by 29.2% with parity greater than four, while 2.8% were nulliparous. A larger proportion of epithelial ovarian neoplasm cases were observed in postmenopausal women (49%), compared with 23% in premenopausal women.

Table 1: Characteristics of different Malignant Ovarian Neoplasm

Type Of Tumor		**Age at diagnosis**			
		Mean ± SD	**Median**	**Minimum**	**Maximum**
Malignant	**Serous Adeno carcinoma**	48.41±10.15	48.00	24	73
	Mucinous	48.00±10.01	51.00	30	60
	Clear call	49.20±7.15	52.00	37	55
	Endometrioid	48.39±9.84	49.0	24	73
Benign (102)	**Serous cyst adenoma**	42.84±1482	40.0	16	71
	Mucinous Cyst adenoma	42.06±11.63	41.00	24	65
	Mature Teratoma	35.58±12.20	34.50	20	66
	Fibroma	27.75±14.17	21.00	20	49
	Endometrioma	33.60±8.14	37.00	21	40
Parity		**Benign**		**Malignant**	
Nullipara		16(15.6%)		2 (2.8%)	
P 1-2		44 (43.1%)		13(18.1%)	
P 3-4		32(31.3%)		36 (50.0%)	
P >4		10 (9.8%)		21(29.2%)	
Menopausal status		**Benign**		**Malignant**	
Pre-menopausal		74 (72.5%)		23(31.9%)	
Post menopausal		28 (27.5%)		49(68.1%)	

Table 2 depicts the pre-treatment serum CA125 levels across different ovarian tumor types (n = 186).

Among benign ovarian tumors, the mean pre-treatment CA125 level in serous cystadenoma was 29.56 ± 43.06 U/mL, while in mucinous cystadenoma it was 91.94 ± 52.62 U/mL. The mean CA125 level in follicular cysts was 13.83 ± 6.39 U/mL, whereas mature cystic teratoma showed a mean value of 48.83 ± 44.99 U/mL. In endometrioma, the mean CA125 level was 83.98 ± 45.45 U/mL, and in ovarian fibroma it was 68.50 ± 55.45 U/mL. A single case of Brenner tumor demonstrated a CA125 value of 98 U/mL.

Among malignant ovarian tumors, the mean pre-treatment CA125 level in serous adenocarcinoma was 852.10 ± 958.30 U/mL, whereas mucinous adenocarcinoma and clear cell carcinoma showed mean values of 454.75 ± 186.60 U/mL and 563.00 ± 503.20 U/mL, respectively. The CA125 level observed in a patient with endometrioid carcinoma was 134 U/mL.

Among other ovarian tumors, the mean CA125 level in sex cord tumors was 397.5 ± 172.8 U/mL, while germ cell tumors and borderline tumors showed mean CA125 levels of 157.5 ± 101.3 U/mL and 164.96 ± 201.35 U/mL, respectively.

Overall, markedly elevated CA125 levels were predominantly observed in malignant ovarian tumors compared with benign lesions.

Table 2: Pretreatment CA125 levels in different ovarian tumors (n=186)

Type Of Tumor	Pretreatment CA125 levels				P value*
	Mean ± SD	Median	Minimum	Maximum	
Serous cystadenoma(n=37)	29.56±43.06	12.00	3.0	220.0	**0.001**
Mucinous cystadenoma(n=18)	91.94±52.62	98.50	16.0	220.0	
Follicular cyst(n=24)	13.83±6.39	11.50	3.0	27.0	
Mature Teratoma(n=12)	48.83±44.99	22.00	2.0	128.0	
Endometrioma(n=5)	83.98±45.45	96.00	7.9	130.0	
Fibroma (n=4)	68.50±55.45	60.00	22.0	132.0	
Brenner Tumor(n=1)	98	-	-	-	
Serous adenocarcinoma(n=54)	852.106±958.30	563.50	150.0	4000.0	**0.002**
Mucinous adenocarcinoma(n=12)	454.75±186.60	455.00	36	730.0	
Clear cell (n=5)	563.00±503.20	876.00	10.0	1009.0	
Endometroid Tumor(n=1)	134	-	-	-	
Sex cord(n=4)	397.5±172.8	149	27	290	
Germ cell (n=3)	157.5±101.3	133	120	220	
Border line (5)	164.96±201.35	88.00	29.8	516.0	

***Kruskal-Wallis test**

Table 3 depicts the pre-treatment serum HE4 levels in different ovarian tumors (n = 102).

