ORIGINAL ARTICLE

Indian Journal of Forensic Medicine and Pathology
Volume 16, Number 4, October-December 2023
DOI: https://dx.doi.org/10.21088/ijfmp.0974.3383.16423.6

A Study of p53 Immunostaining in Breast Carcinomas: Correlation with Histopathological Prognostic Factors

Aninditha Saha[1], Sapna Patel M. C.[2], Harish Srikantegowda[3]

How to cite this article:

Aninditha Saha, Sapna Patel M. C., Harish Srikantegowda. A Study of p53 Immunostaining in Breast Carcinomas: Correlation with Histopathological Prognostic Factors. Indian J Forensic Med Pathol.2023;16(4):267-278.

ABSTRACT

Background: Breast cancer is the most common cancer in women worldwide and 2nd common cancer after cervix in India. Presence of p53 gene alteration like loss of function p53 mutants correlate with poor prognosis and tend to be more frequent in high grade, large size, node positive, and in estrogen-progesterone receptor negative (ER-, PR-) tumors, suggesting that mutant p53 may become increasingly critical for breast cancer progression.

Aims: To determine the frequency of expression of p53 immunostaining (IHC) in breast carcinomas and to correlate its relationship with tumor size, histological grade, lymph node status and with hormonal receptors status wherever possible.

Materials and Methods: Sixty-five breast carcinoma cases were studied for various clinicopathological parameters like tumor size, histological type, grade, axillary lymph node status, lympho vascular invasion, in situ component, Nipple areola complex, deep resected margin involvement, Nottingham Prognostic Index (NPIG) and subjected to p53 immunostaining. 37 out of 65 cases were studied for ER, PR, Human Epidermal Growth Factor Receptor 2 (HER2/neu) antibodies. p53 expression was correlated with known prognostic factors. Furthermore, autopsy studies can be used as supplement to know the disease reservoir.

Results: p53 positivity was found in 72.3% of breast carcinomas with significant association between p53 expression with histological grade (p-value = 0.013) and Nottingham Prognostic Index Groups (NPIG) (p-value = 0.011). No significant correlation was seen between p53 expression and tumor size, lymph node status, hormonal receptor (ER, PR) status and HER2/neu expression.

Conclusion: Our study revealed that the higher the tumor grade, the higher the p53 expression and its association with NPIG. Thus, p53 can be considered as a prognostic marker and useful for management.

Author's Credentials: *[1]Assistant Professor, Department of Pathology, Shaheed Rendo Majhi Medical College and Hospital, Bhangabari, Odisha 766002, India, [2]Associate Professor, Department of Pathology, [3]Professor and HOD, Department of Surgery, JSS Academy of Higher Education and Research, Mysuru, Karnataka 570015, India.*

Corresponding Author: ***Sapna Patel M. C.,*** *Associate Professor, Department of Pathology, JSS Academy of Higher Education and Research, Mysuru, Karnataka 570015, India.*

Email: *drsapnaharish@gmail.com*

Received on: *20-07-2023* ***Accepted on:*** *15-12-2023*

Keywords: Breast carcinoma; Immunohistochemistry; p53; Nottingham Prognostic Index Groups.

Key Message: Timely mammography and self-breast examination may prevent morbidity by early diagnosis and biopsy should be done if physical findings suggest breast cancer, even if mammogram results are negative.

INTRODUCTION

Breast cancer is the most common cancer in women and accounts for 29% of all cancers diagnosed each year worldwide accounting for 19% of the total cancer burden. The development of distant metastasis is the primary cause of death in breast cancer patients. The involvement of axillary lymph nodes, larger size of the tumor, hormone receptor negativity, presence of the mutation of p53 and vascular invasion determine poor prognosis and treatment options upon initial diagnosis.[1]

Predisposition to develop carcinoma breast depends upon several etiological factors like age at menarche, parity, age at menopause, family history, diet, alcohol consumption, socioeconomic status, history of radiation exposure and use of oral contraceptive pills. Recently cigarette smoking also has been identified as a risk factor.[2]

Like other tumors, it is a disease with a complex, heterogeneous genetic and biochemical background. No single genomic or metabolic condition can be regarded as decisive for its formation and progression. However, a few key players can be pointed out and among them is the TP53 tumour suppressor gene, commonly mutated in breast cancer.[1] It leads to an accumulation of non-functioning p53 protein in the cell nuclei, which can be detected by immunohistochemical techniques.[3]

Numerous studies show that in breast cancer, the presence of p53 gene alteration like loss-of-function p53 mutants correlate with poor prognosis and tend to be more frequent in high-grade, large size, node positive, and in estrogen-progesterone receptor negative (ER-, PR-) tumors, suggesting that mutant p53 may become increasingly critical for breast cancer progression.[4]

MATERIALS AND METHODS

It was a cross-sectional study of 2 years (September 2016 to August 2018) undertaken in the Department of Pathology, JSS Medical College and Hospital, JSSAHER, Mysore. All breast carcinomas diagnosed on lumpectomies and mastectomies were included whereas non-epithelial malignant tumors and post chemotherapy cases were excluded from the study. Furthermore, autopsy studies can be used as supplement to know the disease reservoir.

Method of collection of data

All mastectomy specimens of invasive breast carcinoma - NST (IBC – NST) were studied noting the clinical details. After formalin fixation, paraffin embedding and staining with haematoxylin and eosin (H&E), histopathological features were studied. Histopathological grade was assessed using Bloom and Richardson method, modified by Elston and Ellis. Tumor, Node, Metastasis staging (TNM staging) according to AJCC classification 8th edition was used for pathological staging. Immunohistochemistry (IHC) for p53 was performed on all 65 cases on paraffin embedded wax sections.

Procedure for p53 IHC Staining

Paraffin blocks best representing the tumor in each case were selected after reviewing the H&E slides. Three to four µm thick sections were taken on Poly-L-Lysine coated slides and air dried. The slides were baked at 60°C for one hour in hot air oven. Slides were deparaffinised and rehydrated. Retrieval solution (Tris buffer for antigen retrieval) was brought to boil in the pressure cooker. Slides were placed in metal staining racks and lowered into pressure cooker ensuring that the slides were completely immersed in the retrieval solution. When the pressure cooker reached operating temperature and pressure, it was timed for up to two to three whistles. The pressure cooker was removed from the heat source and the slides were allowed to cool for 30 minutes in the same solution. The slides were washed with wash buffer for one minute. Peroxide block was applied for ten minutes and washed with wash buffer for one minute. The sections were incubated with primary antibody (DAKO, FLEX Monoclonal Mouse Anti-Human p53 Protein Clone DO-7 Ready to Use, Code IS616) for 20 minutes and washed twice with wash buffer. The sections were then incubated with LABELLED POLYMER – HRP [Horse radish peroxidase] (DakoEn Vision + Dual Link System – HRP, DAB+ [3,3' Diaminobenzidine], Code K4065) for 20 minutes and washed thrice with wash buffer. The bound antibody was visualized using a DAB-chromogen substrate which was prepared by adding 50µl of DAB Chromogen (DakoEn Vision + Dual Link System – HRP, DAB+, Code K4065) to one ml of DAB buffer (DakoEn Vision + Dual Link System – HRP, DAB+, Code K4065). The sections were washed with wash buffer and counterstained with haematoxylin and again rinsed in water for five minutes. Sections from colorectal carcinoma were taken as positive control.

