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ORIGINAL ARTICLE

IMMUNOHISTOCHEMICAL EXPRESSION OF FORKHEAD BOX (FOX) A1 IN EPITHELIAL OVARIAN CANCER

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ABSTRACT

Keywords: Ovarian cancer, epithelial ovarian cancer, FOX A1, prognostic marker, immunohistochemistry clincopathological data.

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Background: Ovarian cancer (OC) ranks as the fifth leading cause of malignancy-associated mortality in females. **Purpose:** This study aims to evaluate the association between A1 immunohistochemical (IHC) FOX expression in epithelial ovarian cancer (EOC) and its relation to their prognosis, as well as its association with other established prognostic indicators like patient's age, tumor laterality, histological subtype, grade and stage, and aims to validate the role of IHC-FOX A1 expression as a prognostic marker in female patients with EOC in Egypt. **Patients and Methods:** From January 2017 to January 2019 a total of 52 paraffin embedded blocks from patients with EOC were collected. Two serial sections from each tissue block were cut at 4 microns thickness to be used as follows: first section was stained with routine Hematoxylin and Eosin (H&E) to confirm the histologic diagnosis and tumor grade. The second was treated with FOX A1 antibodies to show its expression. **Results:** High significant relation was detected between advanced patient's age, high tumor grade, advanced stages, tumors with ruptured capsule and ascites and FOX A1 expression regardless tumor laterality. **Conclusion:** IHC-FOX A1 in EOC is considered a poor prognostic parameter as it is expressed in more than 70% of cases of EOC, significantly with advanced patient's age, high tumor grade, advanced stage, tumors with ruptured capsule and ascites regardless tumor laterality. The results of the present study indicated that FOX A1 could be considered as a poor prognostic marker.

INTRODUCTION

In 2018, the global estimated number of new cases was 295,414 with 7.8 and 4.9 crude incidence and mortality rates per 100,000 globally. According to the latest Global Cancer Observary (GLOBOCAN), it ranks as the eleventh cause of cancer among Egyptian populations being responsible for 2.1% of new cancer cases and 2.3% of cancer deaths (1).

Studies showed that up to 90% of all OC have epithelial origin. There are several histologic types of EOC, involving serous, endometrioid, clear cell, mucinous, transitional and undifferentiated carcinomas (2).

Forkhead box (FOX) A1 represented a potential candidate gene for therapeutic targeting in human EOC; FOX A1 is a transcription factor that is expressed widely and functions in the development of multiple types of human tissue. FOX A1 served a major function in modulating nuclear steroid receptor activity in breast and prostate cancer, and it was suggested that FOX A1 may be associated with pro-tumorigenic phenotypes. The over-expression of FOX A1 in EOC was proposed to be

associated with clinicopathological features, involving overall survival time. FOX A1 potentially represents a novel biomarker and therapeutic target for EOC (3).

MATERIALS AND METHODS

This prospective and retrospective study was carried out on 52 cases of Egyptian female patients with EOC. These cases were selected from the archives of Pathology Departments, Faculty of Medicine, Sohag and Aswan Universities from January 2017 till January 2019. Cases were collected as paraffin embedded blocks. The selected cases included EOC specimens with definite histopathological diagnosis and fulfilling clinical information, including age, histological type, grade based on the World Health Organization (WHO) criteria, FIGO stage and tumor size.

The study was approved by the Ethics Board of Aswan University. The following cases with EOC were excluded for this study: Inadequate tissue material and cases with missing tissue blocks, benign tumors, borderline tumors and undifferentiated carcinomas, tumors of non-epithelial origin and metastatic tumors, cases treated with neo-adjuvant chemotherapy or radiotherapy, cases with incomplete clinical data, cases with massive necrosis and fibrosis. Clinical and pathological data were collected from pathology reports including age, tumor laterality, capsule rupture, presence of ascites, histological differentiation of tumor and tumor grade, stage at presentation, were studied. Clinical details were collected from case records. Two serial sections from each tissue block were cut at 4 microns thickness to be used as follows: First section was stained with routine H&E to confirm the histologic diagnosis and tumor grade; the second was stained with FOX A1 antibody to show its IHC expression.

