Journal of Carcinogenesis
Covering Genesis to Therapeutics

Journal of Carcinogenesis, ISSN: 1477-3163
2025; Vol 24: Issue 4, https://carcinogenesis.com/

A study on expression of androgen receptor in triple negative breast cancer

N. Naganandhini. [1], Dr. J. Thanka [2], Dr. Sai Sudha.Muddha[3], Dr. S. Nagarajan[4]

[1]Post Graduate,Sree Balaji Medical College and Hospital.

Email ID : nagamedico87@gmail.com ,ORCID ID: 0009-0001-2253-2062

[2]Director and Professor of Pathology Sree Balaji Medical College and Hospital

Email ID : thanka7paul@gmail.com ,ORCID ID: 0000-0002-7499-5552

[3]Associate professor, Department of pathology,Sree Balaji medical college and hospital.

Email ID : radha.5054@gmail.com , Orcid id - 0000-0003-2433-7436

[4]Assistant professor, Department of surgical oncology,Stanley medical college.

Email ID: nagamedico@gmail.com

ABSTRACT

Background: Triple-Negative Breast Cancer (Tnbc) Represents A Biologically Aggressive Subtype Lacking Oestrogen Receptor (Er), Progesterone Receptor (Pr), And Her2 Expression, Leaving Limited Targeted Therapeutic Options. The Androgen Receptor (Ar) Has Emerged As A Potential Biomarker And Therapeutic Target In Tnbc.

Objectives: This Study Aims To Assess Ar Expression In Tnbc And Correlate It With Various Clinico Pathological Parameters.

Methods: A Prospective Observational Study Was Conducted On 56 Histologically Confirmed Tnbc Cases At Sree Balaji Medical College And Hospital From August 2023 To March 2025. Immuno Histochemistry Was Performed Using A Rabbit Monoclonal Anti-Ar Antibody (Clone Ep120). Ar Expression Was Scored Based On Both Intensity And Proportion. Associations With Clinico Pathological Features Were Analyzed Using Fisher's Exact Test And T-Tests. A P-Value < 0.05 Was Considered Statistically Significant.

Results: Ar Positivity Was Observed In 17 Of 56 Tnbc Cases (30%). Significant Associations Were Found Between Ar Expression And Tumor Grade ($P = 0.015$) And Ki-67 Proliferation Index ($P = 0.003$). No Statistically Significant Associations Were Observed Between Ar Status And Age, Tumor Size, Nodal Status, Laterality, Histological Subtype, Lympho Vascular Invasion, Or Tnm Stage.

Conclusion: Ar Expression In Tnbc Is Significantly Associated With Higher Tumour Grade And Increased Proliferative Activity. While Ar Was Not Correlated With Other Clinico Pathological Parameters, Its Expression May Hold Prognostic And Therapeutic Significance. Larger Studies With Standardized Criteria Are Required To Clarify Its Role In Tnbc Management..

Keywords: *Androgen receptor, triple-negative breast cancer, immuno histochemistry, tumor grade, Ki-67, biomarker.*

How to Cite: N. Naganandhini., Dr. J. Thanka , Dr. Sai Sudha.Muddha, Dr. S. Nagarajan, (2025) A study on expression of androgen receptor in triple negative breast cancer, *Journal of Carcinogenesis*, *Vol.24, No.4*, 77-85

1. INTRODUCTION

Breast cancer continues to be one of the most prevalent cancers affecting women globally. It is categorized into five molecular subtypes—luminal A, luminal B, HER2-enriched, basal-like, and normal-like—based on gene expression profiling [1]. This classification is critical in guiding individualized treatment, as each subtype responds differently to hormonal therapies, chemotherapy, or targeted interventions. The presence or absence of specific molecular markers, including the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2), serves as an essential guide for prognosis and therapeutic planning [2].

A notable subset, triple-negative breast cancer (TNBC), is identified by the lack of ER, PR, and HER2 expression, representing roughly 15–20% of all breast cancer diagnoses [3]. Due to the absence of these therapeutic targets, TNBC does not benefit from endocrine or HER2-specific therapies, making chemotherapy the primary treatment option. TNBC is typically associated with a high histologic grade, early tendency for metastasis, and a greater likelihood of recurrence,

all contributing to its poor clinical outcomes [4].

