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Original Article

High FOXA1 immunohistochemical expression level associates with mucinous histology, favorable clinico-pathological prognostic parameters and survival advantage in epithelial ovarian cancer

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Summary

Background. Forkhead box (FOX) A1 is a potential therapeutic biomarker that has been investigated in various human cancers. Limited data exist about FOXA1 biologic role in epithelial ovarian cancer (EOC).

Aim. This study assessed FOXA1 immunohistochemical (IHC) expression and evaluated its association with clinico-pathological parameters in EOC including overall and disease-free survivals (OS, DFS) and patient's outcome.

Methods. Patient's socio-epidemiologic, clinical, radiological, laboratory, surgical, and follow-up data were collected. After histopathologic typing, grading and staging, FOXA1 IHC expression was scored in 98 EOC specimens. Clinico-pathological associations were investigated in high-and low-FOXA1 expression groups using appropriate statistical methods. Kaplan-Meier method was used for survival analysis.

Results. FOXA1 tumor cell nuclear staining was detected in 63.3% of EOC with weak, moderate and strong scores (28.6%, 12.2% and 22.5% respectively). Comparing high- and low-expression groups (34.7% and 65.3% respectively), high FOXA1 was associated with larger tumors, low mean serum CA-125, tumor histopathology (mucinous and low-grade serous), type I EOC, limited tumor's anatomical extent, absence of nodal or distant metastases and omental nodules, earlier FIGO stages, non-recurrent tumors and survival advantage with longer and OS and DFS (all $p \leq 0.05$). Independent predictors of high FOXA1 expression included: omental nodules, tumor's anatomical extent and tumor's size ($p \leq 0.001$, $= 0.046$ and $= 0.023$ respectively).

Conclusion. FOXA1 is frequently expressed in EOC notably mucinous and low-grade serous carcinomas in association with favorable prognostic clinico-pathological parameters and longer OS and DFS. It likely has a suppressor function in EOC and could be recommended as a prognostic and therapeutic biomarker.

Key words: epithelial ovarian cancer, immunohistochemistry, FOXA1, clinico-pathological, survival

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Conflict of interest
The Authors declare no conflict of interest.

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Introduction

Ovarian cancer is a well-established leading cause of female morbidity and mortality worldwide constituting the third common gynecologic cancer after corpus uteri and cervical cancers. 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 Can-

cer 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]. Conspicuously, epithelial ovarian cancer (EOC) comprises the vast majority of clinically encountered ovarian cancers with a tendency for late diagnosis [2,3], culminating in further morbidity and mortality. Due to the limited therapeutic options and the high rate of chemotherapy-resistance in high-grade EOC, there is a compelling need to identify novel biomarkers that can be used to improve patient management [4].

The forkhead box A1/hepatocyte nuclear factor 3α (FOXA1/HNF3α), encoded by FOXA1gene located on at human chromosome 14q21.1, is the founding member of the FOXs family of pioneer or licensing transcription factors and gene regulators that are essential for the normal development of several endoderm-derived tissues [5-8]. FOXAs contain a highly conserved DNA-binding domain or FOX/winged helix domain that shares extremely high homology with that of its namesake, the Drosophila homologue fkh (forkhead) [5]. Essentially, FOXA1 binds to enhancer regions enriched in histone 3 lysine 4 mono- and dimethylation (H3K4me1/me2) of condensed chromatin contributing to chromatin opening to allow other transcription factors to come in close proximity to their target sites and bind to it [9]. It is required for normal development of several organs notably the mammary gland and prostate, and is necessary for hormone receptor-regulated transcription in cancers of these organs. Thus, understanding the functional mechanisms of FOXA1 during development has generated great insights into its function in cancer progression[5], and as a therapeutic target [10,11].

Over the past decade, the biologic role of FOXA1 has been investigated in various human cancers including the mammary [6,12,13], prostatic [8,10], hepatocellular [14], cervical [15], nasopharyngeal[16,17] and colorectal [18] carcinomas, cholangiocarcinoma [19] and ductal carcinoma in situ of the breast [20] using different methodologies, although contradictory data were obtained concerning its prognostic implications and as whether it acts as an oncogene or a tumor-suppressor gene [5,18].

To the best of our knowledge, few previous studies have investigated FOXA1 expression in EOC [21-23], with no certainty about the favorable versus unfavorable impact of FOXA1 expression on patients' outcome and survival. In attempt to fill this gap, this study is addressed to assess the frequency of FOXA1 immunohistochemical (IHC) expression in EOC of different histopathologic types and to evaluate its associations with the clinico-pathological parameters in EOC including overall and disease-free survivals (OS and DFS respectively) and patient's outcome. The possible utility of FOXA1 as a therapeutic target will be addressed.

Patients and methods

Setting

This study was conducted at the Oncology Center Mansoura University (OCMU) and Pathology Laboratory at OCMU during the period from September 2018 to June 2020.

Inclusion and exclusion criteria

A computer-based data search was performed through the OCMU electronic archiving system using the following criteria: a female patient with pathological diagnosis of EOC made during the period from June 2012 to June 2015; availability of complete patient's relevant socio-epidemiologic, clinical, radiological, laboratory, surgical, and follow-up data (including recurrence and metastasis); and a documented fulfilment of a postoperative therapeutic protocol (starting platinum-based first-line chemotherapy for 6 cycles every 21 days, non-platinum-based chemotherapy second-line regimens [e.g. gemcitabine, doxorubicin] were given in the platinum-resistant cases who had a platinum-free interval [PFI] less than 6 months, cases relapsing after 6 PFI months were considered as platinum sensitive and were re-challenged with platinum). Upon achievement of these criteria, accessibility of archived formalin-fixed, paraffin-embedded (FFPE) tumor tissue samples was ascertained. Patients not fulfilling the abovementioned criteria were excluded from the study.

Socio-epidemiologic and clinical data

The following data were collected and tabulated using a code number for each patient: age, tumor diameter, laterality, preoperative serum CA-125 level. Overall survival (OS) and disease-free survival (DFS) were registered in months stating from the date of initial diagnosis till the end of study (by conduction of statistical analysis)/patient mortality for the former, and from the time of first evaluation after the end of primary treatment to recurrence/metastasis (or re-appearance of any signs or symptoms of cancer) for the latter [24].

