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Original Article

To Determine Expression of Pdl-1 in Primary Invasive Breast Carcinoma and Metastatic Lymph Nodes, in Patients Visiting OPD of A Tertiary Care Hospital in Barabanki District, Uttar Pradesh

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ABSTRACT

Introduction: Breast carcinoma remains the most frequently diagnosed malignancy and a leading cause of cancer-related mortality among women worldwide. For India, Global cancer observatory (GLOBOCAN) there were 1.3 million new cancer cases and approximately 850,000 cancer-related deaths by 2020. The advent of immunotherapy, particularly immune checkpoint inhibitors (ICIs), has heralded a new era in oncology. In breast cancer, this approach has shown the most promise in the aggressive triple-negative subtype, leading to the approval of agents targeting the Programmed Death-1 (PD-1)/Programmed Death-Ligand 1 (PD-L1) axis.
Objective: To determine and compare the expression of PD-L1 in primary invasive breast carcinoma and its corresponding metastatic lymph nodes.
Method: This descriptive cross-sectional study was conducted over 18 months in the Pathology Department of HIMS, Safedabad, Barabanki,UP to evaluate the expression of the PD-L1 in triple-negative breast carcinoma (TNBC) and correlate it with clinicopathological features. The investigation focused on a cohort of 30 histopathologically confirmed TNBC cases,
Results: The cohort (mean age 50.5 ± 7.44 years) presented with aggressive, locally advanced disease: 60% had tumors >5 cm (pT3/pT4), 53.3% exhibited lymphovascular invasion (LVI), and 76.7% had lymph node involvement (43.3% with ≥10 nodes). Invasive ductal carcinoma was predominant (90%), and most tumors were intermediate to high grade (90% Grade 2/3). PD-L1 expression was positive in 46.7% of cases. PD-L1 positivity showed notable, though statistically non-significant, trends of association with higher tumor grade (57.1% of positive cases were Grade 3), presence of LVI (71.4%), larger tumor size (>5 cm; 71.4%), advanced T-stage (pT3; 64.3%), and extensive nodal burden (≥10 nodes; 57.1%). A unique U-shaped distribution of PD-L1 was observed across nodal stages (high in N0 and N3). No significant associations were found with patient age or tumor site.
Conclusions: TNBC in this North Indian cohort is characterized by advanced stage and aggressive features at diagnosis. A substantial proportion expresses PD-L1, with its expression showing consistent descriptive trends with markers of tumor aggressiveness and advanced disease. These findings underscore the potential relevance of immune checkpoint inhibitors in this setting and highlight distinct biological patterns warranting further investigation in larger studies.

Keywords: TNBC, IHC, PDL-1, TNM, LVI.

INTRODUCTION

Breast cancer is the most common type of cancer and the second most common cause of cancer death in women around the world. In India, it constitutes 13.5% of new cancer cases and 10.6% of cancer fatalities1. Although early detection and multimodal therapies have progressed, metastatic disease continues to pose significant treatment challenges. Immunotherapy, particularly immune checkpoint inhibitors (ICIs) aimed at the PD-1/PD-L1 axis, has demonstrated potential, especially in triple-negative breast cancer (TNBC)2.PD-L1 expression in the tumor microenvironment is a crucial predictive biomarker for immune checkpoint inhibitor response, evaluated through immunohistochemistry3. However, PD-L1 expression is very different from one tumor to the next (intratumoral), from one primary site to another (spatial), and from one time to the next (temporal)4. This presents a significant clinical challenge: the PD-L1 status of the primary tumor may not accurately represent that of the metastases.Axillary lymph nodes are common first metastatic sites. Studies show that there is a 23.8–37.9% difference between primary tumors and paired lymph node metastases, with bidirectional changes (loss or gain of PD-L1)5,6. Relying only on the assessment of the primary tumor can lead to the wrong treatment, either by not giving patients with PD-L1-positive metastases the right therapy or by giving others too much toxicity. It is very important to get the right information about PD-L1-positive metastatic TNBC because ICIs like atezolizumab and pembrolizumab have been approved for it7. Current diagnostic methods have a lot of problems because of inconsistent expression profiles. This shows how important it is to evaluate the metastatic site to make the best treatment choices.

