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ANDROGEN RECEPTOR EXPRESSION IN ESTROGEN RECEPTOR- AND PROGESTERONE RECEPTOR-NEGATIVE BREAST CARCINOMA: ASSOCIATION WITH HER2 STATUS AND CLINICOPATHOLOGICAL FEATURES

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Abstract

Background: Breast carcinoma is the most frequently diagnosed malignancy among women worldwide. Tumours lacking estrogen receptor (ER) and progesterone receptor (PR) expression are associated with aggressive biological behavior and limited therapeutic options. The androgen receptor (AR) has attracted attention as a potential biomarker that may provide prognostic information and identify additional therapeutic targets in these tumours.

Objective: To determine the immunohistochemical expression of AR in ER- and PR-negative breast carcinoma and evaluate its association with HER2 status and clinicopathological characteristics.

Materials and Methods: This hospital-based observational cross-sectional immunohistochemical study included 50 consecutive cases of ER- and PR-negative breast carcinoma. Clinicopathological data were recorded, and AR and HER2 expression were assessed by immunohistochemistry. Associations between AR expression and clinicopathological variables were analyzed using IBM SPSS Statistics Version 26.0 (IBM Corp., Armonk, NY, USA), with $p<0.05$ considered statistically significant.

Results: All cases were invasive ductal carcinoma. The highest proportion of patients belonged to the 61–70-year age group (32%). Grade III tumours accounted for 68% of cases, while 66% measured 2–5 cm in greatest dimension. AR expression was detected in 56% of tumours, whereas HER2 positivity was observed in 44%. A significant association was identified between AR expression and HER2 status ($p=0.007$), tumour laterality ($p=0.013$), and lymphovascular invasion ($p=0.019$). No significant relationship was observed between AR expression and patient age, tumour size, histological grade, or lymph node involvement.

Conclusion: AR was expressed in more than half of ER- and PR-negative breast carcinomas and showed a significant association with HER2 expression and lymphovascular invasion. These

findings suggest that AR may serve as a useful prognostic biomarker and a potential therapeutic target in hormone receptor-negative breast carcinoma. Larger multicenter studies with long-term follow-up are required to validate its clinical significance.

Keywords: Breast Neoplasms, Androgen Receptors, Immunohistochemistry, Receptor, ErbB-2 (HER2), Carcinoma, Ductal, Breast

INTRODUCTION

Breast carcinoma is the most frequently diagnosed malignancy among women worldwide and continues to represent a major public health challenge. According to GLOBOCAN 2020 estimates, approximately 2.3 million new cases are diagnosed annually, making it the leading cancer among women across many countries. Although advances in early detection, surgical techniques, systemic therapies, and precision medicine have improved survival, the overall burden of breast cancer continues to increase. Factors such as population ageing, obesity, sedentary lifestyles, and changing reproductive patterns have contributed to this rising incidence, underscoring the need for improved molecular characterization and individualized therapeutic strategies.[1] In India, breast carcinoma has overtaken cervical carcinoma as the most common cancer among women, particularly in urban populations, as reported by the National Cancer Registry Programme (NCRP).[2]

The development and progression of breast carcinoma are influenced by complex interactions among steroid hormone receptors and multiple intracellular signaling pathways. Estrogen and progesterone play well-established roles in normal mammary gland development and are important determinants of tumour biology and response to endocrine therapy. However, increasing evidence indicates that androgen signaling also contributes to mammary epithelial differentiation, cellular proliferation, and breast carcinogenesis, suggesting a broader role for steroid hormone receptors in breast cancer than previously recognized.[3]

Assessment of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2) status forms the cornerstone of breast cancer classification and therapeutic decision-making. Tumours lacking ER and PR expression generally exhibit a more aggressive clinical course, higher histological grade, and fewer effective treatment options because they are unlikely to benefit from conventional endocrine therapy. Consequently, the identification of additional biomarkers with prognostic or therapeutic relevance has become an important area of research. Among these, the androgen receptor (AR) has emerged as a promising candidate, particularly in hormone receptor-negative breast carcinomas.[4]

