Section: Pathology

Original Research Article

HISTOPATHOLOGICAL SPECTRUM OF OVARIAN TUMOURS WITH DIAGNOSTIC ACCURACY OF SERUM CA125 AND HE4

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Abstract

Background: Ovarian cancers are the fifth leading cause of cancer mortality among females worldwide. Early detection of cancer greatly increases the chances for a successful treatment. Aim of the present study was to evaluate the diagnostic accuracy of serum cancer antigen 125 (CA125) and human epididymis protein 4 (HE4) in ovarian tumours. **Materials and Methods:** Women between the ages of 22 to 70 years participated in this prospective observational study, and their specimens were collected to determine the levels of HE-4 and CA-125 in the serum using chemiluminescence immunoassay (CLIA). For the histological analysis, ovarian tissues were collected in 10% formalin, processed using standard procedures, and sections were stained with hematoxylin and eosin (H&E). Version 24 of SPSS software was utilized to analyze the results obtained. **Result:** A total of 141 cases of ovarian tumours samples were assessed histopathologically along with serum CA125 and HE4 levels. Among 141 cases of ovarian tumour, 116 (82.26%) were classified as benign, 8 (5.67%) as borderline and 17 (12.05%) as malignant. Surface epithelial tumours were the most prevalent in the present study, comprising 95 cases (67.4%), followed by germ cell tumours with 34 cases (24.1%). Sensitivity of serum CA125 (88.2%) was found higher than serum HE4 (76.5%) for malignant ovarian tumours. Specificity of CA125 was found 56.9% and HE4 was 95.7%. Accuracy associated with malignant ovarian tumours for serum HE4 (93.2%) was found significantly higher than accuracy of serum CA125 (60.9%). **Conclusion:** Serum HE4 complements the utility of CA125 as a tumour marker in ovarian cancer, and the concurrent use of both markers enhances the sensitivity, specificity and diagnostic accuracy of tumour markers for detection of ovarian cancer. Compared to using either marker alone, employing the combination may facilitate improved detection of ovarian cancer.

INTRODUCTION

Ovarian cancers are the fifth leading cause of cancer mortality among females worldwide.[1] Although it constitutes only 2.5% of all female cancer cases in the USA, it is responsible for 5% of cancer-related deaths, underscoring its significant impact on mortality rates.[2] In the context of India, the ovary ranks as the third most common site for cancer in female, following the cervix and breast.[3] The World Health Organization (WHO) classification of tumours of ovary classified ovarian tumours according to the most probable tissue of origin; surface epithelial, germ cell, sex cord-stromal, metastasis, and miscellaneous.[4] Surface epithelial tumours are further classified by cell type (serous, mucinous, endometroid etc) and atypical (benign, borderline) or malignant.

Ovarian cancer often presents no symptoms in its early stages, making early diagnosis challenging. Though risk factors are evident but most ovarian cancer patients (60%) are diagnosed with distant-stage disease, for which 5-year survival is just 29%.[5] Because they are often diagnosed too late, ovarian malignancies have the worst prognosis of any gynaecological cancers.[6] Due to asymptomatic nature, inaccessible location, and the sparse application of several novel procedures, such as cytology and biopsy, make early identification challenging. Therefore, ovarian cancer become the

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promising research area. Non-invasive techniques for early detection of ovarian cancer comprise biochemical marker analysis and radiological imaging. The prognosis and early diagnosis of gynaecological cancers is significantly influenced by tumour markers. In individuals with a pelvic mass, the presence of cancer has been predicted by the use of serum CA125. Nevertheless, CA125's specificity in tissues and organs is poor; concurrently, it also manifests in varying degrees of physiological condition (e.g., pregnancy) and non-malignant diseases. As a result, the use of CA125 as a singular measure for malignancy prediction is uncommon.[7] Serum HE4 is another tumour marker that is expressed at low levels in benign tumours, normal tissues, and neighbouring tissues but at high levels in ovarian and endometrial cancer. HE4 demonstrated the higher specificity of any tumour marker when used alone to identify ovarian cancer.[8] Multiple tumour markers can be detected together, which has been demonstrated to increase diagnostic sensitivity, specificity and accuracy while lowering the rate of misdiagnosis.[9]

