Original Article

Expression of Androgen Receptor in Breast Carcinoma and Its Correlation with Estrogen, Progesterone, and Human Epidermal Growth Factor Receptor-2 Status

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Abstract

Background: Breast cancer is the second most common cancer overall and the most common cancer in women. Immunohistochemistry has become a prerequisite in diagnosing, deciding therapy, and predicting prognosis for carcinoma breast. Despite this, it is still difficult to find targeted therapies for triple-negative breast cancer. Many therapies based on the use of androgen receptor agonists or antagonists are emerging. This study was intended to find the prognostic and therapeutic use of androgen receptors in breast carcinoma cases.

Methods: This study was conducted in the Department of Pathology at Bhagat Phool Singh Government Medical College for Women, Khanpur Kalan, Sonepat, with 45 histologically confirmed breast cancer cases with known status of ER, PR, and HER-2/neu receptors.

Results: The age of the patients ranged from 21 to 70 years, and the mean age was 48.24 years. Overall positivity for AR was observed in 62.22% of cases. An inverse relationship was observed between AR positivity and histologic grade. AR positivity was higher in cases that were ER-positive (72.72%), PR-positive (76.92%), and HER-2/neu positive (73.33%) in contrast to cases that were ER-negative (58.82%), PR-negative (56.25%), and HER-2/neu negative (56.67%).

Conclusions: The authors concluded that AR is a more frequently expressed marker than other biomarkers in breast carcinoma cases. AR is associated with lower histologic grade and a good prognostic group of NPI. AR is expressed in high percentages in ER, PR, and HER-2/neu positive cases.

Keywords:

Androgen Receptor, Estrogen Receptor, Human Epidermal Growth Factor Receptor-2, Progesterone Receptor.

Introduction

Breast cancer is the most common cancer in women worldwide. As per Globocan, the incidence of breast cancer in the year 2020 was 2,261,419. The number of deaths due to breast cancer worldwide was 684,996 [1].

The traditional prognostic and predictive markers of breast carcinoma include the histological subtype, grade of the tumor, and clinical stage of the disease, which is based on tumor size, lymph node status, and the presence or absence of distant metastasis.

In the past two decades, biomarkers such as hormone receptors [estrogen/progesterone receptor (ER/PR)] and HER-2/neu growth factor receptor have gained importance due to implications in prognosis and clinical management. Breast cancers that express estrogen and progesterone receptors can be treated by hormonal manipulation. Targeted therapy towards HER-2/neu has shown great success, and Trastuzumab has been introduced as an adjuvant drug in those showing overexpression of HER-2/neu.

Although the therapeutic options for specific subtypes of breast cancer seem to be efficient, there is a need to counter drug resistance and improve clinical benefit by exploring alternative therapeutic targets for this disease. Additionally, predicting the outcome is difficult in a subgroup of cancers that are ER-negative or triple-negative, and thus, the search for new markers continues [2].

Androgen receptor (AR) is one such emerging biomarker, belonging to the steroid hormone nuclear receptor family, similar to ER and PR. The role of androgens in breast cancer can differ depending on their estrogen and progesterone receptor status. Testosterone induces cell proliferation in ER-positive, but not in ER-negative tumor cells. This has been explained by the high expression of aromatase, which converts androgens to estrogens. In contrast, dihydrotestosterone causes a suppression of cell proliferation in both ER-positive and ER-negative cell lines since dihydrotestosterone is not a substrate for aromatase. Both the stimulatory and inhibitory effects of dihydrotestosterone on AR-positive cell lines disappeared after adding the androgen receptor antagonist, hydroxyflutamide.

Selective AR modulators (SARMs), such as enobosarm, have been preclinically tested, giving favorable results concerning migration and invasion. In vivo studies revealed that SARMs were able to reduce tumor weight by 90%, as well as tumor-induced cachexia, in 5 weeks [3].

Many studies advocate the efficacy of androgen agonists in the treatment of ER-negative, PR-negative, AR-positive, and TNBC, while other studies have shown the efficacy of anti-androgens in the growth restriction of triple-negative breast cancers (TNBC). The efficacy and safety of bicalutamide in ER-negative, PR-negative, AR-positive, and metastatic breast cancer are being investigated in a phase II trial [3].