Histopathological examination: All cases were examined using a light microscope. The following items were recorded: Histopathological type: The revised World Health Organization (WHO) classification of tumors of the female reproductive organs categorized EOC histological types into: serous, mucinous, endometrioid and clear cell (4).

IHC staining: Tissue sections of 4 microns thickness from each case were stained with FOX A1. Negative control was done by omitting the primary antibody. Positive control for FOX A1 was done using breast tissue as recommended in the product datasheet. The primary antibodies used were Rabbit monoclonal antibody against FOX A1 A9793, 1ml concentration with dilution of 1:100.

Evaluation of FOX A1 immunostaining: For evaluation of FOX A1 nuclear staining results, a predefined scoring system based on the product of staining percentage of positive tumor cells was used negative = 0; 1%–50% = 1; 51%–75% = 2; and more than 75% = 3 (5).

Confidentiality of the data:

No encroach on private data of the patient, as blocks of tumor tissue were signed with serial code numbers instead of the name of the patient. Also, the results of the research will be used only in scientific aims.

Statistical analysis of the data:

Data were collected, tabulated and statistically analyzed using a personal computer with Statistical Package for Social Science (SPSS) version 20.0. (6). Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum), mean ± standard deviation and median. Significance of the obtained results was judged at the 5% level (7).

The used tests were:

Chi- square test (X^2- test): was used to compare between qualitative data such as different tumor grade and stage

Probability (p- value): difference considered as follow

Statistically significant (S) when ($p < 0.05$)

Highly significant (HS) when ($p < 0.01$).

Not significant (NS) when ($p > 0.05$).

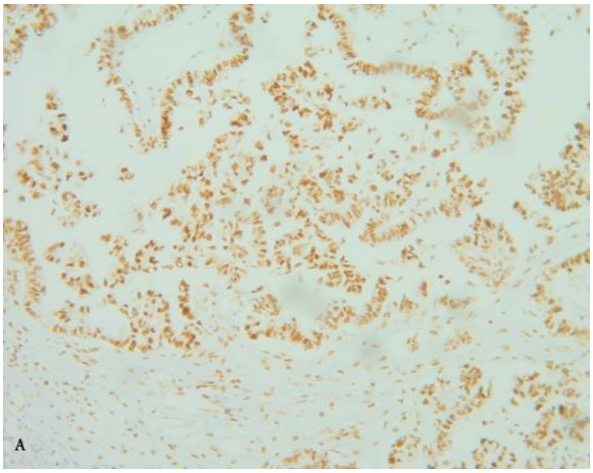

Figure (1): Serous cystadenocarcinoma, grade I, showed strong nuclear IHC FOX A1 expression (X100).

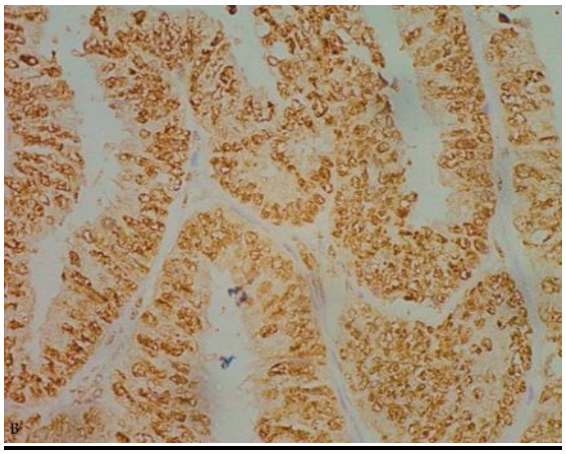

Figure (2): Serous cystadenocarcinoma, grade II, showed strong nuclear IHC FOX A1 expression in tumor cells (X 400)

