Research Article

Immunohistochemical study of FoxA1 in Invasive Duct Carcinoma of the Breast and Corresponding Lymph Node Metastasis

Azza M. Abelzaher, Manal E. Abdelghany, Nehad M. Reda Abdel maqsoud, Heba M. Tawfiq, Wafaa F. Aref.
Department of Pathology, El-Minia Faculty of Medicine

Abstract
FoxA1 (forkhead box protein A1) is one of three members in the FoxA related family (forkhead family of transcriptions factors). FoxA related family proteins are often termed "pioneer factors" because of their ability to bind to highly compacted heterochromatin and making genomic regions more accessible to other transcription factors. This study aimed to evaluate the clinical significance of FoxA1 expression in invasive duct carcinoma of the breast and in corresponding lymph node metastasis. FoxA1 expression was examined in 70 tumor tissues plus 53 paraffin blocks of corresponding lymph node metastasis by immunohistochemistry. The association between expression of this marker and clinicopathologic parameters was analyzed. Also comparison between FoxA1 expression in the primary tumor and corresponding lymph node metastasis was done in 53 pairs. FoxA1 was positive in (45.7%) of cases. FoxA1 expression was associated with tumor grade (p= 0.046), lymph node status (>3 lymph nodes) (p = 0.049), tumor stage (p=0.009), prognostic stage (p=0.001), good Nottingham prognostic index (p=0.042), ER and PR positivity (p=0.028 and 0.033 respectively), Her2 negativity (p= 0.019), Molecular subtypes (p=0.038) and distant metastasis (p=0.004). As regard FoxA1 expression in primary tumor and corresponding lymph node metastasis, 96.2% of pairs revealed a concordance between the primary tumor and corresponding lymph node metastasis and only 2 cases (3.8%) were nonconcordant but the results weren't statistically significant. These findings suggest that FoxA1 expression can be considered as a good prognostic and therapeutic marker for targeted therapy of breast cancer patients.
Keywords FoxA1

Introduction
Invasive breast cancer is the most common malignancy in women around the world accounting for 25 % of all types of cancers and the incidence has more than doubled worldwide in the last 25 years[1]. It is the second leading cause of cancer death in females following lung cancer. It accounts for 14% of all deaths related to cancers with nearly 2.1 million new cases occurring among women worldwide each year and 626,679 deaths per year[2, 3]. Breast cancer in young women is associated with more proliferative disease, worse prognosis, and higher mortality[4].

FoxA1 is one of three members in the FoxA related family. It regulates tissue-specific transcriptional programs and plays critical roles in cell growth, proliferation, apoptosis, differentiation and development of a number of organs including the pancreas, prostate, and breast[5,6].

FoxA1 can direct cellular differentiation during organ development, during cell fate programming in vitro as well as during pathological reprogramming events such as oncogenic transformation[7, 8]. FoxA1 has been shown to have a dual role, either as a growth stimulator or a repressor. It functions as a tumor promotor in initial stages, but as a tumor repressor in the later stages[9]. Its role in human malignancies seems to depend on the cancer type[10]. FoxA1 exhibits tumor suppressive functions in breast cancer and high FoxA1 expression is correlated with favorable prog-nosis in ER-positive breast tumors[11]. However, the precise role of FoxA1 in breast cancer and the molecular mechanisms underlying its effects are not clear[12].

Materials and Methods

Patients

This study enrolled 70 paraffin blocks of invasive ductal carcinoma plus 53 paraffin blocks of corresponding lymph node metastsis which were chosen from the archives of Minia oncology center in the period between January 2013 to February 2018. The mean age is 48.06 years with an age range of 24 to 75 years. All patients were graded according to Scarff-Bloom-Richardson (SBR) grading system and the clinical stage was determined bases on the TNM classification. The patient's clinocopathological characteristics are showed in table 1, Figure (A,B)

Immunohistochemical staining and scoring

The specimens were subjected to routine Haematoxylin and eosin (H&E) staining to revise the histopathological diagnosis and revision of the positive charged slides to confirm the ER, PR, HER2 and Ki67 status. Four µm sections were prepared on positive charged slides. Immunohistochemical staining for FoxA1 primary antibody was performed by utilising the avidin biotin-peroxidase complex method with diaminobenizidine (DAB) chromagen detection system was done using Universal immunostaining kit (Abcam).