Method of reporting IHC

Brown granular nuclear reactivity was taken as positive. Staining characteristics of the tumors were agreed upon by two people involved in the study. Results of the immunohistochemical and histological analysis were recorded. The entire section was screened to determine the region with the maximum proportion of stained nuclei. p53 over expression was recorded as intensity of staining and score based on the percentage of the cells staining positively. First, percentages of the total number of tumor cells are assigned to one of six categories:

Scores

0 for < 5%, 1 for 5-30%, 2 for 30-50%, 3 for 50-70%, 4 for 70-90%, 5 for > 90%.

Second, the intensity of p53 immunostaining is scored as follows:

Staining Intensity

1+ for week, 2+ for moderate, 3+ for intense.

Then the scores of percentages and staining intensity are multiplied to produce a weighted score for each tumor, as a score of < 8 designated as low expression and >8 as high expression.

The immunohistochemical staining technique using a modification of the avidin-biotin method was used for estrogen receptors, progesterone receptors and HER-2/neu and staining was performed with primary antibodies against ER, PR and HER-2/neu (A0485, Dako Corp., diluted 1:1,600). Scoring for ER, PR and HER-2/neu was done by All Red Method.

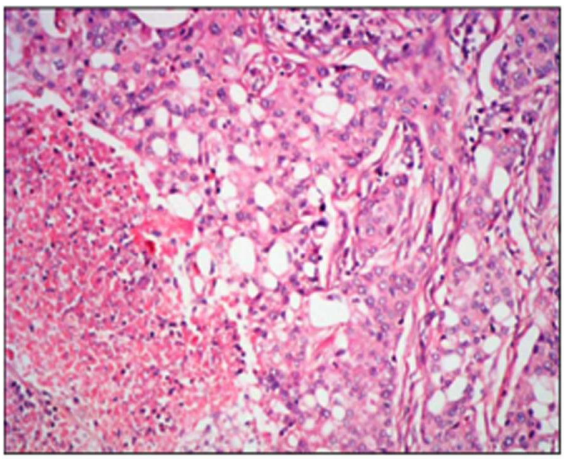

Fig. 1c: Invasive breast carcinoma of No Special Type, Grade 3: Areas of necrosis with tumour cells showing marked pleomorphism and atypical mitosis (H&E, x200)

STATISTICAL ANALYSIS

The statistical analysis was performed using statistical package for social sciences (SPSS) version 22. Percentages was used for categorical data and charts were generated using Microsoft excel 2007. The Association between p53 immunostaining and clinicopathological features including tumor size, nodal status, tumor grade, Estrogen/Progesterone receptors and HER-2/neu status were evaluated using the chi-square test. p-value <0.05 was considered statistically significant.

RESULTS

The age group of patients ranged from thirty years to seventy-five years, with mean age of fifty years. Maximum number of patients (24/65) were in sixth decade of life accounting for 36.9% of total cases. Right side breast was most affected with thirty-seven cases (56.92%) of tumor in the right breast and in twenty cases (43.07%) in the left breast [Fig./Table 1a & 1b].

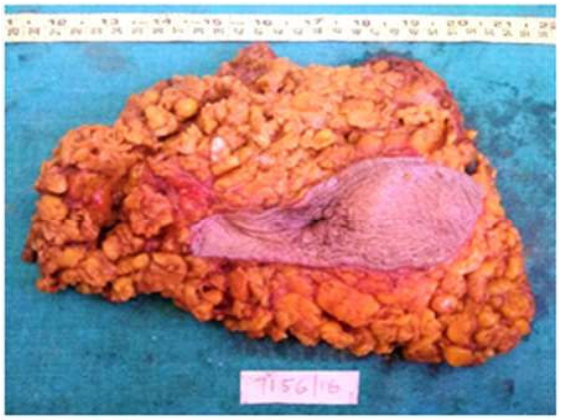

Fig. 1a: Gross picture of modified radical mastectomy specimen showing nipple retraction

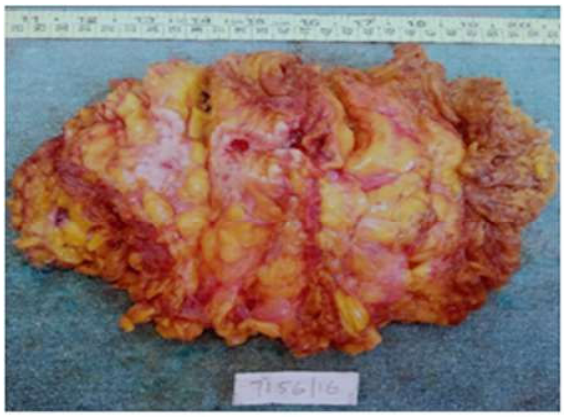

Fig. 1b: Cut section of modified radical mastectomy specimen showing a grey white infiltrating tumour

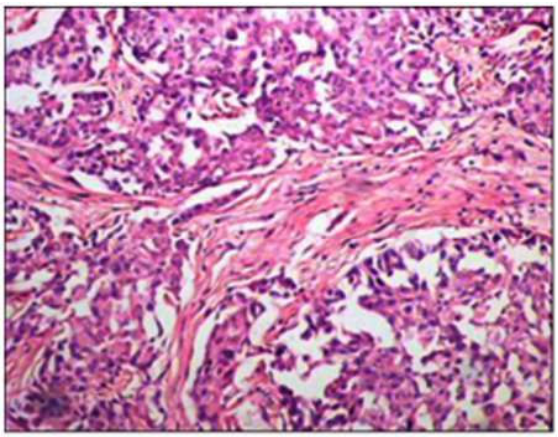

Fig. 1d: Invasive breast carcinoma of No Special Type, Grade 2: Tumour cells showing mild to moderate pleomorphism with areas of desmoplasia (H&E, x200)

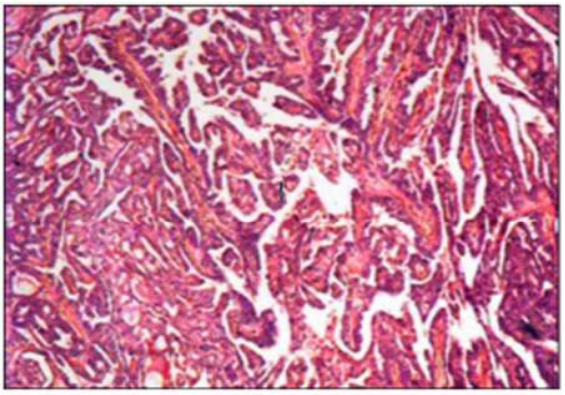

Fig. 1f: Invasive papillary carcinoma: Tumour cells arranged in papillary pattern with central fibrovascular core (H&E, x100)

Histopathological diagnoses included fifty-seven cases (87.69%) of invasive breast carcinoma No Special Type (IBC – NST) [Fig./ Table 1c & 1d], three cases of invasive lobular carcinoma [Fig./ Table 1e], two cases of invasive papillary carcinoma [Fig./ Table 1f] and one case each of mixed ductal and lobular carcinoma.