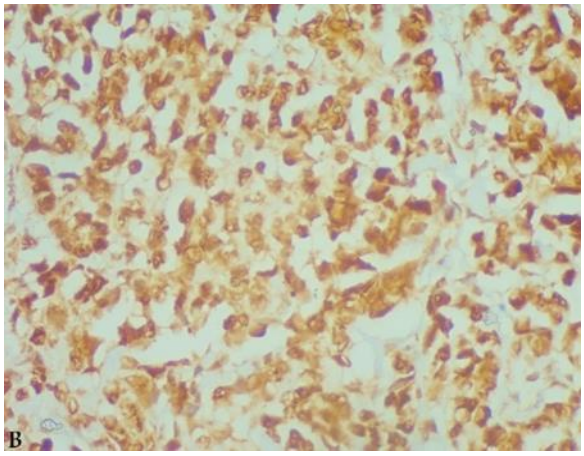

Figure (3): Serous cystadenocarcinoma, grade III, showed strong nuclear IHC FOX A1 expression in tumor cells (X 400)

RESULTS

Assessment IHC expression of FOX A1 was done in malignant epithelial cells for staining status, pattern and intensity. All clinicopathological data are showed in table (1).

For IHC FOX A1 expression; all positive cases showed nuclear pattern of expression. Out of 52, 38 cases (73.1%) showed positive expression (table2).

High significant association was found between FOX A1 expression, patient's age (p=0.010), histologic type (p=0.016), tumor grade (p<0.001), tumor stage (p= 0.022), cases with ruptured capsule (p=0.001) and ascites (p <0.001) (table2).

No significant statistical association could be detected between FOX A1 expression and tumor laterally in the studied cases (table2).

Table (1): Distribution of the studied group as regard age, tumor laterally, histologic type, histologic grade, tumor stage, status of capsule and ascites

Variable	**Number of cases (No)**	**Percentage of cases (%)**
Age (years)		
Total	52	100%
Min. – Max.	31:71	
Mean ± SD.	52.27± 11.55	
Median	53	
Tumor laterality		
Unilateral	21	40.4%
Bilateral	31	59.6%
Stage		
I	22	42.3%
II	9	17.3%
III	11	21.2%
IV	10	19.7
Histologic type		
Serous	37	71.2%
Mucinous	12	23.1%
Endometrioid	1	1.9%
Clear cell	2	3.8%
Grade		
Grade I	14	26.9%
Grade II	18	34.6%
Grade III	20	38.5%
Capsule rupture		
Yes	30	57.7%
No	22	42.3%
Ascites		
Yes	29	55.8%
No	23	44.2%

Table (2): Relation between FOX A1 expression and age, tumor laterality, histologic type, histologic grade, tumor stage, status of capsule and ascites

		IHC FOX A1 Expression		**Significance**	**p value**
		No.	%		
		Positive (n=38)	Negative (n=14)		
Age (years)	Mean ± SD.	54.74±10.85	45.57±11.04	**t= -2.690**	**p= 0.010**
Laterality	Unilateral	14 (66.67%)	7 (33.33%)	X^2 0.736	p= 0.391
	Bilateral	24 (77.42%)	7 (22.58)		
Histologic type	Serous	27 (71.1%)	10(28.9%)		**0.016**
	Mucinous	11 (28.9%)	1(71.1%)		
	Endometrioid	-	1		
	Clear cell	-	2		
Grade	Grade I	3 (21.43%)	11 (78.57%)		**<0.001**
	Grade II	16 (88.89%)	2(11.11%)		
	Grade III	19 (95%)	1 (5%)		
Stage	I II III IV	12 (54.5%) 7 (77%) 10 (90.9%) 9 (90%)	10(45.5%) 2(23%) 1(9.1%) 1 (10.0%)		**0.022**
Capsule rupture	No	11 (50%)	11 (50%)	X^2 **10.322**	**0.001**
	Yes	27 (90%)	3 (10%)		
Ascites	No	11 (47.83%)	12 (52.17%)	X^2 **13.365**	**<0.001**
	Yes	27 (93.1%)	2 (6.9%)		

