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Expression of cyclooxygenase-2 in epithelial ovarian cancers: A study of 101 cases with high proportionof non-serous carcinoma

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Expression of cyclooxygenase-2 in epithelial ovarian cancers: A study of 101 cases with high proportion of non-serous carcinoma

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Background : *Recent studies have reported that expression of cyclooxygenase-2 (COX-2) is elevated in ovarian cancer and may involve in carcinogenesis. Elevated COX-2 expression has also been identified as an independent prognostic factor which is associated with reduced survival.*

Objective : *To investigate COX-2 expression with clinicopathological correlation including survival in a single institutional series of epithelial ovarian cancer.*

Design : *Descriptive study*

Setting : *Department of Obstetrics and Gynecology and Department of Pathology, Faculty of Medicine, Chulalongkorn University*

Methods : *COX-2 was evaluated as high and low expression by immunohistrochemical study of paraffin-embedded tissue from 101 patients with epithelial ovarian cancer (16 serous carcinomas, 19 mucinous carcinomas 40 endometrioid carcinomas, and 26 clear cell carcinomas) who underwent primary surgery at the King Chulalongkorn Memorial Hospital between 2000 to 2002. The relationships between COX-2 expression and clinicopathologic characteristics including survival were determined.*

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Results : *High COX-2 expression was observed in 67 of 101 epithelial ovarian cancers (66.3 %), and it was associated with histologic type and grade. Mucinous ovarian cancer has the highest rate of COX-2 expression, while high COX-2 expression was the least common in endometrioid histologic type. Univariate analysis displayed statistical significance in recurrence and COX-2 expression. In multivariate Cox regression, a significant effect of high COX-2 expression on prolonged survival was obtained.*

Conclusion : *High COX-2 expression was observed in 66.3 % of epithelial ovarian cancers and was associated with the histologic type and grade, but was not associated with worsen prognosis.*

Keywords : *Cyclooxygenase-2, Epithelial ovarian cancer, Non-serous carcinoma.*

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พธู ตัณฑ์ไพโรจน์, ชวิษฐ์ จันทรานุวัฒน์, สุรางค์ ตรีรัตนชาติ, ประเสริฐ ตรีวิจิตรศิลป์, สมชัย นิรุตติศาสน์.การศึกษา Cyclooxygenase-2 ในมะเร็งรังไข่ชนิดเยื่อบุผิวในผู้ป่วยจำนวน 101 ราย. จุฬาลงกรณ์เวชสาร 2550 เม.ย; 51(4): 203 - 16

เหตุผลของการทำวิจัย : *จากรายงานก่อนหน้านี้พบมีการแสดงออกของ Cyclooxygenase-2 เพิ่มขึ้นในมะเร็งรังไข่ และคาดว่าอาจเป็นสาเหตุที่ก่อให้เกิดมะเร็งรังไข่ นอกจากนี้ยังพบว่ามีความสัมพันธ์กับการพยากรณ์โรคที่ไม่ดี*

วัตถุประสงค์ : *เพื่อสำรวจการแสดงออกของ Cyclooxygenase-2 รวมถึงความสัมพันธ์ทางพยาธิวิทยาคลินิกในมะเร็งรังไข่ชนิดเยื่อบุผิวที่มารับการรักษาที่โรงพยาบาลจุฬาลงกรณ์*

รูปแบบการวิจัย : *การศึกษาเชิงพรรณนา*

สถานที่ทำการศึกษา : *ภาควิชาสูติศาสตร์-นรีเวชวิทยา และภาควิชาพยาธิวิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย*

วัสดุและวิธีการ : *ศึกษาการแสดงออกของ Cyclooxygenase-2 โดยวิธีการย้อม immuno-histochemistry ในชิ้นเนื้อจากบล็อกพาราฟินที่ได้จากผู้ป่วยมะเร็งรังไข่ชนิดเยื่อบุผิว ที่มารับการผ่าตัดที่โรงพยาบาลจุฬาลงกรณ์ในช่วงปี พ.ศ.2543 -2545 จำนวน 101 ราย ซึ่งประกอบด้วย มะเร็งชนิด serous 16 ราย, มะเร็งชนิด mucinous 19 ราย, มะเร็งชนิด endometrioid 40 ราย, และมะเร็งชนิด clear cell 26 ราย โดยแบ่งการแสดงออกของ Cyclooxygenase-2 เป็นประเภทสูงและต่ำ จากนั้นนำมาประเมินความสัมพันธ์ทางพยาธิวิทยาคลินิกรวมถึงอัตราการอยู่รอด*

