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DIAGNOSTIC VALUE OF BIOCHEMICAL PREDICTIVE INDEX IN OVARIAN MALIGNANCY

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Abstract : The aim of the study was to determine if diagnostic performance of CA-125 in ovarian malignancy can be improved by considering age of the patient. The study was a retrospective analysis of the medical records of 306 patients who underwent a CA-125 measurement in our Institute. Of the 306 patients, 31% had malignant ovarian diseases, 45% had benign ovarian diseases, 6% had non-ovarian gynaecological diseases and 18% had non-gynaecological diseases. A positive association was found between age and CA-125 levels in patients with ovarian diseases. Patients were divided into three age groups of 18–37 yrs, 38–56 yrs and 57–74 yrs and were given age scores as 1, 2 and 3 respectively. Biochemical predictive index (BPI) was calculated by multiplying age score and CA-125 value. Among the age groups ranging 38–56 yrs and 57–74 yrs, the CA-125 and BPI values were significantly higher in malignant ovarian diseases compared to benign ovarian diseases. ROC curve analysis revealed a cut-off of 86 for the BPI with the sensitivity, specificity, positive predictive value and negative predictive value as 58%, 78%, 56% and 80% respectively. BPI had better specificity and negative predictive value compared to CA-125, can be used in the screening of ovarian pathology.

Key words : biochemical predictive index CA-125 age score ovarian diseases screening

INTRODUCTION

Ovarian cancer has become one of the commonest malignancy affecting Indian women. A steady increase has been observed in the incidence of the ovarian cancer in several registries (1). Ovarian cancer is the most frequent cause of death from gynecological cancer and the fourth most frequent cause of death from cancer in women in developed countries (2).

Almost 90% of patients are diagnosed with metastatic disease in the pelvis or abdomen, for whom the 5-year survival rates are less than 30%. But, detection at stage I

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ovarian cancer confined to the ovaries have a 5-year survival rate in excess of 90%. Ovarian cancer is characterised by late presentation, poor response to current treatment and a poor prognosis. But, the availability of ultrasonography and CA-125 has made the screening for early diagnosis of ovarian cancer quite effective (3). In recent studies, algorithm based on combination of serum biomarkers has been carried for risk assessment of Ovarian Malignancy. The algorithm included CA125 and human epididymal protein 4 and were found to predict the malignancy with higher sensitivity (4, 5).

CA-125 is a high molecular weight glycoprotein, which is expressed by a most of the epithelial ovarian cancers (6). Its detection using murine OC125 monoclonal antibody was developed by Bast *et al* in 1981. Immunofluorescence was used to detect the presence of CA-125, which had no reaction with non-malignant tissues but reacted with few non-ovarian neoplasms. CA-125 can assist in differentiating the different types of ovarian tumors, as it is increased mainly in the epithelial type (7). CA-125 is also helpful as a prognostic marker. It aids in monitoring response to treatment in patients with epithelial ovarian cancer (8).

In an effort to increase the specificity, combinations of CA-125 with various markers and ultrasonography have been evaluated (2). The best individual performance is found in CA-125 levels at a cut-off of 35 U/ml, (sensitivity of 78% and specificity of 75%), followed by ultrasound score (sensitivity of 75% and specificity of 73%) and menopausal status (sensitivity of 73% and specificity of 69%) (9). Three criteria were combined in a risk of malignancy index (RMI) which was calculated from serum CA 125 level, the ultrasound scan and the menopausal status. RMI is found to be more effective in delineating the malignant and benign ovarian diseases, as it combines the various markers or risk factors (10).

The aim of the study was to determine whether by adding age of the patient as a factor along with CA-125 to improve its diagnostic performance. The objectives were to investigate the benign and malignant cases of ovarian disease who underwent CA-125 measurements and to determine the sensitivity, specificity and predictive values of Biochemical Predictive Index, calculated by multiplying age score and CA-125, in the diagnosis of the above said patients.

