International Journal of Research in Medical Sciences
Gill SS et al. Int J Res Med Sci. 2023 May;11(5):1521-1525
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: https://dx.doi.org/10.18203/2320-6012.ijrms20231018

Original Research Article

Correlation of mitotic activity and Ki 67 with BR score and molecular classification in carcinoma breast

Satyajit Singh Gill[1], Nutan Jumle[2], Aleena Babu[2], Saumya Lal[3], Ritu Sharma[3], Rohit Singh Rajput[3], Dnyanesh Limaye[4]*

[1]Jehangir Hospital, Pune, Maharashtra, India
[2]Department of Pathology, Jehangir Hospital, Pune, Maharashtra, India
[3]Jahangir Clinical Development Centre, Pune, Maharashtra, India
[4]Learning and Development, Jahangir Clinical Development Centre, Pune, Maharashtra, India

Received: 19 December 2022
Revised: 31 March 2023
Accepted: 01 April 2023

***Correspondence:**
Dr. Dnyanesh Limaye,
E-mail: Dnyanesh1in@gmail.com

ABSTRACT

Background: To determine if mitotic activity played a role in classifying breast cancer in terms of its biological behaviour. We investigated the prospect of identifying a more meaningful cell proliferation marker for categorising treatment-naive breast cancer.
Methods: The 150 cases diagnosed as invasive breast carcinoma in the histopathology section were systematically studied for the clinical, gross, and microscopic features.
Results: the 50% patients were grade 2 (75), 41% were grade 3 (71) and 9% (14) were grade 1 in present study. The distribution of intrinsic subtypes was luminal A 25% (38), luminal B 59% (88), HER2 enriched 10% (15), basal 6% (9). Out of 150 cases, 29% (43) cases were T1, T2 were 65% (97), T3 were 2% (4), T4 were 4% (6). Mean Ki 67 was 15.6±8.8 in grade 1, 23.3±15.4 and 38.2± in grade 3. There was significant difference between I and III, and II and III ($p<0.05$). Mean mitotic count in grade 1 was 5.4±2.7, in grade 2 it was 9.7±13.5, in grade 3 it was 16.1±6.9. There was significant difference between grade 1 and 2, grade 2 and 3, grade 1 and 3 ($p<0.05$). There was significant difference between T stages ($p<0.05$).
Conclusions: Ki 67 showed a more significant statistical correlation with prognostic factors as compared to mitotic count; we feel Ki 67 is more superior to mitotic count as a prognostic factor.

Keywords: Breast cancer, KI 67, Carcinoma, Breast biopsy, Mitosis

INTRODUCTION

Breast cancer is the most common cause of cancer-related deaths among women globally, including in India. It is a heterogeneous disease with a wide range of morphological, molecular characteristics, behaviour, and therapeutic response. The current standard of care for breast cancer relies on the availability of reliable clinical and pathological prognostic and predictive indicators to aid clinical and patient decision-making in an era when potentially effective treatment alternatives are becoming more widely available.[1]

Histological grade, which represents the morphological assessment of tumour biological properties and has been found to generate crucial information about the clinical behaviour of breast malignancies, is one of the most well-established prognostic variables in breast cancer.[2] Despite the fact that the reproducibility of pathologists' histologic grades has been questioned, Elston and Ellis' study provides instructions on how to grade breast tumours in a

consistent manner. They added semiquantitative evaluations for tubules, nuclear pleomorphism, and mitotic counts to Scarff-Bloom-Richardson approach.[3]

Nuclear pleomorphism and gland or tubule development are assessed across the entire tumour. In ten successive high-powered fields, mitotic counts are taken in the most mitotically active area of the tumour. The mitotic counts are converted in comparison to a standardised area and the high-powered fields are standardised by measuring the diameter of the microscopic field. The cancer committee of CAP has approved it. Non-targeted chemotherapy is more likely to respond to high-grade and quickly proliferating tumour cells.[4]

Many pathologists employ Ki-67 expression assessed by IHC to try to provide a more precise picture of percent dividing cells. Despite the fact that there is no commonly accepted cut-off for low, middle, or high Ki-67 values, and no standardised approach is used, it is known that high Ki-67 levels indicate rapidly dividing tumour cells and predict response to anthracycline chemotherapy. The luminal classifications system uses Ki 67 to distinguish between subtypes of breast cancer.[5]

Aim

Aim of the study was to correlate of mitotic activity and Ki67 with BR score and molecular classification categoriesin carcinoma breast.

