The value of E-cadherin and EGFR expression in ovarian serous tumors

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Abstract: Purpose: To study the expression of E-cadherin and EGFR in ovarian serous tumors in an attempt to determine the predictor for their biological behavior. **Materials and Methods:** E-cadherin and EGFR immunostaining was performed on paraffin embedded tissue sections of 50 ovarian serous tumors. **Results:** Regarding ovarian serous tumor cases, the incidence of bilaterality in benign, borderline and OSC cases was 16.7 % &. 25% and 83.3% respectively**.** On the other hand, E-cadherin was expressed in all benign ovarian serous tumor cases while it was expressed only in 6 cases (75 %) of borderline tumors.On contrary,only 8 cases (26.7%) of ovarian serous carcinoma (OSC) cases expressed E-cadherin. Regarding EGFR, all benign tumor cases were negative while, only 2 cases (25%) of borderline tumors were positive whereas, 21 cases (70%) of OSC were positive. So for both E-Cad and EGFR, only significant differences were documented between malignant and benign serous tumors but was not evident between borderline and the other two groups. Regarding OSC cases, a statistically significant decrease of E-cadherin expression was observed in both higher tumor grade and advanced stage. Conversely, a statistically significant increase of EGFR expression was observed only in higher tumor grade. Finally, there is high statistically significant differences between the positive and negative EGFR/ E-Cadherin groups. **Conclusion:** Direct relationship between incidence of bilaterality and aggressiveness of the tumor is documented. Regarding OSC, E-cadherin is a good prognostic marker whereas, EGFR is a bad prognostic marker.This inverse correlation represents a potential prognostic marker for OSC and may lead to development of different therapeutic strategies for either low or high - grade OSC.
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1. Introduction:

Ovarian serous tumors constitute over 30% of ovarian surface neoplasm while, OSCs represent about 50% of malignant tumors in this location [1]. Each year, over 22,000 women are diagnosed worldwide with epithelial ovarian cancer and 15,000 die of it. Ovarian cancer is the most lethal of all gynecologic malignancies and accounts for only about 4% of all cancers in the female population but ranks fourth as a cause of cancer-related death. It occurs most frequently in women 50–79 years of age, 90% of them have no family history [2]. Ovarian carcinoma includes several histological and pathogenetic profiles that correlate with survival [3&4].

OSC is the most common subtype of epithelial ovarian cancer, accounting for 50–60% of all cases. The prognosis for OSC patients depends largely on tumor grade and International Federation of Gynacology and Obstetrics (FIGO) stage [5]. Generally, women with low-grade OSC have higher 5-year survival rates than do women with high-grade OSC in addition, survival rate of women with ovarian cancer has shown only a marginal improvement over the past 30 years[6].

The biological characteristics of OSC were found to be associated with altered patterns of proliferation, angiogenesis and expression of cell adhesion molecules that play the critical role in mechanisms of tumor growth and metastasis [7]. The violation of cell adhesion leads to escape of tumor cells from tumor node and their spreading by blood and lymphatic vessels [8].

Epithelial cadherin (E-cadherin) is type I transmembrane glycoprotein. In humans, this protein is encoded by specific gene which is a tumor suppressor gene. Adhesive peculiarities of normal and malignant cells are related to cadherin—catenin complex [8 & 9 & 10]. Cadherins are known as basic and crucial factors of homotypic intercellular adhesion [11 & 12]. E-cadherin also plays important roles not only in cell adhesion and morphogenesis but also in cellular signal transduction in collaboration [13 & 14]. E-cadherin immunoreactivity was detected in all epithelial tissues including human oviduct except for the adrenal cortical and granulosa cells [15]. Recently, classical cadherins are adhesion-activated signalling receptors in cancer cells and loss of E-cadherin activity after epidermal growth factor induction is a major determinant of tumor progression and invasion and is observed in many physiological and pathological processes [16].

