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Role of Cyclooxygenase 2 (COX-2) in Prognosis of Ovarian Cancer

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ABSTRACT
Ovarian cancer (OC) Gynecological cancers have the highest fatality rate. Cisplatin, also known as CDDP (cis dichloro diammine platinum), encourages DNA chain and interchain crosslinks, obstructs DNA replication and stimulates cell death. Platinum has emerged as the key medication for the treatment of OC, whereas CDDP has been utilized as a chemotherapeutic medicine since the end of the 1970s. In order to validate the role of overexpression of COX-2 as a prognostic marker in patients with ovarian cancer in the Indian subcontinent, this study examined the relationship between COX-2 expressions in human ovarian cancer as well as its association with other recognized prognostic indicators like age, menopausal status, tumour size, stage and grade. Using the Western Blot Method, ovarian tissue's COX-2 protein expression was evaluated. In the control group, COX-2 expression was not present. Although the proportion of COX-2 positive tumors was higher in patients over 50 years old [25 (51.0%)], this was not statistically significant ($p = 0.07$); postmenopausal status [36 (73.5%)], $p<0.01$, 7 (14.3%) patients with stage I, 14 (28.5%) patients with stage II, 19 (38.8%) patients with stage III and 9 (18.4%) patients with stage IV disease had COX-2 positive tumors. There were statistically substantially more COX-2 positive tumors in grade I (4 patients, 8.2%), grade II (12 patients, 24.5%) and grade III (33 patients, 67.3%) than in grade I (COX-2 negative tumors). In our investigation, COX-2 was positive in 55.1% (27) of T3 tumors, 20.4% (12) of T2 tumors and 20.4% (10) of T1 tumors. COX-2 expression is associated with established indicators of poor prognosis such as postmenopausal status, age >50 years, advanced stage of disease, large tumour size and higher grade. The risk of COX-2 positivity was found to be 3.54 times greater for postmenopausal status and 6.70 times greater for tumours size T2. As a result, COX-2 expression suggests an aggressive tumor biology and may be a key prognostic indicator.

INTRODUCTION

Ovarian cancer (OC) and gynecological cancers have the highest fatality rate. Cisplatin, also known as CDDP (cis-dichloro diammine platinum), encourages DNA chain and interchain crosslinks, obstructs DNA replication and stimulates cell death. Platinum has emerged as the key medication for the treatment of OC, whereas CDDP has been utilized as a chemotherapeutic medicine since the end of the 1970s. As it is challenging to entirely eradicate the malignancy with surgery, the major treatment for an OC tumor has been cytoreductive surgery along with platinum-based combination chemotherapy. Heterologous anti-cancer medications, including the chemotherapeutic drug combination of paclitaxel and CDDP, have demonstrated some curative potential. Patients with OC still have a 20% to 30% five-year survival rate and 70% of them eventually relapse[1]. Platinum resistance, a growing clinical concern that affects the prognosis of OC patients, is the primary factor in treatment failure. The prognosis of OC patients is significantly impacted by medication resistance and metastasis, which are also hot topics in gynecological oncology research[2]. Tumor micro environmental alteration and aberrant tumor metabolism are believed to be chemoresistance-related variables, while drug resistance mechanisms are yet unknown[3].

In 2013, there will be more than 22,000 new cases of ovarian cancer and more than 14,000 fatalities in the United States, placing ovarian cancer as the sixth leading cause of neoplasm death among women. Most patients have advanced stage tumors at the time of diagnosis since early-stage cancers are frequently asymptomatic, which leads to a worse long-term survival rate[4]. Only about 30% of ovarian cancer patients survive for at least 5 years. Finding predictive biomarkers is essential for accurately assessing patient outcomes given the low survival rates of ovarian cancer.

