Original Article

FOXA1 protein expression and survival of breast cancer patients

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Abstract

Forkhead box protein A1 (FOXA1) is pioneer factor has a dual role either as growth stimulator or a repressor and the significance role of this protein as a prognostic biomarker still controversial, so the aim of this study was to identify the precise role of FOXA1 protein expression in prediction of BC patients outcome and recurrence of BC by immunohistochemical (IHC) staning method. In this study, seventy three tissues samples embedded in wax block were taken from BC patients with (52.71+11.55) years who had surgery between 2014-2020 and thirty eight of normal (non tumoral) breast tissues samples with (54.17+10.84) years as a control group. Negative FOXA1 expression increased significantly in patients with recurrence BC (P=0.006), BC patients with good prognosis and moderate prognosis have a significant correlation with positive FOXA1 expression (P=0.018). Overall survival (OS) was 82.74% in patients with positive expression of FOXA1 which is longer than OS in BC patients with negative expression of FOXA1 (61.31%), (P=0.001). Disease free survival (DFS) was 81.36% in BC patients with positive expression of FOXA1 which also longer than in BC patients with negative expression of FOXA1 (59.8%) (p=0.0001). FOXA1 was a good prognostic marker for survival of BC patients.

Keywords: FOXA1, IHC, OS, Breast cancer.

1. Introduction

The BC incidence rate in Iraq progressively from 8.56% of all newly diagnosed cancers in 2008 to 19.55% in 2020 as the most prevalent cancer in Iraq (ICR, 2020). Numerous studies have shown that FOXA1 expression level and BC prognosis are related. According to several research, FOXA1 was found to be a significant predictive factor of both overall survival (OS) and disease free survival (DFS) (Siegel et al., 2015; Xu et al., 2015). While others suggested not (Albergaria et al., 2009; Rakha et al., 2010). FOXA1 is one of three proteins in the FOXA family. It controls tissue-specific transcriptional programs and is involved in cell growth, proliferation, apoptosis, differentiation, and development in a variety of organs, including the pancreas, prostate, and breast (Bernardo and Keri, 2012). FOXA1 has been demonstrated to play both a growth stimulator and a repressor in BC, in the early stages, it promotes tumor growth, but later, it suppresses tumor growth (Hu et al., 2014).

Expression of FOXA1 correlates with ER positivity (Wolf et al., 2007). Good prognosis in luminal (A) BC which responds well to anti-estrogen treatment (Thorat et al., 2008). Additionally, there was a negative correlation between recurrence and FOXA1 expression (Ademuyiwa et al., 2010). It has been hypothesized that treatments increasing FOXA1 expression or activity, when combined with antiestrogens, may prevent the progress of estrogen independence (Nakshatri and Badve, 2007).

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There have been other studies that looked at FOXA1 predictive value, but their scope was limited (Wolf et al., 2007; Habashy et al., 2008; Albergaria et al., 2009). So, this study carried out to investigate the relationship between FOXA1 expression and survival of BC patients in Iraqi women.

2. 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.

2.1. Collection of Samples:

2.1.1. 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. After getting the BC paraffin block from the private laboratories, the data of death and hormone therapy intake followed up for these blocks from middle Euphrates Center of oncology and obtained from it. The data revealed that there were 28 patients death (events), 11 patients survival (censored) and 34 patients missing. The data are used for concluding the rate of four years OS was a prognosis analysis after diagnosis that measured from the date of surgery until the date of death. Patients who were alive at the date of last follow up were censored. On the other hand, this study measured DFS which calculated from the date of surgery until the date of return of BC recurrence (Jemal et al., 2011).

2.1.2. 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.

2.2. 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.

2.2.1. 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).

2.3. 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. When the P-value was less than 0.05, it was considered significant.

3. Results

3.1 Recurrence of BC and FOXA1 expression:

The cases with no recurrence of BC were increased significantly in positive FOXA1 expression 15 (83.3%) while negative FOXA1 expression increased significantly in patients with recurrence BC 14 (66.7%) (P=0.006) as shown in (Table 3-1).

Table 3-1 : Recurrence of BC & FOXA1 protein expression.