Epithelial growth factor receptor (EGFR) family of tyrosine kinase receptors is another target which has been considered for cancer immunotherapy as it is one of four members of the human epidermal receptor which has been demonstrated to have physiologic and also oncogenic roles in some of malignancies [17]. In addition, different pathways of EGFR take part as proto-oncogenes in several cancers such as gastrointestinal, oral and breast cancers [18]. On the other hand, EGFR immunocytochemistry were detected in normal ovarian surface epithelium also ovarian stromal tissue contained reduced but positive EGFR immunostaining [19]. Rouphly, EGFR is over-expressed in up to 60% of ovarian epithelial malignancies [20] and its activation is associated with increased malignant tumor phenotype and poor patient prognosis [21]. Since EGFR is involved in different parts of cancer growth such as tumor initiation, angiogenesis and metastasis, it represents an attractive target for therapeutic interventions [20, 21].

2. Material and methods:

Archival formalin-fixed, paraffin-embedded tissue specimens from 50 patients diagnosed as ovarian serous tumors at the Department of Pathology, Faculty of Medicine, Al Azhar University, Cairo, Egypt were included in this study during the period between 2011 and 2014.

All specimen were obtained as ovarian cystectomy specimens. Clinical data of the patients including age, results of appropriate radiologic studies (including pelvic and abdominal ultrasonography or MRI) and other data were retrieved from the files of the patients. Hematoxylin and Eosin (H&E) stained sections of all the cases were reviewed to confirm the diagnosis. For grading and staging, the criteria was done in conformity with criterions established in 2003 by IARC nominated work group for female genital tract tumors within World Health Organization [22] were followed. The material of this wok consisted of archival paraffin blocks only and the patients were unknown to the author and so no consent from the patients was required.

E-cadherin & EG FR immunostaining (Table 1):

Paraffin blocks which best represented the lesion were selected in all the cases and were cut into 5-µm-thick sections and dried on coated glass slides. The sections were deparaffinized with standard xylene and hydrated through graded alcohol into water. E-cadherin antigen retrieval was performed by heating the tissue sections for 20 minutes in a microwave oven with citrate buffer (pH 6.0). Tissue slides were incubated overnight at 4°C in a humid chamber with the primary antibody. For EGFR, enzymatic antigen retrieval was performed using trypsin (preheated to 37°C) by directly pipetting the solution onto the tissue on the slide and incubating it for 15 minutes in a 37°C incubator. The two primary antibodies were mouse monoclonal supplied by Neomarkers, Lab Vision Corporation, AR, USA. For EGFR, clone111.6 was used at a dilution of 1: 200; while for E-cadherin, clone 36B5 was used at a dilution of 1: 20. The antigen–antibody reaction was visualized by Thermo Scientific UltraVision LP Detection System (Neomarkers, Lab Vision Corporation, AR, USA). Immunohistochemical reactions were developed with diaminobenzidine and sections counterstained with Harrrishematoxylin as a final step. After staining, the slides were dehydrated through graded alcohol and mounted with a coverslip. All immunostains were processed manually, with appropriate positive and negative controls included for each batch of slides. Positive controls were paraffin embedded sections of normal skin epithelium for E-cadherin and Placenta for EGFR. Only membrane staining was assessed for both E-cadherin and EGFR.

Table 1: E-cadherin & EGFR immunostaining.

Antibody	Clone	Source	Dilution	antigen retrieval	Positive control
E-cadherin	mouse monoclonal	Neomarkers, Lab Vision Corporation	1: 20	citrate buffer (pH 6.0).	Normal skin epithelium
EGFR	mouse monoclonal	Neomarkers, Lab Vision Corporation	1:200	citrate buffer (pH 6.0).	Placenta

Evaluation and quantification of immunostaining

Despite deciding to focus only on membranous staining for both proteins, we use the following different scoring system for each of them because of the difference in antigen nature of both proteins.

For E-cadherin, the expression was scored semiquantitatively according to the staining pattern (membranous staining) on a three-point scale of 0 to 3 (0: complete absence of expression, 1:≤10%, 2:> 10 and ≤50%, 3:> 50%) [23].

