

Correlation of Cytohistological Expression and Serum Level of Ca125 in Ovarian Neoplasm

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ABSTRACT

Context or Background: CA125 is a biomarker that has potential utility across the spectrum: risk assessment, early detection, diagnosis, prognosis, monitoring and therapy.

Aims and Objectives: This study was conducted to establish the validity and reliability of correlation of CA125 serum level with immunochemistry expression in imprint cytology and tissues for diagnostic purpose.

Materials and Methods: A prospective study was done on 50 cases of clinically and radiologically diagnosed ovarian tumor. Imprint smears were made intraoperatively from fresh samples and stained with M.G.G. stain for air dried smears and Papanicolaou stain for alcohol fixed smears. Stained smear was assessed and compared with subsequent histopathology report.

Preoperative blood samples were obtained from all patients and sent for the assay of serum CA125 levels. Analysis of CA125 immunochemistry expression in imprint cytology and tissue was done and correlated with preoperative serum blood CA125 levels.

Results: Significant positive correlation was found between elevated serum CA125 levels and cytohistological expression of CA125. Overall sensitivity was 100%, specificity was 86%, positive predictive value was 74% and negative predictive value 100%. Diagnostic accuracy was 90% with high statistical significance ($p < 0.001$).

Conclusion: We considered 35 U/mL as the cut-off value when evaluating serum CA125 ovarian cancer. Patients with high serum levels show good cytohistological expression.

Keywords: Ovarian tumor, Imprint cytology, CA125

INTRODUCTION

Ovarian neoplasms are a heterogeneous group of benign and malignant tumors of epithelial, stromal and germ cell origin. Most of the ovarian carcinomas are usually detected when they have spread beyond the ovary. Rapid intraoperative imprint cytological diagnosis of the nature of the ovarian tumor in young woman avoids unnecessary removal of the contra lateral ovary and helps preservation of fertility. Bast and colleagues in 1981 first described 200 kd glycoprotein recognised by murine monoclonal antibody OC-125 as marker for epithelial malignancies [1]. A raised level of antigen is detected in ovarian tumors of serous, endometrioid, clear cell type, mucinous carcinoma (endocervical variety) [2]. Seventy nine percent of all ovarian cancers are positive for CA125 [3]. It is elevated in nonovarian carcinoma, including fallopian tube, endometrium, endocervix, pancreas, colon, stomach, gall bladder, kidney, mesothelial lining of pleura, pericardium, apocrine sweat gland etc [4]. It is also elevated in follicular phase of menstrual cycle, those with benign conditions such as cirrhosis, hepatitis, endometriosis, Pericarditis and early pregnancy [5,6]. We had taken preoperative serum values of CA125 of patients with ovarian tumors and compared with CA125 expression in imprint cytology and tissues. The normal value of serum CA125 is considered to be less than 35 U/mL [1,7].

MATERIALS AND METHOD

In this prospective study, materials from 50 cases of ovarian tumors, undergoing surgery was taken. After taking valid consent from the patient, a detailed history was taken and clinical examination was done. Preoperative serum levels of CA125 were taken from all patients. Intraoperative imprint smears were taken from fresh ovarian tissue on plain and poly-L-lysine coated slides. Two slides were air dried and smears stained with May-Grunwald-Giemsa (M.G.G.). Two slides were fixed in 95% ethyl alcohol and stained

with Papanicolaou stain. Stained slides were examined under light Pathology Section microscope and findings were reported. Smears on poly-L-lysine coated slides were wrapped in an aluminium foil and stored in (-) 20°C for CA125 Immunocytochemistry. The resected masses were sent for histopathological examination. After proper processing, histopathological reports were given. Results of imprint cytology and histopathology compared. The corresponding blocks were cut into 3-5µm thick sections on poly-L-lysine coated slides. Immunohistochemistry were performed on these slides. Method used was Heat Induced Epitope Retrieval (HIER) techniques using Cell Marques Trilogy™ in conjunction with a pressure cooker. Primary antibody used was Anti CA125 mouse monoclonal antibody diluted in phosphate buffer saline pH 7.4, with protein base and preserved with Sodium Azide (Cell Marque). Positive staining was observed as brown cytoplasmic and membranous staining. CA125 immunostaining were also done on imprint smear. Serum levels were compared with positive expression of CA125 in cytology and tissues. The immunostaining was scored semi-quantitatively by means of a modified histoscore method, taking into account the staining intensity and the percentage of positive tumor cells. Briefly, for each stained section the estimated percentage of tumour cells was multiplied by the intensity value and the result, named immunohistochemical score (IS), was quantified as 0, 1+, 2+ and 3+: 0 = negative; 1+ = slightly positive immunostaining in 5-30% of isolated tumor cells; 2+ = moderate positive immunostaining in 30-80% of tumor cells; and 3+ = intense positive immunostaining in more than 80% of tumor cells [8].