Pathological classifications and definition criteria

Histopathology

Routinely processed hematoxylin and eosin (H&E)-stained microscopic slides were reviewed by 2 pathol-

ogists. According to the latest World Health Organization (WHOC) classification of EOC [25], 98 female patients with histopathologically confirmed primary EOC encompassing 75 serous (21 low-grade and 54 high-grade), 15 mucinous (7 with expansile and 8 with infiltrative/mixed pattern) and 2 endometrioid carcinomas, 2 clear cell carcinomas (CCC), 2 malignant Brenner tumors and 2 mixed carcinomas (major high-grade serous and endometrioid components) were enrolled. Histopathologic diagnosis was confirmed using immunohistochemistry for cytokeratin 7 and 20, p53, Wilms' tumor1(WT1) and napsin A whenever appropriate.

EOC tumor type

Based on the new models of ovarian carcinogenesis [25], EOC were classified two broad categories: type I (low-grade serous, low-grade endometrioid, mucinous carcinomas, and CCC), and type II (high-grade serous, high-grade endometrioid and high-grade mixed carcinomas with serous and endometrioid components). The 2 malignant Brenner tumors were uncategorized in either type.

Evaluation of Anatomical Extent, FIGO stage, omental nodules and recurrence state

A combination of clinical, radiological, surgical and follow-up data as well as gross pathology reports and microscopic assessments (including histopathologic evaluation of ovarian tumor sections, ascitic/peritoneal wash cytology, peritoneal and lymph node samples routinely collected at time of surgery, biopsies from omental nodules, recurrent ovarian site or peritoneal lesions and metastatic nodal or distant lesions) was implemented for defining the anatomical extent of tumors (T), the state of nodal involvement (N) and presence or absence of metastasis (M). Staging of EOCs was performed according to International Federation of Gynecology and Obstetrics (FIGO) staging criteria [2]. The absence or presence of either radiologically or histopathologically-confirmed recurrence/metastasis during the follow-up period has been documented.

Immunohistochemistry (IHC)

IHC Procedure

About 3-4 µm FFPE, EOC sections mount on positively charged silanized glass slides (VitoGnost SIL adhesive microscope slides) were deparaffinized in descending grades of alcohol then dehydrated. Antigen retrieval was done using 0.01 M citrate buffer (pH 6.0 at 92°C) for 10 min in microwave. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide (room temperature for 15 min.). Slides were incubated for 1 hour at room temperature with a mouse monoclonal anti-human FOXA1 antibody, clone 2F83 directed against N-terminus (Sigma-Aldrich, Germany, Cat. No.: 05-1466, isotype: IgG,100 µg purified mouse monoclonal antibody in buffer containing 0.1 M Tris-Glycine pH [7.4], 150 mM NaCl with 0.05% sodium azide, 1:1,000 dilution). Afterwards, the sections were incubated with appropriate secondary antibody at room temperature for 30 min, washed in phosphate-buffered saline (PBS; pH 7.4) and dehydrated. The avidin-biotin technique was performed using diaminobenzidine (DAB) as a chromogen for visualization and hematoxylin for counterstaining. Slides were dehydrated, mounted and covered. Prostate tissue control sections were stained with the same procedure along each IHC run. Negative controls were prepared with the same procedure but omitting the primary antibody incubation.

Assessment of FOXA1 staining

Two pathologists blinded of patients' clinico-pathological and survival data examined the slides under ordinary light microscope (Leica, DM500). A semi-quantitative scoring was applied. Brownish staining of tumor cell nuclei of moderate to strong intensity was considered as positive. After initial low-power screening, scoring was done using 200x magnification power to determine the percentage of stained tumor cells according to the following scheme: staining of < 5% of tumor cells was arbitrated as negative, 5-30% as weak, 31-70% as moderate and > 70% as strong expression scores. For statistical evaluation, negative and weak scores were categorized as FOXA1 low-expression group, while moderate and strong scores were categorized as FOXA1 high-expression group [23].

Research ethics

FFPE tissue samples were retrieved from the archives of Pathology Laboratory at OCMU, thus posing no influence on sampling/operative procedures or therapeutic decisions. No further medical interventions were applied to the patients as a part of the study that was conducted upon approval of the committed Institutional Research Board (IRB) at Faculty of Medicine, Mansoura University, Egypt (code number: R.18.10.314). Informed consents were obtained from patients or their relatives to use their data. Anonymity and confidentiality were secured throughout and after the study by using a code number for each patient instead of using names. All procedures were done in accordance with the current revision of Helsinki Declaration of medical research involving human subjects [26].

Statistical methods

Statistical analysis was carried out with IBM Corp. SPSS (International Business Machines Corporation Statistical Product and Service Solutions), released 2012 for Windows, Standard Version 21.0. Armonk, NY: IBM Corp., Chicago, USA. The normality of data was first tested with one-sample Kolmogorov-Smirnov test. Qualitative data were described as numbers and percentages. To analyze the association between FOXA1 expression and the clinico-pathological parameters, association with categorical variables was tested using Chi-square $\chi 2$ test, while Fisher's exact and Mann–Whitney tests were applied whenever appropriate. Continuous variables were presented as median, min-max and mean ± SD (standard deviation).

Significant variables on univariate analysis of the predictors for high FOXA1 expression were entered into logistic regression model using forward Wald statistical technique to predict the most significant determinants and to control for possible interactions and confounding effects. Kaplan-Meier test was used for survival analysis and statistical significance of differences among curves was determined by Log-Rank test. For all the above-mentioned statistical tests, the threshold of significance was fixed at 5% level, considering a p-value as significant if ≤ 0.05.

Results

Socio-demographic and Clinico-pathological characteristics

As seen in Table I, the study included 98 EOC female patients aging between 30 and 82 years with mean and median ages of 57.1 and 56 years respectively. Tumors ranged in size from 2 to 22 cm. with mean and median diameters of 10.4 and 10.5cm respectively. About 56% of patients were presented with bilateral tumors. A mean serum CA-125 level of 456.7U/mL was detected and 51% of patients had a CA-125 level above the median (245 U/mL) for this group.