Recurrence	Total Count	FOXA1 protein		Total
		Positive expression	Negative expression	
Recurrence	Count	3	14	17
	%	16.7%	66.7%	43.6%
No Recurrence	Count	15	7	22
	%	83.3%	33.3%	56.4%
Total	Count	18	21	39
	%	100%	100%	100%

P= 0.006

3.2 NPI and FOXA1 protein expression:

As illustrated in (Table 3-2), BC patients with good prognosis and moderate prognosis have a significant correlation with positive FOXA1 expression 17 (81.0%) and 18 (58.1%) respectively, while BC patients with poor prognosis correlated with negative FOXA1 expression 13 patients (61.9%) (P=0.018).

Table 3-2: FOXA1 protein expression & NPI.

Nottingham Prognostic Index	Total Count	FOXA1 Protein		Total
		Positive expression	Negative expression	
Good Prognosis	Count	17	4	21
	%	81.0%	19.0%	100%
Moderate Prognosis	Count	18	13	31
	%	58.1%	41.9%	100%
Poor Prognosis	Count	8	13	21
	%	38.1%	61.9%	100%
Total	Count	43	30	73
	%	58.9%	41.1%	100%

P=0.018

3.3 FOXA1 expression and BC patients survival

3.3.1 Overall survival (OS) in BC patients with positive and negative FOXA1 expression:

In Kaplan-Meier analysis, OS was 82.74% in patients with positive expression of FOXA1 which was longer than OS in patients with negative expression of FOXA1 (61.31%), also, patients with negative FOXA1 expression showed shorter mean OS (16.87 ± 1.11 months) than those with positive FOXA1 expression (30.45 ± 1.64 months) (log-rank P = 0.001) as shown in (Table 3-3) and (Figure 3-1).

Table 3-3: OS in positive &negative FOXA1 expression.

FOXA1 protein expression	Mean OS survival ± SE (months)	Event (Deaths)		OS Survival %
		No.	%	
Positive expression	30.45 ± 1.64	10	35.72	% 82.74
Negative expression	16.87 ± 1.11	18	64.28	% 61.31

P = 0.001

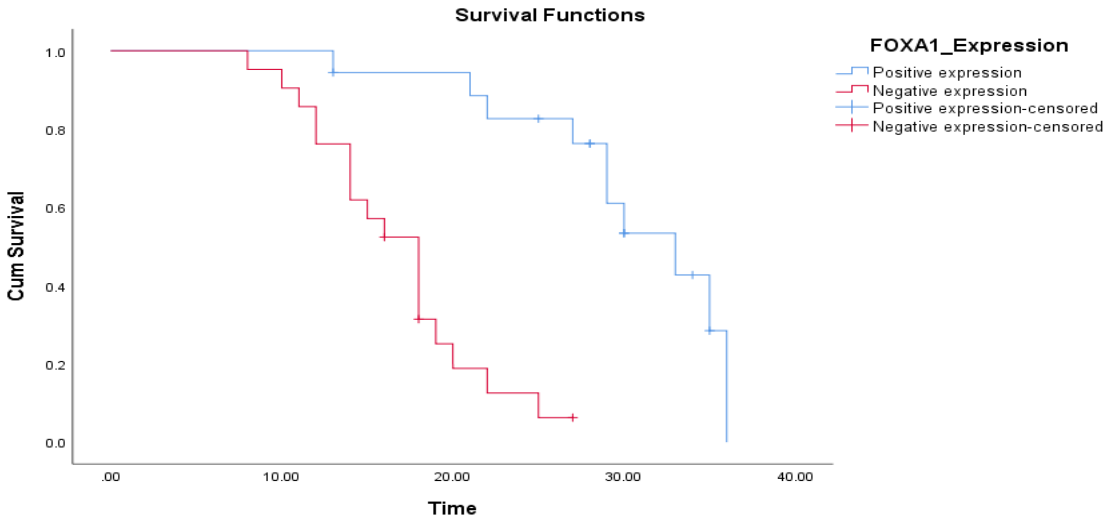


Figure (3-1): OS diagram in BC patients with positive and negative FOXA1 protein expression.