ผลการศึกษา : *ผู้ป่วยมะเร็งรังไข่ชนิดเยื่อบุผิวจำนวน 67 ราย (66.3 %) พบมีการแสดงออกของ Cyclooxygenase-2 สูง มีความสัมพันธ์กับชนิดและเกรดทางจุลพยาธิ โดยที่พบมีการแสดงออกสูงจำนวนมากที่สุดในมะเร็งชนิด mucinous และจำนวนน้อยที่สุดในมะเร็งชนิด endometrioid ในการประเมินความสัมพันธ์ของปัจจัยที่มีผลต่ออัตราการอยู่รอด พบว่าการกลับเป็นซ้ำของโรคและการแสดงออกของ Cyclooxygenase-2 มีผลต่ออัตราการอยู่รอดอย่างมีนัยสำคัญทางสถิติ โดยที่การแสดงออกของ Cyclooxygenase-2 สูงมีความสัมพันธ์กับอัตราการอยู่รอดที่เพิ่มขึ้น*

สรุป : *การแสดงออกของ Cyclooxygenase-2 สูงพบใน 66.3 % ของผู้ป่วยมะเร็งรังไข่ชนิดเยื่อบุผิว และมีความสัมพันธ์กับชนิดและเกรดทางจุลพยาธิแต่ไม่สัมพันธ์กับการพยากรณ์โรคที่ไม่ดี*

คำสำคัญ : *Cyclooxygenase-2, มะเร็งรังไข่ชนิดเยื่อบุผิว, Non-serous carcinoma.*

Ovarian cancer is the sixth most common cancers which are the causes of death from cancer in women worldwide (4.4 % of cases and 4.5 % deaths). Its incidence rates are highest in developed countries, with the rates in these areas exceeding 8 per 100,000. [1] The classification of ovarian tumor is currently based on the histogenesis of a normal ovary. The majority (85 % - 90 %) of malignant ovarian tumors are epithelial. [2] In the western countries, serous type is the most common, comprising >50 % of the cases.[3] However, there is yet still no adequate information on the incidence in Asian countries. Despite recent advances in surgery and chemotherapy, improvement in long-term survival of these patients with epithelial carcinomas has been slight.[4]

Little is known about the molecular events leading to its development and progression. Recent studies have suggested that an increase in the expression of cyclooxygenase-2 (COX-2) may play a significant role in carcinogenesis. The function of cyclooxygenase (COX) enzyme is to catalyze the conversion of arachidonic acid to prostaglandin. Two isoforms of COX are known. COX-1 is constitutively expressed in most types of tissue and has been connected to physiologic function. In contrast, COX-2 is an immediate-early gene, which can be induced by various stimuli including mitogens, proinflammatory cytokines, growth factors, and tumor promoters, and its role has been connected to inflammatory processes, carcinogenesis, and tumor progression. [5-10] Expression of the COX-2 isoform is elevated in a variety of human malignancies and in premalignant lesions. Functionally, COX-2 derived prostenoids have shown to promote angiogenesis, induce invasion, and increase metastasis. [7,8] Because of its high expression in neoplasms, COX-2 constitutes a relevant target in treatment and chemoprevention of cancer.

A number of recent studies have reported that expression of COX-2 is elevated in ovarian cancer. [11-14] Moreover, in ovarian cancer, elevated COX-2 expression has been identified as an independent prognostic factor [15] which is associated with reduced survival [16] and poor response to standard combination chemotherapy.[17] This study was conducted to evaluate COX-2 expression in epithelial ovarian cancer and to assess whether the expression of COX-2 protein is associated with clinicopathological parameters and clinical outcome in Thai women as analyzed by immunohistochemistry.

