How to Cite:
Kumar, Y. R., Sreeramulu, P. N., Hemalata, A., Manjunath, G. N., & Srinivasa, D. (2022). Correlation of prognostic factors of carcinoma breast with Ki 67 proliferation assay. *International Journal of Health Sciences*, *6*(S3), 3315–3323. https://doi.org/10.53730/ijhs.v6nS3.6361

Correlation of prognostic factors of carcinoma breast with Ki 67 proliferation assay

Dr. Yadamreddy Rohit Kumar
Juinor Resident, Department of General Surgery, SDUMC, Kolar

Dr. P. N. Sreeramulu
Professor, Department of General Surgery SDUMC, Kolar

Dr. Hemalata A.
Associate Professor, Department of Pathology, SDUMC, Kolar

Dr. Manjunath G. N.
Associate Professor, Department of Radiation Oncology, SDUMC, Kolar

Dr. Srinivasa D.
Associate Professor Department of General Surgery, SDUMC, Kolar

***Abstract*---**Prognostic factors are important for the diagnosis of breast cancer as it helps in identification of high risk patients. The objective of the study is to assess the proliferation index, Ki-67 and correlate it with other markers. The present study was a cohort study conducted in the Department of General Surgery at Tertiary Care Teaching Hospital over a period of 1 year with a sample size of 98. All the patients meeting the inclusion and exclusion criteria are recruited sequentially by convenient sampling until the sample size is attained, with the agreement of the institutional ethics committee. A total of 98 patients with a mean age of 53.61 ± 12.48 years were studied in the final analysis. The mean duration of lump was 4.62 ± 2.18 months and only 6.12% had the complaint of pain. Majority of them had stage IIIB carcinoma at 43.88%, followed by stage IIA at 27.55%, 15.31% stage IIB, 13.27% stage IIIA. At cut off 20, 69(70.40%) had ki67 proliferation index ≥20 and 29(29.59%) had<20. Correlation of Ki-67 Index with expression of estrogen receptor status had a p value of 0.019 and with progesterone receptor status, p 0.003 which was significant. In the age group of 31 to 60 years, majority of them had ≥20 Ki-67 but age showed no significant association with Ki-67. Duration of lump, menstrual history, physical characteristics of the effected breast, physical characteristics of the lump, size of the lump, stage and lymph node status had no significant association with the

Manuscript submitted: 27 Dec 2021, Manuscript revised: 09 March 2022, Accepted for publication: 18 April 2022

Ki-67 expression. While the estrogen receptor expression had significant association with Ki-67 with p value 0.019, the expression of progesterone receptor showed a significant correlation with Ki-67 with p 0.003.

***Keywords*---**Ki-67, breast carcinoma, cell proliferation, immunohistochemistry, hormone receptor status.

Introduction

The most frequent form of cancer in women is cancer of the breast and is responsible for most of the deaths.[1] It is a multifactorial ailment and several factors contribute to its incidence. Breast cancer is prevalent across the world but its frequency, death rate, and survival rates differ noticeably among various parts of the world. This can be attributed to the type of population, genetic factors and location. [2] Variations in risk factors have led to an upsurge in the frequency of carcinoma breast, which is growing every day. Even though screening people can decrease the burden of breast cancer, over - diagnosis, side effects and expensive costs are the drawbacks of this method. Classification of women depending on the susceptibility of risk factors predisposing them to breast cancer can be effective in improving risk-free methods and designing targeted programs for screening of breast cancer. [3]

According to the World Health Organization (WHO) the prevalence of BC in women, globally is 2.3 million in 2020 and mortality was found in 6,85,000. The death rate in breast cancer is mainly due to extensive metastasis. From the last 5-year data up to 2020, there has been nearly 7.8 Mn w newly diagnosed cases of BC. Therefore, making BC as the most dominant cancer globally. Breast cancer can occur at any age post puberty however, the incidence is greater at older age.[4]. Breast cancer represents numerous entities ranging from carcinoma-insitu to metastatic carcinoma. Breast cancer is often diagnosed through clinical evaluation and special investigations such as fine needle aspiration (FNAC) or core needle biopsy and mammography.[5] Nevertheless, histopathology is the gold standard investigation for breast cancer. Further, the immunohistochemical (IHC) markers help in classifying the type of pathology and directs therapeutic indications.[6]

Prognostic variables are critical in the evaluation of BC as it helps in the identification of high-risk patients.[7] The currently used traditional prognostic factors are successful in identifying approximately 30% of the BC patients. Hence, there is an utmost need for new prognostic markers.[8] Because radiotherapy and various medical hormonal manipulations might cause adverse effects, risk-based refined procedures are necessary to minimize these unwanted effects. Over the last few years, certain additional prognostic factors have been identified.[9] However, clinical confirmation is still required for majority of them.[10]. Tumor markers have received a lot of attention in the search for potential breast cancer prognostic indicators. Invasive breast cancers clinical behavior is heavily influenced by cell proliferation. Cellular Proliferation is associated with a negative prognosis. As actively proliferating cells can be identified by Ki 67 labelling, it is

more sensitive than other techniques. As obtaining a consistent mitotic index requires particular training in counting with the fraction assessed method, mitotic count and Ki 67 proliferation index are regarded as practicable approaches.[11],[12] Ki 67 index has lately sparked renewed interest as a possible marker for predicting chemotherapy response.[13] Ki 67 immuno-staining is more convenient for determining the proliferation index when compared to other markers. Ki 67 immunostaining is a simple and economical technique that is utilized in practically all pathology laboratories. It just takes a little tissue sample, which can be obtained by fine-needle aspirations. In most studies, high Ki 67 levels are linked to a favourable prognosis.[14],[15]. Aims and Objectives: To asses all the prognostic factors of carcinoma breast. To assess the proliferation index (ki67) of each of the patient with carcinoma breast

Materials and Methods

The present study was a cohort study conducted in the Department of General Surgery at Tertiary Care Teaching Hospital over a period of 1 year with a sample size of 98.