On a histopathologic basis, the most frequent tumors were serous carcinomas (75/98 cases; 76.5% of which 21; 28% were low-grade and 54; 72% were high-grade), followed by mucinous carcinomas (15.3%) then endometrioid carcinomas, CCCs, malignant Brenner tumors and mixed carcinomas with major high-grade serous and endometrioid components (about 2% each). These histo-pathogenetic subtypes were further defined based on basis of stage, recurrence status, mortality and survival times (Tab. II), where high-grade serous carcinomas and mixed carcinomas with a high-grade serous component were the most likely carcinomas to present at higher stage disease (66.7% and 100% respectively). There was a significant difference between these histo-pathogenetic subtypes in the recurrence rate, mortality

Table I. Socio-demographic and clinico-pathological criteria of the 98 epithelial ovarian cancer (EOC) patients included in the study and their distribution/statistical associations with Forkhead box A1(FOXA1) immunohistochemical expression.

Criteria	Value	FOXA1 expression		Test of significance	Odds ratio (95%CI)
		Low (n, %)	High (n, %)		
Age/year					
Mean ± SD	57.1 ± 10.9	58.19 ± 11.9	55.18 ± 8.67	t = 1.29 p = 0.198	-
Median (min-max)	56 (30-82)				
< 56	48 (49)	28 (58.3)	20 (41.7)	χ^2 = 2.02 p = 0.16	1.8 (0.8-4.3)
≥ 56 (r)	50 (51)	36 (72)	14(28)		
Tumor diameter/cm					
Mean ± SD	10.4 ± 4.9	9.08 ± 4.44	12.81 ± 4.71	t = 3.87 p ≤ 0.001*	-
Median (min-max)	10.5 (2-22)				
< 10.5 cm (r)	50 (51)	40 (80)	10 (20)	χ^2 = 9.73 p = 0.002*	4.0 (1.6-9.8)
≥ 10.5cm	48(49)	24 (50)	24 (50)		
Laterality (n, %)					
Unilateral	55 (56.1)	33 (60)	22(40)	χ^2 = 1.56 p = 0.21	1.7 (0.7-4.1)
Bilateral (r)	43 (43.9)	31(72.1)	12 (27.9)		

Serum CA-125 U/mL					
Median (Min-Max)	245 (4-4402)	385.5 (4-4402)	130 (10-724)	Z = 2.28 p = 0.022*	-
< 245	48 (49)	26 (54.2)	22 (45.8)	t = 5.15 p = 0.023*	2.7 (1.1-6.3)
≥ 245 (r)	50 (51)	38 (76)	12 (24)		
Histopathology (n, %)					
Low-grade Serous carcinoma	21 (21.4)	2 (9.5)	19 (90.5)	χ^2 = 36.7 p ≤ 0.001*	39 (8-187)
High -grade Serous carcinoma	54 (55.1)	54 (100)	0 (0)	χ^2 = 63.9 p ≤ 0.001*	
Mucinous carcinoma	15 (15.3)	0 (0)	15 (100)	χ^2 = 33.3 p ≤ 0.001*	-
Endometrioid carcinoma	2 (2)	2 (100)	0 (0)	FET p = 0.542	-
Malignant Brenner Tumor	2 (2)	2 (100)	0 (0)	FET p = 0.542	-
Clear cell carcinoma	2 (2)	2 (100)	0 (0)	FET p = 0.542	-
Mixed carcinoma	2 (2)	2 (100)	0 (0)	FET p = 0.542	-
EOC tumor type (n, %)					
Type I	38 (38.8)	4 (10.5)	34 (89.5)	c^2 = 82 p ≤ 0.001*	-
Type II	58 (59.2)	58 (100)	0(0)	FET p ≤ 0.001*	-
Uncategorized (Brenner)	2 (2)	2 (100)	0(0)	FET p = 0.542	-
Tumor's extent (n/%)					
T1	40 (40.8)	16 (40)	24 (60)	T1 vs T2/3(r) χ^2 = 19.1 p ≤ 0.001*	7.2 (2.8-18.2)
T2	10 (10.2)	48 (82.8)	10 (17.2)		
T3	48 (49)				
Nodal status (n, %)					
N0	74 (75.5)	44 (59.5)	30 (40.5)	χ^2 = 4.56 p = 0.033*	3.4 (1.1-10.9)
N1 (r)	24 (24.5)	20 (83.3)	4 (16.7)		
Metastasis (n, %)					
M0	90 (91.8)	56 (62.2)	34 (37.8)	χ^2 = 4.63 p = 0.031*	-
M1	8 (8.2)	8 (100)	0(0)		
FIGO stage (n, %)					
I	34 (34.6)	22 (52.4)	20 (47.6)	I/II vs III/IV (r) χ^2 = 5.42 p = 0.02*	2.7 (1.2-6.4)
II	8 (8.2)				
III	48 (49)	42 (75)	14 (25)		
IV	8 (8.2)				
Omental nodule (n, %)					
No	36 (36.7)	10 (27.8)	26 (72.2)	χ^2 = 35.4 p = ≤ 0.001*	17.5 (6.2-49.7)
Yes (r)	62 (63.3)	54 (87.1)	8 (12.9)		
Recurrence (n/%)					
No	50 (51%)	26 (52)	24 (48)	χ^2 = 7.98 p = 0.005*	3.5 (1.4-8.5)
Yes (r)	48 (49%)	38 (79.2)	10 (20.8)		
Mortality (n/%)					
Survived	48 (49%)	20 (41.7%)	28 (58.3%)	χ^2 = 23.2 p ≤ 0.001*	10.3 (3.7-28.7)
Died (r)	50 (51%)	44 (88%)	6 (12%)		
Total	**98 (100)**	**64 (65.3)**	**34 (34.7)**		

n, number; %, percentage; cm., centimeter; U/mL, units per milliliter; SD, standard deviation; L, low; H, high, (r), reference group; t, χ^2 chi-square test; FET, Fisher's exact test; Z, Mann–Whitney test; CI, confidence interval; p value is significant if ≤ 0.05.

