ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 17, Issue 12, 2024

Online - 2455-3891
Print - 0974-2441

Research Article

COMPARATIVE EVALUATION OF P53 IN NORMAL BREAST, FIBROADENOMA, AND CARCINOMA BREAST

KT ATHULYA KRISHNA KUMAR*, ABHILASH NP, VANISRI HR

Department of Pathology, Chamrajnagar Institute of Medical Sciences, Mysuru, Karnataka, India.
***Corresponding author: KT Athulya Krishna Kumar; Email: nambiarathulya600@gmail.com**

Received: 08 October 2024, Revised and Accepted: 20 November 2024

ABSTRACT

Objectives: The objective of this study was to analyze as well as compare the immunohistochemical expression of P53 in normal breast tissue and cases of fibroadenoma and carcinoma breast.

Methods: The present study was a retrospective analysis over a period of 1 year (2022–2023). A total of 60 cases comprising 10 cases of normal breast tissue, 20 cases of fibroadenoma breast, and 30 cases of carcinoma breast were included. Immunohistochemical staining by P53 antigen was performed and slides were graded accordingly as Grade 0, 1, and 2 depending on the staining intensity and percentage.

Results: Correlation of P53 staining across the spectrum of normal breast, fibroadenoma, and carcinoma breast showed a significant association (p=0.01). There was no significant association between the age and level of P53 expression (p=0.1). On evaluating the association between other clinicopathological variables and grade of P53 expression, we found a significant association with regards to tumor size (p=0.0006), tumor grade (p=0.043), lymph-vascular invasion (p=0.019), and nodal metastasis (p=0.025).

Conclusion: P53 may be a possible prognostic marker, to help in better therapeutic management of cases of breast carcinoma.

Keywords: Fibroadenoma, Carcinoma breast, P53, Immunohistochemistry.

 DOI: http://dx.doi.org/10.22159/ajpcr.2024v17i12.53006. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Globally, the most common malignancy in women is breast cancer, which accounts for 14% of all cancers in India [1]. In view of the increasing breast cancer burden in our country, medical institutes and hospitals have started breast clinics to educate women for self-examination and thereby possible early detection of the same [1]. All approaches aim to categorize breast carcinoma by prognosis and risk. Invasive carcinomas that do not/fail to display characteristics of a specific histologic type are classified as invasive carcinoma of no special type (NST) [2].

Invasive carcinoma of NST (ductal) is the most common histopathological subtype followed by lobular, medullary, mucinous, papillary, tubular, and inflammatory carcinoma [1,2]. The Elston–Ellis modification of the Scarff–Bloom–Richardson grading system is a three-tiered grading system that is of great prognostic relevance in breast carcinoma. This method relies on the evaluation of tubule formation, mitotic activity, and nuclear morphology of the tumor [2]. The TNM system classifies cancers according to local size, lymph node involvement (N category), and distant spread (M category). Clinical as well as pathological staging is important in breast cancer patients to select therapeutic modalities [2].

Breast fibroadenoma is the most common benign breast tumor with the highest prevalence among women of reproductive age group [3-5].

It has a reported incidence of 27.6% in women between the ages of 18 and 40 years [6]. There is a moderately increased risk of breast carcinoma development from fibroadenoma (~2–3-fold) which is reported to be persistent with less variation over time [7].

These tumors are comprised both glandular and stromal components. Histological features and variants of fibroadenoma have been of great interest to both clinicians and pathologists, alike [8]. They are also of clinical significance as a potential mimicker of sonological and clinical features of carcinoma breast. This can lead to diagnostic challenges [6].

Integration of clinical, radiological, and pathological findings helps in the effective management of fibroadenoma breast [5].

P53 was first discovered in the year 1979 and was initially mistaken to be an oncogene [9]. The tumor protein P53 (TP53) gene encodes the P53 protein which regulates the cell cycle at the G1/S checkpoint for deoxyribonucleic acid (DNA) repair, or through induction of apoptosis in damaged cells [10,11]. This hereby confers a genome protective action. Various genetic polymorphisms and mutations can alter the function of P53 protein, and lead to imbalances in cell repair at the genetic level [12]. The suppression of negative growth regulation by p53 occurs in almost all carcinomas. Under normal cellular conditions, p53 signaling appears to be on standby. In response to cellular stresses as well as with the effect of upregulatory cellular kinases, activation of its signaling cascade occurs [9].