MATERIALS AND METHODS

Study Design: This prospective observational study was conducted at department of Pathology of a tertiary care teaching hospital in northern India from January 2021 to December 2023. A total of 141 ovarian tumour cases were included in the study. Written informed consent was given by each study participant. Ethical approval by institutional ethics committee was obtained for the study protocol. We registered 141 participants whose specimens were gathered to assess CA-125 and HE-4 levels for examining ovarian lesions. Pre- and post-operative blood samples (5 ml) were taken in a plain vial. Following that, the blood samples were quickly centrifuged for 10 minutes at about 3000 rpm. The serum supernatant was extracted and sent to measure serum CA125 and HE4 levels. Following surgery, excised ovaries were labelled appropriately, kept in formalin, and sent to the pathology department for histopathological analysis.

Laboratory Techniques and Procedure:

1-Serum CA125 and HE4 determination: The serum levels of CA125 and HE4 were estimated by chemiluminescence immunoassay (CLIA) using diagnostic kits from Roche Diagnostics on cobas e 411. We have taken 35 U/mL and 150 pmol/L as upper limits of normality for serum CA125 and HE4 respectively.

2-Histopathological diagnosis: The tissues were obtained in 10% formalin and processed as per standard techniques for paraffin embedding. Sections were stained with Haematoxylin & Eosin for the histological examination of the tissue.[10] The World Health Organization's categorization system for ovarian cancers was used to classify the tumours. [4]

Statistical Analysis: SPSS software version 24 was used for the analysis of results. Pearson's chi square test was used to compare the categorical variables. Statistical significance was defined as p-value <0.05.

RESULTS

Over a span of two years, a total of 141 cases of ovarian tumours were detected. Among these, 116 (82.26%) were classified as benign, 8 (5.67%) as borderline and 17 (12.05%) as malignant. Age ranged from 22 year to 70 years. Surface epithelial tumours were the most prevalent in the present study, comprising 95 cases (67.4%), followed by germ cell tumours with 34 cases (24.1%), sex cord stromal cell tumours with 10 cases (7.1%), and secondary (metastatic) tumours with 2 cases (1.4%) [Figure1, Table 1]. The most prevalent ovarian tumour was serous tumour, comprising 44% (62 cases) of all ovarian neoplasms. Mature cystic teratoma ranked as the second most common tumour 18% (25 cases).

The results of serum levels of CA125 and HE4 measurements in the different ovarian tumours are shown in Table 2. Higher serum CA 125 levels were found in 88% (15/17) of malignant ovarian tumours, 63% (5/8) of borderline and 43% (50/116) of benign tumours. In comparison to benign ovarian tumours there was statistically significant elevation of serum CA 125 levels in malignant ovarian tumours ($\chi 2$ 12.09, p-value 0.0005). Although serum CA125 levels were elevated in borderline ovarian tumours, however that was statistically not significant ($\chi 2$ 1.14, p-value 0.28). Higher serum HE4 levels were found in 76% (13/17) of malignant ovarian tumours, 25% (2/8) of borderline and 4% (5/116) of benign tumours. In comparison to benign ovarian tumours there was statistically significant elevation of serum HE4 levels in malignant ($\chi 2$ 65.97, p-value 0.00001) and borderline ovarian tumours ($\chi 2$ 6.01, p-value 0.014).