Among benign ovarian tumors, the mean pre-treatment HE4 level in serous cystadenoma was 20.21 ± 15.35 pmol/L, while in mucinous cystadenoma it was 33.05 ± 31.72 pmol/L. The mean HE4 level in follicular cysts was 24.62 ± 23.98 pmol/L, whereas mature cystic teratoma showed a mean value of 31.90 ± 32.56 pmol/L. In endometrioma, the mean HE4 level was 24.20 ± 7.69 pmol/L, while ovarian fibroma demonstrated a mean value of 16.00 ± 5.29 pmol/L. The HE4 level observed in the single case of Brenner tumor was 12 pmol/L.

Among other ovarian tumor types, the mean HE4 level in sex cord tumors was 102 ± 67.2 pmol/L, while germ cell tumors and borderline tumors showed mean HE4 levels of 128.3 ± 9.1 pmol/L and 91.60 ± 28.21 pmol/L, respectively.

Among malignant ovarian tumors, the mean pre-treatment HE4 level in serous adenocarcinoma was 352.25±283.81 pmol/L, whereas mucinous adenocarcinoma and clear cell carcinoma showed mean HE4 levels of 299.58 ± 212.61 pmol/L and 407.0

± 308.98 pmol/L, respectively. The HE4 level observed in a patient with endometrioid carcinoma was 28 pmol/L.

Overall, serum HE4 levels were markedly elevated in malignant ovarian tumors compared with benign lesions.

Table 3: Pretreatment HE4 levels in different ovarian tumors (n=186)

Type Of Tumor	Pretreatment HE4 levels			
	Mean ± SD	Median	Minimum	Maximum
Serous cystadenoma	20.21±15.35	20.00	8.0	98.0
Mucinous cystadenoma	33.05±31.72	21.50	10.0	101.0
Follicular cyst	24.62±23.98	19.50	8.0	99.0
Mature Teratoma	31.90±32.56	22.50	7.8	101.0
Endometrioma	24.20±7.69	27.00	12.0	32.0
Fibroma	16.00±5.29	15.00	11.0	23.0
Brenner Tumor	12	-	-	-
Sex cord	102±67.2	103.5	11	190
Germ cell	128.3±9.1	109	55	221
Border line	91.60±28.21	98.00	54.0	129.0
Serous adenocarcinoma	352.25±283.81	276.00	22.0	1009.0
Mucinous adenocarcinoma	299.58±212.61	340.0	28.0	600.0
Clear cell	407.0±308.98	320.0	45.0	780.0
Endometroid Tumor	28	-	-	-

***Kruskal-Wallis test**

Table 4- depicts Pretreatment CA125 & HE4 levels in different stages of ovarian cancer

The mean pre-treatment CA125 level in patients with stage I disease was 72.81 ± 101.67 U/mL, whereas in stage IV disease it increased markedly to 1865.25 ± 1017.93 U/mL.

