Original Research Paper

Medical Science

Expression of ₚ53 in Breast Lesions, an Immunohistochemical Study

* Dr. Tania R.P	Assistant Professor, Department of Pathology, Meenakshi Medical College Hospital & Research Institute, Kanchipuram , Tamil Nadu, India. * Correspondence Author
Dr. V. Eswari	Associate Professor, Department of Pathology, Meenakshi Medical College Hospital & Research Institute, Kanchipuram , Tamil Nadu, India.
Dr. Geetha Prakash	Professor, Department of Pathology, Meenakshi Medical College Hospital & Research Institute, Kanchipuram , Tamil Nadu, India

ABSTRACT

Aim: *Breast cancer is the most frequent cancer in India. Mutations in the p53 gene are among the common molecular changes detected in breast cancer. p53 mutations and p53 protein accumulation have also been detected in benign breast disease. These observations suggest that p53 changes can occur before the development of breast cancer. The aim of the study is to analyze the expression of p53 in human breast cancer as compared to normal breast tissue and benign breast lesions by immunohistochemistry. Also to assess the usefulness of p53 as a predictor of aggressiveness of breast lesions.* **Materials and methods:** *Formalin fixed paraffin embedded sections of 10 cases of normal breast tissue,20 cases of benign breast lesions and 20 cases of malignant breast lesions were taken up for the study and subjected to immunohistochemistry using p53.* **Results:** *The intensity of p53 immunostaining in normal breast, benign and malignant breast lesions was evaluated and scoring was graded as 0, 1+, 2+, 3+ and +4. Statistical analysis was performed with Chi-Square test and significant differences were noted between these 3 groups. (p value< 0.05).* **Conclusion:** *.p53 expression correlated well with the grade and stage of tumor indicating that p53 positive tumors are biologically aggressive and are associated with poor prognosis but little is known about the implication of genetic alterations of p53 in benign breast lesions.*

KEYWORDS : p53, Immunohistochemistry, breast lesions

I. Introduction

The story of breast cancer is told in the acts and artifacts of the human struggle against disease. The oldest description of breast cancer was in Egypt and dates back approximately to 1600 BC. Breast cancer is the most frequent cancer in India and mortality rates associated with it is higher in India. Many genetic alterations and oncogene protein products which interfere with the mechanism of proliferation and differentiation of tumor growth have been discovered and investigated.

Mutations in the p53 gene are among the most common molecular changes detected in breast cancer. The p53 gene product is a multifunctional transcription factor that is involved in regulating cell cycle arrest and apoptosis, facilitating DNA repair and promoting chromosomal stability. Disruption of p53 function seems to have a pivotal role in carcinogenesis.p53 mutations and p53 protein accumulation have also been detected in benign breast disease. These observations suggest that p53 changes can occur before the development of breast cancer raising the possibility that such changes might be related to the risk of breast cancer development.

II. Materials and Methods
IIa. Experimental Design
The study was conducted at Meenakshi Medical College Hospital and Research Institute, Kanchipuram, Tamil Nadu. Fifty cases were selected , out of which 10 cases of normal breast tissue,20 cases of benign breast lesions and 20 cases of malignant breast lesions. All female cases irrespective of their age and other physical conditions during the period of June 2010 to June 2012 were taken up for the study.

IIb. Selection of cases
1- Study group: Twenty cases of female breast cancer patients were selected randomly, their ages ranging from 20 to 70 years. All of them underwent modified radical mastectomy.

2- Control group: Ten cases of normal breast tissue from patients presenting with breast mass, other than tumor, were selected and regarded as a control group.

3- Comparative group: Twenty cases with benign breast lesions were taken as comparative group to compare the rate of p53 immunoexpression with that in malignant breast lesions. Of the benign cases: 10 cases were fibroadenoma, 10 cases were fibrocystic change. The diagnosis were reconfirmed on hematoxylin and eosin stained sections and the appropriate blocks were subjected to IHC using p53 antibody(Biogenex,USA).

IIc. Immunohistochemical study (IHC) :
Anti-p53 monoclonal antibody
BIOGENEX AM 195-5M p53protein,Monoclonal(BP53-12-1),Mouse Anti-Human p53 Protein, 6 ml, Ready-To-Use was employed using the Avidin Biotin Complex (ABC) detection system.