STATISTICAL ANALYSIS

Histopathological diagnosis of the tumors were taken as the 'gold standard' and the other procedures were statistically analysed using Chi-Square test, Mathews correlation test, Sensitivity, Specificity, Positive predictive value, Negative predictive value and Diagnostic accuracy etc.

RESULTS

Fifty cases were included in this study aged from 5 to 62 years. This includes 32(64%) epithelial tumors and 18(36%) nonepithelial tumor cases. Out of the total cases 16(32%) were benign, 1(2%) case borderline and 33(66%) cases were malignant. Preoperative serum CA125 level was elevated in 19(38%) cases. By histopathological examination 19 cases are further categorised into benign (2 cases) and malignant (17 cases). According to histopathological reports, malignant ovarian tumors having elevated serum CA125 level were serous papillary cystadenocarcinoma (11 cases), mucinous cystadenocarcinoma (4 cases) and metastatic adenocarcinoma (2 cases). Two cases of endometriotic cyst show elevated value of serum CA125. Immunohistochemistry were performed in all 19 cases. Out of the 11 cases of serous papillary cystadenocarcinoma, 10 cases (91%) had significant expression of CA125 in both cytology and tissue. Among the 4 cases of mucinous cystadenocarcinoma, 3 cases (75%) had negative expression for CA125 in both tissue and cytology and only 1 case (25%) had positive expression for CA125. 2 cases of endometriotic cyst showed significant expression for CA125 in both tissue and cytology. Out of 2 cases of metastatic adenocarcinoma, having high serum CA125 level, only 1 case showed significant expression for CA125.

[Table/Fig-1]: showing cytohistological correlation of CA125 immunochemistry with serum level.

Overall sensitivity of serum CA125 level to cytohistological expression is 100% and the specificity is 86% with positive and negative predictive value of 74% and 100% respectively. The kappa statistics for extent of agreement between serum CA125 level to

Histopathology		Ca125 > 35U/mL	Ca125 <35U/mL	Cytohistological +ve expression of ca 125	% of +ve expression
Benign	Benign serous cystadenoma	0	5		
	Mucinous cystadenoma	0	2		
	Mature teratoma	0	4		
	Endometriotic cyst	2	3	2	100
Malignant	Serous papillary cystadenocarcinoma	11	0	10	91
	Mucinous cystadenocarcinoma	4	1	1	25
	Endometrioid carcinoma	0	3		
	Yolk sac tumour	0	2		
	Dysgerminoma	0	2		
	Granulosa cell tumour	0	3		
	Metastatic adenocarcinoma	2	1	1	50
Borderline	Borderline serous cystadenocarcinoma	0	1		

[Table/Fig-1]: Table showing cytohistological correlation of CA125 immunochemistry with serum level

CA125 Status	IHC Grading	IHC Grading	IHC Grading	IHC Grading	Row Totals
	0	1	2	3	
>35 U/mL	5	7	4	3	19
Row %	26.32%	36.84%	21.05%	15.79%	
<35 U/mL	31	0	0	0	31
Row %	100.00%	0.00%	0.00%	0.00%	
Totals	36	7	4	3	50

[Table/Fig-2]: Table Showing IHC grading
Chi-square test 2-tailed p-value < 0.001

cytohistological expression is 0.78 (strong agreement) (p <0.001).

[Table/Fig-2] Table Showing IHC grading.

DISCUSSION

Most widely used tumor marker in ovarian carcinoma, often considered the gold standard is CA125 [9]. It is raised in 90% of ovarian epithelial cancer. In our study preoperative serum levels of CA125 were obtained from all 50 cases of which CA125 level was elevated in 19(38%) cases. Out of those 19 cases, 14 cases showed positive tissue expression, whereas 5 cases with mild elevation of serum CA125 showed negative tissue expression. Diagnostic accuracy was 90% in our study, sensitivity 100%, specificity 86%. Most consistent expression (91%) was found in serous ovarian carcinomas (10 out of 11 cases). Leake J et al., showed 100 % correlation between immunocytochemical findings and elevated serum levels of CA125 in serous tumor in their study [10]. De La Cuesta R et al., studied 50 ovarian carcinoma samples for tissue CA125 along with the serum CA125 levels and got significant higher level of CA125 in malignant tissue (p<0.0001). High CA125 was associated with serous and endometrioid tumors [11].