Subregulation and somatic mutations of the P53 protein may lead to the development of breast cancer [6]. Accumulation of P53 in neoplastic tissues is proportional to the amount of P53 mutations [13]. When this happens, cells with mutated P53 protein have increased chances to accumulate additional chromosomal rearrangements and further mutations. This can assist in the proliferation of mutated cells, which modulates the pathway of benign breast disease cells evolving into breast carcinoma [6]. Loss of heterozygosity of p53 has been postulated to be an initial event in breast carcinomas [12].

The primary objective of this study was to analyze as well as compare the immunohistochemical expression of P53 in normal breast tissue and cases of fibroadenoma and carcinoma breast. We also aimed to analyze P53 expression with other cline pathological parameters.

METHODS

The present study was a retrospective analysis over a period of 1 year (2022–2023). Institutional ethical clearance was obtained. (CIMS/IEC-02/21/2023).

Histopathological specimens of breast lesions were received in the Department of Pathology, and processed. Relevant clinical details were also recorded. Following hematoxylin and eosin staining (H and E), a histopathological diagnosis was made.

Those diagnosed with breast carcinoma were further subtyped, graded, and staged accordingly. Cases of fibroadenoma and invasive ductal adenocarcinoma, not otherwise specified, were included in this study along with normal breast tissue. Ten cases of normal breast tissue, 20 cases of fibroadenoma breast, and 30 cases of carcinoma breast were included. Other special subtypes of breast carcinoma were excluded from this study.

Selected blocks were retrieved and immunohistochemical staining by P53 antigen (Clone DO-7 and DAKO) was performed and graded accordingly. Sections of the tonsil were used as a positive control.

Grading of the immunohistochemical staining [14] was done based on percentage of stain positivity and strength of staining intensity as follows:

- 0–10% stained=negative (−), grade 0
- 10.1–49% stained=positive, grade 1. (heterogeneous and focal staining)
- >50% stained=positive, grade 2. (homogenous and diffuse staining).

The data were entered into an Excel sheet and analyzed using Statistical Packages for the Social Sciences 23 software. Pearson's Chi-square test was used to analyze the level of significance between the degree of P53 expression and various parameters $p<0.05$ were taken as statistically significant.

RESULTS

A total of 60 cases were analyzed in this study comprising 10 normal breasts, 20 fibroadenoma breasts, and 30 cases of invasive ductal carcinoma. The ages of the patients ranged from 19 to 70 years.

Cases of carcinoma breast were maximally seen in the 5th decade. Tumor size ranged from 3 cm to 6 cm. Out of the 30 cases of carcinoma breast, 20% cases (n=06) were of grade 1, 53.33% cases (n=16) were of grade 2, and 26.67% cases (n=08) were of grade 3. 63.33% cases (n=19) displayed lymphovascular invasion and 36.67% cases (n=11) did not have lymphovascular invasion. About 70% of cases (n=21) displayed nodal metastasis and 30% (n=09) cases did not have lymph node metastasis (Table 1).

P53 staining expression was given a three-tiered grading (Fig. 1). Forty percent of normal breast tissue displayed negative P53 staining, and only a single case displayed grade 2 staining (Fig. 2). All cases of fibroadenoma displayed grade 1 or grade 2 staining, with 60% of cases showing grade 2 staining and 40% of cases showing grade 1 staining (Fig. 3).

Maximum number of cases of carcinoma breast (63.33%, n=19) showed strong, diffuse staining with P53 (grade 2). 33.33% (n=10) showed grade 1 staining and a single case showed negative staining (Fig. 4).

Correlation of P53 staining across the spectrum of normal breast, fibroadenoma, and carcinoma breast showed a significant association (p=0.01). There was no significant association between the age and level of P53 expression (p=0.1). On evaluating the association between other clinicopathological variables and grade of P53 expression, we found a significant association with regards to tumor size (p=0.0006), tumor grade (p=0.043), lympho-vascular invasion (p=0.019) and nodal metastasis (p=0.025).