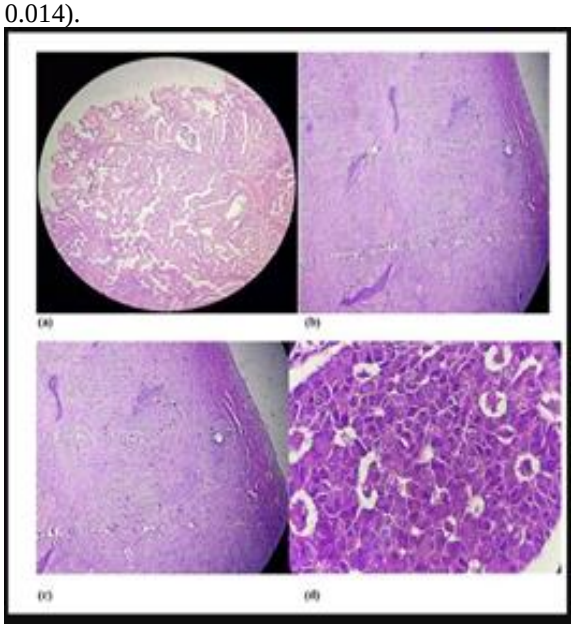

Figure 1: Photomicrographs of Haematoxylin and Eosin-stained section (a) Borderline Serous Tumour,

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(b) Benign Brenner Tumour, (c) Mixed Germ Cell Tumour and (d) Granulosa Cell Tumour. (Magnification x 10)

Table 3 shows the serum CA125 and HE4 diagnostic accuracy in the differential diagnosis of malignant ovarian tumours with benign tumours. Sensitivity associated with malignant ovarian tumours showed by serum CA125 (88.2%) was higher than serum HE4 (76.5%). Specificity of serum CA125 was 56.9% and HE4 was 95.7%. Specificity of HE4 associated with malignant ovarian tumour was significantly higher than specificity of serum CA125. Positive predictive value (PPV) was higher with serum HE4 levels and negative predictive value (NPV) were similar for both marker [Table 3]. Accuracy associated with malignant ovarian tumors for serum HE4 (93.2%) was significantly higher than accuracy of serum CA125 (60.9%).

Table 1: Histopathological distribution of Ovarian Tumours

S. No.	Histopathological Characteristic	Number	Percentage (%)
1	Surface Epithelial Tumours	95	67.4
1a.	Serous Tumours	62	44.0
1b.	Mucinous Tumours	24	17.0
1c.	Brenner Tumours	02	1.4
1d.	Endometrioid Tumours	01	0.7
1e.	Carcinosarcoma	01	0.7
1f.	Mixed epithelial tumours	05	3.5
2	Sex Cord Stromal Tumours	10	7.1
2a.	Fibroma	04	2.8
2b.	Thecoma	01	0.7
2c.	Sclerosing stromal tumours	02	1.4
2d.	Granulosa cell tumours	03	2.1
3	Germ Cell Tumours	34	24.1
3a.	Mature Cystic Teratoma	25	17.7
3b	Immature Teratoma	03	2.1
3c.	Dysgerminoma	01	0.7
3d	Yolk Sac tumour	01	0.7
3e.	Struma Ovarii	02	1.4
3f.	Mixed Germ Cell Tumour	02	1.4
4	Secondaries (Metastatic) tumours	02	1.4
5.	Total	141	100

Table 2: Demography and Serum levels of CA125 and HE4

S. No.	Variables		Groups		
			Benign (N=116) (a)	Borderline (N=8) (b)	Malignant (N=17) (c)
1.	Age (Year)	Mean ± SD	40.3±7.9	45.5±8.1	59.2±10.3
		Median	41	47	61
2.	CA 125 Median (U/mL)		16.4	50.3	201.0
3.	HE4 Median (pmol/L)		63.0	79.0	240.5
4.	CA 125	>35 U/mL	50 (43%)	5 (63%)	15 (88%)
		<35U/mL	66 (57%)	3 (37%)	2 (12%)
		p-value		.28 (a vs b)	.0005 (a vs c)
5.	HE4	>150pmol/L	5 (4%)	2 (25%)	13 (76%)
		<150pmol/L	111 (96%)	6 (75%)	4 (24%)
		p-value		.014 (a vs b)	.00001 (a vs c)