Sections were heated at 60ºC for 10 minutes, dewaxed in two changes of xylene and rehydrated in descending graded alcohol. Sections were then immersed in a 3% solution of hydrogen peroxide and incubated for 30 minutes at room temperature then slides were rinsed gently with buffer solution and were placed in fresh buffer bath for five minutes. Sections were treated in microwave by immersion of the slides in citrate buffer solution (pH 6) for 2 times (10 minutes each), and then slides were allowed to cool, and reach room temperature then washed with PBS buffer for 5 minutes. Slides were then placed side up on a flat level surface in a humidity chamber and slides were incubated overnight at 4 C with the primary antibody as follows: FoxA1 (Monoclonal mouse antibody, ab55178, isotype IgG2a, 100ug, Abcam.) was used at a dilution (3:500 in PBS). Secondary biotinylated antibody was added for each slide for 30 minutes at room temperature. After that, slides were rinsed in buffer solution for 5 minutes; streptavidin reagent was then applied to cover each section for 30 minutes at room temperature. The slides were rinsed gently and placed in PBS for 5 minutes.

Diaminobenzidine tetrachloride (DAB) substrate and chromogen prepared in a ratio of 1:50 and mixed well. DAB substrate-chromagen solution (one or two drops) was applied on sections. Lastly, sections were counterstained in Harris haematoxylin, rinsed gently in distilled water, dehydrated in ascending grades of alcohols (70%, 95% and 100% alcohol), then cleared in xylene and, mounted using an aqueous-based mounting medium, Disterene plasticizer xylene (DPX) and covered slips.

Scoring system

FoxA1 expression was nuclear. The scoring method used for FoxA1 expression was based on a semi-quantitative scoring system**.** In this scoring system, the percentage of staining was calculated as: 0 = no nuclear expression; 1 = 1 to 10% positive tumor nuclei; 2 = 11 to 20%; and so on until a maximum score of 10 = 91 to 100% positive tumor nuclei. The intensity was scored as: 1+ = weak staining; 2+ = moderate staining; and 3+ = strong staining. The numeric final score was obtained by the multiplication product of percentage and intensity of nuclear expression (scoring = percentage × intensity). According to this semiquantitative scoring system, scores under 12 were classified as negative, and scores ≥ 12 to a maximum of 30 were considered positive[13-15]**.**

Statistical analysis

Statistical analysis was conducted using the Statistical Package for Social Sciences (SPSS software version 16). Raw data were compiled and used to determine the means ± standard deviations (SDs), median and range of various features. The Chi- square and Fisher's exact tests were used to compare categorical features. Difference in FoxA1 expression between primary tumour and the corresponding lymph node metastasis were assessed using McNemar test. P value of < 0.05 was considered.

Results

Table (1) Clinicipathological characteristics of invasive duct carcinoma -patients enrolled in this study (n=70)

Clinicopathological Data		NO.	Percent%
Age (years)	**≤ 50**	42	60%
	> 50	28	40%
Laterality	**Right breast**	28	40%
	Left breast	42	60%
Site	**Upper outer quadrent**	37	52.9%
	Lower outer quadrent	5	7.1%
	Upper inner quadrent	8	11.4%
	Lower inner quadrent	6	8.6%
	Retroareaolar	14	20%
Size (cm)	**≤ 2 cm**	8	11.4%
	>2 - ≤5 cm	55	78.6%
	> 5 cm	7	10%
Grade	**II**	44	62.9%
	III	26	37.1%
Lymph node status	**0**	17	24.3%
	1-3	18	25.7%
	> 3	35	50%
Lymph node ratio	**Low risk**	29	41.4%
	Intermediate risk	31	44.3%
	High risk	10	14.3%
Clinical tumor stage	**I**	4	5.7%
	II	43	61.4%
	III	13	18.6%
	IV	10	14.3%
Prognostic tumor stage	**I**	12	17.1%
	II	29	41.4%
	III	10	14.3%
	IV	19	27.1%
NPI	**Good**	16	22.9%
	Moderate	33	47.1%
	Poor	21	30%
ER	**Positive**	43	61.4%
	Negative	27	38.6%
PR	**Positive**	41	58.6%
	Negative	29	41.4%
HER2	**Positive**	18	25.7%
	Negative	52	74.3%
Ki67	**<14%**	28	40%
	>14%	42	60%
Molecular subtypes	**Luminal A**	23	32.9%
	Luminal B	21	30%
	Her2 subtype	14	20%
	Triple negative	12	17.1%
Tumor necrosis	**Present**	11	15.7%
	Absent	59	84.3%
Inflammatory response	**No Inflammatory response**	4	5.7%
	Mild	10	14.3%

	Moderate	23	32.9%
	Severe	33	47.1%
Insitu component	**Present**	30	42.9%
	Absent	40	57.1%
LVI	**Present**	11	15.7%
	Absent	59	84.3%
Distant metastasis	**Present**	12	17.1%
	Absent	58	82.9%
Local Recurrence	**Present**	8	11.4%
	Absent	62	88.6%

- NPI: *Nottingham prognostic index* LVI:*lymphovascular invasion*
- *LNR: lymph node ratio*
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Immunohistochemical expression of FoxA1 and its association with clinicopathological data

In the present study, positive nuclear FoxA1 expression was detected in 32 (45.7%) cases and 38 cases (54.3%) were negative for FoxA1 expression. The association between FoxA1 expression and different clinicopathological features was summarized in table (2) and figure (C).

