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Original Research Article

Assessment of diagnostic performance of HE4 and CA125, individually and combined in epithelial ovarian cancer screening

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ABSTRACT

Background: Epithelial ovarian cancer (EOC) is one of the most lethal gynecological malignancies worldwide, often referred to as a "silent killer" due to its asymptomatic nature in the early stages. Proper diagnosis at early stage of Ovarian malignancy using biomarker is extremely important. The aim of this study was to evaluate the diagnostic accuracy of Human Epididymis Protein 4 (HE4) and Cancer Antigen 125 (CA125).
Methods: This descriptive observational study was conducted in Dhaka Medical College Hospital from January 2021 to December 2021. Data were collected using a pre-designed data collection sheet encompassing demographics, clinical examination, and investigation results. After obtaining informed written consent, clinical examinations and ultrasound of the abdomen were conducted, followed by the collection of venous blood samples.
Results: In our study predominant age group was 41-50 years (32.5%). Serum HE4 and CA125 concentrations were significantly higher in the ovarian cancer patients compared with those seen in patients with benign disease or in the healthy controls ($p<0.05$ in both). In patients with an adnexal mass, the area under the ROC curve was higher when the combination of the markers was used compared with use of CA125 only. Using ROMA, patients could be successfully classified into high- and low-risk group, with 57.7% sensitivity at a specificity of 63.9%.
Conclusions: These findings suggest that measuring serum HE4 concentrations along with CA125 concentrations may provide higher accuracy for detecting early stage of Epithelial ovarian cancer.

Keywords: Benign disease, Cancer antigen 125, Epithelial ovarian cancer, Human epididymis protein 4

INTRODUCTION

Epithelial ovarian cancer (EOC) is one of the most lethal gynecological malignancies worldwide, often referred to as a "silent killer" due to its asymptomatic nature in the early stages.[1] As a result the majority of cases are diagnosed at advanced stages contributing to the high mortality rates associated with this disease.[2] Despite advances in treatment and management, the five-year survival rate for patients diagnosed with late-stage ovarian cancer remains low.[3] Early detection is therefore crucial as it significantly improves the prognosis and survival rates of affected individuals.[4] This highlights the importance of developing effective screening tools that can accurately identify ovarian cancer in its early stages. The

measurement of CA125 (Cancer Antigen 125) levels in the blood is a standard approach in detecting ovarian abnormalities. A cutoff value of 35 KU/ is commonly used, based on its distribution in healthy individuals. Elevated levels of CA125 are often associated with ovarian cancer but can also be present in benign conditions.[2] HE4 (Human Epididymis Protein 4) is a glycoprotein expressed in the cells lining the lungs and reproductive organs, including the ovaries. It is notably produced by most epithelial ovarian cancer cells, making it a valuable tumor marker.[3] In clinical practice, a widely accepted cutoff value for HE4 is 140 pmol/l. Elevated HE4 levels, particularly when used alongside CA125, can improve the accuracy of ovarian cancer screening and diagnosis.

Cancer antigen 125 (CA125) has been widely used as a serum biomarker for ovarian cancer screening and monitoring since its discovery in the early 1980s.[5] Although CA125 has been a cornerstone in ovarian cancer diagnostics, its limitations are well-documented. CA125 levels can be elevated in several benign conditions, such as endometriosis, pelvic inflammatory disease, and other non-gynecological conditions, leading to false-positive results.[6] Moreover, CA125 lacks sensitivity in detecting early-stage ovarian cancer, as its levels may not be elevated in a significant proportion of patients with early-stage disease. These limitations have driven the search for additional biomarkers that could enhance the diagnostic accuracy of CA125 when used in combination.[7]

Human Epididymis Protein 4 (HE4) has emerged as a promising biomarker in the diagnosis of epithelial ovarian cancer.[8] Unlike CA125, HE4 has shown higher specificity for ovarian cancer, with fewer false-positive results in benign gynecological conditions. HE4 is a glycoprotein that is overexpressed in epithelial ovarian tumors but is less commonly elevated in other benign conditions, making it a potentially valuable tool in distinguishing malignant from non-malignant cases.[9]

The U.S. Food and Drug Administration (FDA) has approved HE4 for use alongside CA125 to improve the detection of ovarian cancer, particularly in patients with an ambiguous clinical presentation.[6] Several studies have evaluated the diagnostic performance of HE4 and CA125 individually, as well as their combined use in screening for ovarian cancer.[10] Evidence suggests that while each biomarker has its strengths and limitations, their combined use may significantly improve diagnostic accuracy. The Risk of Ovarian Malignancy Algorithm (ROMA), which incorporates both CA125 and HE4 levels, has been developed as a predictive model to stratify patients into high- or low-risk categories for ovarian cancer.[11] Studies have demonstrated that ROMA has higher sensitivity and specificity compared to either marker alone, providing a more reliable screening tool for clinical practice.[4]

