Prognostic and Predictive Significance of EpCAM and SOX2 expression in Serous Ovarian Carcinoma

Original Article

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ABSTRACT

Background: A prevalent type of cancer in women's reproductive systems is serous ovarian carcinoma. Despite the advance in surgery and chemotherapy, the patient's prognosis is poor due to relapse and chemotherapy resistance. Therefore, we aim to assess the influence of EpCAM and SOX2 expression on tumor behavior and prognosis for improving outcomes and therapy.

Patients and Methods: The expression of EpCAM and SOX2 was evaluated immunohistochemically and also for SOX2 gene expression in 90 cases of ovarian serous carcinoma and their prognostic significance was evaluated.

Results: EpPCAM was statistically correlated with nodal metastases (P=0.002) and highly significant associated with advanced stage (P <0.001). Moreover, it was statistically associated with tumor size (P <0.001). High SOX2 nuclear expression was correlated significantly with high grade of the tumor (P=0.001), lymph-node involvement, and with advanced stage of tumor (P<0.001 for each). The study found that both EpCAM and SOX2 proteins were increased in aggressive ovarian cancers. EpCAM correlated with lymph node spread, larger tumors, and advanced stage. High SOX2 levels were linked to poor overall survival. EpCAM levels seemed to influence SOX2 expression.

Conclusions: EpCAM and SOX2 were overexpressed in ovarian serous carcinoma tissue and substantially related nodal metastasis, TNM stage and poor overall survival making them critical therapeutic targets and prognostic markers in ovarian cancer. The correlation between overexpression of EpCAM and SOX2 expression provides the path for further research into the molecular pathogenesis of tumor progression and the therapeutic target receptors.

Key Words: EpCAM; ovarian serous carcinoma, SOX2.

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INTRODUCTION

Ovarian cancer is a highly common type of cancer that affects the female reproductive system. It is thought to have the greatest death rate among all gynaecological cancers. It is the most fatal type of gynaecological cancer since it is often detected at advanced stage[1].

Despite the advance and improvement in surgery and chemotherapy, the prognosis of these patients is poor due to relapse and chemotherapy resistance. Therefore, finding new molecular therapeutic agents is currently becoming more and more important for the treatment[2].

The antigen known as epithelial cell adhesion molecule (EpCAM) was first identified in human colon cancer. This transmembrane glycoprotein has a mass of 3942 kDa and is calcium-independent. EpCAM participates in the processes of signal transduction, gene regulation, and cell adhesion. The proto-oncogene c-myc is upregulated when EpCAM is expressed, which increases cell proliferation and metabolism. EpCAM expression also plays a role in tissue differentiation via E-cadherin interaction[3].

EpCAM over-expression was observed in many types of tumors as liver and breast and became a unique molecular target for oncologic therapy as a result of its over-expression[4].

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SOX2 belongs to the SOXB1 group in the SOX family. These proteins regulate stem cell maintenance and embryonic development, also they are key factors in transformation a fully differentiated cell to a pluripotent state. Owing to its importance as a stem cell marker, it has also been investigated in human cancers to ascertain whether it plays a part in the development and maintenance of cancer[5].

SOX2 amplification have been noticed in a many cancer types, as bronchogenic carcinoma, rectal cancer and glioblastoma. In ovarian cancer, particularly high-grade cases, the highly expressed SOX2 has been detected. Current data have shown that SOX2 expression was elevated in high-grade ovarian cancer in comparison with normal ovary, borderline and low-grade serous carcinoma, suggesting their role in progression and survival of cases[6].

In order to improve outcomes and therapy, we want to assess the role of EpCAM and SOX2 expression in ovarian serous carcinoma and determine their impact on tumour behaviour and disease prognosis.

PATIENTS AND METHODS

This retrospective-prospective cohort study was conducted at the obstetrics and gynecology departments, pathology department, biochemistry and oncology departments, Faculty of Medicine, Zagazig University. The study included 90 cases of ovarian serous cancer from January 2015 to January 2023. Prior to the collection of samples, written informed consent was given by each patient. The Institutional Review Board of Zagazig University's Faculty of Medicine approved the study. (IRB#:10322/14-2-2023).

