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The Expression and Prognostic Value of p53 Gene in Invasive Ductal Carcinoma Breast: A Study in Mahatma Gandhi Memorial Medical College Indore M.P.

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Abstract: ***The p53 gene is a tumour suppressor gene. The significance of p53 expression is that p53 mutation is correlate with chemo-resistance and transformation to more aggressive disease in infiltrating duct carcinoma breast. In addition, the expression of p53 has been closely correlated with clinicopathological findings like lymph node positivity, recurrence of disease, distal metastasis, and poor outcome. The expression of p53 was studied in 100 cases of invasive ductal carcinoma of the breast, received in department of histopathology in MYH hospital Indore M.P. Detection of p53 done by HRP polymer method if immunohistochemistry. It was found that p53 was expressed in 86% of all the study cases.Expression of other markers like ER/PR, Her2/neu and ki67 also performed on same specimen. Furthermore, its expression was significantly correlated with tumor grade, lymph node status and with other IHC marker for breast. Knowledge of the p53 status may be valuable in making clinical decisions regarding diagnosis, prognosis and therapy.***

Keywords: Breast cancer, p53 expression, clinical prognostic factor

1. Introduction

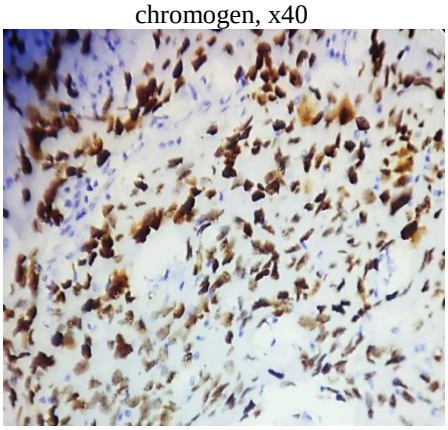
P53 is first tumour suppressor gene to be identified located on chromosome 17[(1)] p53 first described in 1979, initially it was identified as an oncogene. Functions of p53 is to eliminate and inhibit the proliferation of abnormally proliferating tumor cell and thereby preventing neoplastic development. [(2)] The p53 gene is a identified as the most commonly mutated gene in human cancer [(3)] ,tumor where p53 mutation can be found in tumors of the colon [(4),] lung [(5,6)] , breast [(7,8),] ovary [(9),] bladder[(10)], brain[(11)] and other site. P53 gene codes for a nuclear phosphoprotein it controls the progression of cells from the G1 phase to S phase of the cell cycle and play an important role in the regulation of the cell proliferation. Mutations in p53 or deletion chromosome have been shown to be associated with poor prognosis in breast cancer [(12, 13, 14).] Li-Fraumeni syndrome is an inherited mutation of p53 gene and present in various familial forms of breast cancer [(15,16)] Various type of human cancers can be found Li-Fraumeni syndrome that are breast carcinoma, soft tissue sarcomas, osteosarcoma, brain tumors, leukemia and adrenocortical carcinoma. In normal cell wild-type, p53 protein can reduce the tumor generation potential of a cell line [(17,18)]. The mutant form of protein, this could prevent the wild-type subunit from functioning. Therefore, the ratio of mutant to wild-type p53 protein in a cell may be critical in regulation of cell division. Wild-type p53 protein is unable to detect in normal cells by immunohistochemical methods because of its short half-life and to the low amount of detectable p53 protein. Mutations of p53 gene result in stabilisation of the protein and now the levels of p53 protein can be detectable by immunohistochemistry most frequently indicate the expression of a mutant form of p53 gene [(19)] .The aim of this work was to investigate the expression of p53 in infiltrating ductal carcinoma of the breast, as well as to study the clinicopathological associations with p53 expression.