Materials and Methods

Case selection

Of the 136 cases of epithelial ovarian cancer retrieved from surgical pathology files of the Division of Gynecologic Pathology, the Department of Obstetrics and Gynecology, Faculty of Medicine, Chulalongkorn University, from 2000 to 2002; 101 patients who underwent primary surgery without previous chemotherapy which had available clinical follow-up data and paraffin-embedded tissue specimens were included in this study. Hematoxylin-eosin-stained sections from each case were reviewed by two pathologists to confirm the histological diagnosis and assess pathological features and grade.[18] The most representative paraffin block for each case was selected for immunohistochemical analysis.

From a retrospective review of medical records, the patient's demographic and surgical data

were collected. Survival data were retrieved from the database files of the Division of Gynecologic Oncology and the institution computerized clinical information system. Surgical staging was determined with the criteria that are recommended by the International Federation of Gynecology and Obstetrics (FIGO). Optimal surgery was defined as the residual tumor of no greater than 1-2 cm in diameter at any site.[2] Post operative platinum-based chemotherapy was offered to all patients in stage Ib, Ic, and II-IV. The exception of chemotherapy receiving included patients in stage Ic by intraoperative accidental rupture of ovarian tumor and patients with mucinous carcinoma in stage I. COX-2 inhibitor was not administrated in any patient. Survival time was calculated from the date of diagnosis until May 2005.

Immunohistochemical study

We studied COX-2 with goat polyclonal antibody anti-COX-2 (Mo a Hu COX-2, clone: CX-294, Santa Cruz Biotechnology, Santa Cruz, CA; 1:100 dilution), with biotinylated rabbit anti-goat with biotinylated rabbit anti-goat secondary antibody (Dako, Carpenteria, CA; 1:100 dilution). Sections were deparaffinized and subjected to immunohistochemical staining, with standard streptavidin-biotin-peroxidase techniques, and diaminobenzidine (DAB) as the chromogen. Sections of 4 to 5 micron thick underwent antigen retrieval by steam treatment in a citrate buffer. Endogenous peroxidase activity was removed by 3 % hydrogen peroxide (10 minutes). Nonspecific binding sites were blocked with 3 % normal horse serum in phosphate buffered saline solution (PBS) for 20 minutes at room temperature. After incubation with the primary antibodies, slides were rinsed with PBS, and the secondary antibody was applied at 1:100 in 3 % normal horse serum for 30 minutes at room temperature. After rinses with PBS, slides were incubated with streptavidin/ peroxidase at 1:500 in PBS for 30 minutes at room temperature, then rinsed with PBS and incubated for 10 minutes in DAB solution and counterstained with Meyer's hematoxylin. Negative control was performed by substituting the primary antibody with nonimmune sera. Appropriate positive and negative controls were run simultaneously. The immunohistochemically stained sections were evaluated without previous knowledge of the clinical outcome of each patient.

Evaluation of the cyclooxygenase-2 expression

Two investigators individually evaluated the slides blindly under a light microscope. The concordance rate is 90 % between the 2 pathologists. In case of disagreement, the slides were reviewed simultaneously under a multiheaded microscope with a resolution of the different opinion.

Only brown staining in cytoplasm of tumor cells were scored as positive. The staining intensity and the percentage of tumor cells positively stained were analyzed. The proportion of positive cells was scored at low magnification (40x) by evaluating the entire tumor area. The intensity of staining was evaluated subjectively using a scale ranging from 0 (none), 1 (weak), 2 (medium), and 3 (strong). Low expression was defined as intensity 0, 1, 2, or 3 and <10 % cells staining or intensity 0, 1 and <50 % cells staining; high expression was defined as intensity 2, 3, and >10 % of cells staining or intensity 1, 2, 3, and >50 % of cells staining. [19]

Statistical analyses

Statistical analyses were performed with the SPSS for Windows software (version 13; SPSS Inc, Chicago, IL,USA). The association between COX-2 expression and the other prognostic variables was assessed with the Chi-square and Fisher's exact tests. Survival times were estimated in days from the date of diagnosis to the date of death or last follow-up. Survival analysis was computed with Kaplan-Meier method and association with various prognostic variables were made with the log-rank test. A univariate analysis, with Cox proportional hazards models, was used to determine which variables had an effect on clinical outcome. Multivariate survival analysis was performed using Cox proportional hazards model to determine which variables had an independent effect on clinical outcome. Statistical significance was defined as a probability value (P value) <0.05.