The primary treatment for suspected ovarian cancer is usually surgical staging and debulking surgery, followed by chemotherapy in majority but not all patients[7].

If an individual has advanced ovarian cancer and is not a suitable candidate for upfront primary debulking surgery (PDS) because of their advanced age, they should seek neoadjuvant chemotherapy combined with interval debulking surgery[8].

Postoperative recommended treatment options for stage I high grade & stage II–IV comprises 6 cycles of platinum-based IV chemotherapy. Every three weeks on the first day, paclitaxel 175 mg/m2 IV is administered first, and then carboplatin AUC 5.6 IV is administered over 30–60 minutes[9].

Pathological and Immunohistochemical analysis

Sections from the paraffin blocks were cut at 5 µm thick for histopathological evaluation using Hematoxylin and Eosin stain (H&E). According to the FIGO classification 2014, surgical assessment was utilized to determine the clinical stages, grade and metastases existence. Histopathologic grading was performed as follows: Category: High Grade HG and Low Grade[10].

Immunohistochemical analysis

Immunohistochemical staining (IHC) was performed as previously mentioned[11]. Then, 4-µ-thickness sections were incubated with primary monoclonal antibodies as follows: and anti- EpCAM (Mouse monoclonal antibody, 1:100 dilution, Catalogue No: abx019080 (0. 1ml), Dako) and anti- SOX2) Rabbit monoclonal antibody, 1:150 dilution, Catalogue No: # 3579S, DAKO).

Immunohistochemical staining evaluation

Evaluation of the immunohistochemical staining of EpCAM:

EpCAM was considered positive if membranous staining was seen in the cell membrane. Serving as a positive internal control was the ovarian serosal surface. EpCAM scores of expressions were calculated by multiplying the percentage of stained cells (0: 0% & 1 from one to twenty-five percentage & 2 if twenty-six to fifty percentage-stained cells & 3 if fifty-one to seventy-five percentage and 4 if more than seventy-five percentage of cells were stained) by the intensity of staining (0 if no staining; 1 if weak staining; 2 for moderate staining and 3 for strong staining). A total of score ≥ 6 indicated an "EpCAM-high" group, while a score from 0 to 4 indicated an "EpCAM-low" group[12].

Evaluation of the immunohistochemical expression of SOX2:

SOX2 immunohistochemical expression was considered in the nucleus of ovarian carcinoma cells. The score was calculated as follows: According to percentage :0, five percentage positive cells; 1 if five to twenty-five percentage positive cells; 2 if twenty-six to seventy-five percentage positive cells; and 3 if seventy-six positive cells. Staining intensity was scored as: 0, if no staining; 1 with faint-yellow staining; 2, brown–yellow; and 3 if dark-brown staining was seen. The intensity and percentage were added together to get the final score; generating a total score of 6. Scores more than or equal to three were considered high-level expression, while scores less than three were considered low-level expression[13].

SOX2 gene expression analysis using qRT-PCR:

Trizol reagent (Thermo Fisher Scientific; Waltham, MA, United States) was used to extract total RNA. The extracted RNA purity and concentration were assessed by use of Nanodrop spectrophotometer by measuring the

OD at 260 and 280 nm and we accepted A260/A280 at a ratio of 1.8–2.1; this was followed by a two-step real-time PCR to evaluate gene expression with specific SOX2 primer Forward TGTCGGAGACGGAGAAG Reverse GGCAGCGTGTACTTATCC.

The relative expression level of the targeted gene was normalized to that of the housekeeping GAPDH, and the relative fold changes in gene expression were calculated according to the method described by Livak & Schmittgen[14].

Statistical methods

Data were analyzed by IBM SPSS 23.0 for windows (SPSS Inc., Chicago, IL, USA) and also NCSS 11 for windows (NCSS LCC., Kaysville, UT, USA). The quantitative data were considered as mean ± standard deviation (SD); while qualitative data as frequency and percentage.