Table II. The seven histopathologic subtypes of epithelial ovarian carcinoma defined by stage, recurrence status, mortality and survival.

Criteria	Histopathologic types (n, %)							Test of significance
	Low-grade serous carcinoma	High-grade serous carcinoma	Mucinous carcinoma	Endometrioid carcinoma	Clear cell carcinoma	Malignant Brenner tumor	Mixed carcinoma	
FIGO stage (n, %)								
I&II	12 (57.1)	18 (33.3)	8 (53.3)	2 (100)	1 (50)	1 (50)	0 (0)	$\chi^2 = 8.7$ p = 0.193
III & IV	9 (42.9)	36 (66.7)	7 (46.7)	0 (0)	1 (50)	1 (50)	2 (100)	
Recurrence (n/%)								
No	13 (61.9)	22 (40.7)	11 (73.3)	2 (100)	1 (50)	1 (50)	0 (0)	$\chi^2 = 15.6$ p = 0.016*
Yes	8 (38.1)	32 (59.3)	4 (26.7)	0 (0)	1 (50)	1 (50)	2 (100)	
Mortality (n/%)								
Survived	16 (76.2)	18 (33.3)	12 (80.0)	1 (50)	1 (50)	2 (100)	1 (50)	$\chi^2 = 19.03$ p = 0.004*
Died	5 (23.8)	36 (66.7)	3 (20.0)	1 (50)	1 (50)	0 (0)	1 (50)	
OS Median (95%CI)	46 (38-54)	39 (33-45)	40.5 (30-51)	4 (4-4)	5 (5-5)	61 (61-61)	26 (26-26)	Log rank = 81.6 p ≤ 0.001*
DFS Median (95%CI)	44.7 (37-52)	28 (22.9 -33.4)	30 (23-37)	4 (4-4)	5 (5-5)	26 (26-26)	6 (6-6)	Log rank = 95 p ≤ 0.001*

OS, overall survival; DFS, disease-free survival; χ^2 chi-square test; CI, confidence interval; p value is significant if ≤ 0.05.

(p = 0.016 and 0.004 respectively), and both DFS and OS (p ≤ 0.001 for each) as high-grade serous carcinomas were characterized by unfavorable prognostic parameters compared mainly to low-grade serous and mucinous carcinomas.

As categorized by EOC tumor type, most tumors (59.2%) were categorized as type II, less frequently as type I (38.8) and remaining 2% comprising the malignant Brenner tumors were uncategorized to an EOC tumor type. Based on tumor's anatomical extent, most tumors (49%) were involving one or both ovaries and has extended into organs outside the pelvis (T3), 40.8% of tumors were confined to one or both ovaries (T1), and about 10% had tumors involving one or both ovaries with pelvic extension below pelvic brim (T2). About 24.5% of patients had histopathologically confirmed lymph node metastases (N1) and 8.2% had distant metastases including hepatic focal lesions (HFL; all 8 cases of which 3 were confirmed by histopathology and 5 by radiology) and 2 cases had additionally pulmonary nodules (confirmed radiologically). Moreover, 63.3% of patients had omental nodules that were all histopathologically confirmed. Based on FIGO staging criteria, 49% of patients were presented at stage III disease followed by stage I (34.6%) whereas small percentages of patients were at stages I and IV (8.2% each).

The overall survival (OS) ranged from 4 to 94 months, whilst disease-free survival (DFS) ranged from 4 to 67 months. Among the 48 patients who had recurrence (49%), 11 had recurrent pelviabdominal swelling confirmed by histopathology, 10 patients had lymph node recurrence (4 inguinal, 3 iliac, 2 supraclavicular and 1 cervical), 8 patients had malignant ascites confirmed by histopathology, 6 patients had single or multiple omental nodules (3 confirmed by histopathology and 3 by radiology), 4 patients had peritoneal nodules confirmed by histopathology, 4 patients had radiologically confirmed HFLs, 2 patients had radiologically confirmed pulmonary nodules, 2 patients had radiologically confirmed multiple HFLs and omental nodules as well and 1 patient had histopathologically confirmed epigastric and omental masses (in a descending order of frequency). During the follow-up period, 51% of patients died and the remainder survived.

FOXA1 immunohistochemical (IHC) expression in EOC

Figure 1 demonstrates that 63.3% of EOC expressed FOXA1 distributed as 28.6% with weak, 12.2% with moderate and 22.5% with strong expression scores. Accordingly, negative (36.7%) and weak expression were combined in the FOXA1 low-expression group (65.3%) whereas moderate and strong levels were combined in the FOXA1 high-expression group (34.7%; Tab. I).

