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STUDY THE EXPRESSION OF P53 GENE AND ITS CLINICOPATHOLOGICAL CORRELATION IN BREAST CANCER CASES.

Histopathology

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ABSTRACT

Context: Breast cancer is the most common cancer among women and second leading cause of cancer death worldwide. P53 is a tumor suppressor gene located on chromosome 17p13 with germ line mutations that convey a high risk for breast cancer. P53 is antiproliferative, helps in angiogenesis and causes programmed cell death but due to genotoxic stress or mutation, P53 loses its function thereby causing abnormal cell growth. **Aim:** To study the expression of P53 a tumor suppressor gene and its clinicopathological correlation in breast cancer cases. **Settings And Designs:** A Cross Sectional Study (Retrospective and Prospective). **Material and methods:** 50 cases of breast tumors studied over the period of 18 months from January 2021 to June 2022. Samples were received and reported in Department of Pathology, Gandhi Medical College Bhopal, Madhya Pradesh. **Results:** A total of 50 cases of breast carcinoma were included in this study predominated by infiltrating ductal carcinoma (78%). High Immunohistochemistry expression of P53 was statistically significant (p value <0.05) with age, tumor size, tumor grade, lymph node metastasis and ER receptor status whereas no correlation was found with stage of tumor. **Conclusion:** P53 functions as a negative regulator of cell growth. Breast cancer aggressiveness appears to be directly related to the percentage of p53 positive cancer cells and will help in determining prognosis.

KEYWORDS

P53, Breast Cancer, Immunohistochemistry.

INTRODUCTION:

The most common cancer in women is breast cancer [1], which has surpassed lung cancer as the main cause of cancer globally.[2] It is a complex molecular disorder that develops as a result of genetic mutation. An inherited susceptibility gene or genes are thought to be responsible for one-third to one-quarter of breast cancer cases.[3]

p53 is located on chromosome 17p13 being the most frequent mutated gene in many cancers including breast carcinoma. It is antiproliferative, helps in angiogenesis and in programmed cell death(apoptosis). Due to genotoxic stress and mutation of gene it loses it functions thus causing abnormal cell growth.[4] In case of P53, studies have shown over expressions in breast carcinoma patients, in relation to different clinicopathological parameters such as tumor size, TNM stage, lymph node grade, metastasis status, histologic grade and aggressiveness of tumor [5]

In this study, we aimed to study the expressions of p53 genes and investigate the correlation of clinicopathological parameters in patients affected with breast cancer.

MATERIAL AND METHOD:

This was a cross sectional study conducted in the Department of Pathology, Gandhi Medical College & Associated Hospitals, Bhopal, Madhya Pradesh from January 2021 to June 2022. 50 formalin fixed, paraffin-embedded sections of breast carcinoma were studied. Sections were prepared for IHC staining for p53.For all the cases, 4µm histological sections were deparaffinized in xylene and rehydrated in descending dilutions of ethanol and were mounted on positively charged glass slides for immunohistochemical staining and subjected to antigen retrieval. Only nuclear p53 expressions were accepted as specific reactions.[6-7] The staining intensity was evaluated by calculating the percentage of positive cells in 100 malignant cells at objective 40 total magnification: P53 positivity was analyzed by assessing the nuclear positivity. Lymphocytes, stromal cells, and endothelial cells showed negative to p53 expression, therefore were used as internal negative control. [8] Grading was done as follows: **(-): No nuclear reactivity, (+/-): Few focally positive cells (1 to <10% tumor cells), (+): Heterogeneous nuclear reactivity (10 to 50% tumor cells),** (++): **Homogenous intense nuclear reactivity (50 to 100% tumor cells). (-, -+) were considered negative. (+, ++) were considered as positive.**

Male patients with breast carcinoma, Recurrence or patients with neoadjuvant therapy and multiple malignancies were excluded. The institutional ethical committee approved the study.

OBSERVATIONS AND RESULTS

In our study mean age of diagnosis for breast carcinoma was 49.8 years ranging from 27 to 80 years. 28 (56%) cases were below the age of 50 years while 22 cases (44%) were above the age of 50 years.

Table 1: Distribution Of P53 Expression With Relation To Age

AGE	Frequency/ Percentage	P53 Expression			
		Positive		Negative	
		++	+	+-	-
AGE <50	28 (56%)	2278.6(%)	414.3(%)	000(%)	27.1(%)
AGE >50	22 (44%)	941(%)	29(%)	313.6(%)	836.4(%)
TOTAL	50 (100%)	31	6	3	10

Among 28 cases (Out of 50), of age less than 50 years, 26 (92.9%) showed high p53 expression, whereas 22 cases (Out of 50) of age more than 50 years, 11 (50%) showed high p53 expression. There was significant statistical correlation found. (p value=0.04).

