ISSN: 0975-3583,0976-2833 VOL15, ISSUE 9, 2024

EVALUATION OF KI67 EXPRESSION IN NORMAL BREAST AND FIBROADENOMA BREAST: A COMPARATIVE STUDY

K.T ATHULYA KRISHNA KUMAR [1], ASSISTANT PROFESSOR, CHAMRAJNAGAR INSTITUTE OF MEDICAL SCIENCES, KARNATAKA, INDIA
ORCID ID: 0009-0002-9378-1776

ABHILASH N P [2], ASSOCIATE PROFESSOR, CHAMRAJNAGAR INSTITUTE OF MEDICAL SCIENCES, KARNATAKA, INDIA
ORCID ID: 0009-0003-2066-1551

VANISRI H R [3] , PROFESSOR AND HEAD OF DEPARTMENT, CHAMRAJNAGAR INSTITUTE OF MEDICAL SCIENCES, KARNATAKA, INDIA
ORCID ID: 0000-0002-8723-2503

CORRESPONDING AUTHOR:K.T ATHULYA KRISHNA KUMAR
nambiarathulya600@gmail.com
ORCID ID: 0009-0002-9378-1776
Address: Mysuru, Karnataka, India-570004

ABSTRACT

Introduction: Ki-67 is a nuclear protein and a marker of cell proliferation. Expression of Ki-67 is detected by immunohistochemistry. Breast fibroadenomas, are benign tumours with a maximum incidence in the second decade of life . Some studies indicate that that fibroadenoma is a risk factor for development of breast carcinoma. The present study aimed primarily at assessing the expression of Ki-67 in normal breast and fibroadenoma.

Materials and Methods: The study included a total of 30 cases encompassing, 10 cases of normal breast and 20 cases of fibroadenoma. It was conducted over a period of one year (2022-2023). Grading of Ki-67 was performed based on percentage positivity of cells as low proliferative ($\leq$ 20%) and high proliferative (>20%).

Results: Ki-67 displayed a brown nuclear staining in positive cases which was 100%. There was a significant increase in Ki-67 expression on comparative analysis of normal breast and fibroadenomas (p=0.032).

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 9, 2024

Conclusion: Our study showed an increased level of Ki 67 expression in fibroadenoma breast supporting its proliferative nature. Further studies are needed to understand the behavior of this lesion better.

KeyWords: Fibroadenoma, Ki-67, Immunohistochemistry, Benign Breast Lesion

INTRODUCTION

Ki-67 was identified in 1983 by Gerde et al, and was found in the cell line of Hodgkin lymphoma. [1,2]. It is a nuclear protein and a marker of cell proliferation. Expression of Ki-67 is detected by immunohistochemistry [3]. Ki-67 expression reaches a peak during mitosis and it plays a role in ribosomal RNA synthesis. [1] The encoding gene; MKI6 is located on chromosome 10q25. Previous studies have found that Ki-67 shows low expression level in normal breast tissue. [4].

Breast fibroadenomas are benign tumours composed of an admixture of stromal and epithelial elements. Unlike carcinomatous breast lumps ,fibroadenomas are easily mobile , with well defined edges. [5] The incidence of fibroadenoma peaks during the second decade of life.[6]

Besides its classical form, fibroadenomas may be juvenile, giant or complex [7,2], with some studies indicating that that fibroadenoma is a risk factor for development of breast carcinoma. [8]. The present study primarily aimed at assessing the expression of Ki-67 in normal breast, and fibroadenoma, and comparing the expression in both groups.

MATERIALS AND METHODS

The present study included a total of 30 cases encompassing, 10 cases of normal breast and 20 cases of fibroadenoma. The study was a retrospective study over a period of one year

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 9, 2024

(2022-2023) and was approved by the institutional ethics committee. Clinical details, age , sex of the patients were recorded. Specimens received in our histopathology section were grossed following appropriate protocols and processed. Final diagnosis was given after analyzing hematoxylin and eosin (H & E) stained slides. Immunostaining was performed using Ki-67 (Clone Mib-1, DAKO) in these 30 cases Grading was performed based on percentage positivity of Ki-67 as follows [9]:

Low proliferative: ≤ 20% Ki-67-positive cells

High proliferative: > 20% Ki-67-positive cells

Data regarding clinicopathological parameters were tabulated in Microsoft Excel and statistical analysis (SPSS 23) was performed using Chi Square test. Comparison of Ki-67 staining between normal and fibroadenoma breast cases was performed. P value of <0.05 was taken as statistically significant.

