

Osteopontin as a Tumor Marker in Ovarian Cancer

Shikha Rani, Alka Sehgal¹, Jasbinder Kaur², Dilpreet Kaur Pandher¹, Rajpal Singh Punia³

Department of Obstetrics and Gynaecology,
Dr. BR Ambedkar Institute of Medical Sciences, Mohali, Punjab, Departments of
¹Obstetrics and Gynaecology,
²Biochemistry and
³Department of Pathology, Government Medical College and Hospital, Chandigarh, India

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ABSTRACT

Introduction: Ovarian cancer is associated with high morbidity and mortality. This is due to the nonspecific symptoms and no effective screening methods. Currently, carbohydrate antigen-125 (CA125) is used as a tumor biomarker for the diagnosis of ovarian cancer, but it has its own limitations. Hence, there is a need for other tumor biomarkers for the diagnosis of ovarian cancer. Objective of the study was to evaluate the diagnostic test characteristics of plasma osteopontin (OPN) in detecting ovarian malignancy and comparing its performance with CA125. **Materials and Methods:** This is a prospective cross-sectional diagnostic test evaluation. Women with adnexal mass detected by clinical or radiological examination were enrolled as suspected cases. Women who presented with other gynecological conditions were enrolled as controls. OPN and CA125 levels were measured in all enrolled subjects. **Results:** Among 106 women enrolled, 26 were ovarian cancer, 31 had benign ovarian masses, and 49 were controls. Median plasma CA125 levels were higher in subjects with ovarian cancer (298 U/ml; interquartile range [IQR]: 84–1082 U/ml vs. 37.5U/ml; IQR: 17.6–82.9U/ml; $P < 0.001$). CA125 sensitivity, specificity, positive, and negative likelihood ratios were 88.5%, 61.3%, 2.10, and 0.19, respectively. Median plasma OPN levels were higher in subjects with ovarian cancer (63.1 ng/ml; IQR: 39.3–137 ng/ml vs. 27 ng/ml; IQR: 20–52 ng/ml; $P = 0.001$). Sensitivity, specificity, positive, and negative likelihood ratios of OPN were 50%, 87%, 2.58, and 0.62, respectively. **Conclusion:** OPN levels were higher in ovarian cancer than in the benign ovarian mass and had better specificity than CA125. OPN can better differentiate between benign and malignant ovarian mass as compared to CA125.

KEYWORDS: Carbohydrate antigen-125, osteopontin, ovarian cancer, tumor marker

INTRODUCTION

Ovarian cancer is associated with the highest tumor-related mortality among all gynecological malignancies.^[1] Age-standardized rates of ovarian cancer worldwide are 6.6/100,000 females and mortality is 4.4/100,000 females.^[2] This high mortality is due to nonspecific symptoms and ineffective screening methods leading to delay in diagnosis.^[3,4] The best currently available method for early detection of ovarian cancer is the combination of raised carbohydrate antigen 125 (CA125) and transvaginal ultrasonography.^[5,6] However, CA125 has certain limitations as a biomarker for ovarian cancer. It is elevated in less than half of the early-stage ovarian tumors, and it is raised most in

serous histology. CA125 can be false positive in many benign and malignant conditions.^[7,8] In addition, it is influenced by age, race, obesity, smoking, and history of hysterectomy.^[9] However, till now, no other dependable biomarker has been developed to replace CA125 or to further improve its sensitivity and specificity.

Osteopontin (OPN) is an extracellular matrix phosphoglycoprotein which is secreted by osteoblast and epithelial cells of different organs. It is also secreted

Address for correspondence: Dr. Shikha Rani, Department of Obstetrics and Gynaecology, Dr. BR Ambedkar Institute of Medical Sciences, Mohali, Punjab, India. E-mail: shikhataneja2000@gmail.com

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by the macrophages, activated T-lymphocytes, and leukocytes.^[10,11] It regulates physiological processes such as bone resorption, wound healing, immune response, and vascularization. Pathological conditions such as cancer metastasis and wound healing a defect in posttranslational modification cause changes in its functions.^[12] OPN plays a crucial role in cancer progressions such as tumor invasion, angiogenesis, and metastasis.^[13] OPN is increased in various cancers such as ovarian, cervical, breast, colorectal, liver, lung, pancreas, prostate, and melanoma.^[14] Recent studies have shown that combining OPN with CA125 increases the sensitivity and specificity for the detection of ovarian cancer.^[15-18] The levels of biomarkers are influenced by the ethnicity of the population, and no such study has been done in the Indian population. Hence, this study was planned to evaluate the role of OPN and CA125 in subjects with ovarian cancer.