AIM:

This study seeks to compare PD-L1 expression between primary invasive breast carcinoma and its corresponding metastatic lymph nodes. The main goal is to find out how common and what pattern PD-L1 expression is in both places. The secondary objective is to ascertain the correlation between clinicopathological parameters and PD-L1 expression in primary tumors and metastatic nodes.

MATERIAL AND METHOD:

This descriptive cross-sectional study conducted at HIMS, Barabanki, over a duration of 18 months, encompasses 30 cases of triple-negative breast cancer (TNBC) with corresponding primary tumor and metastatic lymph node formalin-fixed, paraffin-embedded (FFPE) blocks. We use consecutive sampling. Using IHC and the Combined Positive Score (CPS), PD-L1 expression is measured. A score of 10 or higher is considered positive. We also looked at Ki-67. The compiled data was methodically evaluated to explore the possible correlation between breast carcinoma and diverse clinicopathological attributes, including tumor grade, lymph node involvement, and hormone receptor status. We used the chi-square test of independence to see if there was a statistically significant relationship between these categorical factors. A p-value of less than 0.05 was considered statistically significant. Positive controls and standardized protocols guarantee the accuracy of staining.

RESULTS:

There were 30 TNBC patients whose histopathology confirmed their diagnosis. The average age was 50.5 years (range: 31–66 years). The majority of patients (43.33%) fell within the 51–60 years age range. Most of them had tumors that were bigger than 5 cm (60%), high-grade histology (Grade 2/3: 90%), and an advanced nodal stage (N2/N3: 70%). Table 1 shows that 46.7% of the cases had PD-L1 expression..

Table 1: Clinicopathological Characteristics of TNBC Patients (n=30)

Parameter	Category	Number of patients	Percentage(%)
Age	<40 years	3	10.00
	41–50 years	9	30.00
	51–60 years	13	43.33
	61–70 years	5	16.67
Tumor size	≤5 cm	12	40.00
	>5 cm	18	60.00
Tumor site	Central	11	36.67

Parameter	Category	Number of patients	Percent age(%)
	Upper inner quadrant	4	13.33
	Upper outer quadrant	3	10.00
	Lower inner quadrant	6	20.00
	Lower outer quadrant	6	20.00
Histologic type	Ductal	27	90.00
	Lobular	3	10.00
Histologic grade	Grade 1	3	10.00
(Nottingham)	Grade 2	15	50.00
	Grade 3	12	40.00
Lymphovascular invasion	Present	16	53.33
	Not identified	14	46.67
Lymph node involvement	0 nodes	7	23.33
	1–3 nodes	2	6.67
	4–9 nodes	8	26.67
	≥10 nodes	13	43.33
Pathologic T stage	pT2	12	40.00
	pT3	17	56.67
	pT4	1	3.33
Pathologic N stage	N0	7	23.33
	N1	2	6.67
	N2	8	26.67
	N3	13	43.33

PD-L1 expression was positive in 14 cases (46.67%) and negative in 16 cases (53.33%) among the 30 cases of primary invasive breast carcinoma, as illustrated in Figure 1.

