Immunohistochemical expression of FOXA1 and Androgen receptor in breast carcinoma in Upper Egypt

Seham Abdelrazik Ismail[a]*, Sabah A.M Fadel[b], Noha A Aboulhagag[b], Mohamed M Wahman[c]

[a]Pathology Department, Faculty of Medicine, South Valley University, Qena, Egypt.
[b]Pathology Department, Faculty of Medicine, Assuit University, Assiut, Egypt.
[c]Clinical Oncology Department, Faculty of Medicine, South Valley University, Qena, Egypt.

Abstract

Background: Breast cancer is the most widespread cancer in women worldwide. Forkhead box A1 (FOXA1) is a forkhead family protein that is encoded by the FOXA1 gene. Recent studies suggest that during the development of cancers, FOXA1 may become an oncogene. Androgen receptor (AR) belongs to the steroid nuclear receptor-ligand-binding superfamily.
Objectives: FOXA1 and AR immunohistochemical (IHC) expression in breast carcinoma showed potential as tumor-specific targets. Their correlation with different histopathological parameters and with each other were assessed.
Patients and Methods: This was a retrospective analytical study carried out at Qena University Hospital from April 2021 to December 2022. The study included 65 formalin-fixed paraffin-embedded tissue blocks from different breast lesions obtained from the Pathology Department at Qena University Hospital.
Results: There were highly statistically significant associations between FOXA1 Score and ER, PR, and Molecular Subtype (Luminal B-like Her2-ve), while the differences between FOXA1 Score and Molecular Subtypes (Her-2-enriched and Triple Negative) were statistically significant. There were statistically significant differences between AR score and ER, PR, and type of operation. Our research showed that AR and FOXA1 are strongly associated.
Conclusion: Our results suggest that in Breast Carcinoma, the expression of FOXA1 has a strong association with the expression of AR. These findings have important clinical significance in selecting a subset of AR+ tumors that are suitable for anti-AR therapies. However, this requires further examination in more extensive cohort studies.
Keywords: Forkhead box A1; FOXA1; Breast carcinoma; Androgen receptor.

DOI: 10.21608/SVUIJM.2024.277714.1829
***Correspondence**: Seham.abdelrazek@med.svu.edu.eg
Received: 30 March,2024.
Revised: 10 April,2024.
Accepted: 9 June,2024.
Published: 10 June, 2024
Cite this article as: Seham Abdelrazik Ismail, Sabah A.M Fadel, Noha A Aboulhagag, Mohamed M Wahman.(2024). Immunohistochemical expression of FOXA1 and Androgen receptor in breast carcinoma in Upper Egypt. *SVU-International Journal of Medical Sciences*. Vol.7, Issue 2, pp: 67-80.

Introduction

Globally, breast carcinoma is the most common type of malignant tumor in women **(Sung et al., 2021**). According to **(Nassar et al., 2020),** BC is the most frequent malignancy in Egypt among females, accounting for 37.7% of the 12,000–13,000 new cases reported every year. Recurrence, resistance to hormone treatment, metastasis, and mortality from breast cancer persist despite advancements in early identification and treatment **(Sung et al., 2021**).

The protein known as forkhead box A1 (FOXA1) is encoded by the FOXA1 gene and belongs to the forkhead family. By transcriptionally activating the genes specific to the liver, albumin, and transthyretin, it was first found to be an essential component for liver development (**Le Lay and Kaestner, 2010**). Based on current studies, FOXA1 may become an oncogene during the growth of cancers such as gliomas, non-small cell lung cancer, and hepatocellular carcinoma **(BenAyed-Guerfali et al., 2019).** It is still not known what is the role that FOXA1 plays in carcinogenesis as well as the functional consequences of FOXA1 mutations (**Horimoto et al., 2020**). Consequently, in cases of breast carcinoma, it is imperative to look into the connection between FOXA1 expression and the development of the disease as well as the therapeutic response to endocrine therapy.

Androgen receptor (AR) is one of the steroid nuclear receptor-ligand binding superfamily. It is nuclear, ligand-dependent transcription factor that is widely expressed all over the body. The sex chromosome Xq12 contains the androgen receptor (AR), which is more than 90 kilobases long and split into 8 (eight) exons **(Jain and Das, 2020).**

The development of male secondary sexual traits and the male reproductive system is the primary physiological function of androgen hormones and their recognized target receptors. The biological functions of androgen receptor (AR) are expressed in many different tissues, including bone, muscle, prostate, adipose tissue, and reproductive, cardiovascular, immune, neurological, and hematopoietic systems **(Davey and Grossmann, 2016).**

Prostate and breast cancers are being studied and treated with AR as a target. Anti-androgens are used in treatment because they attach to the ligand-binding domain of AR and stop other androgens from attaching to the same domain **(Godbole and Njar, 2011).**

Although it has been proposed that the relative ratios of FOXA1, ER, and AR may have an impact on the growth and aggressiveness of cancer cells, the function of FOXA1/AR co-expression in BC has not been studied **(Rangel et al., 2018**).