Despite these promising findings, the use of HE4 and CA125 in combination is still not universally adopted in clinical practice, partly due to variability in results across different populations and the lack of standardization in assay techniques.[12] Moreover, most screening efforts focus on high-risk populations, leaving a gap in the early detection of ovarian cancer in the general population.[13] There is also a need for more comprehensive data on the cost-effectiveness of these combined biomarkers in routine screening, as well as their impact on patient outcomes, including survival rates and quality of life.[14] The objective of this study was to evaluate the diagnostic accuracy of Human Epididymis Protein 4 (HE4) and Cancer Antigen 125 (CA125), both individually and in combination, in the detection and screening of epithelial ovarian cancer.

METHODS

Study place

The study was conducted as a descriptive observational study at Dhaka Medical College Hospital.

Study duration

The study period was from January 2021 to December 2021.

Sample size

The study population consisted of three groups, Group 1 included 34 women aged 20-70 years with suspected malignant ovarian masses, Group 2 comprised 30 women with benign gynecological conditions such as ovarian cysts, endometrioma, and inflammatory tubo-ovarian masses and Group 3 consisted of 19 healthy women with no significant illness.

Sampling method

A purposive sampling method was used due to time and resource constraints as well as the impact of the COVID-19 pandemic.

Inclusion criteria

Inclusion criteria involved women aged 20 to 70 years, both pre- and post-menopausal, suspected of having ovarian cancer awaiting surgery, those with benign gynecological conditions, and healthy women with no history of significant illness.

Exclusion criteria

Exclusion criteria included pregnancy, severe concomitant diseases like chronic heart failure or liver/renal failure, inability to understand written or oral information, and ovarian malignancies other than epithelial ovarian cancer.

Data collection

Data were collected using a pre-designed data collection sheet encompassing demographics, clinical examination,

and investigation results. After obtaining informed written consent, clinical examinations and ultrasound of the abdomen were conducted, followed by the collection of venous blood samples. Serum was separated by centrifugation at 1500 x g for 10 minutes and stored at -70°C until analysis. Serum HE4 and CA125 levels were measured using the ARCHITECT HE4 and CA125 II assays based on chemiluminescence technology. The assays followed a two-step immunoassay process, with HE4 and CA125 levels quantified through a chemiluminescent reaction measured in relative light units (RLUs). Data analysis was performed using SPSS version 26.0, focusing on variables such as age, BMI, parity, menopausal status and serum levels of HE4 and CA125, among others.

Ethical approval

Ethical approval was obtained from the Institutional Review Board of Dhaka Medical College Hospital, with strict adherence to confidentiality and patient consent. Throughout the study, professional assistance was provided by experts in gynecological oncology, histopathology, and immunology, ensuring the accuracy and integrity of data collection and analysis.

RESULTS

This descriptive observational study was conducted in the Gynecological Oncology Unit, Dhaka Medical College Hospital, Dhaka to evaluate the result. According to the inclusion criteria of the study, total 83 participants were recruited. Among them 34 patients were admitted with malignant ovarian tumor (Group 1). 30 Patients were known case of different benign gynaecological diseases (Group 2) and rest 19 were healthy controls (Group 3). Table 1 shows predominant age group was 41-50 years (32.5%). Table 4 shows both CA 125 and HE4 level were sequentially higher in group 1 and group 2 (p value <0.05). Both serum HE4 and CA 125 concentrations where increased in patients with Ovarian cancer, which validated the usefulness of both markers in diagnosis of ovarian cancer.

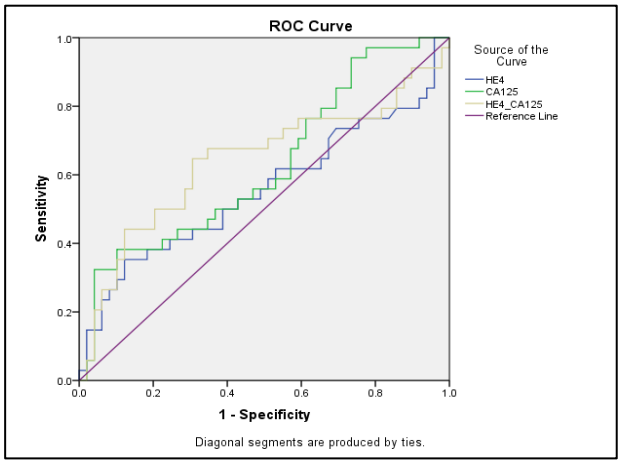

Figure 1: ROC curves for HE4, CA125, and CA125+HE4 for distinguishing ovarian cancer (n=34) from a benign gynaecological disease (n=30).