Materials and Methods

This cross-sectional study was conducted in the Department of Pathology at Bhagat Phool Singh Government Medical College for Women, Khanpur Kalan, Sonepat, after obtaining approval from the Institutional Ethical Committee [reference: BPSGMCW/RC/642/IEC/2021, dated 26/02/2021]. It was a retrospective study that included 45 cases of breast cancer in which ER, PR, and HER-2/neu status had already been recorded. Cases of breast carcinoma for which archival blocks and slides were unavailable were excluded.

Since blocks of cases from previous years were retrieved, there was no interaction with the patients. This research posed no risk to the cases; thus, formal written informed consent was not required, with a waiver granted by the Institutional Ethical Committee. Blocks of known cases of breast carcinoma, along with requisition forms, were retrieved. Patient confidentiality was ensured by de-identifying the data, and patients were assigned a unique numerical code. Additional clinical information and relevant investigation findings were collected from case sheets and requisition forms.

Hematoxylin and eosin-stained sections were studied to determine the histological type and grade of the tumor. Nottingham's modification of the Bloom-Richardson grading system was used to classify the cases into histological grades [4]. Immunohistochemistry slides were examined to determine ER, PR, HER-2/neu, and AR status. The Quick scoring system was

used for ER and PR quantification [5]. The ASCO/CAP (American Society of Clinical Oncology and the College of American Pathologists) guidelines (2018) were used for the interpretation of HER-2/neu, and cases were classified into scores of 0, 1+, 2+, and 3+ [6]. Nottingham's prognostic index (NPI) was used to define the prognosis as good, moderate, or poor [7]. Androgen receptor expression was semi-quantitatively analyzed according to the percentage of cells showing positive staining in the nucleus [Figure 3, Figure 4]. Samples were scored as positive for AR when at least 10% of the nuclei of tumor cells were immunoreactive [8, 9].

Statistical Methodology: Mean ± SD and percentage were used for statistical analysis of quantitative data. Correlation was determined using the relevant coefficients. The Chi-square test was employed, and a P value of < 0.05 was considered statistically significant.

Results

This study was conducted on 45 cases of breast carcinoma in which ER, PR, and Her-2/neu status had already been reported. Out of the total 45 cases, 42 were female and 3 were male. The age of the patients ranged from 21 to 70 years, with a mean age of 48.2 years. The most frequent histological type was infiltrating duct carcinoma – not otherwise specified (IDC-NOS) (88.9%). The majority of cases belonged to histologic grade II (44.44%), followed by grade III (40%), with the least number of patients in grade I (15.56%). According to the Nottingham prognostic index classification, half of the cases belonged to the moderate prognostic group. Axillary lymph nodes were detected in 32 cases, of which 18 cases revealed metastatic deposits [Table 1].

Overall positivity for AR was observed in 28 out of 45 cases (62.22%). The maximum percentage of breast cancer cases positive for AR was in the age group 31-40 years (87.50%), whereas the least was in the age group 61-70 years (50%). The overall positivity for ER, PR, HER-2/neu, and AR was 24.44%, 28.89%, 33.33%, and 62.22%, respectively. AR showed much higher positivity than the other three markers. A total of 67.50% of IDC-NOS cases showed AR positivity. Lymph node-positive cases showed slightly higher AR positivity (61.11%) than lymph node-negative cases (50%). All 7 cases of histologic grade I were positive for AR, followed by 12 cases (60%) of grade II and 9 cases (50%) of grade III. An inverse relationship was observed between AR positivity and histologic grade ($P = 0.06$). According to the Nottingham prognostic index, both cases in the good prognostic group were positive for AR. In the moderate prognosis category, 10 out of 16 cases (62.50%) were positive, whereas in the poor prognostic category, 6 out of 14 cases (42.86%) were positive for AR. A decrease in AR positivity with worsening prognosis was observed, but this correlation was not statistically significant ($P = 0.507$) [Table 2].