Scoring system:
The criteria for positive immunoreaction is dark brown precipitate (nuclear for p53). While the intensity of the staining was assessed as follows:

Scoring according to Sophia K. et al.[18], at objective 40 and as follow:
Score 0: Negative, none or <5% of the cells revealed positivity for the marker.
Score +1: Weak or mild staining, (5-10%) positive of tumor cells.
Score +2: Moderate staining, less than 25% of tumor cells are stained positive.
Score +3: Strong staining, (25-50%) of tumor cells are stained positive.
Score +4: Highly strong staining, over 50% of tumor cells are stained positive.

III. Statistical analyses

Statistical analyses of all results were done by using Chi square test at level of significance p ≤ 0.05 was done.

IV. Ethical concern
Ethical clearance was obtained from the Ethical committee meeting conducted at Meenakshi Medical College and Research Institute, Kanchipuram, Tamil Nadu, India.

V. Results
TABLE 1: Cases included in the study

Type of Breast Tissue	No. of cases	Percentage
Normal	10	20
Benign	20	40
Malignant	20	40
Total	50	100

The cases included in the study were selected irrespective of their age differences. Analysis of the study sample showed that majority of the cases were in the age range of 15 to 45 years which comprised 76% of the sample size.

TABLE 2: Age wise distribution of cases

SERIAL NO	AGE GROUP	NO. OF CASES	PERCENTAGE
1	15-30 YRS	19	38
2	31-45 YRS	19	38
3	46-60 YRS	8	16
4	>60 YRS	4	8
Total		50	100

TABLE 3: The characteristic features of all malignant breast lesions

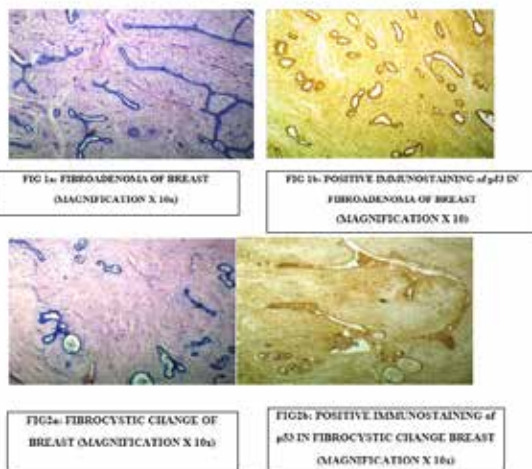| Features		No.of Cases
Grade	I	2
	II	8
	III	10
Stage	0	0
I		0
	II	4
	III	12
	IV	4
Axillary lymph-node	Positive	14
	Negative	6

p53 expression in normal breast tissue:
In immunohistochemical analysis of p53 protein it was observed that none of the 10 cases of normal breast tissue showed immunoreactivity for p53.

p53 expression in benign breast lesions : Out of the 20 cases of benign breast lesions, 10 cases were fibroadenoma and 10 cases were fibrocystic change. Expression of p53 was noted in 5 (25%) of the 20 cases, of which 2 cases were fibroadenoma (fig 1a&1b) and 3 cases were fibrocystic change.(fig 2a& 2b)

TABLE 4: Expression of p53 in benign breast lesions

Types of Benign Breast lesions	No.of cases	p53(+ve)	p53(-ve)
Fibroadenoma	10	2	8
Fibrocystic Disease	10	3	7
Total	20	5	15

FIG 1a: FIBROADENOMA OF BREAST (MAGNIFICATION X 10x)
FIG 1b: POSITIVE IMMUNOSTAINING of p53 IN FIBROADENOMA OF BREAST (MAGNIFICATION X 10)
FIG2a: FIBROCYSTIC CHANGE OF BREAST (MAGNIFICATION X 10x)
FIG2b: POSITIVE IMMUNOSTAINING of p53 IN FIBROCYSTIC CHANGE BREAST (MAGNIFICATION X 10x)

Intensity of staining of p53 in benign breast lesions:
Among the 3 cases of fibrocystic change, 2 cases showed 1+ positivity, 1 case showed 2+ positivity. Among the 2 cases of fibroadenoma, 1 case showed 1+ positivity, 1 case showed 2+ positivity for p53 protein.