Table 1: Frequencies and percentages of various clinicopathological parameters

Variable	Frequency	Percent
Age		
≤42 years	30	50
>42 years	30	50
Size of carcinoma		
≤4.8 cm	13	43.33
>4.8 cm	17	56.67
Grade of carcinoma		
Grade 1	06	20
Grade 2	16	53.33
Grade 3	05	26.67
Lymphovascular invasion in carcinoma		
Absent	11	36.67
Present	19	63.33
Lymph node metastasis in carcinoma		
Absent	09	30
Present	21	70

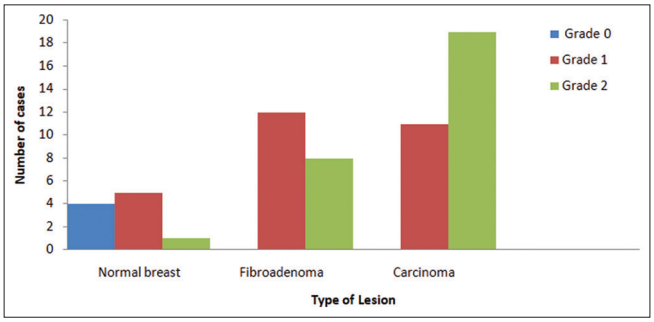

Fig. 1: Bar diagram depicts P53 grade of staining in normal breast, fibroadenoma, and carcinoma breast

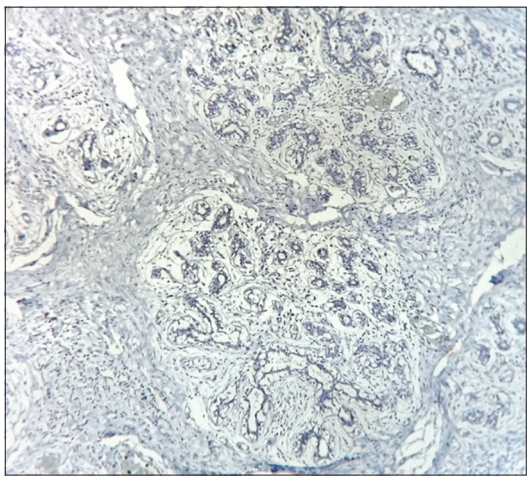

Fig. 2: Photomicrograph displays grade 0 (negative) P53 staining in normal breast tissue (P53, ×10)

DISCUSSION

P53 acts on the G1/S checkpoint in the process of DNA repair. Through a cascade of reactions, P53 prevents abnormal cells from mitosis and completion of cell division [14,15].

Cells having mutated P53 do not have the ability to stop the cell cycle, making them unstable, with the accumulation of additional mutations leading to the proliferation of these mutated cells and subsequent evolution of neoplasms [16-18]. Furthermore, the accumulation of the inactive form of P53 leads to an exponential increase in mutant P53 expression due

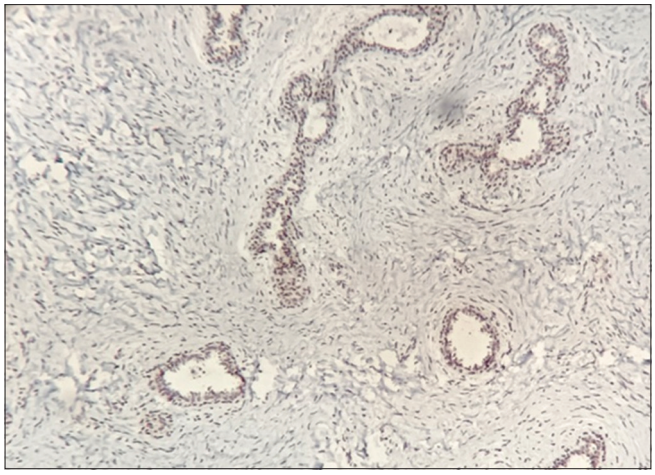

Fig. 3: Photomicrograph displays grade 1 P53 staining in fibroadenoma breast (P53, ×10)

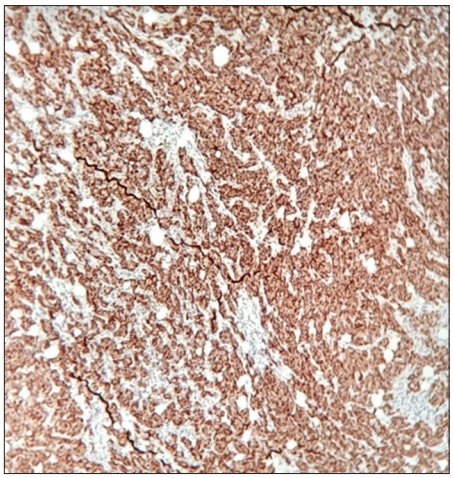

Fig. 4: Photomicrograph displays grade 2 P53 staining in Invasive ductal carcinoma breast (P 53, ×10)

to increased DNA damage. Hence, the accumulation of P53 protein in neoplastic tissues is directly linked to the presence of mutated P53 [19].