Results

COX-2 Immunostaining

Of 101 patients included in our study, the survival data were available in 92 patients. The mean age of the patients was 49.88 years (18 - 83 years). Only 16 patients (15.8 %) had serous carcinomas. Of the 85 non-serous tumors, 19 (18.8 %) were mucinous carcinomas, 40 (39.6 %) endometrioid carcinomas, and 26 (25.7 %) clear cell carcinomas. The mean survival time was 32.8 months with a range of 8 days to 62.9 months. Clear cell carcinoma had the highest mortality rate, while the highest survival was in mucinous carcinoma.

COX-2 expression was low in 33.7 % (34/101) and high in 66.3 % (67/101). No significant COX-2 staining was observed in the stromal component. (Figure 1). There was no significant association between COX-2 expression and patient age, parity, menopausal status, FIGO stage, presence of ascites, surgical and chemotherapy treatment, or recurrence. Regarding histologic features, a significant association was only noted between COX-2 expression and the histologic type and grade (Table 1). Mucinous ovarian cancer has the highest rate of COX-2 expression, while high COX-2 expression was the least common in endometrioid histologic type.

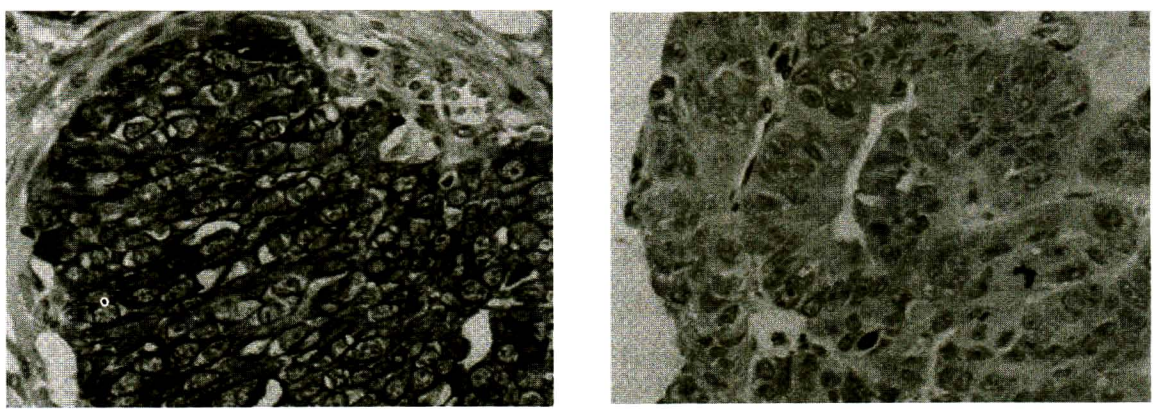

Figure 1. Positive immunuhistochemical staining for COX-2 in two examples of ovarian endometrioid carcinoma is shown. A) High COX-2 expression is characterized by dense cytoplasmic staining. The stromal surrounding the sheet of tumor cell is negative. B) Low COX-2expression demonstrated weak cytoplasmic staining in some tumor cells.

Table 1. Association between clinicopathologic parameters and COX-2 expression.