Regarding EGFR expression, membrane staining was considered to be positive. The staining pattern was further classified as incomplete and complete staining and was scored as score 0: (no staining), score 1: (weak and incomplete staining) of more than 10% of tumor cells, score 2: (moderate and complete

staining) of more than 10% of tumor cells, score 3: (strong and complete staining) of more than 10% of tumor cells [24].

Statistical Analysis

Data were represented as the mean± SD with range for quantitative parametric data and as frequency (number & percent) for qualitative data. For quantitative parametric data, comparison between two different groups was carried out by t-test while comparison between more than two groups was carried out by analysis of variance (ANOVA). Inter-group comparison of categorical data was performed by using pearson's chi square test or fisher exact test. A p value < 0.05 was considered statistically significant. Analysis was done using SPSS for Windows (Version 17.0).

3. Results:

Clinco-pathologic data (Table 2&3):

The age range was **43.20-72.90** years and the mean age was 62.30**±8.09** years. The median age of benign, borderline tumors and OSC patients were 47, 52 and 55 years respectively.

Out of 50 studied ovarian serous tumor cases, 12 cases (24%) were benign, 8 cases (16%) were borderline and the remaining 30 cases (60%) were OSC.

Benign cases were bilateral in 2 cases (16.7 %) and 8 cases (66.7%) were below 10 cm. While, 2 cases (25%) of borderline tumors were bilateral and 5 cases (62.5%) of them were below 10 cm. Regarding OSC cases, 25 cases (83.3%) were bilateral and 8 cases were below 10 cm so there is statistically significant relationship was documented between incidence of bilaterality and aggressiveness of the tumor. Out of 30 studied OSC cases, 12 cases were low -grade and 18 cases were high- grade. In addition, 6 cases (20%) were stage I, 6 cases (20 %) were stage II and the remaining 18 cases (60 %) were stage III/IV.

Table 2: Age distribution in studied groups.

		Benign serous tumors (No.=12)	Border line tumors (No.=8)	OSC (No.=30)	P
Age	Mean±SD	58.72±6.27	60.20±5.76	64.30±8.78	0.09
	Range	49.30-(69.20	53.02-71.30)	43.20-72.90	

Data expressed as mean ±SD or frequency

Table 3: Clinicopathologic parameters of studied groups.

Parameter		Benign serous tumors (No.=12)	Border line tumors (No.=8)	OSC (No.=30)	P1	P2	P3
Tumor size (cm)	<10	8 (66.7%)	5(62.5%)	8(26.7%)	1.00	0.03*	0.09
	>10	4(33.3%)	3(37.5%)	22(73.3%)			
Laterality	Unilateral	10(83.3%)	6(75%)	5(16.7%)	1.00	<0.001**	0.004 *
	Bilateral	2(16.7%)	2(25%)	25(83.3%)			

P1: significance between benign tumors & border line tumorsP2:significance between benign tumors & OSC tumors P3: significance between border lines & OSC tumors. *: significance≤0.05, P value. **: high significance<0.001, P value.

Immunohistochemical findings (Table 4 & 5 & 6 & 7 & 8):

In the current study, E-cadherin membranous expression was documented in all benign ovarian serous tumors 12 /12 cases (100 %) and the expression pattern of all cases had score 3 (Fig. 1) while in borderline tumors cases, E-cadherin was expressed in 6 out of 8 cases (75%) as the expression pattern of 1 cases had score 1 and 1 cases had score 2 while the remaining 4 cases showed score 3 (Fig.2).

Regarding OSC cases, E-cadherin was expressed only in 8 out of 30 cases (26.7%) and the expression pattern was as follows: 3 cases had score 1 and 3 cases had score 2 while the remaining 2 cases showed score 3 (Fig.3 & 4).

Conversely, EGFR membranous expression was not observed in any cases of benign ovarian serous tumor (Fig. 5) while it was expressed in 2 out of 8 (25%) borderline tumor cases and the expression pattern was as follows: one case had score 2 and the other case showed score 3 (Fig. 6). On the hand, EGFR was expressed in 21 /30 (70%) of OSC cases and the expression pattern was as follows: 5 cases had score 1, six cases had score 2 (Fig.7) while the remaining 10 cases showed score 3 (Fig. 8). So for both E-cadherin and EGFR expression, only significant differences were observed between malignant and benign ovarian serous tumors but was not evident between borderline and the other two groups.