AR positivity was higher in cases that were ER positive (72.72%, $P = 0.408$), PR positive (76.92%, $P = 0.277$), and HER-2/neu positive (73.33%, $P = 0.279$), compared to cases that were ER negative (58.82%), PR negative (56.25%), and HER-2/neu negative (56.67%) [Table 2]. The correlation between AR expression and other IHC markers was not statistically significant. All four IHC markers showed the highest positivity in grade I, with decreasing positivity in grade II and grade III cases [Figure 1]. Maximum AR positivity was observed in the luminal B category (75%), followed by luminal A (71.43%). In the HER-2/neu enriched category, AR positivity was 70%. The lowest AR positivity was seen in triple-negative breast cancer cases (47.62%) [Figure 2].

Discussion

Breast cancer is the most common cancer among women, affecting 2.1 million women every year, and also causes the highest number of cancer-related deaths among women. Although ER, PR, and HER-2/neu promote the genesis and development of breast cancer and are associated with its prognosis, there is a need to identify and validate new biomarkers for better prediction and

prognostication. TNBC is a heterogeneous group that is associated with accelerated growth, high recurrence rates, and frequent metastasis, constituting 15-20% of all breast carcinomas. At present, the standard treatment for triple-negative breast carcinoma is nonspecific cytotoxic chemotherapy. New classifications have isolated a subset of TNBC that expresses AR positivity. Similar to estrogen-targeted therapy for hormone-positive breast cancers, antiandrogen therapies can show promising results in such patients.

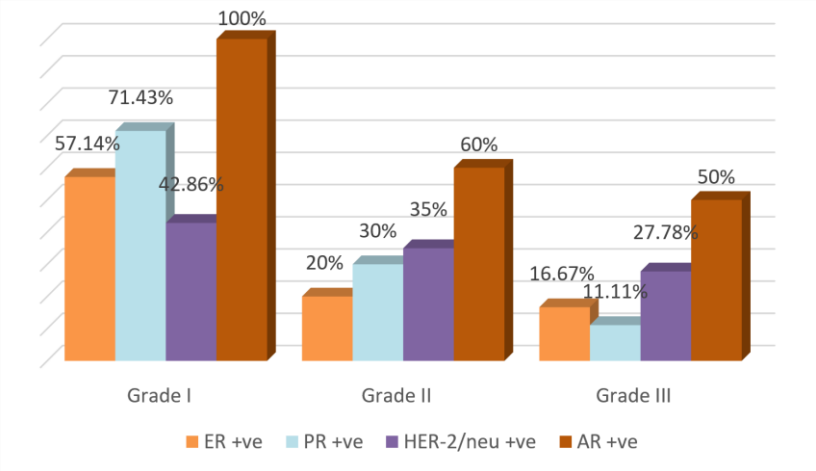

Figure 1: Histologic grade wise distribution of IHC marker positive cases (n=45)

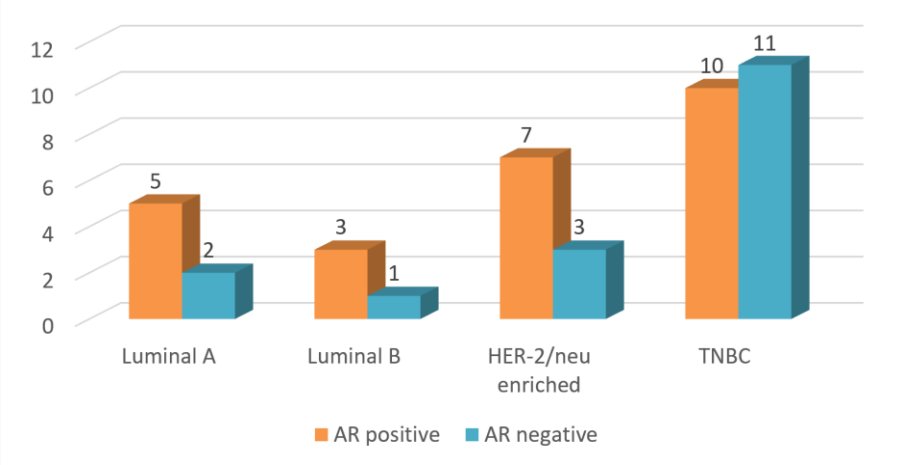

Figure 2: Distribution of AR status with molecular subtypes (n=45)

In this study, AR expression was studied with respect to demographic parameters, histologic grade, prognostic markers, and other IHC markers. AR positivity in patients aged <50 years was 66.67%, while in those aged ≥50 years, it was 55.56%. Similar to our study, higher AR positivity was seen in the age group <50 years in studies by Yu et al. [10] (74.71%) and H.A. Alshenawy [11] (84.62%). Studies by Agoff et al. [12], Astvatsaturyan et al. [13], and Vellaisamy et al. [14] had lower AR positivity in the age group <50 years.

