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To compare the expression of p53 with hormonal receptor status in breast carcinoma: An immunohistochemical study

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Abstract
Background: Breast carcinoma is the most common malignancy in women in the world.
This study evaluated the expression of ER, PR and p53 in breast carcinoma and its correlation with age, size, lymph node status and other parameters.
Methods: 103 histologically proven Breast carcinoma cases were subjected to IHC for ER,PR and p53.
Result: Total cases of breast cancer were 103. All the cases were between 21-60 years with tumour size varying from 0.5->5cm. Maximum cases were of grade III. Metastatic carcinomatous deposits in lymph nodes were seen in 53 cases. Tumors were separated into 4 categories depending on ER/PR expression with ER-ve/PR-ve category having maximum number of cases. As the tumour grade increased lesser was the ER PR expression. P53 positivity was noted in 60 cases comprising of 58.3%. Significant correlation was noted while comparing p53 with lympho vascular invasion (p=0.042) but not with age, tumour size, tumour grade and lymph node status. Significant correlation was also noted while correlating p53 and ER/PR expression with tumour size (p = 0.036) but not with tumour grade, lympho vascular invasion and lymph node status.
Conclusion: In the present study it was observed that ER PR status is inversely proportional to p53 expression. It is concluded that p53 has direct relationship with tumour grade, tumour size, lymphovascular invasion. Thus p53 helps to provide prognostic information and better treatment options

Keywords: lymphovascular invasion, IHC for ER, PR

Introduction
Carcinoma of the breast is the most common malignancy in women in the world. It is a multifactorial disease comprising of different biological subtypes, diverse natural history, variable clinical, pathologic and molecular features and variable prognostic and therapeutic implications. Pathological variables like tumor size, histological type and grade, lymph node metastasis, vascular invasion, tumor cell proliferation and ductal carcinoma in situ are the prognostic predictors and also forecast the need for adjuvant therapy [1].
Screening detects some cancers before they have metastasized, hence by using screening methods such as ultrasound and mammography, conducting self-examination, the age of breast cancer incidence has reduced in the last few years [2]. However, reappearance may be seen in 20%-30% of breast cancer patients despite the employment of the effective therapies based on presented prognostic factors [3].
Tumor markers are molecules occurring in tissues that are associated with cancer. Identification of these markers is useful in diagnosis and treatment [2]. Prognostic factors include patient's age, tumor type, tumor size, tumor grade, number of involved lymph nodes at the time of tumor diagnosis [2]. Predictive factors are distinguished from prognostic factors in that the latter can be measured and are associated with the nature of the disease, whereas the former determine the response to treatments [4]. Some factors are both prognostic and predictive, these include estrogen receptor (ER) and progesterone receptor (PR) status, p53 mutation status, and human epidermal growth factor receptor (HER2/neu) overexpression. Patients with ER/PR positive tumors are hormone receptive and consequently have a considerably better prognosis as compared with the patients with ER/PR negative tumours [4]. Nearly one third of breast cancers have mutation in p53, a tumor suppressor gene p53 is the first tumor suppressor gene to be identified. It is located on short arm of chromosome 17.

It is the chief regulator of genomic stability through regulation of the cell cycle. The most frequent genetic alteration in breast cancers and in other malignancies such as ovarian, esophageal and GIT is the over expression of p53 caused by TP 53 mutation.[4] Mutated p53 accumulates in the nucleus of tumor cells and consequently it can be detected by immunohistochemistry.[1] Breast tumors expressing a high amount of p53 are more frequently ER-ve/PR-ve and are also associated with a high proliferation rate, high histological and nuclear grades, aneuploidy and poorer survival [5].

Materials and Methods

The study was conducted on 103 proven cases of breast carcinoma in the department of pathology, Sri Guru Ramdas Institute of Medical Sciences and Research, Amritsar. The tissue was formalin fixed and paraffin embedded and was then stained for haematoxylin and eosin for histopathological confirmation, typing and grading. All the cases then were subjected to immunohistochemistry for ER, PR, and P53 expression. Positive controls were run with every batch of IHC.

Results and Discussion

A total of 103 patients were taken in study. Out of this 101 were female patients and 2 were male patients. Age of the patients varied from 21-60 years. Maximum cases were from 41-60 years of age. One patient was <30 years old.

