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Assessing p53 Expression In Breast Carcinoma: A Comprehensive Immunohistochemical Study At Tertiary Care Centre

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ABSTRACT

Breast cancer is a leading cause of cancer-related deaths among women globally and is becoming increasingly common in India. It accounts for 22% of all female cancers worldwide. Major risk factors include age, estrogen exposure, and genetic mutations such as BRCA1, BRCA2, and TP53, while early pregnancy and prolonged breastfeeding are protective. Breast cancer is heterogeneous and classified into subtypes—Luminal A/B, HER2-enriched, and Triple Negative (TNBC) expression. TNBC is the most aggressive subtype with poor prognosis. The p53 protein, a key tumor suppressor, regulates the cell cycle and DNA repair, and is commonly mutated in cancers. In breast carcinoma, p53 mutations are linked to high-grade tumors, hormone receptor negativity and the basal-like subtype. To study the immunohistochemical (IHC) expression of p53 in breast carcinoma and correlate with various clinicopathological parameters. This observational study was conducted over one year (Aug 2022–23) in the Department of Pathology, PGIMS Rohtak, including 60 cases of modified radical mastectomy. Specimens were formalin-fixed, paraffin-embedded, and stained with H&E and IHC markers (ER, PR, HER2, Ki-67, and p53). p53 expression was evaluated based on nuclear staining in 100 tumor cells and scored from 0–6 as per Arora et al.; >3 score was taken positive. Inadequate biopsies and incomplete cases were excluded.Breast cancer is a heterogeneous disease influenced by various clinicopathological and molecular features. This observational study analyzed 60 primary breast carcinoma cases to assess p53 expression and its correlation with clinicopathological parameters. The mean age of patients was 52.7 years, with the majority being postmenopausal. Most tumors were 2–5 cm in size, and invasive ductal carcinoma (IDC-NOS) was the predominant histological subtype. Grade II tumors were most common, and lymph node involvement was seen in 50% of cases.ER, PR, and HER2 were positive in 45%, 38.33%, and 20% of cases, respectively. High Ki67 expression was seen in 56.67% of tumors. Triple-negative breast cancer (TNBC) was the most prevalent molecular subtype (38.3%). p53 overexpression (score ≥3) was observed in 60% of cases, with 41.67% showing strong expression. While p53 positivity was higher in older age groups and grade II tumors, no statistically significant association was observed with tumor size, grade, lymph node status, or menopausal status. Interestingly, stronger p53 expression was more frequent in node-negative and lymphovascular invasion-negative cases.Although no significant correlation was found between p53 expression and NPI-based prognostic groups, the findings align with previous literature indicating p53's potential role in breast cancer progression and its variability across molecular subtypes. The variability in p53 association across studies may reflect differences in scoring methods, tumor heterogeneity, and molecular subtype distribution. Further large-scale studies are warranted to validate p53 as a prognostic biomarker and therapeutic target in breast carcinoma. Immunohistochemical (IHC) evaluation of p53 in breast cancer shows potential as an independent prognostic marker, particularly in identifying subgroups with high tumor aggressiveness and poor prognosis, including triple-negative breast cancers. While no significant association was observed between p53 expression and clinicopathological parameters or molecular subtypes, a significant correlation was noted in expression intensity with hormone receptor status, Ki67 index, and molecular subtypes. These findings suggest that p53 may serve as a poor prognostic indicator. Larger studies incorporating follow-up and survival data are needed to validate its clinical utility and guide tailored treatment strategies.

Keywords: p53, Breast Carcinoma, Immunohistochemical

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INTRODUCTION

Breast cancer is one of the widespread cancer in females and the leading cause of death in the western world. Breast cancer is becoming more common in India and is approaching that of the western world. Breast cancer is second biggest cause of death among women in India, after carcinoma of the cervix. Carcinoma of the breast accounts for 22% of all female cancers worldwide, 26% in affluent countries [1].

The most important risk factors are age (rare in women younger than age 25 and increases in incidence rapidly after age 30), lifetime exposure to estrogen, genetic inheritance, and, to a lesser extent, environmental and lifestyle factors. High-risk genes associated with familial breast cancer include several involved with DNA repair and genomic stability, most notably BRCAI, BRCA2, and TP53.The major factors that decrease risk are early pregnancy (prior to 20 years of age) and prolonged breastfeeding [2].

Breast carcinoma is a heterogeneous disease. According to the PAM50 classification, depending on them RNA levels of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (Her2), we can recognize four different subtypes of breast carcinoma that express at least one of these three receptors: -

Luminal type, which express ER and/or PR, lack Her2: they can be further divided into A and B subtypes that differ for the expression ofKi67 (low levels in luminal A and high in B).

Her-2 enriched breast carcinoma, which are PR negative, usually ER negative but express high Her2 rRNA levels and are characterized by a more aggressive phenotype and worse prognosis compared with luminal breast carcinoma.

Basal-like that are for the most part ER, PR, and Her2 negative [triple negative TNBC] which are associated with the worst outcome among the breast carcinomas [3].

Compared to other subtypes of breast cancer, TNBC is more biologically aggressive and has higher recurrence rate, higher frequency of metastasis and worse survival. The clinicopathological parameters of this subgroup consist of large tumors size, multiple apoptotic cells, high proliferative index, highly undifferentiated, central necrosis and high positivity of lymph node involvement . The major histological type of TNBC is ductal and less commonly the medullary.

It has been analyzed that almost all the human cancers including breast show dysregulated pathways of p53. So, p53 is important protein involved in cell cycle and control pathways which are frequently targeted in human tumorigenesis.

