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Correlation of FOXA1 with some clinicopathological features, lymph node, grade, stage and tumor size

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***Abstract*---**The Forkhead box (FOX) protein family includes FOXA1, and is a transcription factor (TF). It work as a pioneer factor, interacting with compressed chromatin to allow ER to bind more easily later (Nagaraj and Ma, 2021). It significantly contributed to the development and invasiveness of endocrine-resistant cells by changing the ER dependent transcriptome in numerous endocrine-resistant preclinical BC cell types (Chun *et al.*, 2017). The present study is done during the period September 2021 and May 2022 at the University of Kufa's, Faculty of Medicine, Middle Euphrates Unit for Cancer Research. In this study, Seventy three tissues samples embedded in wax block taken from BC patients, who had surgery between 2014-2020 and thirty eight of normal breast tissues samples. as a control group. These samples gathered from archives of the two private laboratories and re-evaluted by two pathologist. Positive nuclear IHC expression of FOXA1 is significantly reduced in 43 BC cases (58.9%) compared to 38 control persons (100%) while negative nuclear IHC expression of FOXA1 is significantly reduced in BC patients, 30 cases (41.1%) compared to 0 control persons (0.0%), Odd ratio 1.66, 95% CI (1.37-1.98), P=0.0001. Moreover, FOXA1 positivity was elevated significantly in patients with grade I and II (P=0.002) and absent of lymph node metastasis (p=0.027). This study concluded, positive FOXA1 expression correlated with grade II while negative FOXA1 expression correlated with grade III, and negative correlation with lymph node metastasis.

***Keywords*---**FOXA1, IHC, breast cancer

Introduction

The breast cancer (BC) is etiologically, multifactorial disease causes mortality and forms The most prevalent cancer in females all over the world and in Arab countries (Yahia, *et al.*, 2014). Breast Carcinoma is abnormal growth and uncontrolled division of the cells in the breast lining lobules or ducts in multistep pattern mostly occurred in females (Fazilaty and Mehdipour,2014). Women's tumors are most commonly caused by lung cancer, followed by BC. It accounts for 14% of all cancer-related deaths, Each year, almost 2.1 million women are diagnosed with BC, and 626,679 deaths. (Bray *et al.*, 2018; Zafar *et al.*, 2019). BC was the most common type of cancer in Iraq. It makes up 19.5% of all cancer cases (4,996 cases) and 34.3% of all cancers in women (4922 cases). In 2016, 897 women died from this disease. It was the leading cause of death from cancer among Iraqi women (23.6%), and it was the second leading cause of death from cancer overall (12.1%), after lung cancer (Alwan *et al.*, 2019). FOXA1, initially identified for its function in liver development as a transcriptional activator, also was expressed in BC (Shou *et al.*, 2016). FOXA1 interacts to certain DNA sequences within the nucleosome core and decondenses chromatin to allow the interaction of other transcription factors to gene promoters. FOXA1 is required for the expression of 50% of ER-related genes and thus plays an important role in BC pathogenesis (Hurtado *et al.*, 2011). (Shou *et al.*, 2016). In BC, studies of global gene expression revealed high expression of FoxA1 mRNA in tumors expressing of estrogen receptor (ER) (Yamaguchi *et al.*, 2008). So, we carried out this research to investigate the relationship between FOXA1 expression with the tumor size, stage, grade and lymph node metastasis in BC Iraqi women.

Materials and Methods:

This research was carried out between September 2021 and May 2022 at the University of Kufa's Faculty of Medicine, Middle Euphrates Unit for Cancer Research.

Collection of Samples

Patients Group

This study is carried out on seventy three cases of BC their Paraffin blocks gathered from surgical BC patients between 2018 and 2022. The archives of two private laboratories were used to collect the data retroactively. Each pathologic specimen was histologically reevaluated by two pathologists, Electronic medical records of these laboratories are used to obtained the clinicopathological characteristics of BC patients which includes tumor size, stage, grade and lymph node metastasis to investigation the correlation between FOXA1 and these clinicopathological features.

Control group

Thirty eight blocks of normal non-tumoral breast tissue has been gathered randomly from archives of three private laboratories during the collection of BC tissue blocks, and also, re-evaluated by two pathologist to ensure their normality.

IHC Procedure

One hundred eleven blocks of Paraffin were prepared for FOXA1 by Labeled Streptavidin Biotin (LSAB) method, Five µm thickness sections have been cut from paraffin-embedded blocks and set on positively charged slides.