RESULTS

30 cases were analyzed in this study, which included 10 cases of normal breast and 20 cases of fibroadenoma (Fig.1).

Histopathological examination followed by immunohistochemical staining with Ki-67 was performed. The mean age was found to be 28. 9 years. Fibroadenoma was noted in the age group of 20- 32 years, with a mean lesion size of 2.5cm. Following immunohistochemistry, Ki-67 displayed a brown nuclear staining in positive cases. Positive Ki-67 expression in epithelial cells only were considered in all 30 cases. In the present study all cases (100%) showed Ki-67 positivity.

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 9, 2024

A two tier grading system was followed based on the percentage positivity of cells. In cases of normal breast a majority of cases (90%, n=09) showed low proliferative Ki 67 staining . (Fig 2) In fibroadenoma breast low proliferative Ki 67 staining was seen in 50% cases (n=10) (Fig 3) and 50% cases showed high proliferative staining (n=10). (Fig 4, Fig 5).

There was a statistical significance in the Ki-67 expression on comparative analysis (Fig 6) of normal breast , and fibroadenoma (p=0.032). Out of the 8 cases of fibroadenoma breast having >2.5cm greatest dimension, high proliferative Ki 67 expression was seen in 04 cases. We did not find statistical significance with regard to age and Ki 67 staining percentage (p=0.26) as well as with regards to lesion size and Ki 67 expression. (p=0.12).