Table 2: Distribution Of P53 Expression With Relation To Tumor Size

TUMOR SIZE	FREQUENCY / PERCENTAGE	P53 EXPRESSION			
		POSITIVE		NEGATIVE	
		++	+	+-	-
<2CM	06 (12%)	3(50%)	0(00%)	0(00%)	3(50%)
2-5CM	16 (32%)	4(25%)	03(18.7%)	03(18.7%)	6(37.5%)
>5CM	28 (56%)	24(85.7%)	03(10.7%)	00(00%)	01(3.6%)
TOTAL	50 (100%)	31	06	03	10

Among 6 (12%), cases (out of 50) of less than 2 cm, 3(50%) cases were positive for p53 expression. Among 16(32%) cases (out of 50) between the tumor size of 2- 5 cm, 7(43.7%) were positive for p53 expression.

Among 28 (56%) cases (out of 50) of tumor size more than 5 cm, 27(96.4%) were positive for p53 expression. There was significant statistical correlation between tumor size and p53 expression. (p= 0.02)

Table 3: Distribution Of P53 Expression With Relation To Lymph Node Metastasis

LYMPH NODE STATUS	FREQUENCY	P53 EXPRESSION			
		positive		negative	
		++	+	+-	-
POSITIVE	33 (66%)	24(72.7%)	5(15.1%)	2(6.1%)	2(6.1%)
NEGATIVE	17 (34%)	7(41.2%)	1(5.9%)	1(5.9%)	8(47%)
TOTAL	50 (100%)	31	06	03	10

In our study cases that showed p53 high expression, 87.8% had lymph node metastasis, and 47.1% showed no lymph node metastasis. There was a significant statistical correlation found between them. (p=0.02)

Table 4: Distribution Of P53 Expression With Relation To Various Histologic Grades Of Breast Carcinoma

GRADE	FREQUENCY/PERCENTAGE	P53 EXPRESSION			
		POSITIVE		NEGATIVE	
		++	+	+-	-
GRADE I	10 (20%)	220(%)	110(%)	110(%)	660(%)
GRADEII	14 (28%)	750(%)	17.1(%)	214.3(%)	428.6(%)
GRADE III	26 (54%)	2284.6(%)	415.4(%)	000(%)	000(%)
TOTAL	50 (100%)	31	6	3	10

In Our study out of 50 cases, grade I tumors showed 3(30%) cases positive for p53 expression. Grade II tumors, showed 8(57.1%) cases positive for p53 expression. All 26 (100%) grade III tumors were positive for p53 expression. There was significant statistical correlation between histologic grade and P53 expression. (p=0.03)

Table 5: Distribution Of P53 Expression With Relation To Various Stages Of Tumor

STAGE	FREQUENCY/ PERCENTAGE	P53 EXPRESSION			
		POSITIVE		NEGATIVE	
		++	+	+-	-
I	04 (8%)	125(%)	000(%)	000(%)	375(%)
II	06 (12%)	350(%)	000(%)	116.67(%)	233.33(%)
III	40 (80%)	2767.5(%)	0615(%)	0205(%)	0512.5(%)
TOTAL	50 100	31	06	02	24

Among 4(10%) cases (out of 50) of stage I, 1(25%) was positive for p53 expression. Among 6 (12%) cases of stage II (out of 50), 3 (50%) were positive for p53 expression and among 40 (80%) cases of stage III (out of 50), 33(82.5%) were positive for p53 expression. However, there was no significant statistical correlation between stage of tumor and p53 expression.

Table 6: Distribution Of P53 Expression With Relation To Er Receptor Status

RECEPTOR STATUS	FREQUENCY	P53 EXPRESSION			
		POSITIVE		NEGATIVE	
		++	+	+-	-
ER positive	26 (52%)	1661.5(%)	000(%)	13.8(%)	934.6(%)
ER negative	24 (48%)	1562.5(%)	625(%)	28.3(%)	14.2(%)
TOTAL	50 (100%)	31	6	3	10

Among 26 (52%) cases showing ER receptor positivity (out of 50), 16(61.5%) were positive for p53 expression. Out of 24 (48%) cases showing ER receptor negativity (out of 50), 21 (87.5%) were positive for p53 expression. There was significant statistical negative correlation between ER receptor status and P53 expression. (p=0.02).