In our study, we found an increased P53 expression from normal breast tissue to carcinoma breast, with cases of carcinoma breast maximally displaying strong diffuse expression. This is in accordance with numerous previous studies, where there is increased expression of P53 in carcinoma breast when compared to cases of benign breast disease including cases of fibroadenoma [20-23].

In the cases of invasive ductal carcinoma breast, we found a significantly increased P53 expression with respect to increasing tumor size, grade, presence of lymphovascular invasion, and nodal metastasis. This points to the association of P53 with clinicopathological parameters having a worse prognosis.

Dash *et al.* [24] and Gupta *et al.* [25] found an increasing P53 expression in breast carcinoma with the presence of lymph nodal metastasis. Gupta *et al.* [25] and Yamashita *et al.* [26] also found a significant correlation of P53 expression with increasing tumor grade. This was comparable to the findings of our study.

Li *et al.* [27] discovered a significant association between increased P53 expression and the presence of lymphovascular invasion and tumor grade. They concluded that P53 was an independent factor for worse prognosis in breast cancer patients [27].

CONCLUSION

TP53 mutations are found in 30% of breast carcinoma. It is now established that P53 expression is associated with adverse prognosis. It has also been reported that P53 accumulation is linked to endocrine therapy resistance. In the present study, we found maximum p53 expression in breast carcinoma when compared to fibroadenoma, along with a direct correlation with worse clinicopathological factors in the cases of invasive ductal carcinoma breast. Further research to understand the functions regulated by the P53 gene in tumor cells will help to pinpoint its exact role and pave the path for opportunities for targeted therapies *in vivo* P53 may be used as a prognostic marker in the future, to help in the better therapeutic management of cases of breast carcinoma.

ACKNOWLEDGMENT

None.

AUTHOR'S CONTRIBUTION

All authors contributed equally to the conception, data collection, analysis, and drafting of the manuscript.

CONFLICT OF INTEREST

None.

REFERENCES

1. Raj S, Soni S, Aden D, Yadav A, Raj S. Survivin and p53 expression in benign and malignant lesions of breast. IP J Diagn Pathol Oncol. 2022;7(4):229-32. doi: 10.18231/j.jdpo.2022.054
2. Berg JW, Hutter RV. Breast cancer. Cancer. 1995;75(1):257-69. doi: 10.1002/1097-0142(19950101)75:1+<257:aid-cncr2820751311>3.0.co;2-y
3. Guirguis MS, Adrada B, Santiago L, Candelaria R, Arribas E. Mimickers of breast malignancy: Imaging findings, pathologic concordance and clinical management. Insights Imaging. 2021;12(1):53. doi: 10.1186/s13244-021-00991-x, PMID: 33877461
4. Ajmal M, Khan M, Van Fossen K. Breast fibroadenoma. In: StatPearls. Treasure Island, FL: StatPearls Publishing; 2022. Available from: https://www.ncbi.nlm.nih.gov/books/NBK535345 [Last accessed on 2024 Jun 12].
5. Stachs A, Stubert J, Reimer T, Hartmann S. Benign breast disease in women. Dtsch Ärztebl Int. 2019;116(33-34):565-74. doi: 10.3238/arztebl.2019.0565, PMID: 31554551
6. Greenberg R, Skornick Y, Kaplan O. Management of breast fibroadenomas. J Gen Intern Med. 1998;13(9):640-5. doi: 10.1046/j.1525-1497.1998.cr188.x, PMID: 9754521
7. Li J, Humphreys K, Ho PJ, Eriksson M, Darai-Ramqvist E, Lindström LS, *et al*. Family history, reproductive, and lifestyle risk factors for fibroadenoma and breast cancer. JNCI Cancer Spectr. 2018;2(3):pky051. doi: 10.1093/jncics/pky051, PMID: 31360866
8. Costa RS, Silva IF. P53 Expression in benign breast disease development: A systematic review. Asian Pac J Cancer Prev. 2020;21(9):2485-91. doi: 10.31557/APJCP.2020.21.9.2485, PMID: 32986343
9. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature. 2000;408(6810):307-10. doi: 10.1038/35042675, PMID: 11099028
10. Agarwal ML, Taylor WR, Chernov MV, Chernova OB, Stark GR. The p53 network. J Biol Chem. 1998;273(1):1-4. doi: 10.1074/jbc.273.1.1, PMID: 9417035
11. Pharoah PD, Day NE, Caldas C. Somatic mutations in the p53 gene and prognosis in breast cancer: A meta-analysis. Br J Cancer. 1999;80(12):1968-73. doi: 10.1038/sj.bjc.6690628, PMID: 10471047
12. Casey G, Lopez ME, Ramos JC, Plummer SJ, Arboleda MJ, Shaughnessy M, *et al*. DNA sequence analysis of exons 2 through 11 and immunohistochemical staining are required to detect all known p53 alterations in human malignancies. Oncogene. 1996;13(9):1971-81. PMID: 8934544
13. Gogoi S, Das B, Borgohain M, Gogoi G, Das J. Ki67 and P53 expression in breast cancer and their correlation with clinicopathological parameters. Indian J Pathol Oncol. 2021;8(4):478-84. doi: 10.18231/j.ijpo.2021.099
14. Levine AJ. p53, the cellular gatekeeper for growth and division. Cell. 1997;88(3):323-31. doi: 10.1016/s0092-8674(00)81871-1,