The androgen receptor is a ligand-dependent nuclear transcription factor belonging to the steroid hormone receptor superfamily. Following activation by testosterone or dihydrotestosterone, AR regulates the expression of genes involved in cell growth, differentiation, apoptosis, and survival.[5] Although traditionally associated with prostate cancer, AR expression has also been demonstrated in several molecular subtypes of breast carcinoma, including luminal, HER2-enriched, and triple-negative tumours.[6] In ER- and PR-negative breast carcinomas, AR may identify a distinct molecular subgroup with biological characteristics resembling molecular apocrine tumours,

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raising the possibility of targeted treatment using anti-androgen agents such as bicalutamide and enzalutamide.[6]

Experimental and clinical studies have further suggested functional interactions between AR and HER2 signaling pathways. HER2-mediated activation of the PI3K/AKT and MAPK pathways may influence AR signaling, while AR activation may, in turn, modulate HER2 expression and downstream cellular responses. This reciprocal relationship has generated interest in combined therapeutic approaches targeting both pathways in selected patients.[7]

In view of these observations, the present study was undertaken to evaluate the immunohistochemical expression of androgen receptor in ER- and PR-negative breast carcinoma and to determine its association with HER2 status and clinicopathological characteristics. A better understanding of AR expression in this subset of breast carcinomas may help refine prognostic assessment and identify patients who could benefit from emerging targeted therapeutic strategies.

MATERIALS AND METHODS

Study Design and Setting

This hospital-based observational immunohistochemical study was conducted in the Department of Pathology at a tertiary care teaching hospital in North India after obtaining approval from the Institutional Ethics Committee. Patient confidentiality was maintained throughout the study, and all procedures conformed to the ethical principles of the Declaration of Helsinki.

Study Population

The study included 50 consecutive histopathologically confirmed cases of estrogen receptor (ER)-negative and progesterone receptor (PR)-negative breast carcinoma received in the Department of Pathology during the study period. Clinicopathological information, including patient age, tumour size, tumour laterality, anatomical location, histological grade, lymphovascular invasion (LVI), and lymph node status, was obtained from pathology records and histopathology reports. Sample size was based on all eligible cases received during the study period because of the relatively low frequency of ER-/PR-negative breast carcinoma.

Eligibility Criteria

Inclusion Criteria

Histopathologically confirmed cases of breast carcinoma. Tumours negative for both estrogen receptor (ER) and progesterone receptor (PR) on immunohistochemistry. Mastectomy specimens with adequate tissue available for histopathological and immunohistochemical evaluation.

Exclusion Criteria

ER-positive and/or PR-positive breast carcinomas. Tru-cut biopsy specimens. Mastectomy specimens showing marked autolytic changes. Tissue blocks with insufficient material for immunohistochemical analysis.

Histopathological Evaluation

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All surgical specimens were fixed in 10% neutral buffered formalin for 12–24 hours and processed routinely using standard paraffin-embedding techniques. Sections measuring 4 μm in thickness were prepared and stained with hematoxylin and eosin (H&E) for histopathological examination.

Tumours were classified according to the World Health Organization (WHO) Classification of Breast Tumours. Histological grading was performed using the Nottingham modification of the Scarff-Bloom-Richardson grading system, based on tubule formation, nuclear pleomorphism, and mitotic activity, categorizing tumours into Grade I, Grade II, or Grade III. Additional pathological variables, including tumour size, laterality, anatomical quadrant, lymphovascular invasion, and axillary lymph node involvement, were assessed on routine H&E-stained sections.

Immunohistochemical Analysis

Immunohistochemistry (IHC) was performed using commercially available antibodies (Biocare Medical, USA) to determine the expression of androgen receptor (AR), estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2).