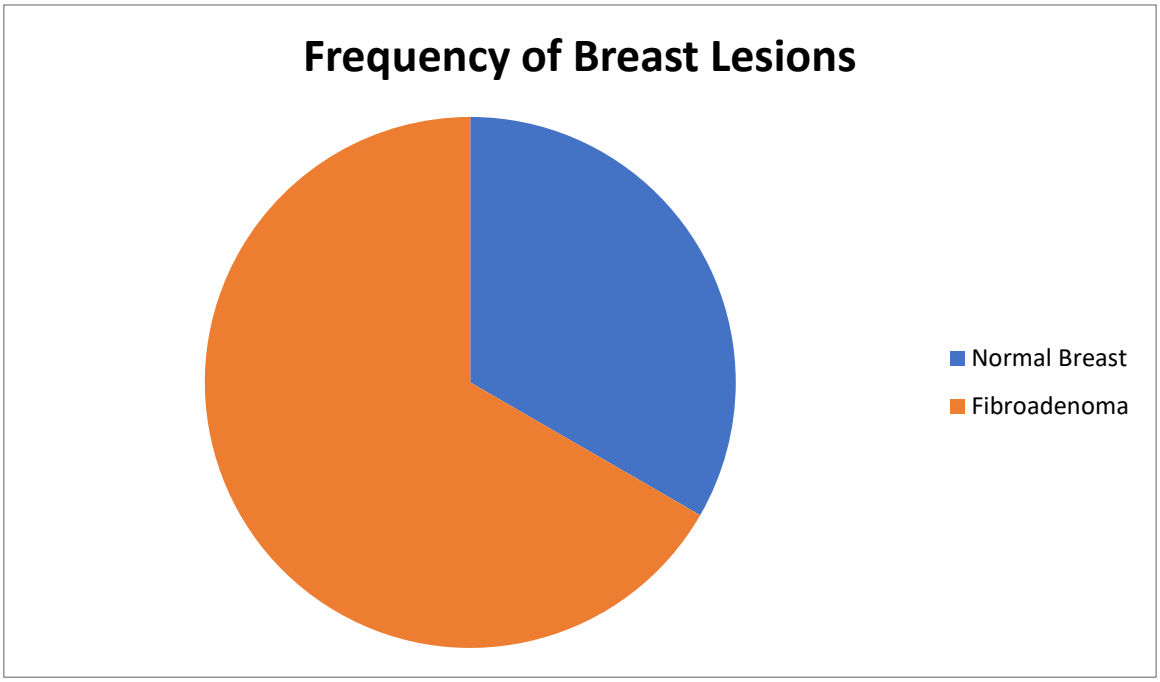

Figure 1 : Frequency of Breast lesions

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 9, 2024

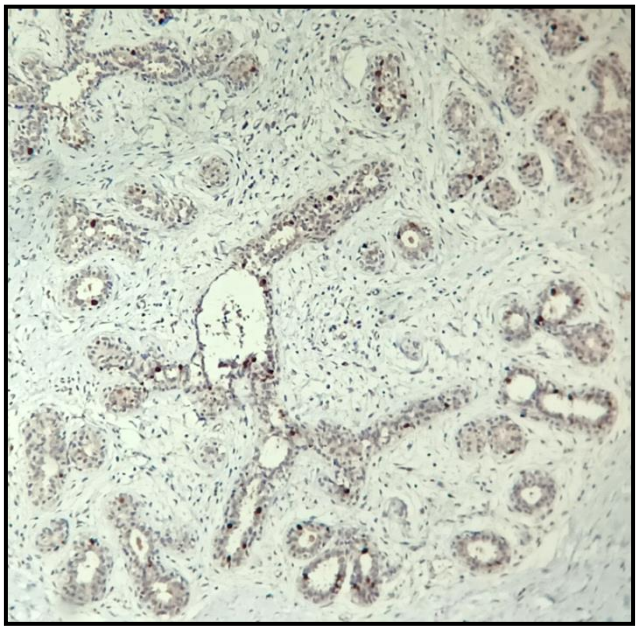

Figure 2: Ki-67 positive staining (<20%) in normal breast (Ki-67, 10x)

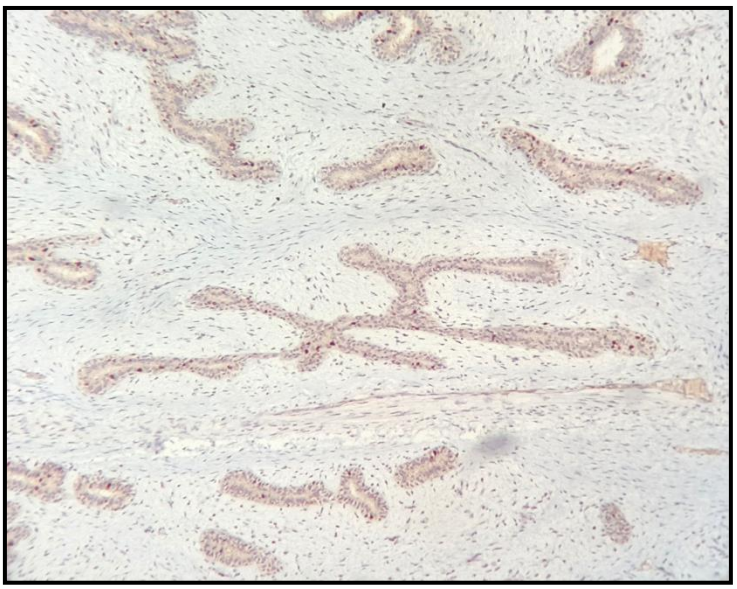

Figure 3: Ki-67 nuclear staining (<20%) in fibroadenoma breast (Ki-67, 10x)

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 9, 2024

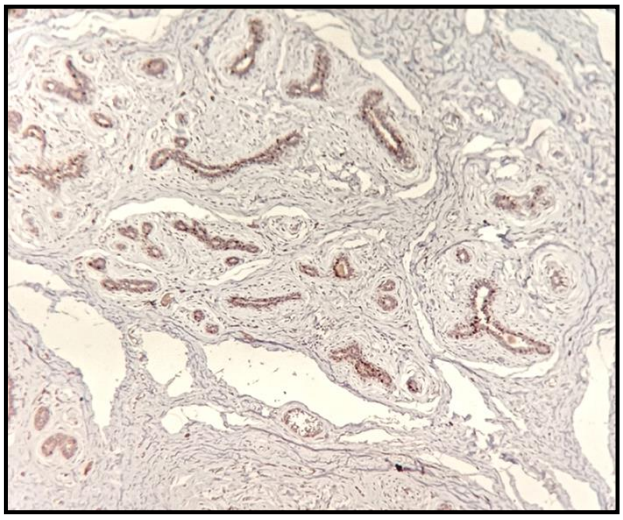

Figure 4: Ki-67 staining (>20%) in fibroadenoma breast (Ki-67, 04x)