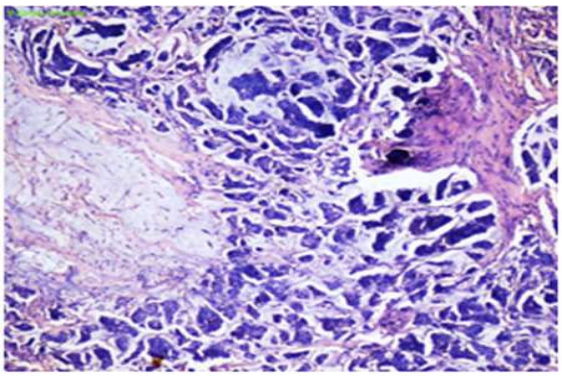

Fig. 2c: Mucinous carcinoma: Tumour cells arranged in clusters in a pool of abundant mucin (H&E, x200)

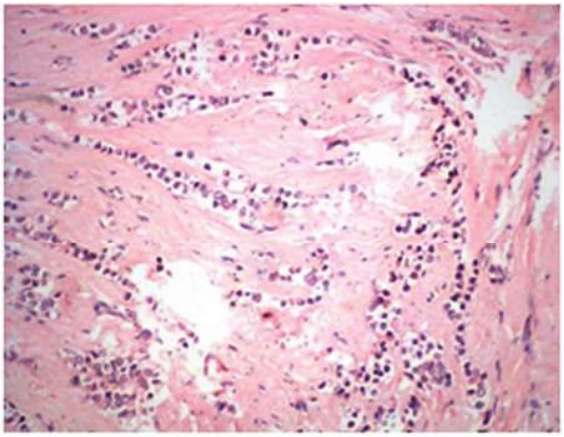

Fig. 1e: Invasive lobular carcinoma with typical Indian file arrangement of tumour cells (H&E, x100)

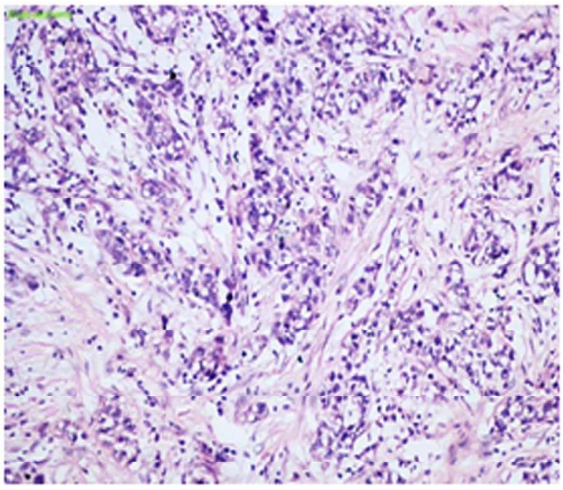

Fig. 2a: Mixed ductal and lobular carcinoma (H&E, x100)

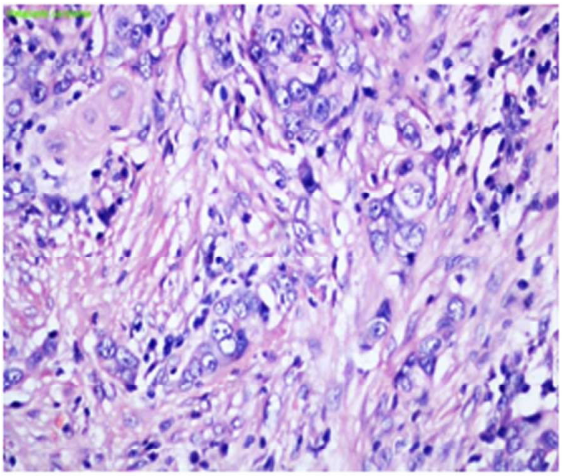

Fig. 2b: Metaplastic carcinoma: Tumour cells arranged in nests with focal area of squamous differentiation (H&E, x400)

[Fig./Table 2a], metaplastic carcinoma [Fig./ Table 2b], and mucinous carcinoma [Fig./ Table 2c].

Histopathological prognostic markers

Histologic grade was assessed in all cases of IBC – NST and metaplastic carcinoma using Elston and Ellis modification of the Bloom-Richardson grading system. Maximum number of cases were found to be of grade 3 accounting for 55.2% cases followed by grade 2 (44.8%). There was no case of grade 1 tumor.

The size of tumors ranged from two cm to twelve cm in greatest dimension with a mean size of 4.2 cm. The tumors were divided into four categories based on tumor size using TNM system of staging. Maximum number of cases were of pT2 category accounting for 67.7%.

Out of all, in four cases axillary lymph nodes could not be assessed. From rest, axillary lymph node involvement was seen in forty cases. Twenty cases showed metastasis in 1-3 lymph nodes (pN1),

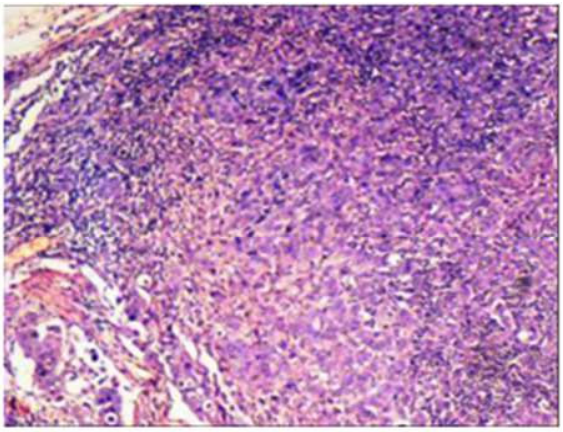

Fig. 2d: Lymph node: Tumour cells showing metastatic deposit (H&E, x100)

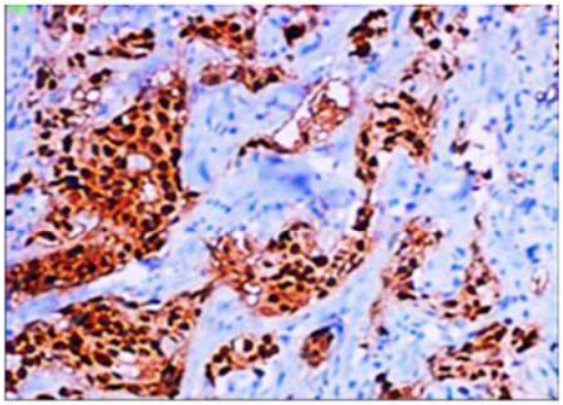

Fig. 2e: ER Positive: Tumour cells showing nuclear positivity with estrogen receptor immunostain (IHC, x200)

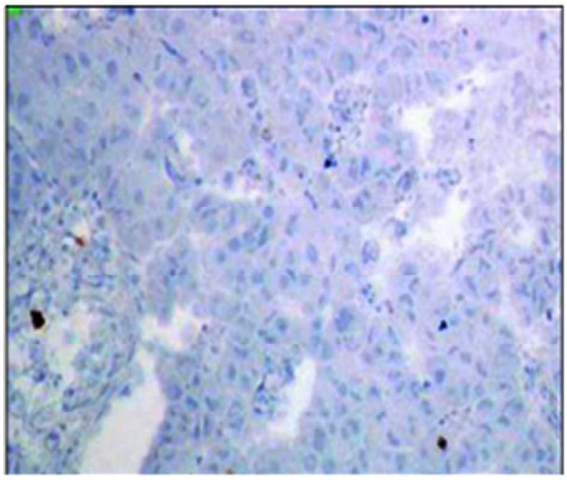

Fig. 2f: ER Negative: Tumour cells showing no staining with estrogen receptor immunostain (IHC, x200)

7 cases showed metastases in 4-9 lymph nodes (pN2) and 13 cases showed metastases in >10 lymph nodes (pN3) [Fig./ Table 2d].