Study on breast cancer by Patnayak *et al*. reported a mean age of 50.7 years [6]. Piplani *et al*. reported maximum number of cases in age group 41-50 years [7]. Due to amplified screening of the breast cancer and sophisticated imaging techniques, there has been an rise in incidence of breast cancer in premenopausal age group.

Left side was more commonly involved (55.3% cases) than right side (44.7% cases). Upper outer quadrant was most commonly involved (63% cases). Least involved quadrant was upper inner (6% cases). Few cases of retroareolar growths were also seen (11% cases). Amer MH has reported upper outer quadrant involvement in 52.5% cases [8].

Tumour size is a well-established prognostic factor. In present study, tumour size varied from 0.5cm to >5 cm. In maximum number of cases, size varied from 2-5cm (74.8% cases) followed by 16 cases (15.5%) with >5cm size and 10 cases with <2cm (9.7%) size. This is in concurrence with the results obtained by Patnayak *et al*. [6], Piplani *et al*. [7], Gupta *et al*. [19], Arora *et al*. [1] and Neharika *et al*. [10] who found 79.6%, 67.7%, 63.9% and 59.6% and 53.9%. Another Study conducted by Prabhu *et al*. showed maximum number of cases with tumour size between 2-5 cm in size [11].

Histological type is one of the main prognostic factors in breast carcinoma. In this study,out of 103 cases, 96 cases (93.2%) were of IDC-NOS type, 4 cases (3.9%) were of mucinous type, 2 cases (1.9%) were of Invasive lobular type, 1 case (1.0%) was of carcinosarcoma histological type. This was in concordance with observations of Patnayak *et al*. [6], Gupta *et al*. (86.1%) [9], Arora *et al*. (84.6%) [1] and Sheikpour *et al*. (84.6%) [12]. Which had IDC-NOS as the most common histological type.

Bloom Richardson grading (Nottingham modification) is the method for grading of breast tumour. It includes three criteria namely tubule formation, nuclear pleomorphism and mitosis. In present study, 45 cases (43.7%) were of Grade II (Figure 1) and 51 cases (49.5%) were Grade III (Figure 2). No patient with Grade I cases was encountered. Grade was not granted to Mucinous, lobular and carcinosarcoma histopathological types and these constituted 7cases (6.8%). Similar observations were made by Piplani *et al*. (64.6%) [7] and Neharika *et al*. (46%) [10] with grade III being the most common grade. However observations by Shoukouh *et al*. (54.8%) [2], Gupta *et al*. (45.8%) [9] and Patnayak *et al*. (60.9%) [6] reported a higher frequency of grade II tumours. Due to lack of routine mammographic screening, there is difference in tumour grades in different studies.