Table 5 shows the sensitivity of serum HE4 for identifying epithelial ovarian cancer with an adnexal mass was estimated to be 51.74% at specificity of 95% and 44.68% at specificity of 98%. CA125 vs CA125+HE4, p=0.0031*HE4 vs CA125+HE4, p=0.0521CA125 vs HE4, p=0.0022*. The ROC curve analysis revealed that when serum HE4 and CA125 were used in combination, it has higher discriminating power for distinguishing ovarian cancer from benign mass than if CA125 used alone (CA125 vs CA125+HE4, p=0.0031).

Table 1: Socio demographic characteristics of the study subjects (n=83).

	Age distribution			
Variables	**Group 1 n=34**	**Group 2 n=30**	**Group 3 n=19**	**Total**
Age (in years)				
20-30	11 (13.3%)	8 (9.6%)	2 (2.4%)	21 (25.3%)
31- 40	8 (9.6%)	10 (12.0%)	6 (7.2%)	24 (29.9%)
41- 50	10 (12.0%)	10 (12.0%)	7 (6.4%)	27 (32.5%)
51-60	5 (6.0%)	1 (1.2%)	4 (4.8%)	10 (12.0%)
61-70	0	1 (1.2%)	0	1 (1.2%)

Table 2: Detection of serum CA125 in each subset of the study population.

Characteristics	N (%)	CA125, U/ml (Median, Range)	P value
All	83 (100%)	36.75 (20.70-733.00)	
Disease status			
Group 1 (Ovarian cancer)	34 (40.96%)	41.30 (24.20-717.90)	
Group 2 (Benign disease)	30 (36.14%)	38.95 (20.70-731.30)	0.023*
Group 3 (Healthy control)	19 (22.89%)	29.40 (22.60-41.10)	0.041*

Data are expressed as "median (range), *p<0.05, P=0.041 with respect to the healthy controls, p=.023 with respect to benign gynaecological diseases.

Table 3: Detection of serum HE4 in each subset of the study population.

Characteristics	N (%)	HE4 pmol/l (Median, Range)	P value
All	83 (100%)	54.20 (24.40-211.40)	
Disease status			
Group 1 (Ovarian cancer)	34 (40.96%)	58.90 (29.00-177.10)	
Group 2 (Benign disease	30 (36.14%)	57.35 (24.40-127.60)	0.013*
Group 3 (Healthy control)	19 (22.89%)	51.10 (37.10-198.70)	0.004*

Data are expressed as "median (range), *p<0.05, P=0.004 with respect to the healthy controls, p=.013 with respect to benign gynaecological diseases.

Table 4: Comparison of serum HE4 and CA125 in each subset of the study population.

Parameters	Number	HE4, pmol/l (Median, Range)	CA125, U/ml (Median, Range)
All	83	55.30 (24.40-507.30)	37.30 (20.70-753.70)
Group 1	34	57.40 (30.80-507.30)	39.95 (24.20-742.10)
Group 2	30	57.35 (24.40-127.60)	42.85 (20.70-753.70)
Group 3	19	51.05 (37.10-235.80)	29.45 (22.20-44.80)
P value		0.012*	0.003*

*P value, calculated using the Wilcoxon rank-sum test, because the data was not distributed normally.

Table 5: Tumor marker accuracy and sensitivity at 90%, 95%, 98% specificity for ovarian cancer vs benign disease.

Markers	ROC-AUC (95% CI)	P value for comparison of ROC-AUC to CA125	P value for comparison of ROC-AUC to HE4	Sensitivity, %		
				At 90% specificity	At 95% specificity	At 98% specificity
All women						
CA125	55.6 (42.2-69.0)		0.0022*	49.36	41.55	37.56
HE4	62.6 (50.2-75.0)	0.0022*		55.13	51.74	44.68
CA125+HE4	64.1 (51.2-77.1)	0.0031*	0.0521	59.03	53.44	49.03

*P value, calculated using the Wilcoxon rank-sum test, because the data was not distributed normally.

DISCUSSION

This study is aimed to investigate the diagnostic value of serum CA 125 along withHE4 for detection of Epithelial ovarian cancer and other benign gynecological conditions. CA 125 is a conventional biomarker used for diagnosis of Malignant Ovarian Mass. It is widely known that serum CA 125 is significantly affected by benign gynaecological conditions. Moreover, according to previous studies, the serum CA 125 level is frequently elevated in ovarian cancer patients in advanced stages.[15]

HE 4 is a novel serological marker developed for aiding diagnosis of ovarian cancer. Developing a new biomarker to complement CA 125 in clinical practice has been focus of many research studies. A number of studies concluded that combination of serum CA 125 and HE 4 results in a higher accuracy of ovarian cancer diagnosis than if either marker is used alone.[16] Similar findings were observed in a study done by Kim YM et al. among Korean females.[17]

