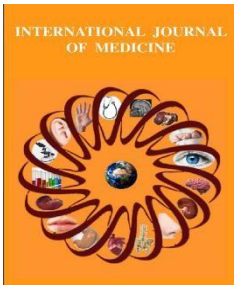

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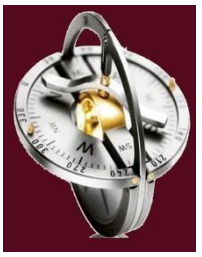

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Original Article

Prognostic and therapeutic implications of lysyl oxidase and cyclooxygenase 2 expressions in epithelial ovarian carcinoma

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Abstract

Background: New prognostic and predictive biomarkers for better choice and improving the current therapies for ovarian cancer are greatly needed. This study aimed to investigate the prognostic and therapeutic importance of lysyl oxidase (LOX) and cyclooxygenase 2 (COX2) expressions in epithelial ovarian carcinoma.

Methods: We performed immunohistochemical analysis on formalin-fixed paraffin sections of epithelial ovarian tumors. The association between their expressions in epithelial ovarian carcinoma (EOC) with the survival as well as response to chemotherapy was analyzed.

Results: The frequency of the nuclear expression of LOX and cytoplasmic expression of COX2 was significantly higher in malignant tumors than in benign and borderline tumors. Also, there were statistically significant relationships between pathological grades and both LOX and COX2 positivity in EOC being at the higher end for poorly differentiated tumors. Both LOX and COX2 expressions were also correlated significantly with higher tumor stage. Overexpression of either LOX or COX2 in EOC was significantly associated with poor survival. Moreover, LOX and COX2 positive expressions in EOC were associated with poor response to chemotherapy.

Conclusions: The increased expressions of LOX and COX2 in EOC are associated with several adverse clinicopathologic parameters, including reduced survival and chemotherapy resistance thus suggesting a role for such biomarkers in disease progression.

Keywords: cancer, cyclooxygenase 2, lysyl oxidase, ovary, prognosis

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Introduction

Ovarian cancer (OC) is one of the most common female malignancies worldwide. In Egypt, it represents the most common gynecologic cancer and the sixth cause of cancer death in females [1]. On the other hand, the majority of all ovarian tumors (60 -70%) are of the epithelial type and their malignant forms represent >90% of ovarian cancers [2]. High-grade serous carcinomas (HGSCs) account for approximately 70% of all epithelial ovarian carcinomas (EOCs) [3]. The main line of treatment in early EOCs is surgery with adjuvant chemotherapy, the 5-year survival in early stages ranges between 80% and 93%, while in late stages it is less than 30% [4]. Unfortunately, the majority of patients (60%-70%) present with an advanced stage due to non-specificity of symptoms and lack of reliable screening biomarkers [5]. This situation calls for the investigation of EOC and identification of new prognostic and predictive markers for use as targets in treatment.

Cyclooxygenases (COXs) catalyze the first rate-limiting step in the conversion of arachidonic acid to prostaglandins. Two COX enzymes have been identified: COX1 is constitutively expressed in most tissues whereas COX2 is not detectable in normal tissues. COX2 can be seen in various tumors and rapidly induced by various stimuli such as inflammatory reactions [6]. In addition to its upregulation in malignancy, COX2 expression also plays an important role in malignant transformation of the epithelial cells of premalignant lesions [7]. Furthermore, several functions of COX2 have been described in the pathogenesis of various carcinomas such as increased cell proliferation, inhibition of apoptosis and stimulation of angiogenesis [8]. Thus, COX2 overexpression has the potential to promote tumor progression and the development of new specific inhibitors may lead to new concepts in cancer chemoprevention.

Lysyl oxidase (LOX) is a copper-dependent amine oxidase that catalyzes the cross-linking of different types of collagen and elastin, two basic components of the extracellular matrix (ECM), thus controlling the tensile strength of tissues. It has been recently found to be up-regulated during ovarian carcinogenesis [9]. LOX family of oxidases has a critical role in the transition of tumors from indolent to aggressive disease through regulating epithelial to mesenchymal transition (EMT) transcription factors, a critical e vent in cancer cell invasion and metastasis [10]. The role of such EMT has been documented in ovarian carcinogenesis [11].

The current study aimed to determine LOX and COX2 expressions in EOC and to assess their prognostic role regarding the survival and response to chemotherapy.

Methods

Patients

This study was performed in the Departments of Obstetrics and Gynecology, Pathology and Clinical Oncology, Faculty of Medicine, Zagazig University and included 70 patients with epithelial ovarian tumors who were registered in the period from January 2012 to December 2016. Clinicopathological characteristics of the patients were obtained. Patients were classified according to criteria of the International Federation of Gynecology and Obstetrics (FIGO) staging system [12]. This study was conducted in accordance with the Declaration of Helsinki and written consent was obtained from each participant. Also, the present study was permitted by the Ethical Committee of our university.

Regarding tumor histology and differentiation degree, 70 blocks of formalin-fixed-paraffin-embedded ovarian tissue (one for each case) were evaluated. Cases diagnosed with EOC were examined by two independent pathologists, classified and graded according to th e World Health Organization (WHO) classification system [13]. Other types of ovarian cancers rather than EOC were excluded. All patients were followed up for survival data and the mean follow up period was three years. Regarding patients with EOC, clinical follo w-up was done every three months to all cases, and patients who received any form of chemotherapy were assessed for response according to Response Evaluation Criteria in Solid Tumors (RECIST) [14]. Among the 40 EOC patients, only 14 patients with the resectable e arly-stage disease were treated with radical surgery and adjuvant chemotherapy. In addition, three patients with the initial advanced-stage disease who achieved a complete response to neoadjuvant chemotherapy followed by interval cytoreductive surgery. These patients were followed and assessed for the presence or absence of relapse.