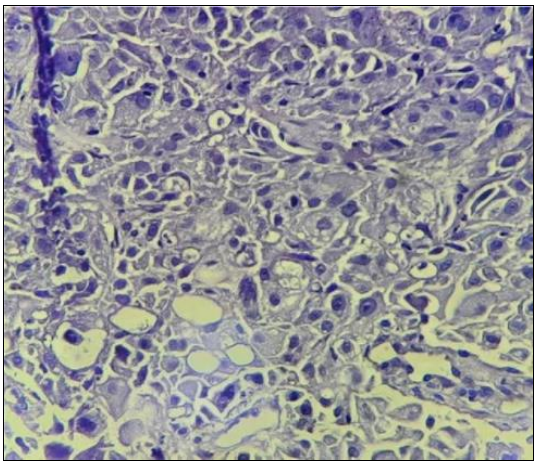

Fig 1: IDC Grade II

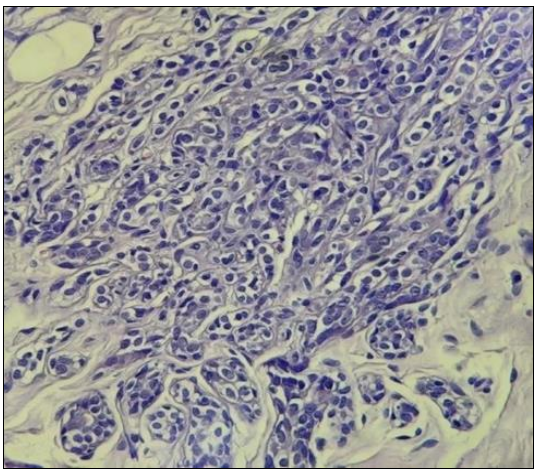

Fig 2: IDC Grade III

In present study, Lymph nodes were recovered in 97 cases (94.17%). Axillary lymph node metastatic carcinomatous deposits were present in 53 cases (48.62%). Out of this 21 cases (20.38%) were of N1 stage (1-3 positive nodes), 18 cases (17.47%) were of N2 stage (4-9 positive nodes) and 14 cases (13.59%) were of N3 stage (>10 positive nodes). Reactive lymph nodes were present in 44 cases (42.7%).

On correlating tumour size with lymph node status, 6 cases of size <2cm, 39 cases of size 2-5cm and 8 cases with size >5cm showed axillary metastatic carcinomatous deposits. 25 cases with grade II, 27 cases with grade III and 1 case with no grade showed axillary metastatic carcinomatous deposits. No significant correlation was noted while correlating tumour grade (p=0.212) and tumour size (p=0.166) with lymph node status. However observations made by Piplani *et al*.[7] and Shokouh[2] *et al*. showed a direct relationship between increased tumour size and lymph node status.

Lymphovascular invasion (LVI) was seen in 87 cases (84.5%) and 16 cases (15.5%) were negative for LVI.While correlating the LVI with the histopathological grade of the

tumour, it was seen that 25 cases in grade II and 12 cases in grade III were positive for LVI. A p value of 0.003 was obtained which indicated a significant correlation. As the tumor size increased more cases were negative for LVI. (Table 1).

Table 1: Showing correlation of lympho vascular invasion with tumour grade

Histological grade	LVI Present	LVI Absent	Total	Chi value	P value
No grade	4	3	7	11.17	0.003
Grade II	25	20	45		
Grade III	12	39	51		
Total	41	62	103		

ER/PR Scoring

ER and PR are nuclear receptors. In Allred system of scoring, (Tables 2, 3, 4).

1. Score 0-5 is given to the cells depending on the proportion of cells which are stained (proportion score [PS]) and
2. Score 0-3 is given depending on the staining intensity (intensity score [IS]).
3. By adding the PS and IS, we can calculate the final Allred score (PS + IS = AS)

Table 2: Proportion score

Score	Percentage of stained cells
0	No cells are ER positive
1	<1% cells are ER positive
2	1-10% cells are ER positive
3	11-33% cells are ER positive
4	34-66% cells are ER positive
5	67-100% cells are ER positive

Table 3: Intensity Score

Score	Intensity of staining
0	Negative
1	Weak
2	Intermediate
3	Strong

Table 4: Allred score (allred score = proportion score + intensity score)

Allred score	Effect of hormone therapy
0-1	No effect
2-3	Small(20%) chance of benefit
4-6	Moderate(50%)chance of benefit
7-8	Good(75%)chance of benefit

In present study ER positivity (Figure 3) was noted in 41 cases (42.23%) and Progesterone receptors (figure 4) were found to be positive in 62 cases (63.86%). On the basis of ER/PR expression, tumours were categorized into 4 categories that is ER+ ve/PR+ ve, ER+ ve/PR-ve, ER-ve/PR-ve, ER-ve/PR+ve. It was observed that out of 103 cases, 59 cases (57.3%) were ER-ve/ PR-ve and 38 cases (36.9%) were ER+ve/ PR+ve (Table 5). Arora *et al.*, [1] Neharika *et al.*, [10] Piplani *et al.* [7] and Patnayak *et al.* [6] reported a prevalence of 23.1%, 38.16%, 41.5% and 41.6% ER positive cases respectively. Piplani *et al.*, [7] Arora *et al.* [1] and Patnayak *et al.* [6] reported 40%, 36.5% and 48.8% PR positive cases respectively.