Control of IHC

In IHC study , two controls were used:

A: Positive control: Paraffin blocks of BC were prepared and dyed as positive control for FOXA1 in every run.

B: Negative control: Untreated slides with primary antibody were regarded as negative controls for each slides set.

Results of Staining

Only tumor cells that have nuclear positivity are included for assessment. Evaluation takes into account both staining ratio (the percentage of stained cells) and intensity. The staining ratio is recorded as 0 (0%), 1 (>0% to 25%), 2 (>25% to 50%), 3 (>50% to 75%), and 4 (>75%), whereas the intensity was marked as 0 (negative), 1 (weakly positive), 2 (moderately positive), or 3 (strongly positive). The overall protein expression score = staining intensity score × staining extent score, which was graded and divided into five scores according to this equation, 0 (0%), 1 (1–25%), 2 (26–50%), 3 (51–75%) and 4 (76–100%). (Yuan *et al.*,2020).

Statistical Analysis

The data were analyzed using SPSS version 21. Numeric variables were reported as mean ± SD and nominal variables as number and percent when variables were regularly distributed, the Student t-test is used to compare means between two groups, whereas the Mann Whitney U test was used to do so, when the distribution of the variables was not normal. When the chi-square test cannot be used because it is invalid, the corrected chi-square test was utilized. Odd ratios with a 95% confidence interval (CI) and were used to estimate risk.

Results

Nuclear expression of FOXA1 protein in BC patients and some clinicopathological features.

Nuclear IHC positive expression of FOXA1 was considerably reduced in the 43 patients in the patient group (58.9%) in comparison with control group 38 persons (100%) while the negative expression of FOXA1 was considerably reduced in patients group 30 patients (41.1%) in comparison to the control group 0 individuals (0.0%) (P=0.0001). On the other hand, Odd ratio for this protein was 1.66 95% CI (1.37-1.98) as shown in (Table 1) and (Figures 1 to 3). The majority of patients were in stage II (41.1%) while the percentage of BC patients with Stage I and stage III were equal (24.7%), while (9.6%) of BC patients was stage IIII. The larger percentage of BC (61.6%) was lymph nodes metastasis while the rest of BC

cases without lymph nodes involvement (38.4%). According to BC grades, the distribution prevalence of cases in this study were 13 (17.8%), 38(52.1%) and 22(30.1%) as grade I, grade II and grade III of BC consequently. The percentage of BC patients with tumor size equal or less than 5 centimeters (cm) was (64.4%) which higher than BC patients with tumor size more than 5 cm (35.6%). All these result shown in (Table 2).

Table 1: FOXA1 nuclear expression in two groups of the study.

Nuclear IHC of FOXA1 expression	**Control Group**		**Patients Group**		**Odd ratio (CI^+ %95)**
	No.	**%**	**No.**	**%**	
Positive expression	38	100.00	43	58.9	1.66 (1.37-1.98)
Negative expression	0	0.0	30	41.1	
Total	38	100.00	73	100.00	

P=0.0001, CI^+= Confidence interval, Pearson Chi-Square Test

Table 2: The clinicopathological characteristics of BC patients

Clinicopathological features	**No.**	**%**
TNM stage:		
Stage I	18	24.7
Stage II	30	41.1
Stage III	18	24.7
Stage IIII	7	9.6
Lymph node metastasis:		
Present	45	61.6
Absent	28	38.4
Grade:		
Grade I	13	17.8
Grade II	38	52.1
Grade III	22	30.1
Tumor size:		
≤ 5 cm	47	64.4
>5 cm	26	35.6