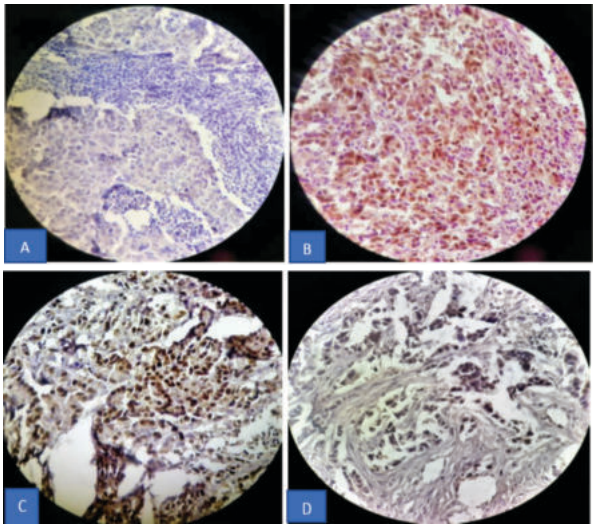

Figure 1: Immunohistochemical Staining Of P53 Showing A)No nuclear staining in Medullary Carcinoma Breast 40x B) And C) Homogenous staining in Invasive Ductal Carcinoma 40x D)Heterogenous staining in Invasive Ductal Carcinoma 40x

DISCUSSION:

In the present study 92.9% showed high p53 expression in age less than 50 years and 50% showed high p53 expression in age more than 50 years. There is significant correlation found between age of patient and high p53 expression (p value=0.04). Study showed that higher incidence of p53 positive accumulation occur in the nuclei of younger patients than in the older patients. Also incidence of higher grade in younger patients in our study attributed to this result. Similar results were seen in the study conducted by **AW Al-bideri et al** [8] (p=0.03). **Salmani H et al** [9] did not find any correlation (p value 0.3). In contrast, **Sheikhpour R et al** [10] found that higher p53 expression was seen in older patients rather than younger patients (p=0.04).[11]

In the present study we found that 96.4 % of tumor size > 5cm showed high p53 expression compared to tumor size between 2-5 cm (43.7%) and less than 2cm (50%) with a significant difference (p= 0.02). Large tumor size and p53 overexpression is an indicator of more aggressive cancer and poor prognosis. **Jindal B et al** [12] found similar results but **AW Al bideri et al** [8.] **Salmani H et al** [9], **Sheikhpour R et al** [10] found no correlation between tumor size and high p53 expression (p= 0.9).

In the present study we found that 87.8% of lymph node positive metastasis and 47.1% of lymph node negative metastasis showed high p53 expression. There was a significant correlation between high p53 expression and lymph node metastasis. (p=0.02). P53 expression is significantly associated with lymph node involvement, and this may be attributed to the aggressive behavior of lymph node positive breast cancer. Studies done by **AW Al bideri et al** [8] **Dash et al** [13] and **Jindal B et al** [12] found similar results whereas **Salmani H et al** [9], **Wakasugi E et al** [16] found no correlation between them.

The present study revealed that p53 high expression was more frequently seen in grade III tumors (84.6%) than in grade II and grade I (50%,20%) respectively. There was a significant correlation between high grade and high p53 expression p value(p=0.03). This reflects that the more abnormally accumulated p53 protein in nuclei represents an indicator of the accumulation of mutations which present in cases with high grade. **AW Al bideri et al** [10] and **Wakasugi E et al** [14] found similar results whereas **Sheikhpour R et al** [10] and **Song HS et al** [15] found no significant correlation between the two factors.

In our present study stage III showed 82.5 % of high p53 expression as compared to stage II (50%) and stage I (25%). There was no statistically significant correlation found between tumor stage and high p53 expression (p=0.4). This can be attributed to the small sample size in our study. IHC markers exhibit significant variation in their expression depending on race, ethnicity, and geographic distribution. Similar, findings were seen by **Salmani H et al** [9], **Song HS et al** [15]

(p= 0.06) and **Sheikhpour R et al**. [10] On the contrary **AW Al bideri et al** [8] found significant correlation between high tumor stage and p53 expression.

In the present study 61.5% of ER positive status and 87.5% of ER negative status shows high p53 expression. There was statistically significant negative correlation found between ER receptor and high p53 expression (p=0.02). An ER-negative tumor status is associated with aggressive tumor growth, increased invasiveness, poor patient prognosis, and loss of p53 function.[16] **Wakasugi E et al** [14], **Gurkan et al** [17] found similar results whereas **Salmani H et al** [9] and **Song HS et al** [15], found no significant correlation.

CONCLUSION

This is a hospital-based study and may not represent the true incidence of the disease in the community. P53 functions as a negative regulator of cell growth, and alterations in the gene lead to loss of this negative growth regulation and more rapid cell proliferation. Breast cancer aggressiveness appears to be directly related to the percentage of p53 positive cancer cells.[18] High expression of P53 was associated with, patients of age less than 50 years, high tumor size, high grade, lymph node metastasis and ER receptor negative status showing poor prognosis and progression of breast carcinoma which was in concordance with other studies.

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