2. Materials and Method

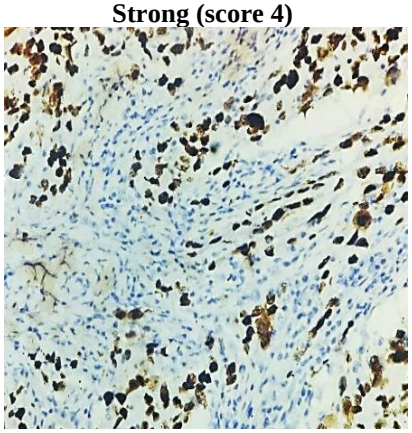
A total of 100 records of patients with infiltrating ductal carcinoma of the breast were submitted in department of pathology in MYH hospital in Indore, from 2016 to 1018. The clinical data obtained from hospital record the records. Histopathology and Immunohistochemistry was performed for histologic type and tumor grade, estrogen receptor status, progesterone receptor status, HER2/neu, Ki67 and p53 status. Ethical approvals were obtained. Fresh samples of breast cancer tissue obtained from the operations theatre were fixed in 10% formalin within 13 hours at room temperature. Older tissue samples in paraffin wax blocks were obtained from the Departments of Pathology of MYH hospitals Indore.For the tissue detection of p53, 4 µm breast cancer tissue sections were deparaffinised and rehydrated. The sections were heated in a pressure cocker in 0.01 M sodium citrate buffer, pH 6.0. A mouse anti-human p53 antibody (prediluted), was added and incubated for one hour.Ihc performed by HRP polymer method. Nuclear staining marked the positive expression of p53 (Fig1). The Pearson Chi-square test (Pearson $\chi 2$) and Spearman rank correlation were measured using SPSS software.Immunoreactivity scoring of p53-(C.J. Fishser*et al.*, 1994: Problem with p53 immunohistochemical staining: the effect of f variation in the methods of evaluation)

Score	Staining character
Strong 4	Dark nuclear staining that is easily visible with a low power objective and involves > 5o% of cells
Moderate 3	Focal darkly staining areas, (< 5o% of cells) or moderate nuclear staining of > 5o% of cells
Weak 2	Focal moderate staining in < 5o% of cells or pale nuclear staining in any proportion of cell not easily seen under a low power
Scattered 1	Dark nuclear staining of widely scattered cell
Negative 0	Tumor that show none of above

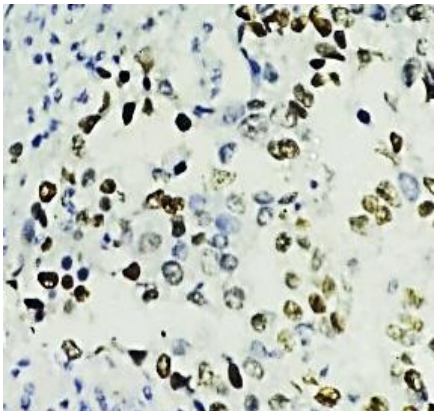
Figure 1: Anti-p53- poly horseradish peroxidase- DAB chromogen, x40



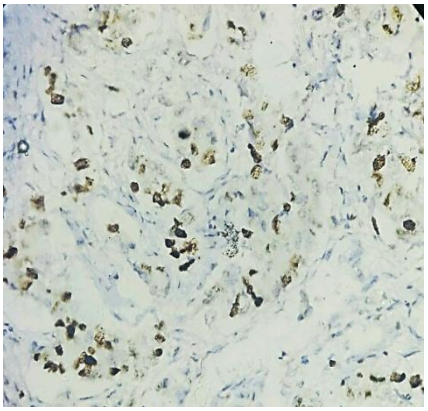
Strong (score 4)



Moderate (Score 3)



Weak (score2)



Scattered (score 1)

3. Result

Table 1: Association between P53 and ER/PR

			ER/PR		Total
			Negative	Positive	
p53	0	Count	0	7	7
		% within ER	0.0%	23.3%	14.0%
	1	Count	0	7	7
		% within ER	0.0%	23.3%	14.0%
	2	Count	0	6	6
		% within ER	0.0%	20.0%	12.0%
	3	Count	9	3	12
		% within ER	45.0%	10.0%	24.0%
	4	Count	11	7	18
		% within ER	55.0%	23.3%	36.0%
Total		Count	20	30	50
		% within ER	100.0%	100.0%	100.0%

Pearson Chi Square = 22.801,df = 4, P value = 0.000, Significant.