Table 3: Evaluation of serum CA125 and HE4 accuracy in the ovarian tumours

S. No.		CA125	HE4
1.	Sensitivity	88.2%	76.5%
2.	Specificity	56.9%	95.7%
3.	Positive Predictive Value	23.1%	72.2%
4.	Negative Predictive Value	97.1%	96.5%
5.	Accuracy	60.9%	93.2%

DISCUSSION

In present study a total of 141 cases of ovarian tumours samples were assessed histopathologically along with serum CA125 and HE4 levels. There were 116 cases (82.3%) of benign tumours, 8 cases (5.7%) of borderline tumours, and 17 cases (12%) of malignant tumours. Couto et al. and Pilli et al. also reported similar results, with a higher proportion of benign tumours compared to malignant tumours.[11,12] This study represented 44.8% of serous tumours, which is comparable to results from studies by Misra et al. and Maheshwari et al., who found 49% and 46.01%, respectively.[10,13] Granulosa cell tumours were detected in 2.1% of cases, a percentage that closely aligns with the findings of the research conducted by Ramachandra et al.[14,15] The most common germ cell tumour, mature cystic teratoma, accounted for 17.7% of all neoplastic lesions. This is similar to the results of Gupta et al. (23.13%) and Tyagi et al. (18.46%).[15,16]

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In the current study, it was observed that sensitivity, specificity, PPV, NPV and accuracy of serum CA 125 in malignant ovarian tumours were 88.2%, 56.9%, 23.1%, 97.10% and 60.9% respectively. Similar to present study many studies reported PPV in the diagnosis of ovarian cancer in asymptomatic women ranging from 10% to 21%.[17-21] Although sensitivity of serum CA125 is good enough to use it as screening marker, however the major drawback of using serum CA 125 as an initial step in such a screening strategy is its low specificity and PPV. Specificity is a significant issue for serum CA 125 as abnormal serum levels of CA 125 may be found in benign ovarian tumours and malignant diseases other than ovarian malignancy.[17-21] Despite these issues, CA125 is used as a prognostic factor in the early diagnosis of recurrence or to assess response to treatment.[20-22]

In the current study, it was observed that sensitivity, specificity, PPV, NPV and accuracy of serum HE4 in malignant ovarian tumours were 76.5%, 95.7%, 72.2%, 96.5% and 93.2% respectively. These findings are consistent with the results reported by Hamed et al. .[23] HE4 is a tumour marker with higher accuracy than CA125. However, the main problem to use HE4 alone in the differential diagnosis of ovarian tumours is its low sensitivity (76.5%). The sensitivity of CA 125 (88.2%) is higher than HE4 (76.5%) and specificity of CA125 (56.9%) is less than that of HE4 (95.7%). HE4 is reported a higher specificity than CA 125 in benign and malignant ovarian tumours. Many studies on serum HE4 have been published indicating that serum HE4 sensitivity and specificity in ovarian tumours are better than CA125 and both the markers are complementary to each other. [22,24] Results of present study confirm the previous studies and indicated that the use of HE4 could be important in the differential diagnosis of ovarian tumours.

Results obtained in present study can be summarised as in our population, surface epithelial tumours were the most prevalent, comprising 95 cases (67.4%), followed by germ cell tumours with 34 cases (24.1%), sex cord stromal cell tumours with 10 cases (7.1%), and secondary (metastatic) tumours with 2 cases (1.4%). In this study we found sensitivity of CA125 is better than HE4 and specificity & accuracy of HE4 is better than CA125. Therefore, CA 125 and HE4 should be used as complementary tests to improve the diagnosis of ovarian cancer.

CONCLUSION

Concurrent use of serum CA125 and serum HE4 as a tumour marker in ovarian cancer cases, improves the sensitivity, specificity and diagnostic accuracy of tumour markers for the detection of ovarian cancer. Compared to using either marker alone, employing the combination may facilitate improved detection of ovarian cancer.

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