The p53 protein is a nuclear phosphoprotein, composed of 393 amino acids in human. It has five structural and functional domains involved in the regulation of DNA binding. P53 is activated by stresses such as DNA damage and assists in DNA repair by causing GI arrest and inducing the expression of DNA repair genes. Of human tumors, 70% demonstrate biallelic mutations in TP53. Mutations in the p53 gene can appear at either the initial stage or the late-stage during tumorigenesis depending on the origin of cancer types, and strongly facilitate the onset or progression of cancers. Most p53 missense mutants acquire oncogenic gain-of-function activities [2].

The p53 gene is found to have mutated in approximately 20% to 40% of all breast carcinoma cases depending on the tumor size and stage of the disease. Some studies have found that abnormal p53 immunochemical expression is associated with more aggressive tumor features, a higher tumor grade, negative ER/PR status, the more aggressive basal subtype and an appealing target for therapeutic strategies. [4, 5].

Aim

To study the immunohistochemical expression of p53 in breast carcinoma.

Objectives

Primary Objective:

To study the immunohistochemical expression of p53 in various molecular subtypes of breast carcinoma.

Secondary Objective

To correlate the p53 expression with various clinicopathological parameters in breast carcinoma.

MATERIAL AND METHODS

Case selection

The present study was performed in Department of Pathology, Pt. B. D. Sharma, PGIMS, Rohtak. This was an observational study, spanned over a period of one year (August 2022-23).

All the modified radical mastectomy specimens received in pathology department were grossed and fixed in 10% Neutral Buffered Formalin. Processing of tissue and preparation of paraffin embedded tissue blocks was done as per standard protocol [6, 7]. The sections obtained from these blocks were stained with routine haematoxylin and eosin stain. The representative section from each case diagnosed as breast carcinoma was subjected to ER, PR, Ki67, Her2/neu and p53 IHC staining as per standard protocol and assessed.

Sample size: A minimum sample of 60 patients of modified radical mastectomy.

Study tool: Clinical and histopathology.

Exclusion criteria

- Inadequate biopsies
- Cases with incomplete information

IHC analysis

Immunohistochemistry was assessed by subjecting one section each from representative block to p53 and other IHC markers (ER, PR, HER-2neu, and Ki-67) for molecular profiling. IHC stains were performed using standard technique.

P53 staining

P53 expression was assessed by the presence of nuclei staining, counting at least 100 cells and scored according to the guidelines proposed by Arora et al [8]

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Total score obtained by the sum of intensity score and percentage score, ranging from 0-6

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

P53 expression was considered positive at a score of ≥ 3 and further graded as moderate and strong expression based on score 3-4 and 5-6 respectively.

Control

Positive and negative control were applied with each batch of IHC staining. Appendix served as external positive tissue control and negative control was obtained by substituting the primary antibody with antibody of non- specific relevance.

ER/PR staining

Brown diffuse or grainy nuclear staining was taken as positive for ER/PR and it was assessed by Quick scoring based on assessment of proportion and intensity [9, 10].

The scores was summed to give a maximum of 8. Patients with tumors scoring 2 or less was regarded as ER/ PR negative

HER2neu staining

HER2/neu was assessed by HER2/neu scoring system. Brown membranous staining was taken as positive. IHC analysis showing uniform, intense membrane staining of >10% of the tumor cells was taken as positive.

The p53 expression was correlated with various clinicopathological parameters such as age, tumor size, tumor type, axillary lymph node status, histological grade, NPI score and ER, PR and HER2/neu.

Ki67 % Score

Inwald et al defined Ki67 (nuclear antigen) score as the percentage of positively stained tumor cells among the total number of malignant cells assessed. A ki67 cut-off point of 15% was considered and >15% was considered as high Ki67 index [7].

Statistical Analysis

A descriptive study was carried out for all the variables included in the study. The whole data was subjected to statistical analysis using SPSS 20 software. P value less than 0.05 was accepted as statistically significant.

RESULTS

The age ranged from 26-74 years with mean of age in years(SD) was 52.73. Maximum number of the patients had age: 41-50 years (31.66%) (Table 1). Out of 60 cases, 43 cases were post menopausal.

Table 1: Distribution of cases according to age of the patient. (N = 60)

Age of the patient	Number of cases	Percentage
21 – 30 years	3	5%
31 – 40 years	6	10%
41 – 50 years	19	31.66%
51 – 60 years	13	21.67%
61 – 70 years	15	25%
71 – 80 years	4	6.67%
Mean age of the patient in years (SD)	52.73 (11.95)	
Range	26 - 75	

Morphological evaluation

All the cases were divided according to tumor size into 3 subgroups (<2cm, 2- 5cm, and >5cm). Majority of the cases 41(68.33%) belonged to subgroup 2-5cm followed by 10 (16.67%) of the cases

belonging to group >5cm. Tumours were categorized into different histologic subtypes. Invasive carcinoma that failed to exhibit significant characteristics were classified as invasive ductal NOS which was most common histologic subtype(90%). Other histologic subtypes included were mucinous carcinoma, invasive carcinoma with comedo pattern and invasive carcinoma with medullary features including two cases in each group (3.33%). All the cases were graded using Modified Scarff Bloom Richardson grading system. Sixty percent of total cases were of Grade II (Moderately differentiated). Grade III tumors were least common (13.34% of total cases). Lymph nodes were involved in 30 (50%) cases. 23.33% of the cases had 1-3 Nodes involved. 21.67% of the cases had 4-9 Nodes. 5% of the cases had ≥10 Nodes. Twenty six (43.33%) cases revealed lymphovascular invasion while in 34 cases (56.67%) lymphovascular invasion could not be identified. Only 5 (8.33%) cases revealed perineural invasion. Out of 60 cases, 5 (8.33%) cases had associated ductal carcinoma in- situ component. All the cases were categorized based on tumor size, grade and lymph node status. Majority of the cases 37 (61.67%) belonged to moderate prognostic group.