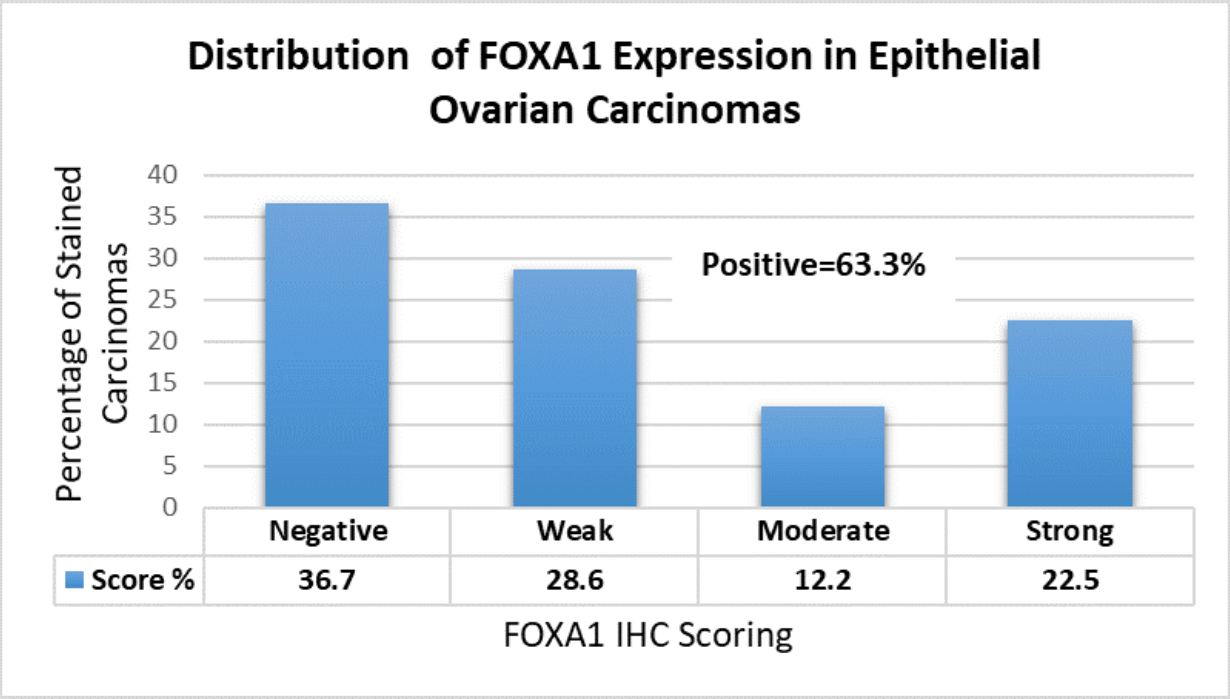

Figure 1. Distribution of Forkhead box A1(FOXA1) immunohistochemical (IHC) expression scores among the 98 (36, negative and 62, positive) studied epithelial ovarian cancers (EOC).

FOXA1 expression association with socio-demographic and clinico-pathological characteristics

Patient's age revealed no significant associations when compared between FOXA1 low- and high- expression groups. There was a significant direct association between FOXA1 expression and tumor size ($p \leq 0.001$ for mean and = 0.02 for median diameters), as 80% of tumors less than 10.5 cm had low expression while 50% of tumors larger than the median diameter had high expression. Unilateral tumors had a tendency to express higher level of FOXA1 (40%) as compared to bilateral ones (27.9%), however this difference did not reach statistical significance. Concerning serum CA-125, tumors with high FOXA1 expression revealed a significantly lower mean CA-125 (233.4 U/mL) when compared to tumors with low FOXA1 expression (575.38 U/mL, $p = 0.022$), and most (76%) of patients with a CA-125 serum level above 245U/mL had tumors with low FOXA1 expression rendering a significant difference ($p = 0.023$) when compared to patients with serum CA-125 below this level.

Among the seven histopathologic subtypes, high FOXA1 expression was observed in all (100%) of mucinous carcinomas and most (90.5%) of low-grade serous carcinomas, while all tested high-grade serous (44.4% weak and 55.6% negative) endometroid (negative), clear cell (negative) and mixed carcinomas (weak) as well as malignant Brenner tumors (negative) were included in the FOXA1-low expression group imparting a statistically significant difference in FOXA1 expression among the histopathologic types of EOC ($p \leq 0.001$) with mucinous carcinoma followed by low-grade serous carcinoma being the most frequently FOXA1-expressing histopathologic subtypes. Moreover, there was a significant difference in FOXA1 expression between EOC tumor types ($p \leq 0.001$*) as most type I tumors (89.5%) revealed high FOXA1 expression, while all type II tumors exhibited a low FOXA1 expression (Fig. 2).

There were significant inverse associations between anatomical extent (T), nodal involvement (N), presence of distant metastasis (M), FIGO stage, the presence of positive omental nodules or recurrence ($p \leq 0.001$, = 0.033, = 0.031, = 0.02, ≤ 0.001 and = 0.005 respectively). Most (60%) tumors confined to the ovary/ies (T1), about 40% of tumors without nodal metastases (N0), 37.8% of those without distant metastases (M0), 47.6% of early FIGO stages I/II tumors, 72.2% of tumors not associated with omental nodules and 48% of tumors with no recurrence had high FOXA1 expression as compared to 17.2% of tumors extending beyond the ovary/ies (T2/T3), 16.7% of tumors with nodal metastases, 0% of tumors with distant metas-