In the current study, a statistically significant association was found between FoxA1 expression and tumor grade (p= 0.046). FoxA1 expression was detected in grade II tumors more than grade III tumors. Cases of grade II and III showing positive FoxA1 expression were 24 (54.5%) and 8 (30.8%) respectively. There was a statistically significant association between FoxA1 expression and lymph node status (p= 0.049). Large percentage of cases with negative lymph node involvement showed positive FoxA1 expression (11 cases; 64.7%), followed by the cases with positive lymph nodes < 3 (10 cases; 55.6%) then tumors with >3 positive lymph node metastasis were 11 cases (31.4%).

Regarding tumor stage, FoxA1 expression showed a statistically significantly association with tumor stage (p=0.009). There was decrease in FoxA1 in high tumor stage. The cases with tumor stage I, II, III and IV that showed positive FoxA1 expression were 2 cases (50%), 26 cases (60.5%), 3 cases (23.1%) and 1 cases (10%) respectively. Also there was a statistically significant association between FoxA1 expression and prognostic tumor stage (p=0.001). The cases with stage I, II, III and IV that showed positive FoxA1 expression were 8 cases (66.7%), 19 cases (65.5%), 2 (20%) and 3 cases (15.8%) respectively.

In the present study FoxA1 was more detected in cases with good NPI. Cases with good, moderate and poor NPI positive for FoxA1 were (10 cases; 62.5%), (17 cases; 51.5%) and (5 cases; 23.8%) respectively. There was statistically significant association between FoxA1 expression and NPI (P=0.042).

There was statistically significant association between FoxA1 expression and ER and PR expression (p=0.028 and p=0.033 respectively). FoxA1 expression was detected more in ER positive tumors. ER positive tumors showed positive FoxA1 expression were (24 cases (55.8%) while only 29.6% of ER negative tumors showed positive FoxA1 expression (8 cases). Also FoxA1 expression was detected more in PR positive tumors. PR positive tumors showing positive FoxA1 expression were (23 cases; 56.1%), while the PR negative tumor cases showing positive FoxA1 expression were 9 cases (31%). Also there was statistically positive association between FoxA1 expression and Her2 expression (p=0.019). Her2 negative tumors showed positive FoxA1 expression were (28 cases; 53.8%), while Her2 overexpressing tumors showing positive FoxA1 expression were only 4 cases (22.2%).

Regarding Molecular subtypes, luminal A, luminal B, Her2 subtype cases and triple negative cases with positive FoxA1 expression were 9 cases (39.1%) and 15 cases (71.4%), 4

cases (28.6%) and 4 cases (33.3%) respectively. There was a statistically significant positive association was found between positive expression of FoxA1 and molecular subtypes (p= 0.038).

In the present study, there was a statistically significant association between cases with distant metastasis and FoxA1 expression (p=0.004). It was detected that cases that were negative for distant metastasis and positive for FoxA1 expression were (31 cases; 53.4%) comparing to only one case (8.3%) with distant metastasis showed positive FoxA1 expression.

To assess whether the expression of FoxA1 undergoes changes during breast cancer progression, 53 pairs of primary tumor and their corresponding metastasis to lymph nodes were compared in table (3). FoxA1 positive primary tumors were 21 (39.6%) while FoxA1 positive metastatic lymph nodes were 19 (35.8%). These two rates aren't statistically different (McNemar test, p=5.00). Of total 53 pairs, 51 pairs (96.2%) revealed a concordance between the primary tumor and corresponding lymph node metastasis (figure D, E).

Table (2): Association between FoxA1 expression and clinicopathological data for the patients with invasive duct carcinoma (n=70).

Clinicopathological Data	NO.	FoxA1 expression		P value
		Negative expression (%) (N=38)	Positive expression (%) (N=32)	
Age (years)				
≤ 50	42	24 (57.1)	18(42.9)	0.366
> 50	28	14 (50)	14 (50)	
Laterality				
Right breast	28	17 (60.7)	11 (39.3)	0.263
Left breast	42	21 (50)	21 (50)	
Site				
Upper outer quadrant	37	20 (54.1)	17 (45.9)	0.693
Lower outer quadrant	5	3 (60)	2 (40)	
Upper inner quadrant	8	6 (75)	2 (25)	
Lower inner quadrant	6	3 (50)	3 (50)	
Retroareolar	14	6 (42.9)	8 (57.1)	
Size (cm)				
≤ 2 cm	8	5 (62.5)	3 (37.5)	0.745
>2 - <5 cm	55	30 (54.5)	25 (45.5)	
> 5 cm	7	3 (42.9)	4 (57.1)	
Grade				
II	44	20 (45.5)	24 (54.5)	**0.046***
III	26	18(69.2)	8 (30.8)	
Lymph node status				
0	17	6 (35.3)	11 (64.7)	**0.049***
1-3	18	8 (44.4)	10 (55.6)	
> 3	35	24 (68.6)	11 (31.4)	
Lymph node ratio				
Low risk	29	16 (55.2)	13 (44.8)	0.891
Intermediate risk	31	16 (51.6)	15 (48.4)	
High risk	10	6 (60)	4 (40)	
Tumor stage				
I	4	2 (50)	2 (50)	**0.009***
II	43	17 (39.5)	26 (60.5)	
III	13	10 (76.9)	3 (23.1)	
IV	10	9 (90)	1 (10)	