Immunohistochemistry (IHC)

The immunohistochemical staining procedure was done using the streptavidin-biotin immunoperoxidase technique (Dako-Cytomation, Glostrup, Denmark) [15]. Sections of 3-5 µm from the formalin-fixed-paraffin-embedded blocks were cut and mounted on positively charged slides then deparaffinized by xylene and rehydrated in graded alcohol. Thereafter, sections were boiled in buffered citrate (pH 6.0) for about 20 minutes then washed in phosphate-buffered saline (PBS) (pH 7.3). Then, endogenous peroxidase activity was blocked with 6% hydrogen peroxide in methanol. Slides from all blocks were incubated overnight with rabbit polyclonal COX2 (Thermo scientific, Fermont, USA, 1:50), others were incubated with mouse monoclonal LOX (GeneTex International Corporation GTX84182, Dilution 1:50) antibodies. After rinsing in PBS, the slides were immersed with a biotin-conjugated secondary antibody (Lab Vision Corporation, Fermont,

USA). DAB was used as a chromogen and Mayer's Hematoxylin was used as a counterstain and then the slides were washed with distilled water and PBS. Positive and negative controls were stained with the same setting of the studied cases. Lung carcinoma and breast cancer tissue sections were used as a positive control for COX2 and LOX respectively. For negative control, primary antibodies were replaced by non-immune normal serum.

Immunohistochemical scoring

Cytoplasmic stain as positive for COX has been considered positive and the distribution of COX2 was scored independently and in blinded manner by two investigators on the following scale; 0, no staining; 1, weak cytoplasmic staining (may contain stronger intensity in <10% of the cancer cells); 2, moderate cytoplasmic staining in >10% of the cancer cells; and 3, strong cytoplasmic staining in >50% of cancer cells [16].

Nuclear staining has been considered positive for LOX and the scoring criteria for LOX intensity were: no color, 0; light yellow, 1; yellow, 2; and brown, 3. For the percentage of tumor positivity, the following scoring was used: negative, 0; 1-25%, 1; 25-50%, 2; and >50%, 3. Both the staining intensity and percentage positivity scores were summed and tumors with scores ranging from 0 to 9 were assi gned to: negative (0-1), + (2), ++ (3-4), +++ ≥5 [17].

Table 1. Clinicopathological features, immunohistochemical markers of 70 patients with ovarian tumors

	All (N=70)	
	N	(%)
Age (years)		
Mean ± SD	46.11	±11.02
Median (Range)	45	22-67
≤50 years	44	62.9%
>50 years	26	37.1%
Tumor type		
Benign tumor	20	28.6%
Borderline tumor	10	14.3%
Malignant tumor	40	57.1%
Histological type		
Serous type	51	72.9%
Mucinous type	15	21.4%
Endometrioid type	4	5.7%
COX2		
Negative	27	38.6%
Positive	43	61.4%
LOX		
Negative	33	47.1%
Positive	37	52.9%
COX2/LOX		
Negative/Negative	22	31.4%
Negative/Positive	5	7.1%
Positive/Negative	11	15.7%
Positive/Positive	32	45.7%

Statistical analysis

Continuous variables were expressed as the (mean±SD) and median (range) and the categorical variables were expressed as a number (percentage). The continuous variables were checked for normality by using the Shapiro-Wilk test. Mann Whitney U test was used to compare between two groups of non-normally distributed variables. Kruskal Wallis H test was used to compare between more than two

groups of normally distributed variables. Percent of categorical variables were compared using Pearson's Chi-square test or Fisher's exact test when appropriate. Disease Free Survival (DFS) was calculated as the time from date of surgery to relapse or the most recent follow-up in which no relapse was detected. Overall survival (OS) was calculated as the time from diagnosis to death or the most recent follow-up contact (censored). Stratification of DFS and OS was done according to immunohistochemical markers. These time-to-event distributions were estimated using the method of Kaplan-Meier plot and compared using two-sided exact log-rank test. A *p*-value of ≤0.05 was considered significant. All statistics were performed using SPSS 22.0 for Windows (SPSS Inc., Chicago, IL, USA) and MedCalc Windows (MedCalc Software bvba 13, Ostend, Belgium).

Table 2. The comparison between benign, borderline and malignant ovarian tumors

	All (N=70)		**Benign ovarian tumors (N=20)**		**Borderline ovarian tumors (N=10)**		**Malignant ovarian tumors (N=40)**		***p*-value**
	N	(%)	N	(%)	N	(%)	N	(%)	
Age (years)									
Mean ± SD	46.11	±11.02	38.50	±8.40	41.80	±11.39	51	±9.57	<0.001•
Median (Range)	45	22-67	39.50	22-55	39.50	22-63	52.50	28-67	
≤50 years	44	62.9%	19	95%	8	80%	17	42.5%	<0.001‡
>50 years	26	37.1%	1	5%	2	20%	23	57.5%	
Histological type									
Serous type	51	72.9%	15	75%	7	70%	29	72.5%	0.437‡
Mucinous type	15	21.4%	5	25%	3	30%	7	17.5%	
Endometrioid type	4	5.7%	0	0%	0	0%	4	10%	
COX2									
Negative	27	38.6%	14	70%	5	50%	8	20%	0.001‡
Positive	43	61.4%	6	30%	5	50%	32	80%	
LOX									
Negative	33	47.1%	15	75%	6	60%	12	30%	0.003‡
Positive	37	52.9%	5	25%	4	40%	28	70%	
COX2/LOX									
Negative/Negative	22	31.4%	12	60%	3	30%	7	17.5%	0.001‡
Negative/Positive	5	7.1%	2	10%	2	20%	1	2.5%	
Positive/Negative	11	15.7%	3	15%	3	30%	5	12.5%	
Positive/Positive	32	45.7%	3	15%	2	20%	27	67.5%	

• Kruskal Wallis H test, ‡ Chi-square test.

Results

Patients' characteristics

The present study included 70 patients with epithelial ovarian tumors: 20 benign, 10 borderline and 40 EOC. The age distribution of patients with benign tumors ranged between 22-55 years with a mean age of 38.5 (±8.4) while the age of patients with borderline tumors ranged between 22-63 years with a mean age of 41.8 (±11.39) (Tables 1, 2).