Twenty-nine cases showed in situ component, 2 cases showed involvement of nipple and areola and 7 cases showed involvement of deep resected margin. ER and PR status were analysed only in 37 cases. 73% of cases (27 cases) were positive for ER [Fig./Table 2e & 2f] and 70% of cases (26 cases) were positive for PR [Fig./ Table 3a & 3b].

HER2/neu status was analysed only in 37 cases. 32.4% of cases (12 cases) were positive for HER2/ neu with a score of 3. Twenty-five cases were

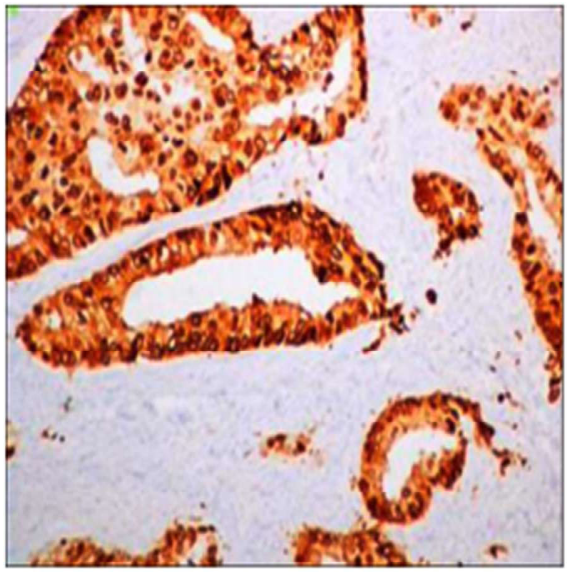

Fig. 3a: PR positive: Tumour cells showing nuclear positivity with progesterone receptor immunostain (IHC, x200)

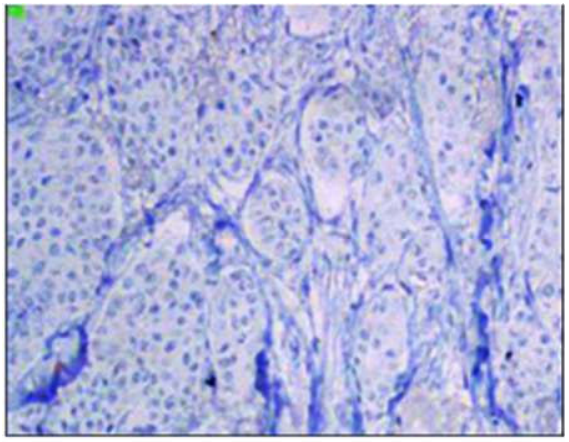

Fig. 3b: PR negative: Tumour cells showing no staining with progesterone receptor immunostain (IHC, x200)

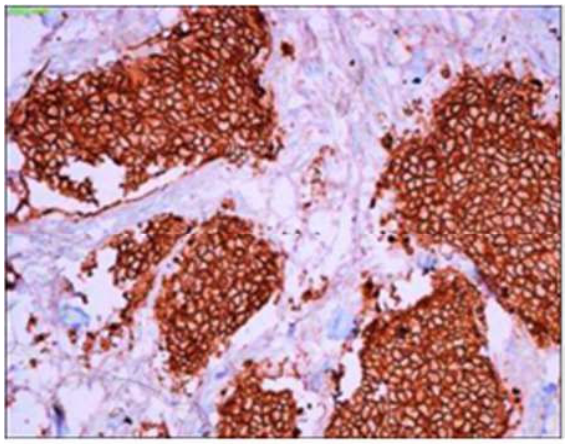

Fig. 3c: HER2/neu Score 3: Tumour cells showing strong membrane positivity with HER2/neu receptor immunostain (IHC, x200)

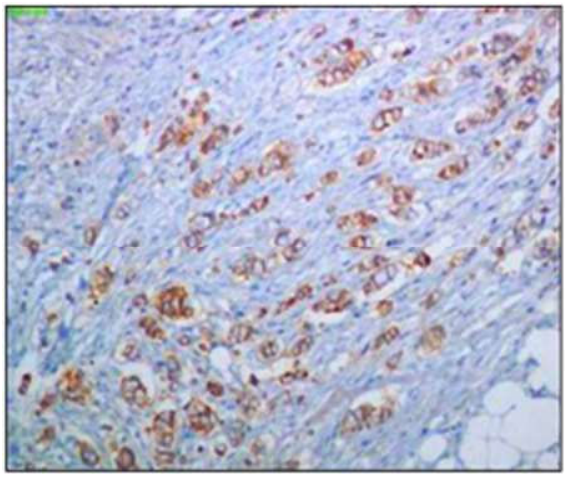

Fig. 3d: HER2/neu Score 3: Tumour cells showing moderate staining of >10% of cells with HER2/neu receptor immunostain (IHC, x200)

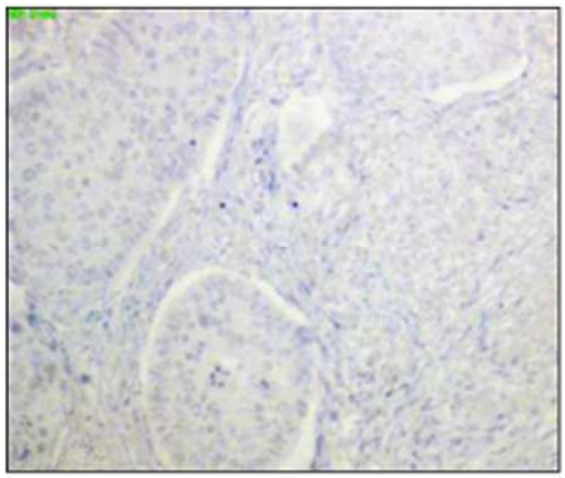

Fig. 3e: HER/neu Score 0: Tumour cells showing no staining with HER2/neu receptor immunostain (IHC, x200)

negative (12 had a score of 0 and 4 had a score of 1) and 9 cases were inconclusive (score 2) [Fig./ Table 3c, 3d &3e]

NPI was calculated by {size (cm) x 0.2} + {lymph node stage (1 – 3)} + {grade (1 – 3)}. Patients were categorised into 3 groups. Maximum number of cases were in moderate group (50%)

p53 Expression in breast carcinomas

p53 expression was found in nuclei of tumor cells and the percentage of tumor cells showing p53 positivity and intensity of p53 positivity varied between tumors. The expression was assessed and evaluated by applying scoring system used by Jin-Woo *et al*.[5] All the cases were evaluated by applying this scoring system. 72.3% of cases (47 cases) showed p53 positivity and 27.7% of cases (18 cases) were negative for p53. Out of 47 positive cases, maximum number of cases (15 cases) were with score 1 (5-30% tumor cells showing positivity) and minimum (5 cases) were with score 5 (>90% tumor cells showing positivity). Intense staining (intensity score of 3+) was noted in 26 cases (55.3% cases) and weak staining (intensity score of 1+) was noted in 10 cases (21.3%) whereas 25 cases (53.2% of cases) showed high expression (weighted score >8) and 22 cases (46.8% of cases) showed low expression (weighted score <8) of p53. So, 38.5% of cases showed p53 high expression while 33.8% of cases were with low expression. p53 was not expressed in