Prognostic stage				
I	12	4 (33.3)	8 (66.7)	
II	29	10 (34.5)	19 (65.5)	**0.001***
III	10	8 (80)	2 (20)	
IV	19	16 (84.2)	3 (15.8)	
NPI				
Good	16	6 (37.5)	10 (62.5)	**0.042***
Moderate	33	16 (48.5)	17 (51.5)	
Poor	21	16 (76.2)	5 (23.8)	
ER				
Positive	43	19 (44.2)	24 (55.8)	**0.028***
Negative	27	19 (70.4)	8 (29.6)	
PR				
Positive	41	18 (43.9)	23 (56.1)	**0.033***
Negative	29	20 (69)	9 (31)	
Her2				
Positive	18	14 (77.8)	4 (22.2)	**0.019***
Negative	52	24 (46.2)	28 (53.8)	
Ki67				
<14%	28	18 (64.3)	10 (35.7)	0.130
>14%	42	20 (47.6)	22 (52.4)	
Molecular subtypes				
Luminal A	23	14 (60.9)	9 (39.1)	
Luminal B	21	6 (28.6)	15 (71.4)	**0.038***
Her2 subtype	14	10 (71.4)	4 (28.6)	
Triple negative	12	8 (66.7)	4 (33.3)	
Tumor necrosis				
Present	11	4 (36.4)	7 (63.6)	0.166
Absent	59	34 (57.6)	25 (42.4)	
Inflammatory response				
No inflammatory response	4	1 (25)	3 (75)	
Mild	10	6 (60)	4 (40)	0.488
Moderate	23	11 (47.8)	12 (52.2)	
Severe	33	20 (60.6)	13 (39.4)	
Insitu component				
Present	30	17 (56.7)	13 (43.3)	0.459
Absent	40	21 (52.5)	19 (47.5)	
LVI				
Present	11	3 (27.3)	8 (72.7)	0.051
Absent	59	35 (59.3)	24 (40.7)	
Distant metastasis				
Present	12	11 (91.7)	1 (8.3)	**0.004***
Absent	58	27 (46.6)	31 (53.4)	
Local Recurrence				
Present	8	3 (37.5)	5 (62.5)	0.262
Absen**t**	62	35 (56.5)	27 (43.5)	

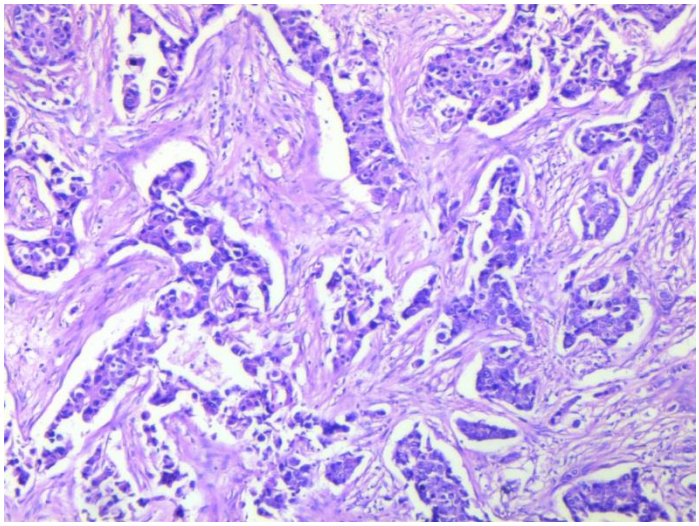
Test of significance: Chi-Square and Fisher's exact tests.