A study done by Montagnana M et al found the median CA 125 and HE 4 serum concentration significantly higher among EOC patients than in healthy females (both p<0.05).[18] Kim YM et al. in their study found that the median serum HE 4and CA125 concentrations were higher in patients with ovarian cancer (HE4 80.0 pmol/l, CA125 216.8 U/ml) compared with those in healthy women (HE4 35.3 pmol/l, CA125 11.5 U/ml) or patients with benign diseases (HE4 29.8 pmol/l, CA125 21.3 U/ml).[17] These differences were significantly different among groups (p<0.0001 in both). Serum HE4 was lower in the patients with benign disease compared to those seen in healthy individuals (29.8 vs. 35.3 pmol/l), whereas the opposite was true for serum CA125 (21.3 vs. 11.5 U/ml).

Among healthy individuals, both serum HE4 and CA125 concentrations in postmenopausal women differed significantly from those in premenopausal women. Serum HE4 was higher in the postmenopausal women (37.9 vs 33.3 pmol/l, p-0.001) and serum CA125 was higher in the premenopausal women (14.1 vs 9.7 pmol/l, p=0.0001).

In our study, median serum concentration of HE 4 and CA125 were found higher in ovarian cancer patients than those of healthy controls (57.40 pmol/l vs 51.05 pmol/l, 37.30U/ml vs 29.45 U/ml, p<0.05). This finding is in agreement with other studies. Compared to healthy women, patients with benign diseases showed higher CA 125 concentration and HE 4 level. But KimYM et al, found lower HE4 level in patients with benign diseases.[17]

In present study, both HE4 and CA 125 level was found higher in postmenopausal women compared to premenopausal in group 1 and group 2 (p<0.05), but in group 3 statistically significant difference between pre and postmenopausal healthy women was not observed.

A positive correlation between CA 125 and HE4 concentration (r=0.70, p <0.0001) was observed in patients with ovarian malignancy in the study done by Montagnana M et al. Similar findings was observed in our study also.[18]

Hellstrom I et al, observed that serum CA 125 is significantly affected by benign gynaecological diseases.[19] KimYM et al also found higher CA 125 level in the benign condition than that in healthy individuals.[17] Half of the cases with benign gynaecological diseases had serum CA125 concentrations higher than 35 U/ml. However, serum HE4 concentration was not increased in benign gynaecological conditions as compared to that in healthy subjects. But in our study, median value of HE 4 level in benign gynaecological conditions was found higher than that in healthy controls. The cut of value of HE 4 concentrations in healthy control of Bangladeshi population is not yet determined by well validated study.

The diagnostic accuracy of the combination of CA 125 and HE 4 was evaluated using ROMA.[20] They found that ROMA had a sensitivity of 88.7% at a specificity of 75%. In another study, when specificity was set at 93.8%, ROMA achieved a sensitivity of 87.5%.[17] In our study, in premenopausal females' sensitivity was 49.3% at specificity of 64.1% and in postmenopausal women, sensitivity was 67.6% at specificity of 59.4%.

The ROC curve analysis revealed that when serum HE 4 and CA 125 were used in combination, the combination had higher discriminating power for distinguishing ovarian cancer from a benign mass than if CA 125 used alone enhanced diagnostic performance of the combination of markers has been reported previously in this study sensitivity and specificity was more pronounced in post-menopausal group than premenopausal group. Cause of level of sensitivity and specificity of combined HE 4 and CA 125 in diagnosis of ovarian epithelial malignancy needs further study.

Biomarker expression is more strongly associated with the ovarian carcinoma subtypes than with the stage.[21] Among the epithelial ovarian carcinoma, the predominant histological type was serous tumours followed by mucinous tumors in this study. Further investigation is necessary to explain the relationship between serum HE 4 concentrations and the histological types of ovarian cancer.

The sample size of participants was relatively small, which may have a lower statistical power. Equal sample size could not be possible to collect due to Covid 19 pandemic. The small sample size may account for the discrepancies with other studies which have been observed during the comparison of various parameters. Duration of study period was relatively short. The study subjects were selected from single hospital, so that the study result may not represent the exact picture of the general population of the country.

CONCLUSION

Serum CA125 and HE4 level, both are increased in Epithelial ovarian carcinoma compared to benign gynaecological diseases. Diagnostic accuracy of HE4 for detecting EOC increases- significantly when it is used in combination with CA125. HE4 improves the utility of CA125 as a tumour marker in EOC, and using both markers simultaneously increases the tumour marker sensitivity. The use of this combination might enable to improve detection of Epithelial ovarian cancer as compared with use of either marker alone for in differentiating of benign from malignant ovarian lesions.

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