TABLE 5: Percentage expression of intensity of staining in benign breast lesions for p53

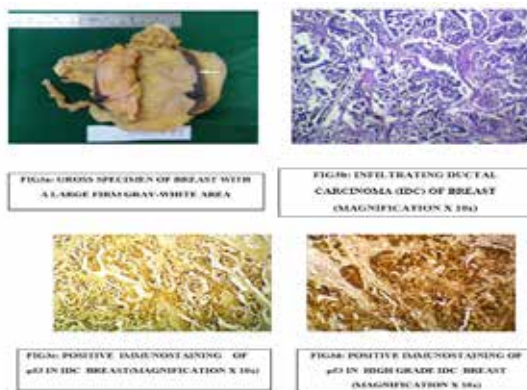| INTENSITY OF STAINING OF p53	NO. OF CASES	PERCENTAGE
1+	3	60
2+	2	40
3+	0	0
4+	0	0

p53 expression in malignant breast lesions:
Out of the 20 cases of malignant breast lesions, expression of p53 was noted in 12 cases(60%).fig (3a.3b,3c &3d)

Intensity of staining of p53 in malignant breast lesions:
Among the 12 cases, 2 cases showed 1+ positivity, 1 case showed 2+ positivity, 4cases showed 3+ positivity and 5 cases showed 4+ positivity (p<0.05) for p53 protein.

TABLE 6: Percentage expression of intensity of staining in malignant breast lesions for p53

INTENSITY OF STAINING OF p53	NO. OF CASES	PERCENTAGE
1+	2	17
2+	1	8
3+	4	33
4+	5	42

FIG3a: GROSS SPECIMEN OF BREAST WITH A LARGE FIRM GRAY-WHITE AREA
FIG3b: INFILTRATING DUCTAL CARCINOMA (IDC) OF BREAST (MAGNIFICATION X 10x)
FIG3c: POSITIVE IMMUNOSTAINING OF p53 IN IDC BREAST(MAGNIFICATION X 10x)
FIG3d: POSITIVE IMMUNOSTAINING OF p53 IN HIGH GRADE IDC BREAST (MAGNIFICATION X 10x)

p53 overexpression and grade of tumor

p53 immunohistochemical analysis in relation to grade of tumor revealed that none of grade I was positive, 4 (50%) out of 8 cases of grade II were positive, 8 out of 10 (80%) cases of grade III were positive for p53. It seems that the detection rate of p53 is well correlated to the

grade of tumor, (p<0.05)

TABLE 7: Expression of p53 in relation to grade of the tumor

Grade of Tumor	p53 immunostaining		Total
	positive	negative	
I	0	2	2
II	4	4	8
III	8	2	10

p53 Overexpression and stage of tumor

p53 immunohistochemical expression was reported in 25% (1 out of4) of stage II, in 66% (8 out of 12) of stage III, and in 75% (3 out of 4) cases of stage IV .

There was significant positive correlation between p53 overexpression and the stage of tumor (p value <0.05), and a higher proportion of cases was found in stage III and IV.

TABLE 8: Expression of p53 in relation to the stage of the tumor

Stage of Tumor	p53 immunostaining		Total
	positive	negative	
TO	0	0	0
I	0	0	0
II	1	3	4
III	8	4	12
1V	3	1	4

p53 immunohistochemical expression and axillary lymphnode involvement:

10 out of 14 cases of node-positive breast cancer found to have p53 over expression (71%),while only two out of 4 cases of node-negative breast cancer showed p53 over expression (33%) ,with significant difference between these two groups (p value <0.05)

VI. Discussion:
p53 over expression and breast lesions:

The current study demonstrated that there was complete absence of expression of p53 protein in normal breast tissue although 5 cases of benign breast lesions showed immunoreactivity for p53. However there was a significant over expression of p53 among the 12 investigated breast carcinoma (p value < 0.05) The results have clarified that 60 % of 20 cases of breast cancer expressed p53 immunostaining in their histological sections.This result is slightly higher than those of Noranizah W et al [1] and K.J. Ranade et al [2] who reported that 50% and 50.6% respectively of primary breast carcinoma were p53 positive. Out of the 20 cases of benign breast lesions, expression of p53 was noted in 5 cases (25%). This finding is slightly higher than those of Sirotkovic Skerlev M et al [3], K.J. Ranade et al [2] who reported that 19.6%, 13% respectively of benign breast lesions were positive for p53 protein.In this study p53 over expression was detected in 60% of breast cancer patients and 25% of benign breast lesions was found to be p53 immuno reactive, with a significant difference between these groups (p<0.05).