Similarly, the mean pre-treatment HE4 level in stage I disease was 161.81 ± 195.85 pmol/L, while in stage IV disease it was 579.00 ± 357.00 pmol/L, demonstrating a progressive increase with advancing stage of disease

Table 4- Pretreatment CA125 & HE4 levels in different stages of ovarian cancer

Stages	Pretreatment CA125 levels				P value*
	Mean ± SD	Median	Minimum	Maximum	
I	72.81±101.67	20.00	10.0	309.0	**0.012**
II	253.90±255.88	156.50	23.0	789.0	
III	928.05±865.52	619.60	33.0	4000.0	
IV	1865.25±1017.93	1953.50	545.0	3009.0	
Stages	**Pretreatment HE4 levels**				
	Mean ± SD	**Median**	**Minimum**	**Maximum**	
I	161.81±195.85	110.0	27.0	700.0	
II	175.20±199.57	77.00	22.0	599.0	
III	400.67±261.63	399.00	28.0	1003.0	
IV	579.00±357.00	558.50	190.0	1009.0	

***Kruskal-Wallis test**

Figure 1 shows the receiver operating characteristic (ROC) curve analysis of serum CA125 for diagnosing malignant epithelial ovarian neoplasms.

The area under the curve (AUC) for CA125 was 0.910, indicating strong and statistically significant discriminatory ability for differentiating malignant epithelial ovarian tumors from benign lesions.

At an optimal cutoff value of 39 U/mL, serum CA125 demonstrated a sensitivity of 86.1% and specificity of 65.8%. The positive predictive value (PPV) and negative predictive value (NPV) were 72.9% and 67.3%, respectively. The overall diagnostic

accuracy of CA125 in detecting malignant epithelial ovarian neoplasms was 71.1%

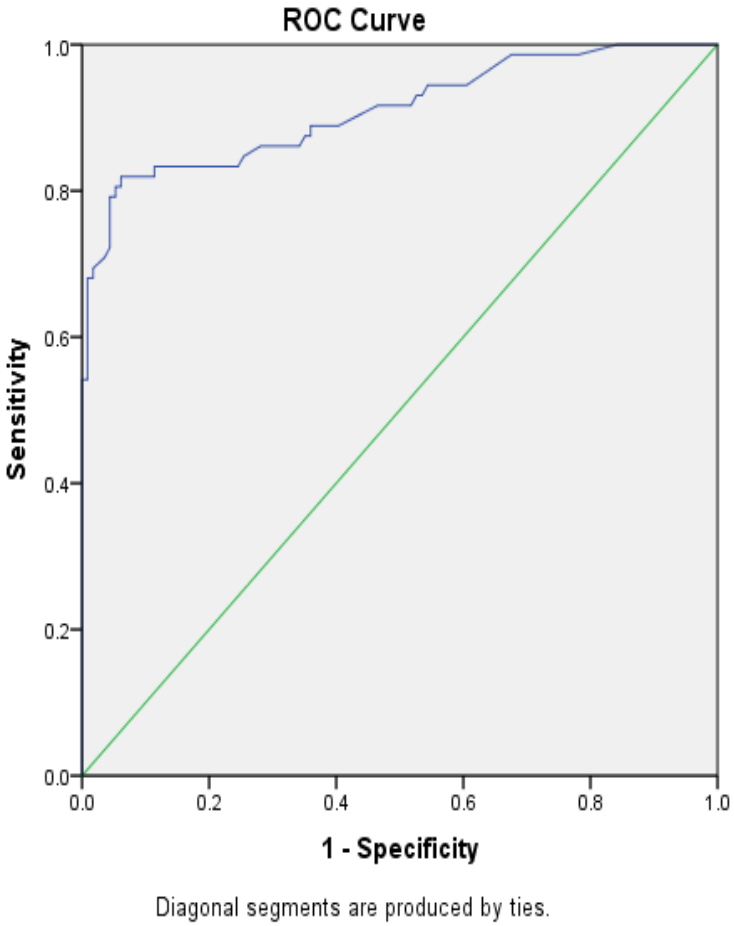

Fig 1: ROC for CA125 predicting Malignant epithelial ovarian tumors.