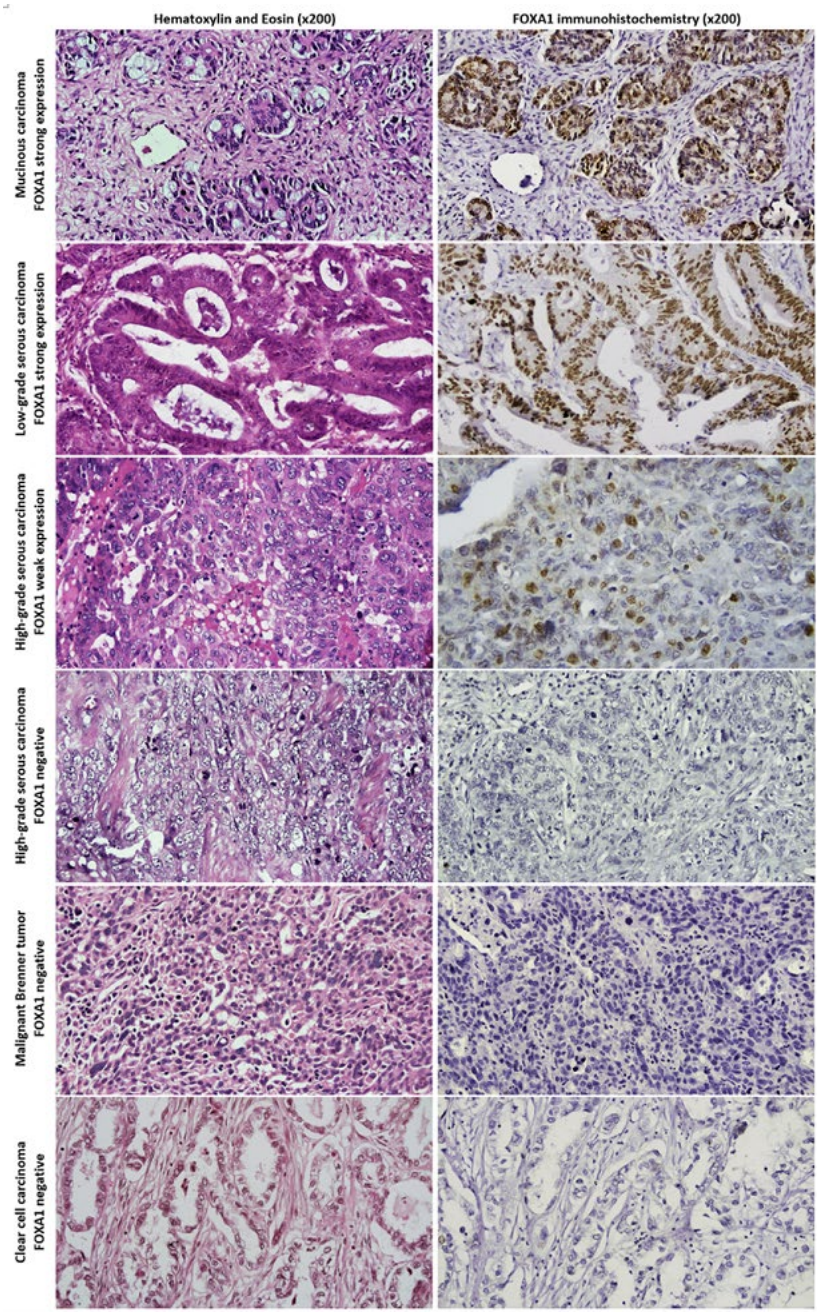

Figure 2. Representative examples of epithelial ovarian cancers (EOC) of different histopathologic types and grades with the corresponding FOXA1 immunohistochemical expression scores.

tases, 25% of tumors with advanced FIGO stages III/IV, 12.9% of tumors associated with positive omental nodules and 20.8 of recurrent tumors respectively. Concerning mortality, 88% of patients who died during the follow-up period had tumors with low FOXA1 expression, meanwhile 58.3% of patients who survived had tumors with high FOXA1 expression conferring a significant difference between both groups ($p \leq 0.001$).

Predictors of-high FOXA1 expression in EOC

Among tested variables (Tab. III), logistic regression analysis revealed that omental nodules, anatomical extent and tumor diameter were independent predictors

Table III. Logistic regression analysis of independent predictors of high Forkhead box A1(FOXA1) expression.

Independent predictors		β	p-value	OR (95%CI)
Omental nodule	Yes (r), No	2.97	≤ 0.001	14.1 (3.5-57)
Tumor's anatomical extent	T1 T2&3 (r)	2.21	0.046	9.1 (1.2-8.4)
Tumor diameter/ cm	< 10.5 (r) ≥ 10.5	1.36	0.023	3.9 (1.04-78)

(r), reference group; CI, confidence interval; p value is significant if ≤ 0.05.

Table IV. Kaplan-Meier survival for FOXA1 as a predictor for mortality (overall survival; OS) and recurrence (disease free survival; DFS) in epithelial ovarian cancer.

	Mortality (OS)		Recurrence (DFS)	
FOXA1 Expression	Low	High	Low	High
Median Survival/ months	43.68	75.14	34.75	53.18
Std. Error	4.21	4.42	3.24	2.81
95% CI	35.4-51.9	66.8-83.8	28.4-41.1	47.7-58.7
p-value	≤ 0.001	0.002		

CI, confidence interval; p value is significant if ≤ 0.05.

of high FOXA1 expression in EOC (p ≤ 0.001, = 0.046 and = 0.023 respectively), where the probability of high FOXA1 expression was found to decrease with the presence of omental nodules, tumors extending beyond the ovary/ies and tumors smaller than 10.5 cm (reference groups).

Survival analysis

The OS and DFS of patients in the FOXA1 low-expression and high-expression groups differed significantly (p ≤ 0.001 and 0.002 respectively; Tab. IV, Fig. 3). Patients with high FOXA1 expression demonstrated both OS and DFS advantages contrasting to those with low FOXA1 expression with evidence of direct association between FOXA1 expression and longer OS and DFS times in EOC.

Discussion

In the era of personalized cancer medicine, the development of putative biomarkers for cancer prognosis and therapy has become a sine qua non for successful treatment. To exemplify for this, the relationship between FOXA1 expression and several prognostic factors has been investigated in various cancers in the past few years. However, the influence of FOXA1 expression on prognosis was found to differ according to the primary site and tumor type [27]. Overall, both oncogenic and tumor-suppressive roles have been reported for FOXA1, which suggests that its precise

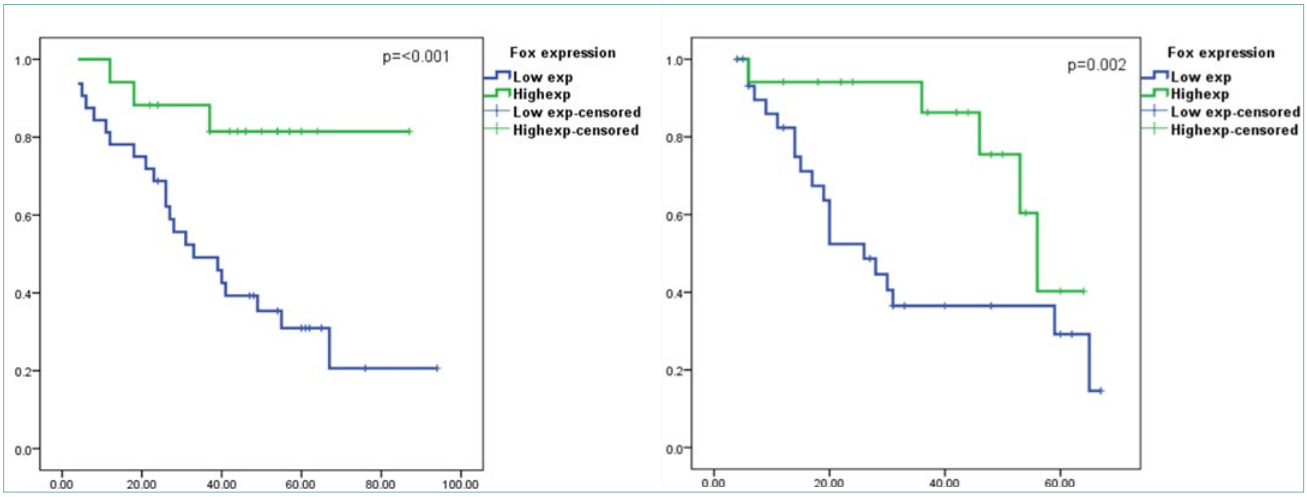

Figure 3. Kaplan-Meier survival curves for patients with epithelial ovarian cancer (EOC) stratified by FOXA1 high or low expression. Both overall survival (OS; left panel) and disease-free survival (DFS; right panel) are significantly longer in patients with high FOXA1 expression compared to those with low expression (p < 0.001 and p = 0.002 respectively).

contribution to cancer development or progression may be dependent on several other factors [28].