Objectives

Objectives were to obtain mitotic count and Ki-67 labelling index in needle biopsy and surgical resection specimens by average method. To correlate Ki-67 labelling with the histological tumor grade. To obtain ER, PR and Her 2 status and assign each case into specific molecular subtype. To correlate mitotic count with molecular subtype. To correlate mitotic count and Ki-67 with TNM stage of the tumor individually. To understand the relevance of mitotic activity in grading of breast cancer in the context of its biological behavior as understood by molecular classification.

METHODS

Study design

This is a prospective observational study.

Study population

The department of pathology, Jehangir hospital, Pune.

Inclusion criteria

All the diagnosed cases of invasive breast carcinoma (on core biopsies as well as surgical specimens) at Jehangir hospital histopathology laboratory.

Exclusion criteria

In-situ carcinoma was not taken in present study. All male breast cases. Cases with incomplete data post neoadjuvant chemotherapy (post NACT) cases are excluded.

Sample size

The 150 patients were included in the study.

Study duration

Study conducted from June 2018 to March 2020.

Data collection method

All the cases diagnosed as invasive breast carcinoma in the histopathology section were systematically studied for the clinical, gross, and microscopic features like tumor grade with respect to tubule formation, mitotic count per 10 high power field and nuclearpleomorphism apart from lymph vascular invasion along with immunohistochemistry markers of ER, PR, Her2neu and Ki-67 and data will be collated as per the research proforma. Block selection for Immunohistochemistry and Haematoxylin and Eosin stain: a) Core biopsy: Block with maximum percentage of tumour. b) Resection specimen: Representative block of tumour with adjacent breast tissue. Standard immunohistochemistry protocol was performed using rmAb clone SP6 by hermoscientific (diluted to 1:100) to obtain Ki-67 expression. Ki67 labelling index: Sections of 4-micron thickness were selected and all the tumor cell nuclei with brown staining of any intensity were counted as positive in per 1000 tumor cells and result will be given in percentage. Immunohistochemistry slides were reviewed and Ki-67 labelling index was assessed by following methods. Average method: manually counting the positive tumor cells in the three microscopic fields and calculating the average percentage of positive tumor cells. MI procedure: Section thickness of 4 microns will be selected for mitotic count per 10 high power fields in tumor cell nuclei. To reduce the inter-observer variability, the fields for ki67 index was selected by a single observer. Assessment of Ki-67 and mitotic count was done under 40x objective with a field diameter of 0.4 mm under a fixed microscope.

Data were analysed using the SPSS software for Windows (version 26.0, IBM corporation,USA).

RESULTS

The mean Ki 67% and mitosis/10 HPF were compared between grades using a one-way ANOVA as shown in the Table 1. Because the data was non-homogeneous, the Welch correction was applied. As the data was non-normal, the Kruskal-Wallis test was applied. Between grades, there was a significant difference in mean Ki 67%

and mitosis/10 HPF. In mitosis/10 HPF between all grades, a post-hoc test revealed a significant difference in mean Ki 67% between grades I and II andgrade II and III. Because the data was non-normal, the Kruskal-Wallis test was applied. Overall, there was a significant change in mean Ki (67%) but not in mean mitosis/10 HPF between tumor stages, according to the findings. There was no significant change in mean KI-67% and mean mitosis /10 HPF between N stages, according to the findings.

Table 1: Ki 97, and mitotic count.