In Danish 'MALOVA' ovarian carcinoma study, Estrid VS Hogdall studied 778 Danish women with ovarian tumors. Significantly more CA125 expression positive tumors (no expression vs. expression) was noted in the serous subtype compared to the percentage of positive tumors in mucinous, Endometrioid and other subtypes of patients with borderline ovarian tumors and with ovarian carcinoma (p<0.00001) [12]. Our study also corroborate with this finding bearing p value of <0.001.

In our study benign conditions like endometriotic cyst having elevated serum CA125 level also had significant tissue expression suggesting that the non malignant conditions had elevated CA125 level in serum. A recent published study has shown that elevated serum CA125 (>35 U/mL) could be found in many benign conditions such as menstruation, pregnancy, functional cyst, pelvic infection and endometriosis [13-15].

Medeiros LR et al., studied serum Ca125 level for diagnosis of ovarian tumors and compared them with paraffin embedded sections as gold standard on 2,374 cases of ovarian tumors and got a pooled sensitivity of 80% and specificity of 75% for diagnosis of ovarian carcinoma [16].

In our study 5 cases having elevated serum CA125 level did not reveal tissue expression of CA125. Possible explanations for this phenomenon are loss of CA125 in tissues during formalin fixation and paraffin embedding or heterogeneity within carcinomas and between different metastasis. Therefore assessment of CA125 by immunohistochemistry technique requires ample sampling of tumor tissue [17]. Again Breitenacker G et al., found that 54% of cases had discordant results for CA125 staining in different tumor metastasis [18].

When ovarian carcinoma is diagnosed in stage I, up to 90% of the patient can be cured by radiotherapy or chemotherapy [19]. At present <25% are diagnosed at the stage I [18]. To increased fraction of ovarian carcinoma detected at an early stage, screening strategy has been devised that utilise rising serum CA125 level to trigger the transvaginal sonography [17]. Serum level of CA125 is used to monitor response to chemotherapy, relapse and disease progression [20,21].

Gundogdu F et al., studied serum CA125 level and CA125 tissue expression positivity in prediction of recurrence of the stage III and stage IV epithelial ovarian tumors. The relationship between CA125 values and recurrence was significant in stage III/IV patients (p=0.041/p=0.006). The relationship between CA125 tissue expression positivity and recurrence development was significant in stage III/IV patients (p=0.041/p=0.029). It was

significantly correlated with recurrence in the patients with serous tumor, endometrioid tumor, clear cell tumor, mucinous tumor, and undifferentiated tumor. It was insignificant in mucinous tumor ($p=0.667$) [9].

LIMITATIONS

CA125 has limited specificity for ovarian cancer because elevated CA125 levels can be found in individuals with other cancers, including endometrial cancer, fallopian tube cancer, lung cancer, breast cancer and gastrointestinal cancer. The specificity of CA125 is particularly low in premenopausal women because many benign conditions that cause fluctuations in CA125 levels, such as menstruation, pregnancy, and pelvic inflammatory disease, are seen in this population.

FUTURE SCOPE

Monitoring CA125 blood serum levels is useful for determining how ovarian cancer is responding to treatment and for predicting a patient's prognosis after treatment [22]. This is because the persistence of high levels of CA125 during therapy is associated with poor survival rates in patients [22]. Also, an increase in CA125 levels within individuals in a remission is a strong predictor of the recurrence of ovarian cancer [23].

In April 2011 the UK's National Institute for Health and Clinical Excellence (NICE) recommended that women with symptoms that could be caused by ovarian cancer should be offered a CA125 blood test [24].

CONCLUSION

Detection of ovarian carcinoma at an early stage by serum CA125 level and immunocytochemistry by CA125 in a large no of case can impact greatly on patient survival. CA125 was measured preoperatively in serum, correlated well with the tissue expression. Significant higher level of CA125 express is seen in mostly malignant tissue in our study.

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