In recent years, the androgen receptor (AR) has been recognized as a promising biomarker with both diagnostic and therapeutic potential in breast cancer, including the triple-negative subtype (TNBC). AR is a nuclear steroid hormone receptor that mediates the physiological effects of androgens, such as testosterone and dihydrotestosterone. While extensively studied in prostate cancer, AR expression has also been observed in approximately 35% of TNBC cases, suggesting a potential role in tumor biology. This discovery has facilitated the identification of a distinct molecular variant of TNBC, referred to as the luminal androgen receptor (LAR) subtype, which exhibits unique gene expression profiles, reduced proliferative activity, and AR-dependent tumor behavior.

In light of these findings, we conducted a prospective study to evaluate androgen receptor expression in TNBC cases and to investigate its association with various clinicopathological parameters

2. METHODS AND MATERIALS:

This **prospective observational study** was conducted in the Department of Pathology at **Sree Balaji Medical College and Hospital (SBMCH), Chromepet**, between **August 2023 and March 2025**, following approval from the **Institutional Human Ethics Committee of SBMCH**. A total of **56 cases** of **triple-negative breast cancer (TNBC)** were included. The study focused on assessing **androgen receptor (AR) expression** and its correlation with various **clinicopathological parameters**.

We included all patients diagnosed with TNBC, confirmed by **immunohistochemistry (IHC)** in our department. A total of **56 formalin-fixed, paraffin-embedded (FFPE) tissue sections**, obtained from **surgically resected specimens and trucut biopsies**, were processed during the study period. poorly processed or non-representative tissue, specimens with inadequate material, and sections exhibiting extensive necrosis or hemorrhage were excluded.

The study variables analyzed were patient **age**, **sex**, **tumor site**, **type of specimen**, **histological subtype and grade**, **tumor stage**, and **AR expression status**. Tissue samples were fixed in **10% neutral buffered formalin**, processed using an **automated tissue processor (Leica)**, and embedded in paraffin. Sections of **4 µm thickness** were prepared for routine **hematoxylin and eosin (H&E)** staining, while **3 µm sections** were used for **immunohistochemical staining** on **positively charged slides**, prepared with **polylysine coating** to enhance adhesion.

For H&E staining, sections were deparaffinized in xylene, rehydrated through graded alcohols, and stained with **Harris hematoxylin** followed by **eosin**. The slides were then dehydrated, cleared in xylene, and mounted using **DPX (Dibutyl Phthalate Polystyrene Xylene) [5]**.

Immunohistochemistry was performed using a **rabbit monoclonal primary antibody** against AR (clone EP120, IVD class, Rabbit IgG, nuclear localization), with **prostate tissue** serving as the positive control. The detection was carried out using the **PolyExcel HRP/DAB universal detection kit**, which includes H_2O_2, target binder, PolyHRP, DAB substrate buffer, and DAB chromogen. Antigen retrieval was achieved by heating sections in **TRIS-EDTA buffer (pH 9)** using a pressure cooker for 15 minutes. After blocking endogenous peroxidase activity with hydrogen peroxide, slides were incubated sequentially with target binder, PolyHRP, and DAB chromogen. The reaction produced a **brown nuclear stain** indicating AR positivity. Slides were counterstained with hematoxylin, dehydrated, and mounted.

Immunoreactivity scoring was based on both **staining intensity** and **extent**. Intensity was graded on a scale from 0 to 3: 0 (negative), 1+ (weak), 2+ (moderate), and 3+ (strong). The extent of staining was scored as 0 (<1% cells), 1 (1–9%), 2 (10–50%), and 3 (>50%).

Statistical analysis was performed using descriptive statistics to evaluate demographic and pathological data, stratified by **AR expression status (positive or negative)**. **Fisher's Exact Test** was used for assessing associations between categorical variables, with a **p-value < 0.05** considered statistically significant. Continuous variables such as age were summarized using **means**, **standard deviations**, and **95% confidence intervals (CI)**. All analyses were carried out using appropriate statistical software.