In the present study, the comparison of receptor positivity with histological grade showed that the positivity for androgen receptors decreased as the grades increased. In terms of grade-wise distribution of AR positivity, our findings were most comparable to the study by H.A. Alshenawy [11], which had 90.91% AR positivity in grade I, 72.41% in grade II, and 46.67% in grade III. A similar

trend of decreasing AR positivity with increasing grade is seen in studies by Agoff et al. [12], Park et al. [8], and Vellaisamy et al. [14].

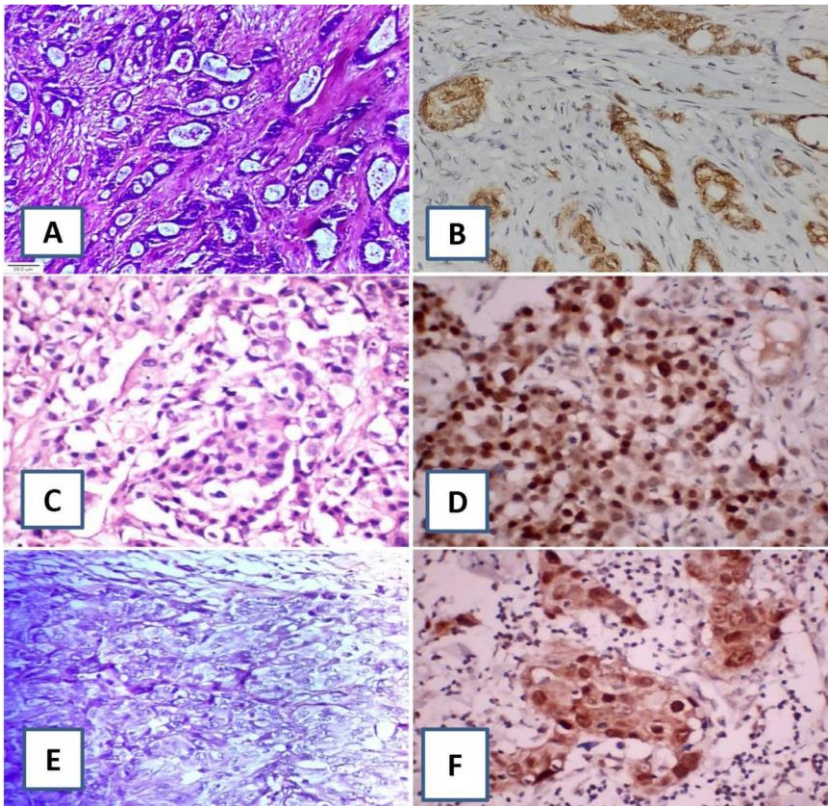

Figure 3: Photomicrograph showing infiltrating ductal carcinoma-not otherwise specified, A) Grade I (H&E X400), B) Grade I (AR X 200), C) Grade II (H&E X400), D) Grade II (AR X 400), , E) Grade III (H&E X400), F) Grade III (AR X 400).