We performed these tests:

- Chi-square and fisher exact for analysis of qualitative data; while Kaplan-Mayer test for survival analysis.
- Probability (*P-value*): If *P-value* <0.05; it was considered significant, if <0.001; this was highly significant and if *P-value* was >0.05; this is insignificant.

RESULTS

Clinicopathological data

This study included 90 ovarian serous carcinoma cases. Sixty-one cases were high grade while 29 cases were low grade. Fifty-four cases showed lymph-node metastasis. Regarding stage grouping ;13 cases were stage I, twenty-two cases were stage II, 38 cases were stage III, while 17 were stage IV. Regarding the size of tumor; 46 cases were more than 10 cm, while 44 cases were less than 10 cm. Fourty one cases of ninety showed serum CA125 level more than 500 U\ml and Fourty nine were less than 500 U\ml.

EpCAM expression and its relationship with clinicopathological variables

The EpCAM membranous expression was high in 53 cases, while 37 cases showed low expressed (Table 1, Figures 1 A,B). EPCAM was statistically correlated with lymph node metastases (P = 0.002) and highly significant correlated with advanced tumor staging (P <0.001) with 17 of 17 cases of stage IV had high expression. Moreover, it was statistically associated with tumor size (P <0.001). Tumor grade and CA125 were not associated with EPCAM expression.

SOX2 expression and its relationship with the clinicopathological variables

The nuclear expression of SOX2 was detected highly in 57 cases of 90 while it was low in 33 cases of 90 (Table 2, Figure 1 C,D). High SOX2 immunohistochemical expression was significantly related with high tumor grade (46 of 57 cases) (P=0.001). Moreover, a highly significant correlation with lymph-node involvement (42 of 57 cases) and advanced tumor stage (13 of 17 cases) (P <0.001 for each) was found. The level of CA125 in serum did not correlate with SOX2 expression.

Correlation between the immunohistochemical expression EpCAM and SOX2

EpCAM expression and SOX2 expressions were significantly correlated (Table 1). As shown in table 1, forty-one cases of 57 was highly expressed with EPCAM (P = 0.002).

Correlation between the immunohistochemical expression of EpCAM and SOX2 gene expression

Cases of high EPCAM expression show highly significant increase in SOX2 gene expression (3.56 ± 0.95), and SOX2 gene expression in low level of EPCAM expression was (2.17 ± 1.24) (Table 1).

Correlation between the immunohistochemical expression SOX2 and SOX2 gene expression

A highly significant positive correlation was found between immunohistochemical expression of SOX2 and SOX2 gene expression (Table 2).

Correlation between CA125 level and SOX2 gene expression

Cases of high level of CA125 show increase in SOX2 gene expression but not significant (Table 3).

Association of EpCAM and SOX2 expression and survival: Eight-year Overall Survival (OS) and event-Free Survival (PFS) were used as the primary outcomes.

Correlation between the expression of SOX2 and the prognosis of ovarian cancer patients

1- Correlation between the expression of SOX2 and overall survival

High expression of SOX2 (973.7%) was correlated with

significant poor overall survival than low SOX2 expression (97.0%), 69.7 months Vs 79.7 months respectively (p = 0.008). (Figure 2: A)

2-Correlation between the expression of SOX-2 and disease-free survival

Longer disease-free life (86.5 months versus 75.6 months) was linked to higher expression of SOX-2 (96.5%) than low expression (90.9%), although the difference was not statistically significant. (p-value=0.118). (Figure 2; B).

Correlation between the expression of EpCAM and the prognosis of ovarian cancer patients

1-Correlation between the expression of EpCAM and overall survival

The mean overall survival is prolonged in low EPCAM expression (89.2%), with statistically significant difference than high expression (77.4%), 83.3 months versus 71.8 months respectively, *p-value* (0.02). (Figure 2; C).