3. RESULTS:

This study included a total of **56 patients diagnosed with triple-negative breast cancer (TNBC)** .Out of these, **17 cases (30.35%)** demonstrated **androgen receptor (AR) positivity**, while **39 cases (69.64%)** were negative for AR expression. Androgen Receptor Expression and Staining Intensity Scores summarised in Table 1.

Table 1:Androgen Receptor Expression and Staining Intensity Scores

Androgen Receptor Expression Category		
Negative(<1%)	39	69.64%

1-9%	5	8.92%
10-50%	8	14.28%
>50%	4	7.14%
Androgen Receptor Staining Intensity Score		
0(Negative)	39	69.64%
1+	7	12.5%
2+	6	10.71%
3+	4	7.14%

Characteristics of Patients by AR Status:

Characteristics	**Total Patients (n=56)**	**AR+ (n=17)**	**AR- (n=39)**	**P-value**
Total Number of Patients	56	17	39	
Age at Diagnosis				0.91
< 50 years	27	8	19	
≥ 50 years	29	9	20	
Mean Age at Diagnosis				0.85
Mean	49.75	49.35	49.92	
SD	10.36	10.71	10.34	
Histological Subtype				0.13
IDC – NST	51	15	36	
IDC – Mucinous	2	1	1	
IDC – Micro Papillary	2	0	2	
IDC – Oncocytic	1	1	0	
Laterality				0.91
Left	27	19	8	
Right	29	20	9	
Tumor Grade				**0.015**
Grade 1	6	2	4	
Grade 2	37	7	30	
Grade 3	13	8	5	
Tumor Size				0.48
≤ 20 mm (T1)	1	0	1	
21–50 mm (T2)	25	6	19	

> 50 mm (T3)	30	11	19	
Nodal Status				0.6
N0	15	5	10	
N1	37	12	25	
N2	1	0	1	
N3	3	0	3	
Ki-67 Score				0.75
Low (<10%)	8	5	3	
Intermediate (10–20%)	21	4	17	
High (>20%)	27	8	19	
Lympho-Vascular Space Invasion				0.11
Present	24	10	14	
Absent	32	7	25	
Tumor Stage				0.38
I	14	3	11	
II	3	1	2	
III	35	13	22	
IV	4	0	4	

Demographic analysis revealed no statistically significant association between **age and AR expression**. Patients were divided into two age groups: <50 years and ≥50 years. The <50 group included **8 AR-positive** and **19 AR-negative** cases, while the ≥50 group had **9 AR-positive** and **20 AR-negative** cases (**p = 0.91**). The overall **mean age** at diagnosis was **49.75 years**, with a mean age of **49.35 years in AR-positive** and **49.92 years in AR-negative** patients (**p = 0.85**).

Analysis of **histological subtypes** showed that **51 cases were invasive ductal carcinoma of no special type (IDC-NST)**, comprising **15 AR-positive** and **36 AR-negative** cases. Two cases each of **mucinous** and **micropapillary carcinoma** and one **oncocytic carcinoma** were also observed; however, this distribution showed **no significant association** with AR status (**p = 0.15**). Tumor **laterality** was almost equally distributed, with **left-sided tumors** in **19 AR-negative** and **8 AR-positive** patients, and **right-sided tumors** in **20 AR-negative** and **9 AR-positive** patients (**p = 0.91**).

A **statistically significant association** was observed between **AR expression and tumor grade** (**p = 0.015**). **Grade 3 tumors** were more common among AR-positive cases (**8/17**), while **Grade 2 tumors** predominated in AR-negative cases (**30/39**). Grade 1 tumors were less frequent in both groups.

Tumor size showed no significant correlation with AR status (**p = 0.48**). The majority of cases were classified as **T2 (21–50 mm)** or **T3 (>50 mm)**, with **6 AR-positive** and **19 AR-negative** tumors in the T2 category, and **11 AR-positive** and **19 AR-negative** in the T3 group. Only one T1 tumor (<20 mm) was AR-negative.

Nodal involvement (N stage) also showed no statistical association (**p = 0.60**). **Five AR-positive** and **10 AR-negative** patients were node-negative (N0), while the majority fell under the **N1** category. Only one case was N2 (AR-negative), and three were N3 (all AR-negative).