Regarding OSC cases, E-cadherin expression was documented in 6 low grade cases (Fig 3) while it was observed only in 2 high grade cases (Fig.4).Thus mild statistically significant (p=0.03) decrease of E-

cadherin protein expression was observed with higher tumor grade. On the other hand, E-cadherin expression was documented in 5 cases of stage I as compared to 2 cases and one case of stage II and III/IV respectively. Thus statistically significant (p2=0.001) decrease of E-cadherin expression was found with higher tumor stage. Conversely, EGFR expression was documented in only 5 low - grade cases (Fig.7) while it was documented in 16 high - grade cases (Fig.8) thus a mild statistically significant (p=0.01) increase of EGFR protein expression was observed with higher tumor grade. On the other hand, EGFR expression was documented in 5 cases of stage I as compared to 4 cases and 12 cases of stage II and III/IV respectively hence, no statistically relation was found between EGFR expression and tumor stage. Finally, there is high statistically significant (P<0.001***) differences between the positive and negative EGFR/ E-Cadherin groups.

Table 4: Detailed ImmunohistochemicalEexpression of E-cadherin in studied groups

GROUPS	E-cadherin Eexpression					
	NO	%	Scoring Of Immunoreactivity			
			-ve	+ve		
			0	1	2	3
Benign serous tumors	12/12	100%	0	0	0	12
Borderline tumor	6/8	75%	2	1	1	4
OSC	8/30	26.7%	22	3	3	2
P1	0.7					
P2	0.018*					
P3	0.11					

P1: significance between benign tumors & border line tumors. P2: significance between benign tumors & OSC tumors.
P3: significance between border line tumors & OSC tumors. *: significance≤0.0 5 P value.

Table 5: Detailed Immunohistochemical Eexpression of EGFR in studied groups.

GROUPS	EGFR Eexpression					
	NO	%	Scoring Of Immunoreactivity			
			-ve	+ve		
			0	1	2	3
Benign serous tumors	0/12	0%	12	0	0	0
Borderline tumor	2/8	25%	6	0	1	1
OSC	21/30	70%	9	5	6	10
P1	0.19					
P2	0.006*					
P3	0.29					

P1: significance between benign tumors & border line tumors, P2: significance between benign tumors & OSC tumors.
P3: significance between border line tumors & OSC tumors. *:significance≤0.05 P value.

Table 6: Relationship between both E-cadherin & EGFR Expression and tumor grade.

		E-cadherin expression				P	EGFR expression				P
		Negative		Positive			Negative		Positive		
		No	%	No	%		No	%	No	%	
Grade	Low grade (No.= 12)	6	27.3%	6	75.0%	0.03*	7	77.8%	5	23.8%	0.01*
	High grade (No.= 18)	16	72.7%	2	25.0%		2	22.2%	16	76.2%	

* Mild statistical y significant P value.

Table 7: Relationship between both E-cadherin & EGFR Expression and tumor stage.

		E-cadherin expression				EGFR expression			
		Negative		Positive		Negative		Positive	
		No	%	No	%	No	%	No	%
FIGO stage	Stage I (No.= 6)	1	4.5%	5	62.5%	1	11.1%	5	23.8%
	Stage II (No.= 6)	4	18.2%	2	25.0%	2	22.2%	4	19.1%
	Stage III/IV (No.= 18)	17	77.3%	1	12.5%	6	66.7%	12	57.1%
P1		0.24				1.00			
P2		0.001*				0.6			
P3		0.14				1.00			

P1: comparison between Stage I & Stage II, P2: comparison between Stage I & Stage III/IV.
P3: comparison between Stage II & Stage III/IV. *: Mild statistically significant P value

Table 8: Immunohistochemical differences between positive and negative EGFR/ E-Cadherin groups.