Paraffin-embedded tissue sections (3–5 μm thick) were mounted on poly-L-lysine-coated slides, deparaffinized, and rehydrated through graded alcohols. Heat-induced antigen retrieval was performed using a Decloaker at 95°C for 60 minutes. Endogenous peroxidase activity was blocked, followed by incubation with a protein blocking solution to minimize nonspecific staining. Sections were subsequently incubated with the primary monoclonal antibody, followed by a polymer-based detection system. Immunoreactivity was visualized using 3,3′-diaminobenzidine (DAB) as the chromogen, and the slides were counterstained with hematoxylin, dehydrated, cleared, and mounted. Appropriate positive and negative controls were included with each staining run. Normal prostate tissue served as the positive control for AR immunostaining.

Interpretation of Immunohistochemistry

Androgen Receptor

AR expression was evaluated by the presence of brown nuclear staining in invasive tumour cells. An immunoreactive score was obtained by multiplying the staining intensity (graded 1–3) by the proportion of positively stained tumour cells (graded 0–4). A final score greater than 2 was considered positive for AR expression, whereas a score less than 1 was considered negative.

HER2 Evaluation

HER2 expression was interpreted according to the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) recommendations.

Score 0: No staining or incomplete, faint membrane staining in ≤10% of tumour cells.

Score 1+: Incomplete faint membrane staining in >10% of tumour cells.

Score 2+: Weak to moderate complete membrane staining in >10% of tumour cells or intense complete membrane staining in ≤10% of tumour cells (equivocal).

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Score 3+: Complete, intense circumferential membrane staining in >10% of invasive tumour cells (positive).

Outcome Measures

The primary outcome was the immunohistochemical expression of androgen receptor in ER- and PR-negative breast carcinoma.

Secondary outcomes included the association of AR expression with HER2 status, patient age, tumour size, histological grade, tumour laterality, anatomical site, lymphovascular invasion, and axillary lymph node status.

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using IBM SPSS Statistics version XX (IBM Corp., Armonk, NY, USA). Categorical variables are presented as frequencies and percentages. Associations between AR expression and clinicopathological variables were evaluated using the Chi-square test or Fisher's exact test, as appropriate. All statistical tests were two-tailed, and a p-value <0.05 was considered statistically significant.

RESULTS

Clinicopathological Characteristics

A total of 50 female patients with histopathologically confirmed estrogen receptor (ER)-negative and progesterone receptor (PR)-negative invasive breast carcinoma were included in the study. The patients ranged in age from 29 to 77 years, with a mean age of 53 years. The highest proportion of cases was observed in the 61–70 years age group (32.0%, n=16). Tumours were slightly more common in the left breast (52.0%, n=26) than in the right breast (48.0%, n=24). The upper outer quadrant was the most frequent tumour location, accounting for 40.0% (n=20) of cases (Table 1).

Tumour size ranged from less than 2 cm to more than 5 cm. Most tumours (66.0%, n=33) measured 2–5 cm, while 10.0% (n=5) were larger than 5 cm and 24.0% (n=12) measured less than 2 cm (Table 1).

Histopathological grading demonstrated a predominance of high-grade tumours. Grade III carcinoma constituted 68.0% (n=34) of cases, whereas 32.0% (n=16) were classified as Grade II. No Grade I tumours were identified. Lymphovascular invasion was present in 54.0% (n=27) of cases, and axillary lymph node metastasis was also observed in 54.0% (n=27) of patients (Table 1).

Immunohistochemical Findings

Immunohistochemical analysis revealed androgen receptor (AR) positivity in 56.0% (n=28) of tumours, while 44.0% (n=22) were AR-negative. Among the AR-positive cases, variable staining intensities were observed, ranging from mild to strong nuclear immunoreactivity in the tumour cells. Representative photomicrographs demonstrating mild AR expression (Figure 1), moderate

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AR expression (Figure 2), and strong AR expression (Figure 3) are shown. HER2 overexpression (3+) was identified in 44.0% (n=22) of cases, whereas 56.0% (n=28) were HER2-negative (Table 2).

Association *of* AR Expression with Clinicopathological Parameters

AR expression showed a statistically significant association with HER2 status (p=0.007), indicating that AR positivity was more frequently observed in HER2-positive tumours (Table 3).