MATERIALS AND METHODS

This prospective cross-sectional diagnostic test evaluation study was done in the department of obstetrics and gynecology in collaboration with the department of biochemistry and the department of pathology of a tertiary care hospital. The study was approved by the institutional ethics committee. The study was done from December 2016 to August 2018. Written informed consent was obtained from all the enrolled subjects. Subjects were enrolled consecutively with consideration of inclusion and exclusion criteria. During the study period, a total of 106 subjects were enrolled. Fifty-seven out of 106 subjects, with the following criteria, were enrolled as suspected cases. Subjects with newly suspected or diagnosed adnexal mass had any of the following: complex adnexal mass, adnexal mass which is not decreasing in size on conservative management of adnexal mass which had ultrasound features suggestive of malignancy. Subjects who were already on treatment for cancer ovary or tube and whose ultrasound features were not suggestive of adnexal malignancy were excluded from the study.

Forty-nine out of 106 subjects, with gynecological problems other than adnexal mass or any other gynecological cancer, were included in the control group.

Detailed history and examination were performed in all enrolled subjects. Ten ml of blood sample was obtained for measurement of plasma OPN, serum CA125, and other routine investigations. Pelvic ultrasonography was performed in all subjects. Computed tomography or magnetic resonance imaging of pelvis and abdomen was done as per clinical indication. Further management such

as fine-needle aspiration cytology (FNAC) or surgery was done as per the clinical protocol of the department. Samples of OPN and CA125 were taken preoperatively. Treating team was blinded to values of OPN till the report of FNAC or histopathology was received. Thus, case treatment was not influenced by OPN levels or because of the study. After the cases were operated, they were followed up with examination, ultrasound, and CA125 levels.

Processing of sample

Estimation of carbohydrate antigen-125 and osteopontin levels

A plain vial blood sample of 5 ml for CA125 and 2-ml EDTA blood sample for OPN were immediately sent to the department of biochemistry. Samples were stored at -20°C . Stored serum for CA125 and blood for OPN levels were used for estimating their levels later. However, no more than one freeze and thaw cycle was permitted. CA125 was measured by chemiluminescence immunoassay kits on ADVIA Centaur Siemens. OPN was measured using enzyme-linked immunoassay methods. Human OPN ELISA (RayBiotech, Norcross, Georgia) was used as per the manufacturer's protocol for the estimation of OPN.

Sample size calculation and statistical analysis

Due to the low incidence of ovarian cancer, a minimum number of 57 consecutive subjects who attended the outpatient department obstetrics and gynecology with ovarian mass were enrolled as suspected cases. Forty-nine subjects without any complaints of adnexal mass were enrolled in the control.

Data were entered in Epi Info. Data analysis was done in Stata 16.1 (StataCorp, College Station, Texas USA) Performance of diagnostic tests was carried out by kappa statistics, sensitivity, specificity, positive predictive value, negative predictive value, receiver operating characteristic (ROC) curve, and area under curve (AUC). About 95% of confidence interval was calculated.

RESULTS

This prospective cross-sectional diagnostic test evaluation study was done in the department of obstetrics and gynecology in collaboration with the department of biochemistry and the department of pathology of a tertiary care hospital.

Baseline variables

During the study period, a total of 106 subjects were enrolled. After the final histopathology or cytology, the suspected cases and controls were further reallocated into groups. Group 1 had who were proven as ovarian cancer on histopathology or cytology. Group 2 had subjects who were suspicious of ovarian cancer but were

proven as benign ovarian masses on histopathology or cytology. Group 3 had subjects who were originally in the control group.