2- Correlation between the expression of EpCAM and disease-free survival

High expression of EpCAM (90.6%) is associated with significant poor and shorter disease-free survival than low expression (100%), 41.2 months versus 63.1 months. (*p-value*=0.006). (Figure 2; D).

Table 1: Relation between EPCAM level and tumor characteristics of the studied groups.

N		High N=53		Low N=37		X^2	*P*
		%	N	%			
Age	Mean ± SD	50.6 ± 11.1		48.5 ± 11.1		1.03	0.301 NS
Tumor grade	High	40	75.5	21	56.8	3.49	0.062 NS
	Low	13	24.5	16	43.2		
LN involvement	Positive	38	71.7	16	43.2	7.35	0.002 S
	Negative	15	28.3	21	56.8		
Stage	I	0	0.0	13	35.1	45.7	<0.001 HS
	II	6	11.3	16	43.2		
	III	30	56.6	8	21.6		
	VI	17	32.1	0	0.0		
Tumor size	>10 cm	36	67.9	10	27.0	14.8	<0.001 HS
	<10 cm	17	32.1	27	73.0		
CA 125 level	>500 U\ml	23	43.4	18	48.6	0.242	0.671 NS
	<500 U\ ml	30	56.6	19	51.4		
SOX-2 level	Low	12	22.6	21	56.8	14.8	0.002 S
	High	41	77.4	16	43.2		
SOX-2 gene expression	Mean ± SD	3.56 ± 0.95		2.17 ± 1.24		5.84	<0.001 HS
Chemotherapy platinum	Sensitive	18	34.0	25	67.6	9.82	0.002 S
	Resistant	35	66.0	12	32.4		
Interval Debulking surgery		12	22.6	4	10.8	2.09	0.124 NS
Relapse		5	9.4	0	0.0	F	0.07 NS
Mortality	Died	12	22.6	4	10.8	2.09	0.149 NS

Table 2: Relation between SOX-2 level and tumor characteristics of the studied groups.

N		High N=57		Low N=33		X^2	*P*
		%					
Age	Mean ± SD	52.04 ± 9.66		45.5 ± 11.89		2.68	0.01 S
Tumor grade	High	46	80.7	15	45.5	11.9	0.001 S
	Low	11	19.3	18	54.5		
LN involvement	Positive	42	73.7	12	36.4	12.3	<0.001 HS
	Negative	15	26.3	21	63.6		
Stage	I	0	0.0	13	39.4	27.7	<0.001 HS
	II	14	24.6	8	24.2		
	III	30	52.6	8	24.2		
	VI	13	22.8	4	12.1		
Tumor size	>10 cm	36	63.2	10	30.3	9.03	0.003 S
	<10 cm	21	36.8	23	69.7		
CA 125 level	>500 U\ml	27	47.4	14	42.4	0.206	0.651 NS
	<500 U\ ml	30	52.6	19	57.6		
Chemotherapy platinum	Sensitive	21	36.8	22	66.7	7.42	0.006 S
	Resistant	36	63.2	11	33.3		
Interval Debulking surgery		11	19.3	5	15.2	0.249	0.624 NS
Relapse		2	3.5	3	9.1	F	0.354 NS
Mortality	Died	15	26.3	1	3.0	7.75	0.005 S
SOX-2 gene expression	Mean ± SD	3.69± 0.84		1.75 ± 0.87		10.4	<0.001 HS

Table 3: Relation between CA-125 level and tumor characteristics of the studied groups.