**** P - value < 0.05 is considered statistically significant***

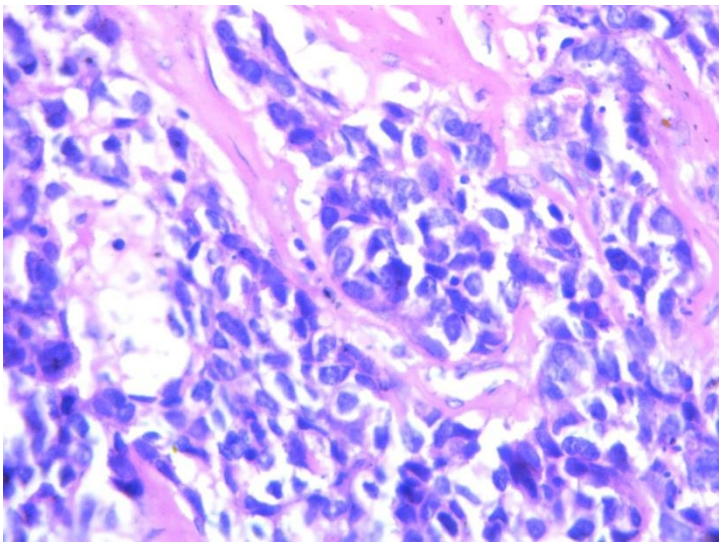
- NPI: *Nottingham prognostic index* LVI:*lymphovascular invasion*
- *LNR: lymph node ratio*

Table (3): Comparison in the expression of FoxA1 in 53 pairs of primary tumor and corresponding lymph node metastasis

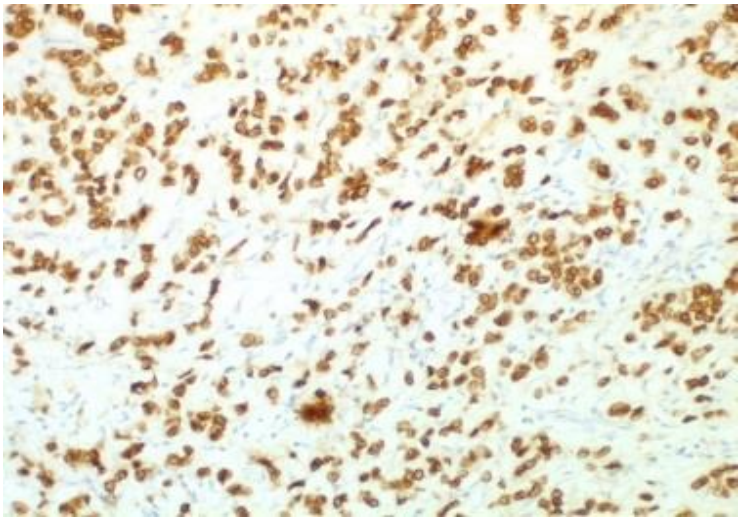
FoxA1 expression in the primary tumor	FoxA1 expression in lymph node metastasis (n= 53)		Total (%)	P value
	Negative (%)	Positive (%)		
-Negative	32 (60.3)	0 (0)	32 (60.3)	
-Positive	2 (3.8)	19 (35.8)	21 (39.6)	0.500
Total	34 (64.2)	19 (35.8)	53 (100)	



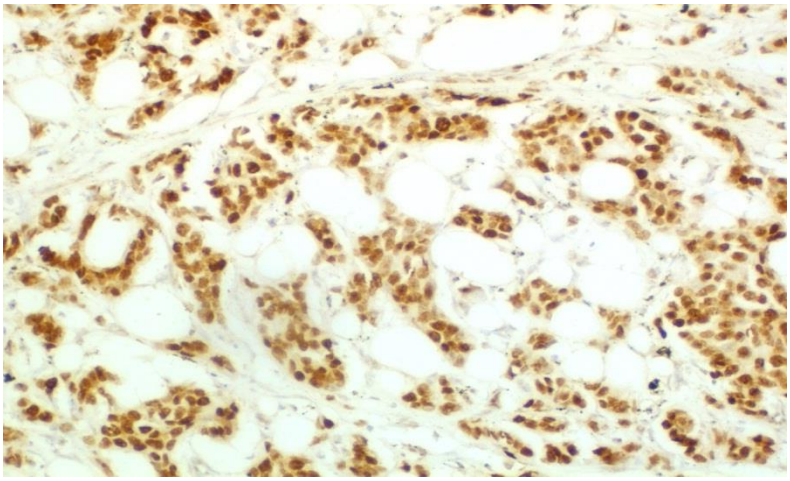
A) Grade II invasive duct carcinoma of breast (Streptavidin-biotin-immunoperoxidase X400)