Regarding EOC patients, the age at the time of initial diagnosis ranged from 28 to 67 years with a mean age of 51 (±9.57) years. Fourteen of these 40 patients (35%) presented with early-stage disease and underwent radical surgery followed by adjuvant chemotherapy. On the other hand, twenty-six of EOC patients (65%) presented with advanced bulky disease or with significant comorbidities which precluded primary debulking surgery. These patients had received adjuvant chemotherapy and evaluated for response according to RECIST criteria. The median follow-up time was 26.5 months (range: 12-36 months) and during the follow-up period, 29.4% of the patients were disease-free without relapse. Recurrence occurred in 70.6% of the patients, while 62.5% of the patients died during follow -up. The clinicopathological characteristics of the 40 patients with EOC are summarized in Table 3.

Table 3. Clinicopathological features, immunohistochemical markers and outcome of 40 patients with malignant ovarian tumors

Characteristics	All (N=40)		Characteristics	All (N=40)	
	N	(%)		N	(%)
Age (years)			**LOX**		
Mean ± SD	51	±9.57	Negative	12	30%
Median (Range)	52.50	28-67	Positive	28	70%
≤50 years	17	42.5%	**COX2/LOX**		
>50 years	23	57.5%	Negative/Negative	7	17.5%
Histological type			Negative/Positive	1	2.5%
Serous type	29	72.5%	Positive/Negative	5	12.5%
Mucinous type	7	17.5%	Positive/Positive	27	67.5%
Endometrioid type	4	10%	**Response**	(N=26)	
Grade			Complete response	3	11.5%
Grade I	7	17.5%	Partial response	2	7.7%
Grade II	18	45%	Stationary disease	7	26.9%
Grade III	15	37.5%	Progressive disease	14	53.8%
Distant metastasis			Overall response	5	19.2%
Absent	27	67.5%	No response	21	80.8%
Present	13	32.5%	**Follow-up duration (months)**		
Stage			Mean ± SD	26.72	±8.60
Stage I	5	12.5%	Median (Range)	26.50	12-36
Stage II	9	22.5%	**Relapse**	(N=17)	
Stage III	13	32.5%	Absent	5	29.4%
Stage IV	13	32.5%	Present	12	70.6%
COX2			**Mortality**		
Negative	8	20%	Alive	15	37.5%
Positive	32	80%	Died	25	62.5%

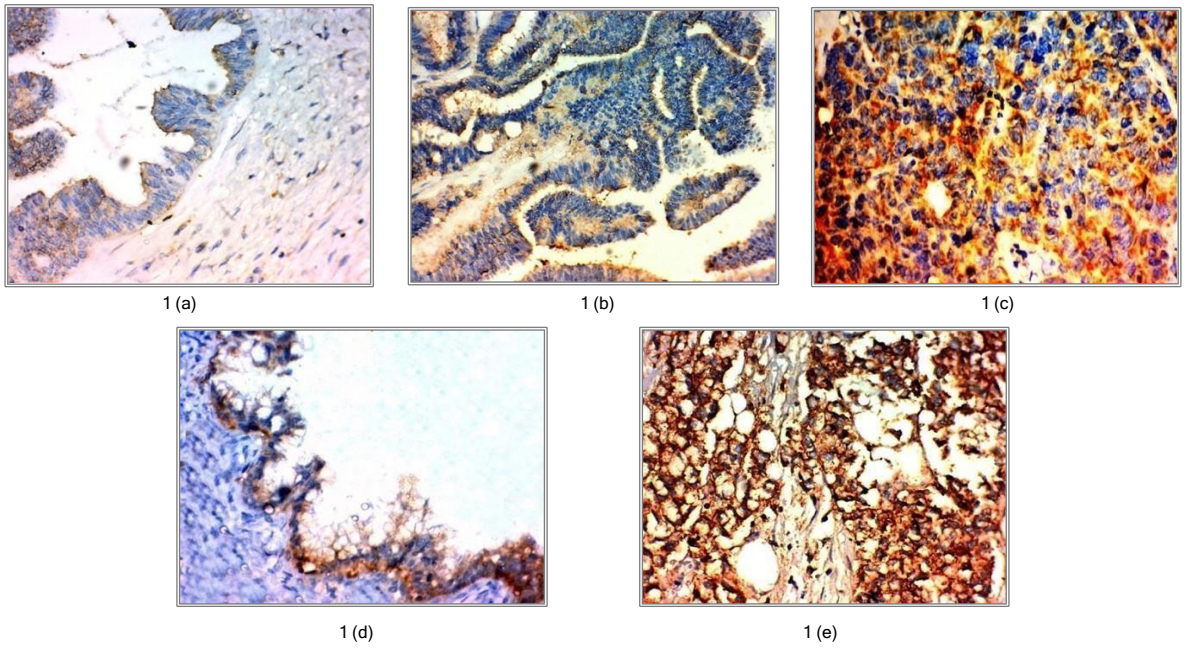


Figure 1. COX2 expressions in ovarian tumors: a) Weak (score 1) immunoreactivity in borderline serous, b) Moderate immunoreactivity (score 2) in serous ovarian adenocarcinoma (grade 1), c) Strong immunoreactivity (score 3) in serous ovarian adenocarcinoma (grade 3), d) Weak (score 1) in benign mucinous adenoma, e) Strong (score 3) in high grade mucinous adenocarcinoma (X400).