χ_2, and p values for Chi square test

DISCUSSION

Ovarian cancer is 2nd most common type of gynecological cancer worldwide, and it is a major cause of cancer- associated mortality in women (8). There were an estimated 300,000 new OC cases diagnosed worldwide in 2018 that was corresponding to 3.4% of all cancer cases between women. However, there is a substantial geographic variation in the burden of OC (rates are varying from 5.0 per 100,000 person/years in Africa to 9.5 per 100,000 person/years in Europe) (9). The more practically accepted concept of classification of ovarian carcinomas is to be categorized into 5 main histological types as follow: high-grade serous carcinoma (HGSC), clear-cell carcinoma, endometrioid carcinoma, mucinous carcinoma, and low-grade serous carcinoma (LGSC). This classification depends on differences according to their biology, clinical presentation, and response to chemotherapy (10).

The aim of the current study was to evaluate IHC expression of FOX A1 in tumor cells and its relation to prognosis of EOC.

In this study, cases were females ranging in age from 31 to 71 years. The mean age of the studied cases was 52.27± 11.55 years. This age finding was comparable to those reported by Sheta H et al., (11) who made a study on 98 cases of primary EOC. They reported that the mean age of the patients was 57.1 ± 10.9 years.

In the present work, bilateral tumors were the most frequent 59.6%. This result was comparable to those reported by Sheta H et al., (11) as they reported that 43.9% of tumors were bilateral. For histologic type, the current study denoted that more than 70% of studied cases were serous carcinoma. Sheta H et al., (11) reported that 55.1% of tumors were HGSC. Also, Amanullah et al., (12) reported that 48.3% were serous tumors.

Regarding tumor grade, high grade tumors were found to represent the highest percentage 73.0% of cases in the current research. This is in line with a study done by Ndukwe et al., (13) who reported that 66% of tumors were high-grade neoplasms and 34% of case were low-grade neoplasms.

The state of capsule and presence of ascites are a powerful prognostic factor and are routinely used to determine the stage of the tumor. In the current study, 57.7% of cases had ruptured capsule and 55.8% of cases had ascites, compared with 73% of cases had ruptured capsule in the study of Amanullah et al., (12).

Regarding IHC expression of FOX A1 in EOC specimens; the present study demonstrated high expression of FOX A1 in 73.1% of EOC tissues. The study that was carried out by Sheta H et al., (11) reported that FOX A1 expression was seen in about 63.3% of EOC tissues. However Li-Li Wang et al., (14) reported that FOX A1 expression in EOC was about 32.03% of cases. According to the relation between FOX A1 expression and tumor P-stage; FOX A1 was positive in 90.48% of cases at stages III and IV. This result agreed with Zhang et al., (15) who detected FOX A1 in 84.7% of cases at stages III and IV.

In this study a significant statistical relation could be detected between FOX A1 expression and tumor grade as 92.1% of tumors of grade II and grade III showed positive expression of FOX A1. This was concomitant with the study of Wang et al., (16) who observed that 41/62 cases of moderately differentiated and poorly differentiated EOC showed strong FOX A1 expression of tumors cells and that was statistically significant. These findings suggest that FOX A1 expression was associated with poor prognostic parameters in EOC.

In the current work; 66.7% of serous carcinomas showed positive FOX A1 expression and there was significant relation between FOX A1 expression and histologic type of tumor. These results agreed with Wang et al., (16) who reported that 55% of serous carcinomas were stained positive with FOX A1. According to the current work, there was high significant association between FOX A1 expression and tumors with ruptured capsule and ascites.

CONCLUSION

FOX A1 immune-expression in EOC is considered as a poor prognostic parameter as it is expressed in more than 70% of EOC specimens. This expression is significantly associated with advanced patient's age, high tumor grade, advanced stages, tumors with ruptured capsule and ascites regardless tumor laterality. The results of the present study indicated that FOX A1 could be a poor prognostic marker.

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