PARAMETER	CA125
AUC	0.910
ST. ERROR	0.024
95% Confidence Interval	0.862-0.957
p Value	0.0001
Cut off	39.0
Sensitivity%	86.1%
Specificity%	65.8%
PPV	67.3%
NPV	72.9%
Diagnostic accuracy	71.1%
Youden's Index	0.519

Figure 2 illustrates the receiver operating characteristic (ROC) curve analysis of serum HE4 for diagnosing malignant epithelial ovarian neoplasms.

The area under the curve (AUC) for HE4 was 0.948, indicating excellent and statistically significant discriminatory ability in differentiating malignant epithelial ovarian tumors from benign lesions.

At an optimal cutoff value of 86.5 pmol/L, serum HE4 demonstrated a sensitivity of 76.4% and specificity of 87.7%. The positive predictive value (PPV) and negative predictive value (NPV) were 64.4% and 78.1%, respectively. The overall diagnostic

accuracy of HE4 in detecting malignant epithelial ovarian neoplasms was 82.9%.

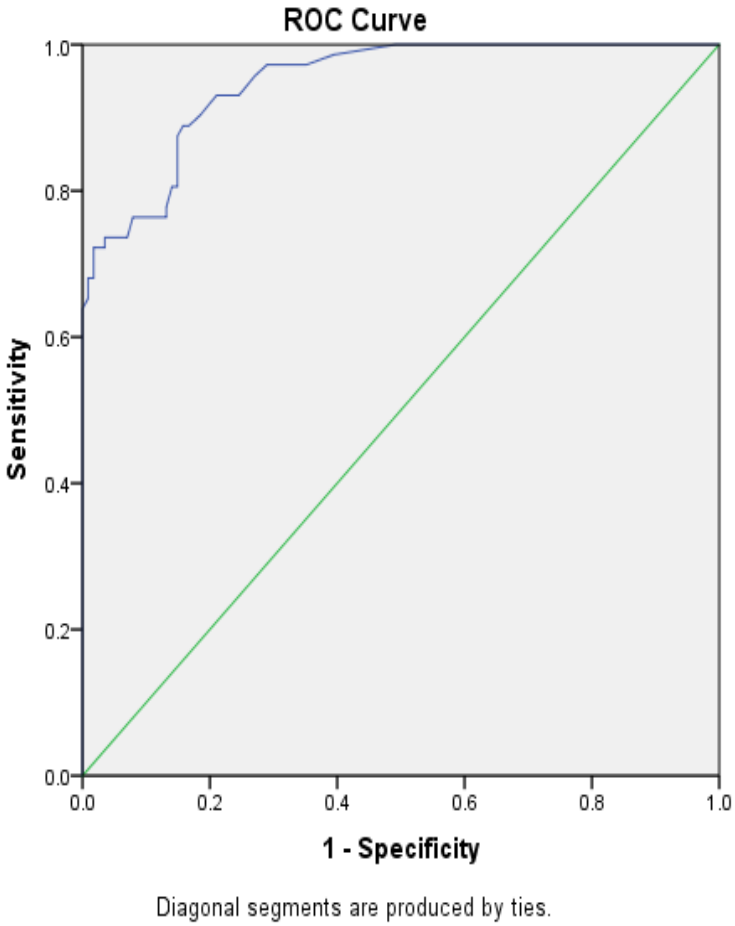

Fig 2: ROC for HE4 levels predicting Malignant epithelial ovarian tumors.

PARAMETER	HE4
AUC	0.948
ST. ERROR	0.014
95% Confidence Interval	0.920-0.976
p Value	0.0001
Cut off	86.5
Sensitivity%	76.4%
Specificity%	87.7%
PPV	78.1%
NPV	64.4%
Diagnostic accuracy	82.9%
Youden's Index	0.641

Figure 3 demonstrates the receiver operating characteristic (ROC) curve analysis of the combined use of serum CA125 and HE4 for diagnosing malignant epithelial ovarian neoplasms.