Prostaglandin-endoperoxide synthase-2 (PTGS-2) is another name for cyclooxygenase-2 (COX-2), which is also implicated in inflammatory and carcinogenic processes[5,6]. Additionally, during carcinogenesis, COX-2 dependent prostaglandin production can reduce immunological activation and antigen presentation. Most solid tumors, including colorectal, lung, pancreatic, liver and ovarian cancer, were found to overexpress COX-2 and The predictive significance of COX-2 expression in ovarian cancer has been documented in some research, albeit the findings are inconsistent and even contradictory[7,8]. We gathered the pertinent literature and carried out a meta-analysis to evaluate the predictive significance of COX-2 expression in ovarian cancer patients in order to explain the issues.

The most common cause of mortality for women with gynecological cancers is ovarian cancer. Additionally, it ranks as the seventh most common cause of mortality for females overall[9]. The majority of instances are discovered when the disease has already progressed, which results in dismal consequences. The limited predictive value of the current screening tests adds to this anguish.

MATERIALS AND METHODS

Patient selection: This was a prospective study. The patients were divided into two groups. The Comprehensive ovarian Clinic & ovarian Cancer Research Unit, IPGME&R/SSKM Hospital, Kolkata, West Bengal, India between 2010 and 2012.

Sample Size: 65

Tissue processing: The samples were divided into minute pieces, submerged in collagenase at 37°C for 4-6 hrs and then rinsed with phosphate buffered saline (PBS). The tissue was chopped and then incubated with collagenase for 10 min. Cells were homogenized in a ripalysis buffer mixture (1:3) at 4°C to extract the total protein, which was then determined spectrophotometrically using Lowry's technique.

Immunoblotting: To obtain the cytosolic fraction, cells were lysed in buffer (10 mM Hepes, pH 7.9, 1.5 mM MgCl2, 10 mM KCl and 0.5 mM dithiothreitol). To get the nuclear fraction, the pellet was spun down at 12000 g for 30 min while being suspended in buffer (20 mM Hepes, pH 7.9, 0.4 M NaCl, 1.5 mM MgCl2, 0.2 mM EDTA and 0.5 mM dithiothreitol). Cells were suspended and homogenized in buffer (100 mM Tris-Cl, pH 7.4, 300 mM NaCl, 1% NP-40 and 0.25% sodium-deoxycholate) to create whole cell lysates. Mixtures of protease and phosphatase inhibitors were added to all the buffers. For a direct western blot analysis, the cell lysates or specific fractions were separated by SDS polyacrylamide gel electrophoresis, transferred to nitrocellulose membrane and probed with designated antibodies, such as -COX-2 made by Santa Cruz (Santa Cruz, CA, USA). The immunoblots were then detected by chemiluminescence. A-actin antibody from Santa Cruz was used to check that the proteins were loaded equally.

RT-PCR assay: Reverse transcription was performed on two mg of total RNA that had been extracted from cells using the TRIzol reagent (Invitrogen, Carlsbad, Carlsbard, CA, USA) before GeneAmpPCR 2720 (Applied Biosystems, Foster City, CA, USA) was used for PCR. The COX-2-specific primers (5-TGA TCGAAGACTACG TGCAACA-3/5-GCG GATGCCAGTGAT

AGAGTG-3) and GAPDH (internal control)-specific primers (5-CA-GAACATCATCCCTGCC TCT-3 /5-GCTTGAC AAAGTGGTCGTTGAG-3) were used to amplify the cDNAs.