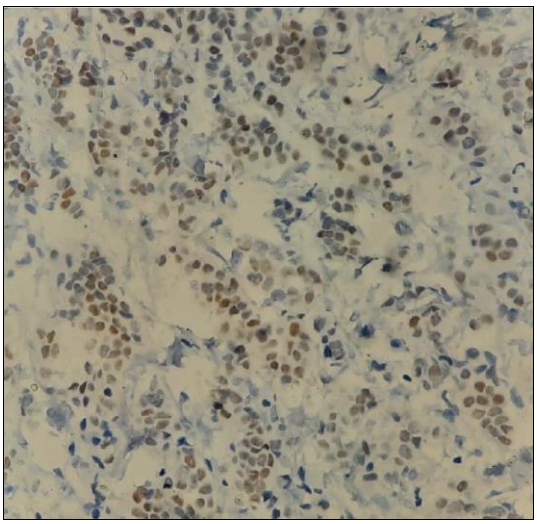

Fig 3: ER Positivity

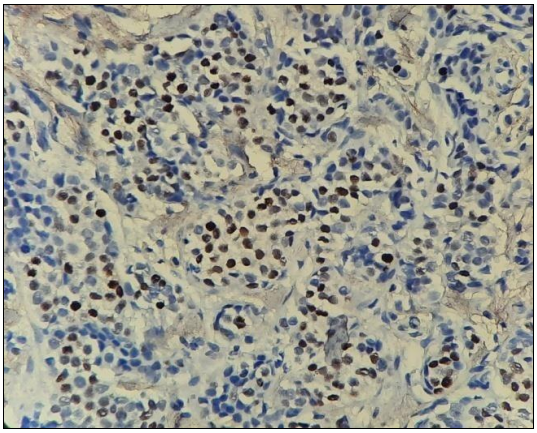

Fig 4: PR Positivity

Table 5: Showing hormone receptor positivity

Combination of ER, PR	No. of cases	Percentage
ER + PR +	38	36.9%
ER - PR -	59	57.3%
ER + PR -	3	2.9%
ER - PR +	3	2.9%

While comparing ER PR expression with tumour size, it was observed that ER positivity was 60% in cases with tumour size <2cm, 36.4% in cases with tumour size 2-5cm and 43.7% in cases with tumour size >5cm. PR positivity for three groups was 50%, 39% and 37.5% respectively. Tumours with size 2-5 cm had maximum ER PR positive cases as well as ER PR negative cases. As the tumour size increased, lesser was the ER PR expression. A statistically significant (p=0.010) correlation was noted between the variables. (Table 6)

Piplani *et al.* [7] observed ER positivity of 52.6%, 36.4% and 50% in cases with tumour size <2cm, 2-5 cm and >5cm respectively. Similarly PR positivity for three groups was 57.9%, 31.8% and 50% respectively.

When ER, PR was correlated with tumour grade. ER positivity was noted in 53.33% cases with grade II and in 25.49% cases with grade III. PR positivity was noted in 55.6% cases with grade II and in 23.5% cases with grade III. As no grade I cases were encountered, 57.14% cases with no grade were ER positive and 57.1% cases with no grade were PR positive. 22 cases of Grade II were ER + ve PR + ve and 38 cases of grade III were ER –ve PR -ve. As the tumour

grade increased lesser was the ER PR expression. Statistically significant correlation (p = 0.021) was noted amongst these them (Table 7).This was in concordance with study by Piplani *et al*. [7]. They observed ER positivity to be 53% in grade II tumours and 28.6% in grade III tumours. PR positivity in their study was 58.8% in grade II tumours and 23.8% in grade III tumours.

Table 6: Showing correlation of ER/PR with tumour size

Size	ER + PR +	ER - PR -	ER + PR-	ER - PR+	Total	Chi value	P value
<2cm	4	3	2	1	10	16.62	**0.010**
2-5cm	28	47	0	2	77		
>5cm	6	9	1	0	16		
Total	38	59	3	3	103		

Table 7: Showing correlation of ER, PR with tumour grade

Grade	ER+ PR+	ER-PR-	ER+ PR-	ER- PR+	Total	Chi Value	p-value
No grade	4	3	0	0	7	14.83	0.021
Grade II	22	18	2	3	45		
Grade III	12	38	1	0	51		
Total	38	59	3	3	103		

On comparing LVI with ER/PR expression it was observed that LVI was absent in maximum number of cases which were ER+PR + (31 cases). Non-significant statistical correlation was noted between ER PR expression and LVI (p=0.717).