N		High N=41		Low N=49		X^2	P
		%					
Age	Mean ± SD	51.1 ± 11.8		48.5 ± 10.5		1.09	0.281 NS
Tumor grade	High	27	65.9	34	69.4	0.129	0.721 NS
	Low	14	34.1	15	30.6		
LN involvement	Positive	27	65.9	27	55.1	1.08	0.381 NS
	Negative	14	34.1	22	44.9		
Stage	I	6	14.6	7	14.3	3.67	0.291 NS
	II	10	24.4	12	24.5		
	III	14	34.1	24	49.0		
	VI	11	26.8	6	12.2		
Tumor size	>10 cm	22	53.7	24	49.0	0.193	0.653 NS
	<10 cm	19	46.3	25	51.0		
CA 125 level	>500 U\ml	27	47.4	14	42.4	0.206	0.651 NS
	<500 U\ ml	30	52.6	19	57.6		
Chemotherapy platinum	Sensitive	23	56.1	20	40.8	2.09	0.146 NS
	Resistant	18	43.9	29	59.2		
Interval Debulking surgery		9	22.0	7	14.3	0.899	0.344 NS
Relapse		2	4.9	3	6.1	F	0.794 NS
Mortality	Died	5	12.2	11	22.4	1.61	0.208 NS
High SOX-2		27	65.9	30	61.2	0.206	0.65 NS
SOX-2 gene expression	Mean ± SD	3.11 ± 0.98		2.96 ± 0.92		0.116	0.809 NS
High EPCAM		23	56.1	30	61.2	0.242	0.632 NS