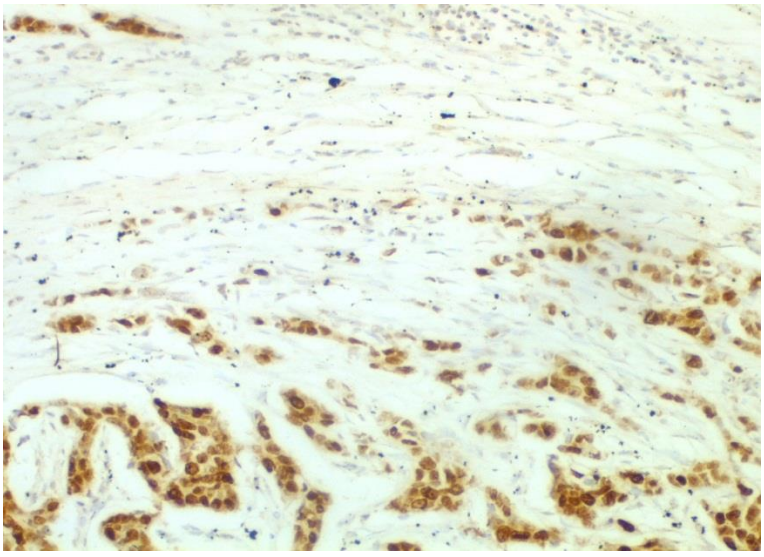
B) Grade III invasive duct carcinoma of breast (Streptavidin-biotin-immunoperoxidase X400)



C) Nuclear expression of FoxA1 (Streptavidin-biotin-immunoperoxidase X400)



D) FoxA1 expression in primary tumor (Streptavidin-biotin-immunoperoxidase X400)



E) FoxA1 expression in corresponding lymph node (Streptavidin-biotin-immunoperoxidase X400)

Discussion

Breast cancer is a heterogeneous disease and up till now it isn't known how ERα-positive luminal breast cancer (LBC) could lose their ERα and progress toward basal like breast cancer (BLBC). FoxA1 is associated with LBC with subsequent good prognosis. However, its role in breast cancer progression needs further research[16]. In this study, there was clinical evidence has been provided to indicate that FoxA1 is associated with good prognosis in breast cancer.

Seventy patients in our study were aged between 24 and 75 years (mean 48.06 years). The patients were divided into two age groups: patients aged up to or equal 50 years and patients older than 50. We used the age of 50 (the median age of patients in our study) as a cutoff point as many previous studies indicated[17-21].

In the present study, 60% of the cases were diagnosed with breast cancer at the age of 50 and less. This was in line with[17, 21, 22]. On the other hand,[18, 20] reported that most of females diagnosed with breast cancer were older than 50 years old with a percentage of 59.6% and 70% respectively. The disease occurring at younger age in current study can be explained in terms of racial and social differences. Young patients with breast cancer in Egypt constitute a unique group of patients as they have special tumor characteristics. This group has more advanced presentation, with much more aggressive biologic behavior, and has a significantly higher axillary lymph node involvement[23]. Breast cancer risk factors such as age at menarche, age at first full term birth, age at menopause, parity, obesity, and oral contraceptive pills use have all been shown to demonstrate quantitative and qualitative age interactions with regard to breast cancer risk[24].

In the present study, most of the tumors were (>2 - ≤5 cm) in diameter (78.6%). This was concordant with[25-29] who reported tumors that were (>2cm - ≥5cm) ranged from 50% to 74.5%. While[21, 30] reported that the percentage of tumors sized ≤ 2 cm in diameter were 68% and 52.8% and this is probably due to screening programs leading to early detection. Regarding tumor grade, most of the tumors were grade II (62.9%) and the remaining cases were grade III (37.1%). This was consistent with other reports by[17,21,22,31-33] who reported that high percentage of the grade II tumors with a percentage of 69%, 55%, 59.7%, 58.4%, 74.5% and 50% respectively. On the other hand,[26, 27, 34] found that the high percentage of the tumors were of grade III (61.4%, 56.1% and 78.5% respectively) and this was probably because difference in number of cases included in these studies.

Regarding the lymph node status, 50% of tumor cases had > 3 positive lymph nodes and 18 cases (25.7%) had ≤ 3 positive. Our results were in agreement with other study by[26,29] found that 30.7% and 36.3% had > 3 positive lymph nodes respectively and 17.3% and 26.3% had ≤ 3 positive respectively. But[22, 32] found that include 69% and 65.3% of patients included in their study with negative lymph node metastasis. This may be due to advanced screening programs in the developed countries leading to early detection.

Regarding tumor stage, the smallest percentage was stage I (5.7%) while stage II, III and IV were 61.4%, 18.6% and 14.3% respectively. This was concordant with the study[31] in which the percentage of stage I and IV were 3.3% and 13.3% respectively but stage II and stage III cases were 38.3% and 45% respectively.

Also[22, 30] studies included high number of stage II cases that were 45% and 61.1% respectively. However,[33, 35, 36] found that stage II cases were 15.3%, 40% and 23% respectively, this difference may be due to difference in number of cases included in each study. According to prognostic stage, in our study, most of cases were stage II (41.4%) while stage I, III and IV were (17.1%, 14.3% and 27.1% respectively) this was in the line with[37] who reported stage I, II, III and IV were 29.4%, 53%, 2.4% and 13.9% respectively. But[38] reported most of cases -were stage I (70.1%) followed by stage II (20.2%) this was due to prognostic stage integrates biomarkers into TNM staging system and most of cases in their study were of stage I (61.4%) and ER positive (71.5%) and PR positive (60.2%) and this explain why most of their cases were of stage I prognostic stage.