Table 2: Distribution of cases according to molecular subtypes. (N = 60)

Molecular subtype	Number of cases	Percentage
Luminal A	20	33.34%
Luminal B	7	11.66%
Her-2 enriched	10	16.66%
Triple negative/Basal	23	38.34%

IHC analysis

ER expression was assessed by Allred scoring taking into account percentage and intensity of staining. Score of ≥ 3 was considered as positive expression. 45% percent of the total cases were ER Positive while 55% were negative. Twenty three (38.33%) cases were PR Positive. HER2/neu status was assessed by HER2/neu Scoring System. Complete membrane staining in >10% of invasive component of tumor was taken as positive. 12 (20%) cases were positive for HER2/neu and 75% of cases showed HER2/neu negativity while 3 cases revealed equivocal expression which was considered negative based on FISH study. Ki 67 cut-off point of 15% was considered high expression. 34 (56.66%)cases revealed high Ki67 expression. Triple Negative/Basal like was the most common molecular subtype (38.34%) among all the cases and Luminal B was least common subtype (11.66%). Twenty cases (33.34%) were of Luminal A subtype. (Table 2)

P53 expression was observed in the term of proportion of nuclear stained cells and staining intensity on a scale 0 to 3 each. Total aggregate p53 score was obtained on a scale from 0 to 6 by adding proportion and intensity score and score of equal and more than 3 was considered positive expression. Based on total score, 24 patients were seen in the score of 0-2 and were considered negative for p53 expression (40%).Out of 36 positive cases, 25 cases revealed 5-6 expression which was considered as strong expression (69.4%)

Following table (3) shows distribution of cases according to total intensity and proportion score

	Proportion				Intensity			
	0 (0)	>0-10% (1)	10-50% (2)	>50% (3)	No stain (0)	Weak stain (1)	Moderate stain (2)	Strong stain (3)
No. of cases	11	15	14	20	11	17	11	21
Percent-age	18.33%	25%	23.33%	18.33%	18.33%	28.33%	18.33%	35%

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Table 4: Distribution of cases based on total aggregate score of p53 expression

(Total score)	P53 expression			
	Negative/weak (0-2)	Positive		
		Moderate (3-4)	Strong (5-6)	Total
No. of cases	24	11	25	**36**
Percentage	40%	18.33%	41.67%	**60%**

Correlation of P53 with clinicopathological parameters

The maximum number of cases that showed nuclear positivity for p53 fell in the age group of 41-50 years. No significant statistical association of p53 seen with age (p = 0.534). P53 positive cases were more in post menopausal breast carcinoma patients however difference was not statistically significant (67.45%). No correlation of p53 expression was seen with the laterality and tumor size.

In infiltrating ductal carcinoma NOS, 63% cases revealed positive p53 expression. Both mucinous and invasive carcinoma with medullary features revealed strong positive p53 expression and one of 2 cases of invasive carcinoma with comedo pattern revealed strong positive expression. However, statistical significance could not be ascertained due to small number of cases in categories other than IDC NOS. No Correlation of p53 expression was seen with tumor grade. Although strong expression was seen in all grade III cases whereas 66.67% of cases with strong positive expression of p53 in grade I and II tumor grade but difference was not statistically significant.

P53 expression was seen more commonly in patients with > 3 lymph node involvement but difference was not statistically significant. Also no correlation was seen with lymphovascular as well as perineural invasion.

Positive p53 expression was seen in 62.5%, 53.57% and 68.75% of cases in good, moderate and poor NPI score respectively and the difference was not statistical significant. Fifty one percent of ER positive patients and 66.67% of ER negative patients revealed positive p53 expression. There was no clinical correlation of p53 expression with ER status. However, in patients with negative ER status revealed strong p53 expression(81.82%) and difference was statistically significant. Fifty six percent of PR positive patients and 62.17% of PR negative patients revealed positive p53 expression. There was no clinical correlation of p53 expression with PR status However, patients with negative PR status revealed strong p53 expression(86.96%) and difference was statistically significant. Seventy five percentage Her2 negative cases, 58.33% were positive for p53 expression and difference was not statistically significant. Out of 31 cases with high Ki67 expression,21(67.75%) revealed positive p53 expression. In patients with low Ki67 index, 51.73% were positive for p53 expression and difference was not statistical significant. However, in patients with positive p53 expression, more number of patients revealed strong expression with high Ki67 index and this difference was statistical significant. Her2 enriched tumour cases revealed highest degree of p53 expression with maximum number of cases (85.72%) revealing strong expression. There was no difference in p53 expression between luminal A,B and triple negative subtypes. Triple negative tumour cases also revealed greater degree of p53 expression with 12 out of 14 positive cases revealing strong expression and this difference was statistical significant.

Table 5: Correlation of patient characteristics with p53 status. (N = 60)

		Weak p53	Moderate p53	Strong p53	p-value Neg vs Positive	p- value moderate vs strong
Age of the patient	21 – 30 y	2 (66.67%)	0 (0%)	1 (13.33%)	0.534	0.478
	31 – 40 y	2 (33.33%)	0 (0%)	4 (66.67%)		
	41 – 50 y	9 (47.37%)	5 (26.31%)	5 (26.32%)		
	51 – 60 y	5 (38.47%)	3 (20.51%)	5 (41.02%)		
	61 – 70 y	6 (40%)	2 (22.22%)	7 (77.78%)		
	71 – 80 y	0 (0%)	1 (25%)	3 (75%)		