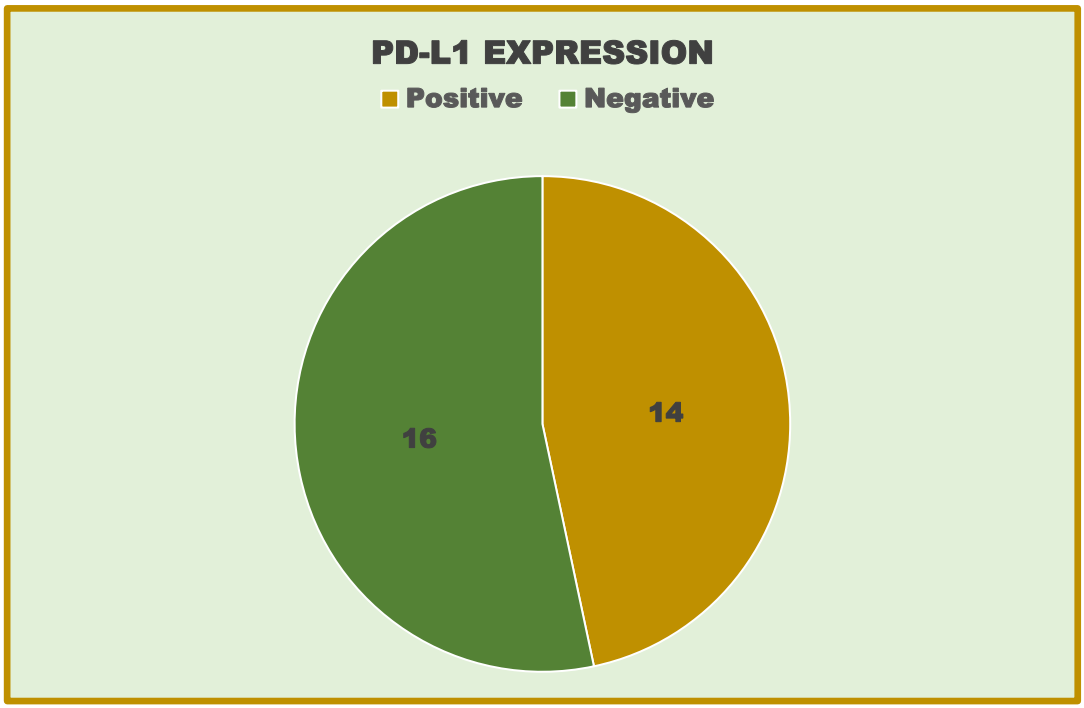

Figure1: Distribution of cases according to PD-L1 expression (n=30)

The correlation between PD-L1 expression and diverse clinicopathological parameters was assessed using the chi-square test. Table 2 shows that PD-L1 positivity was more common in larger tumors (>5 cm), higher histologic grades (Grade 3), lymphovascular invasion, and advanced nodal stages (pN3). However, none of these relationships were statistically significant ($p > 0.05$ for all).

Table 2: Association Between PD-L1 Expression and Clinicopathological Variables

Variable	Subgroup	PD-L1 Positive (%)	PD-L1 Negative (%)	P-value
Age	<40	14.28	6.25	0.75
	41–50	35.71	25.00	
	51–60	35.71	50.00	
	61–70	14.28	18.75	
Tumor site	Central	35.71	37.50	0.155
	Upper inner	21.42	18.75	
	Upper outer	35.71	6.25	
	Lower inner	7.14	18.75	
	Lower outer	0.00	18.75	
Tumor size	≤5 cm	28.57	50.00	0.232
	>5 cm	71.42	50.00	

Variable	Subgroup	PD-L1 Positive (%)	PD-L1 Negative (%)	P-value
SHistologic grade	Grade 1	14.28	6.25	0.106
	Grade 2	28.57	68.75	
	Grade 3	57.14	25.00	
Lymphovascular invasion	Present	71.42	37.50	**0.063**
	Not identified	28.57	62.50	
Lymph node count	0	28.57	10.00	0.135
	1–3	0.00	12.50	
	4–9	14.28	37.50	
	≥10	57.14	31.25	
pT stage	pT2	28.57	50.00	0.322
	pT3	64.28	50.00	
	pT4	7.14	0.00	
pN stage	N0	28.57	10.00	0.135
	N1	0.00	12.50	
	N2	14.28	37.50	
	N3	57.14	31.25	

Chi-square test; borderline significance (p=0.063) for LVI.

PD-L1 positivity exhibited a marginal correlation with lymphovascular invasion (71.4% vs. 37.5%, p=0.063) (illustrated in Figure 2) and was more prevalent in Grade 3 tumors (57.1% vs. 25.0%, p=0.106) (depicted in Figure 3) and in cases with ≥10 involved lymph nodes (57.1% vs. 31.2%, p=0.135) (represented in Figure 3), although these findings did not achieve statistical significance.

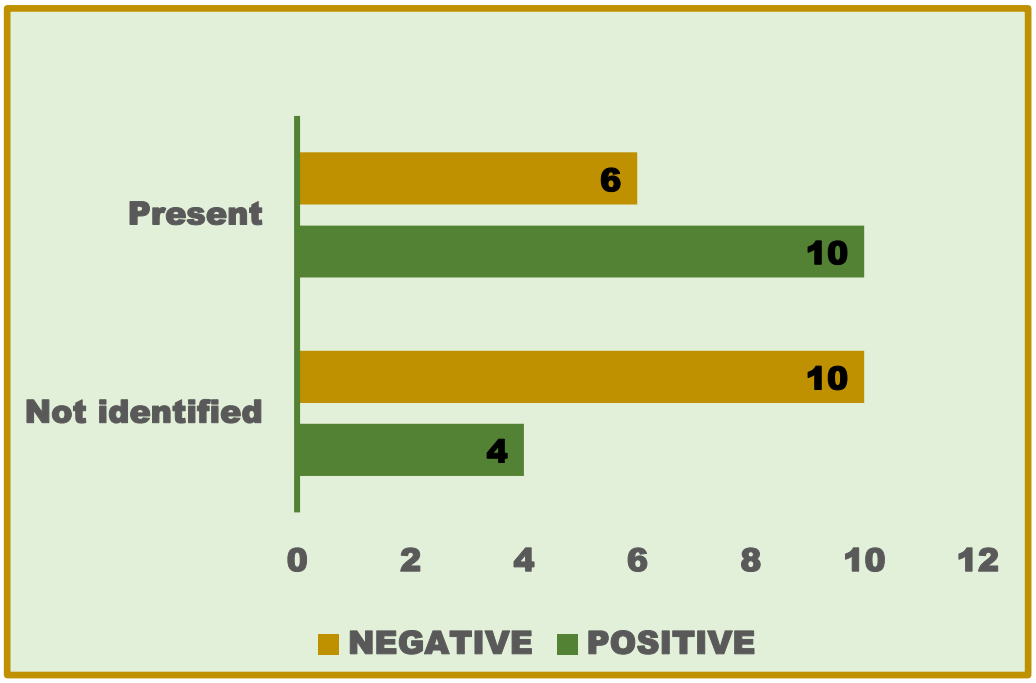

Figure2: Association between PD-L1 expression & LVI

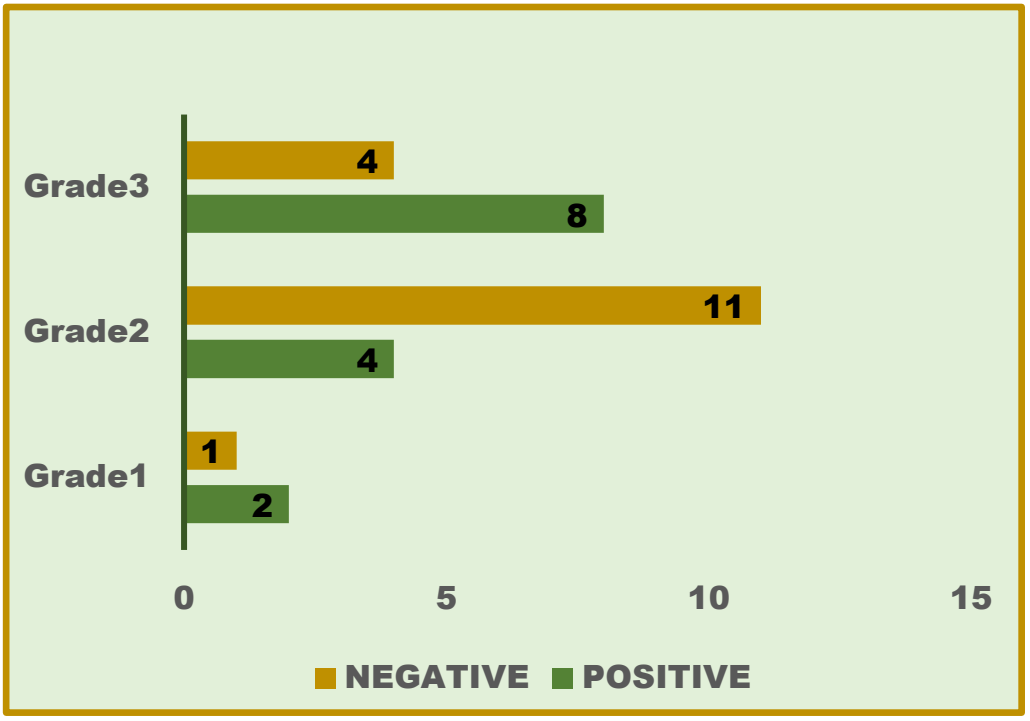

Figure3: Association between PD-L1 Expression & Histologic Grade

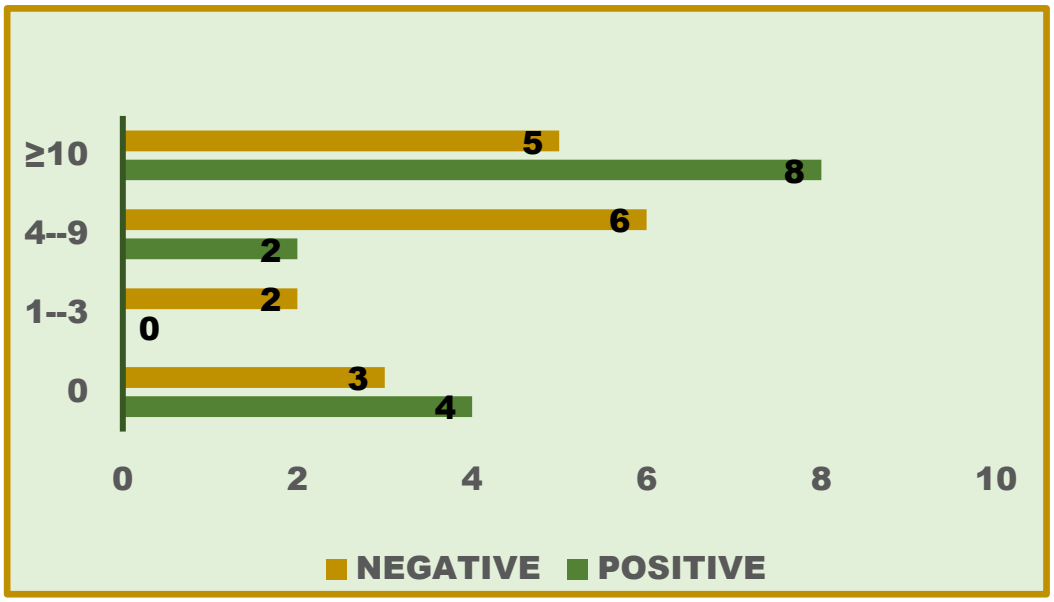

Figure4: Association between PD-L1 Expression and the number of Lymph Node

Table 3, shows the link between PD-L1 expression and quantitative biomarkers (CPS and Ki-67). Tumors that were PD-L1 positive had much higher mean CPS and Ki-67 proliferation indices than tumors that were PD-L1 negative.

Table 3: PD-L1 Association with CPS and Ki-67 Scores

PD-L1 Status	Mean CPS Score (SD)	Mean Ki-67 % (SD)
Positive (n=14)	36.35 (±13.66)	43.35 (±23.64)
Negative (n=16)	2.46 (±2.93)	29.31 (±19.61)

Immunohistochemical staining patterns are illustrated in Figures 5, 6 and 7.

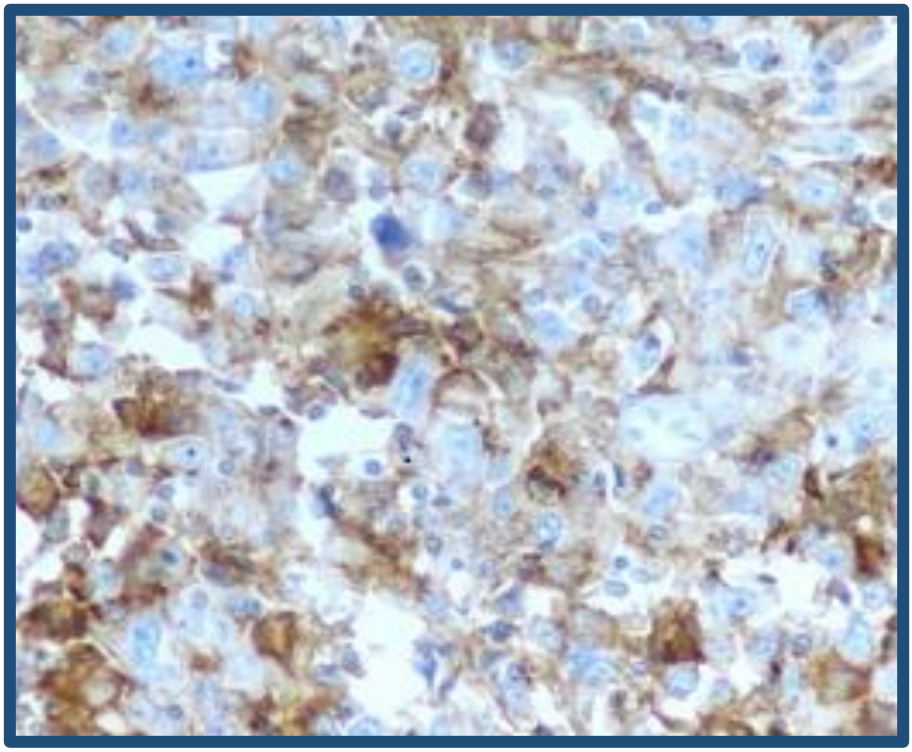

Figure 5: Membranous expression of PD-L1 in tumor cells in Invasive carcinoma of no special type (ductal), Grade 2 (IHC 40x)

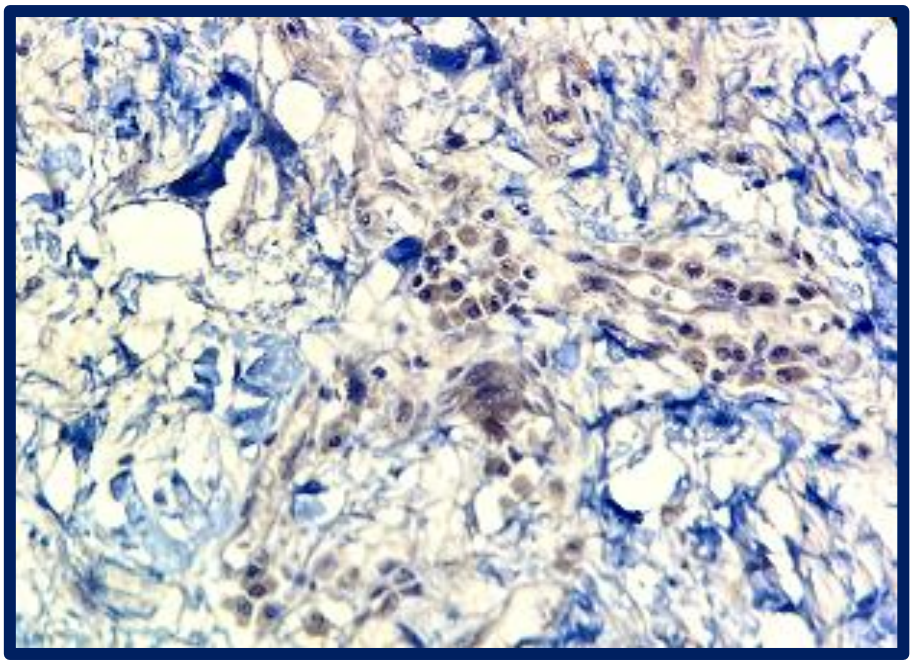

Figure 6: PD-L1 expression in tumor infiltrating lymphocytes (IHC 40x)

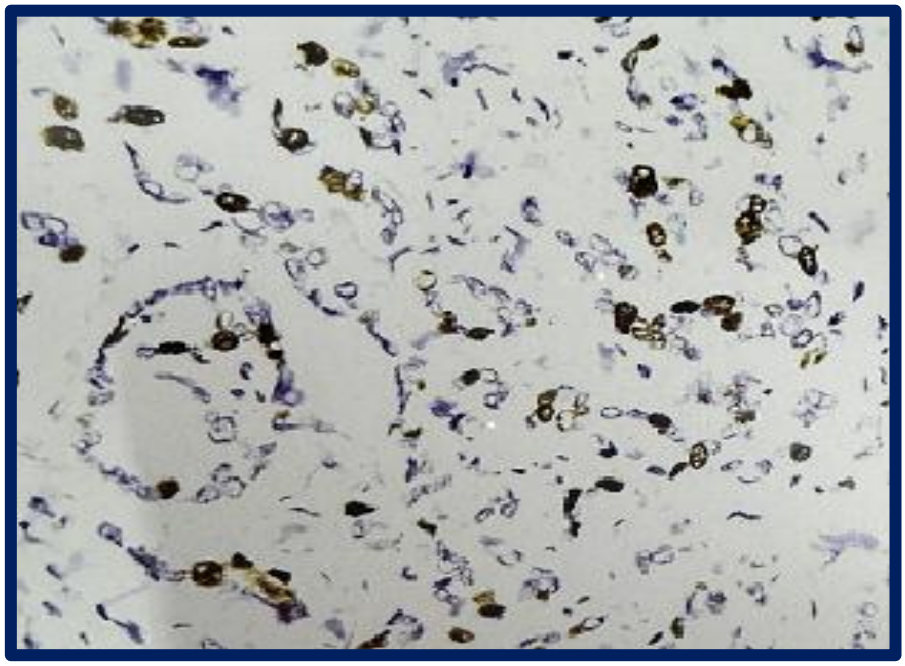

Figure 7: Ki-67 expression in tumor cells (IHC 40)

DISCUSSION:

In this group of 30 patients with histopathologically confirmed triple-negative breast carcinoma (TNBC), the average age was 50.5 years, and a large number of them were younger than 50 (40%). This age distribution corresponds with studies conducted in sub-Saharan Africa and other resource-constrained environments8, highlighting the necessity for targeted early detection initiatives in younger demographics9.

A notable observation was the prevalence of locally advanced disease: 60% of patients exhibited tumors exceeding 5 cm, and 56.7% were categorized as pT3. This is very different from studies on general invasive breast carcinoma, where tumors that are 5 cm or smaller make up 71.8–97.9% of the cases10-12. Likewise, central breast involvement (36.7%) was predominant in our cohort, contrasting with the usual preference for the upper outer quadrant noted in other studies13-15.These differences may reflect unique anatomical or biological behavior in TNBC or delayed presentation in our setting. Histologically, invasive ductal carcinoma of no special type (IDC-NST) constituted 90% of cases, aligning with established literature13,16-20. Grade 2 tumors were the most common (50%), followed by Grade 3 tumors (40%), which is in line with earlier studies10,12,19,21-23.

Lymphovascular invasion (LVI) was observed in 53.3% of cases, aligning with certain studies (45–69%)10,20, yet exceeding others (25.8%)16. Advanced nodal involvement was significant: 43.3% exhibited ≥10 positive lymph nodes (pN3), and 70% presented with pN2–pN3 disease. This is different from studies where pN0 is the most common (44–65%)12,16,24,25, which shows that our group has a more aggressive nodal presentation.

In 46.7% of cases, PD-L1 expression was positive. PD-L1 positivity, while not statistically significant, exhibited notable trends correlating with elevated histologic grade (57.1% in Grade 3), the presence of lymphovascular invasion (71.4%), and the involvement of ten or more nodes (57.1%). PD-L1 positive tumors exhibited significantly elevated mean CPS (36.35 vs. 2.46) and Ki-67 proliferation index (43.35% vs. 29.31%), indicating a correlation with aggressive tumor biology. In this group of 30 patients with histopathologically confirmed triple-negative breast carcinoma (TNBC), PD-L1 expression was found in 46.7% of the cases. This prevalence is consistent with the extensive range of 25.9–72% documented in the literature26-30, with variations arising from disparities in antibody clones, assay platforms, and positivity thresholds31-32.

The 41–60 years age group had the highest percentage of PD-L1 positive cases (71.4%), which is in line with studies by Babu S et al.33 and Salih SS et al.32. Anatomically, PD-L1 positive tumors were uniformly distributed between the central region and the upper outer quadrant, each comprising 35.7%. The predominance of upper outer quadrant tumors is consistent with previous studies34-35; however, the significant prevalence of central tumors in this cohort indicates a potentially unique anatomical pattern of PD-L1 expression in TNBC.

In terms of tumor size, 71.4% of PD-L1 positive cases exhibited tumors exceeding 5 cm, supporting the findings of Gupta A et al.31 while contradicting studies that indicate PD-L1 predominance in smaller tumors36, thereby emphasizing the heterogeneous relationships across populations.

A robust correlation was identified between PD-L1 positivity and elevated histologic grade: 57.1% of PD-L1 positive tumors were classified as Grade 3, succeeded by Grade 2 (28.6%) and Grade 1 (14.3%). This pattern is consistent with several studies14,27,31-32,37-38; however, some reports have indicated a predominance of PD-L1 in Grade 2 tumors39, suggesting variability in the grade–PD-L1 relationships.

Lymphovascular invasion (LVI) was observed in 71.4% of PD-L1 positive cases, aligning with studies that indicate LVI in 53–64% of PD-L1 positive cohorts14,22,40, although one study reported a significantly lower rate (25.1%)27.

In terms of pathological tumor stage, PD-L1 positivity was most common in pT3 tumors (64.3%), then in pT2 tumors (28.6%), and finally in pT4 tumors (7.1%). This is consistent with Muenst S et al.41 but not with Yassin FE et al.37, who found that pT2 cases were more common.

Nodal status analysis indicated that 57.1% of PD-L1 positive cases were categorized as pN3, succeeded by pN0 (28.6%), pN2 (14.3%), and pN1 (0%). This robust correlation with advanced nodal metastasis indicates that PD-L1 mediated immune evasion may promote tumor dissemination11,24-25. The prevalence of pN3 disease in PD-L1 positive cases highlights the aggressive biological characteristics of this subgroup.

Our findings indicate a mean CPS of 36.35 ± 13.66 in PD-L1 positive cases and 2.46 ± 2.93 in negative cases, aligning with the biological characteristics of aggressive breast cancers, where elevated CPS values are predominantly seen in TNBC and luminal B subtypes42.

A notable correlation exists between PD-L1 positivity and elevated Ki-67 expression (43.35% vs. 29.31%), corroborating and augmenting results from various recent studies in breast cancer research. We found that patients who were PD-L1 positive had a much higher proliferation index. The relationship between PD-L1 and Ki-67 expression identified in our study supports the conclusions of Botti et al.43 and is further substantiated by subsequent research44-45.

CONCLUSION:

In this tertiary care cohort, triple-negative breast cancer predominantly affected middle-aged women (mean 50.5 years) with locally advanced, high-grade tumors. PD-L1 expression was seen in 46.67% of cases, supporting potential immunotherapy eligibility. While no significant associations emerged with age, tumor size, or stage, trends included borderline higher PD-L1 positivity with lymphovascular invasion (p=0.063). Notably, PD-L1 positivity was high in node-negative (N0) and extensive nodal metastasis (N3) but low in limited-to-moderate nodal disease (N1/N2), suggesting a non-linear relationship. These findings require validation in larger, multi-center studies to clarify PD-L1's role in TNBC immune evasion and metastasis.

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