The area under the curve (AUC) for the combined biomarkers was 0.929, indicating very strong and statistically significant discriminatory ability in differentiating malignant epithelial ovarian tumors from benign ovarian lesions.

The combined use of CA125 and HE4 showed a sensitivity of 81.3% and specificity of 76.8%. The positive predictive value (PPV) and negative predictive value (NPV) were 68.5% and 73.4%, respectively. The

overall diagnostic accuracy of the combined biomarkers was 80.01%.

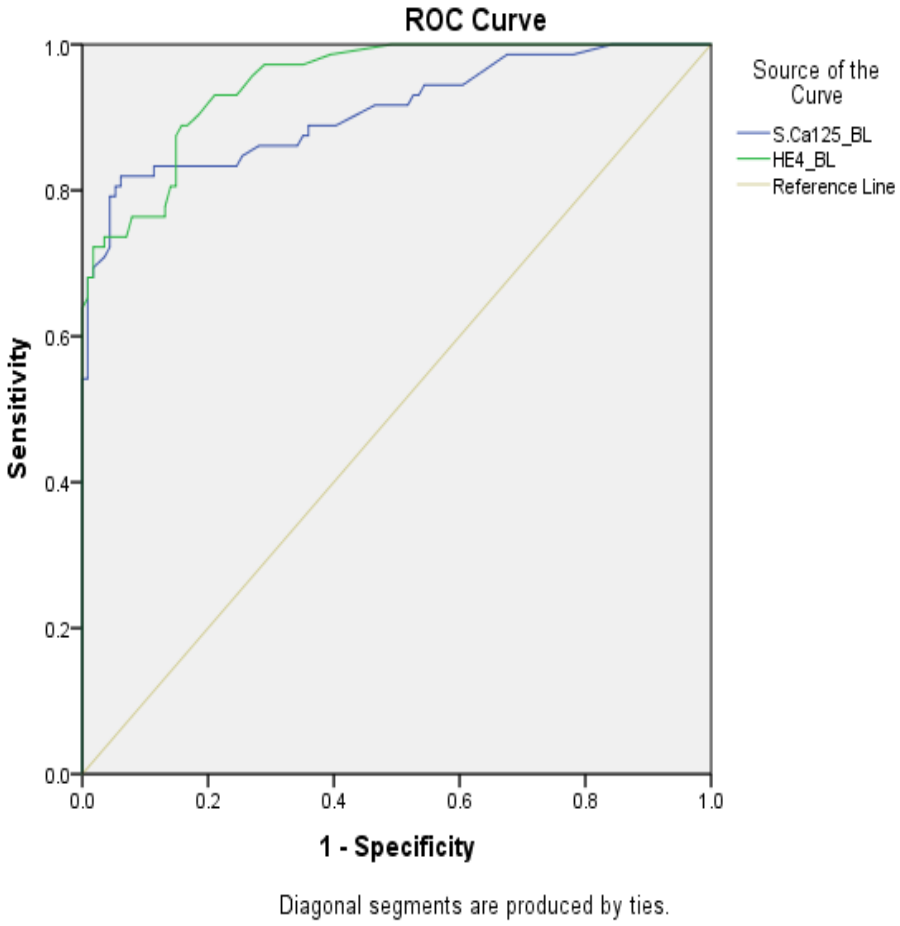

Fig 3: ROC for combined CA125 and HE4 for diagnosing malignant epithelial ovarian tumors.

PARAMETER	CA125 and HE4
AUC	0.929
ST. ERROR	0.019
95% Confidence Interval	0.891-0.967
p Value	0.002
Sensitivity%	81.3%
Specificity%	76.8%
PPV	73.4%
NPV	68.5%
Diagnostic accuracy	80.01%
Youden's Index	0.58

DISCUSSION

In the present study, benign ovarian tumors accounted for the majority of cases (54.8%), followed by malignant tumors (42.4%) and borderline tumors (2.7%). This distribution is consistent with previous studies which showed predominance of benign lesions among surgically managed ovarian masses. The higher proportion of malignant cases in our study may be because our institute is tertiary care referral centre catering to more complex and high-risk patients.