Menopausal state	Pre	10(58.82%)	1 (14.28%)	6 (85.72%)	0.061	0.298
	Post	14(32.55%)	10(34.48%)	19(65.52%)		
Laterality	Left	12(44.44%)	4 (26.67%)	11(73.33%)	0.525	0.669
	Right	12(36.36%)	7 (33.33%)	14(66.67%)		
Tumor size	< 2 cm	1 (11.11%)	3 (37.5%)	5 (62.5%)	0.158	0.890
	2 – 5 cm	17(44.73%)	6 (28.57%)	15(71.43%)		
	> 5 cm	6 (46.15%)	2 (28.57%)	5 (71.43%)		
Histological type	IDC-NOS	20 (37%)	12 (35.3%)	22 (64.7%)	NA	NA
	IDC-Mucinous	0 (0%)	1(50%)	1 (50%)		
	Invasive	1 (25%)	0 (0%)	3 (75%)		
LN status	0 nodes	12 (40%)	2 (11.11%)	16 (8.89%)	0.345	0.093
	1 – 3 nodes	8 (57.14%)	3 (50%)	3 (50%)		
	4 – 9 nodes	3 (23.07%)	5 (50%)	5 (50%)		
	≥ 10 nodes	1 (33.33%)	1 (50%)	1 (50%)		
MBR Grading	Grade I	7 (43.75%)	3 (33.33%)	6 (66.67%)	0.294	0.487
	Grade II	12(33.33%)	8 (33.33%)	16(66.67%)		
	Grade III	5 (62.5%)	0 (0%)	3 (100%)		
NPI scoring	Good	6 (37.5%)	5 (50%)	5 (50%)	0.596	0.678
	Moderate	13(46.42%)	8 (53.33%)	7 (46.67%)		
	Poor	5 (31.25%)	4 (36.36%)	7 (63.64%)		
ER	Positive	13(48.15%)	7 (50%)	7 (50%)	0.244	0.043
	Negative	11(33.33%)	4 (18.18%)	18(81.82%)		
PR	Positive	10(43.47%)	8 (61.53%)	5 (38.47%)	0.665	0.002
	Negative	14(37.83%)	3 (13.04%)	20(86.96%)		
Her2neu	Positive	4 (33.33%)	2 (25%)	6 (75%)	0.598	0.699
	Negative	20(41.67%)	9 (32.14%)	19(67.85%)		
Perineural invasion	Present	2 (40%)	1 (33.33%)	2 (66.67%)	1.000	0.913
	Absent	22 (40%)	10(18.18%)	23(69.68%)		
Lymphovascular invasion	Present	12(46.15%)	8 (57.14%)	6 (42.85%)	0.395	0.006
	Absent	12(35.29%)	3 (13.63%)	19(86.37%)		
Molecular subtype	Luminal A	9(45%)	7(63.63%)	4(36.37%)	0.883	0.039
	Luminal B	3(42.85%)	1(25%)	3(75%)		
	Her2 rich	3(10%)	1(14.28%)	6(85.72%)		
	TNBC	9(39.13%)	2(14.28%)	12(85.72%)		
Ki67	Low	14(48.27%)	8(53.33%)	7(46.64%)	0.206	0.012
	High	10(32.25%)	3(14.28%)	18(85.72%)		

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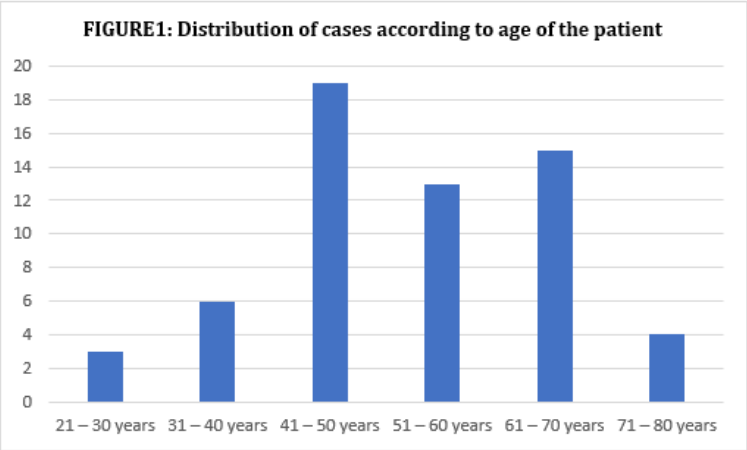