It was observed that out of 38 ER+ve/PR+ve cases, 59 ER-ve/PR-ve, 3 ER+ve/PR-, 3 ER-/PR+ cases, cases which showed axillary secondary carcinomatous deposits were 18 cases (47.4%), 32 cases (54.23%), 2 cases (66.7%), I case (33.3%) respectively. No statistically significant correlation was noted between ER/PR expression and lymph node status (p=0.938). Study by Piplani *et al*. [7] revealed 42.1% cases in ER+ve/PR+ve category and 72.8% cases in ER-ve/PR- category showed secondary carcinomatous deposits.

P53 scoring

P53 staining takes into account the intensity of staining and the percentage of nuclei stained. Total score was then obtained by the sum of intensity score and percentage score, ranging from 0-6. (Table 8)

Table 8: P53 scoring

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

P53 positivity (figure 5) was noted in 60 cases comprising of 58.3% while 42 cases (41.7%) were p53 negative. Percentage of positive cells varied from <10% to >50% with weak, intermediate and strong intensity. In many other studies by Arora *et al*., [1] Patnayak *et al*., [6] Sekar *et al*., [13] Radha *et al*., [14] Neharika *et al*. [10] p53 positivity was observed in 61.5%, 69.2%, 71.6%, 22%, 47.4% cases respectively. Variability in the p53 expression may be attributed to the diverse range of procedures and reagents used by different investigators.

Maximum number of cases with p53 positivity were >60 years of age comprising 21 cases (65.6%). While 15 cases each of age group 41-50 (55.6%) years and 5160(55.6%) years were p53 positive No significant correlation (p=0.681) was noted amongst the two. Piplani *et al*. [7] observed high p53 expression in age group 41-50 years.

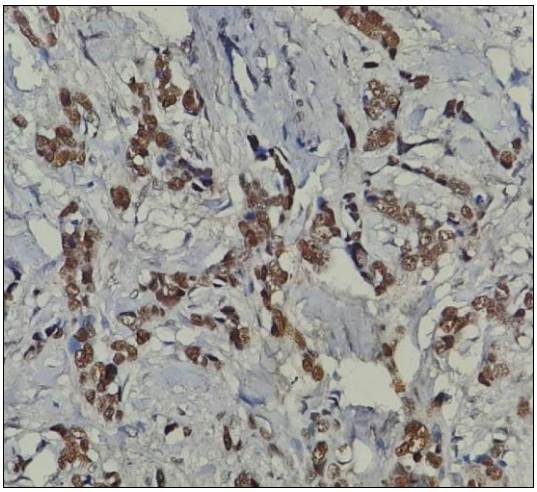

Fig 5: P53 Positivity

In this study p53 expression demonstrated a reverse relationship with patients having tumour size 2-5cm showed showing higher p53 positivity (59.7%). The results were however not significant statistically (p=0.363). Similar results were obtained by Piplani *et al*. [7] (73.3%) and Gupta *et al*. [9] (63.9%) in their study.

In our study p53 expression in grade II and grade III tumours was found to be

57.8% and 54.9% respectively. No significant correlation was noted between the two (p=0.299). This is in concordance with Sheikhpour *et al*. [12]. However in contrast to our study many studies by Neharika *et al*., [10] Shoukouh *et al*., [2] Piplani *et al*. [7] Sekar *et al*. [13] and Gupta *et al*. [9] have reported significant positive association between tumour grade and p53 expression.

While correlating p53 expression with lymph node status, out of the 60 cases showing p53 positivity, 32(53.3%) cases had secondary carcinomatous deposits, 24cases (40%) had reactive lymph nodes and in 4cases(6.6%) no lymph nodes were recovered . While in p53 negative category, out of 40 cases, 21cases (52.3%) had secondary carcinomatous deposits, 20cases (50%) had reactive lymph nodes and no lymph nodes were recovered in 9 cases (22.5%) No

significant correlation was noted between p53 expression and lymph node status (p=0.770). This was in concordance with Shoukouh *et al.* [2] and Piplani *et al.* [7] In contrast to our study, Gupta *et al*., [9] Abdollahi *et al*. [15] and Neharika [10] *et al*. found a significant correlation between lymph node status and p53 positivity.