The **Ki-67 proliferation index** revealed low (<10%) expression in **5 AR-positive** and **3 AR-negative** cases, intermediate (10–20%) in **4 AR-positive** and **17 AR-negative**, and high (>20%) in **8 AR-positive** and **19 AR-negative** tumors. No statistically significant difference was noted (**p = 0.75**).

Lymphovascular invasion (LVI) was present in **10 AR-positive** and **14 AR-negative** cases and absent in **7 AR-positive** and **25 AR-negative** cases (**p = 0.11**), showing no significant association.

Regarding **pathological staging**, the majority of cases were **Stage III**, with **13 AR-positive** and **22 AR-negative** patients. Stage I included **3 AR-positive** and **11 AR-negative** cases; Stage II included **1 AR-positive** and **2 AR-negative**; and **Stage IV** was observed only in **4 AR-negative** cases. These differences were not statistically significant (**p = 0.38**).

The only **clinicopathological parameter** that demonstrated a **statistically significant correlation** with **AR expression** was **tumor grade** (**p = 0.015**). All other parameters, including **age**, **histological subtype**, **laterality**, **tumor size**, **nodal status**, **Ki-67 index**, **lymphovascular invasion**, and **stage**, showed **no significant association** with AR status.

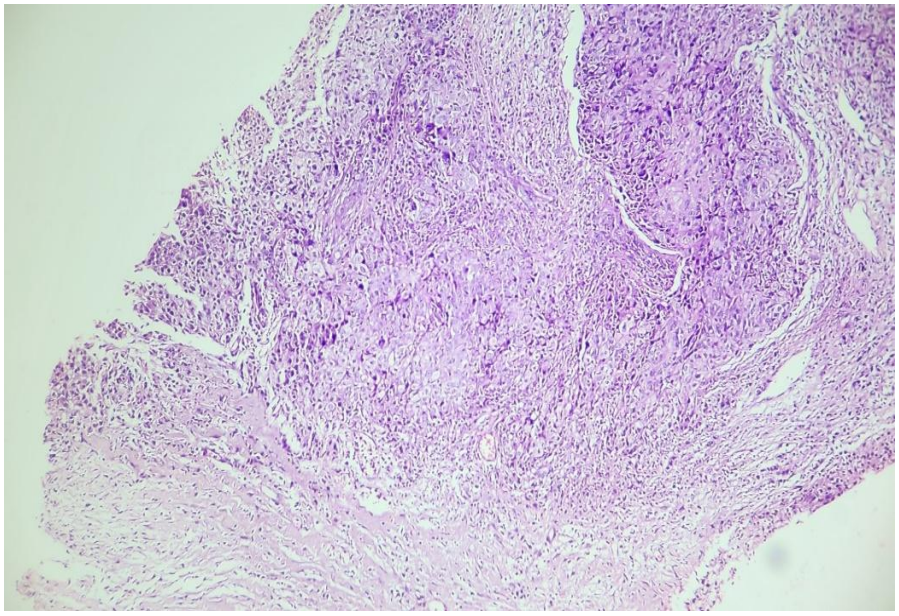

Fig 1: Invasive Breast Carcinoma – No Special Type (H&E X 100)