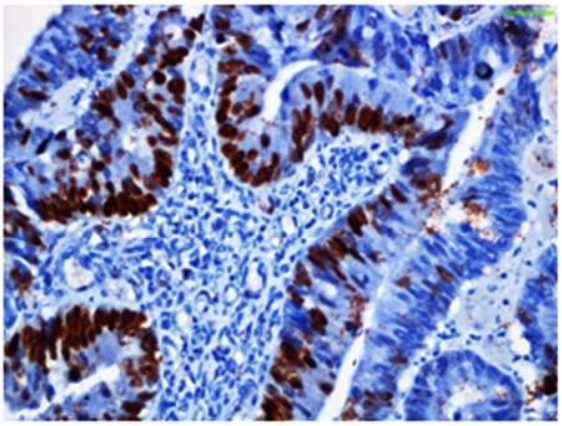

Fig. 4a: p53 nuclear positivity with high expression in carcinoma colon (control) (IHC, x200)

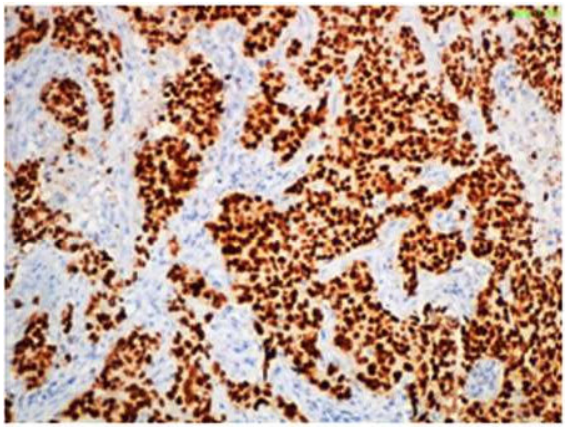

Fig. 4b: p53 nuclear positivity with high expression (weighted score 5x3 = 15) (IHC, x100)

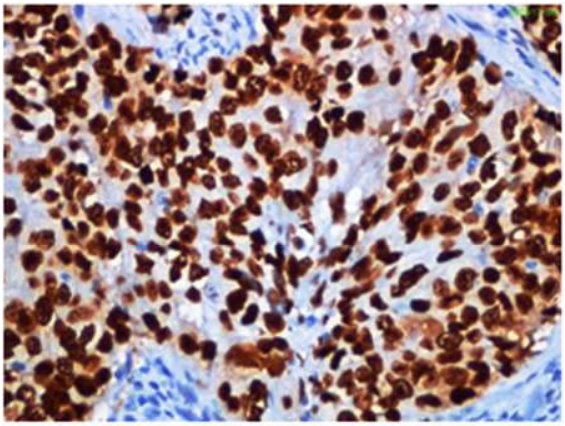

Fig. 4c: p53 positivity with high expression (weighted score 5x3 = 15) (IHC, x200)

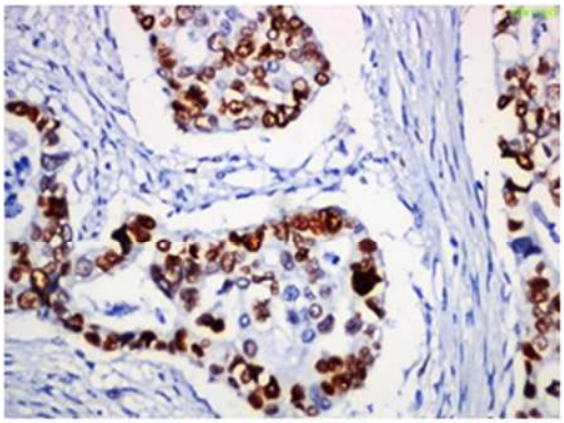

Fig. 4d: p53 positivity with high expression (weighted score 4x3 = 12) (IHC, x200)

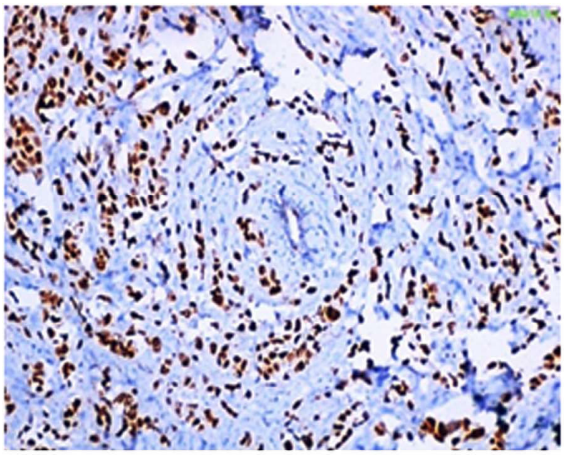

Fig. 4e: p53 positivity with high expression (weighted score 3x3 = 9) (IHC, x40)

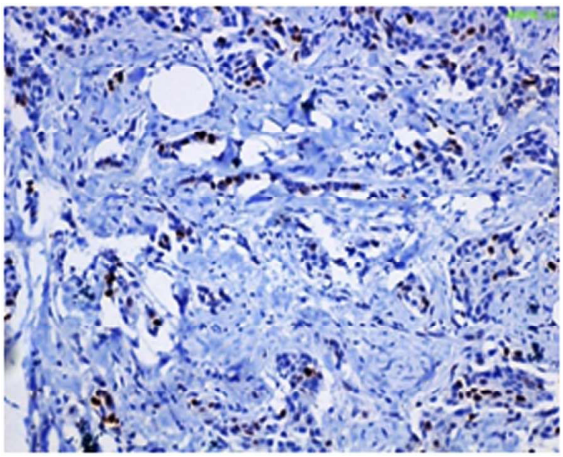

Fig. 4f: p53 positivity with low expression (weighted score 2x3 = 6) (IHC, x40)

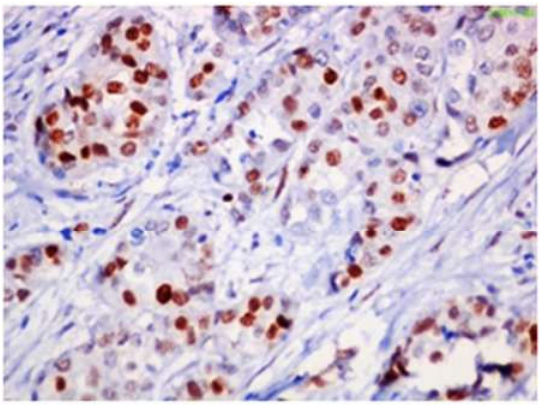

Fig. 5a: p53 positivity with low expression (weighted score 2x2 = 4) (IHC, x200)

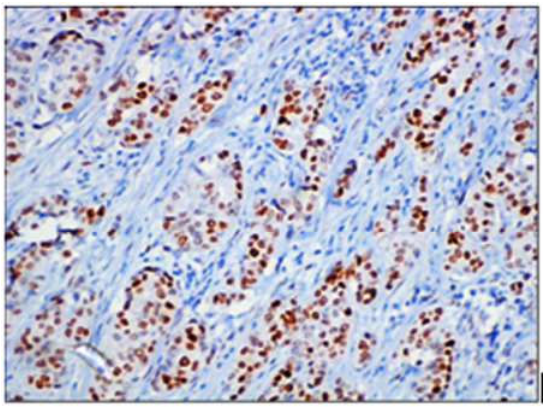

Fig. 5b: p53 positivity with low expression (weighted score 3x1 = 3) (IHC, x40)