Table 4. The relation between clinicopathological features and immunohistochemical markers of 40 patients with malignant ovarian tumors

Characteristics	All (N=40)		COX2				*p*-value	LOX				*p*-value
			Negative (N=8)		Positive (N=32)			Negative (N=12)		Positive (N=28)		
	N	(%)	N	(%)	N	(%)		N	(%)	N	(%)	
Age (years)												
Mean ± SD	51	±9.57	44.50	±8.94	52.62	±9.14	0.050•	48.58	±9.84	52.03	±9.44	0.360•
Median (Range)	52.50	28-67	46	28-56	54	35-67		51	28-65	53.50	35-67	
≤50 years	17	42.5%	6	35.3%	11	64.7%	0.053‡	6	35.3%	11	64.7%	0.530‡
>50 years	23	57.5%	2	8.7%	21	91.3%		6	26.1%	17	73.9%	
Histological type												
Serous type	29	72.5%	4	13.8%	25	86.2%	0.195‡	7	24.1%	22	75.9%	0.409‡
Mucinous type	7	17.5%	2	28.6%	5	71.4%		3	42.9%	4	57.1%	
Endometrioid type	4	10%	2	50%	2	50%		2	50%	2	50%	
Grade												
Grade I	7	17.5%	6	85.7%	1	14.3%	<0.001§	6	85.7%	1	14.3%	<0.001§
Grade II	18	45%	2	11.1%	16	88.9%		6	33.3%	12	66.7%	
Grade III	15	37.5%	0	0%	15	100%		0	0%	15	100%	
Distant metastasis												
Absent	27	67.5%	8	29.6%	19	70.4%	0.037‡	12	44.4%	15	55.6%	0.004‡
Present	13	32.5%	0	0%	13	100%		0	0%	13	100%	
Stage												
Stage I	5	12.5%	5	100%	0	0%	<0.001§	4	80%	1	20%	<0.001§
Stage II	9	22.5%	3	33.3%	6	66.7%		5	55.6%	4	44.4%	
Stage III	13	32.5%	0	0%	13	100%		3	23.1%	10	76.9%	
Stage IV	13	32.5%	0	0%	13	100%		0	0%	13	100%	
COX2												
Negative	8	20%						7	87.5%	1	12.5%	<0.001‡
Positive	32	80%						5	15.6%	27	84.4%	
LOX												
Negative	12	30%	7	58.3%	5	41.7%	<0.001‡					
Positive	28	70%	1	3.6%	27	96.4%						

• Mann Whitney U test, ‡ Chi-square test, § Chi-square test for trend.

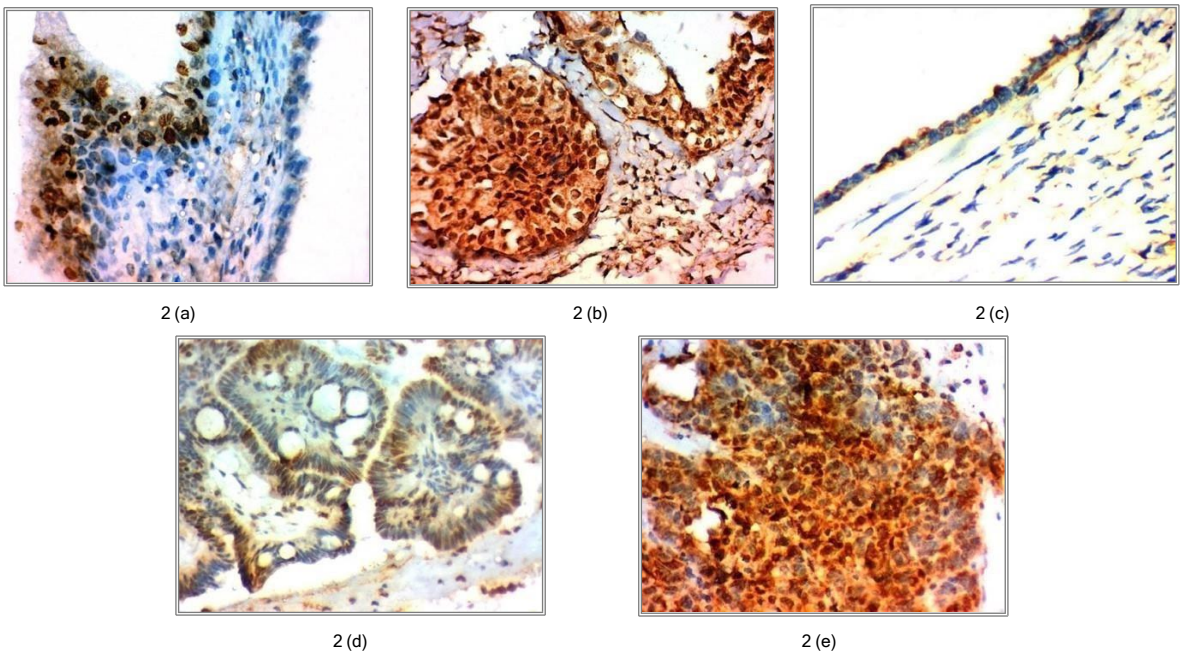
Association of LOX and COX2 expression with clinicopathological parameters

Regarding EOC patients, positive LOX expression was observed in 70% of the patients and positive COX2 IHC staining was observed in 80% of the patients (Tables 4, 5). COX2 was stained in the cytoplasm of cancer cells (Figure 1) while LOX was stained in the nuclei of cancer cells (Figure 2). Both biomarkers were significantly correlated with tumor grade ($p<0.001$ for both). In addition, a significant association was observed between FIGO stage and LOX IHC staining, with 100% of the patients with stage IV disease having posi tive staining versus 20% of the patients with stage I disease ($p<0.001$). Furthermore, a significant association between FIGO stage and COX2 was observed, in which 100% of the patients with stage IV disease had positive staining versus 0% of the patients with stage I disease. In the same way, a significant association between FIGO stage and LOX/COX2 IHC staining was observed, in which 100% of the patients with stage IV had positive staining versus 0% of the patients with stage I disease. Moreover, there was a significant association between LOX and COX2 IHC staining, in which 96.4% of the patients with positive staining for LOX showed positive staining for COX2 versus 58.3% of the patients with negative staining for LOX showed negative staining for COX2.