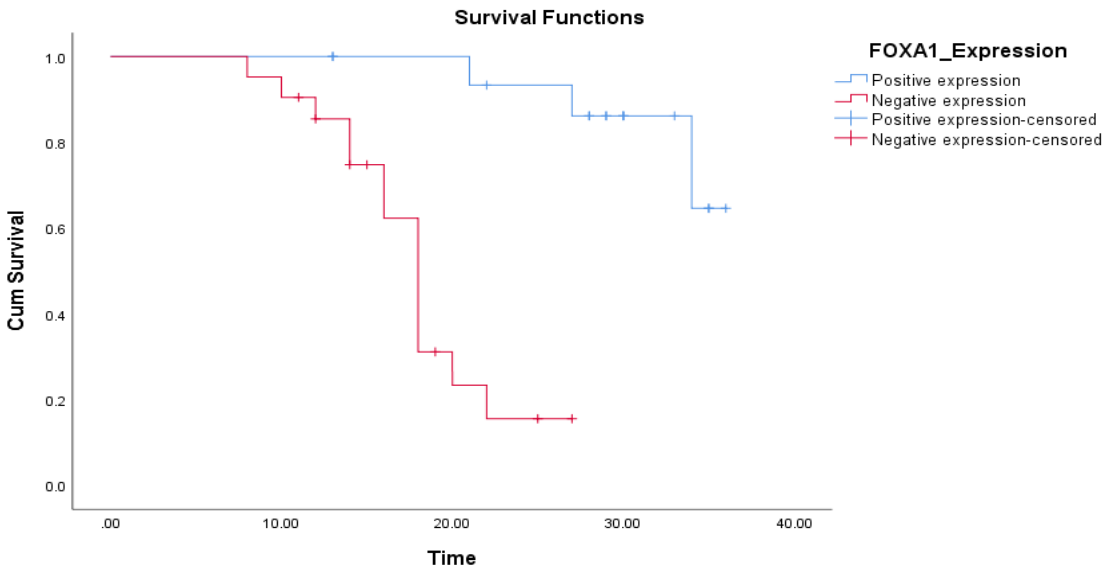
3.3.2 DFS in BC patients with positive and negative FOXA1 expression:

In Kaplan-Meier analysis, DFS is 81.36% in patients with positive expression of FOXA1 and 59.8% in patients with negative expression of FOXA1, also, patients with negative FOXA1 expression showed shorter OS (18.03 ± 1.21 months) than those with positive FOXA1 expression (33.92 ± 1.13 months) (log-rank P = 0.001) as shown in (Table 3-4) and (Figure 3-2).

Table 4-20 : DFS in positive & negative FOXA1 expression.

FOXA1 protein expression	Mean DFS survival ± SE (months)	Event (Death)		DFS Survival %
		N	%	
Positive expression	33.92 ± 1.131	3	17.64	%81.36
Negative expression	18.03 ± 1.215	14	82.35	59.8%

P = 0.0001



Figure(3-2) : DFS diagram for BC patients with positive and negative FOXA1 protein expression.

4. Discussion:

There was a significant relationship between negative FOXA1 expression with BC recurrence, these results were in line with those of previous studies (Bernardo et al., 2010; Hurtado et al., 2011). Also, FOXA1 was significant correlated with NPI, most cases of positive FOXA1 was good prognosis and moderate prognosis, while BC patients with poor prognosis was negative FOXA1. This finding was in agreement with those obtained by (Albergaria et al., 2009; Abelzaher et al., 2022) and in disagreement with the results concluded by Thorat et al.(2008). The function of FOXA1 in normal breast tissue remains unknown. Two-week-old FOXA1 mice are alive and have been thoroughly examined for prostate development. Animals with the FOXA1 mutation have a significantly changed ductal pattern devoid of developed or mature luminal epithelial cells. FOXA1, in conjunction with the androgen receptor, has been implicated in the differentiation of prostate epithelium by modulating the expression of genes including Nkx3.1, Shh, and FOXA2. By analogy, FOXA1 and ER may have a role in the morphogenesis and differentiation of the mammary ducts. In stromal and ER-negative luminal cells, ER-expressing cells have been demonstrated to influence proliferation and gene expression pattern via paracrine pathways. FOXA1 may play a role in ER's ability to do this function (Gao et al., 2005).

The present study revealed that the FOXA1 positive was a significant predictor for OS and DFS in BC patients. This also accords with our earlier observations, FOXA1 was found to be a significant predictor of OS (Ijichi et al., 2012; Shou et al., 2016) and DFS (Kawase et al., 2015; Shou et al., 2016; Horimoto et al., 2020). Mehta et al. (2012) indicated that FOXA1 act as tumor repressor and inhibited BC cell proliferation and migration via downregulating SOD2 and IL6 expression and decreased BC progression. This outcome is contrary to Thorat et al. (2008) who found no correlation between FOXA1 expression and OS and DFS of BC patients . A possible explanation for this conflicting results might be because sample size, living environment contribute to bias (Hu et al.,2014). This study revealed that FOXA1 protein positive expression is independent predictors of prolonger BC patients survival

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