Among malignant epithelial ovarian neoplasms, the serous subtype was the most common (75%), followed by mucinous (16.6%), clear cell (6.9%), and endometrioid (1.3%) tumors. Our results are in agreement with studies by Nahar et al.[1] and Jha et al.[2],

who also reported serous adenocarcinoma as the predominant histological subtype, although with a relatively lower proportion (approximately 46.2%). With respect to germ cell tumors (n = 3), one case each of dysgerminoma, yolk sac tumor, and choriocarcinoma was identified. Previous literature, including Husaini et al[3]., has reported dysgerminoma as the most common malignant germ cell tumor of the ovary, accounting for approximately 1–2% of all ovarian malignancies. Variations in histopathological distribution across studies may reflect regional differences in the epidemiology of ovarian cancer.

Age distribution in our cohort demonstrated that benign ovarian tumors were most frequently observed in the 31–40-year age group, whereas malignant tumors were predominantly seen in patients aged 41–50 years. Notably, the mean age at presentation for malignancy in our study was approximately a decade higher than that reported by Khound et al.[4] and Vasanthamani et [5]al., but was comparable to findings by Jha et al.[2] and Farag et al.[6] This variation may be due to regional differences in disease epidemiology, healthcare access of the studied population.

In our study 79.2% of patients with malignant epithelial ovarian neoplasms were multiparous (parity >3) and only 2.8% were nulliparous in contrast to study by Gaitskell et al.[7], which demonstrated an increased risk of ovarian cancer among nulliparous women and Toufakis et al.[8], which demonstrated a protective effect of childbirth across various histological subtypes. The absence of a protective association of higher parity in our cohort might be because of small sample size which predominantly included multiparous women.

In our study, majority of women diagnosed with epithelial ovarian neoplasm were postmenopausal (49%), while 23% were premenopausal. Our finding are in concordance with Hada A. et al.[9] who in their study observed that malignancy was more common in the postmenopausal group compared to the premenopausal group (<0.001).

The primary objective of our study was to assess the diagnostic performance of CA125 and HE4. Receiver operating characteristic (ROC) analysis for CA125 yielded an area under the curve (AUC) of 0.910, indicating good discriminative ability and statistical significance. Using a cut-off value of 39 U/L, CA125 demonstrated a sensitivity of 86.1% and a specificity of 65.8%. The positive predictive value and negative predictive value were 67.3% and 72.9%, respectively. These findings are comparable to those reported by Funston et al.[10], who observed that at a threshold of ≥35 U/mL, CA125 achieved a sensitivity of 77.0% (95% CI: 72.8–80.8%) and a specificity of 93.8% (95% CI: 93.6–94.0%), with an AUC of 0.92 (95% CI: 0.90–0.93) for the detection of ovarian cancer. The lower specificity observed in our cohort may reflect the inclusion of benign conditions known to elevate CA125, highlighting its limitation as a standalone diagnostic marker.

Conversely, HE4 exhibited superior diagnostic performance, with an area under the ROC curve (AUC) of 0.948. At a cut-off value of 86.5 U/L, HE4 showed a sensitivity of 76.45% and a specificity of 87.7%. The positive predictive value and negative predictive value were 78.1% and 64.4%, respectively, with an overall diagnostic accuracy of 82.9%.

Our findings are in concordance with study by Kim et al.[11], who reported that a substantial proportion of patients with benign gynecological conditions exhibited elevated CA125 levels (>35 U/L), whereas HE4 levels largely remained within the normal range. This observation reinforces the higher specificity of HE4 and its superior ability to differentiate malignant ovarian tumors from benign conditions. Barr et al.[12] reported an AUC of 0.92, with a sensitivity of 90.2% and specificity of 88.4%, along with a positive predictive value of 66% and a negative predictive value of 90%. These findings are comparable to those observed in our study,

reinforcing better diagnostic performance of HE4.