A significant relationship was also found between AR expression and tumour laterality (p=0.013) as well as lymphovascular invasion (p=0.019). In contrast, AR expression was not significantly associated with patient age, tumour size, histological grade, axillary lymph node status, or tumour location within the breast (p>0.05 for all comparisons).

Table 1: Distribution of Clinical and Histopathological Parameters

Parameter	Category	Frequency (n=50)	Percentage (%)
Age Group	29–40 Years	13	26%
	41–50 Years	13	26%
	51–60 Years	8	16%
	61–70 Years	16	32%
Tumour Size	< 2 cm	12	24%
	2–5 cm	33	66%
	> 5 cm	5	10%
Histological Grade	Grade II	16	32%
	Grade III	34	68%
Lymph Node Status	Negative (N0)	23	46.0%
	Positive (N1)	13	26.0%
	Positive (N2)	11	22.0%
	Positive (N3)	3	6.0%

Table 2: Expression Profile of AR and HER2/NEU

Biomarker	Status	Frequency (n=50)	Percentage (%)
Androgen Receptor (AR)	Positive	28	56%
	Negative	22	44%

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HER2/NEU Status	Positive	22	44%
	Negative	28	56%

Table 3: Correlation Between AR Expression and HER2/NEU

	HER2/NEU Positive	**HER2/NEU Negative**	**Total**
AR Positive	17 (77.3%)	11 (39.3%)	28
AR Negative	5 (22.7%)	17 (60.7%)	22
Total	22	28	50

A statistically significant association was observed between these two markers ($p = 0.007$).

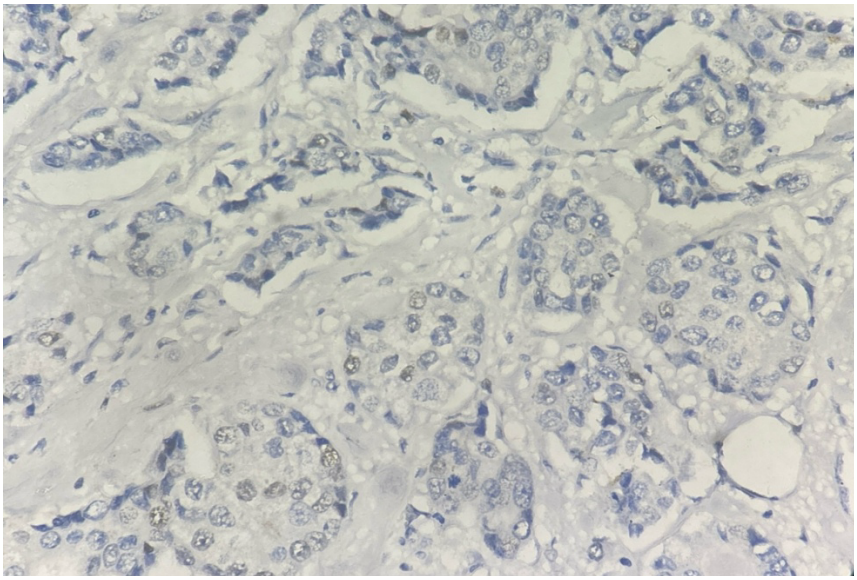

Figure 1: Representative photomicrograph showing mild nuclear androgen receptor immunoreactivity in invasive breast carcinoma cells (IHC, DAB, ×400).