		EGFR expression						P
		Negative		Positive		Total		
		No	%	No	%	No	%	
E-cadherin expression	Negative	5	18.5%	19	82.6%	24	48.0%	<0.001**
	Positive	22	81.5%	4	17.4%	26	52.0%	
	Total	27	100.0%	23	100.0%	50	100.0%	

**: high statistically significant P value.

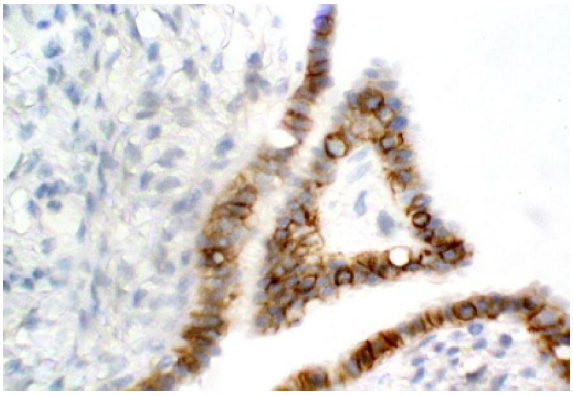

Figure 1: A case of benign ovarian serous tumor, showing strong membranous expression of most of tumor cells for E-cadhrine (score 3) (ABC, counterstained with Hx. ×200).

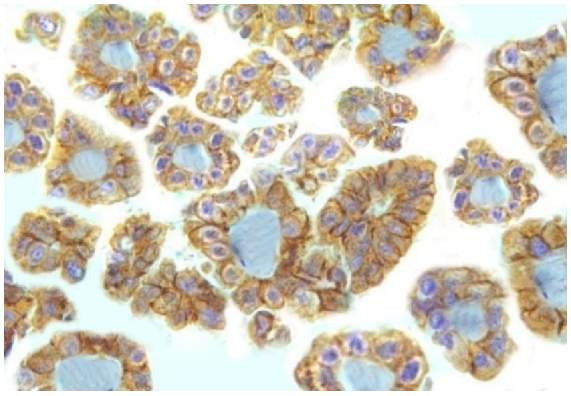

Figure 2: A case of borderline ovarian serous tumor, showing strong membranous expression of most of tumor cells for E-cadhrine (score 3) (ABC, counterstained with Hx. ×200)

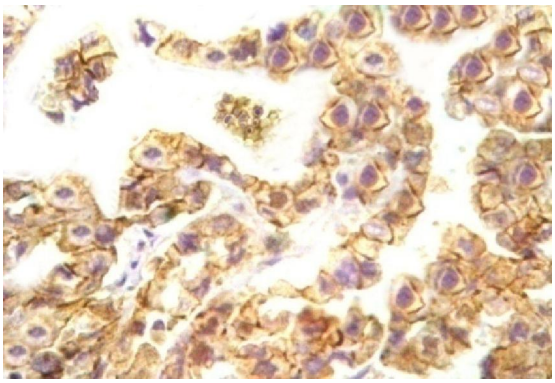

Figure 3: A case of low grade OSC, showing strong membranous expression of most of tumor cells for E-cadhrine (score 3) (ABC, counterstained with Hx. ×400).

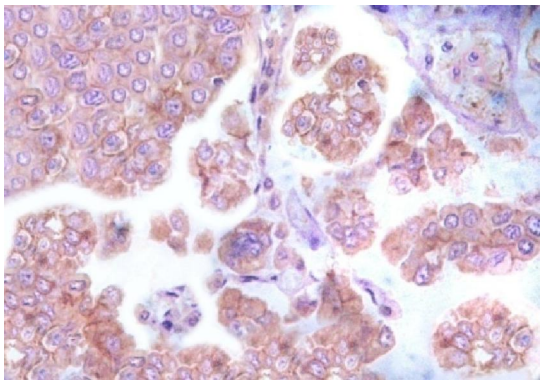

Figure 4: A case of high grade OSC, showing strong membranous expression of most of tumor cells for E-cadhrine (score 3) (ABC, counterstained with Hx. ×400).