The expression of FOXA1 and AR were examined in a group of breast cancer cases with different histopathological varianets due to their potential as tumor-specific targets.

Patients and Methods

Sixty-five formalin-fixed paraffin-embedded tissue blocks from different breast lesions (IDC, mixed ductal and lobular carcinoma and benign breast lesions) were selected from the Lab of Pathology Department, Qena University Hospital in the period from April 2021 to April 2022. They were 50 IBC, 10 benign breast lesions, and 5 cases of normal breast used as a control. Tissue specimens were obtained by modified radical mastectomy (MRM) (48 cases), conservative breast surgery (2 cases), excisional biopsy specimens (10 cases), and 5 cases of normal breast obtained from reduction mastectomy. The study received approval from the Ethical Committee of Qena Faculty of Medicine, Ethical approval code: SVU-MED-PAT005-2-21-4-178.

The clinicopathological data were obtained from the pathology reports of the cases. Tumors were re-evaluated for the following parameters: histological subtype and tumor grade. Lymphovascular invasion (LVI), Lymph node metastasis (LNM), and pathological stage were also assessed. Multiple tissue samples from the specimens were obtained and formalin-fixed, paraffin-embedded tissue blocks were prepared. Three tissue sections from each block were prepared; one section was stained with hematoxylin and eosin (H&E) stain and the remaining two sections were subjected to IHC staining with antibodies against FOXA1 and AR. The Nottingham Histological Score System was used for the histopathological grading; a modification of the Scarff-Bloom-Richardson grading system **(Elston and Ellis, 1998)**. Tumors were staged using the AJCC TNM staging system, 8th eighth edition **(Amin et al., 2017).**

Immunohistochemistry: Tumor blocks were representative, formalin-fixed, paraffin-embedded, and sectioned at 4-µm thickness. Immunohistochemistry (IHC) was carried out utilizing the avidin-biotin technique. Before applying the antibodies, the antigen was extracted. Using 0.3% hydrogen peroxide in deionized water, endogenous peroxidase was inhibited. The following clones were used: THERMO SCIENTIFIC Corporation, Fremont, USA, clone 1512, FOXA1 mouse monoclonal antibody against human (7 ml, prediluted, Catalog number (Cat #) MC0275RTU7). Human antigen-specific rabbit polyclonal antibody (7 ml, prediluted; Cat # FNab00388, Fine Test Corporation, Fremont, USA). Hematoxylin was used to stain the nuclei and 3,3'-diaminobenzidine was used to visualize the reaction's end product. When evaluating FOXA1 expression, nuclei labeling in more than 1% of neoplastic cells was regarded as a positive result (sample scoring was conducted without knowledge of clinical or pathological data for patients). An expression of 1-49% was considered low-level staining, and an expression of equal to or greater than 50% was considered high-level staining **(Sasahara et al., 2014).** For the evaluation of AR expression, tumor cell nuclei were scored and the occurrence of positive nuclei was divided into three groups, 0% (−); 1–10% (+), and >10% (++) **(Hilborn et al., 2016).** Two senior pathologists independently evaluated all microscopic slides.

We simply re-evaluated the expression of ER, PR, and HER2 that had been done outside for every case**.** A threshold of over 1% in stained cancerous cells was chosen to indicate positive ER and PR immunoreactivity **(Safarpour, Pakneshan and Tavassoli, 2014).**

The presence and intensity of membranous staining were assessed in order to determine HER2 overexpression. A score of 0 or 1+ was regarded as negative, a score of 3+ as strong positive, and a score of 2+ as weak positive. If a tumor had an IHC score of 3+ or 2+ and had HER2 amplification (ratio >2.0) according to fluorescence in situ hybridization (FISH), it was classified as HER2-positive **(Qaiser et al., 2018).**

Statistical analysis

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 20. The qualitative data were presented as number and percentages while quantitative data were presented as mean, standard deviations and ranges when their distribution found parametric.

The comparison between two groups with qualitative data were done by using *Chi-square test* and/or *Fisher exact test* was used instead of Chi-square test when the expected count in any cell was found less than 5.

The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following: $p > 0.05$ = non-significant (NS). $p < 0.05$ = significant (S). $p < 0.001$ = highly significant (HS).

Results

Association of FOXA1 and AR IHC expression with the clinico-pathological characteristics

The clinical and histopathological characteristics of the cases examined are reported in (**Table.1)** as follows**.** There were 25 cases whose age is less than or equal to 50 years and 25 cases whose age is more than 50 years. There were 58.0% of cases had cancer in the left breast and 42.0% of cases had cancer in the right breast. MRM was done in 96.0% of the studied cases. Eight percent (8.0%) of the studied patients had a tumor of the size I, 68.0% had a tumor of the size II and 24.0% had a tumor of the size III. IDC was the most histological type among studied cases. LVE was found in 76.0% of of the studied patients, perineural invasion was found in 10.0% of the studied patients, LN metastasis was found in 34.0% of of the studied patients. ER+ tumor was found in 74.0% of of the studied patients, PR+ tumor was found in 84.0% of the studied patients and Her2+ tumor was found in 8.0% of the studied patients. Regarding to FOXA1 score, 66.0% of the studied cases had high score and 26.0% had low score, while 26.0% of the studied cases had high AR score and 56.0% had low AR score. There were 40.0% of the studied cases had muscle invasion, 48.0% had skin invasion, 54.0% had prominent TILs and 40.0% had necrosis.