Expression of p53 in normal breast tissue

In immunohistochemical analysis of p53 protein it was observed that none of the 10 cases of normal breast tissue showed immunoreactivity for p53. This fact corresponds to studies done by Noranizah W *et al*[1], ,Alfredo Ribeiro-Silva et al [4]) but differs from the studies carried out by Rita Kandel , Thomas E.Rohan *et al*. [5,6] who reported p53 mutation in normal appearing breast tissue.

Expression of p53 in benign breast lesions:

Out of the 20 cases of benign breast lesions, expression of p53 was noted in 25% of the cases.

This finding corresponded to and is slightly higher than those of Sirotkovic Skerlev M et al, K.J. Ranade et al[3,2] who reported that 19.6%, 13% respectively of benign breast lesions were positive for p53 protein but differs from the study which was carried out by Alfredo Ribeiro-Silva et al [4] Anne loakin Liossi[7] who found that expression of p53 was negative in benign breast lesions.

Expression of p53 in malignant breast lesions:

Positive over expression of p53 in paraffin- embedded tissue of infiltrating ductal carcinoma was found in 60% of patients.

This finding is higher than those of Sirotkovic Skerlev M et al[3], Anne loakin Liossi [7] who reported that 34% and 45% respectively of primary breast carcinoma were p53 positive and slightly higher than those of Noranizah W [1] K.J. Ranade et al [2]who reported that 50% and 50.6% respectively of primary breast carcinoma were p53 positive.

p53 over expression and grade of tumor

p53 immunohistochemical analysis in relation to grade of tumor revealed that none of grade I were positive, 50% of grade II were positive, 80% of grade III were positive for p53. There was highly significant positive correlation between p53 over expression and grade of breast cancer (p<0.05).This finding agrees with Ryu JW et al[9]and AL-Janabi AA et al[8] , but differs from that of Hong S et al who documented no significant correlation between p53 over expression and grade of tumor [10]

p53 Overexpression and stage of tumor

p53 immunohistochemical expression was reported in 25 of stage II, in 66% of stage III, and in 75% of stage IV .There was significant positive correlation between p53 over expression and the stage of tumor (p value <0.05). This finding was in agreement with Brano T et al., and Davidoff AM et al who suggested that there was a significant correlation between tumor p53 and stage of breast cancer[11,12], and differs from Kalfon B et al [13], AL-Janabi AA et al[8] and Hong S et al [10]who found no statistical significant correlation between p53 and stage of tumor .p53 was more expressed in those with advanced stage which reflects the aggressive behavior of the tumor.

p53 over expression and age.

The results revealed that p53 immunoexpression was not always increased with increasing age. There was no significant difference among these age groups (p>0.05). This may be corresponding to the natural frequency of breast cancer. This finding is consistent with that of Branes DM et al [14] Sasa M et al[15],, who found that there is no correlation between age of the patient and p53 expression, but differs from Kalfon B et al [13]who had documented a significant correlation of p53 and age exists.

p53 and Axillary lymph node involvement

p53 over expression is higher in node positive breast cancer than in node negative breast cancer with significant difference between these two groups (p value <0.05). This finding agreed with that of Kourea HP et al[16]and AL-Janabi AA et al[8] and differs from that of Hong S et al [10] and Mohamed T et al[17] that p53 expression is not significantly associated with lymph node involvement and this may be attributed to the aggressive behavior of node positive breast cancer.

Conclusion.

Results of this study showed that no normal, epithelial or stromal breast tissue expressed immunohistochemically detectable levels of p53. However expression of p53 was noted in benign breast lesions immunohistochemically. The clinical significance of p53 expression in benign breast lesions remains to be determined. Further research may be necessary to evaluate whether these markers could serve as useful adjuncts in evaluation of the malignant potential of benign breast lesions.

VII. Conclusion

In this study, we conclude that p53 over expression was significant in

all grades and stages of breast cancer (p<0.05). p53 correlated well with the grade and stage of tumor indicating that p53 positive tumors are biologically aggressive and are associated with poor prognosis. Nonetheless resolution of the role of p53 in the genesis of breast cancer requires larger sample size with long term follow up studies.

VIII. References

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