Variables	Ki 67 (Average)	Mitotic count (Average)
Grade		
I	15.6±8.8	5.4±2.7
II	23.3±15.4	9.7±3.5
III	38.2±22.5	16.1±6.9
Luminal subtype		
Luminal A	10.9±2	9.7±5.3
Luminal B	28.8±16.1	12.1±6.4
Her 2 neu enriched	50.6±14	10.4±4.7
Triple negative	59.5±17.1	17.2±5.9
T stage		
T1	20.4±14.2	10.5±5.4
T2	32.7±21.4	12.3±5.8
T3	18.3±8.8	8.5±2.6
T4	28.7±15.4	18.2±14.2
Nodal stage		
N0	90 (60)	25.7 ± 18.9
N1	34 (22.7)	37.3 ± 21.3
N2	9 (6)	26.4 ± 16.2
N3	17 (11.3)	27.9 ± 20.6

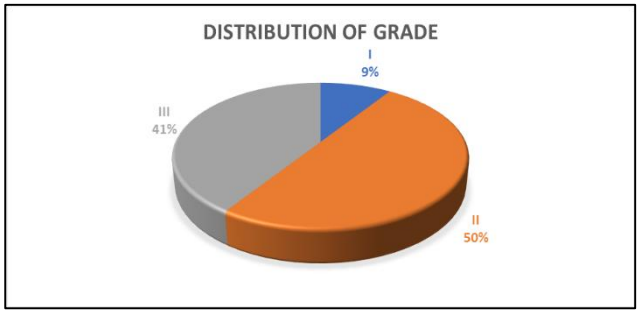

Figure 1: Distribution of grades.

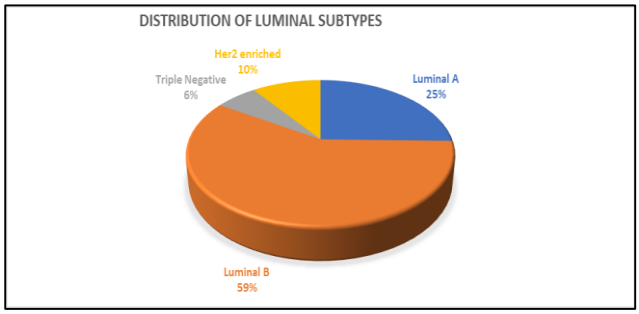

Figure 2: Distribution of luminal subtypes.

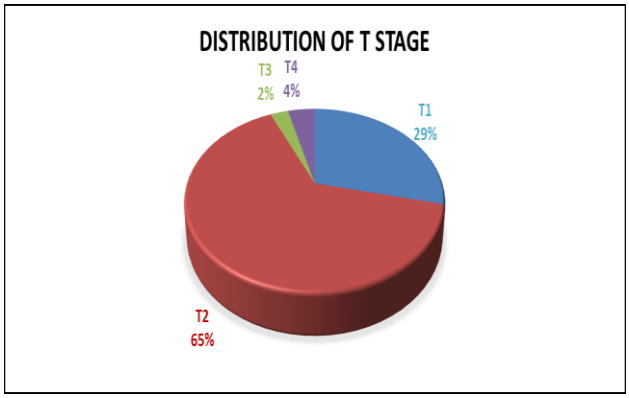

Figure 3: Distribution of T stage.

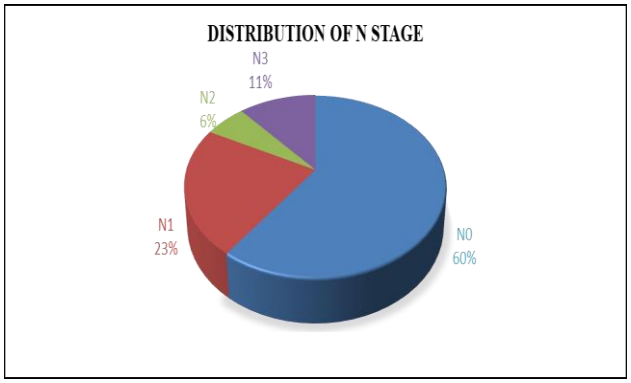

Figure 4: Distribution of N stage.