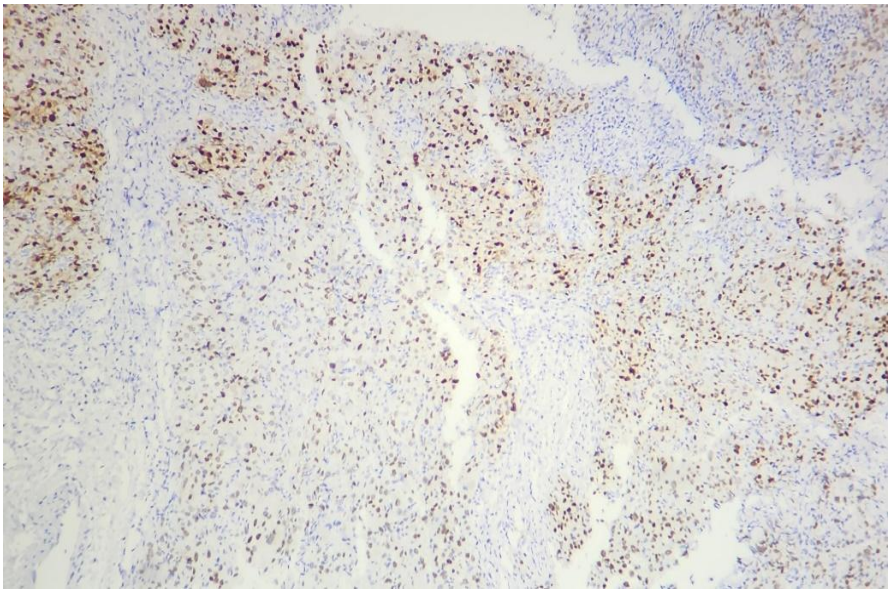

Fig 2: IHC For Androgen Receptor (IHC X 100)- Invasive Breast Carcinoma – No Special Type Showing Nuclear Expression

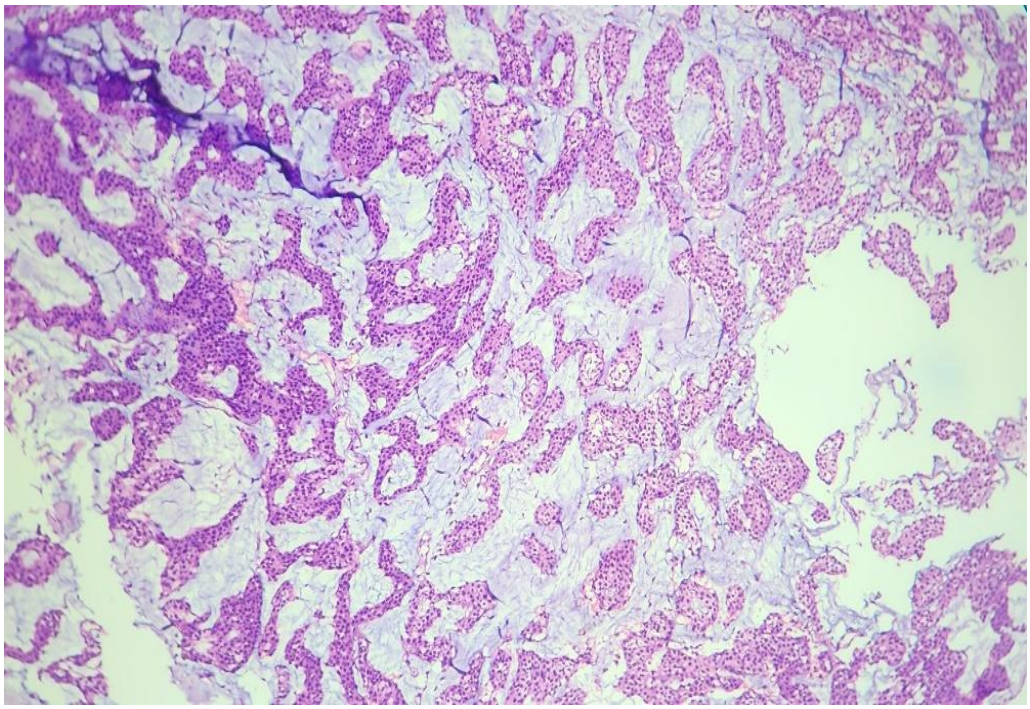

Fig 3: Invasive Mucinous Carcinoma (H&E X 100)