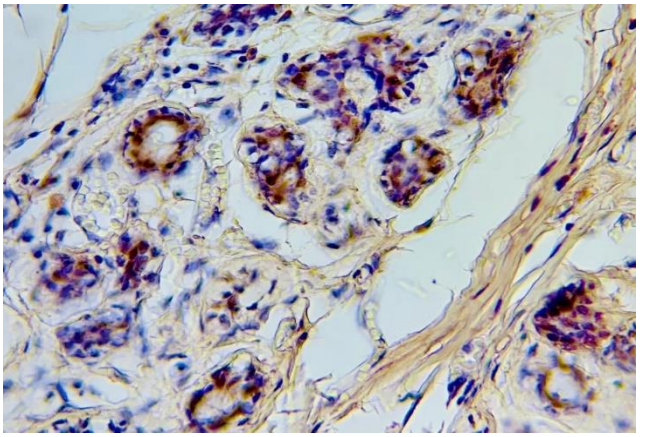

Figure (1): Normal tissue, positive nuclear stain for FOXA1 by IHC technique 400X.
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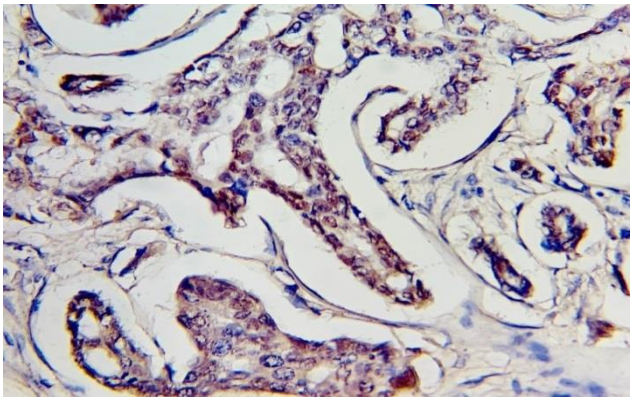

Figure (2): Moderately differentiated IDC, positive nuclear stain for FOXA1 by IHC technique 400X.

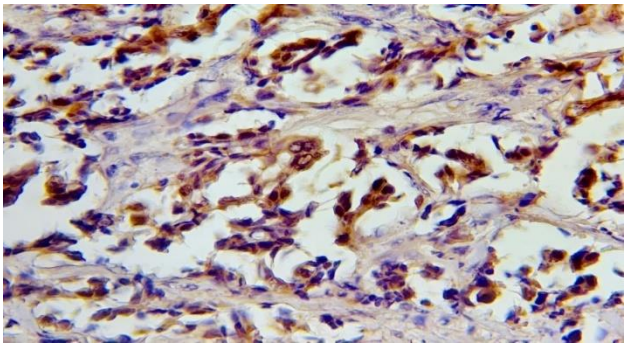

Figure (3): Poorly differentiated IDC with focus of in situ carcinoma, diffuse positive nuclear stain for FOXA1 by IHC technique. 400X

BC Stages and FOXA1 protein expression

There was no significant association between FOXA1 protein expression and different Stages of BC (P=0.096) as clarified in (Table 3).

Table 3: BC Stages and FOXA1 protein expression.

Stage	Total Count	FOXA1 Protein		Total
		Positive expression	Negative expression	
Stage I	Count	11	7	18
	%	61.1%	38.9%	100.0%
Stage II	Count	22	8	30
	%	73.3%	26.7%	100.0%
Stage III	Count	7	11	18
	%	38.9%	61.1%	100.0%
Stage IIII	Count	3	4	7
	%	42.9%	57.1%	100.0%
Total	Count	43	30	73
	%	58.9%	41.1%	100.0%

P=0.096, Pearson Chi-Square Test

Lymph Nodes and FOXA1 protein expression

Positive FOXA1 expression in BC patients increased significantly in BC patients with absent lymph node metastasis 21 cases (75.0%) related with BC patients with lymph node metastasis 22 cases (48.9%) (P=0.027) as illustrated in (Table 4).

Table 4: Lymph Nodes involvement and FOXA1 protein expression.

Lymph Nodes Involvement	Total Count	FOXA1 Protein		Total
		Positive expression	Negative expression	
Present	Count	22	23	45
	%	48.9%	51.1%	100.0%
Absent	Count	21	7	28
	%	75.0%	25.0%	100.0%
Total	Count	43	30	73
	%	58.9%	41.1%	100.0%

P=0.027, Pearson Chi-Square Test

Tumor Size and FOXA1 protein expression:

There was no significant correlation between FOXA1 protein expression and tumor size of BC (P=0.514) as seen in (Table 5).

Table 5: BC Tumor Size and FOXA1 protein expression

Tumor Size	Total Count	FOXA1 Protein		Total
		Positive expression	Negative expression	
≥5 cm	Count	29	18	47
	%	61.7%	38.3%	100.0%
<5 cm	Count	14	12	26
	%	53.8%	46.2%	100.0%
Total	Count	43	30	73
	%	58.9%	41.1%	100.0%

P=0.514, Pearson Chi-Square Test

BC grades and FOXA1 protein expression:

There was a significant relationship (P= 0.002) between FOXA1 positive expression and grades I 6 cases (61.5%) and grade II 29 cases (76.3%) of BC in contrast to its relation with grade III in which negative FOXA1 expression increased significantly 16 cases (72.7%) as shown in (Table 6).