DISCUSSION

Breast cancer is the most frequent type of cancer in women. Breast cancer incidence in developed countries is higher, while relative mortality is greatest in less developed countries Breast cancerhas ranked number one cancer among Indian females.[6] With age adjusted rate as high as 25.8 per 100,000 women and mortality 12.7 per 100,000 women. Data reports from various latest national cancer registries were compared for Incidence, mortality rates.[7] An early age has been found as a major risk factor for breast cancer in Indian women. Breast cancer projection for India during time periods 2020 suggests the number to go as high as 17,97,900.[8] Changes in risk factors have resulted in an increase in the prevalence of breast cancer, which is on the rise.[8] Women can be classified depending on their risk factors for breast cancer, which can help improve risk-free procedures and build tailored breast cancer screening programs.[9,10] Many factors have been suggested as factors influencing breast cancer risk in a younger female population, with varying effect sizes and degrees of modifiability. Some risk factors for breast cancer development are obviously "inherent," meaning that an individual's decisions cannot impact the risk factor, such as the germline genome or prenatal development. Other risk variables that are possibly "modifiable" include physical activity, body weight/habit, and alcohol use, all of which are impacted by personal choice.[11,12]

Factors relating to the hormonal (estrogen) milieu to which the breast is exposed from menarche to the cessation of ovulation at menopause play a major role. They are-Early age at menarche nulliparity late age at first birth late age at any birth, low parity, late menopause.[13,14]

The link between socioeconomic status and breast cancer risk is well known, with women in higher socioeconomic groups at a higher risk. Exogenous hormone exposure, such as oral contraceptives and hormone replacement treatment. Dietary fat consumption is the true relationship between fat consumption and breast cancer does not appear to be especially strongor constant.[15]

In the present study showed that there was a link between Ki-67 labelling and histology tumor grade. It was attempted to assess the significance of mitotic activity in breast cancer grading in the context of its biological behavior as defined by molecular categorization and mitotic activity as determined by a more direct way than a mitotic count by determining the Ki-67LI. Between mitotic index and Ki-67 LI, it was investigated, the prospect of identifying the more relevant cell proliferation marker in classifying treatment-naive breast cancer into categories that better depict biological behavior of the illness. A total of 150 cases were included in the investigation. The histologic grade is based on a combination of gland development, nuclear pleomorphism, and mitotic numbers. Each category was scored from 1 to 3, for a total score of 3-9 divided into three grades: grade 1, grade 2, and grade 3. In our study, 50% of the patients were in grade 2 (75), 41% were in grade 3 (71), and 9% (14) were in grade 1. In a study of 1,61,708 instances of breast cancer from the SEER program, grade 2 was assigned the most frequently: 70,477 cases; 65,443 cases were categorized as grade 3 (40.4%); and only 25,788 cases were listed as grade 1 (15.9%). Oddo et al looked at 151 breast cancer samples.