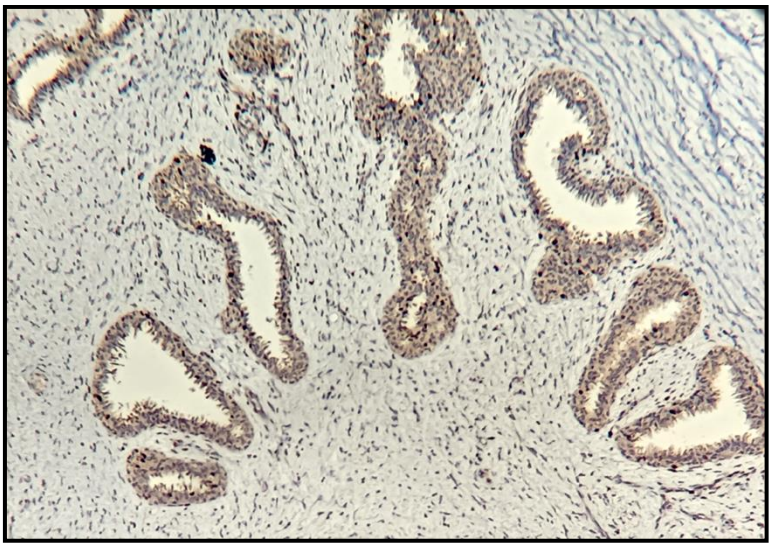

Figure 5: Ki-67 staining (>20%) in fibroadenoma breast (Ki-67, 10x)

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 9, 2024

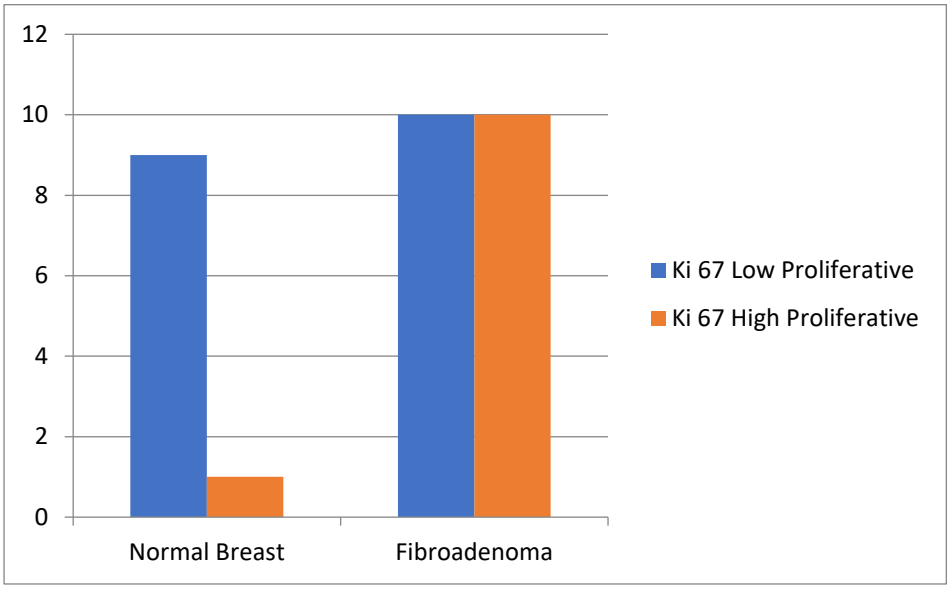

Figure 6: Proportion of positive Ki-67 staining with respect to normal breast and fibroadenoma breast.