Table 6: BC Grades and FOXA1 protein expression.

Grade	Total Count	FOXA1 Protein		Total
		Positive expression	Negative expression	
Grade I	Count	8	5	13
	%	61.5 %	38.5%	100.0%
Grade II	Count	29	9	38
	%	76.3 %	23.7%	100.0%
Grade III	Count	6	16	22
	%	27.3 %	72.7%	100.0%
Total	Count	43	30	73
	%	58.9 %	41.1%	100.0%

P=0.002, Pearson Chi-Square Test

Discussion

Positive FOXA1 expression decreased significantly in BC patients than control with normal breast tissue. The findings of this study indicate that the majority of BC cases was positive FOXA1 expression 58.9% while negative FOXA1 expression 41.1%. Other studies showed, positive nuclear FOXA1 expression was detected in 32 cases (45.7%) while 38 cases (54.3%) were negative for FOXA1 expression (Abelzaher *et al.*, 2022). Besides, positive FOXA1 expression was detected in 84.6% of cases while negative FOXA1 expression was 15.4% of cases (Rangel *et al.*, 2018). Other finding claimed, positive FOXA1 expression was 74% in BC caces (Badve *et al.*, 2007). Furthermore Habshy *et al.* (2008), Mehta *et al.* (2012), Guiu *et al.* (2018) revealed that positive FOXA1 expression were (55%), (85%), and (55%) respectively. As a result of overexpression, mutation, or amplification, the expression and function of FOXA1 are not working as they should (Razavi *et al.*, 2018, Fu *et al.*, 2019). In the current study, there was a substantial relationship between positive FOXA1 and low grade (I and II) of BC while negative expression increased in high grade (III). This result was an agreement with several obvious studies (Wolf *et al.*, 2007; Albergaria *et al.*, 2009; Mangia *et al.*, 2019). Histone deacetylase 7 (HDAC7) forms a ternary complex with FOXA1 and ER that is required for estrogen-directed repression of the cell cycle suppressor (Malik *et al.*, 2010). Transducin-like enhancer protein 3 (TLE3), a different corepressor, via binding to FOXA1, indirectly interacts with chromatin at ER-responsive genes when estrogens were not present. Suppress gene expression by TLE3 recruits HDAC2. TLE3 was associated with approximately 50% of ER-FOXA1 interaction events in the luminal MCF7 cell line, indicating that TLE3 and FOXA1 work cooperatively to suppress expression. In fact, TLE3 was brought in by FOXA1 to the chromatin to suppress the transcription of the estrogen-responsive gene (Jangal *et al.*, 2014). As a result, FOXA1 is a crucial regulator of hormone response in BC, serving as an actual pioneering factor to develop ER binding patterns (Robinson *et al.,* 2013; Carroll, 2016; Jozwik *et al.*, 2016; Glont *et al.,* 2019). In this study, there was a negative correlation between positive FOXA1 expression and lymph nodes metastasis.This result also accords with these observations, which showed that the same findings (Albergaria *et al.*, 2009; Hu *et al.*, 2014; Abelzaher *et al.*, 2022). By affecting the expression of the BRCA1-associated cell cycle suppress p27 and encouraging the development of CDH1, it has been demonstrated that FOXA1 high expression can halt the course of metastatic disease. The CDH1 was directly stimulated by FOXA1, and the resulting increasing the expression of CDH1 reduces the ability of BC cells to migrate (Hu *et al.*, 2014). Similarly, regulation CDH1 expression by FOXA1 involved in EMT. FOXA1 controls EMT in cancer cells by elevating CDH1 expression and reducing vimentin protein levels. (Abelzaher *et al.*, 2022). In contrast, some studies found there was no relationship between FOXA1 expression and nodal status (Habashy *et al.*, 2008; Liu *et al.*, 2010; Ijichi *et al.*, 2012). This study concluded, positive FOXA1 expression correlated with grade II while negative FOXA1 expression correlated with grade III, and negative correlation with lymph node metastasis.

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