Table 1. Distribution of the studied cases according to different clinicopathological data

Variables		**No.**	**%**
Age	**Less than or equal to 50 years**	25	50.0%
	More than 50	25	50.0%
Laterality	**Left**	29	58.0%
	Right	21	42.0%
Type of operation	**MRM**	48	96.0%
	Breast conservative surgery with axillary dissection	2	4.0%
Size	**I**	4	8.0%
	II	34	68.0%
	III	12	24.0%
Histological type	**IDC**	43	86.0%
	ILC	3	6.0%
	Mixed ductal and lobular carcinoma	4	8.0%
P staging	**T1**	4	8.0%
	T2	28	56.0%
	T3	18	36.0%
Grading	**I**	0	0.0%
	II	49	98.0%
	III	1	2.0%
LVE	**Negative**	12	24.0%
	Positive	38	76.0%
Perineural invasion	**Negative**	45	90.0%
	Positive	5	10.0%
LN metastasis	**0**	13	26.0%
	1	17	34.0%

	2	20	40.0%
ER	**Negative**	13	26.0%
	Positive	37	74.0%
PR	**Negative**	8	16.0%
	Positive	42	84.0%
Her2	**Negative**	46	92.0%
	Positive	4	8.0%
Ki67	**High**	30	60.0%
	Low	20	40.0%
Molecular subtype	**Her-2 enriched**	2	4.0%
	Luminal A-like	9	18.0%
	Luminal B-like Her2 -ve	32	64.0%
	Triple Negative	7	14.0%
FOXA1 score	**High**	33	66.0%
	Low	13	26.0%
	Negative	4	8.0%
AR	**High (++)**	13	26.0%
	Low (+)	28	56.0%
	Negative	9	18.0%
Margins	**Free**	31	62.0%
	Positive	19	38.0%
Muscle invasion	**Absent**	30	60.0%
	Present	20	40.0%
Skin invasion	**Absent**	26	52.0%
	Present	24	48.0%
TILs	**Minimal**	23	46.0%
	Prominent	27	54.0%
Necrosis	**Absent**	30	60.0%
	Present	20	40.0%

Regarding to FOXA1 score 92% of the studied cases were positive for FOXA1, (**Fig.1**) and 8% were negative, while 82% of the studied cases had positive AR score and 18% had negative score, (**Fig.2**).

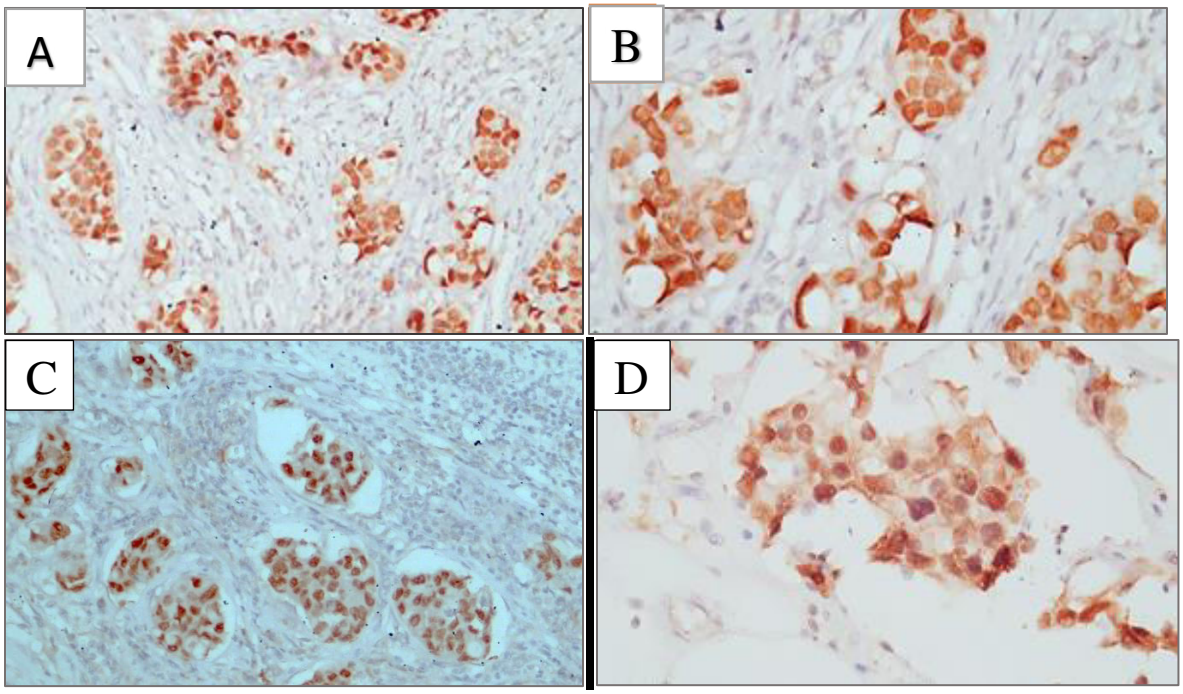

Fig.1. A and C: IDC-NST (grade II) with strong nuclear FOXA1 expression (X200). B and D: IDC-NST (grade II) with strong nuclear FOXA1 expression (X400).