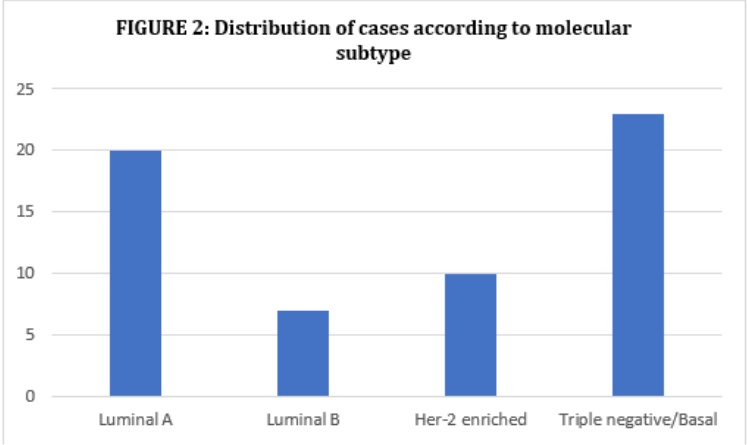

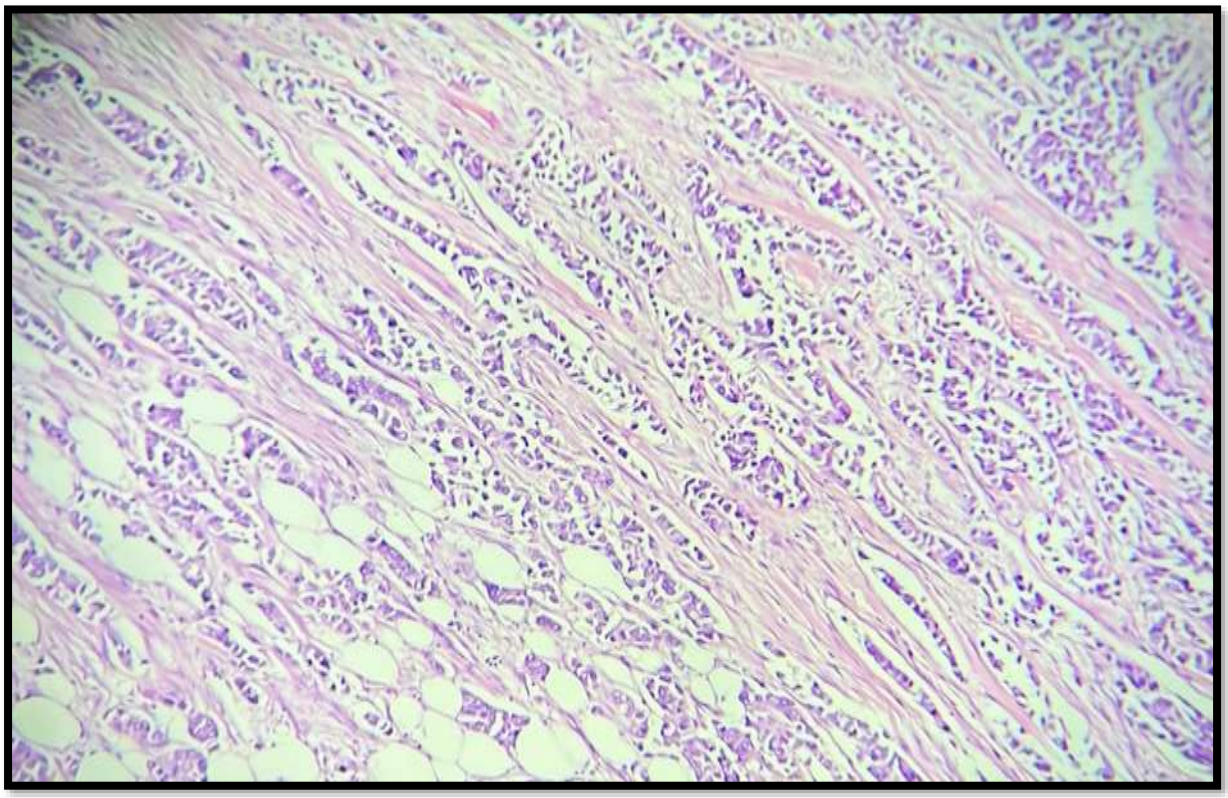

FIGURE 1: Infiltrating Ductal Carcinoma, (H&E,100X)

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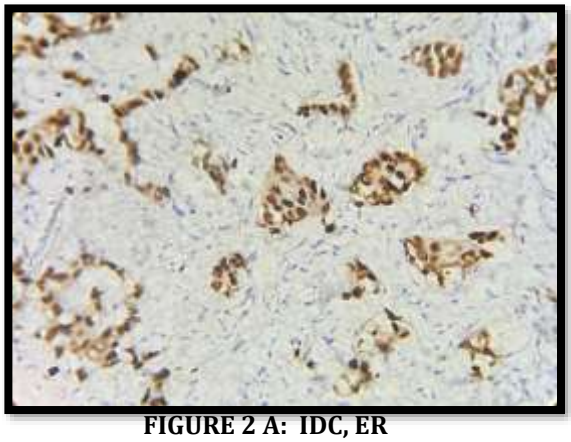
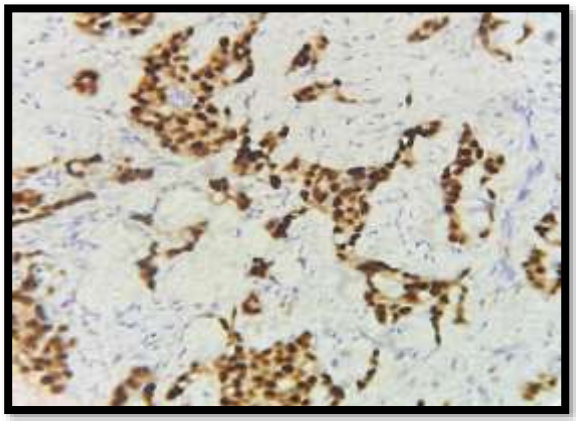


FIGURE 2 A: IDC, ER POSITIVE,(IHC,400X),



B: PR POSITIVE (IHC,400X)

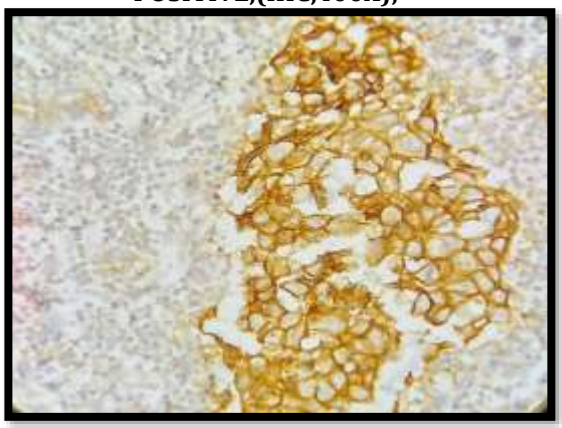


FIGURE 3: HER2 3+, (IHC, 400X)

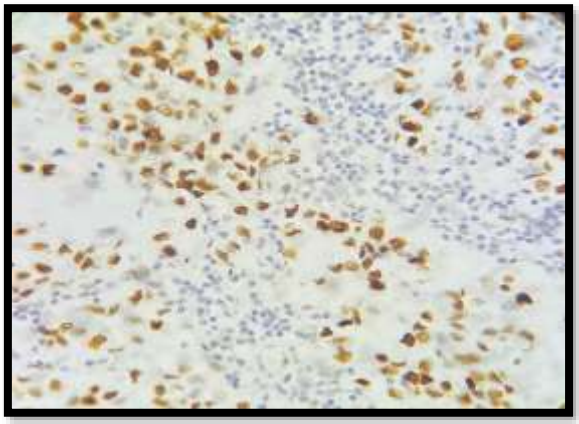


FIGURE 4: Ki67- 20%,(IHC,400X)