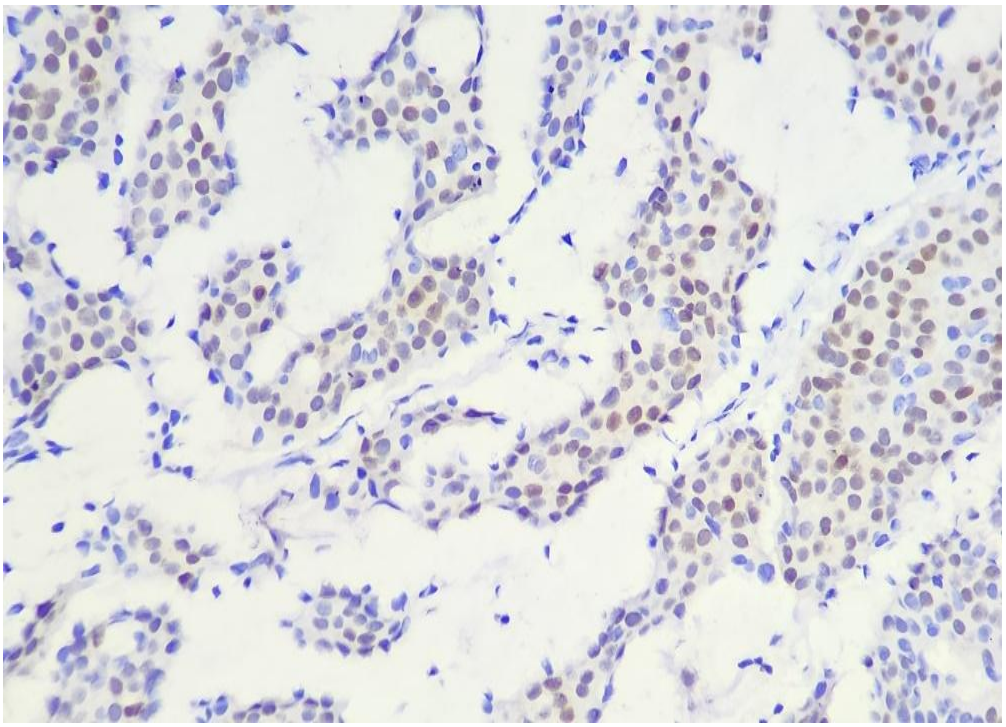

Fig 4: IHC For Androgen Receptor (IHC X 400)- Invasive Mucinous Carcinoma Showing Nuclear Expression

4. DISCUSSION:

In this study, a total of 56 patients diagnosed with breast carcinoma were evaluated. The mean age at diagnosis was 49.8 years, with an age range of 32 to 71 years. This finding aligns with the report by Titiloye et al., who observed the highest frequency of breast malignancies in the fifth decade of life, with a mean age of 50.26 years [6]. Similarly, Soares et al. reported a mean age of 53 ± 13.1 years, while Pathak et al. documented a mean diagnostic age of 49.29 years, comparable to our findings [7].

With respect to tumor laterality, 27 cases (48.2%) involved the left breast, and 29 cases (51.7%) involved the right breast, showing no significant side predominance. Comparable trends were reported by Titiloye et al. and Pathak et al., where involvement was nearly symmetrical between both breasts.

Histological grading, based on the Nottingham system, showed that 6 cases (10.7%) were Grade I, 37 (66%) were Grade II, and 13 (23.2%) were Grade III. In comparison, Titiloye et al. found a higher frequency of Grade III tumors (49%), while Pathak et al. reported Grade II as the most common (76.79%). Similarly, Oluogun et al. found that Grade II tumors accounted for 71%, followed by Grade III (22%) and Grade I (7%) [8].

In terms of histological subtype, Invasive Carcinoma of No Special Type (NST) was the predominant type in our study, constituting 91% of cases. Micropapillary and mucinous carcinomas each accounted for 3.5%, while the oncocytic subtype was observed in 1.7%. This distribution is consistent with data from Titiloye et al. and Soares et al., who also reported NST as the most common subtype (87.5% and 88.7%, respectively).

Regarding tumor size (T stage), 1 case (1.7%) was categorized as T1, 25 cases (44.6%) as T2, and 30 cases (53.5%) as T3. These findings are comparable to those by Pathak et al., where T2 tumors were most common (67%), and by Oluogun et al., who reported T3 (39.1%) and T2 (51.9%) as the predominant stages. Der et al. observed a wide tumor size range (0.5–24 cm), with the majority falling into the T3 category [9].

Nodal staging in our cohort showed 15 cases (26.7%) as N0, 37 cases (66%) as N1, with 1.7% (1 case) and 5.3% (3 cases) as N2 and N3, respectively. In contrast, Oluogun et al. reported 76.1% as pN0, while Der et al. observed 44% of cases with 1–3 positive nodes, and Boder et al. found the highest proportion in N2 category (21.8%) [9].