DISCUSSION

Immunohistochemistry (IHC) is utilized to understand the expression of various intracellular /cell surface proteins in tissues. [10]. KI-67 is a non-histone nuclear protein expressed throughout the S, G1 and M phases of the cell cycle . A critical characteristic of malignant tissues is their uncontrolled capacity to multiply and progress [11,12]. Being a marker of cell proliferation Ki-67 is seen to be significantly up regulated in cancer of various organ systems. [12]

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 9, 2024

Some authors have described fibroadenoma breast as a hyperplasic lesion due to its clonality. The evolution of neoplasia is reinforced by its monoclonal origin.

Kuijper et al proposed that fibroadenoma can have a bidirectional evolution, that is, either in an epithelial direction leading to carcinoma in situ and subsequently breast carcinoma or in a stromal direction forming a phyllodes tumour. [13]

In our knowledge, presently, there is a great paucity of studies regarding comparative analysis of Ki-67 expression in normal and fibroadenoma breast..

Shoker BS et al (2001) found an increase of Ki-67 expression from normal breast tissue (3.2% percentage positivity) to fibroadenoma breast (9.5% percentage positivity) [14]

Ekundina OV et al, (2022) found a positivity rate of nuclear Ki-67 expression of 20% in normal cases ,which increased progressively to 36% in cases of fibroadenoma . [15]

We found similar results in the present study with an increased expression rate of Ki 67 in cases of fibroadenoma as compared to cases of normal breast.

Prior literature (Lelle et al, 1987) revealed that the growth fractions among various other benign condition of the breast such as cystic breast disease, fibroadenoma and blunt duct adenosis did not vary significantly (average of 3.1% $\pm$ 2.2%). [16]

On clinical diagnosis of fibroadenoma , further evaluation by sonography and fine needle aspiration cytology are advocated, as 30% breast neoplasms initially diagnosed as fibroadenoma, are found to be other lesions post surgery. In women less than age of 35 a more conservative approach to fibroadenoma is indicated with a follow up protocol every 6 months to detect any lesional change. In women older than 35 years mammography is also recommended in view of increased risk of developing carcinoma breast. [17]

Surgical excision is however recommended for fibroadenomas that do not completely regress or remain as is even by the age of 35 years. [17,18] Woman with presence of multiple

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 9, 2024

fibroadenomas are treated in the same manner as those with a single lesion. [17, 18] Katie T et.al. in 2010 assessed the methylation status in a panel of 11 genes in order to learn more about fibroadenoma biology. [19]

CONCLUSION

Our study showed an increased level of Ki 67 expression in fibroadenoma breast supporting its proliferative nature. Further studies with a larger sample size are warranted to understand the behavior and biology of this lesion.

ACKNOWLEDGEMENTS

None

CONFLICTS OF INTEREST

None

REFERENCES

1. Ragab HM, Samy N, Afify M, El Maksoud NA, Shaaban HM. Assessment of Ki-67 as a potential biomarker in patients with breast cancer. *J Genet Eng Biotechnol*. 2018;16(2):479-84. DOI: 10.1016/j.jgeb.2018.03.005

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 9, 2024

2. Sukumar C, Yadav SK, Singh G, Singh S, Sarin N. Comparison of P-53, Ki-67 and Cd-10 Expression Between Fibroadenoma, Benign Phyllodes Tumor and Malignant Phyllodes Tumor. *Ann Pathol Lab Med*. 2021;8(1). DOI: 10.21276/apalm.2007
3. Rego MF, Navarrete MA, Facina G, Falzoni R, Silva R, Baracat EC, Nazario AC. Analysis of human mammary fibroadenoma by Ki-67 index in the follicular and luteal phases of menstrual cycle. *Cell Prolif*. 2009;42(2):241-7. DOI: 10.1111/j.1365-2184.2008.00585.x
4. Gerdes J, Schwab U, Lemke H, Stein H. Production of a mouse monoclonal antibody reactive with a human nuclear antigen associated with cell proliferation. *Int J Cancer*. 1983;31(1):13-20. DOI: 10.1002/ijc.2910310105
5. Gerdes J, Lemke H, Baisch H, Wacker HH, Schwab U, Stein H. Cell cycle analysis of a cell proliferation-associated human nuclear antigen defined by the monoclonal antibody Ki-67. *J Immunol*. 1984;133(4):1710-5. DOI: 10.4049/jimmunol.133.4.1710
6. Scholzen T, Gerdes J. The Ki-67 protein: from the known and the unknown. *J Cell Physiol*. 2000;182(3):311-22. DOI: 10.1002/(SICI)1097-4652(200003)182:3<311::AID-JCP1>3.0.CO;2-6
7. Urruticoechea A, Smith IE, Dowsett M. Proliferation marker Ki-67 in early breast cancer. *J Clin Oncol*. 2005;23:7212-20. DOI: 10.1200/JCO.2005.02.046
8. Cole P, Elwood JM, Kaplan SD. Incidence rates and risk factors of benign breast neoplasms. *Am J Epidemiol*. 1978;108(2):112-20. DOI: 10.1093/aje/108.2.112
9. 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.2021.089