Kim et al. [11] reported that the combined use of CA125 and HE4 yielded an AUC of 0.904 ($p = 0.0017$), with a sensitivity of 79.6% and specificity of 90%. While their specificity was slightly higher, the sensitivity closely approximates that observed in our study. Consistent with these findings, our results demonstrate that CA125 alone has lower diagnostic accuracy (71.1%) compared with HE4 (82.9%) and the combined use of both markers (80.0%). these findings highlight the consistent and reliable diagnostic performance of HE4 across different study populations and emphasize the added value of combining HE4 with CA125, which is in agreement with the results of the present study.

Similarly, Ahmed et al.[13] reported that the combined use of HE4 and CA125 achieved a sensitivity of 75.8%, specificity of 93.5%, and an AUC of 0.940, with an overall accuracy of 85.7%. Fawzy et al.[14] also demonstrated comparable findings, with an AUC of 0.935, sensitivity of 88.3%, and specificity of 85%. In our cohort, the combined use of both biomarkers resulted in a sensitivity of 81.3%, specificity of 76.8%, and diagnostic accuracy of 80.01%, which is broadly in agreement with these studies. Taken together, these observations reinforce the complementary roles of CA125 and HE4 for diagnosis of ovarian tumor.

An additional finding of our study was the progressive rise in serum CA125 and HE4 levels with advancing stage of ovarian cancer. Mean pre-treatment levels of both biomarkers were significantly higher in advanced-stage disease compared to early-stage cases, suggesting a potential role in reflecting tumor burden and disease severity. Comparable findings have been described by Bandiera et al[15]. and Molina et al[16]., reinforcing the association between elevated biomarker levels and advanced stages of disease.

The combined assessment of CA125 and HE4 in our study demonstrated a sensitivity of 81.3% and specificity of 76.8%, with an AUC of 0.929, indicating superior overall diagnostic performance compared to either marker alone. This improvement can be attributed to the complementary characteristics of the two biomarkers, where CA125 offers higher sensitivity and HE4 provides greater specificity. Combined use of both marker yields a more balanced approach for the preoperative evaluation of ovarian masses. These findings further suggest that CA125 and HE4 may have utility not only in diagnosis but also in disease stratification and monitoring

Overall, the findings of the present study add to the existing evidence that HE4 demonstrates greater specificity than CA125, while the combined use of both biomarkers provides superior diagnostic performance compared to either marker alone. This combined approach may enhance the preoperative distinction between benign and malignant adnexal masses.

Limitations

Present study was a single-center study with limited sample size, larger multicentric studies are required to validate these findings.

CONCLUSION

In conclusion, HE4 demonstrates higher specificity compared to CA125, while the combined use of CA125 and HE4 provides superior diagnostic accuracy for differentiating malignant from benign ovarian tumors. The integration of these biomarkers into routine clinical practice may enhance preoperative evaluation and improve patient stratification. Future large-scale, multicentric studies incorporating biomarker combinations with clinical and imaging parameters are needed to further improve diagnostic algorithms and for better patient care

Authors' Contribution: Concept, design- SA, ER (Dr Shuchi Agrawal, Eva Raman) , definition of intellectual content -SA,ER literature search- SA,ER,PA clinical studies- SA,ER,PA(Preeti Agrawal) experimental

studies- SA, ER,PA data acquisition- SA,SK. data analysis- SA,ER statistical analysis- ER,SA manuscript preparation-ER,SA editing -ER,SA,PA,SS (Shalini singh) manuscript review -ER,SS,PA,AA,SS.SG. Manuscript has been read and approved by all the authors, that the requirements for authorship have been met, and that each author believes that the manuscript represents honest work, manuscript has not been submitted or presented elsewhere.

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Conflict of Interest: Nil

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