Regarding TNM staging, Stage III was the most frequent in our study, comprising 62.5% of cases. Stage I was observed in 25%, Stage II in 5.3%, and Stage IV in 7.1%. This staging pattern closely mirrors that reported by Der et al., where Stage III accounted for 39.1%, and Stages III and IV together represented 62.8% of cases.

Lymphovascular invasion (LVI) was noted in 24 cases (42.8%), while 32 cases (57.2%) had no evidence of LVI. Pathak et al. reported a higher LVI rate of 62.5%, highlighting variability in LVI incidence across populations.

Androgen Receptor Expression and Clinicopathological Correlation:

Among the 56 TNBC cases, AR positivity was observed in 30% of tumors, a rate consistent with prior studies reporting prevalence between 18.8% and 41%. While our study utilized a <1% cut-off for AR negativity, some investigations have used <10%, which may account for variations in AR positivity rates. Studies employing a 10% threshold have reported AR positivity ranging from 17.1% to 38%.

The correlation between AR expression and clinical parameters remains controversial. For instance, Dubreva et al. found a significant association between AR expression and age, though this was not observed in our study or others, including Pathak et al. A multicenter study comparing Nigerian and UK populations found significantly lower AR positivity in Nigerian patients (8.3%) than in UK patients (54.9%), suggesting ethnic and genetic factors may influence AR expression [10].

In terms of histology, some studies reported a higher frequency of ductal carcinoma in AR-negative TNBC, though our results did not show significant differences between IDC-NST cases by AR status. Notably, tumor grade was the only parameter in our study significantly associated with AR expression ($p = 0.015$), with AR-positive tumors more frequently of Grade III—a finding contrary to many studies which report higher grades in AR-negative tumors.

There was no significant association between AR status and tumor size, TNM stage, nodal involvement, or laterality. Although some literature suggests AR-negative tumors are more aggressive, our findings suggest otherwise in terms of tumor grade.

Ki-67 index, a marker of proliferation, has been inversely correlated with AR in some studies, with AR-negative tumors showing higher Ki-67. However, our study did not find a statistically significant relationship between AR and Ki-67 index [11-12].

Similarly, LVI, often linked with poor prognosis, showed no correlation with AR status in our cohort, in contrast to some reports suggesting a higher incidence of LVI in AR-positive tumors.

A Malaysian study involving 97 TNBC patients reported several tumor features with prognostic significance, yet our data demonstrated no significant correlation between AR status and most clinicopathological variables, with the exception of tumor grade [13].

Conflicting evidence from studies using both 1% and 10% AR positivity thresholds further complicates the prognostic value of AR. While many studies associate AR negativity with larger tumor size, higher grade and stage, nodal metastasis, and higher proliferation index, our findings diverged—highlighting higher grade tumors among AR-positive cases[14-15].

These discrepancies may stem from variations in methodology, including antibody clones, scoring systems, cut-off values, and sample sizes. Therefore, establishing standardized criteria for AR evaluation is essential for determining its clinical

and prognostic utility in TNBC.

5. CONCLUSION:

Androgen receptor (AR) is an emerging biomarker in breast carcinoma, with growing relevance in **triple-negative breast cancer (TNBC)** where therapeutic options remain limited. Although traditionally associated with prostate cancer, AR expression in breast tumors is gaining attention in the context of molecular subtyping and personalized oncology.

In our study, **AR expression was identified in 30% of TNBC cases**, aligning with the broad range reported in previous literature. A **statistically significant association** was observed between AR expression and **histological tumor grade** (**p = 0.015**), as well as with the **Ki-67 proliferation index** (**p = 0.003**). These findings suggest that AR may be linked to both tumor differentiation and proliferative activity.

Conversely, **no significant correlations** were found between AR status and other clinicopathological variables, including **tumor size (T stage), nodal involvement (N stage), metastatic status (M stage), overall AJCC stage, histological subtype, lymphovascular invasion, focality, laterality**, or **patient age**. This lack of association with traditional prognostic indicators points to a more nuanced, possibly subtype-specific role of AR in breast cancer biology.