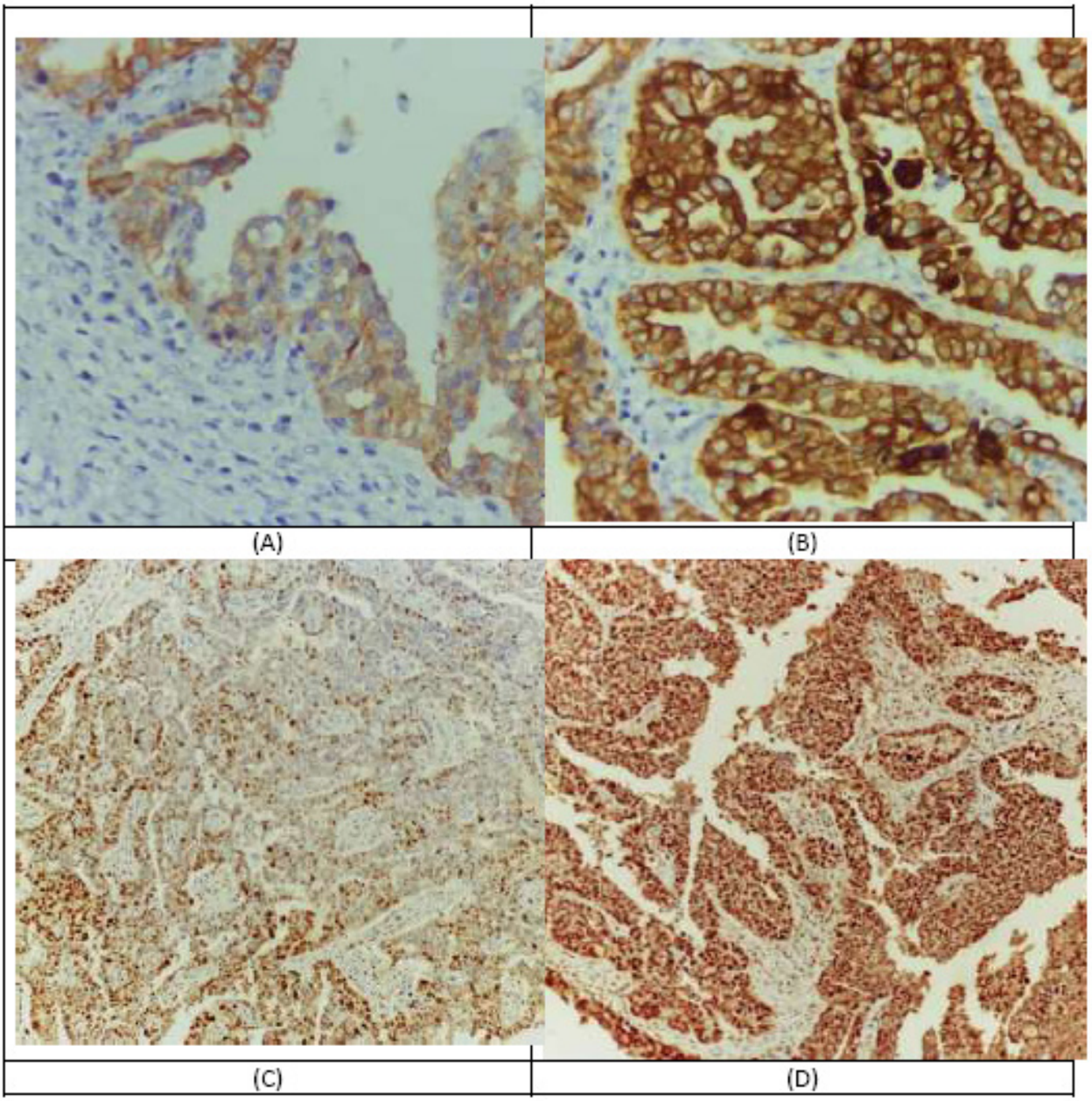

Fig. 1: Representatve samples of EpCAM and SOX2 Immunohistochemical expression in ovarian serous carcinoma:
(A) Low EpCAM immunoreactiviy. (B) High EpCAM immunoreactiviy
(C) Low SOX2 immunoreactivity. (D) High SOX2 immunoreactiviy (x 400).