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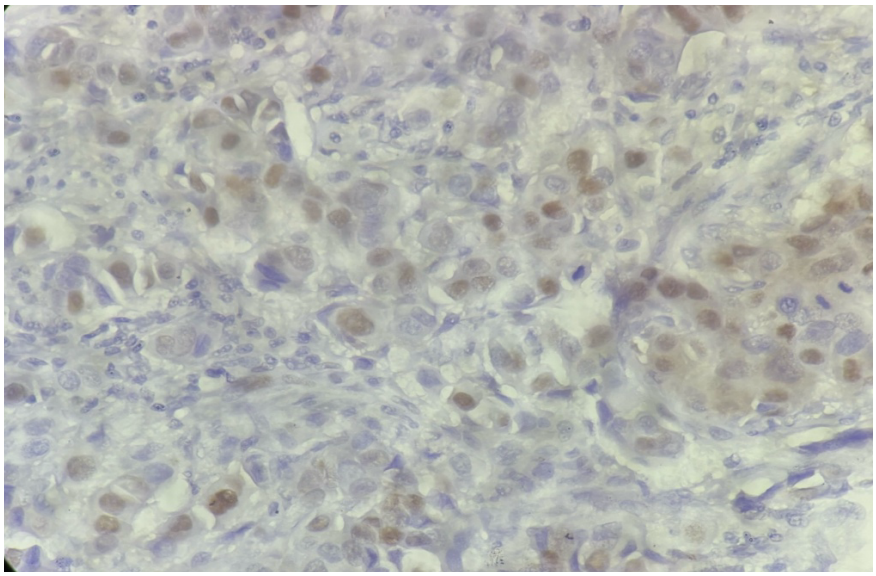

Figure 2: Representative photomicrograph showing moderate nuclear androgen receptor immunoreactivity in invasive breast carcinoma cells (IHC, DAB, ×400).

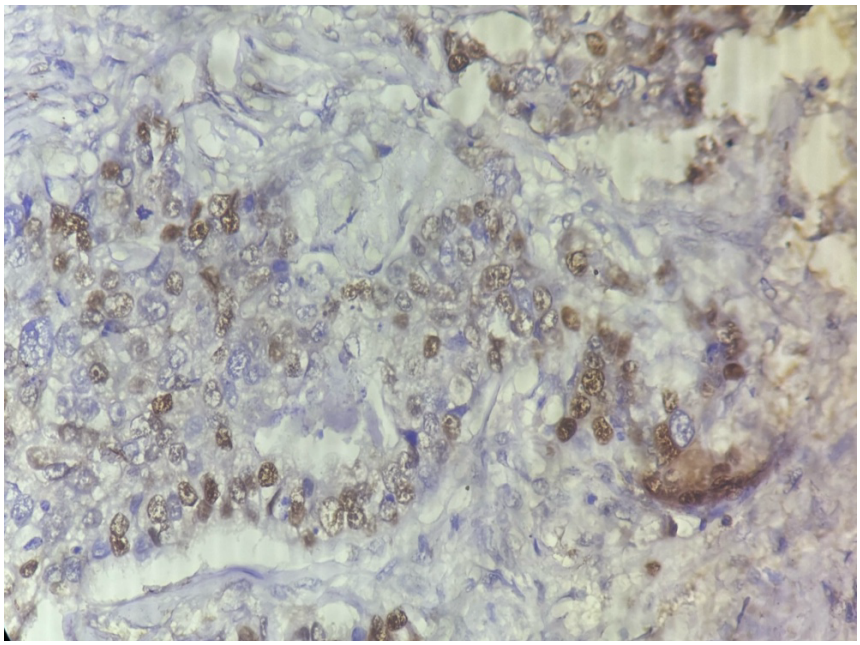

Figure 3: Representative photomicrograph showing strong diffuse nuclear androgen receptor immunoreactivity in invasive breast carcinoma cells (IHC, DAB, ×400).

DISCUSSION

In the present study, 56% of ER- and PR-negative breast carcinomas demonstrated androgen receptor (AR) expression, indicating that AR is retained in a substantial proportion of hormone receptor-negative tumours. This finding is consistent with previous reports from both Indian and international studies. Sidhu et al.[4] observed AR positivity in 63.8% of hormone receptor-negative breast carcinomas in a North Indian cohort, while Micello et al.[8] reported a comparable prevalence

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of 56.6%. The similarity across these studies suggests that loss of ER and PR expression does not necessarily indicate the absence of steroid hormone receptor activity. Instead, AR expression may define a biologically distinct subgroup of breast carcinomas with potential prognostic significance and emerging therapeutic relevance.