MATERIALS AND METHODS

The study was a retrospective analysis of the medical records of 306 patients who underwent a CA-125 measurement between March 2010 to July 2011 in our Institute, which was approved by the Institute Scientific Committee and the Ethics Committee. The patients were categorized based on the histopathlogical findings. Patients with ovarian diseases who underwent CA-125 measurements were categorized into three groups - ovarian diseases, non-ovarian gynaecological diseases and non-gynaecological diseases. The CA-125 assays performed in the clinical chemistry laboratory were considered, from which an abnormal result was taken to be 35 U/litre or above. CA-125 assay was done by two-site sandwich immunoassay in chemiluminescence system, Advia Centaur CP, Japan. The assay

uses two monoclonal mouse antibodies specific for CA 125. The first antibody is directed toward the M11 antigenic domain, and is labeled with acridinium ester. The second antibody is directedtoward the OC 125 antigenic domain and is labeled with fluorescein. The immunocomplex formed with CA 125 is captured with monoclonal mouse anti-fluorescein antibody coupled to paramagnetic particles in the Solid Phase. A direct relationship exists between the amount of CA 125 present in the patient sample and the amount of relative light units (RLUs) detected by the system. The sensitivity is 93.5% and CV% is 2.3.

Statistical analysis

Statistical analysis was done using SPSS software. Kolmogrov Smirnov test was used to analyze the normality of distribution. One way ANOVA with post hoc Tukey analysis was done for comparison between groups. Mann Whitney test was used to compare the levels of CA-125 and Biochemical Predictive Index (BPI) in malignant and benign ovarian diseases. Receiver operating characteristic (ROC) curve analysis was done to find the diagnostic significance of CA-125 and BPI. Sensitivity was defined as the proportion of patients with ovarian cancer correctly identified by CA-125 or BPI, and specificity as the proportion of patients without ovarian cancer correctly identified by CA-125 or BPI. Youden index was used as the summary measure of ROC curve. It helped to measure the effectiveness of CA-125 / BPI and enabled the selection of an optimal threshold value for the marker.

RESULTS

Of the 306 patients, 31% had malignant ovarian diseases, 45% had benign ovarian diseases, 6% had non-ovarian gynaecological diseases and 18% had non-gynaecological diseases. The age of patients with malignant ovarian diseases was significantly higher than that of benign ovarian diseases ($P<0.001$). The CA – 125 values were significantly higher in malignant ovarian diseases compared to the benign ovarian diseases ($P<0.001$) and non-ovarian gynaecological diseases ($P<0.001$) (Table I).

A positive association was found between age and CA-125 levels in patients with ovarian diseases.($r = 0.19$, $p = 0.005$) (Fig. 1) Based on this observation, patients were divided into three age groups of 18–37 yrs, 38–56 yrs and 57–74 yrs. Age score was given based on the age groups, 1 for 18–37 yrs, 2

TABLE I: Values of CA-125 and biochemical predictive in the four groups.

Type of disease	*% (N)*	*Age (years)*	*CA-125 (U/ml)*	*Biochemical predictive index*
Malignant ovarian diseases	31 (95)	47±13	205±242	451±559
Benign ovarian diseases	45 (138)	41±13**	59±129**	102±248**
Non-ovarian gynaecological diseases	6 (19)	45±13	67±126**	118±247**
Non-gynaecological diseases	18 (54)	47±12	125±221	278±532

N represents the number of patients in each group. Data is expressed as Mean ± S.D.**$p < 0.001$. p values represent comparison with the malignant ovarian diseases group. Analysis was done by One way ANOVA with post-hoc Tukey analysis.