Parameters	N (%)	COX-2 expression (n)		P value
		Low	High	
Age				
■ <60 yr	81	31 (38.3 %)	50 (61.7 %)	0.064
■ ≥60 yr	20	3 (15.0 %)	17 (85.0 %)	
Parity				
■ Nulliparous	47	17 (36.2 %)	30 (63.8 %)	0.676
■ Multiparous	54	17 (31.5 %)	37 (68.5 %)	
Menopause				
■ Premenopause	56	22 (33 %)	34 (60.7 %)	0.209
■ Postmenopause	45	12 (26.7 %)	33 (73.3 %)	
Stage				
■ I	49	14 (28.6 %)	35 (71.4 %)	
■ II	13	7 (53.8 %)	6 (46.2 %)	0.309
■ III	30	9 (30.0 %)	21 (70.0 %)	
■ IV	9	4 (44.4 %)	5 (55.6 %)	
Ascites				
■ No	27	6 (22.2 %)	21 (77.8 %)	0.161
■ Yes	74	28 (37.8 %)	46 (62.2 %)	
Optimal surgery				
■ No	21	10 (47.6 %)	11 (52.4 %)	0.193
■ Yes	80	24 (30.0 %)	56 (70.0 %)	
Recurrence				
■ No	60	22 (36.7%)	38 (63.3 %)	0.191
■ Yes	38	9 (23.7%)	29 (76.3 %)	
Histologic type				
■ Serous	16	5 (31.3 %)	11 (68.8 %)	
■ Mucinous	19	2 (10.5 %)	17 (89.5 %)	0.044*
■ Endometrioid	40	19 (47.5 %)	21 (52.5 %)	
■ Clear cell	26	8 (30.8 %)	18 (69.2 %)	
Histologic grade				
■ I (Well)	48	10 (20.8 %)	38 (79.2 %)	
■ II (Moderate)	15	9 (60.0 %)	6 (40.0 %)	0.012*
■ III (Poor)	38	15 (39.5 %)	23 (60.5 %)	

* Statistically significant

Univariate Analysis

Cumulative survival curves were calculated according to Kaplan-Meier method, and differences in survival were assessed using log-rank test. Among the clinical parameters analyzed, high FIGO stage (p = <0.001), advance stage (p = <0.001), presence of ascites (p = 0.001), suboptimal surgery (p = 0.008), recurrence (p = <0.001), as well as histologic type (p = 0.024), and high histologic grade (p = 0.016) demonstrated a significant prognostic effect on survival. However, the COX-2 expression in tumor samples revealed no significant effect on patient survival (p = 0.367) (Figure 2). In contrast, the univariate analysis displayed statistical significance in recurrence (p = <0.001) and COX-2 expression (p = 0.004).

Multivariate Analysis

A multivariate progression analysis based on Cox's proportional hazard model was performed to test the independent value of potential parameters predicting survival. Parameters accepted by the multiple Cox regression with forward stepwise selection were only FIGO stage IV, suboptimal surgery, recurrence, and cox-2 expression (Table 2). Forward stepwise selection revealed a hazard ratio (HR) of 0.34 (95 % confidence interval, CI: 0.156 - 0.740; p = 0.007) for high expression of COX-2 in the tumors, showing that the risk to die was reduced by a factor of 0.34 for patients with high COX-2 expression.