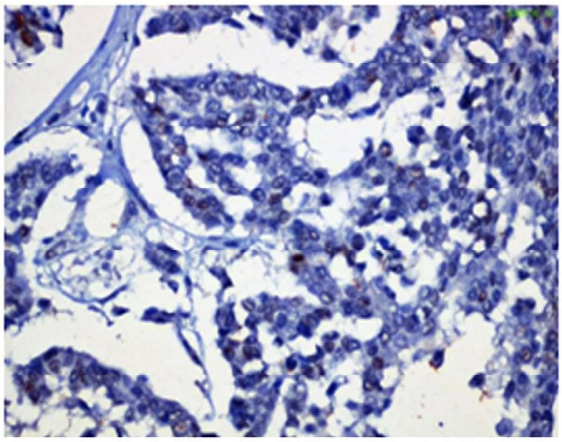

Fig. 5c: p53 positivity with low expression (weighted score 1x1 = 1) (IHC, x100)

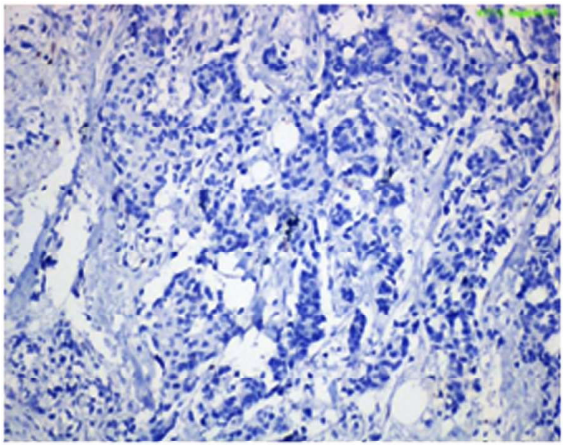

Fig. 5d: p53 immunostaining negative (IHC, x40)

Table 1: Correlation of p53 expression with known histopathological prognostic factors

Histopathological Parameters		No. of Patients (%)	p53			P – value	Significance
			High Expression No. (%)	Low Expression No. (%)	Negative Expression No. (%)		
Tumour size	T1	7 (10.8)	1 (14.3)	4 (57.1)	2 (28.6)	0.601	Not significant
	T2	44 (67.7)	19 (43.2)	13 (29.5)	12 (27.3)		
	T3	11 (16.9)	3 (27.3)	4 (36.4)	4 (36.4)		
	T4	3 (4.6)	2 (66.7)	1 (33.3)	0		
Tumour grade	2	26 (44.8)	5 (19.2)	11 (42.3)	10 (38.5)	0.013	Significant
	3	32 (55.2)	18 (56.3)	9 (28.1)	5 (15.6)		
Lymph node status	N0	21 (34.43)	4 (19.05)	10 (47.62)	7 (33.33)	0.175	Not significant
	N1	20 (32.79)	10 (50)	3 (15)	7 (35)		
	N2	7 (11.47)	3 (42.86)	3 (42.86)	1 (14.28)		
	N3	13 (21.31)	5 (38.46)	6 (46.15)	2 (15.38)		

Table to cont...

NPIG	Good	10 (17.9)	0 (0)	3 (30)	7 (70)		
	Moderate	28 (50)	13 (46.4)	10 (35.7)	5 (17.9)	0.011	Significant
	Poor	18 (32.1)	8 (44.44)	7 (38.9)	3 (16.7)		
ER Status	Negative	10 (27)	5 (50)	3 (30)	2 (20)	0.809	Not significant
	Positive	27 (73)	10 (37)	9 (33.3)	8 (29.6)		
PR Status	Negative	11 (29.73)	5 (45.5)	4 (36.4)	2 (18.2)	0.812	Not significant
	Positive	26 (70.3)	10 (38.5)	8 (30.8)	8 (30.8)		
HER2/Neu Status	0	12 (32.4)	6 (50)	2 (16.7)	4 (33.3)		
	1	4 (10.8)	0	3 (75)	1 (25)	0.435	Not significant
	2	9 (24.3)	3 (33.3)	3 (33.3)	3 (33.3)		
	3	12 (32.4)	6 (50)	4 (33.3)	2 (16.7)		

the remaining 27.7% of cases. [Fig./ Table 4a, 4b, 4c, 4d, 4e, 4f, 5a, 5b, 5c, 5d]

Correlation of p53 expression with prognostic factors

Significant correlation of p53 expression was found with tumor grade and Nottingham Prognostic Index Groups with p-value of 0.013 and 0.011 respectively.

DISCUSSION

Breast carcinoma is one of the most common malignancies and the leading cause of cancer death in women. Early and correct diagnosis is the most important factor for treatment outcome of the patient. There are various known prognostic factors for breast carcinoma like tumor size, histopathological grade and lymph node status.[6] Now-a-days these known prognostic factors are supplemented with measurements of steroid hormone receptors, proliferation index, tumor suppressor genes, oncogenes and oncoproteins for predicting outcome of the disease and decision making for optimal treatment. Recent attention has been directed at molecular classification and molecular and genetic testing are found to have greater prognostic and predictive value but still they are expensive and not yet widely available.[5]

The p53 mutation remains the most common genetic change identified in human neoplasia. The p53 gene encodes for 53 kDa nuclear phosphoprotein, which has been implicated in controlling cell cycle regulation, differentiation, DNA repair and apoptosis. Unlike normal p53, non-functional mutated p53 accumulates in the nucleus of tumor cells and can be detected by IHC. According to many studies, p53 mutation is associated with greater degree of progression of tumor, high proliferation rate, more aggressive disease, greater probability of micro metastases and worse overall survival in breast carcinoma.[7-10] Careful studies with micro dissected tumor material have shown that p53 mutation may occur in ductal carcinoma in situ (DCIS) before the development of invasive cancer and the frequency increases from around zero in low grade DCIS to 30 – 40% in high grade DCIS.[11-13] These results emphasize the important role of p53 alterations early in the carcinogenesis of breast.[14]

In this study total 65 cases of breast carcinomas were studied for expression of p53 and out of 65, 37 cases were studied also for expression of hormonal receptors like ER and PR and for HER2/neu. p53 expression was correlated with known histopathological prognostic factors. There was significant correlation between p53 expression with histological grade and NPIG but not with tumor size, lymph node status, hormone receptors status or HER2/neu status.

p53 Expression

p53 IHC positivity was identified by distinct brown colour nuclear staining due to oxidised DAB. The result of IHC was quantified as scores.[15] As there is no specific scoring system for evaluation of p53 expression, different scoring systems were used by different studies. The present study followed the scoring system used by Ryu *et al.*[16] Which showed p53 positivity in 72.3% of cases. This is like studies done by Patnayak *et al.*[17] (69.2%), Sekar *et al.*[18] (71.67%) and Gupta *et al.*[19] (88.9%) but according to many other researchers the frequency of positivity of p53 is comparatively less. These variations can be explained by the effect of genetic

and environmental factors in the study population, the type of tissue used for the study (formalin fixed, frozen, stored etc.), the type of antibody used and interpretation of results.[20]

Correlation of p53 expression with histological prognostic factors

Tumor size is an independent prognostic factor in breast carcinoma and survival rate decreases with increasing size of the tumor.[21,22] Hence the p53 expression was correlated with the size of tumor in the present study. The tumor size ranged from 2 cm to 12 cm with maximum number of tumors between (T2) 2 – 5 cm in size. This can be explained as most of the breast carcinomas are relatively painless, they usually get ignored by the patients till they reach a significant palpable size.[18] There was no significant correlation between tumor size and p53 expression in this study (p-value = 0.601) which was comparable to the findings of Gupta *et al.*[19] AI – Joudi *et al*.[20] and Sheipour *et al*.[23] and Lebe *et al*.[24]

Histologic grade of breast carcinoma is validated to be a poor prognostic factor by multiple independent studies. In the present study grade 3 tumors were more in number in comparison to grade 2 which was similar to findings of Cass *et al.*[25] It was found that there was a significant correlation between histological grade and p53 expression (p-value – 0.013) in the study. The studies done by Gupta *et al*.[19] Cass *et al*.,[26] Sirvent *et al.*[27] AI-jaudi *et al*.[20] Prabal *et al*.[15] and Singh *et al*.[28] also yielded similar result.