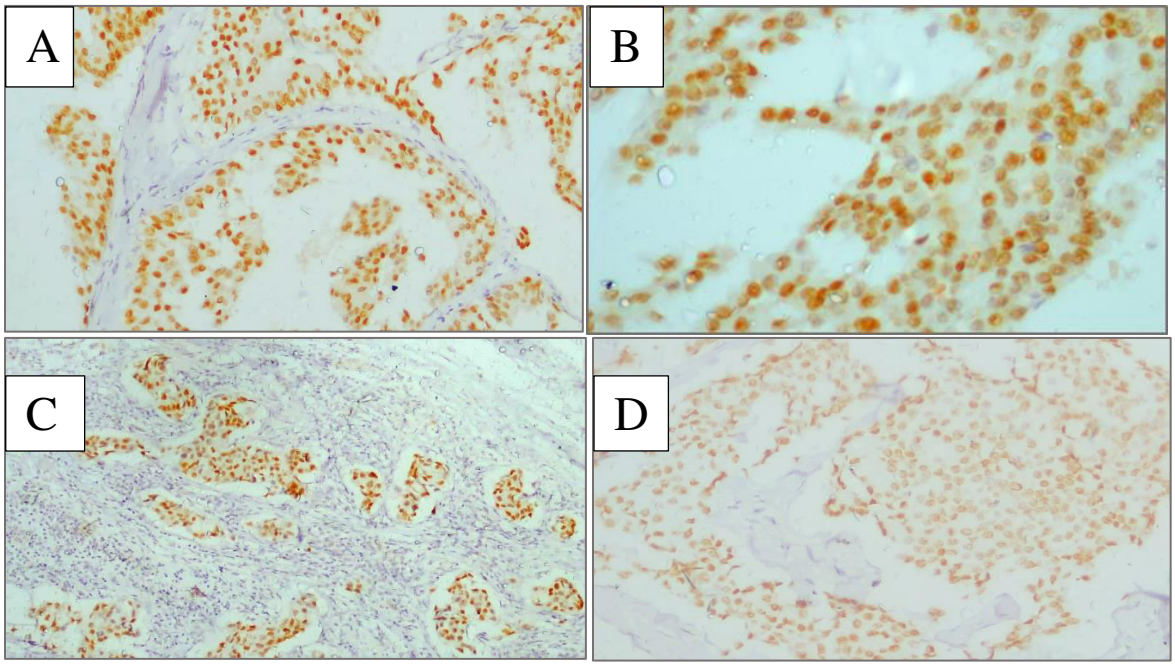

Fig.2.A: IDC-NST (grade II) with strong nuclear AR expression (X200), B: IDC-NST (grade II) with strong nuclear AR expression (X400). C: IDC-NST (grade II) with strong nuclear AR expression (X100), D: IDC-NST (grade II) with moderate nuclear AR expression (X200).

Relation Between FOXA1 Score and Other Parameters

There were positive correlations between FOXA1 Score and ER, PR. There were highly statistically significant differences between FOXA1 Score and Molecular Subtype (Luminal B-like Her2 – ve), while there were statistically significant differences between FOXA1 Score and Molecular Subtypes (Her-2 -enriched and Triple Negative), while there were no statistically significant differences between FOXA1 Score and Age, Laterality, Type of Operation, Size, Histological Type, P Staging, Grading, LVE, Perineural Invasion, LN Metastasis, Her2, Ki67, Margins, Muscle Invasion, Skin Invasion, TILs, and Necrosis as shown in (**Table.2)**.

Table 2. Relation between FOXA1 Score and different clinicopathological parameters

Variables		**FOXA1 score**						**Test value**	**P-value**	**Sig.**
		High		**Low**		**Negative**				
		No.	**%**	**No.**	**%**	**No.**	**%**			
Age	Less than or equal to 50 years	16	48.5%	8	61.5%	1	25.0%	1.723*	0.423	NS
	More than 50	17	51.5%	5	38.5%	3	75.0%			
Laterality	Left	23	69.7%	4	30.8%	2	50.0%	5.916	0.059	NS
	Right	10	30.3%	9	69.2%	2	50.0%			
Type of operation	MRM	32	97.0%	12	92.3%	4	100.0%	0.709	0.702	NS
	Breast conservative surgery with axillary dissection	1	3.0%	1	7.7%	0	0.0%			
Size	I	3	9.1%	1	7.7%	0	0.0%	0.418	0.981	NS
	II	22	66.7%	9	69.2%	3	75.0%			
	III	8	24.2%	3	23.1%	1	25.0%			
Histological type	IDC	29	87.9%	10	76.9%	4	100.0%	6.807	0.146	NS
	ILC	3	9.1%	0	0.0%	0	0.0%			
	Mixed ductal and lobular carcinoma	1	3.0%	3	23.1%	0	0.0%			
P staging	T1	3	9.1%	1	7.7%	0	0.0%	1.848	0.764	NS
	T2	17	51.5%	9	69.2%	2	50.0%			