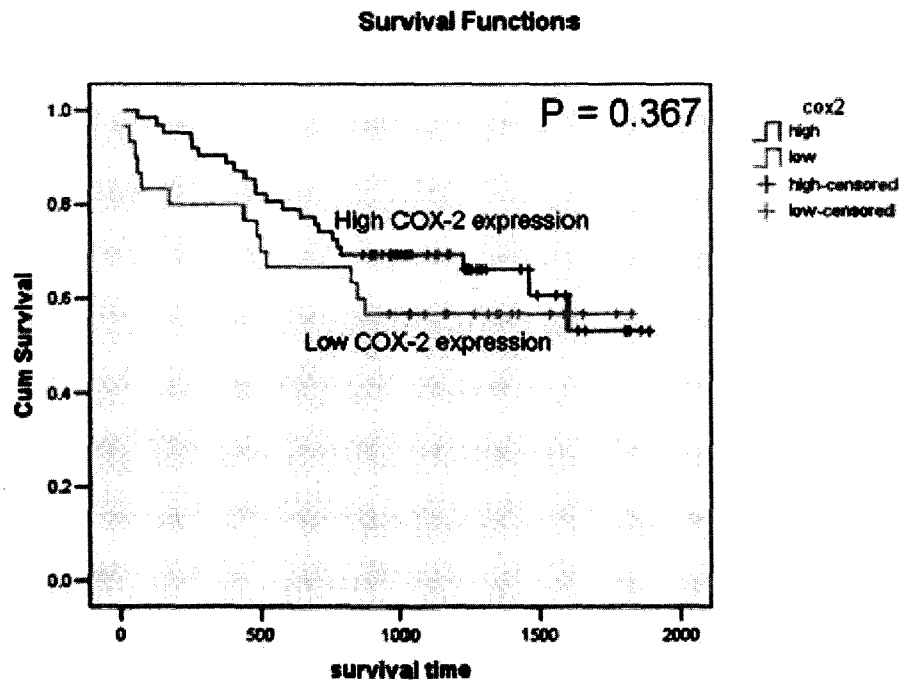

Figure 2. Survival graph of high and low COX-2 expression.

Table 2. Multivariate Cox regression with the influential parameters accepted by the forward stepwise selection model.

	HR	95%CI	P value
FIGO stage IV	52.40	11.67-235.31	<0.001
Recurrence	12.33	4.51-33.71	<0.001
Suboptimal surgery	2.70	1.05-6.93	0.039
High COX-2 expression	0.34	0.15-0.74	0.007

Discussion

In most western countries, ovarian carcinoma is the fifth most commpn malignancy and raks fourth in cancer mortality. Serous carcinoma is the most common type of ovarian cancer and account for approximately 50 % of malignant ovarian neoplasms.[20] However, the serous carcinoma is much less common in our institute with the incidence of only 25 -30 % of malignant ovarian tumors. [21,22]

Chemical carcinogenesis experiments and epidemiological and clinical studies have collectively identified prostaglandins and their rate-limiting enzymes, COX-2, as molecules involved in the process of carcinogenesis through a number of mechanisms. These include increased proliferation, reduced apoptosis, and stimulation of metastases and angiogenesis. COX-2 expression plays a pathogenic role in a number of malignancies such as gastric, pancreatic, colorectal, mammary, prostatic, and hepatocellular carcinomas, and to our particular interest, cervical, endometrial, and ovarian carcinomas. [23-28] The development of selective COX-2 inhibitors clearly adds a novel potential pharmacological agent for cancer prevention and treatment.

The prevalence of COX-2 expression in epithelial ovarian cancer varied greatly, depended on the difference of histologic type and positive-staining criteria. However, the overall percentage of high COX-2 expression in our study was quite similar to those previously reported. [19,29, 30] In this study, only histologic type and grade were significantly associated with high COX-2 expression. Surprisingly, high COX-2 expression was demonstrated as an independent prognostic factor inversely associated with risk of death by the multivariate analysis. This strange result may be explained by the different proportion of histologic types. Our population was mainly composed of nonserous ovarian cancer (84.2 %) with rather high proportion of the clear cell carcinoma (25.7 %). Microscopically, clear cell carcinomas display several different patterns and cytoplasmic features. The latter can range from scant amount in hobnail-like cells to moderate amount of clear cytoplasm. Because the brown staining within the cytoplasmic substance is required for the interpretation of positive staining, the clear cytoplasm due to accumulation of abundant glycogen in clear cell carcinomas tend to make them interpreted as low expression. Together with the fact that clear cell carcinoma has the highest mortality rate compared

to the other histologic types (Table 3), these may explained the conflicting results in our study. After we excluded the cases of clear cell carcinomas, the multivariate analysis of the COX-2 expression displayed no statistically significant correlation with the survival (p = 0.653).