Regarding to the Nottingham prognostic index, the maximum percentage in this study was of the moderate prognostic index group (47.1%) followed by the poor prognostic group (30%). The smallest percentage was of the good prognosis (22.9%). This was in line with[26, 29, 30] which reported that the percentage of moderate prognostic groups was 42.9%, 49.8% and 48% respectively. NPI is calculated depending on size, grade and stage of the tumor. This explains why most of the cases were of moderate and poor prognostic indices because most of the cases in our study were of size ≥ 2 cm, grade II and III and stage II-III.

Regarding the hormone receptor status, ER positivity was detected in 61.4% of the cases. This was in line with other previous studies in which 72.5%, 65.3%, 70.8%, 51.5% and 100% of tumors were ER positive[29, 30, 36, 39, 40]. On the other hand,[41, 42] reported that ER positive cases were only 27.2% and 26.2% respectively. This may be due to difference in cutoff point (they used ≥ 10% positive cells for ER as cutoff). PR positivity was detected in 58.6% of the cases. This was in accordance with[30, 32, 36, 39, 40] who found that 60%, 62.7, 63.4%, 66.6% and 60% of tumors were PR positive**.** While in[18, 41, 43] studies, 1.9%, 24.3% and 40.4% of tumors were PR positive respectively due to different cut-off for PR scoring of staining (at least 2% of tumor cells showing positive nuclear staining for PR) and difference in number of cases included in each study. Her2 positivity was detected in 25.7% of the tumors and this was in the line with previous studies by[36, 40, 44] which reported that 17.3%, 21.6% and 24.1% of the tumors were Her2 positive. However,[32] found that 79% of tumors were Her2 positive.[45] reported that many demographic and pathologic variables can contribute to differences in the percentage of Her2-positive breast cancers in different geographic regions including age, race/ethnicity, marital status, AJCC stage, tumor size, grade, histologic subtype and hormone receptor status.

In the present study, most of tumors were luminal A and luminal B (32.9% and 30% respectively). Twenty percent of cases were Her2 subtype and only 17.1% of the tumors were triple negative. This was in agreement with a previous studies by[31,46] who reported that luminal A and luminal B, Her2 subtype and triple negative were (28.3%, 25%, 30% and 16.7% respectively) and (36.2%, 29.9%, 22.8% and 11% respectively.[41, 43] found that 81.4% and 56.3% of the tumors were triple negative. This variation is probably due to different study population. Breast cancers in black races tend to be more aggressive represented triple negativity than in western countries.

In the present study, distant metastasis was found in only 17.1% of cases and this was in concordant with[40,42,47] who found that 4.4%, 16.2% and 11.6% of cases studied were positive for distant metastasis respectively. Also local recurrence at the site of the operation was found in only 11.4% of cases and this also was in the line with[40,47] who found 14.2% and 4.4 of cases with local recurrence respectively. On the other hand,[48] reported 43.2% and 49.5% of cases with distant metastasis and local recurrence respectively. This can be explained by cases in their study were not randomly selected as the aim of their study was to identify prognostic factors for long-term outcomes among patients with isolated locoregional recurrence of breast cancer with subsequent probability of distant metastasis as their first failure event.

In this study, we determined FoxA1 expression in invasive duct carcinoma using immune-histochemistry; the association between FoxA1 expression and clinicopathologic features.

FoxA1 is a crucial transcription factor, which opens chromatin to permit transcription in the ER signaling that occurs in breast tumors. It is reportedly responsible for luminal cell differentiation and patients with tumors showing high FoxA1 protein expressions tend to have better outcomes[49]

In the present study, 32 out of 70 cases (45.7%) showed FoxA1 expression. This was in line with previous study[42] who found 43% of cases with positive FoxA1. On the other hand[43,50] found that 84.6% and 92.6% of the cases expressed FoxA1, this may be due to difference in number of cases included in each study and difference in scoring method for FoxA1 (they considered with > 1% positive cells for FoxA1 as positive).

FoxA1 expression was significantly associated with lower tumor grade. Only thirty cases of grade III express FoxA1 compared to 54.5% of grade II. This was in accordance with [42,51,52] in which FoxA1 was negatively associated with tumor grade. These features suggest that tumors with high FoxA1 expression are less aggressive than tumors with low FoxA1 expression while[43] found that FoxA1 positivity was weakly-associated with nuclear grade, but the relationship was not statistically significant.