Table 5. The relation between clinicopathological features and immunohistochemical markers of 40 patients with malignant ovarian tumors

Characteristics	All (N=40)		COX2/LOX								*p*-value
			Negative/Negative (N=7)		Negative/Positive (N=1)		Positive/Negative (N=5)		Positive/Positive (N=27)		
	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	
Age (years)											
Mean ± SD	51	±9.57	44.14	±9.59	47		54.80	±6.76	52.22	±9.56	0.237•
Median (Range)	52.50	28-67	45	28-56			54	46-65	54	35-67	
≤50 years	17	42.5%	5	29.4%	1	5.9%	1	5.9%	10	58.8%	0.164‡
>50 years	23	57.5%	2	8.7%	0	0%	4	17.4%	17	73.9%	
Histological type											
Serous type	29	72.5%	3	10.3%	1	3.4%	4	13.8%	21	72.4%	0.533‡
Mucinous type	7	17.5%	2	28.6%	0	0%	1	14.3%	4	57.1%	
Endometrioid type	4	10%	2	50%	0	0%	0	0%	2	50%	
Grade											
Grade I	7	17.5%	5	71.4%	1	14.3%	1	14.3%	0	0%	<0.001§
Grade II	18	45%	2	11.1%	0	0%	4	22.2%	12	66.7%	
Grade III	15	37.5%	0	0%	0	0%	0	0%	15	100%	
Distant metastasis											
Absent	27	67.5%	7	25.9%	1	3.7%	5	18.5%	14	51.9%	0.026‡
Present	13	32.5%	0	0%	0	0%	0	0%	13	100%	
Stage											
Stage I	5	12.5%	4	80%	1	20%	0	0%	0	0%	<0.001§
Stage II	9	22.5%	3	33.3%	0	0%	2	22.2%	4	44.4%	
Stage III	13	32.5%	0	0%	0	0%	3	23.1%	10	76.9%	
Stage IV	13	32.5%	0	0%	0	0%	0	0%	13	100%	

• Kruskal Wallis H test, ‡ Chi-square test, § Chi-square test for trend.



2 (a) 2 (b) 2 (c)

2 (d) 2 (e)

Figure 2. LOX expressions in ovarian tumors: a) Positive (score 2) immunoreactivity in mucinous ovarian adenocarcinoma (low grade), b) Positive immunoreactivity (score 3) in mucinous ovarian adeno carcinoma (high grade), c) Negative immunoreactivity (score 0) in ovarian serous adenoma, d) Positive (score 2) in serous ovarian carcinoma (grade I), e) Positive (score 3) in high grade serous adenocarcinoma (X400).

Table 6. The relation between each immunohistochemical marker and outcome of 40 patients with malignant ovarian tumors

Characteristics	All		COX2				*p*-value	LOX				*p*-value
			Negative		Positive			Negative		Positive		
	N	(%)	N	(%)	N	(%)		N	(%)	N	(%)	
Response	(N=26)				(N=26)			(N=3)		(N=23)		
Complete response	3	11.5%			3	11.5%		3	100%	0	0%	
Partial response	2	7.7%			2	7.7%		0	0%	2	8.7%	<0.001‡
Stationary disease	7	26.9%			7	26.9%		0	0%	7	30.4%	
Progressive disease	14	53.8%			14	53.8%		0	0%	14	60.9%	
Overall response	5	19.2%			5	19.2%		3	100%	2	8.7%	0.004‡
No response	21	80.8%			21	80.8%		0	0%	21	91.3%	
Relapse	(N=17)		(N=8)		(N=9)			(N=12)		(N=5)		
Absent	5	29.4%	4	50%	1	11.1%	0.131‡	5	41.7%	0	0%	0.245‡
Present	12	70.6%	4	50%	8	88.9%		7	58.3%	5	100%	
Disease free survival												
Mean (month) (95% CI)	30.12 months (27.58-32.66)		34 months (32.45-35.55)		26.67 months (23.45-29.88)			32.17 months (29.87-34.46)		25.20 months (20.46-29.94)		
Median (months)	31 months		34 months		26 months		0.006†	32 months		24 months		0.013†
2-year DFS	76.5%		100%		55.6%			91.7%		40%		
3-year DFS	29.4%		50%		11.1%			41.7%		0%		
Mortality	(N=40)		(N=8)		(N=32)			(N=12)		(N=28)		
Alive	15	37.5%	8	100%	7	21.9%	<0.001‡	12	100%	3	10.7%	<0.001‡
Died	25	62.5%	0	0%	25	78.1%		0	0%	25	89.3%	
Overall survival												
Mean (month) (95% CI)	26.73 months (24.09-29.36)		36 months		24.41 months (21.65-27.17)			36 months		22.75 months (20.12-25.38)		
Median (months)	24 months		Not reached		24 months		<0.001†	Not reached		22 months		<0.001†
1-year OS	95%		100%		93.8%			100%		92.9%		
2-year OS	50%		100%		37.5%			100%		28.6%		
3-year OS	37.5%		100%		21.9%			100%		10.7%		

‡ Chi-square test, † Log rank test.

Association between LOX and COX2 expression and response to adjuvant chemotherapy

Among the twenty-six patients who were inoperable at the time of diagnosis (either due to advanced bulky disease or presence of comorbidity), three patients (11.5%) had complete recovery (CR), two patients (7.7%) had partial recovery (PR), seven patient s (26.9%) had stationary disease (SD) and fourteen (53.8%) patients had progressive disease (PD). LOX/COX2 (positive/positive) staining was significantly associated with poor response to chemotherapy ($p<0.001$) (Tables 6, 7).

Association between LOX and COX2 expression and survival

We analyzed the disease free survival (DFS) and overall survival (OS) of EOC patients using the Kaplan -Meier method (Figure 3). Regarding DFS, a significant difference existed between patients with negative versus those with positive LOX expressions. The mean DFS for patients with negative expression was significantly longer than that with positive expression (32.17 versus 25.2 months, $p\leq0.013$) and the 3-year DFS was 41.7 % versus 0%, respectively. With respect to mortality rate, a significant association between LOX IHC staining was observed, where 89.3% of the patients with positive staining died versus 0% of the patients with negative staining ($p\leq0.001$). Similarly, a significant association between COX2 IHC staining and mortality was observed, in which 78.1% of the patients with positive staining died versus 0% of patients with negative staining.