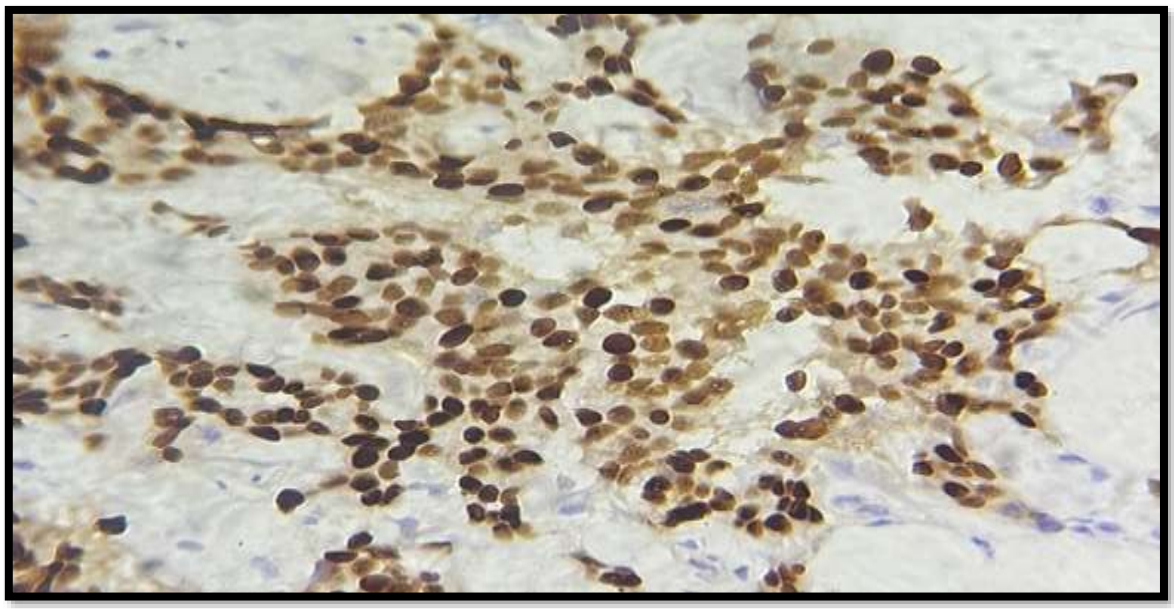


FIGURE 5: INFILTRATING CARCINOMA NOS,P53- INTENSITY-3, PERCENTAGE-3 (TOTAL SCORE=6 STRONG), (IHC,400X)

DISCUSSION

Identifying biomarkers and gene expression patterns is key to advancing early detection and prognosis of breast cancer, while also ensuring that treatments are tailored to the specific molecular characteristics of each patient. Research has revealed that prognostic and predictive biomarkers are critical

in regulating important cellular processes, including cell growth, apoptosis, angiogenesis, metastasis and therapeutic resistance.

The present study was a observational study conducted in the department of Pathology, Pt. B.D.S. PGIMS, Rohtak. Sixty cases of primary breast cancer were taken up for study (MRM specimen). Specimens were examined for different clinic-pathological parameters and correlation with p53 was done.

Out of a total of 60 cases of primary breast carcinoma, the patient's age ranged from 26-74 years. The mean age was 52.7 years. Majority of cases in our study were ≥ 40 years with 71.67% of cases being post menopausal. The mean age of various other studies was variable. Our data was similar to Li et al[11], Rana et al[12] and Dash et al [13]. Studies done previously indicating that evidence of cancer increases significantly with age.

Based on tumor size, all the cases were divided into 3 subgroups; tumor size less than 2 cms, 2-5 cms and more than 5 cms. Largest group with 68.3% of the cases was in category of 2-5 cm followed by 16.6% of the cases in >5cm. Only 15% were less than 2 cm in size. The results of Pan et al [7] , Lee et al [14] and Ali et al [15] were similar to ours with maximum number of the cases of size 2-5 cm.

Invasive Ductal Carcinoma (NOS) constituted the largest group in our study (90.0%). The results of our study are concordant with those of Dash [13], Rana et al [12] and Pan et al [7]. Other types included were invasive carcinoma with medullary features, with comedo pattern and mucinous carcinoma with 2 cases in each category.

The histologic grading was done using Nottingham modification of Bloom-Richardson grading system [16]. In our study, maximum number of cases were found to be of grade II(60%), Grade I and III constituted 26.66% and 13.34% of cases respectively. Our results are in concordant with studies Li et al [11], Ali et al [15] and Pan et al [7] however discordant with Milicevic et al [17] and Lee et al [14].

Lymph node involvement was seen in 30 out of 60 cases and out of 30 positive cases 16 (26.67%) revealed ≥ 4 involved lymph nodes and 14 cases revealing 1-3 lymph node involvement. Variable results have been reported with some of the studies showing more number of cases with no nodal involvement. The results of Dendale et al [18] and Lee et al [14] were similar to our study with maximum number of cases showing no nodal involvement.

Based on tumor size, histologic grade and lymph node status, tumors were categorized into different prognostic groups as per NPI scoring system. Majority of patients in our study were in moderate prognostic group(61.67%) while 21.67% were in good prognostic group and 16.67% in poor prognostic group and is in accordance with previous studies [13].

ER, PR and HER2NEU Expression

Out of 60 cases, positive ER and PR immunoexpression was seen in 27% and 23% cases respectively. Our results were in line with Dendale et al [18] in contrary to others studies which included more in of cases with positive ER/PR expression [15, 19-21].

Positive Her2neu expression was seen only in 12 cases while 45 cases were negative and 3cases revealed equivocal expression and were included in negative expression after FISH study. Our results are in concordance with Yang et al [20], Ali et al [15].