The above table shows association between P53 and ER.. The difference was found to be statistically significant ($p<0.05$).

Table 3: Association between P53 and HER 2/neu

			HER 2/neu		Total
			Negative	Positive	
p53	0	Count	7	0	7
		% within HER2/neu	21.9%	0.0%	14.0%
	1	Count	6	1	7
		% within HER2/neu	18.8%	5.6%	14.0%
	2	Count	5	1	6
		% within HER2/neu	15.6%	5.6%	12.0%
	3	Count	6	6	12
		% within HER2/neu	18.8%	33.3%	24.0%
	4	Count	8	10	18
		% within HER2/neu	25.0%	55.6%	36.0%
Total		Count	32	18	50
		% within HER2/neu	100.0%	100.0%	100.0%

Pearson Chi Square = 10.352,df = 4, P value = 0.035, Significant

The above table shows association between P53 and HER 2/neu. The difference was found to be statistically significant ($p<0.05$).

Table 4: Association between P53 and Lymph Node Status

P53		Lymph Node Status		Total
		Negative	Positive	
0	Count	4	3	7
	%	22.2%	9.4%	14.0%
1	Count	2	5	7
	%	11.1%	15.6%	14.0%
2	Count	2	4	6
	%	11.1%	12.5%	12.0%
3	Count	5	7	12
	%	27.8%	21.9%	24.0%
4	Count	5	13	18
	%	27.8%	40.6%	36.0%
Total	Count	18	32	50
	%	100.0%	100.0%	100.0%

Pearson Chi Square = 2.240,df =42, P value = 0.692, Non Significant

The difference was found to be statistically non significant (p<0.05).The table shows higher percentage of score 4 of P53 for positive Lymph Node Status. (40.6%) whereas score 3 and score 4 of P53 were equally higher (27.8%) each for negative Lymph Node Status patients.

Table 5: Association Between p53 and Histological Grade

P53		Histological Grade			Total
		Grade I	Grade II	Grade III	
0	Count	5	2	0	7
	%	35.7%	9.5%	0.0%	14.0%
1	Count	5	2	0	7
	%	35.7%	9.5%	0.0%	14.0%
2	Count	3	3	0	6
	%	21.4%	14.3%	0.0%	12.0%
3	Count	1	6	5	12
	%	7.1%	28.6%	33.3%	24.0%
4	Count	0	8	10	18
	%	0.0%	38.1%	66.7%	36.0%
Total	Count	14	21	15	50
	%	100.0%	100.0%	100.0%	100.0%

Pearson Chi Square = 28.529,df =82, P value = 0.000, Significant

The above table shows association between P53 and Histological Grade. The difference was found to be statistically significant (p<0.05).The table shows higher percentage of p53 of grade III for Histological Grade (66.7%) whereas score 0 and score 1 are equally higher in Grade I (35.7%) and score 4 for Grade II (38.1%) for Histological Grade.

Table 6: Association between P-53 and Lymph Node Status

			Lymph Node Status		Total
			Negative	Positive	
P-53	0	Count	4	3	7
		%	22.2%	9.4%	14.0%
	1	Count	2	5	7
		%	11.1%	15.6%	14.0%
	2	Count	2	4	6
		%	11.1%	12.5%	12.0%
	3	Count	5	7	12
		%	27.8%	21.9%	24.0%
	4	Count	5	13	18
		%	27.8%	40.6%	36.0%
Total		Count	18	32	50
		%	100.0%	100.0%	100.0%

Pearson Chi Square = 2.240,df = 4, P value = 0.692, Non Significant

The difference was found to be statistically non-significant (p<0.05).