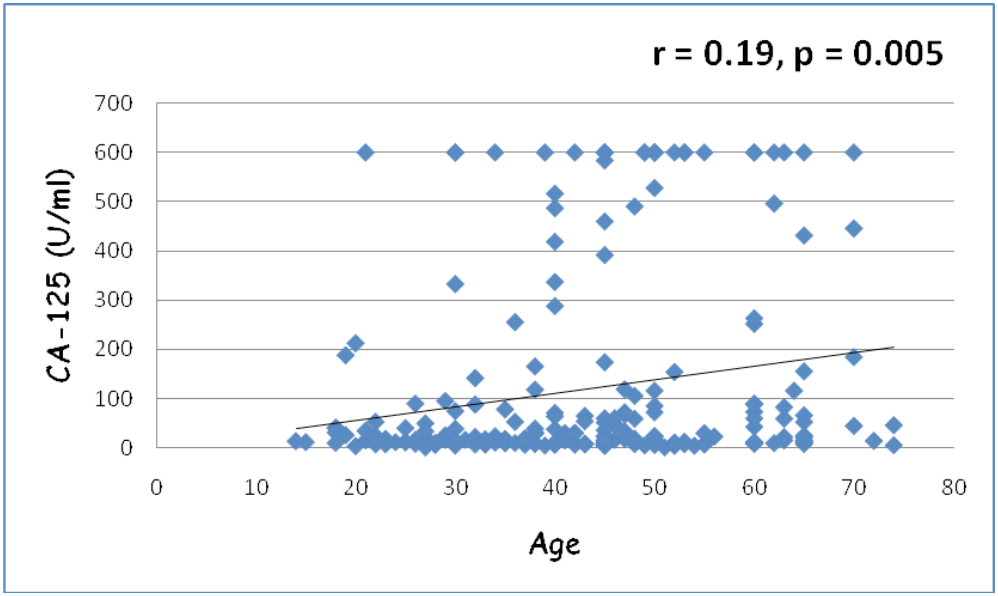

Fig. 1: Correlation between Age and CA-125 in patients with ovarian diseases.

for 38–56 yrs and 3 for 57–74 yrs. Biochemical predictive index was defined as age score multiplied by CA-125 value. The biochemical predictive index values were significantly higher in malignant ovarian diseases compared to the benign ovarian diseases (P<0.001) and non- ovarian gynaecological diseases (P<0.001), similar to CA-125. (Table I) Among the age groups ranging 38 – 56 yrs and 57–74 yrs, the CA-125 and BPI values were significantly higher in malignant ovarian diseases compared to benign ovarian diseases (Table II).

At a cut-off of 35 U/ml of CA-125, the sensitivity, specificity, positive predictive value and negative predictive value were 62%, 72%, 60% and 73% respectively. ROC curve analysis showed a cut-off of 38 U/ml for CA-125, which has sensitivity, specificity, positive predictive value and negative predictive value of 63%, 74%, 62% and 74% respectively. When the cut-off is raised from 35 U/ml to 38 U/ml, there is an increase in sensitivity, specificity and predictive values. ROC curve analysis revealed a cut-off of 86 for the Biochemical Predictive Index with

TABLE II: CA-125 values in different age groups.

Age group	*Age score*	*Malignant Ovarian Diseases*			*Benign Ovarian Diseases*		
		No	*CA-125*	*BPI*	*No*	*CA-125*	*BPI*
18–37	1	19	100±183	100±183	53	58±126	58±126
38–56	2	52	230±257**	459±514**	66	59±129	117±257
57–74	3	25	233±233*	700±699*	16	62±146	187±437

Data is expressed as Mean±S.D. **P<0.001, *P<0.01. Analysis was done by Mann Whitney U test.

TABLE III : Statistical differences of BPI and CA-125 using ROC analysis for differentiating benign and malignant ovarian disease.

	Cut-off	*Sensitivity*	*Specificity*	*PPV*	*NPV*	*Youden index*	*AUC±SEM*	*95% CI*	*P*
BPI	85.50	58	78	56	80	0.37	0.695±0.035	0.627–0.764	<0.001
CA-125	37.85	63	74	62	74	0.37	0.678±0.036	0.609–0.748	<0.001

AUC±SEM : Area under the curve±Standard Error of Mean
CI:Confidence interval
PPV:Positive predictive value
NPV:Negative predictive value