Cut- off point of 15% was considered to divide tumours with low and high Ki67 index. Based on the cut off, 34 cases (56.67%) revealed high Ki67 index which is concordant with studies [7, 15, 19, 21]. The difference may be due to different cut off values included in various studies.

Triple Negative/Basal like subtype constituted the largest group in our study (38.3%), followed by Luminal A type (33.3%). Ten cases were of Her2 enriched type (16.6%) and 7 were of Luminal B type (11.6%). Dendale et al [18], Pan et al [7] also mentioned the more or less similar findings whereas studies by Miller et al [22], Kikuchi et al [19], Nishimura et al [21] and Ali et al [15] had more percentage of luminal A/B cases.

Nuclear expression of p53 was observed and scored on a scale of 0-6 by adding proportion and intensity score on a scale of 0-3 each. Positive expression was considered when total score was ≥ 3 and further graded as moderate if score was 3-4 and strong if score was 5-6.

Positive p53 expression was seen in 36 out of 60 cases(60%) out of which strong expression was seen in 25 (41.67%) cases. The result of various studies demonstrated p53 positivity ranging from 14-82.6%. The difference in percentage positivity may be explained due to difference in the patient selection criteria, different clone used and variability of scoring methods.

In this study, the expression of p53 was correlated with various clinicopathological parameters including age, tumor size, lymph node status, histological grade, NPI, histological type and hormone receptor, Ki67 expression and molecular subtypes.

Thirty six out of 60 cases revealed positive p53 expression including all cases of >71 years, with 75% of them revealing strong expression where as only 33.33% of cases in age group 21-30 years revealed strong expression while in all other age groups positive expression was seen in 50-66% of cases only with almost variable difference in intensity of expression in different age group. There was no significant statistical association of p53 expression in different age group including with menopausal status [1, 12, 15, 23].

A study by Meera Balakrishnan [1], it has been observed that those with tumor on left side had a higher chance of showing P53 expression whereas no difference in p53 expression seen with laterality in our study.

No association of p53 expression was seen with tumor size in our study as 88.89% of tumour <2cm and 55.27% and 53.85% of tumour with 2-5 and >5 cm respectively revealed positive p53 expression with no difference in intensity of expression with increasing size of tumours. Our results are similar to other studies [6, 7, 14, 24] but are contrary to other who reported significant difference in p53 with increasing size [1, 19, 21]. The variation may be due to different cut off used and different molecular subtypes included in various studies.

The most common histological subtype noted in our study was IDC-NOS (90%) and 34 out of 54 of cases observed in this type revealed positive p53 expression, with 64.70% of positive cases revealing strong expression. Two cases each of invasive carcinoma with medullary features and mucinous carcinoma observed in our study revealed positive p53 expression whereas one out of 2 cases of invasive carcinoma with comedo pattern revealed strong expression of p53. Both cases of invasive carcinoma with medullary features revealed strong expression.

The exact statistical association could not be calculated because of small number of cases in categories other than IDC NOS. Similar results have been observed in other studies which also observed as IDC as most common subtypes [7, 12, 13]. Dendale et al [18] did study of p53 expression only in medullary carcinoma patients and observed 57% of tumour revealing positive expression, with 91% of positive p53 revealing strong expression. More number of cases involving each histologic types are needed further to evaluate p53 expression and its correlation with histologic subtypes.

P53 showed higher positivity in grade II (66.67%) than in grade I (56.25%) and least positivity in grade III (37.45%) tumors and all grade III tumours however revealed strong expression. No statistically significant association seen between histologic grade and P53 expression. Studies done by Lee et al [14] and Dash et al [13] also showed no significant association between these two parameters. However, significant relationship between p53 expression and histologic grade has been observed in few studies.[11,15,17] The variable correlation between p53 expression and histologic grade in various studies may be related to limited population size and also due to variable molecular subtypes included in the study.

Lymph node involvement may be an indicator of more advanced and metastatic disease and associated with poor prognosis. In our study 60% p53 positive cases showed no nodal involvement and no statistical difference in p53 expression was observed between negative and positive cases and even strong expression was seen more frequently in node negative cases(88.89%). Variable results have been observed in previous studies with some observing significant association with lymph node involvement [7,11,14] while not in others [13, 21].

Also, we did not observe difference in p53 expression between cases with or without lymphovascular invasion. On the contrary show strong positivity was seen in patients without lymphovascular invasion and difference was statistically significant.

Variable results may be due to different methods used as well as selection of patients. Moreover detection of mutant TP53 using IHC may not be an accurate substitute for complex mutations. Also mutant p53 contributes tumour progression via a dual mechanism, the loss of tumour suppression activity and a gain of oncogenic activity. Wild type p53 protein associated with strong IHC signal may be overexpressed as a compensatory mechanism to repair DNA damage occurring during tumorigenesis and may serve as a favourable prognostic indicator.

In our study, p53 positivity in good and poor prognostic groups was 62.5% and 68.75%. Difference in percentage positivity and intensity of expression was not statistically significant. However, Dash et al [13] observed significant association with NPI indicating more aggressive course and poor prognosis. Further studies including more number of patients for correlation with NPI status are needed.