Overall, AR expression may hold value as a **prognostic and potentially therapeutic marker**, particularly in relation to **tumor grade and proliferation** in TNBC. However, the absence of broader associations highlights the need for **further large-scale, multi-institutional studies** with **standardized AR assessment protocols** to fully define its clinical significance and therapeutic utility in breast carcinoma.

REFERENCES

[1] Abd El-Rehim, D.M.; Ball, G.; Pinder, S.E.; Rakha, E.; Paish, C.; Robertson, J.F.; Macmillan, D.; Blamey, R.W.; Ellis, I.O. High-throughput protein expression analysis using tissue microarray technology of a large well-characterised series identifies biologically distinct classes of breast cancer confirming recent cDNA expression analyses. Int. J. Cancer 2005, 116, 340–350.

[2] . Harbeck N, Gnant M. Breast cancer. Lancet. 2017;389(10074):1134–50

[3] . Bianchini G, Balko JM, Mayer IA, Sanders ME, Gianni L. Triple-negative breast cancer: Challenges and opportunities of a heterogeneous disease. Nat Rev Clin Oncol. 2016;13(11):674–90

[4] . Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, et al. Triple-negative breast cancer: Clinical features and patterns of recurrence. Clin Cancer Res. 2007;13(15):4429–34.

[5] Bancroft JD, Gamble M, editors. Theory and practice of histological techniques. Elsevier health sciences; 2008.".

[6] . Titiloye NA, Bedu-Addo K, Atta Manu E, Ameh-Mensah C,Opoku F, Duduyemi BM. Breast lesions and cancer :histopathology and molecular classification in a referral

[7] hospital in Ghana. Alex J Med [Internet] . 2021;57(1):130 –6.

[8] Available from: http: / /dx.doi .org/10.1080/20905068.2021.1907960

[9] . . Pathak R, Jha A, Neupane PR, Chal ise S, Basnyat AS. Histopathological evalua ion of carcinoma of breast . J Pathol Nepal [Internet] . 2016;6(11):922–7.

[10] Available from: http: / /dx.doi .org/10.3126/ jpn.v6i11.15674.

[11] . Oluogun WA, Adedokun KA, Oyenike MA, Adeyeba OA. Histological classification, grading, staging, and prognostic indexing of female breast cancer in an African populat ion: A 10-year retrospect ive study. Int J Heal th Sci (Qassim). 2019;13(4):3–9.

[12] . Prognostication of Breast Cancer in Ghanaian Women Receiving Modified Radical Mastectomy: A Retrospective Histopathological Study at Korle -Bu Teaching Hospital .Accra,Ghana.

[13] . Dubrava AL, Kyaw PSP, Newman J, Pringle J, Westhuyzen J, La Hera Fuentes G, Shakespeare TP, Sakalkale R, Aherne NJ. Androgen Receptor Status in Triple Negative Breast Cancer: Does It Correlate with Clinicopathological Characteristics? Breast Cancer (Dove Med Press). 2023 May 11;15:359-371. doi: 10.2147/BCTT.S405719. PMID: 37197610; PMCID: PMC10184857.

[14] . Soliman NA, Yussif SM. Ki-67 as a prognostic marker according to breast cancer molecular subtype. Cancer Biol Med. 2016;13(4):496–504. doi:10.20892/j.issn.2095-3941.2016.0066

[15] . Hu XQ, Chen WL, Ma HG, Jiang K. Androgen receptor expression identifies patient with favorable outcome in operable triple negative breast cancer. Oncotarget. 2017;8(34):56364–56374. doi:10.18632/oncotarget.16913.

[16] . Teoh PY, Tan GC, Mahsin H, Wong YP. Androgen receptor expression in triple negative breast carcinoma

and its association with the clinicopathological parameters. Malays J Pathol. 2019;41(2):125–132.30.

[17] . Cabezas-Quintario MA, Zenzola V, Arguelles M, Perez-Fernandez E. Androgen receptor as prognostic marker in triple-negative breast cancer patients. J Med SurgPathol. 2018;3(4):1–6. doi:10.4172/2472-4971.1000170

[18] Astvatsaturyan K, Yue Y, Walts AE, Bose S. Androgen receptor positive triple negative breast cancer: clinicopathologic, prognostic, and predictive features. Public Library Sci One. 2018;13(6). doi:10.1371/journal.pone.01978279