In the current work, we verified that EOCs frequently express FOXA1 (63.3%) in different grades of immunopositivity, and that high scores of FOXA1 IHC expression associates with the favorable clinico-pathological characteristics and an OS as well as DFS advantages for EOC patients. These inferences were compatible with the data observed in breast carcinoma [6,7,13], cholangiocarcinoma [19], salivary duct [27], hepatocellular [14], nasopharyngeal and endometrial carcinomas [29]. In contrast, unfavorable associations had been observed in prostatic [8,28], colorectal [18], and cervical [15] carcinomas, supporting the notion that FOXA1 may act as a tumor-suppressor gene or as an oncogene.

In the study by Karpathiou et al. [21], 19% of EOC strongly and diffusely expressed FOXA1and 75% of these EOC were of the mucinous followed by serous histology, but endometrioid and CCCs were completely negative. In agreement with the former study, 34.7% of our samples exhibited high FOXA1 expression and all mucinous carcinomas were in this category. Likewise, none of our included endometrioid carcinomas and CCCs was FOXA1 positive, suggesting that FOXA1 could possibly act as a biomarker for mucinous differentiation in EOC in a manner similar to that in which Wilms' tumor-1 protein (WT1) works for serous differentiation. On the contrary, Wang et al., [23] detected FOXA1 more frequently in EOC tissues (94.5%), with 73.6% high-expression and reported no association between FOXA1 expression and the histopathological subtype.

One of the major challenges in establishing prognostic and predictive biomarkers in EOC is the heterogeneity associated with the clinico-pathological and molecular levels of the disease [4]. Therefore, EOC has been recently classified into the good prognostic type I carcinomas that are typically low-grade, relatively indolent carcinomas arising from well-characterized precursor lesions and harboring few somatic activating mutations of Ras, Raf, β-catenin, phosphatase and tensin homolog (PTEN), erythroblastic oncogene B (ERB) B2 and Phosphatidylinositol-4,5-Bisphosphate 3-kinase catalytic subunit alpha (PIK3CA); and the poor prognostic type II carcinomas that are typically aggressive, high-grade neoplasms, arising from intraepithelial carcinomas, associated with a higher serum baseline CA-125 and are driven by frequent p53 gene mutations and/or epigenetic alterations in BRCA1 or BRCA2 connected with chromosomal instability and are responsible for 90% of ovarian cancer deaths [24,25]. In this context, we documented FOXA1 high-expression occurring significantly in type I compared to type II EOC, suggesting its linkage to the aforementioned good prognostic indicators integral to type I EOC. In custody with the expanded revised new EOC model that divides type I tumors into three groups based on their origin: i) endometriosis-related tumors that include endometrioid, clear cell, and seromucinous carcinomas; ii) low-grade serous carcinomas; and iii) mucinous carcinomas and malignant Brenner tumors and conforms with the original model in that type II tumors are composed, for the most part, of high-grade serous carcinomas [30], we separated low-grade from high-grade serous carcinomas yielding seven histo-pathogenetic subtypes of EOC for each of which we compared stage, relapse, and survival, as well as FOXA1 expression level. Using this approach, we have confirmed the significant pathogenetic difference between mucinous and low-grade carcinomas on one hand (favorable prognostic parameters & FOXA1 high-expression) and high- grade serous carcinomas on the other side (unfavorable prognostic parameters and FOXA1 low-expression).

On exploring more FOXA1 prognostic associations in EOC, we were able to detect inverse significant associations between FOXA1 expression and CA-125 serum level, anatomical tumor extent (T), nodal involvement (N), presence of distant metastasis (M), FIGO stage, the presence of positive omental nodules, recurrence and mortality. Moreover, logistic regression analysis revealed that omental nodules, anatomical extent and tumor diameter are considered as independent predictors of high FOXA1 expression in EOC in this cohort. Although FOXA1 high-expression was significantly associated with larger size EOC in this study, understanding the fact that mucinous carcinomas (the most frequently FOXA1 high-expressing EOC) are usually larger-size tumors than other EOC [25], would ultimately clarify this seemingly odd finding.

The above-mentioned favorable prognostic findings seem logical as epigenetic studies revealed that FOXA1, as a "pioneer" interacts with and inhibits DNA methyltransferases (DNMTs) that are responsible for epigenetic inactivation of tumor suppressor genes. At the same time, decreasing the expression of FOXA1 was found to promote cell growth and inhibit apoptosis. Thus, FOXA1 could be a potential demethylation target for prevention and treatment of cancer [7].

FOXA1 behaves similarly in breast cancer as its upregulation is associated with good prognosis owing to its preferential upregulation in the ER-positive tumors and in luminal A subtypes contrasting to triple-negative carcinomas [7,13]. It also negatively correlates with high histological grade and Ki-67 index and concomitant ER/FOXA1-negative sates carry increased risk of breast cancer recurrence, wherein the crosstalk between FOXA1 and ER seems to favor the expression

of differentiation-associated genes rather than proliferation-associated genes. It also promoting E-cadherin expression, thus blocking the migratory capacity and metastasis in breast cancer[6]. Therefore, it is not surprising that FOXA1is highly expressed in ductal carcinoma in situ as well, which by definition is non-invasive carcinoma [20].