ER and PR expression in p53 positive cases showed an inverse relationship. More number of ER/PR negative cases were there and majority revealed strong expression. The difference in intensity was also statistical significant. Our findings are in concordance with other studies [12, 15, 19, 21]. However, in study done by Yang et al [20] no association was seen with estrogen and progesterone receptor negative status which may be due to limited sample size and selection bias. More studies with larger sample size needed to confirm our observation of association with ER/PR status. (Table 6)

Table 6: Comparison of correlation of p53 with ER and PR of present study with previous studies

STUDY	NO. OF CASES	P53	ER +	ER -	P value (ER)	PR +	PR -	P Value (PR)
Yang et al[20] (2013)	97	+ -	72.6% 64.7%	27.4% 35.3%	0.491	62.1% 37.9%	37.9% 62.1%	0.379
Abubakar et al[23] (2019)	7226	+ -	71.6% 80.9%	28.4% 19.1%	1.64	70.2% 76.8%	29.8% 23.2%	2.31
Nishimura et al[21] (2020)	4463	+ -	10% 90%	49.9% 50.1%	0.0001	8.8% 91.8%	39.1% 60.9%	0.0001
Ali et al[15] (2022)	86	+ -	75.51% 24.49%	91.89% 8.11%	0.047	75.51% 24.49%	91.89% 8.11%	0.047
Present study	60	+ -	56% 44%	62.86% 18.18%	0.043	56.63% 43.47%	62.17% 37.83%	0.002

No difference in p53 expression was seen in our study between Her2neu positive and negative cases. This is correlated with the studies done by[15,20]. However, our findings are in contrary to other studies [12, 21] which suggest that coexistence of overexpression of Her2neu and p53 protein accumulation is a strong prognostic marker in breast cancer. Studies involving more number of patients are needed for confirmation.

Table 7: Comparison of p53 with Her2neu in previous studies and present study

STUDY	NO. OF CASES	P53	HER2NEU +	HER2NEU -	P Value
Yang et al[20] (2013)	97	+ -	51.7% 44.1%	48.3% 55.9%	0.513
Nishimura etal[21] (2020)	4463	+ -	43.6% 56.4%	13.2% 86.8%	0.0001
Ali etal[15] (2022)	86	+ -	33.8% 13.33%	66.2% 86.67%	0.117

Present study	60	+ -	66.67% 33.33%%	58.33% 41.67%	0.598

Ki67 is a proliferative marker associated with poor histological parameter. Various studies done previously revealed p53 expression correlation with Ki67 status. However, in few no association has been seen [7, 11].

In our study also no association was p53 positive and negative expression in low and high Ki67 index. However, statistical association has been observed in intensity of expression between low and high Ki67 index with more number of cases showing strong expression(85.72%) with high Ki67 index. The variability of results may be due to different molecular subtypes included in various studies and different P53 and Ki67 cut off taken.

Breast cancer is divided into molecular subtypes based on hormone receptor and Her2neu status with therapeutic significance including Luminal A, Luminal B, Her2neu enriched and basal like(Triple negative). All of these have targeted therapies except triple negative breast cancer in which chemotherapy is the mainstay of treatment and has worst prognosis. Variable results of p53 expression have been observed in relation with molecular subtypes with most of the studies showing high p53 expression in TNBC and Her2neu enriched type but with variable expression in Luminal A and B subtypes [15].

It has also been stated that p53 IHC can be used to identify a P53 positive Luminal A like breast cancer phenotype with high Ki67 index and more frequently expressing basal markers.[20]

In our study, Her2neu enriched tumour cases revealed highest degree of p53 expression with maximum number of cases(85.72%) revealing strong expression. There was no difference in percentage positivity p53 expression between Luminal A, B and triple negative subtypes. Triple negative tumour cases however revealed greater degree of p53 expression with 12 out of 14 positive cases revealing strong expression and this difference was statistical significant.

Further studies may be needed for role of p53 IHC in various molecular subtypes as well as further refining luminal A like breast cancer into subgroups with prognostic and possibly therapeutic implications.

Table 8: Correlation of p53 with molecular subtypes in previous studies and present study

STUDY	P53	LUMINAL A	LUMINAL B	HER2 enriched	TRIPLE NEGATIVE	p-Value
Kikuchi et al[19] (2013)	+ -	9% 91%	37% 63%	51% 49%	52% 48%	0.0001
Nishimura et al[21] (2020)	+ -	2.1% 97.9%	13.4% 86.6%	53.1% 46.9%	48.6% 51.4%	0.0001
Dash et al[13] (2021)	+ -	62.5% 37.5%	25% 75%	83.4% 16.6%	61.5% 38.5%	0.075
Ali et al[15]	+ -	68.57% 31.43%	92.86% 7.14%	91.67% 8.33%	92% 8%	0.045
Present study	+ -	55% 45%	57.15% 42.85%	70% 30%	60.87% 39.13%	0.039

Limitations of the study

One of the main limitations of this study was relatively small sample size with few number of cases in each category and no direct evaluation of the prognostic value of p53 due to lack of information on clinical outcomes.

The majority of the cases were categorized as histological Grade II, with fewer cases in Grades I and III. This imbalance in the distribution of histological grades made it difficult to establish a statistically significant association. To strengthen the findings and achieve more reliable conclusions, a larger sample size with a more even distribution of cases across all histological grades would be necessary.

CONCLUSION

Breast cancer is the leading cause of death in female worldwide. IHC has provided enormous benefits in evaluating prognostic and therapeutic markers in breast cancer.

P53 IHC has been suggested to further refine molecular subtype with prognostic and, probably, therapeutic implications. However, evidences are not strong enough for p53 status to be recommended as a routine marker in clinical practice. Variable association between IHC p53 expression and clinicopathological parameters as well as molecular subtypes has been observed. We observed no significant difference in p53 expression with various clinicopathological parameters, hormone receptor expression and molecular subtypes, however, statistical significant difference in intensity of expression was seen with hormone receptor, Ki67 index and molecular subtypes indicating it can be used as poor prognostic marker in breast cancer.

IHC p53 may defines a subgroup of breast carcinoma patients with high tumor aggressiveness and poor prognosis. As a novel independent prognostic marker p53, might aid in identifying subgroups of patients who are more likely to have a poor outcome and to whom specific therapies might be directed including TNBC category.

Further researches on p53 expression in breast carcinoma should be conducted on a larger scale, incorporating follow-up and survival data, to validate the role of p53 in the etiology and progression of breast cancer, enabling the design of prognostic groups and tailored treatment strategies.

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