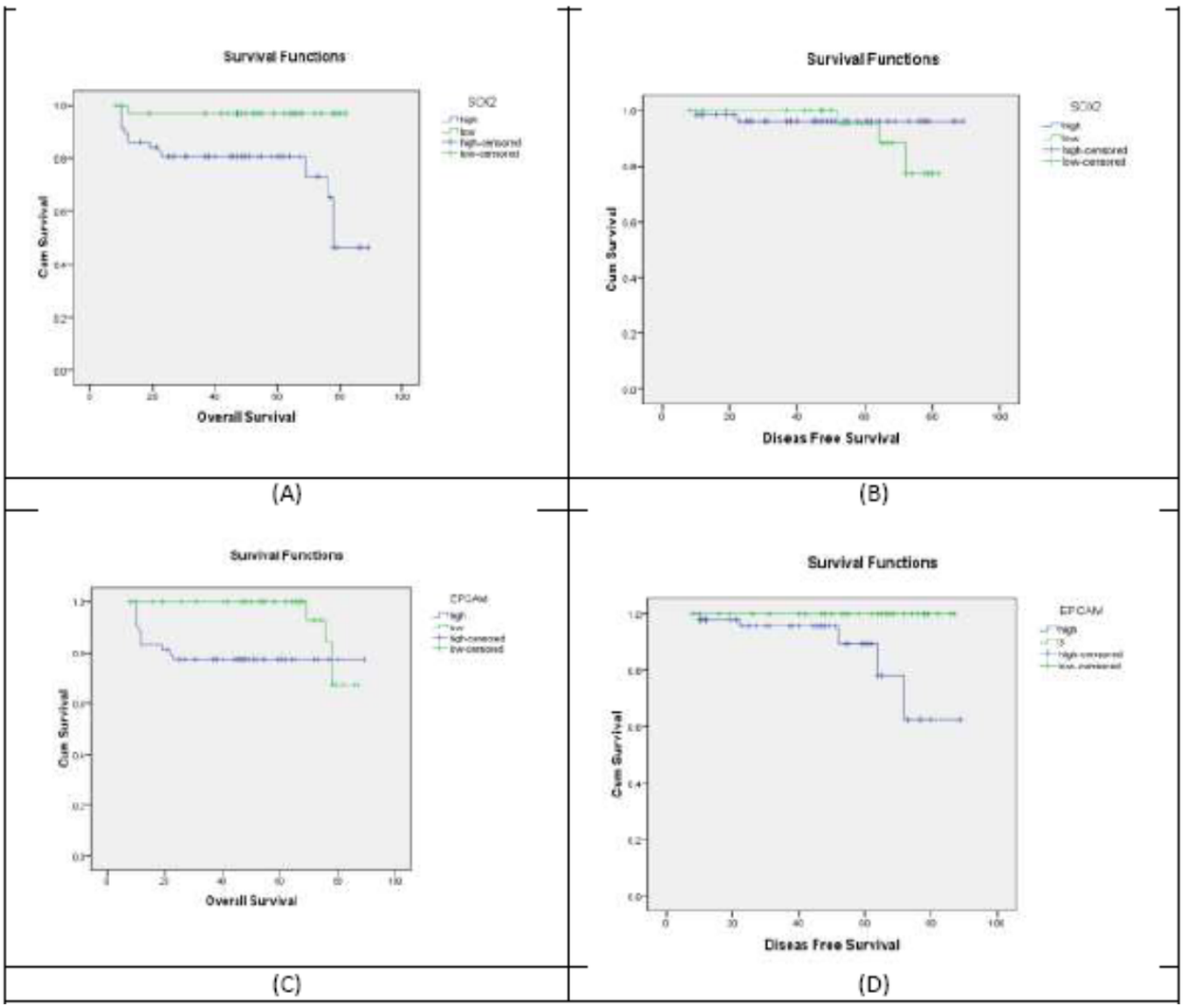

Fig. 2: (A): Correlation between the expression of SOX2 and Overall survival. (B): Correlation between the expression of SOX-2 and disease-free survival (C): Correlation between the expression of EPCAM and Overall survival. (D): Correlation between the expression of EPCAM and disease-free survival

DISCUSSION

Epithelial ovarian cancers (EOC) remain the second commonest death cause from gynecological cancers and the 5th most common fatal gynecologic cancer, about 48% five-year relative survival rate. On world wide scale, there are 1.6% new cases and 2.1% death of all cancer annually from ovarian cancer. Most cases experience disease with recurrence despite several treatment lines. About 80% of ovarian cancer cases with late stages have a dismal overall survival rate, regardless of therapy[15,16].

In carcinogenesis, EpCAM promotes tumor cell proliferation, tumor-initiating potential and survival. It has been found overexpressed in many tumors associated with a poor prognosis, treatment failure, and early tumor recurrence[17].

In the current study, EpCAM was highly expressed in 58.8 % of cases, which agreed with the results attained by Mostafa *et al* and Tayama *et al* who stated that high EpCAM expression in 54.7%,57.7% of their cases respectively[4,12].

Our research revealed a high significant association between EpCAM and staging and lymph node metastasis (P<0.001 for each). These results are in harmony with Zheng *et al*, who showed a significant correlation between EpCAM expression and the FIGO stage (*p-value* 0.05), but these results opposed with Woopen *et al*., and Tayama *et al*., who found that EpCAM expression and FIGO stage were not significantly correlated[3,12,18].

Regarding to tumor grade, our study showed no significant correlation with EpCAM expression (*p-value* 0.062). Woopen *et al*., was agreed with our results, who reported that EpCAM was not correlated with tumor

grade, but Mostafa *et al* and Zheng *et al* disagreed with our results, they found out a statistically significant correlation between EpCAM expression and tumor grade (*p-value* 0.03, 0.05 respectively)[3,4,18].

In our study, lower expression of EPCAM was related with better significant overall and disease-free survival *p-value* (0.02), (0.006). respectively, Ibrahim *et al* reported that EpCAM showed high expression in sixty percentage of the cases with a significant correlation with higher tumor grade, lymph node metastasis, and advanced stage ($p < 0.001$ for each). The high EpCAM showed a significant correlation with chemotherapy response ($p= 0.043$), relapse ($p < 0.001$) shorter OS ($p=0.006$) and DFS ($p< 0.001$)[19].

The overexpression of EpCAM has been linked to better progression-free survival also with increased platinum-based treatment response rates ($p=0.040$ and $p=0.048$, respectively). EpCAM was considered as an independent prognostic indicator for overall survival ($p=0.022$)[18].