PMID: 9039259

15. Rivoire WA, Corleta HE, Silva IS.Molecular biology of cervical cancer. Rev Bras Cancerol. 2001; 47(2): 179-84. doi: 10.32635/2176-9745.RBC.2001v47n2.2332
16. Bourdon JC, de Laurenzi V, Melino G, Lane D. p53: 25 years of research and more questions to answer. Cell Death Differ. 2003;10(4):397-9. doi: 10.1038/sj.cdd.4401243, PMID: 12719714
17. Bourdon JC. p53 and its isoforms in cancer. Br J Cancer. 2007;97(3):277-82. doi: 10.1038/sj.bjc.6603886, PMID: 17637683
18. Oliveira AM, Ross JS, Fletcher JA. Tumor suppressor genes in breast cancer: The gatekeepers and the caretakers. Am J Clin Pathol. 2005;124:S16-28. doi: 10.1309/5XW3L8LU445QWGQR, PMID: 16468415
19. Dowell SP, Wilson PO, Derias NW, Lane DP, Hall PA. Clinical utility of the immunocytochemical detection of p53 protein in cytological specimens. Cancer Res. 1994;54(11):2914-8. PMID: 8187077
20. Ekundina OV, Ejelue CC, Apulu OP, Ayodele OO. Cell proliferation, contact inhibition, and apoptosis profiling in a benign and malignant breast lesion. Int J Oncol Res. 2022;5:034. doi: 10.23937/2643-4563/1710034
21. Angèle S, Jones C, Reis Filho JS, Fulford LG, Treilleux I, Lakhani SR, *et al*. Expression of ATM, p53, and the MRE11-Rad50-NBS1 complex in myoepithelial cells from benign and malignant proliferations of the breast. J Clin Pathol. 2004;57(11):1179-84. doi: 10.1136/jcp.2004.017434, PMID: 15509680
22. Niezabitowski A, Lackowska B, Rys J, Kruczak A, Kowalska T, Mitus J, *et al*. Prognostic evaluation of proliferative activity and DNA content in the phyllodes tumor of the breast: Immunohistochemical and flow cytometric study of 118 cases. Breast Cancer Res Treat. 2001;65(1):77-85. doi: 10.1023/a:1006457304526, PMID: 11245343
23. Ioakim-Liossi A, Markopoulos C, Karakitsos P, Safioleas M, Gogas J, Vaiopoulos G. p53 protein expression in benign and malignant breast lesions. Acta Cytol. 1998;42(4):918-22. doi: 10.1159/000331968, PMID: 9684577
24. Dash SS, Alaka S, Anupa T. P53 expression in breast carcinoma and its association with tumor aggressiveness. J Datta Meghe Inst Med Sci Univ. 2021;16(2):266-72.
25. Gupta KK, Dash AK, Mishra DP. Correlation of p53 expression with clinicopathological characteristics of breast carcinoma. Ann Pathol Lab Med. 2016;3:A162-70.
26. Yamashita H, Nishio M, Toyama T, Sugiura H, Zhang Z, Kobayashi S, *et al*. Coexistence of HER2 over-expression and p53 protein accumulation is a strong prognostic molecular marker in breast cancer. Breast Cancer Res. 2004;6(1):R24-30. doi: 10.1186/bcr738, PMID: 14680497
27. Li JP, Zhang XM, Zhang Z, Zheng LH, Jindal S, Liu YJ. Association of p53 expression with poor prognosis in patients with triple-negative breast invasive ductal carcinoma. Medicine (Baltimore). 2019;98(18):e15449. doi: 10.1097/MD.0000000000015449, PMID: 31045815