	T3	13	39.4%	3	23.1%	2	50.0%			
Grading	II	32	97.0%	13	100.0 %	4	100.0%	0.526	0.769	NS
	III	1	3.0%	0	0.0%	0	0.0%			
LVE	Negative	7	21.2%	5	38.5%	0	0.0%	2.894	0.235	NS
	Positive	26	78.8%	8	61.5%	4	100.0%			
Peri neural invasion	Negative	31	93.9%	11	84.6%	3	75.0%	1.988	0.370	NS
	Positive	2	6.1%	2	15.4%	1	25.0%			
LN metastasis	0	7	21.2%	6	46.2%	0	0.0%	7.000	0.136	NS
	1	12	36.4%	2	15.4%	3	75.0%			
	2	14	42.4%	5	38.5%	1	25.0%			
ER	Negative	3	9.1%	8	61.5%	2	50.0%	14.635	0.001	HS
	Positive	30	90.9%	5	38.5%	2	50.0%			
PR	Negative	3	9.1%	2	15.4%	3	75.0%	11.536	0.003	HS
	Positive	30	90.9%	11	84.6%	1	25.0%			
Her2	Negative	31	93.9%	12	92.3%	3	75.0%	1.741	0.419	NS
	Positive	2	6.1%	1	7.7%	1	25.0%			
Ki67	High	18	54.5%	10	76.9%	2	50.0%	2.127	0.345	NS
	Low	15	45.5%	3	23.1%	2	50.0%			
Molecular subtype	Her-2 enriched	0	0.0%	1	7.7%	1	25.0%	6.430	0.040	S
	Luminal A-like	5	15.2%	3	23.1%	1	25.0%	0.541	0.763	NS
	Luminal B-like Her2 -ve	25	75.8%	7	53.8%	0	0.0%	9.673	0.008	HS
	Triple Negative	3	9.1%	2	15.4%	2	50.0%	4.987	0.043	S
Margins	Free	21	63.6%	7	53.8%	3	75.0%	0.691	0.708	NS
	Positive	12	36.4%	6	46.2%	1	25.0%			
Muscle invasion	Absent	17	51.5%	10	76.9%	3	75.0%	2.916	0.233	NS
	Present	16	48.5%	3	23.1%	1	25.0%			

Skin invasion	Absent	16	48.5%	7	53.8%	3	75.0%	1.029	0.598	NS
	Present	17	51.5%	6	46.2%	1	25.0%			
TILs	Minimal	14	42.4%	5	38.5%	4	100.0%	5.163	0.076	NS
	Prominent	19	57.6%	8	61.5%	0	0.0%			
Necrosis	Absent	17	51.5%	9	69.2%	4	100.0%	4.118	0.128	NS
	Present	16	48.5%	4	30.8%	0	0.0%			

P-value >0.05: Non significant(NS); P-value <0.05: Significant(S); P-value< 0.01: highly significant(HS) *: Chi-square test

There was an association between FOXA1 score and AR score as 75% of the cases negative for FOXA1 score were negative for AR score, 53.8% of cases with low score for FOXA1 were also low for AR score. 60.6% of cases which was high FOXA1 score were low for AR score. This indicate that there was a positive correlation between the FOXA1 score and the AR score (p-value=0.032) as shown in (**Table.3**).

Table 3. Relation between FOXA1 Score and AR

Variables		**FOXA1 score**						**Test value***	**P-value**	**Sig.**
		High		**Low**		**Negative**				
		No.	**%**	**No.**	**%**	**No.**	**%**			
AR score	**High**	9	27.3%	4	30.8%	0	0.0%	9.900	0.032	S
	Low	20	60.6%	7	53.8%	1	25.0%			
	Negative	4	12.1%	2	15.4%	3	75.0%			

P-value >0.05: Non significant(NS); P-value <0.05: Significant(S); P-value< 0.01: highly significant(HS) . *: Chi-square test

Association Between AR Score and Other Parameter

There were statistically significant differences between AR Score and ER, PR and Type of Operation while there were no statistically significant differences between AR Score and Age, Laterality, Size, Histological Type, P Staging, Grading, LVE, Perineural Invasion, LN Metastasis, Her2, Ki67, Molecular Subtypes, Margins, Muscle Invasion, Skin Invasion, TILs, and Necrosis as shown in (**Table. 4).**