Histology and Immunohistochemistry: Measurements of the tumor size were made after the ovarian cancer tumors were fixed in 10% neutral-buffered formalin for 24 hrs. The lymph nodal status and grade were then evaluated after sectioning the tumor and embedding it in paraffin. To prepare tumor paraffin slices for immunohistochemistry, they were washed repeatedly with xylene, 100% ethanol, a phosphate buffer [10 mm], (pH 7.4) and 0.138 M saline with 2.7 mM KCl. Diluted antigen retrieval buffer (DAKO Corp.) was used to retrieve the antigen. 3% hydrogen peroxide was used to inhibit endogenous peroxidase. Slides were then rinsed in PBS/KCl, exposed to 10% normal horse serum, the primary antibody (rabbit anti-ER or rabbit anti-PR or rabbit anti-c-erbB2; HER-2/neu) and allowed to sit in the incubation mixture for an overnight period at 4°C. After that, the slides underwent a 45 min incubation with a biotinylated secondary antibody, followed by an ABC reagent and diaminobenzidine reaction. Hematoxylin was used for the counterstain. By washing sections with 95% ethanol, 100% ethanol and xylene in that order, sections were dehydrated. Slides were mounted using Paramount and coverslips. Using an Olympus microscope with a SPOT digital camera, digital images of stained and un-stained cells were captured.

Statistical analysis: Statistical analysis was performed with help of Epi Info (TM) 3.5.3. EPI INFO is a trademark of the Centers for Disease Control and Prevention (CDC). Using this software, basic cross-abulation, inferences and associations were performed. Chi-square test was used to test the association of different study variables with the expression of COX-2. Test of proportions was used to test the significant difference between two proportions and corresponding standard normal deviate (Z-values) were calculated with corresponding p-values. Odds ratio (OR) with 95 % Confidence Interval (CI) was calculated to measure the different risk factors under univariate analysis. Under multivariate analysis Logistic Regression was used to find the risk factors. $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

This was a prospective study. The patients were divided into two groups. The Comprehensive ovarian Clinic and ovarian Cancer Research Unit, IPGME&R/SSKM Hospital, Kolkata, West Bengal, India between 2010 and 2012. Total 65 patients were include in this study.

Ovarian cancer is the most prevalent cause of death for women with gynecological malignancies. It also comes in at number seven on the list of all causes of death for females.[9]. The majority of instances are discovered when the disease has already progressed, which results in dismal consequences. The limited predictive value of the current screening tests adds to this anguish.

Only about 30% of ovarian cancer patients survive for at least five years[10]. Finding predictive biomarkers is essential for accurately assessing patient outcomes due to the low survival rates of ovarian cancer.

Neither the RT-PCR method nor the western blot method detected COX-2 expression in the control group. The patients' median age was 50 years, with a mean age (mean standard deviation) of 57.71 8.81 years and a range of 37-75 years.

Our research revealed that Stage-I tumors with a T1 tumor size did not significantly differ in COX-2 expression. The expression of COX-2 did not significantly differ between ductal carcinoma in situ and lobular carcinoma according to histology. The percentage of COX-2 positive cases versus COX-2 negative cases were noticeably greater for all other factors. The proportion of COX-2 positive tumours in postmenopausal patients [36 (73.5%)] was significantly higher than in the pre-menopausal group [13 (26.5%)], ($p<0.001$). Likewise, the proportion of COX-2 positive tumours in patients of age >50 years [25 (51.0%)] was higher than those of age $\leq$50 years [24 (49.0%)] but this was not statistically significant ($p = 0.07$). COX-2 positive tumors were present in 7 (14.3%) patients with stage I disease, 14 (28.5%) patients with stage II illness, 19 (38.8%) patients with stage III disease and 9 (18.4%) patients with stage IV disease. There were statistically substantially more COX-2 positive tumors in grade I (4 patients, 8.2%), grade II (12 patients, 24.5%) and grade III (33 patients, 67.3%) than in grade I (COX-2 negative tumors) (Table 1 and 2).

In our investigation, COX-2 was positive in 55.1% (27) of T3 tumors, 20.4% (12) of T2 tumors and 20.4% (10) of T1 tumors.

Our research revealed that post-menopausal patients had a 3.54 [OR-3.54 (1.00, 6.54; $p = 0.0023$] times higher risk of COX-2 positive and T2 tumors had a 6.70 [OR-6.70 (3.17, 15.39; $p = 0.0482$] times higher risk. Both risks were statistically significant. The odds of having COX-2 positive were 2.40 [OR-2.40 (0.84, 7.53); $p = 0.07$] and 2.42 [OR-2.42 (0.71, 8.64); $p = 0.950$] times greater for patients who were older (>50 years) than those who were younger (50 years), respectively, although the odds were not statistically significant.