Subjects in different groups were Groups 1, 2, and 3 had 26, 31, and 49 subjects, respectively. Mean age in Groups 1, 2, and 3 had 51 ± 12 years, 43.2 ± 11 years, and 44.5 ± 12 years, respectively ($P = 0.005$). Median parity in Groups 1, 2, and 3 was 3 (3–4), 2 (1–3), and 2 (2–3), respectively, and is not statistically different ($P = 0.080$). Distension abdomen (16/26) and pain abdomen (18/26) were the most common symptoms reported by the Group 1 subjects. Weight loss was seen in (5/26) subjects of Group 1.

Table 1 shows the final histopathology in Groups 1 and 2. Serous adenocarcinoma followed by serous cystadenoma was the most common histopathology found in Group 1 and 2, respectively. As per FIGO staging, the distribution of patients was in Stage 1 (3/26), Stage 2 (5/26), Stage 3 (15/26), and Stage 4 (3/26).

The median values of the CA125 and OPN are shown in Table 2. The median value and interquartile range for risk of malignancy index-2 (RMI2) scores in Group 1 and 2 were 2846 (252–9738) and 82 (29–256), respectively, and were statistically different ($P < 0.004$).

To calculate the diagnostic performance of CA125 and OPN, a cutoff of 42 U/ml for CA125 and 64 ng/ml for OPN was taken. CA125 value >42 U/ml was the 75% centile of the values in Groups 2 and 3 combined. OPN value >64 ng/ml which was calculated by the 90th percentile of Groups 2 and 3.

Table 1: Final histopathology of subjects in Groups 1 and 2

Histopathology	Number of subject=57
Serous adenocarcinoma	18
Mucinous adenocarcinoma	7
Clear cell carcinoma	1
Serous cystadenoma	13
Mucinous cystadenoma	9
Granulosa cell tumor	3
Fibroma	2
Mature cystic teratoma	1
Sclerosing cell tumor	1
Borderline mucinous cystadenoma	2
Borderline serous cystadenoma	1

Table 2: Carbohydrate antigen-125 and osteopontin values in different groups

Variable	Group 1 (n=26)	Group 2 (n=31)	Group 3 (n=49)	P
CA-125 (U/ml) ^a	298 (84-1082)	37.5 (17.6-82.9)	14 (10-35.6)	<0.001
OPN (ng/ml) ^a	63.1 (39.3-137)	27 (20-52)	23 (17.5-36.3)	0.001

^aValues are represented in median (IQR). CA-125: Carbohydrate antigen-125, IQR: Interquartile range, OPN: Osteopontin

Diagnostic performance of osteopontin

The sensitivity and specificity of OPN were 50% and 87%, respectively, with AUC 0.75 (0.62–0.89). Positive and negative likelihood ratios of OPN were 2.58 and 0.62, respectively.

Comparison of osteopontin with other markers

CA125 sensitivity and specificity was 88.5% and 61.3%, respectively, with AUC 0.83 (0.72–0.93). Combined CA125 and OPN had higher sensitivity of 93% but reduced specificity of 39.3%. An RMI2 value of >200 had the sensitivity and specificity of 80% and 67%, respectively. The combination of OPN with RMI2 had almost comparable sensitivity to CA125 (84%) and higher specificity 67% and the AUC of 0.87 (0.79–0.96)

Sensitivity and specificity of the CA125 and OPN alone, combined CA125 and OPN, either CA125 or OPN, and a combination of OPN with RMI2 are shown in Table 3. Figure 1 shows the ROCs of CA125 and OPN.