[16] Classifying 24 as histological grade I, 74 as histological grade II, and 53 as Histological Grade The 150 cases were categorized into four luminal subtypes: luminal A, luminal B (ER, PR, HER2 Ki67 14%), HER 2 enriched (ER-, PR-, HER2 ; high Ki67) and basal (ER-, PR-, HER2; low Ki67) (ER-, PR-, HER2-). The proportion in our study was luminal A 25%, luminal B 59 percent (88), HER2 enriched 10% (15), and basal 6%. (9). The main tumor was classified as T1, T2, T3, or T4 according to its stage. In our analysis, T1 accounted for 29% of the cases, T2 accounted for 65%, T3 accounted for 2%, and T4 accounted for 4%. This is in line with previous research. In each grade group, Ki 67 and grade Ki 67 were evaluated, and the mean Ki 67 was computed. In grade 1, the mean Ki 67 was 15.6 8.8, in grade 2 it was 23.3 15.4, and in grade 3 it was 38. Between grades 1 and 3, as well as 2 and 3, there was a substantial difference. This favorable link between grade and Ki 67 has been demonstrated in numerous research. According to a study by Aziz et al, Ki 67 levels rise as grades rise.[17] In their study, the median Ki 67 in grades 1-2 was 11.8 percent, but it was 30 percent in grades 3 and 4. 69 ref Ki 67 increased with grade, according to Oddo et al.[16] The Tamhane post hoc test revealed that most histological grade I breast cancers had much lower Ki67 values than histological grade 2 or 3 tumors. Thangaraj et al found a strong connection between grade and Ki 67 in a retrospective examination of the Ki-67 index and its prognosis.[18] Significance in Over 800 primary breast cancer cases. Ki-67 expression levels were shown to be substantially linked with histological grade in all patients in research by Liang et al ($p=0.001$).[19] 77th reference count and grade of mitosis each patient's mitotic count was measured, and the mean mitotic count for each grade was computed. The mean mitotic count in grade 1 was 5.42.7, 9.7 13.5 in grade 2, and 16.1 6.9 in grade 3 (Table 1). Between grades 1 and 2, grade 2 and 3, and grade 1 and 3, there is a big variation. With each passing grade, there was an upward trend. This is consistent with other standard investigations, which have shown that mitotic count increases with grade. The median mitotic count increased with grade in a study by Aziz et al with the mean mitotic count of grade 1-2 being 1.68 and grade 3 being 5.47.[17] So, like most historical data, both Ki 67 and mitotic count exhibited a positive connection with grade in our investigation. The socioeconomic status of the subjects in our study was more akin to that of the Aziz et al found that cases with 1-3 lymph nodes had a mean Ki 67 of 13.2 while cases with 4 lymph nodes had a mean Ki 67 of 14.8.[17] Mitotic count revealed a substantial difference between grades, but not between all intrinsic subtypes, but rather between luminal A and Her2 enriched, luminal B and triple negative, and trriple negative and Her 2 enriched. Compared to mitotic count, Ki 67 has a stronger connection with numerous parameters such as grade, intrinsic subtypes, and T stage, according to this study. These characteristics, such as intrinsic subtypes and grade T stage, have been proven to have predictive value in various investigations. Positive association and significant difference between tumor grades, intrinsic subtypes,and T stage in the study clearly reveals that Ki 67 is more closely associated to biological behavior of the disease. Mitotic index has only showed a link with tumor grade, which is to be expected given that mitotic count is one of 3 criteria in breast cancer grading.