LVI was present in 87 cases, out of this 47 cases (54.0%) were p53 positive and 40 cases (46.0%) were p53 negative. p53 expression was seen more in cases with LVI than p53 negative cases. Hence a significant correlation (p=0.042) was noted amongst the two (Table 9). In contrast to this study no correlation was observed between these variables in a study by Arora *et al.* [11]

Table 9: Correlation of p53 with lymphovascular invasion

	P53 positive		P53 negative		Total	Chi value	P value
Lymphovascular Invasion	**N**	**% age**	**N**	**% age**		**4.110**	**0.042**
Present	47	54.0%	40	46.0%	87		
Absent	13	81.3%	3	18.8%	16		
	60	58.3%	43	41.7%	103		

While correlating p53 with ER/PR it was observed that p53 expression was found to be higher in ER-ve/PR- vecategory (57.6%) as compared to patients who were hormonally positive i.e. ER+ ve/ PR + ve but no significant correlation(p=0.119) was noted amongst them (Table 10). ER-ve/PR-ve group constituted the largest group of p53 positive cases and showed that EP, PR and p53 are inversely related. Similar inverse relation has been demonstrated by Piplani *et al.* [7] However Neharika *et al*., [10] Arora *et al*., [1] Shoukouh *et al.* [2] found a significant correlation between ER, PR and p53 expression.

Table 10: Showing correlation of ER and Pr with p53

	P53 positive		P53 negative		Total	Chi Value	p value
ER PR status	**N**	**%age**	**N**	**%age**		**5.849**	**0.119**
ER + PR+	25	65.8%	13	34.2%	38		
ER-PR-	34	57.6%	25	42.4%	59		
ER+PR-	1	33.3%	2	66.7%	3		
ER-PR+	0	0.0%	3	100.0%	3		
Total	60	58.3%	43	41.7%	103		

Since the maximum number of cases were of tumour size 2-5cm i.e 77 cases, out of this, 27 cases of ER –ve/PR-ve category were p53 positive and 20 cases of ERve/PR-ve category were p53 negative. Significant correlation (p=0.036) was noted while comparing ER, PR and p53 with tumour size (Table 11).

Table 11: Showing correlation of p53 and ER, PR with tumour size

Size	P53 Positive				P53 negative				Total	Chi value	P value
	ER+PR+	**ER PR**	**ER+PR**	**ER PR+**	**ER+PR+**	**ER PR**	**ER+PR**	**ER PR+**		**22.087**	**0.036**
<2cm	3	3	1	0	1	0	1	1	10		
2-5cm	19	27	0	0	9	20	0	2	77		
>5cm	3	4	0	0	3	5	1	0	16		
Total	25	34	1	0	13	25	2	3	103		

As Grade III cases were maximum in this study i.e. 51, out of these 51 cases of grade III, 7 ER+ve/PR+ve cases, 21 ER-ve/PR-ve cases were p53 positive and 5 ER+ve/PR+ve cases 17 ER-ve/PR-ve cases and 1 ER+ve/PR-ve case were p53 negative. In this study, it was observed that out of 51 cases of Grade III tumours, 28 cases showed p53 positivity (54.9%) and 21 cases showed ER/ PR negativity (41.2%). In grade II tumours, out of 45 cases, 26 showed p53 positivity (57.8%) with 11 ER/PR negative cases (26.4%). This shows as the grade of tumour increases p53 positivity increases and ER/PR positivity decreases suggesting that ER/PR are inversely related to p53 status. But No statistically significant correlation was noted amongst them (p=0.082).

Maximum number of cases with LVI were p53 postive and ER/PR negative. No significant correlation was noted amongst them (p=0.566).

P53 positivity was seen in maximum number of cases with axillary secondary carcinomatous deposits. When this was correlated with ER/PR status, it was non-significant (p=0.997).

Conclusion

Thus it is concluded that p53 has direct relationship with tumour grade, tumour size and lymphovascular invasion. It is highlighted that all breast carcinoma cases should be stained for p53, if found positive, aggressive intevention is required. Thus p53 helps to provide prognostic information and better treatment modalities.

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Competing Interests

Nil

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