Osteopontin as a Tumor Marker in Ovarian Cancer

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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 CA 125 is used as a tumor biomarker for the diagnosis of ovarian cancer, but it has its own limitations. So, there is need for other tumor biomarkers for the diagnosis of ovarian cancer. To determine the diagnostic test characteristics of plasma osteopontin (OPN) in detecting ovarian malignancy and comparing its performance with carbohydrate antigen-125 (CA 125).

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 CA 125 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 CA 125 levels were higher in subjects with ovarian cancer (298 U/ml; IQR 84-1082 U/ml vs. 37.5U/ml; IQR 17.6-82.9U/ml; $P<0.001$). CA 125 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. 27ng/ml; IQR 20-52ng/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.

Introduction

Ovarian cancer is associated with highest tumor-related mortality amongst all gynecological malignancies. [1] Age standardized rates of ovarian cancer worldwide is 6.6 per 100,000 females and mortality is 4.4 per 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 combination of raised carbohydrate antigen 125(CA 125) and transvaginal ultra-sonography (TVS).[5,6] However, CA 125 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. CA 125 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 CA-125 or to further improve its sensitivity and specificity.

Osteopontin (OPN) is an extracellular matrix phosphoglycoprotein. [10] It regulates physiological processes like bone resorption, wound healing, immune response, and vascularization. In pathological

conditions like cancer metastasis and wound healing a defect in posttranslational modification causes changes in its functions.[11] OPN plays a crucial role in cancer progression like tumor invasion, angiogenesis and metastasis.[12] OPN is increased in a various cancer like ovarian, cervical, breast, colorectal, liver, lung, pancreas, prostate and melanoma.[13] Recent studies have shown that combining OPN with CA 125 increases the sensitivity and specificity for detection of ovarian cancer.[14-17] The levels of biomarkers are influenced by ethnicity of the population and no such study has been done in Indian population. So, this study was planned to evaluate the role of OPN and CA125 in subjects with ovarian cancer.

Material And Methods

This prospective cross-sectional diagnostic test evaluation study was done in the Department of Obstetrics & Gynecology in collaboration with the Department of Biochemistry and Department of Pathology of a tertiary care hospital. Study was approved by the institutional ethics committee (IEC2017/003). All the principle of Declaration of Helsinki was followed during the study. 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 and 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 CA 125 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 like fine needle aspiration cytology (FNAC) or surgery was done as per clinical protocol of the department. Samples of OPN and CA125 were taken preoperatively. Treating team was blinded to values of osteopontin till the report of FNAC or histopathology was received. Thus, case treatment was not influenced by OPN levels or because of study.

Processing Of Sample

Estimation of CA 125 and OPN levels

A plain vial blood sample of 5 ml for CA125 and 2 ml EDTA blood sample for OPN was immediately sent to the department of Biochemistry. Samples were stored at -20⁰C. Stored serum for CA 125 and blood for OPN levels was used for estimating their levels later. But no more than one freeze and thaw cycle were permitted. CA 125 was measured by chemiluminescence immunoassay kits on Advia Centaur Siemens. OPN was measured using enzyme linked immunoassay methods. Human OPN ELISA (RayBiotech, Norcross, Georgia) were used as per the manufacturer's protocol for estimation of OPN.

Sample size calculation & Statistical analysis

Due to the low incidence of ovarian cancer a minimum number of 57 consecutive subjects who attended the out- patient department Obstetrics & Gynecology with ovarian mass were enrolled as suspected cases. Forty-nine without any complaints of adnexal mass were enrolled in the control.

Data was entered in Epi info. Data analysis was done in Stata 16.1 (Stata Corp) Performance of diagnostic tests were carried out by kappa statistics, sensitivity, specificity, positive predictive value, negative predictive value, receiver-operating characteristic (ROC) curve and Area under curve (AUC). 95% confidence interval was calculated.

Results

This prospective cross-sectional diagnostic test evaluation study was done in the Department of Obstetrics & Gynecology in collaboration with the Department of Biochemistry and 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 group 1, 2 and 3 had 26, 31 and 49 subjects, respectively. Mean age in group 1, 2 and 3 had 51±12yrs, 43.2± 11yrs and 44.5±12yrs respectively (p= 0.005). Median parity in group 1, 2 and 3 was 3(3-4), 2(1-3) and 2(2-3) respectively and is not statistically different (p value=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 group 1 & 2. Serous adenocarcinoma followed by serous cystadenoma was the most common histopathology found in group 1 and 2 respectively.

Median values of the CA125 and OPN are shown in table 2. Median value and IQR for risk of malignancy index-2 (RMI 2) score in group 1 and 2 was 2846 (252-9738) and 82(29-256) respectively and was

statistically different (p value <0.004).