DISCUSSION

In our study, we found a statistically significant difference in the values of OPN in Groups 1 and 2. CA125 is a more sensitive biomarker for detecting ovarian cancer as compared to OPN. However, the specificity of OPN is much better and thus can differentiate better between benign and malignant ovarian masses. Sensitivity of CA125 further improved if we combined CA125 and OPN or either of them is positive but specificity decreases significantly. OPN alone is the most specific

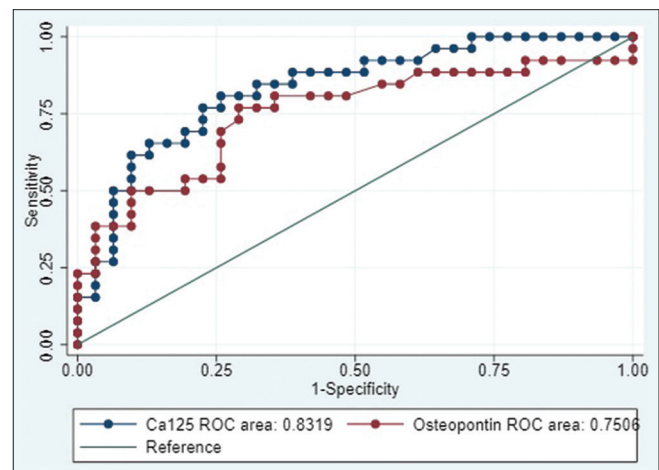


Figure 1: Shows the receiver operating characteristics of CA125 and OPN. OPN: Osteopontin, CA125: Carbohydrate antigen-125

Table 3: Sensitivity and specificity of carbohydrate antigen-125 and osteopontin

Variable	True positive	True negative	Sensitivity (%)	Specificity (%)	AUC (95% CI)
CA-125	23/57	19/57	88.5	61.3	0.83 (0.72-0.93)
OPN	13/57	4/57	50	87	0.75 (0.62-0.89)
Combined CA-125 + OPN	12/57	28/57	93	39.3	-
CA-125 or OPN	8/57	30/57	90	46.1	-
Combined OPN + RMI 2	9/57	28/57	84	67	0.87 (0.79-0.96)

Cutoff used for CA-125 was 42 U/ml and OPN was 64 ng/ml. CA-125: Carbohydrate antigen-125, OPN: Osteopontin, RMI-2: Risk of malignancy index-2, AUC: Area under curve, CI: Confidence interval

marker as compared to CA125, CA125+OPN, or RMI2. OPN with RMI2 has almost comparable sensitivity and specificity to CA125.

Kim *et al.* were the first researcher to find the role of OPN as a tumor marker for ovarian cancer. They enrolled 251 subjects, of which 51 were epithelial ovarian cancer. Values of OPN were significantly higher in ovarian cancer patients. Furthermore, immunolocalization of the OPN on the tissue sample had demonstrated higher levels in invasive and borderline ovarian tumors compared to normal and benign ovarian tissue.^[15]

Nakae *et al.* did preoperative plasma OPN levels as complementary to CA125 in predicting ovarian cancer. A total of 127 were enrolled, of which 32 subjects were of ovarian cancer. The sensitivity of CA125, OPN, and either CA125 or OPN was 84.4%, 81.3%, and 93.8%, respectively. The specificity of CA125, OPN, and either CA125 or OPN was 54.7%, 66.3%, and 87.4%, respectively. They reported combining OPN and CA125 can better predict cancer ovary. We have found higher specificity of OPN and slightly better sensitivity of CA125. Cutoff taken for CA125 was almost like our study (35 U/ml). The OPN cutoff taken by Nakae *et al.* was 498 ng/ml which was 95th percentile of healthy women. However, the highest value of OPN in Group 3 of our study was 36.3 ng/ml.^[17]

Moszynski *et al.* studied the role of OPN in differentiating benign and malignant ovarian tumors. They found that the OPN levels were raised in all histologic types of ovarian cancer as compared to CA125. Furthermore, OPN was less elevated in endometriosis cyst as compared to CA125. As in our data, we do not have any subjects with endometriotic cysts so this conclusion cannot be replicated. They found almost similar diagnostic accuracy of CA125, OPN, and ultrasonographic markers. Researchers proposed that the OPN can better differentiate endometriosis cysts and has better utility in the detection of ovarian cancer at places where access to ultrasonography is difficult. Further, they had shown the ability of OPN to diagnose ovarian cancer is similar to combined ultrasonography and CA125 levels. However, they have emphasized that as

ultrasonography is operator-dependent, so it should be done by an experienced sonologist.^[19]