4. Discussion

JolantaKupryja et al[20] in 1992 found expression of p53 was 22% in sporadic and 34% in familial breast cancer, In present study we found 86% cases nonspecific invasive duct carcinoma breast express p53.Prabha B. Rajan et al in 1997[21]was found that expression of p53 was maximum with higher tumor grades. In our study we found a significant association between p53 expression and tumor grades (p<0.05) score 3 and 4 of p53 are associated with higher tumor grade Grade III tumor.Rakha EA et al[22]2007 studied that grade I, 9 cases of grade II and 256 cases of grade III. P53 positivity was 28% and lymph node positivity was found in 104 cases. Triple negative tumor are more associated with high tumor grade. (p<0.001) In our study we also found similar significant association (p<0.005) triple negative 0 with grade I, 6 (54.2%) with grade II and 5 (45.5%) with grade III tumor. Triple negative breast cancer are more commonly associated with higher tumor grades.F S Al-Joudi et al[23] in 2008 also found similar association between p53 and lymph node invasion.K.J. Ranade et al[24]in 2009 studied thatp53 was observed that in IDC I, II and III, the distinct nuclear expression of p53 was seen in 33%, 52% and 67% of the cases, respectively.In our study p53 positivity is 64.2% in grade I tumor, 90.5% in grade II tumors and 100% in grade III tumor. There is no statistically significant association is found between p53 expression and tumor grade.We did not found a significant correlation between hormone status of tumor and lymph node involvement but they found a significant correlation between them (p= 0.004, p= 0.022 respectively).Sumita A. Jain et al[25]in 2014 Her-2/neu protein receptor over-expression was present is 18.18% in grade I tumor, 27.18% grade II tumors and 52.27% grade III tumors. It is 11.1% in grade I and 44.4% in grade II and grade III. Her-2/neu protein receptor overexpression was high with high grade tumor which was significant statistically (p = 0.001). We did not get a significant association between them (p=0.08). Her-2/neu protein receptor positivity was not significantly associated with (p value >.05) of lymph node involvement. ToghipourZahirShokouh et al[26]studied thatgrade II was the most common that are 54.8% . In our study that are 42% are grade II and 28% are grade I.In our study lymph node involvement is 64% in all three grades of invasive ductal carcinoma breast. ER expression were available 64.2% of tumors, it is 60% in our study, PR expression were available in 57%, and it is 60% in our study. HER2/neu overexpression was observed in 35.4% of the tumors, it is 36% in our study. These results are in favor of present study. There is a significant correlation between tumor grade and HER2/neu overexpression, and HER2/neu was more frequently expressed with higher tumor grades in this study. We also got the similar results

5. Conclusion

100 cases of IDC were studied for histological grade, lymph node status and IHC expression for various prognostic

markers like ER/PR, HER2/neu, ki67 and p53 were correlated with parameters like lymph node involvement and grade of tumour.IDC was the most common histologic type of breast cancer. Majority of the patients at the time of presentation had grade II that is 42%.We observed for immunohistochemistry 60% cases are ER and PR positive. 36% are her2/neu. 92% tumor are positive for Ki67 and 86% for p53. Grade II and III tumors are more commonly are Her2/neu enriched and triple negative type. This association is statistically significant (P=0.005). p53 and Ki67 expression scores are more with triple negative and Her2/enriched type and less with Luminal type tumors. This association is statistically significant (P=0.05).In this study Her2/neu over expression is more common with higher grades of tumor, Higher score of p53 and Ki67 expression and this association is statistically significant (P<0.05). Her2/neu expression is more commonly associated with high incidence of lymph node involvement, But not statistically significant (p>0.05). In present study p53 scores are more with tumors having lymph node positivity. There is no statistically significant association was observed between these two.(P>0.05).

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