Limitations

Our study was done only in 150 cases diagnosed as invasive breast carcinoma. For Ki-67 still there are no gold standard recommendations. In spite of these limitations, our study adds valuable information for Ki-67's use as molecular biomarker in the breast cancer paradigm.

CONCLUSION

Further research into better techniques for improving the accuracy of automated Ki 67 assessment, particularly in identifying and detecting tumor cells only, as well as lowering the cost of this technique and making it more

widely available, could help to establish automated Ki 67 assessment as the most accurate and standard method, allowing universal agreement on a standard cut off that better distinguishes prognostic subgroups and correlates with the molecular classification. Ki-67 proliferation indices provide an accurate measurement of breast cancer cells' proliferativepotential. Although Ki-67 is frequently used in histopathological evaluation, discrepancies in assessment methodology, a lack of gold standard recommendations, and uneven acceptance of multigene panels integrating Ki-67 all compromise the biomarker's reliability and standardization in clinical practice. Digital image analysis, supplementation with microRNAs, or radiomic techniques may be used in the future to improve Ki-67's use as a molecular biomarker in the breast cancer paradigm.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Łukasiewicz S, Czeczelewski M, Forma A, Baj J, Sitarz R, Stanisławek A. Breast Cancer-Epidemiology, Risk Factors, Classification, Prognostic Markers, and Current Treatment Strategies-An Updated Review. Cancers. 2021;13(17):4287.
2. Sancho-Garnier H, Colonna M. Épidémiologie des cancers du sein. La Presse Médicale. 2019;48(10):1076-84.
3. Momenimovahed Z, Salehiniya H. Epidemiological characteristics of and risk factors for breast cancer in the world. Breast cancer (Dove Medical Press), 2019;11:151-64.
4. Daly AA, Rolph R, Cutress RI, Copson ER. A Review of Modifiable Risk Factors in Young Women for the Prevention of Breast Cancer. Breast cancer (Dove Medical Press), 2021;13:241-57.
5. Yi-Sheng S. Risk Factors and Preventions of Breast Cancer. Int J Biological Sci. 2017;13(11):1387-97.
6. Kamińska M, Ciszewski T, Łopacka-Szatan K, Miotła P, Starosławska E. Breast cancer risk factors. Przeglad menopauzalny Menopause Rev. 2015;14(3):196-202.
7. Sayed S, Fan S, Moloo Z. Breast Cancer Risk factors in Relation to Molecular Subtypes in Breast Cancer Patients from Kenya. Research Square. Risk Factors and Prevention for Breast Cancer. ZenOnco.io. 2021.
8. Lyndin M, Hyriavenko N, Sikora V, Lyndina Y, Soroka Y, Romaniuk A. Invasive Breast Carcinoma of No Special Type with Medullary Pattern: Morphological and Immunohistochemical Features. Turk Patoloji Derg. 2021;12.
9. Agaimy A. Pleomorphic (giant cell) carcinoma revisited: A historical perspective and conceptual reappraisal. Semin Diagn Pathol. 2021;38(6):187-92.
10. Peña-Jaimes L, González-García I, Reguero-Callejas ME, Pinilla-Pagnon I, Pérez-Mies B, Albarrán-Artahona V, et al. Pleomorphic lobular carcinoma of the breast with osteoclast-like giant cells: a case report and review of the literature. Diagn Pathol. 2018;13(1):62.
11. McCart Reed AE, Kalinowski L, Simpson PT, Lakhani SR. Invasive lobular carcinoma of the breast: the increasing importance of this special subtype. Breast Cancer Res. 2021;23(1):6.
12. Metzger-Filho O, Ferreira AR, Jeselsohn R, Barry WT, Dillon DA, Brock JE. Mixed Invasive Ductal and Lobular Carcinoma of the Breast: Prognosis and the Importance of Histologic Grade. Oncologist. 2019;24(7):e441-9.
13. Limaiem F, Ahmad F. Mucinous Breast Carcinoma. In: StatPearls. Treasure Island (FL): StatPearls Publishing. 2022.
14. Dai D, Shi R, Wang Z, Zhong Y, Shin VY, Jin H, Wang X. Competing Risk Analyses of Medullary Carcinoma of Breast in Comparison to Infiltrating Ductal Carcinoma. Scientific Rep. 2020;10(1):560.
15. Reddy TP, Rosato RR, Li X, Moulder S, Piwnica-Worms H, Chang JC. A comprehensive overview of metaplastic breast cancer: clinical features and molecular aberrations. Breast Cancer Res. 2020;22(1):121.
16. Oddó D, Domínguez F, Gómez N. Granulomatous lobular mastitis associated with ductal carcinoma in situ of the breast. SAGE Open Med Case Rep. 2019;7:1-5.
17. Aziz S, Wik E, Knutsvi G. Evaluation of Tumour Cell Proliferation by Ki-67 Expression and Mitotic Count in Lymph Node Metastases from Breast Cancer. PLoS ONE. 2010;11(3):e0150979.
18. Thangaraj S, Shyamsundar V, Krishnamurthy A. Deregulation of extracellular matrix modeling with molecular prognostic markers revealed by transcriptome sequencing and validations in Oral Tongue squamous cell carcinoma. Sci Rep. 2021;11:250.
19. Liang Q, Ma D, Gao R. Effect of Ki-67 Expression Levels and Histological Grade on Breast Cancer Early Relapse in Patients with Different Immunohistochemical-based Subtypes. Sci Rep. 2020;10(1):7648.

Cite this article as: Gill SS, Jumle N, Babu A, Lal S, Sharma R, Rajput RS et al. Correlation of mitotic activity and Ki 67 with BR score and molecular classification in carcinoma breast. Int J Res Med Sci 2023;11:1521-5.