Milivojevic *et al.* studied the diagnostic accuracy of OPN and OPN with CA125 in differentiating benign and malignant ovarian masses. In 79 subjects, they had 48 ovarian cancer and 31 benign cysts. Sensitivity of OPN alone and in combination with CA125 was 62.5% and 74.9%, respectively, with a predefined specificity of 90%.^[20]

Researchers have also used various multipaneled biomarker tests to improve the detection of ovarian cancer. OPN has been widely used as one of the tumor biomarkers in these panels. Meinhold-Heerlein *et al.* in their study did the serum samples of 67 subjects for 5 biomarkers of cancer ovary (OPN, CA125, kallikrein, matrix metalloproteinase-7, and secretory leukoprotease inhibitor). They reported the highest sensitivity and specificity, 88% and 100%, respectively.^[18]

Mor *et al.* evaluate the role of four biomarkers (leptin, prolactin, OPN, and insulin-like growth factor-II) for the early detection of ovarian cancer. A total of 86 subjects were enrolled, of which 18 were newly diagnosed with ovarian cancer and 40 with recurrent ovarian cancer. They reported a sensitivity of 95% and a specificity of 95% for this panel of markers.^[16]

Zhang *et al.* evaluated the expression of OPN in normal (15), benign (20), borderline (20), and malignant (40) ovarian tissue by immunohistochemistry. They reported significantly higher expression of OPN in ovarian cancer compared to borderline and normal ($P < 0.05$). Positive rates of OPN expression were significantly more in poorly differentiated ovarian cancer, stage III and IV ($P < 0.05$), and lymph node metastasis. OPN expression was not influenced by age or histologic type.^[21]

It has been reported in the literature that certain conditions such as weight loss, exercise, Vitamin K supplements, diabetes mellitus, cardiovascular disease, and renal stones can influence the serum OPN levels.^[22-24] Furthermore, these conditions can be present in patients with ovarian cancer. However, no subgroup

analysis has been done in studies evaluating the role of these conditions on OPN levels in patients with ovarian cancer.

Hu *et al.* did a systematic review of 13 studies to determine the diagnostic accuracy of OPN in ovarian cancer. They reported a sensitivity of sensitivity, specificity, and AUC are 0.66 (95% confidence interval [CI], 0.51–0.78), 0.88 (95% CI, 0.78–0.93), and 0.85 (95% CI, 0.81–0.88), respectively.^[25] They found that ethnicity and the type of test used were significant sources of heterogeneity among the included studies. This is the reason for different values of OPN in our population. Researchers further opined that 12 of 13 studies were from Europe or North America so the results were more specific for these populations.

Difference in the diagnostic performance of CA125 and OPN can be due to different cutoff values, ethnic diversity influencing values of OPN, and diverse histology and stage of ovarian cancer. We propose that the CA125 and RMI2 both markers have very good sensitivity and adding a tumor biomarker of higher specificity can better predict whether a mass is malignant or not. We further propose that women with an ovarian mass should first undergo CA125 and RMI and if a diagnosis of a suspicious ovarian mass is made, then OPN levels should be done for better categorization of mass. However, histopathology remains the gold standard.

Our studies also found comparable results with a little lower specificity. This is the first kind of study done in the Indian population is the strength of our study. However, due to the small number of ovarian cancer subjects in our study, the effect of stage and histology of cancer on levels of OPN could not be well established. We recommended further large multi-centric studies on larger sample sizes are required to be conducted for evaluating and establishing the role of osteopontin as a diagnostic tumor biomarker in ovarian cancer along with long-term follow-up in ovarian cancer before OPN levels can be used in routine clinical practice.

CONCLUSION

OPN has higher specificity compared to CA125 in detecting ovarian cancer. OPN can better differentiate between benign and malignant ovarian cancer as compared to CA125.

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Conflicts of interest

There are no conflicts of interest.

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