In our study, COX-2 was activated in 75% cases of human ovarian carcinoma as analyzed by western blotting and RT-PCR, where as COX-2 was undetectable

Table 1: Clinico-pathological details according to COX-2 status

	COX-2 positive (49)		COX-2 negative (16)			
	No. of patients	Percentage	No. of patients	Percentage	Z-value	p-value
Menopausal status						
Premenopausal	13	26.5	7	43.7	5.49	0.0278
Postmenopausal	36	73.5	9	56.3	9.56	<0.0001
Age (years)						
≤50	25	51.0	9	56.3	6.61	0.0060
>50	24	49.0	7	43.7.	8.80	0.0022
Stage						
I	7	14.3	4	25.0	1.63	>0.0500
II	14	28.5	3	18.7	3.53	<0.0001
III	19	38.8	8	50.0	9.94	<0.0001
IV	9	18.4	1	6.3	2.67	<0.0001
Tumor size (cm)						
T1	10	20.4	2	12.5	1.60	>0.0500
T2	12	24.5	11	68.7	5.74	<0.0001
T3	27	55.1	3	18.8	9.62	<0.0001
Grades						
I	4	8.2	7	43.8	2.35	<0.0500
II	12	24.5	5	31.2	3.24	<0.0001
III	33	67.3	4	25.0	11.71	<0.0001

Table 2: Different risk factors under univariate analysis according to COX-2 status

	COX-2 negative		COX-2 positive			
	No. of patients	Percentage	No. of patients	Percentage	Odds ratio with 95% CI	p-values
Menopausal status						
Premenopausal	13	26.5	11	68.8	3.54 (1.00,6.54)	0.0023
Postmenopausal	36	73.5	5	31.2		
Age (years)						
≤50	25	51.0	9	56.3	2.40 (0.84, 7.53)	0.0700
>50	24	49.0	7	43.7		
Stage						
Early (I, II)	21	42.9	7	43.8	2.30 (0.81, 6.57)	0.9500
Advance (III, IV)	28	57.1	9	56.2		
Tumor size (cm)						
T1	18	36.7	2	12.5	6.70 (3.17, 15.39)	0.0482
T2	31	63.3	14	87.5		

in the control group. COX-2 has been shown to be expressed in both ductal carcinoma in situ and invasive ductal carcinoma but not in normal ovarian tissue in several research articles[11-13]. In this study, COX-2 expression was seen more frequently in postmenopausal patients.

Numerous investigations have shown that the expression of COX-2 was substantially connected with large tumor size and advanced illness stage[14-17]. We demonstrated that COX-2 expression was higher in advanced ovarian cancer than in early ovarian carcinoma. Large-sized and high-grade tumors were substantially linked in this study with COX-2 activation. In ovarian cancer patients, COX-2 overexpression is associated with aggressive phenotypic traits like high grade, large tumor size status. It is conceivable that COX-2 expression could be a crucial biomarker for determining tumor aggressiveness in therapeutic settings. Novel treatment approaches that reduce COX-2's abnormal expression or activity may play a significant role in the prevention of human ovarian cancer if COX-2 expression represents an early initiating event in the development of ovarian cancer.

CONCLUSION

In ovarian cancer, COX-2 expression has been linked to factors such postmenopausal status, age greater than 50, advanced disease stage, large tumor size and higher grade. This also shows that COX-2 expression is linked to an aggressive tumor biology and can predict tumors with a bad prognosis. Therefore, inhibiting COX-2 expression may slow tumor growth and stop ovarian carcinogenesis, which may have significance for ovarian cancer prevention in high-risk individuals.

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