Axillary lymph node status is an important prognostic factor in breast carcinoma. The risk of distant recurrence is directly related to number of positive lymphnodes.[26] Furthermore, the mortality rate in node positive cases is four to eight times higher than node negative cases with lymphnode involvement being a valuable indicator of long term survival.[27, 29] In the present study there was no correlation between p53 expression and lymphnode status which was similar to findings of Han *et al.*,[30] AI–Jaudi *et al*.[20] Sirvent *et al.*[27] and Sheikhpour *et al.*[23] However, the studies conducted by Gupta *et al.*[19] Singh *et al.*[28] and Ivkovic – Kapicl *et al*.[31] contradicted this. The variation in result can be explained as in the present study, lumpectomy specimens were also considered and in 4 cases lymph nodes could not be assessed. Hence large number of cases are required to find out the association between p53 expression and axillary lymph node status.

Nottingham prognostic index is a numerical index used for prognostication of breast cancer. It depends on tumor size, grade of the tumor and nodal status. In the study, NPI has significant association with p53 status which is contradicting the study done by Kurshumilu *et al*.[32] and Esin *et al.*[33]

Correlation of p53 expression with ER/PR status and Her2/neu status

Hormonal receptors have been known to be very important for therapy and prognosis in breast cancer. The receptor positive patients are considered to have better prognosis than negative patients who present lower survival rate and more tumor relapse.[34,35,36] In the present study there was no correlation between p53 expression and hormonal receptor status which was comparable with studies done by AI – Jaudi *et al.*[20] and Sheikhpour *et al.*[23] However, studies done by Sirvent *et al.*[27] Han *et al*.,[30] and Alireza *et al.*[37] showed significant relationship between these two. The difference can be due to small study population.

The breast cancers positive for HER2/neu are very aggressive in nature and have higher chances of tumor progression and metastasis.[38] There was no significant correlation between p53 expression and HER2/neu status in the present study. The similar result was found by Han *et al.*,[30] Sirvent *et al.*[27] and Shokouh *et al.*[39] but contradicted by Rasheed *et al*.[40] and Patnayak *et al.*[17] The variation in result may be due to a smaller number of cases taken in the study.

Use of Autopsy Series to know the Disease Reservoir-How much more Breast Carcinoma Cases can be Detected?

Autopsy studies may be used to estimate the prevalence pool of incidental cancer (and pre-cancerous lesions) among people not known to have specific cancers during their life. A substantial reservoir of insitu carcinomas is undetected during life. How hard we the pathologists look for the disease and, perhaps, our threshold for making the diagnosis are potentially important factors in determining how many cases of insitu lesions will be diagnosed. The latter has important implications for what it means to have the disease.

CONCLUSION

Carcinoma breast is considered a major concern these days since it is contributing to a large part of mortality and morbidity worldwide. Many

researches from the last decade have correlated a significant association between the development of carcinoma breast and polymorphism of p53, which is the well-established and most studied tumor suppressor gene.

The percentage of tumor cells stained with p53, and the intensity of staining varied among cases in the study which can be explained on the basis of genetic variation in the population. The level of p53 expression increased with increase in grade of the tumor that is most of the grade 3 tumors showed positivity with high expression. However, association was not that evident with tumor size and axillary lymph node status, hormone receptors expression or HER2/neu status.

In conclusion, studying the p53 expression with more molecular markers, larger number of cases and with longer duration will provide a better idea about its prognostic significance and usefulness in treatment. And autopsy studies may be used as an adjunct to estimate the prevalence pool of incidental carcinoma cases.

ACKNOWLEDGEMENT

The authors are immensely grateful to the technical staff of the hospital for their skilful assistance in this study. We are also thankful to the Head of the Department for the valuable support throughout our study.

Conflict of Interest: *Nil*

Source of Funding: *Nil*

REFERENCES

1. ***Walerych D, Napoli M, Collavin L and Sal GD.***
The rebel angel: mutant p53 as the driving oncogene in breast cancer. Carcinogenesis 2012;33(11):1-11.
2. ***Singh S et al. p53 gene.***
Mutation and immunohistochemical analysis in patients with invasive ductal Biotechnol 2013;9(4): 395-403.
3. ***Khaliq T, Afghan S, Naqi A, Haider MR.***
p53 Mutations in Carcinoma Breast - a Clinicopathological Study. JPMA 2001;51:21-25.
4. ***Abdollahi A, Sheikhbahaei S, Safinejad S and Jahanzad I.***
Correlation of ER, PR, HER- 2 and p53 Immunoreactions with Clinico-Pathological Features in Breast Cancer. J Korean Med Sci 2013; 8(3):147-152.
5. ***Taylor CR, Shi SR, Barr NJ, Wu N.***
Techniques of immunohistochemistry: principles, pitfalls, and standardization. Diagnostic immunohistochemistry. 2nd edition, Churchill Livingstone. Elsevier; 2006: 3-44.
6. ***Pan Y, Yuan Y, Liu G, Wei Y (2017)***
P53 and Ki-67 as prognostic markers in triple- negative breast cancer patients. PLOS ONE 12(2): e0172324.
7. ***Acroix M, Toillon RA, Leclercq G.***
p53 and breast cancer, an update. Endocr Relat Cancer. 2006;13(2):293-325.
8. ***Lane DP.***
Cancer: p53, guardian of the genome. Nature. 1992;358(6381):1516.
9. ***Vogelstein B, Kinzler KW.***
p53 function and dysfunction. Cell. 1992;70(4):523-6.
10. ***Elledge R.M., Fuqua S.A.W., Clark G.M., Pujol P. and Allred D.C.***
The role and prognostic significance of p53 gene alterations in breast cancer. Breast Cancer Res. Treat. 1993;27(1-2):95-102.
11. ***Ho GH, Calvano JE, Bisogna M, et al.***
In microdissected ductal carcinoma in situ, HER-2/neu amplification, but not p53 mutation, is associated with high nuclear grade and comedo histology. Cancer 2000;89(11):2153-60.
12. ***Done SJ, Eskandarian S, Bull S, Redston M, Andrulis IL.***
p53 missense mutations in microdissected high-grade ductal carcinoma in situ of the breast. J Natl Cancer Inst 2001;93(9): 700-704.
13. ***Malamou-Mitsi V, Gogas H, Dafni U, et al.***
Evaluation of the prognostic and predictive value of p53 and bcl-2 in breast cancer patients participating in a randomized study with dose-dense sequential adjuvant therapy. Ann Oncol 2006; 17 (10): 1504-11.
14. ***Borresen-Dale AL.***
TP53 and breast cancer. p53 review article. Hum Mutat 2003; 21: 292-300.
15. ***Deb P, Deshpande GU, Rai R.,***
Correlationship of hormone receptor status, p53 mutation and c – erb B – 2 overexpression with nuclear grading in breast cancers. Med J Armed Forces India. 2000;56(4):305-308.
16. ***Ryu JW, Lee MC, Jang WC.***
Detecting p53 gene mutation of breast cancer and defining differences between silver staining PCR-SSCP and immunohistochemical staining. J Korean Med Sci. 2000;15(1):73-77.
17. ***Patnayak R, Jena A, Rukmangadha N, Chowhan A, Sambasivaiah K, Phaneendra B, et al.***
Hormone receptor status (estrogen receptor, progesterone receptor), human epidermal growth factor-2 and p53 in South Indian breast cancer patients: A tertiary care center experience. Indian J Med Paediatr Oncol. 2015;36(2):117-22.
18. ***Sekar P, Bharti JN, Nigam JS, Sharma A, Soni PB.***
Evaluation of p53, HoxD10, and E-Cadherin Status in Breast Cancer and Correlation with Histological Grade and Other Prognostic Factors. Journal of Oncology. 2014;2014:1–4.
19. ***Gupta K.***
Correlation of p53 Expression with clinicopathological characteristics of breast carcinoma. Annals of Pathology and Laboratory Medicine. 2016;03(03):162 -170.
20. ***Al-Joudi FS, Iskandar ZA, Rusli J.***
The expression of p53 in invasive ductal carcinoma of the breast: a study in the North-East States of Malaysia. Med J Malaysia. 2008;63(2):96–99.
21. ***Fitzgibbons PL, Page DL, Weaver D,***
Thor AD, Allred DC, Clark GM, et al. Prognostic Factors in Breast Cancer: College of American Pathologists Consensus Statement 1999. Arch Pathol Lab Med. 2000 Jun;124:966–78.
22. ***Michaelson JS, Silverstein M, Sgroi D, Cheongsiatmoy JA, Taghian A, Powell S et al.***
The effect of tumor size and lymph node