It is well known that FIGO stage is the powerful predictor of prognosis in ovarian cancer and the stage distribution of ovarian cancer varies by histologic type. The highest proportion of FIGO stage I cases is found among mucinous carcinomas, in approximately 50 % of cases, while only 16 % of serous carcinomas are diagnosed in stage I. [18] Most cases in our study population were non-serous carcinomas (84.2 %) and mainly in FIGO stage I (Table 4). Another explanation for the inverse relationship of mortality and the high COX-2 expression in this study is that patients with mucinous carcinomas in FIGO I have the highest survival rate and the highest rate of high COX-2 expression by immunohistochemical study. In our study, 84.2 % of mucinous carcinoma are in stage I, almost survived (93.8 %) and mostly displayed high COX-2 expression (82.4 %). These findings may interfere with the overall survival, resulting in longer survival time in the high COX-2 expression group. The rather high staining of COX-2 in mucinous carcinoma in this study is also different from the previos study of Seo SS [30] which found very low staining in mucinous tumor (5.6 %). This result may be due to different antibody type and method of immunohistochemical staining used, including the different criteria to evaluate immunostaining.

Table 3. Association between histologic type and death (n = 92).

Histologic type	Death		P value
	No	Yes	
Serous	7 (63.6 %)	4 (36.4 %)	
Mucinous	15 (83.3 %)	3 (16.7 %)	
Endometrioid	24 (64.9 %)	13 (35.1 %)	0.048
Clear cell	11 (42.3 %)	15 (57.7 %)	

Table 4. Histologic type and distribution of FIGO stage (n = 101).

Histologic type	FIGO stage				Total
	I	II	III	IV	
Serous	3 (18.8 %)	2 (12.5 %)	9 (56.2 %)	2 (12.5 %)	16
Mucinous	16 (84.2 %)	0 (0 %)	1 (5.3 %)	2 (10.5 %)	19
Endometrioid	15 (37.5 %)	7 (17.5 %)	15 (37.5 %)	3 (7.5 %)	40
Clear cell	15 (57.7 %)	4 (15.4 %)	5 (19.2 %)	2 (7.7 %)	26

Chi-square test: p = 0.008

Most of the published reports were investigated in the population composed mainly of serous carcinoma (range from 42.2 - 75.0 %), and clearly demonstrated a significant correlation between high COX-2 expression and worse prognosis, including metastasis and poor response to the treatment. [15,17,29-32] However, serous carcinoma composed only 15.8 % of cases in this study. Thus, we believed that this low incidence reflected closely to the actual incidence of the serous ovarian cancer in our country because our hospital is a large refereal institute that received ovarian cancer patients from around the country without exclusion. Similar low incidence of serous carcinoma also noted in other referral centers in Thailand. [33,34] Our study is the first to show that COX-2 expression was not definitely associated with unfavorable prognosis, especially in the population with high proportion of non-serous carcinoma.

It is important to recognize that this study has limitations including the limited number of cases, the only one institutional experience, and the staining interpretation is semiquantitative. However, despite of these limitations, it adds to our understanding the association between COX-2 expression and clinical parameters in the different population of the epithelial ovarian cancer. Our findings suggest that more caution in the immunohistochemical scoring and analysis is required in the further studies, especially in the patients with clear cell carcinoma. A larger cohort study that in addition to immunohistochemistry evaluation of COX-2 with more sensitive techniques, such as PCR-based analyses, is clearly warranted to elucidate the role of COX-2 in the epithelial ovarian cancer, especially in nonserous carcinoma.

As previously mentioned, high COX-2 expression was observed in more than half of the patients with epithelial ovarian carcinoma. A number of recent trials indicated that nonsteroidal anti-inflammatory agents (NSAIDs) or selective inhibitors of COX-2 may have a role in cancer treatment and even the chemoprevention in some cancers such as cancer of the breast and colon. [10,24,35,36] Therefore, a study of combining COX-2 inhibitors and paclitaxel in epithelial ovarian cancer cells showed no additive or synergistic. [37] However, COX-2 inhibitors still merit further development and studies as they could become alternative agents for treatment of invasive disease or cancer prevention.

In conclusion, high COX-2 expression in tumor tissue was observed in 66.3 % of the patients with epithelial ovarian carcinoma and was associated with the histologic type and grade. However, high COX-2 expression in our population was not associated with worsen prognosis. This result may be influenced by the high proportion of the clear cell carcinoma with difficulty in the staining interpretation and the presence of many FIGO stage I mucinous carcinomas with high COX-2 expression. More studies on COX-2 expression especially in the population with high proportion of non-serous carcinomas are needed.

Acknowledgments

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