Moreover, a significant difference was observed between patients with negative LOX expression and those with positive expression with respect to OS, where the mean OS for negative patients was significantly longer than that of positive patients (36 versus 22. 75 months, p<0.001) and 3-year OS was 100% versus 10.7%, respectively. In addition, a significant difference between negative COX2 and positive COX2 patients with respect to OS, where the mean OS for negative patients was significantly longer than the mean OS for positive patients (36 versus 24.41 months, p<0.001) and the 3-year OS was 100% versus 21.9%, respectively. Furthermore, a significant association between LOX/COX2 IHC staining and mortality was found, in which 92.6% of the patients with positive/positive staining died versus 0% of patients with negative/negative staining (Figure 3, Tables 6, 7).

Table 7. The relation between both immunohistochemical markers and outcome of 40 patients with malignant ovarian tumors

Characteristics	**All**		**COX2/LOX**								***p*-value**
			Negative/Negative		Negative/Positive		Positive/Negative		Positive/Positive		
	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	
Response	(N=26)						(N=3)		(N=23)		
Complete response	3	11.5%					3	100%	0	0%	<0.001‡
Partial response	2	7.7%					0	0%	2	8.7%	
Stationary disease	7	26.9%					0	0%	7	30.4%	
Progressive disease	14	53.8%					0	0%	14	60.9%	
Overall response	5	19.2%					3	100%	2	8.7%	0.004‡
No response	21	80.8%					0	0%	21	91.3%	
Relapse	(N=17)		(N=7)		(N=1)		(N=5)		(N=4)		
Absent	5	29.4%	4	57.1%	0	0%	1	20%	0	0%	0.046§
Present	12	70.6%	3	42.9%	1	100%	4	80%	4	100%	
Disease free survival											
Mean (month) (95% CI)	30.12 months (27.58-32.66)		34 months (32.23-35.77)				29.60 months (25.66-33.54)		23 months (20.47-35.53)		<0.001†
Median (months)	31 months		Not reached				30 months		22 months		
2-year DFS	76.5%		100%		100%		80%		25%		
3-year DFS	29.4%		100%		0%		20%		0%		
Mortality	(N=40)		(N=7)		(N=1)		(N=5)		(N=27)		
Alive	15	37.5%	7	100%	1	100%	5	100%	2	7.4%	<0.001§
Died	25	62.5%	0	0%	0	0%	0	0%	25	92.6%	
Overall survival											
Mean (month) (95% CI)	26.73 months (24.09-29.36)		36 months				36 months		22.26 months (19.71-24.81)		<0.001†
Median (months)	24 months		Not reached				Not reached		22 months		
1-year OS	95%		100%		100%		100%		92.6%		
2-year OS	50%		100%		100%		100%		25.9%		
3-year OS	37.5%		100%		100%		100%		7.4%		

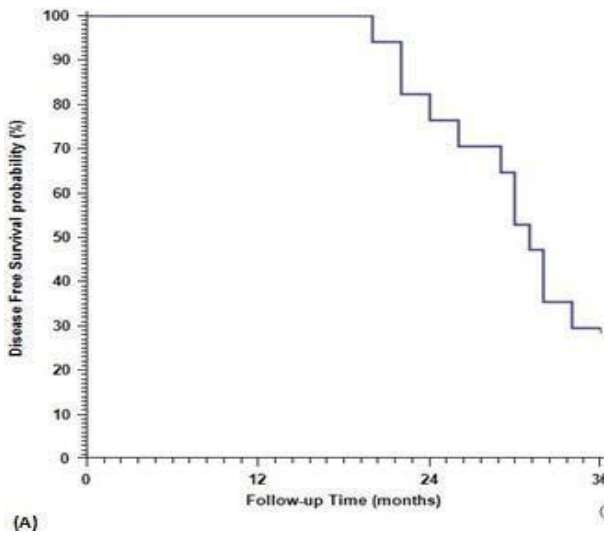
‡ Chi-square test, § Chi-square test for trend, † Log rank test.

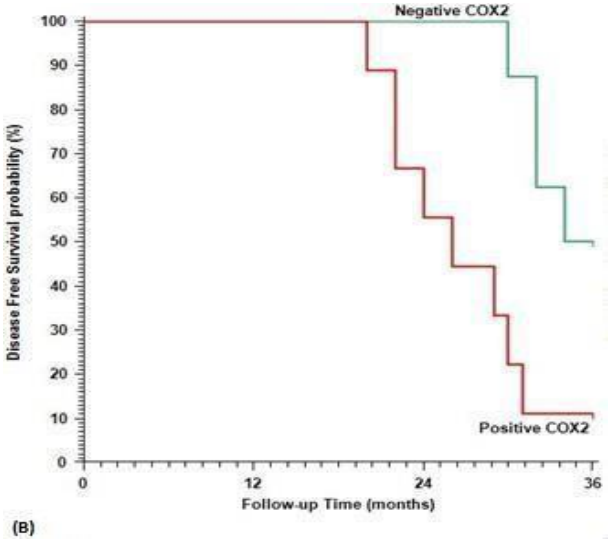
Association between LOX and COX2 expression and tumor relapse

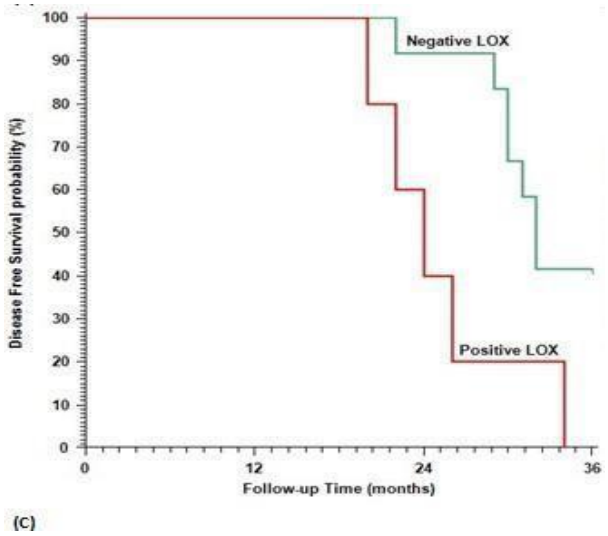
As demonstrated in Table 7, a significant association between LOX/COX2 IHC staining and relapse was observed, in which 100 % of the patients with positive/positive staining relapsed versus 42.9% of the patients with negative/negative staining.