Table 4. Relation between AR Score and different clinicopathological parameters

Variables		AR score						Test value	P-value	Sig.
		High		Low		Negative				
		No.	%	No.	%	No.	%			
Age	Less than or equal to 50 years	7	53.8%	13	46.4%	5	55.6%	0.331	0.848	NS
	More than 50	6	46.2%	15	53.6%	4	44.4%			
Laterality	Left	5	38.5%	18	64.3%	6	66.7%	2.769	0.250	NS
	Right	8	61.5%	10	35.7%	3	33.3%			
Type of operation	Breast conservative surgery with axillary dissection	2	15.4%	0	0.0%	0	0.0%	5.929	0.042	S
	MRM	11	84.6%	28	100.0%	9	100.0%			
Size	I	1	7.7%	3	10.7%	0	0.0%	1.393	0.845	NS
	II	9	69.2%	19	67.9%	6	66.7%			
	III	3	23.1%	6	21.4%	3	33.3%			
Histological type	IDC	12	92.3%	23	82.1%	8	88.9%	2.611	0.625	NS
	ILC	0	0.0%	3	10.7%	0	0.0%			
	Mixed ductal and lobular carcinoma	1	7.7%	2	7.1%	1	11.1%			
P staging	T1	1	7.7%	3	10.7%	0	0.0%	1.313	0.859	NS
	T2	7	53.8%	16	57.1%	5	55.6%			
	T3	5	38.5%	9	32.1%	4	44.4%			
Grading	II	12	92.3%	28	100.0%	9	100.0%	2.904	0.234	NS
	III	1	7.7%	0	0.0%	0	0.0%			
LVE	Negative	3	23.1%	8	28.6%	1	11.1%	1.147	0.564	NS
	Positive	10	76.9%	20	71.4%	8	88.9%			
Peri neural invasion	Negative	13	100.0%	24	85.7%	8	88.9%	2.028	0.363	NS
	Positive	0	0.0%	4	14.3%	1	11.1%			
LN metastasis	0	3	23.1%	9	32.1%	1	11.1%	2.850	0.583	NS
	1	6	46.2%	8	28.6%	3	33.3%			
	2	4	30.8%	11	39.3%	5	55.6%			
ER	Negative	2	15.4%	6	21.4%	5	55.6%	5.152	0.046	S
	Positive	11	84.6%	22	78.6%	4	44.4%			
PR	Negative	1	7.7%	3	10.7%	4	44.4%	6.668	0.036	S
	Positive	12	92.3%	25	89.3%	5	55.6%			
Her2	Negative	12	92.3%	26	92.9%	8	88.9%	0.148	0.929	NS

	Positive	1	7.7%	2	7.1%	1	11.1%			
Ki67	High	8	61.5%	14	50.0%	8	88.9%	4.309	0.116	NS
	Low	5	38.5%	14	50.0%	1	11.1%			
Molecular subtype	Her-2 enriched	0	0.0%	1	3.6%	1	11.1%	1.740	0.419	NS
	Luminal A-like	3	23.1%	5	17.9%	1	11.1%	0.517	0.772	NS
	Luminal B-like Her2 -ve	9	69.2%	19	67.9%	4	44.4%	1.829	0.401	NS
	Triple Negative	1	7.7%	3	10.7%	3	33.3%	3.475	0.176	NS
Margins	Free	9	69.2%	17	60.7%	5	55.6%	0.467	0.792	NS
	Positive	4	30.8%	11	39.3%	4	44.4%			
Muscle invasion	Absent	8	61.5%	17	60.7%	5	55.6%	0.093	0.955	NS
	Present	5	38.5%	11	39.3%	4	44.4%			
Skin invasion	Absent	7	53.8%	14	50.0%	5	55.6%	0.108	0.947	NS
	Present	6	46.2%	14	50.0%	4	44.4%			
TILs	Minimal	6	46.2%	12	42.9%	5	55.6%	0.442	0.802	NS
	Prominent	7	53.8%	16	57.1%	4	44.4%			
Necrosis	Absent	8	61.5%	17	60.7%	5	55.6%	0.093	0.955	NS
	Present	5	38.5%	11	39.3%	4	44.4%			

p-value >0.05: Non significant(NS); p-value <0.05: Significant(S); p-value< 0.01: highly significant(HS) *: Chi-square test