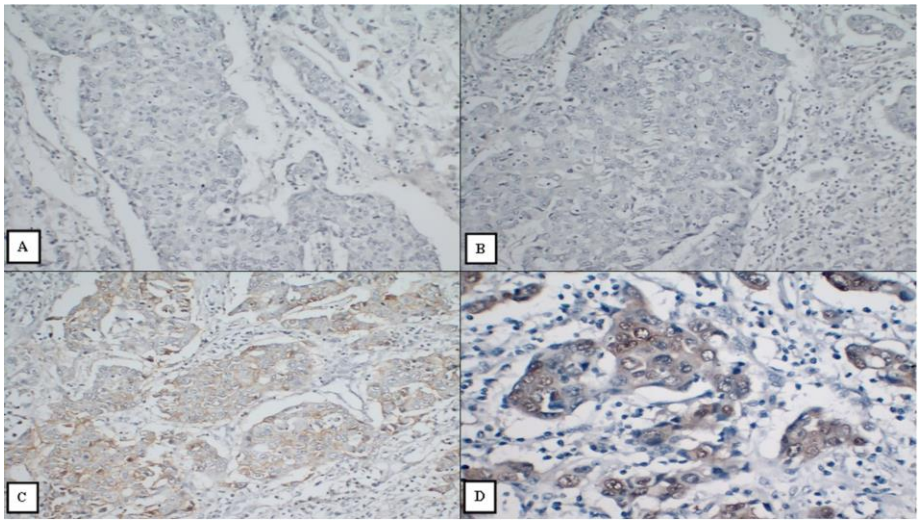

Figure 4: Photomicrographs showing infiltrating ductal carcinoma, grade III showing immunostaining A- ER negative [X400], B- PR negative [X400], C- HER-2/neu negative [X400] and D- AR positive

Table 1: Demographic and histological findings of carcinoma breast cases

Demographic and Histological findings	**No. of cases(n)**			**Percentage (%)**		
Total cases	45			100		
Age						
<50 Years	27			60		
>50 Years	18			40		
Mean age:	48.2			-		
Median age:	50			-		
Range:	21-70			-		
Gender						
Male	03			6.67		
Female	42			93.33		
Grade-wise distribution according to histological type (n)						
	GI	GII	GIII	GI	GII	GIII
Infiltrating ductal carcinoma-not otherwise specified (40)	6	19	15	13	42.22	33.3
Metaplastic carcinoma (3)	0	0	3	0	0	6.66
Papillary carcinoma (1)	1	0	0	2.2	0	0
Invasive lobular carcinoma (1)	0	1	0	2	2.22	0
Axillary Lymph node involvement in the study group (n=32)						
Lymph node positive	18			56.25		
Lymph node negative	14			43.75		
Distribution of Breast carcinoma cases according to Nottingham's Prognostic Index(n=32)						
Good (NP1<3.4)	2			6.25		
Moderate (NPI>3.4 to <5.4)	16			50		
Poor (NPI >5.4)	14			43.75		
Distribution of cases of breast carcinoma with respect to IHC (n=45)						
	Overall positivity					
	n			%		
ER	11			24.44		
PR	13			28.89		
Her-2/neu	15			33.33		
AR	28			62.22		

With respect to NPI, 100% positivity was seen in cases with the good prognostic group, followed by 62.50% positivity in cases with moderate prognosis, and 42.86% positivity in cases with poor prognosis in our study, implying that AR was more associated with good prognosis. Although no study is available that compares AR status with NPI, certain studies have compared AR status with other prognostic factors like staging and tumor proliferation rate. Agrawal et al. [15] studied the relation of AR with various prognostic and predictive factors and concluded that the therapeutic efficacy of adjuvant hormone therapy was higher in the group of AR-positive patients than in AR-negative ones. Studies conducted by Isola et al. [16] compared AR status with other prognostic factors like hormone receptors and tumor proliferation rate. They concluded that AR receptor positivity may have value as a prognostic factor and predictor of response to endocrine therapy.

Overall positivity for ER, PR, HER-2/neu, and AR was 24.44%, 28.89%, 33.33%, and 62.22%, respectively, with AR showing the highest positivity among all four markers. A similar trend was seen in studies by Park et al. [8], Yu et al. [10], H.A. Alshenawy [11], and Anand et al. [17] with AR expression in 72.9%, 72.5%, 71.11%, and 56% of breast carcinoma cases, respectively.

Table 2: Distribution of AR status with various histological parameters

Category	Subcategory	AR Positive		AR Negative	
		n	**%**	**n**	**%**
Histological Type (n)	Infiltrating duct carcinoma - not otherwise specified (40)	27	67.50	13	32.50
	Metaplastic carcinoma (3)	0	0	3	100
	Papillary carcinoma (1)	0	0	1	100
	Invasive lobular carcinoma (1)	1	100	0	0
Histological Grade (n)	Grade I (7)	7	100	0	0
	Grade II (20)	12	60	8	40
	Grade III (18)	9	50	9	50
Axillary Lymph Node Status	Lymph node positive	11	61.11	7	38.89
	Lymph node negative	7	50	7	50
AR Status with NPI	Good (NPI < 3.4)	2	100	0	0
	Moderate (NPI > 3.4 to < 5.4)	10	62.50	6	37.50
	Poor (NPI > 5.4)	6	42.86	8	57.14
ER Status (n)	ER positive (11)	8	72.72	3	27.28
	ER negative (34)	20	58.82	14	41.18
PR Status (n)	PR positive (13)	10	76.92	3	23.08
	PR negative (32)	18	56.25	14	43.75
Her-2/neu Status (n)	Her-2/neu positive (15)	11	73.33	4	26.67
	Her-2/neu negative (30)	17	56.67	13	43.33