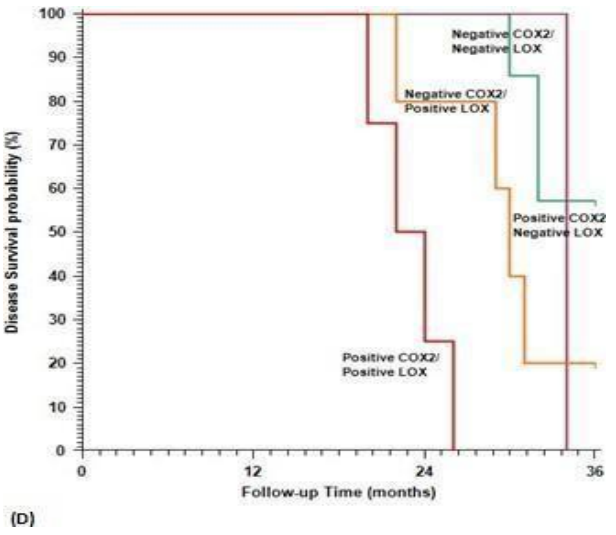
Discussion

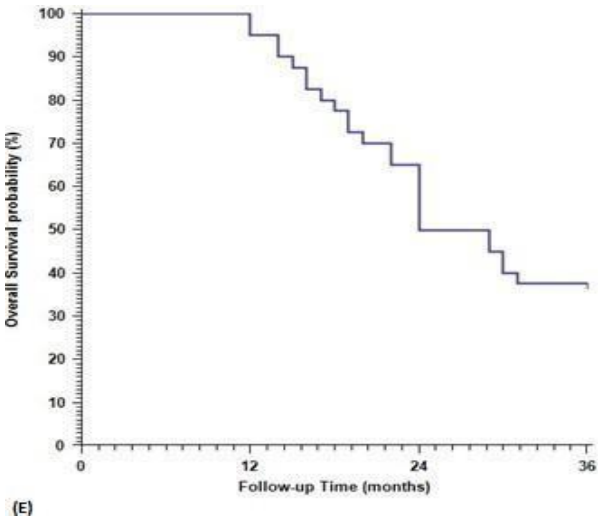
It has been established that ovarian cancer development reflects a loss of tissue organization and differentiation. These processes have been associated with increases in LOX expression and activity that regulate EMT transcription factors [11].

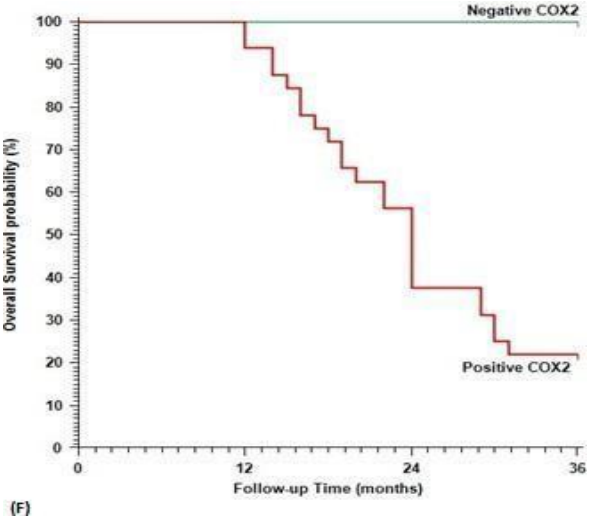

Disease Free Survival probability (%)
Follow-up Time (months)
(A)


Negative COX2
Positive COX2
Disease Free Survival probability (%)
Follow-up Time (months)
(B)


Negative LOX
Positive LOX
Disease Free Survival probability (%)
Follow-up Time (months)
(C)


Negative COX2/
Negative LOX
Negative COX2/
Positive LOX
Positive COX2
Negative LOX
Positive COX2/
Positive LOX
Disease Survival probability (%)
Follow-up Time (months)
(D)


Overall Survival probability (%)
Follow-up Time (months)
(E)


Negative COX2
Positive COX2
Overall Survival probability (%)
Follow-up Time (months)
(F)