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 9, 2024

10. Ahmed ST, Ahmed AM, Musa DH, Sulayvani FK, Al-Khyatt M, Pity IS. Proliferative index (Ki67) for prediction in breast duct carcinomas. *Asian Pac J Cancer Prev*. 2018;19:955. DOI: 10.22034/APJCP.2018.19.4.955
11. Suciu C, Muresan A, Cornea R, Suciu O, Dema A, Raica M. Semi-automated evaluation of Ki-67 index in invasive ductal carcinoma of the breast. *Oncol Lett*. 2014;7(1):107-14. DOI: 10.3892/ol.2013.1611
12. Hashmi AA, Hashmi KA, Irfan M, Khan SM, Edhi MM, Ali JP, Hashmi SK, Asif H, Faridi N, Khan A. Ki67 index in intrinsic breast cancer subtypes and its association with prognostic parameters. *BMC Res Notes*. 2019;12(1):605. DOI: 10.1186/s13104-019-4572-4
13. Kuijper A, Buerger H, Simon R, Schaefer KL, Croonen A, Boecker W, Van Der Wall E, Van Diest JP. Analysis of the progression of fibroepithelial tumours of the breast by PCR-based clonality assay. *J Pathol*. 2002;197:575-81. DOI: 10.1002/path.1187
14. Shoker BS, Jarvis C, Davies MP, Iqbal M, Sibson DR, Sloane JP. Immunodetectable cyclin D(1) is associated with oestrogen receptor but not Ki67 in normal, cancerous and precancerous breast lesions. *Br J Cancer*. 2001;84(8):1064-9. DOI: 10.1054/bjoc.2001.1766
15. Victor O, Ekundina E, Chiamaka A, Ondokeyi A, Ore O. Cell Proliferation, Contact Inhibition, and Apoptosis Profiling in a Benign and Malignant Breast Lesion. *Int J Oncol Res*. 2022;5:1-6. DOI: 10.11648/j.ijor.20220501.11
16. Lellé RJ, Heidenreich W, Stauch G, Wecke I, Gerdes J. Determination of growth fractions in benign breast disease (BBD) with monoclonal antibody Ki-67. *J Cancer Res Clin Oncol*. 1987;113(1):73-7. DOI: 10.1007/BF00306543

ISSN: 0975-3583,0976-2833 VOL15, ISSUE 9, 2024

17. Sasidharan S, Cerin AA, Sithalakshmi M, Joseph AM. Stromal Expression of P53 and Ki 67 in Fibroadenoma: A Prospective Evaluation Study. *Int J Cad Med Pharm*. 2023;5(6):774-6. DOI: 10.11648/j.ijcmp.20230506.12
18. Ron G, Yehuda S, Ofer K. Management of breast fibroadenoma. *J Gen Intern Med*. 1998;13(9):640-5. DOI: 10.1046/j.1525-1497.1998.00197.x
19. Katie T, Alexander D, Max Y, Rooshdiya Z, Karim C, Soon L. DNA methylation profiling of phyllodes and fibroadenoma tumors of the breast. *Breast Cancer Res Treat*. 2010;124(2):555-65. DOI: 10.1007/s10549-010-0852-7