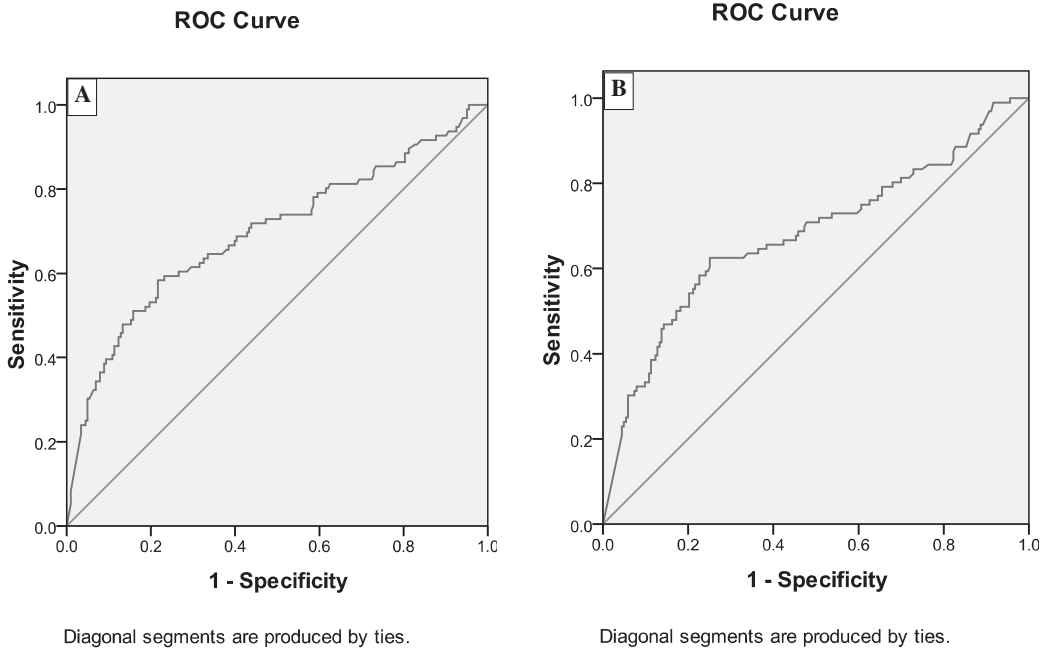

Fig. 2 : ROC curve characteristics of (A) Biochemical Predictive Index and (B) CA-125 for malignant ovarian disease.

the sensitivity, specificity, positive predictive value and negative predictive value as 58%, 78%, 56% and 80% respectively. Biochemical Predictive Index has better specificity and negative predictive value compared to CA-125. (Table III; Fig. 2).

DISCUSSION

Though CA-125 is a very good marker for epithelial ovarian cancer, it has a high false positivity as it is increased in non-ovarian neoplasms like carcinoma of pancreas, liver, etc and also in benign diseases like benign ovarian cysts, ectopic pregnancy etc. Since ovarian cancer is predominantly a disease occurring in the older age, considering the age as in 'risk of malignancy index' is useful (2). Therefore, specificity of CA-125 is poor, which may be due to the common cut-off of 35 U/ml which is used in all age groups. Even in the 'risk

of malignancy index', age is not directly taken into consideration. Instead only the menopausal status has been incorporated. Maybe it is time for us to change this concept and study deeply about the variations in CA-125 values in different age groups. We have made an effort in this direction, which led us to framing a new index incorporating the CA-125 value and age.

In the present study, compared to the benign ovarian diseases and non-ovarian gynaecological diseases, malignant ovarian diseases have a significantly higher CA-125 levels. This reinstates the role of CA-125 as a marker of malignant ovarian diseases, inspite of its high false-positive rate.

Age has a role in the ovarian cancer, with post menopausal women having more predispositions (2). Two-third of the women with ovarian cancer are found to be above 55 yrs (11). Though the results of this study didn't exactly follow this distribution, we found a significant positive correlation between age and CA-125, which led us to investigate the CA-125 levels in different age groups. We defined a Biochemical Predictive Index, which can be obtained by multiplying the age score with the CA-125 value.

There wasn't any significant difference in CA-125 and BPI levels between benign and malignant ovarian diseases in the age group 18–37 yrs. Maybe this can be attributed to an improper matching of sample size in this age group, unlike the other two age groups. But, one notable fact is that the sample size is comparable in the other two age groups.

The CA-125 and BPI levels are significantly higher in the malignant ovarian diseases than in the benign ovarian diseases in the age groups 38–56 yrs and 57–74 yrs. Among the two age groups, the 38–56 age group shows more significant difference. Maybe the CA-125 and BPI levels can help in differentiating the benign and malignant ovarian diseases in age group 38–56 yrs better than in other age groups.

Most ovarian cancers occur after menopause when the ovaries have no physiological role. Since abnormal ovarian function causes no symptoms after menopause and due to the anatomical location of the ovaries deep in the pelvis, ovarian cancers become symptomatic only after they reach a large size or after metastasis (3).