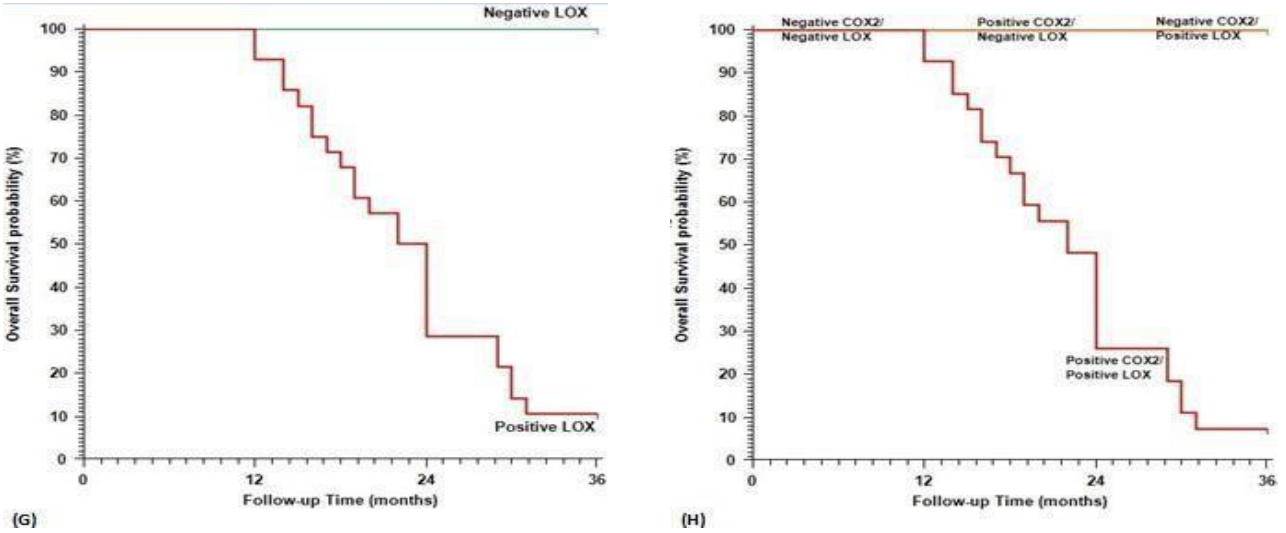


Figure 3. Kaplan Meier Survival Plots, Left Panel: Disease Free Survival (DFS); Right Panel: Overall Survival, (A) and (E): All studied malignant ovarian tumor patients, (B) and (F) Stratified by COX2 IHC staining, (C) and (G) Stratified by LOX IHC staining, (D) and (H) Stratified by COX2/LOX IHC staining.

Given the potential link of LOX to specific localization, De Donato et al [17] assessed only the nuclear expression of LOX in ovarian tumors, while Ji et al investigated mainly its cytoplasmic expression [18]. In this study, we examined the nuclear expression of LOX in EOC. LOX overexpression was detected in EOC tumor tissues compared with non-cancer tissues with a statistically significant difference which agree with other studies [18]. Many cancers showed LOX overexpression compared with their normal or non-aggressive neoplasms [19,20].

It has been documented that LOX protein structure and function are so complex and involve vital biological processes, such as cell movement, signal transduction and gene regulation [21]. Our results revealed a positive relationship between LOX expression and grade of malignancy. This is consistent with other studies [18,22]. Polymorphism of LOX was associated with increased risk of EOC and promoted the ovarian cancer progression and correlated with a lower tumor differentiation degree and with lymph node metastasis [22]. On the other hand, Wang et al reported that overexpression of LOX suppressed the proliferation and invasion of ovarian cancer cells, demonstrating the tumor suppressor function of the LOX gene [21].

LOX plays a critical role during the formation of premetastatic niches by stimulating collagen cross -linking and fibronectin synthesis, facilitating tumor-stromal interactions that are important for tumor progression and metastasis [10]. This role has been extensively studied in breast cancer in which LOX was considered to be important for late-stage tumor progression to metastasis [19]. Our results were consistent with Ji et al [18] who reported that LOX expression was positively correlated with advanced stage. These results demonstrated that LOX overexpression is an independent prognostic factor of a worse outcome in EOC patients.

Regarding the response to chemotherapy, our findings are similar to that of De Donato et al who reported an association between LOX expression and chemotherapy resistance [17]. LOX activity modulates the physical barrier function of the ECM for small molecule drugs thus influencing their therapeutic efficacy [23]. Therefore, therapeutic targeting of LOX may one day improve the clinical course of metastatic ovarian cancer patients.

With respect to survival analysis, we found that EOC patients with high LOX expression had a poor outcome as indicated by low rates of OS and DFS. This is in accordance with other studies which demonstrated that LOX overexpression was significantly associated with decreased OS (p=0.009) and DFS (p=0.048) in EOC [24].

However, there is still some debate regarding the association of LOX overexpression and the pathogenesis of invasive carcinomas [25]. On one hand, we have increased LOX expression in several types of cancer which is considered an independent prognostic marker of a worse outcome in gastric cancer and in lung adenocarcinoma [26]. On the other hand, LOX appears to act as a tumor suppressor in other cancers such as human osteosarcoma [27]. Therefore, the suggestion of whether LOX enhances or suppresses tumor progression in various tumors is still controversial. An explanation for such seemingly paradoxical role for LOX in cancer is likely the existence of multiple forms and the differential localization of LOX. Moreover, LOX expression may vary during the different stages of transformation and wit h different tissue types [28].

The COX2 has been established to be frequently expressed in human adenocarcinomas and its inhibition suppresses tumor progression [16]. In the present study, COX2 expression was detected in 80% of EOC cases which differed significantly from benign adenoma and borderline tumors (p=0.001). These results are consistent with other studies [7,16] where COX2 positive EOC cases were significantly

correlated with poor differentiation, advanced stage, short survival time and resistance to chemotherapy as compared to patients with tumor stained negative for COX2.

The current study showed a significant association between COX2 overexpression and high tumor grades. Magnowska et al [29] reported discordant results. This might be attributed to that there is no single accepted grading system for ovarian cancer. This is because the same characters cannot be applied to all histological subtypes. Therefore, we used the FIGO system which is the most widely accepted determinant method [30].

Regarding survival analysis, we found that EOC patients with high COX2 had decreased DFS and OS. In contrary to our results, other studies reported that there is no significant association between COX2 overexpression with any clinicopathological parameters and therapy response [31,32]. This discordance might be due to the use of different antibodies, different scoring criteria and different grading system conducted in their material and selection of the patients.

It has been conducted that the increased expression of LOX and COX2 in EOC are associated with each one another and with several adverse clinicopathological parameters of epithelial ovarian carcinoma including chemotherapy resistance and reduced survival. Further investigations of the exact prognostic and therapeutic implications of COX2 and LOX overexpression are strongly recommended.

Conflict of interest

All authors declare that they have no conflict of interest.

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