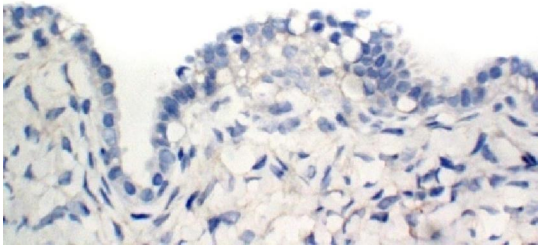

Figure 5: A case of benign ovarian serous tumor, showing negative expression for EGFR (score 0) (ABC, counterstained with Hx. ×200)

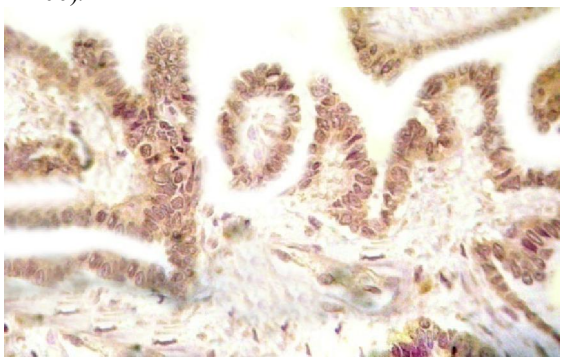

Figure 6: A case of borderline ovarian serous tumor, showing strong membranous expression of most of tumor cells for EGFR (score 3) (ABC, counterstained with Hx. ×200).

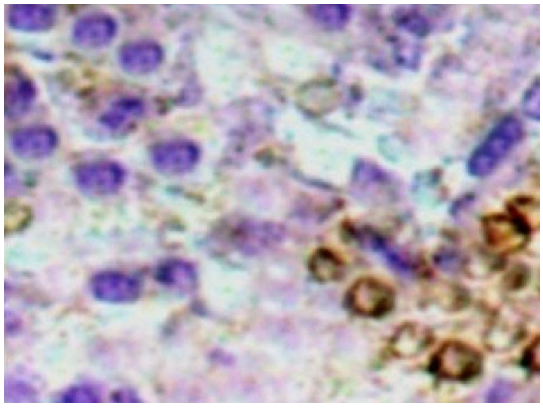

Figure 7: A case of low grade OSC, showing membranous expression of~ 20% of tumor cell for EGFR (score 2) (ABC, counterstained with Hx x400).

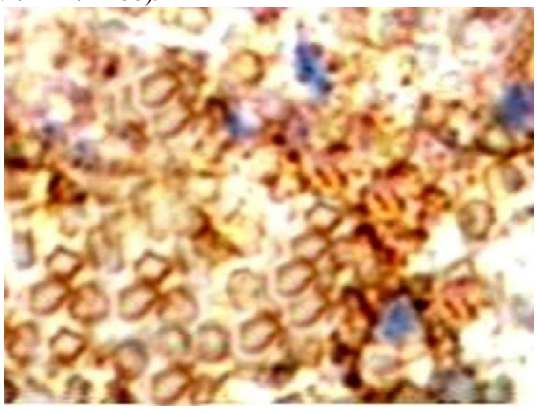

Figure 8: A case of high grade OSC, showing strong membranous s expression o f most of tumor cells for EGFR (score 3) (ABC, counterstained with Hx. ×400)

4. Discussion:

Ovarian serous tumors make up about one-fourth of all ovarian tumors. Most cases occur in adults. A morphologic spectrum of proliferation exists in these tumors, at one end is the benign serous tumor. At the other end is the OSC and In between there are borderline ovarian serous tumors. OSC is characterized by initial local growth followed by spreading into the peritoneal cavity at later stages of tumor progression. Recently, dual concept of ovarian carcinogenesis showed that low-grade OSC are the result of progression of a benign ovarian serous tumors to borderline and then malignant tumor while high-grade OSC occur most frequently de novo also disappearance or impaired function of E-cad have often been associated with tumor formation and invasion [1] conversely, EGFR expression is used to identify benign/borderline tumors with progression potential and the malignant aggressive tumors and the expression is frequently elevated in OSC which have a poor prognosis and the expression of both E-cadherin and EGFR may have therputic implications [25 & 26].