The initiation of epithelial carcinogenesis due to ovulation may start from 20-30 yrs of age, tumor promotion may occur after age 30 either due to ovulation or due to the high levels of hormones associated with it. These events may culminate in the development of a tumor which may become clinically apparent after 30 (12). A relationship has been found between ovulation and the mutant p53 overexpression in epithelial ovarian cancer (13).

ROC analysis revealed 86 as cut-off for BPI, which has a better specificity and negative predicitive value than CA-125 alone. This index will be helpful in differentiating benign and malignant ovarian diseases, as it has more specificity. Even the marginal improvement in specificity is crucial, as it can eliminate the false positivity which might be quite alarming for the patient.

The limitation of our study was unavailability of data on ultrasound and menopausal state of the study subjects in their case records. These details would have helped us in calculating RMI score and to compute its association with Biochemical Predictive Index. We consider this as major limitation of our study.

Hence we conclude that Biochemical Predictive Index, which has a better specificity and negative predictive value than CA-125, can be used in the screening of ovarian pathology. Further large-scale studies on this regard will provide a cost-effective biochemical screening strategy.

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REFERENCES

1. Murthy NS, Shalini S, Suman G, Pruthvish S, Mathew A. Changing Trends in Incidence of Ovarian Cancer - the Indian Scenario. *Asian Pac J Cancer Prev* 2009; 10: 1025–1030.
2. Jacobs I, Menon U. Progress and challenges in screening for early detection of ovarian cancer. *Mol Cell Proteomics* 2004; 3: 355–366.
3. Jacobs I, Davies AP, Bridges J, Stabile I, Fay T, Lower A, et al. Prevalence screening for ovarian cancer in postmenopausal women by CA 125 measurement and ultrasonography. *BMJ* 1993; 306: 1030–1034.
4. Moore RG, Miller MC, Disilvestro P, Landrum LM, Gajewski W, Ball JJ et al. Evaluation of the diagnostic accuracy of the risk of ovarian malignancy algorithm in women with a pelvic mass. *Obstet Gynecol* 2011; 118: 280–288.
5. Kadija S, Stefanovic A, Jeremic K, Radojevic MM, Nikolic L, Markovic I et al. The utility of human epididymal protein 4, cancer antigen 125, and risk for malignancy algorithm in ovarian cancer and endometriosis. *Int J Gynecol Cancer* 2012; 22: 238–244.
6. Moss EL, Hollingworth J and Reynolds TM. The role of CA-125 in clinical practice. *J Clin Pathol* 2005; 58: 308–312.
7. Bast RC Jr, Feeney M, Lazarus H, Nadler LM, Colvin RB, Knapp RC. Reactivity of a monoclonal antibody with human ovarian carcinoma. *J Clin Invest* 1981; 68: 1331–1337.
8. Bast RC Jr, Klug TL, St John E, Jenison E, Niloff JM, Lazarus H et al. A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. *N Engl J Med* 1983; 309: 883–887.
9. Torres JC, Derchain SF, Faundes A, Gontijo RC, Martinez EZ, Andrade LA. Risk-of-Malignancy index in preoperative evaluation of clinically restricted ovarian cancer. *Sao Paulo Med J* 2002; 12: 72–76.
10. Jacobs I, Oram D, Fairbanks J, Turner J, Frost C, Grudzinskas JG. A risk of malignancy index incorporating CA 125, ultrasound and menopausal status for the accurate preoperative diagnosis of ovarian cancer. *Br J Obstet Gynaecol* 1990; 97: 922–929.
11. Clarker-Pearson DL. Clinical practice. Screening for ovarian cancer. *N Eng J Med* 2009; 361: 170–177.
12. Purdie DM, Bain CJ, Siskind V, Webb PM, Green AC. Ovulation and risk of epithelial ovarian cancer. *Int J Cancer* 2003; 104: 228–232.
13. Schildkraut JM, Bastos E, Berchuck A. Relationship between lifetime ovulatory cycles and overexpression of mutant p53 in epithelial ovarian cancer. *J Natl Cancer Inst* 1997; 89: 932–928.