Discussion

The most common malignant tumor in women is breast cancer, affecting approximately 2 million of them every year worldwide (**Metovic et al., 2022**). More than 600,000 women lost their lives due to breast cancer globally in 2018 alone, accounting for 15% of all female cancer deaths **(Harbeck et al., 2019).** In the current study, the expression of FOXA1 and AR in BC were assessed. It was found that FOXA1 expression showed variation in its expression among infiltrating breast carcinoma specimens (Figure 1). FOXA1 was expressed in 46/50 (92%) of IBC specimens and showed negative or no expression in 4/50 (8%) of IBC specimens. This was consistent with the results of (**Mehta et al., 2012**) who found FOXA1 positivity in 86% of cases which was higher expression than that was recorded in previous studies ranging from 42-60% **(Albergaria et al., 2009; Guiu et al., 2018**). FOXA1 was highly expressed in 33/46 (72%) of FOXA1positive cases and low expressed in 13/45 (28%) of positive FOXA1 cases. We also found that the expression of FOXA1 and AR showed positive correlation as 75% of the cases negative for FOXA1 score were negative for AR score, 53.8% of cases with low score for FOXA1 were also low for AR score. 60.6% of cases which was high FOXA1 score were low for AR score. This was consistent with the studies of **(Sasahara, 2014; Rangel, 2018**), There were positive correlations between FOXA1 Score and ER, PR as the study of (**Metovic et al., 2022**) which documented a high level of FOXA1 expression has been associated to a better outcome in ER+BC. There were association between FOXA1 Score and Molecular Subtype (Luminal B-like Her2 –ve), **(Hisamatsu et al., 2012; Ijichi et al., 2012; Mehta et al., 2012**) and the expression of FOXA1 showed statistically significant differences between FOXA1 Score and Molecular Subtypes (Her-2 enriched and Triple Negative). There were no statistically significant differences between FOXA1

Score and Age, Laterality, Type of Operation, Size, Histological Type, P Staging, Grading, LVE, Perineural Invasion, LN Metastasis, Her2, Ki67, Margins, Muscle Invasion, Skin Invasion, TILs, and Necrosis. This was consistent with many previous studies **(Hisamatsu et al., 2012; Ijichi et al., 2012; Mehta et al., 2012).**

We also assessed the expression of AR in BC. Prior research found that the frequency of AR+ breast cancer cases ranged from 58.8 to 90.5%. In this study, AR expression was found in 82% of the studied tumors (**Niemeier et al., 2010; Hu et al., 2011; Park et al., 2011; Elebro et al., 2015**) The utilization of various antibodies, frozen or paraffin-embedded sections, and different cutoff values can all affect the various percentages of AR + tumors. We found that there were statistically significant differences between AR Score and ER, PR, and Type of Operation while there were no statistically significant differences between AR Score and Age, Laterality, Size, Histological Type, P Staging, Grading, LVE, Perineural Invasion, LN Metastasis, Her2, Ki67, Molecular Subtypes, Margins, Muscle Invasion, Skin Invasion, TILs, and Necrosis. This was consistent with the studies of (**Gonzalez-Angulo et al., 2009; Niemeier et al., 2010; Hu et al., 2011; Park et al., 2011; Elebro et al., 2015**) Research indicates that AR expression in BC is linked to the enrichment of hormone-regulated pathways, including steroid synthesis and androgen/estrogen metabolism **(Lehmann et al., 2016).** Pre-clinical studies revealed that signaling pathways involving FOXA1 and GATA3 regulate the transcriptional activity of AR **(Seachrist, Anstine and Keri, 2021)).**We have investigated the co-expression of FOXA1 and AR and found a strong association between them.

Conclusion

Our results suggest that in BC the expression of FOXA1 has a strong association with expression of AR and we think that knowledge of the IHC expression of AR and FOXA1 may play a key role in the development of targeted anticancer treatment for breast carcinoma which needs further studies and researches. These findings have important clinical implications in identifying a subpopulation of AR (+) tumors, which can be targeted for anti-AR therapies. However, this needs further investigation in larger cohort studies.

References

- **Albergaria A, Paredes J, Sousa B, Milanezi F, Carneiro V, Bastos J, et al. (2009).** Expression of FOXA1 and GATA-3 in breast cancer: the prognostic significance in hormone receptor-negative tumours. Breast cancer research, 11: 1-15.
- **Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, et al. (2017).** The eighth edition AJCC cancer staging manual: continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. CA: a cancer journal for clinicians, 67(2): 93-99.
- **BenAyed-Guerfali D, Dabbèche-Bouricha E, Ayadi W, Trifa F, Charfi S, Khabir A,et al. (2019).** Association of FOXA1 and EMT markers (Twist1 and E-cadherin) in breast cancer. Molecular biology reports, 46(3): 3247-3255.
- **Davey RA, Grossmann M. (2016).** Androgen receptor structure, function and biology: from bench to bedside. The clinical biochemist reviews, 37(1): 3.
- **Elebro K, Borgquist S, Simonsson M, Markkula A, Jirström K, Ingvar C, et al. (2015).** Combined Androgen and Estrogen Receptor Status in Breast

Cancer: Treatment Prediction and Prognosis in a Population-Based Prospective CohortAndrogen and Estrogen Receptor Status in Breast Cancer. Clinical cancer research, 21(16): 3640-3650.