As a putative biomarker for favorable prognosis in a set of other cancers, low FOXA1 expression was significantly correlated with non-endometrioid histology, high histologic grade, loss of ER and PR expression in endometrial carcinomas [29]. Likely, high FOXA1 expression is associated with lower (T) classification in salivary duct carcinoma [27]. Moreover, FOXA1-over-expressing cholangiocarcinoma cells exhibit a significant reduction in proliferative, invasive and spheroid formation abilities, and its down-regulation subsequently reduces microRNA (miR)-122 expression and induces epithelial-mesenchymal transition (EMT) and disease progression [19]. In nasopharyngeal carcinoma, FOXA1 overexpression associates with a non-aggressive behavior and favorable prognosis in early TNM stages [16], and suppresses growth, migration and invasion of the carcinoma cells via downregulation of miR-100-5p or miR-125b-5p [17]. As a tumor suppressor, FOXA1 was found to inhibit PI3K/Akt signaling pathway in hepatocellular carcinoma [14].

In contradiction to our findings, the potential oncogenic role of FOXA1 in EOC development and progression has been proposed by the virtue of its effect cyclin-dependent kinase 1, phosphatidylinositol-3 kinase, E2F transcription factor 1, B-cell lymphoma 2, and vascular endothelial growth factor A protein pathways, leading to EOC cell proliferation, migration, independent growth and resistance to apoptosis and chemotherapeutic agents [22]. In the same vein, Wang et al. [23], disclosed that increased FOXA1 expression is associated with increased EOC tumor grade and poorer differentiation irrespective of age, histopathological type, tumor size or location, and that EOC of advanced stages (III/IV) express FOXA1 at higher levels than those with low stages (I/II). It is to be noted that the prevailing few views on FOXA1 expression in EOC have not reached a consensus, with contrasting evidence seen in different cohorts of cancer patients.

A similar dilemma does exist in prostatic cancer. An earlier study denoted the unfavorable prognostic implications of FOXA1 in prostatic cancer based on the findings that FOXA1 is expressed at high levels in metastatic and castration-resistant prostate cancers (CRPC), and that FOXA1 levels correlates with higher (T) stages and Gleason scores and with faster biochemical disease progression in patients with low androgen receptor (AR) levels [31]. However, other subsequent studies demonstrated the ability of FOXA1 to inhibit prostatic cancer cell motility and EMT through AR-independent mechanism. The later studies reported FOXA1 upregulation in localized prostate cancer being able to inhibit metastasis, and its downregulation in CRPC supporting its tumor suppressor effects [10,32].

One of the most important findings in this study was the association between higher FOXA1 expression and both OS and DFS advantages in EOC. Patients with EOC within the FOXA1 high-expression group exhibited a significantly longer OS ($p \leq 0.001$) and DFS ($p = 0.002$) times compared to those with EOC within the FOXA1 low-expression group, suggesting that FOXA1 expression may positively predict survival time in patients with EOC. Although this finding has been contradicted in the study by Wang et al. [23], who reported an inverse association between high FOXA1 expression and OS, several other studies have further supported our notion. For example, high FOXA1 expression was found to significantly associate longer 3-year OS and DFS in in salivary duct carcinoma [27], low FOXA1 protein expression was significantly associated with reduced DFS in endometrial carcinoma [29], and FOXA1 positivity was associated with prolonged OS and DFS rates in nasopharyngeal carcinoma [16]. Furthermore, FOXA1 was identified as a predictor for longer OS and DFS in breast cancer [6], and as an independent factor involved in the OS rate in cholangiocarcinoma patients as low FoxA1 expression was related with short survival rates [19].

Despite recent advances in the surgical and pharmaceutical therapies, survival rates of EOC remain poor mostly due to late presentation of the disease, suboptimal tumor debulking, resistance to standard chemotherapies and lacking predictive biomarkers. Accordingly, tumor immune landscape and microenvironment targeting may offer novel avenues for actionable immune-based biomarkers [4]. As a potential therapeutic target, FOXA1 was shown to be induced by interferon-beta (IFN-β), resulting in generation of FOXA1+ regulatory T cells (FOXA1+Treg). Upon IFN-β treatment, favorable clinical outcomes were observed in relapsing-remitting multiple sclerosis patients suggesting that FoxA1 induced by IFN-β can support the differentiation and suppressive function of FOXA1+Treg [33]. Based on our findings, it would be interesting to study the therapeutic potentialities of FOXA1 in EOC.

In conclusion, the present study revealed that FOXA1 is frequently expressed in EOC notably mucinous followed by low-grade serous carcinomas. FOXA1 high-expression is associated with favorable prognostic clinico-pathological parameters in EOC including

mucinous histopathology, type I EOC, low CA-125 serum level, low T, absence of nodal or distant metastases and omental nodules, earlier FIGO stages and non-recurrent tumors. Longer OS and DFS were associated with high FOXA1 IHC expression scores. Therefore, FOXA1 may have an imperative suppressor function in EOC progression and could be recommended as a biomarker for therapy and prognosis. However, further investigations concerning the regulatory mechanisms and functions of FOXA1 in EOC should be sought for.

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Compliance with ethical standards

Ethical approval was obtained from Institutional Research Board (IRB) at Faculty of Medicine, Mansoura University, Egypt (code number: R.18.10.314). All procedures were done in accordance with the current revision of Helsinki Declaration of medical research involving human subjects.

Author contributions

Heba Sheta: conceptualization, provision of study materials, collection of histopathology data, histopathologic evaluation of the H&E and IHC slides, tabulation of histopathology data, slide photography, review and approval of the manuscript.
Amal Abd El hafez: conceptualization, provision of study materials, share in histopathologic evaluation of the H&E and IHC slides, preparation of final results and figure work-art, literature review, manuscript writing and submission for publication.
Maha Saif and Alyaa R. Elsergany: conceptualization, provision of study materials, clinical data collection and interpretation, review and approval of the manuscript.
Doaa Al emam: provision of study and materials, statistical analysis of data, review and approval of the manuscript.
Mahmoud M. Abdelrazik: conceptualization, provision of study materials, clinical data collection and interpretation, review and approval of the manuscript.

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