The demographic profile of the present study showed a mean patient age of 53 years, with the highest proportion of cases occurring in the 61–70-year age group. These findings are in agreement with those reported by Pollán et al.[9], who also observed that breast carcinoma occurs predominantly in postmenopausal women. The increased frequency of disease in this age group is likely related to the cumulative effect of age-associated genetic alterations, prolonged exposure to hormonal and environmental risk factors, and changes in breast tissue biology, all of which contribute to breast carcinogenesis but contrasting with the shift toward younger demographics noted by Brinton et al. [10] Anatomically, the upper outer quadrant was the most frequent site (40%), consistent with Shah et al.[11] while a slight left-sided predominance (52%) was observed, supporting the data from Al Saad et al.[12] and Pistelli et al.[13]Aggressive tumour biology was evident, as 66% of cases measured 2–5 cm and 68% were classified as Grade III , with a high prevalence of lymphovascular invasion (LVI) at 54% .

Most notably, a strong correlation was found between AR expression and HER2/neu status ($p = 0.007$), with 77.3% of HER2-positive cases also expressing AR. This significant biological link, also documented by Sidhu et al.[4] ($p < 0.00002$), Micello et al. [8]($p < 0.005$), and Agoff et al.,[14] ($p = 0.003$), suggests that the androgen receptor is not only a viable prognostic marker but also a potential therapeutic target for patients with HER2-positive or triple-negative breast cancers who currently face limited treatment options. These findings suggest that AR and HER2 may represent complementary therapeutic targets in selected patients (Table 4).

Table 4: Comparison of Androgen Receptor Positivity Rates Reported in Hormone Receptor-Negative Breast Carcinoma

Study	AR+ in Her2+ (%)	AR- in Her2+ (%)	AR+ in Her2- (%)	AR- in Her2- (%)	p-Value
Present Study	77.3%	22.7%	39.3%	60.7%	0.007
Sidhu et al.[4]	89.2	10.8	45.8	54.2	0.00002
Micello et al.[8]	76.7	23.3	30.4	69.6	< 0.005
Agoff et al.[14]	81.2	18.8	38.5	61.5	0.003

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Park et al.[7]	74.4	25.6	37.9	62.1	< 0.001

The present study demonstrated a significant association between AR expression and lymphovascular invasion (LVI) (p=0.019), suggesting that AR may be involved in the biological processes underlying tumour invasion. Experimental evidence indicates that AR signaling can influence pathways associated with epithelial-mesenchymal transition (EMT), extracellular matrix remodeling, and tumour cell migration, which may facilitate vascular invasion and metastatic dissemination. In contrast to ER-positive breast carcinomas, where AR expression has often been linked to more favorable pathological features, our findings suggest that in ER- and PR-negative breast carcinoma, AR expression may be associated with a more invasive tumour phenotype.

A statistically significant association was also observed between AR expression and tumour laterality (p=0.013). However, the biological significance of this finding remains uncertain and may reflect the limited sample size rather than a true biological difference. Further studies involving larger patient cohorts are required to clarify this observation.

No significant associations were identified between AR expression and patient age (p=0.38), tumour size (p=0.73), histological grade (p=0.23), lymph node status, or tumour location. Nevertheless, the significant relationships observed with lymphovascular invasion and HER2 status suggest that AR may contribute to tumour behaviour in a subset of hormone receptor-negative breast carcinomas.

The present study has several limitations, including its single-center design, relatively small sample size, and lack of molecular subtyping, survival analysis, and follow-up data. These factors may limit the generalizability of the findings.

CONCLUSION

The present study demonstrated that AR was expressed in more than half of ER- and PR-negative breast carcinomas and showed significant associations with HER2 positivity and lymphovascular invasion. These findings support the potential role of AR as an adjunct prognostic biomarker and a promising therapeutic target in hormone receptor-negative breast carcinoma. Larger prospective multicenter studies incorporating molecular profiling and survival outcomes are warranted to validate these observations.

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Conflict of Interest: The authors declare no conflict of interest.

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