- **Elston C, Ellis I. (1998).** Assessment of histological grade. The breast, 13: 356-384.
- **Godbole AM, Njar VC. (2011).** New insights into the androgen-targeted therapies and epigenetic therapies in prostate cancer. Prostate cancer, 2011.
- **Guiu S, Mollevi C, Charon-Barra C, Boissière F, Crapez E, Chartron E, et al. (2018).** Prognostic value of androgen receptor and FOXA1 co-expression in non-metastatic triple negative breast cancer and correlation with other biomarkers. British journal of cancer, 119(1): 76-79.
- **Harbeck N, Penault-Llorca F, Cortes J, Gnant M, Houssami N, Poortmans P, et al. (2019).** Breast Cancer Nat Rev Dis Primers 5: 66.
- **Hilborn E, Gacic J, Fornander T, Nordenskjöld B, Stål O, Jansson A. (2016).** Androgen receptor expression predicts beneficial tamoxifen response in oestrogen receptor-α-negative breast cancer. British journal of cancer, 114(3): 248-255.
- **Hisamatsu Y, Tokunaga E, Yamashita N, Akiyoshi S, Okada S, Nakashima Y, et al. (2012).** Impact of FOXA1 expression on the prognosis of patients with hormone receptor-positive breast cancer. Annals of surgical oncology, 19: 1145-1152.
- **Horimoto Y, Sasahara N, Sasaki R, Hlaing MT, Sakaguchi A, Saeki H, et al. (2020).** High FOXA1 protein expression might predict late recurrence in patients with estrogen-positive and HER2-negative breast cancer. Breast Cancer Research and Treatment, 183(1): 41-48.
- **Hu R, Dawood S, Holmes MD, Collins LC, Schnitt SJ, Cole K, et al. (2011).** Androgen Receptor Expression and Breast Cancer Survival in Postmenopausal WomenAndrogen Receptor Expression and Breast Cancer Survival. Clinical cancer research, 17(7): 1867-1874.
- **Ijichi N, Shigekawa T, Ikeda K, Horie-Inoue K, Shimizu C, Saji S, et al. (2012).** Association of double-positive FOXA1 and FOXP1 immunoreactivities with favorable prognosis of tamoxifen-treated breast cancer patients. Hormones and Cancer, 3: 147-159.
- **Jain S, Das A. (2020).** Virtual screening of natural compounds as combinatorial agents from indian medicinal plants against estrogen positive breast cancer. International Journal on Integrated Education, 3(10): 266-275.
- **Le Lay J, Kaestner KH. (2010).** The Fox genes in the liver: from organogenesis to functional integration. Physiological reviews, 90(1): 1-22.
- **Lehmann BD, Jovanović B, Chen X, Estrada MV, Johnson KN, Shyr Y, et al. (2016).** Refinement of triple-negative breast cancer molecular subtypes: implications for neoadjuvant chemotherapy selection. PloS one, 11(6): e0157368.
- **Mehta RJ, Jain RK, Leung S, Choo J, Nielsen T, Huntsman D, et al. (2012).** FOXA1 is an independent prognostic marker for ER-positive breast cancer. Breast Cancer Research and Treatment, 131: 881-890.
- **Metovic J, Borella F, D 'Alonzo, M, Biglia N, Mangherini L, Tampieri C, et al. (2022).** FOXA1 in breast cancer: a luminal marker with promising

prognostic and predictive impact. Cancers, 14(19): 4699.

- **Nassar A, Abouelhoda M, Mansour O, Loutfy SA, Hafez MM, Gomaa M, et al. (2020).** Targeted next generation sequencing identifies somatic mutations in a cohort of Egyptian breast cancer patients. Journal of Advanced Research, 24: 149-157.
- **Niemeier LA, Dabbs DJ, Beriwal S, Striebel JM, Bhargava R. (2010).** Androgen receptor in breast cancer: expression in estrogen receptor-positive tumors and in estrogen receptor-negative tumors with apocrine differentiation. Modern Pathology, 23(2): 205-212.
- **Park S, Koo J, Kim M, Park H, Lee J, Kim, S, et al. (2011).** Androgen receptor expression is significantly associated with better outcomes in estrogen receptor-positive breast cancers. Annals of Oncology, 22(8): 1755-1762.
- **Qaiser T, Mukherjee A, Reddy PbC, Munugoti SD, Tallam V, Pitkäaho T, et al. (2018).** Her 2 challenge contest: a detailed assessment of automated her 2 scoring algorithms in whole slide images of breast cancer tissues. Histopathology, 72(2): 227-238.
- **Rangel N, Fortunati N, Osella-Abate S, Annaratone L, Isella C, Catalano MG, et al. (2018).** FOXA1 and AR in invasive breast cancer: new findings on their co-expression and impact on prognosis in ER-positive patients. BMC cancer, 18(1): 1-9.
- **Safarpour D, Pakneshan S, Tavassoli FA. (2014).** Androgen receptor (AR) expression in 400 breast carcinomas: is routine AR assessment justified? American Journal of Cancer Research, 4(4): 353.
- **Sasahara M, Matsui A, Ichimura Y, Hirakata Y, Murata Y, Marui E. (2014).** Overexpression of androgen receptor and forkhead-box A1 protein in apocrine breast carcinoma. Anticancer Research, 34(3): 1261-1267.
- **Seachrist DD, Anstine LJ, Keri RA. (2021).** FOXA1: a pioneer of nuclear receptor action in breast cancer. Cancers, 13(20): 5205.
- **Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. (2021).** Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians.