

Original research article**The expression of p53 in triple negative breast carcinoma: An immunohistochemical study****¹Dr. Gurleen Singh, ²Dr. Menka Khanna, ³Dr. Karamjit Singh Gill, ⁴Dr. Sanjay Piplani and ⁵Dr. Harminder Kaur Hare**^{1, 2, 3, 4, 5} Department of Pathology, Sri Guru Ram Das Institute of Medical Sciences and Research, Sri Amritsar, Punjab, India**Corresponding Author:**

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Abstract

Background: Triple negative breast carcinomas (TNBC) are a very aggressive type of breast carcinoma. In this study the expression of p53 in triple negative breast carcinoma cases was observed and correlated with age, tumor size, grade, lymph node status and other parameters.

Methods: In the present study 52 cases of histologically and immunohistochemically proven Triple negative breast cancer cases were taken and subjected to IHC for p53 expression.

Result: A total of 52 female patients with TNBC were taken for the study. The age of the patients varied from 30 - 81 years old and the maximum number of cases was seen in the age group of 41- 50 years old. Left side was involved in 57.7% of the cases. The upper outer quadrant was most involved in 63.5% of all cases. The tumor size varied from 1 cm to 8 cm. Maximum number of cases presented with a size variation of 2 - 5 cm (78.8%cases). Of all these cases, 92.3% were of IDC- NOS type. In our study 59.6% cases were of grades III and 34.6% were of grade II while 5.8% were not assigned any grade. There were no cases of grade I. Lympho- vascular invasion was seen in 82.7% cases. As the grade of the tumor increased, LVI also increased. This correlation was statistically significant (p value 0.042). Lymph nodes were recovered in 94.2% cases. Lymph node metastasis was seen more commonly in premenopausal age groups (<50) as compared to postmenopausal age groups (>50). This correlation was statistically significant (p value 0.025). Metastatic carcinomatous Lymph node deposits were present in 55.1% cases. No correlation was found when LN status was correlated with tumor size, and perineural invasion. The expression of p53 was noted in 61.5% cases. The p53 expression was most seen in the age group of 41-50 years. A direct correlation was found between p53 expression and LVI. This correlation was statistically significant (0.011). No statistically significant correlation was noted while correlating p53 expression, its intensity and cumulative score with age, tumor size, tumor grade, and perineural invasion.

Conclusion: In the present study we established that cases with p53 overexpression were mostly high grade and had a higher potential for metastasis. Thus p53 helps to provide better prognostic evaluation and can become a novel antigen for targeted therapy.

Keywords: IHC, p53, Triple Negative Breast Cancer, Breast Cancer

Introduction

Carcinoma of the breast is the most commonly prevalent cancer among women. It is estimated that about 23 lakh new cases of breast cancer were diagnosed globally and the world mourned 6,85,000 deaths due to breast cancer in 2020, making it the second leading cause of cancer related deaths in women. The tumors of breast generally start from ductal hyper proliferation, that can then develop either into benign, or malignant and then to even metastatic carcinomas depending upon stimulation by various oncogenic factors. It is related to numerous risk factors such as gender, ageing, estrogen exposure and levels, family history, gene mutations, unhealthy lifestyle, and many more which can increase the possibility and risk of developing breast cancer ^[1].

The prognosis of breast carcinoma has a multifaceted dependence, with the most important and commonly considered factors being age, tumor size, nodal metastasis, type, presence of hormonal receptors, molecular type, grade, invasion into skin or muscle, distant metastasis, etc. The most important diagnostic modalities for breast cancer are mammography, FNAC, histopathology, and immunohistochemistry to help in the prediction of the prognosis and direction of treatment. Histopathology is the most important investigation for a definite diagnosis of breast cancer; along with Immunohistochemistry (IHC) which has now become an essential investigation for breast carcinomas ^[2, 3].

Immunohistochemistry helps to type the tumors depending on the presence of receptors like Estrogen Receptor (ER), Progesterone Receptor (PR), Her2neu, p53, Cytokeratins, etc. The receptors ER, PR and

Her-2neu are the most tested antigens on a newly diagnosed case of breast cancer. Depending on the presence or absence of these receptors a breast tumor can be classified as either luminal type A/B, Her2 enriched, basal, or triple negative type. Determining these types helps in guiding the oncologist in planning an appropriate treatment regimen, as these types have different treatment options and modalities. For instance, luminal types respond well to hormonal therapies while triple negative tumors need to be treated with chemotherapy^[4].

Triple negative breast cancers (TNBC) lack all three main receptors namely ER, PR and Her2/neu. It is an aggressive cancer with high recurrence rate and accounts for 15% of all breast cancers. Due to the absence of conventional receptors p53 is considered the target marker for triple negative breast cancers^[5].

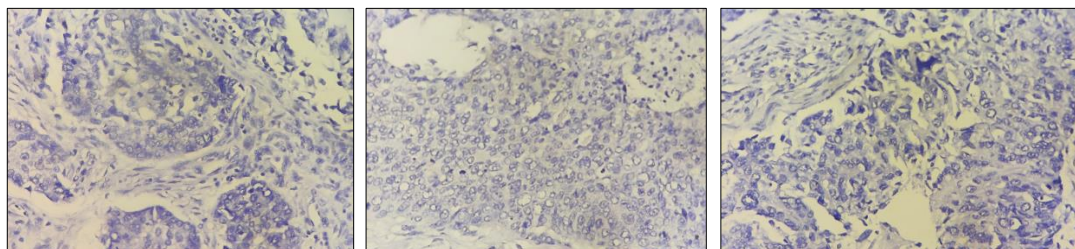


Fig 1: Microphotograph of ER, PR and her2neu negative

The p53 gene is known as the policeman of the genome. This is the gene to most frequently undergo mutation in breast cancers especially in triple negative breast cancer. The expression of p53 is seen in almost 80% of the triple-negative breast carcinomas. Presence of high expressions of p53 is considered as a poor prognostic sign. Due to the high prevalence of p53 and paucity of other receptors in triple negative breast carcinomas p53 can become a potential target for the treatment and it also holds a significant position in predicting the prognosis in TNBC^[6,7].

Materials and Methods

The study was conducted on 52 cases presented within the time period of April 2021 to June 2022 at Sri Guru Ram Das Institute of Medical Sciences and Research, Sri Amritsar. ER, PR and Her-2neu is routinely done for the cases of breast cancer, from these TNBC cases were taken, detailed clinical data was recorded as per the performa attached. The tissues were formalin fixed, paraffin embedded and were then stained with hematoxylin and eosin for histopathological typing and grading. All these cases were then subjected to Immunohistochemistry for p53 expression.

Results and Discussion

The present study was conducted in the pathology department of Sri Guru Ram Das institute of medical sciences and research, Sri Amritsar on 52 cases of triple negative breast carcinoma which were evaluated as triple negative (ER -ve, PR -ve, and HER-2neu -ve) on IHC. These cases were analysed by light microscopy and immunohistochemistry for the expression of p53 using primary antibody – Rabbit Monoclonal Antibody (Biocare Medical).

The age of the patients in our study was mostly seen from 30-81 years. The 41-50 years age group had the maximum number of patients, comprising 48% of the total patients and the mean age came out to be 52 years and majority of the patients belonged to the premenopausal age group.

Similar study done by Sood *et al.*^[8] on 36 TNBC patients found the mean age of the patients to be 45.18 years. Rao *et al.*^[9] in their study on 50 TNBC cases concluded that the mean age for TNBC patients was 46.8 years and maximum patients were from the age group of 41-50 years. This increased incidence in the premenopausal age group can be attributed to increased screening for breast cancer and advancement in imaging techniques.

In our study Left side was more commonly involved (57.7%) of cases as compared to 42.3% tumors presenting in the right side. The upper outer quadrant was the most involved site seen in 63.5% of the total cases.

Size of the tumor is one of the most important prognostic factors in breast carcinoma. The tumor size varied from 1 cm to 8 cm in our study. Majority of the patients had tumor size in the 2-5 cm range (78.8%). In a similar study done by Rao *et al.*^[9] on 50 cases of triple negative breast cancer concluded that 68% of the tumors were in the size range of 2-5 cm. In a study done by Saleh and Abdeen on 166 cases they found the tumor size between 2-5 cm was reported in 53.6% of all cases^[10].

In the present study 94.2% cases were Infiltrating Ductal Carcinoma and 1.9% were of special types (one each of carcinosarcoma, lobular and mucinous carcinoma). Similar results were reported by a study done by Rao *et al* where IDC was seen in 88% of the total 50 cases^[9].

Nottingham modification of Bloom Richardson system is the most used grading system. This includes criteria namely tubule formation, nuclear pleomorphism and mitosis. In the present study on 52 TNBC

cases majority were of grade III (59.6%). Grade II cases comprised 34.6% of all cases. The remaining 5.8% cases were not graded. Similar results were seen in a study done by Shet *et al* on 11780 cases concluded that grade III tumors comprise 70% of all the cases.¹¹ However, in a study done by Sood *et al.*¹⁸ grade II tumors constituted 47% and grade III were 38% of the total (36) cases of TNBC.

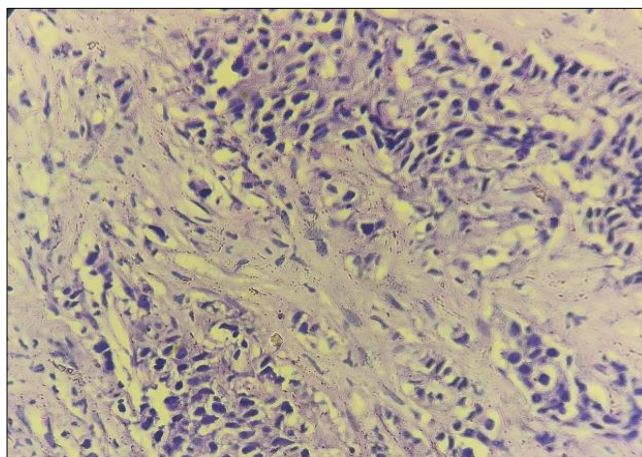


Fig 2: Microphotograph of idc grade II

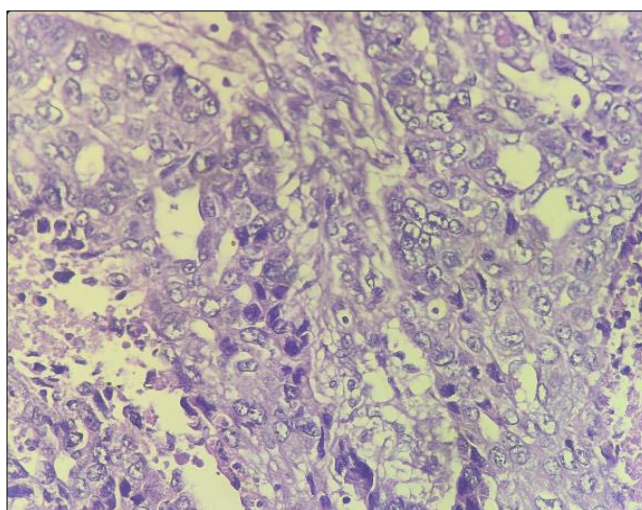


Fig 3: Microphotograph of idc grade III

In the present study metastasis in axillary lymph nodes was seen in 27 (55%) out of the 52 cases. A study done by Suhani *et al.*^[12] concurred with our findings where lymph node involvement was seen in 59.2% cases of TNBC. A study done by Jindal B reported similar results in their study with the involved nodes being 55.56% of the total 50 cases^[13]. Status of the axillary lymph nodes in invasive carcinoma is the most important prognostic factor. Sentinel lymph node biopsy has now emerged as a potential alternative to axillary dissection for staging of breast cancer. It is found to be very sensitive and specific in predicting the status of axillary metastasis.

In our study it was observed that lymph nodal metastasis was seen in 72.73% of the pre-menopausal patients, as compared to 40.74% in post-menopausal patients. A statistically significant correlation was found between them (p value 0.025) (Table 1). Similarly in a study done by Saghir N S *et al* on 1320 patients, found that the premenopausal patients had a higher lymph nodal involvement^[14].

Table 1: Correlation of lymph node involvement with menopausal status

Age Group	Metastasis Absent		Metastasis Present		Total
	No	%age	No	%age	
<50 years	6	27.27%	16	72.73%	22
≥50 years	16	59.26%	11	40.74%	27

p value:- 0.025- Significant Chi Square

In the present study, TNBC cases with tumor size < 2 cm, all (100%) showed lymph node involvement, however cases with tumor size between 2 - 5 cm, 51% showed lymph node deposits and the cases with tumor size >5 cm, 57% showed lymph node deposits. However no significant correlation between tumor

size and lymph node involvement was observed.

In our study on TNBC cases, the lymph node involvement was seen in 55.6% of cases presenting with Grade II tumors, and in 60.7% of patients with Grade III tumours. This showed that the higher grade was related with higher lymph nodal metastasis, however no statistically significant correlation between grade of tumor and lymph node status was obtained. The axillary lymph node status is one of the most important predictors of disease free and overall survival in breast cancer cases.

LVI was seen in 82.7% of the 52 TNBC cases in the present study. While correlating lymphovascular invasion with grade of the tumor, it was observed that 77.78% (14/18) grade II and 90.32% (28/31) grade III cases showed lymphovascular invasion. The LVI increased with increase in grade of the tumor. Correlating LVI with the grade of the tumor a statistically significant correlation was obtained (p value 0.042) (Table 2). Similar results were obtained in a study by Emad A R *et al*, on 3812 patients of breast carcinoma. They concluded that LVI had a direct correlation (p value 0.001) with grade of tumor, seen as an increase in lymphovascular invasion with increase in the grade of the tumor^[15].

Table 2: Correlation of lympho-vascular invasion with grade

Grade of Tumor	Lymphovascular Invasion Absent		Lymphovascular Invasion Present		Total
	No	%age	No	%age	
Grade I	0	-	0	-	
Grade II	4	22.22%	14	77.78%	18
Grade III	3	9.68%	28	90.32%	31
Special types	2	66.67%	1	33.33%	3

p value-0.042-Significant Chi Square

The expression of p53 in the present study was observed in 61.5% of TNBC cases. The frequency of positive cells varied from <10%, 10%-50% and >50% with weak, intermediate and strong intensities. The p53 scoring was done as per semi quantitative method as described by Arora K *et al*, which takes into account the intensity of staining and the percentage of nuclei stained.

Table 3: Showing p53 cumulative score

Intensity score	
No stain	0
Weak stain	1
Intermediate stain	2
Strong stain	3
Percentage score	
<10%	1
10-50%	2
>50%	3

Total cumulative score was then obtained by the sum of intensity score and the percentage score, ranging from 0-6^[16].

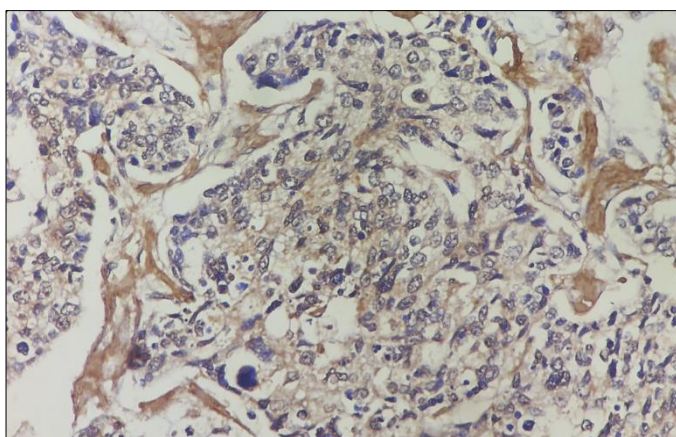


Plate 4: Microphotograph of p53 weakly positive

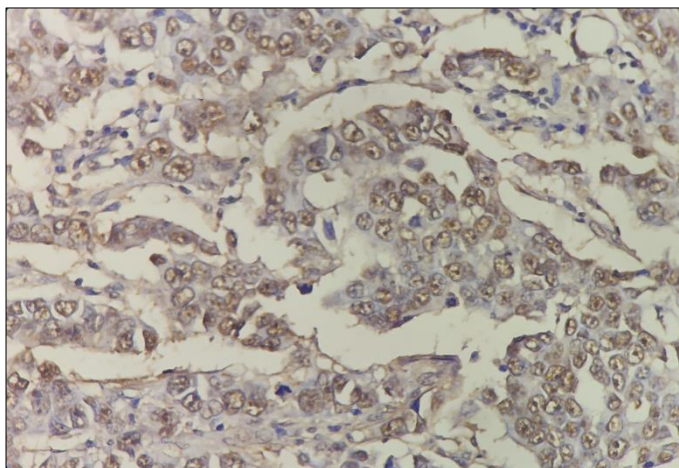


Plate 5: Microphotograph of p53 intermediate intensity

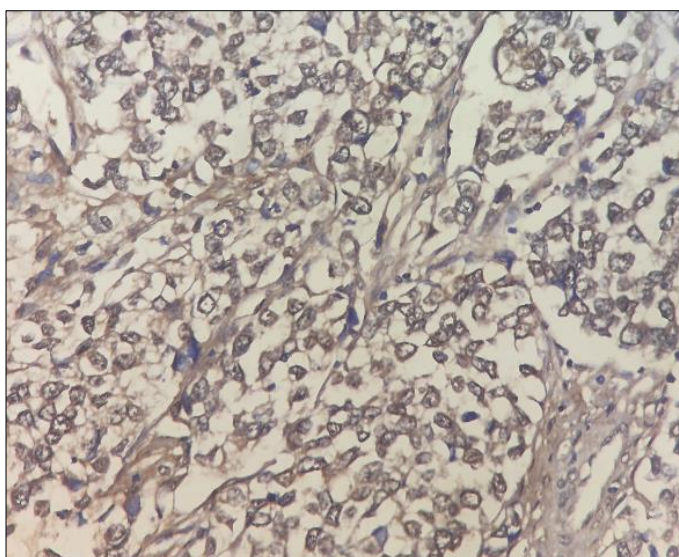


Plate 6: Microphotograph of p53 intermediate expression

In our study on TNBC patients it was seen that maximum p53 expression (68.86%) was seen in patients of the age group 41-50. Similarly Pan Y *et al* in their study on 115 cases observed the expression of p53 was higher in age groups of less than 60 years of age ^[17].

In this study all the patients with tumor size <2 cm expressed p53, in 60.97% of the patients with tumor between 2-5 cm, and in 50% of the patients with tumor larger than 5cm. This indicated a reverse relationship of tumor size with the p53 expression; this was however not significant statistically. However Pan Y *et al* in their study reported maximum patients in the size range of 2-5 cm (55.7%) ^[17]. In a study conducted by Hashmi AA it was noted that higher p53 expression was seen in cases with larger tumor size ^[6].

In our study on grade I tumors were not seen. The p53 expression in grade II and grade III tumors was found to be 61.1% and 61.2% respectively. No correlation was found between the two. In contrast, studies by Hashmi AA *et al.* ^[6], Jing- Ping li *et al.* ^[18] reported significant positive association between tumor grade and p53 expression.

In our study on p53 positive TNBC cases. 51.7% cases had lymph node deposits. While 60% p53 negative cases had secondary carcinomatous deposits. No statistically significant correlation was noted between p53 expression and lymph node status. Contrary to this study, studies by Hashmi AA *et al.* ^[6] and Jing-Ping L *et al.* ^[18] where they found a positive correlation between p53 expression and lymph node stage.

Amongst 43 cases with Lymphovascular invasion p53 expression was seen in 55.8% cases. The LVI was seen more in cases with p53 expression as compared to p53 negative cases. Thus a statistically significant correlation (0.011) was noted between them (Table 4). This was in concurrence with studies done by Hashmi AA *et al.* ^[6] and Jing-Ping L *et al.* ^[18].

Table 4: Correlation of cumulative score with lymphovascular invasion

Score	Vascular Invasion Absent		Vascular Invasion Present		Total
	No	%age	No	%age	
0	1	5.00%	19	95.00%	20
2	0	0.00%	8	100.00%	8
3	2	50.00%	2	50.00%	4
4	3	50.00%	3	50.00%	6
5	3	37.50%	5	62.50%	8
6	0	0.00%	6	100.00%	6

p value: 0.011 Significant Chi Square

Conclusion

In this study we established that the p53 expression was seen in 61.5% of TNBC cases. These cases with p53 over-expression were mostly high grade and were more associated with lymphovascular invasion, therefore these tumors are more aggressive and are associated with poor prognosis. The status of presence of p53 expression can help us to provide prognostic information and enhance the treatment modalities that are available for the patients of triple negative breast carcinomas. Moreover, TNBC patients in contrast to ER, PR or Her-2neu positive cases are deprived of hormonal or herceptin treatment and are also exposed to aggressive chemotherapy. It is emphasized that all cases of triple negative breast carcinoma should be tested for the expression of p53. It has a potential to become a novel antigen for targeted therapy and evaluation of triple negative breast carcinomas.

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Competing interests: Nil

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