Numerous studies revealed that pathways linked to EpCAM can upregulate c-myc, stimulate tumour growth, and encourage cell proliferation. Furthermore, in *vitro* data suggest a role for EpCAM in immunosuppression and tumour cell growth[20].

Clinical research showing that in dual-phenotype hepatocellular carcinoma, overexpression of EpCAM and CD133 is correlated with poor outcomes[21].

EpCAM display as a good prognostic marker in some tumour entities. Although it is linked to a poor prognosis in others, EpCAM's involvement in carcinogenesis appears to be closely linked to the tumour biology in various tumours types.

Only few data are reported on EpCAM expression in ovarian cancer. Tayama *et al* found a clinical importance of EpCAM in chemotherapy resistance[12].

Van Der Gun *et al*. did not find a significant impact of overexpression on survival. They reported that downregulating EpCAM expression inhibits breast cancer cell growth, but there is no effect on ovarian cancer cell development. These findings are not consistent with to our results. This may be explained by heterogeneity of histopathological and clinical characteristics of the studied cases[22].

Another study by Mohamed *et al*. reported that over-expression of EpCAM is associated with the increased malignant potential of the tumor & implying that EpCAM expression could be a novel biological therapeutic target for advanced-stage disease and a biomarker for monitoring the course of epithelial ovarian cancer[23].

Our findings showed that SOX2 was expressed in 63.3 % of the ovarian cancer cases, Wen *et al*, and Hasana *et al* showed SOX 2 high expression in 83.5% and 81.8% of ovarian cancer cases respectively which are in agreement with our study.

Our results showed a highly significant correlation between SOX2 expression, lymph node metastasis and staging ($p<0.001$). High expression of Sox-2 was associated with poor overall survival (*p-value* 0.008). But high expression of SOX-2 is associated with prolonged disease-free survival than low expression, but difference was non statistically significant (*p-value*=0.118).

The adverse survival was similar with Baath *et al* who reported that advanced stage HGSOC patients with macroscopic residual tumour following first surgery, SOX2 expression is a powerful prognostic factor that affects both progression-free and overall survival. On the other hand, after radical surgery, there is no predictive effect for patients with advanced stage disease[6].

Wen *et al*. found that SOX 2 was essential for the maintenance of cancer stem cells in ovarian cancer. SOX2 inhibits ovarian cancer cell metastasis and increases cisplatin sensitivity. Down-regulation of SOX2 lowers colony formation and ovarian cancer stemness[24].

Zhang *et al*. studied 540 ovarian carcinoma cases, the majority were high grade serous carcinoma, for SOX2 expression by immunohistochemistry. In univariate survival analysis, SOX2 was linked to a shorter disease-free survival but not in multivariate survival analysis[25].

In the study of 53 tumours, SOX2 protein expression by IHC was linked to poor OS, PFS, and chemoresistance[24].

In one study, SOX2 expression by IHC was associated with a longer disease-free survival for patients with stage II–IV ovarian cancer, which may be correlated with better outcomes[26].

In Belotte analysis, The SOX2 amplification demonstrated a significant survival advantage in both the Cox and K-M predictive models. While the precise interpretation of the correlation between improved outcomes and SOX2 amplification remains uncertain, such associations are frequently observed[27].

Mirzaei, studied SOX2 role in different tumors, SOX2 has both tumour-suppressive and tumour-promoting properties in cancer, and its importance varies depending on the setting. Moreover, SOX2 may perform both roles in some cancer types. Thus, before SOX2 is targeted

for cancer therapy, its expression in each tumour and its relationship to tumour progression/suppression should be ascertained. Thereafter, SOX2 therapeutic targeting is a promising strategy[28].

CONCLUSION

EpCAM and SOX2 were overexpressed in ovarian serous carcinoma tissue and substantially related to lymph node metastasis, TNM stage and poor overall survival making it a critical prognostic biomarker and therapeutic target in ovarian cancer. The correlation between overexpression of EpCAM and SOX2 expression in ovarian cancer provides the path for further research into the molecular mechanisms of tumor progression and the therapeutic target receptors.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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