In our study, 72.72% of ER-positive and 76.92% of PR-positive breast cancer cases were positive for AR. Similar to our study, high AR positivity was reported in ER-positive cases in studies conducted by Agoff et al. [12] (89.47%), Park et al. [8] (83.39%), Qi et al. [9] (84.25%), Yu et al. [10] (88.82%), H.A. Alshenawy [11] (64.29%), and Vellaisamy et al. [14] (72.97%). Likewise, high AR positivity was seen in PR-positive cases in studies by Agoff et al. [12] (92.86%), Park et al. [8] (82.03%), Yu et al. [10] (78.23%), H.A. Alshenawy [11] (59.65%), Agrawal et al. [15] (57.89%), Anand et al. [17] (73.91%), and Vellaisamy et al. [14] (69.70%). In our study, 73.33% of HER-2/neu-positive breast cancer cases were positive for AR. Comparable findings were reported by Agoff et al. [12] (77.78%), Park et al. [8] (81.48%), Yu et al. [10] (65.91%), H.A. Alshenawy [11] (54.84%), Agrawal et al. [15] (52.63%), Anand et al. [17] (60.3%), and Vellaisamy et al. [14] (70%). The relationship between AR and HER-2/neu was not statistically significant in any of these studies.

Maximum AR positivity was seen in luminal B (75%), followed by luminal A (71.43%). In the HER-2/neu-enriched category, 70% AR positivity was seen. The least AR positivity was seen in triple-negative breast cancer cases (47.62%). In relation to various molecular subtypes, the findings of our study were similar to those of Yu et al. [10], Qi et al. [9], and Anand et al. [17]. In the study by Yu et al. [10], AR positivity in luminal A, luminal B, HER-2/neu-enriched, and TNBC categories were 83.8%, 75.6%, 55.8%, and 39%. In the study by Qi et al. [9], AR positivity in luminal A, luminal B, HER-2/neu-enriched, and TNBC categories were 89.69%, 79.83%, 65.55%, and 53.17%. In the study by Anand et al. [17], AR positivity in luminal A, luminal B, HER-2/neu-enriched, and TNBC categories were 77.8%, 63.3%, 55.1%, and 30%. These studies depict higher AR positivity in TNBC compared to TNBC.

Conclusion

Breast carcinoma accounts for the most common cancer among females in India. Being diverse in behavior, response to therapy, and outcome, there is a need for new biomarkers for breast carcinoma, especially for TNBC cases that can aid in the development of newer targeted therapies to enhance response and survival in breast cancer patients. AR is one such emerging biomarker that belongs to the steroid hormone receptor family. In our study, AR is a more frequently expressed marker than the other three biomarkers, associated with lower histologic grade and a good prognostic group of NPI. AR is expressed in high percentages in ER, PR, and HER-2/neu-positive cases and in a significant number of triple-negative breast cancer cases. These observations suggest that AR can serve as a potential target for treatment in breast carcinoma patients, including TNBC and ER-negative/PR-negative cases. AR assessment should be incorporated routinely along with ER, PR, and HER-2/neu in all cases of breast carcinoma.

Author Contributions:

Conceptualization: AR, SK, HS, KS

Data Curation: NM, AR

Formal Analysis: AR, NM, SK, KS, GMK

Investigation: NM, AR, SK

Methodology: AR, SK, KS, HS

Project Administration: AR

Resources: AR, NM, SK, HS, GMK

Software: NM, AR

Supervision: AR, HS

Validation: AR, KS

Visualization: NM, AR, HS

Writing - Original Draft Preparation: NM, AR, HS

Writing - Review and Editing: NM, AR, HS

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