status on breast carcinoma lethality. Cancer. 2003 Nov 15;98(10):2133-43.

23. ***Sheikhpour R, Ghassemi N, Yaghmaei P, Ardekani JM, Shiryazd M.***
Immunohistochemical Assessment of p53 Protein and its Correlation with Clinicopathological Characteristics in Breast Cancer Patients. Indian Journal of Science and Technology. 2014;7(4):472-9.

24. ***Lebe BA, Canda TÜ, Tuna BU, Sagol O, Ozer E.***
The evaluation of p53 and Ki67 expressions in invasive micropapillary carcinoma of the breast and its relation with other prognostic parameters: Thirty two cases. Turk J Cancer. 2002; 32(2):48-56.

25. ***Rakha EA, El-Sayed ME, Lee AHS, Elston CW, Grainge MJ, Hodi Z, et al.***
Prognostic significance of Nottingham Histologic Grade in invasive breast carcinoma. J Clin Oncol. 2008;26(19):3153-58.

26. ***Cass J, Varma S, Day A, Sangrar W, Rajput A, Raptis L et al.***
Automated Quantitative Analysis of p53, Cyclin D1, Ki67 and pERK Expression in Breast Carcinoma Does Not Differ from Expert Pathologist Scoring and Correlates with Clinico-Pathological Characteristics. Cancers. 2012;4(3):725742.

27. ***Sirvent JJ, Salvado MT, Santafe M, Martinez S, Brunet J, Alvaro T, Palacios J.***
p53 in breast cancer. Its relation to histological grade, lymph-node status, hormone receptors, cell-proliferation fraction (ki-67) and c-erbB-2. Immunohistochemical study of 153 cases. Histol Histopathol 1995; 10:531-9.

28. ***Singh S, Rajput S, Mishra P, Mohan G, Kumar M, Singh R et al.***
P53 gene: Mutation and immunohistochemical analysis in patients with invasive ductal carcinoma of breast. American Journal of Biochemistry and Biotechnology. 2013;9(4):395-403.

29. ***Soerjomataram I, Louwman MJ, Ribot JG, Roukema JA, Coebergh JW.***
An overview of prognostic factor for long term survivors of breast cancer. Breast Cancer Res Treat 2008;107(3):309-30.

30. ***Han J, Cao D, Molberg K, Sarode V, Rao R, Sutton L et al.***
Hormone Receptor Status Rather Than HER2 Status Is Significantly Associated With Increased Ki-67 and p53 Expression in Triple-Negative Breast Carcinomas, and High Expression of Ki-67 but Not p53 Is Significantly Associated with Axillary Nodal Metastasis in Triple-Negative and High-Grade Non–Triple Negative Breast Carcinomas. Am J of Clin Pathol. 2011;135(2):230-237.

31. ***Ivkovic-Kapicl T, Panjkovic M, Nincic D, Knezevic-Usaj S.***
Factors correlating with lymph node metastases in patients with T1 ductal invasive breast cancer. Arch oncol. 2006;14(1-2):19-22.

32. ***Kurshumliu F, Gashi-Luci L, Kadare S, Alimehmeti M, Gozalan U.***
Classification of patients with breast cancer according to Nottingham Prognostic Index highlights significant differences in immunohistochemical marker expression. World J Surg Oncol. 2014;12(1):243-7.

33. ***Cengiz-Boduroglu E, Irkkan Ç, Bilir G.***
Is Nothingham Prognostic Index correlated with apoptosis and P53 expression in infiltrating ductal carcinoma of the breast?. Pathology & Oncology Research. 2003;9(2):100-103.

34. ***Howell A, Harland R, Bramwell V, Swindell R, Barnes D, Redford J et al.***
Steroid-hormone receptors and survival after first relapse in breast cancer. The Lancet. 1984;323(8377):588-591.

35. ***McGuire W, Horwitz K, Pearson O, Segaloff A.***
Current status of estrogen and progesterone receptors in breast cancer. Cancer. 1977;39(6):2934-2947.

36. ***Pertschuk L, Eisenberg K, Carter A, Feldman J.***
Immunohistologic localization of estrogen receptors in breast cancer with monoclonal antibodies. Correlation with biochemistry and clinical endocrine response. Cancer. 1985;55(7):1513-1518.

37. ***Alireza A, Sheikhbahaei S, Safinejad S, Jahanaz I. Correlation of ER, PR,***
HER - 2 and P53 Immunoreactions with Clinico - Pathological Features in Breast Cancer. Iranian Journal of Pathology. 2013;8(3):147-152.

38. ***Mortenson ED, Fu YX.***
Adaptive Immune Responses and HER2/neu-Positive Breast Cancer. Curr Pathobiol Rep. 2013 Mar 1;1(1):37-42.

39. ***Shokouh T, Ezatollah A, Barand P.***
Interrelationships Between Ki67, HER2/neu, p53, ER, and PR Status and Their Associations With Tumor Grade and Lymph Node Involvement in Breast Carcinoma Subtypes. Medicine. 2015;94(32):e1359.

40. ***Rashed MM, Ragab NM, Galal MK.***
The association of HER-2/neu overexpression in relation to p53 nuclear accumulation, hormonal recceptor status and common clinico-pathological prognostic parameters in a series of Egyptian women with invasive ductal carcinoma. European Journal of General Medicine. 2007;4(2): 73–79.