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

Diagnostic performance of OPN

Sensitivity and specificity of OPN was 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 OPN 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 osteopontin had higher sensitivity of 93% but reduced specificity of 39.3%. A RMI 2 value of >200 had the sensitivity and specificity of 80% and 67% respectively. 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 & OPN, either CA125 or OPN and combination of OPN with RMI2 had been shown in table 3. Figure 1 shows the receiver operating characteristics of CA125 and OPN.

Discussion

In our study we found a statistically significant difference in the values of OPN in group 1 and 2. CA125 is a more sensitive biomarker for detecting ovarian cancer as compared to OPN. But the specificity of OPN is much better 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 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. Also, immunolocalization of the OPN on the tissue sample had demonstrated higher levels in invasive and borderline ovarian tumor compared to normal and benign ovarian tissue. [14]

Nakae et al did preoperative plasma OPN levels as complementary to CA 125 in predicting ovarian cancer. A total of 127 were enrolled of which 32 subjects were of ovarian cancer. Sensitivity of CA-125, OPN and either CA-125 or OPN were 84.4%, 81.3% and 93.8% respectively. Specificity of CA 125, OPN and either CA-125 or OPN were 54.7%, 66.3% and 87.4% respectively. They reported combining OPN and Ca-125 can better predict cancer ovary. We have found higher specificity of OPN and slightly better sensitivity of CA125. Cut off taken for CA125 was almost like our study(35U/ml). The OPN cut off taken

by Nakae et al was 498 ng/ml which was 95th percentile of healthy women. But the highest value of OPN in group 3 of our study was 36.3ng/ml. [16]

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. Also, OPN was less elevated in endometriosis cyst as compared to CA125. As in our data we do not have any subject with endometriotic cyst 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 cyst and has better utility in detection of ovarian cancer at places where access to ultrasonography is difficult. [18]

Milivojevic M 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 cyst. Sensitivity of OPN alone and in combination with CA125 was 62.5% and 74.9% respectively with a predefined specificity of 90% [19]

Researchers have also used various multipanel biomarker tests to improve the detection of ovarian cancer. OPN has been widely used as one of the tumor biomarkers in these panels. Meinhold et al in their study did the serum samples of 67 subjects for 5 biomarkers of cancer ovary (OPN, CA 125, kallikerin, matrix metalloproteinase-7 and secretory leukoprotease inhibitor). They reported highest sensitivity and specificity, 88% and 100%, respectively. [17]

Mor et al evaluate the role of four biomarkers (leptin, prolactin, osteopontin, and insulin-like growth factor-II) for early detection of ovarian cancer. A total of 86 subjects were enrolled of which 18 new diagnosed ovarian cancer and 40 with recurrent ovarian cancer. They reported a sensitivity of 95% and a specificity of 95% for this panel of markers. [15]

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% CI, 0.51–0.78) ,0.88 (95% CI, 0.78–0.93) and 0.85 (95%CI, 0.81–0.88) respectively. [20] They found that ethnicity and type of test used were the significant source 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 cut off values, ethnic diversity influencing values of OPN and diverse histology and stage of the ovarian cancer. We propose that the CA125 and RMI 2 both markers have a 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 Indian population is the strength of our study. However, the effect of stage and histology of cancer on levels of OPN could not be found due to the small number of ovarian cancer subjects in our study

Conclusion

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

Declarations

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Conflict of interest: The authors have no relevant financial or non-financial interests to disclose.

AUTHOR CONTRIBUTIONS

Protocol/project development: SR, AS and JK Laboratory tests: JK and RPS Data collection or management: SR, DKP Data analysis: SR Manuscript writing/editing: SR AS, JK, DKP and RPS

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Tables

Table 1 Final histopathology of subjects in group 1 and 2

Histopathology	Number of Subject =57
Serous adenocarcinoma	18
Mucinous adenocarcinoma	07
Clear cell carcinoma	01
Serous cystadenoma	13
Mucinous cystadenoma	09
Granulosa cell tumor	03
Fibroma	02
Mature cystic teratoma	01
Sclerosing cell tumor	01
Borderline mucinous cystadenoma	02
Borderline serous cystadenoma	01

Table 2 CA-125 and Osteopontin values in different groups

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

Values in ^a are represented in median (IQR)

Table 3 Sensitivity and Specificity of CA-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 CA125+ OPN	12/57	28/57	93%	39.3%	-
CA125or OPN	08/57	30/57	90%	46.1%	-
Combined OPN +RMI 2	9/57	28/57	84%	67%	0.87(0.79-0.96)

Cut off used for CA125 was 42U/ml and OPN was 64 ng/ml

Figures

Figure 1

shows the receiver operating characteristic of CA125 and OPN