The present study investigated the expression of E-cadherin and EGFR in benign, borderline and malignant ovarian serous tumors in an attempt to determine the predictor for their biological behavior. Out of 50 studied ovarian serous tumor cases, 12 cases were benign, 8 cases were borderline and the remaining 30 cases were OSC. The age range of studied cases was 28-70 years and the mean age was 52.3 ± 11.4 years. Honestly, we could not say that median age is higher or lower in a group compared to another because of p value for comparing age among 3 studied groups was 0.09.

Our results observed that, the incidence of bilaterality in benign, borderline and OSC cases was 16.7 % &. 25% and 83.3% respectively so the incidence of bilatrality is more frequent in OSC cases as compared to benign and borderline ovarian serous tumors. These results were in keeping with those of **Tavassoli and Devilee** who reported that there is direct relationship between incidence of bilaterality and aggressiveness of the tumor [22].

E-cadherin in this study was expressed in 52% of studied cases as all benign tumor cases are E-cadherin positive and 75 % of borderline cases exhibited positive expression.This finding is In contrast to those of **Sundfeldt et al.,** who reported that benign and borderline tumors were uniformly expressed E-cadherin [27]. Also in this work, E-cadherin was expressed only in 26.7% of OSC cases so a statistically significant decrease of E-cadherin protein expression in OSC cases as compared to benign ovarian serous tumors but no significant differences was documented between borderline and the other two groups.Similar findings were reported by **Daraï et al., and Koensgen et al.,** who reported that E-Cadherin expression was reduced in ovarian serous carcinoma cases as compared to benign ovarian serous tumors [28 & 29].

Wong et al., and Yuecheng et al., observed that low-grade OSC cases expressed significantly higher levels of E-cadherin than did high-grade OSC cases [30& 31].This results were in keeping with our finding that showed 6 cases of low - grade OSC express E- cadherin as compared to only 2 cases of high - grade OSC. Moreover, our results showed decrease in E-cadherin expression with higher tumor stage. This finding is in accordance with those of **Ryabtseva et al.,** who found a correlation between decreased E-cadherin expression and advanced tumor stage [32].

Regarding EGFR expression, our results showed about 46% of examined cases were EGFR postive and despite it was not expressed in any case of the benign tumors, it was expressed in 25% and 70% of borderline and OSC cases respectively so, only significant differences were documented between malignant and benign ovarian serous tumors but was not evident between borderline and the other two groups. Nearly similar study was obtained by **Brustmann** who reported that EGFR expression was scored negative in all benign and borderline ovarian serous tumors however, membranous EGFR expression was determined in 64% of ovarian serous carcinoma cases [33].

Brustmann and Lassus et al., observed direct relationship between EGFR expression and tumor grade [33&34]. Our results also agree with this finding and showing 16 cases of high - grade OSC express EGFR as compared to only 5 cases of low - grade OSC. Conversely, no relation was found between EGFR expression and tumor stage. This finding agree also with **Brustmann** who reported that EGFR expression was not correlated with tumor stage [33].

Finally,.our results observed high statistically significant differences between the positive and negative EGFR/ E-Cadherin groups also, in this current work we found that about 70% of OSC cases express EGFR whereas only 26.7 % of cases express E- cadherin. This findings were concordant with those of **Voutilainen et al.**, who observed that EGFR expression is frequently elevated in OSC cases and this often causes disruption of adherent junctions and reduces E-cadherin protein levels in tumor tissue [26].

In summary, the result of the work supports the direct relationship between incidence of bilaterality and aggressiveness of the tumor and also documents the diagnostic and prognostic role of E- cadherin and

EGFR expression in ovarian serous tumors as for E-Cad and EGFR expression, only significant differences were documented between malignant and benign ovarian serous tumors but was not evident between borderline and the other two groups. These differences may lead to the development of different therapeutic strategies for women with either low or high - grade OSC also EGFR can be used to identify benign/borderline tumors with progression potential and to detect malignant aggressive tumors.

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2/9/2016