Regarding lymph node status, FoxA1 expression was positively associated with less lymph node involvement. Positive FoxA1 expression was detected in 64.7% of tumors with negative lymph node metastasis, 55.6% of tumors with 1-3 positive LNs and 31.4% of tumors with > 3 positive LNs. This was in concordant with[42, 51, 53]. On the other hand,[43] found no significant association between FoxA1 expression and lymph node metastasis and this was probably due to most of cases included in their study were of negative for lymph node metastasis (80%) and only 4.7% of cases with positive lymph nodes >3.

In the line with[42] FoxA1 was negatively associated with tumor stage suggesting that FoxA1 decreases tumor progression in breast cancer. In our study, ninety percent of stage IV tumors were negative for FoxA1. This indicates that patients with negative expression of FoxA1 have poor prognosis. Also,[54] detect high FoxA1 levels in nasopharyngeal tumors at TNM I-III. However,[43] found no significant association between them this may be due to difference in number of studied cases. To the best of our knowledge, no previous studies detect the relationship between FoxA1 expression and prognostic tumor stage.

Regarding NPI, it showed a statistically significant association with FoxA1 expression. Large percentage of good prognostic group tumors were FoxA1 positive (62.5%) while only 23.8% of the poor prognostic group tumors showed positive FoxA1 expression, this was in the line with[13, 55] but[51] found no significant association between them due to difference in number of cases included in each study.

Hormonal status is determinant for breast tumors characterization and a key predictive biomarker for patient management. We showed that, ER and PR positive tumors that were FoxA1 positive were 55.8% and 56.1% respectively. FoxA1 was positively associated with ER, AR and the molecular subtype. Similarly, in the medical literature, most publications report a very strong correlation between FoxA1 positivity in breast cancers and the expression of the hormone receptors ER and PR [13,42,51,52,56] confirmed by two meta-analysis[53,57] . In the current study, 53.8% of Her2 negative tumors FoxA1 positive and their association were statistically significant. This was in agreement with[51]. These results are suggesting that FoxA1 didn't enrich in aggressive breast carcinomas which are represented by ER and PR negativity and Her2 overexpression. But[56] found that FoxA1 expression was not associated with Her2 expression may be due to difference in cutoff point.

In the present study, there was a statistically significant association between FoxA1 expression and molecular subtypes. Tumors with high FoxA1 were mainly of luminal A and B subtypes; few Her2 subtype and triple-negative tumors showed high FoxA1 expression and this was in the line with [52, 56].

Several studies demonstrated that FoxA1 contributes to epithelial mesenchymal transition (EMT) mainly through regulating E-cadherin expression in pancreatic cancer and lung cancer[58,59]. Similarly, in gastric cancer cells, FoxA1 regulates the EMT in cancer cells, by inducing the E-cadherin expression and decreasing the vimentin protein level[60]. To the best of our knowledge, this was the first time to detect association between FoxA1 expression and distant metastasis in breast cancer. Ninety one % of cases with distant metastasis were negative for FoxA1.[13, 15, 51, 55] found no association between them in breast cancer so further studies is needed to explain the relation between them.

Collectively, the significant associations between FoxA1 with tumor grade, lymph node status, lower stage and prognostic stage, good

NPI, ER and PR positivity, HER2 negativity and molecular subtypes (luminal A and Luminal B) and absence distant metastasis suggest that FoxA1 expression is a marker of good prognostic value. This result is supported by other studies by[9,36,61].

There was no statistically significant association between FoxA1 expression and other clinicopathological data and this was in agreement with previous studies by[13,43]. FoxA1 expression was associated with a less aggressive breast cancer phenotype and a better patient prognosis so evaluation of FoxA1 expression may provide a cost-effective strategy in the risk stratification of breast cancer patients.

Metastatic lymph node involvement is still the most powerful prognostic factor for recurrence and death, but lymph node dissection does not affect patients survival[62]. It is still the matter of debate if positive lymph nodes are able to metastasize[63]. Our work explored patterns of conversion in FoxA1 between primary breast tumors and corresponding synchronous axillary lymph node metastases to determine whether phenotypic variability is associated with different clinical outcome. We found that the rate of FoxA1 expression is higher in the primary tumor than corresponding lymph node metastasis but the results weren't statistically significant. Only 2 cases (3.8%) changed from positive expression in tumor to negative expression in lymph nodes and this confirm that FoxA1 is marker of good prognosis and these results are supported by previous studies[42, 53]. But[43] found that the rate of FoxA1 expression in the primary tumor was equal to that of corresponding lymph node.

Conclusion

Our findings suggest that FoxA1 represent a marker for good prognosis in breast cancer. FoxA1 expression was associated with a less aggressive breast cancer phenotype and a better patient prognosis so evaluation of FoxA1 expression may provide a cost-effective strategy in the risk stratification of breast cancer patients. Further studies are needed to detect the role of FoxA1 as a prognostic factor for EMT.

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