Inclusion criteria

- All biopsy proven carcinoma breast were included in this study

Exclusion criteria

- Patients who are treated with neoadjuvant chemotherapy.
- Male carcinoma breast patients.
- Recurrent carcinoma breast patients.
- Patients with distant metastasis.

Methodology

Patients admitted with diagnosis of carcinoma of breast were included in the study. Specimen was sent in 10% buffered formalin. The paraffin blocks of primary tissue and metastatic lymph node was sent for tumour marker study using IHC. The value of ki67 were studied and compared with other prognostic markers using appropriate statistical analysis methods.

Statistical Methods

Descriptive analysis was carried out by mean and standard deviation for quantitative variables, frequency and proportion for categorical variables. All quantitative variables were checked for normal distribution. For normally distributed quantitative parameters the mean values were compared using independent sample t-test. Categorical outcomes were compared using Chi square test /Fisher's Exact test (If the overall sample size was < 20 or if the expected number in any one of the cells is < 5, Fisher's exact test was used.). P value < 0.05 was considered statistically significant**.** Data was analyzed by using S PSS software, V.22.

Results

A total of 98 subjects were considered in the study.

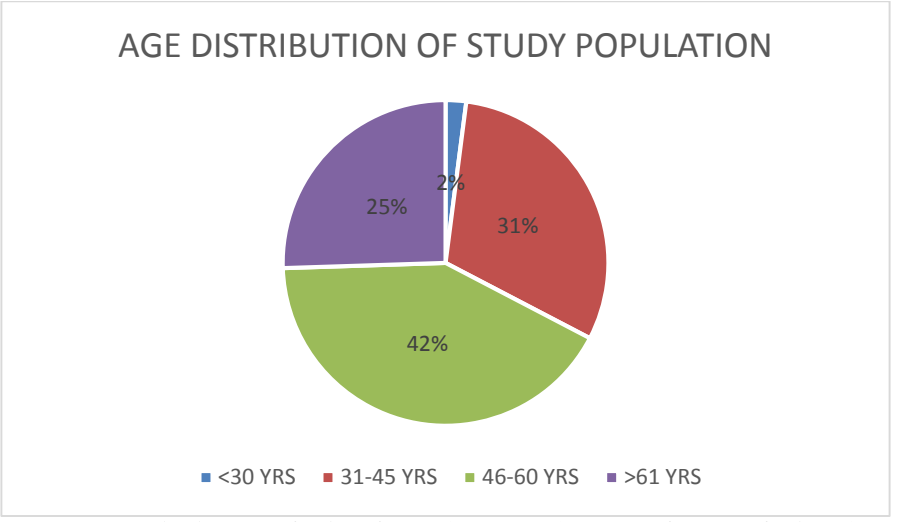

Figure 1. Descriptive Analysis of Age (In Years) In Study Population (N=98)

The study population consisted of patients aged between 30 to 80 years with a mean age of 53.61 ± 12.48 years. (Figure 1)

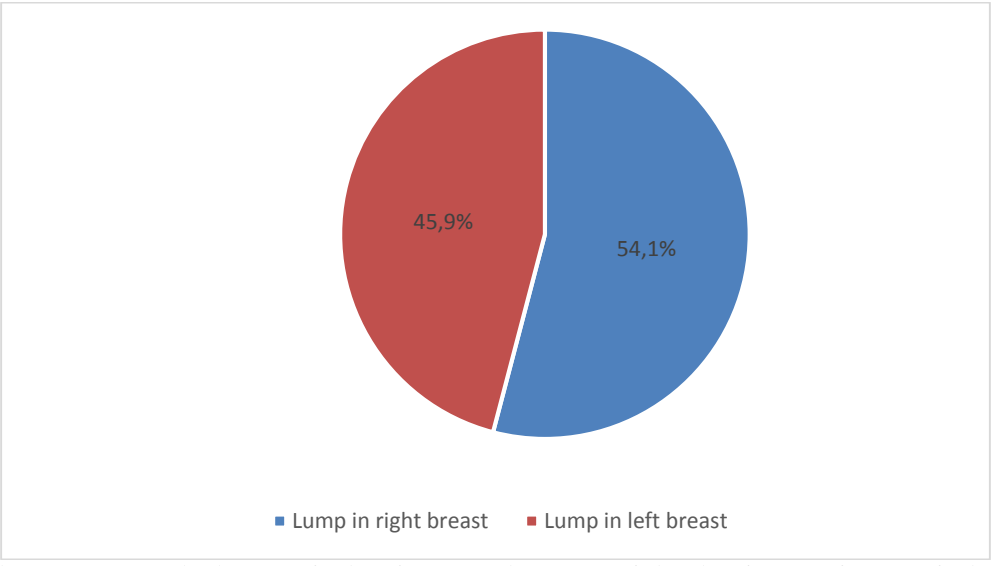

Figure 2. Descriptive Analysis of Presenting Complaint in the Study Population (N=98)

Among presenting compliant, 53 (54.08%) had lump in right breast and 45 (45.92%) had lump in left breast. (Figure 2)

Table 1
Descriptive Analysis of Size of Breast Lump in the Study Population (N=98)

Parameter	Mean ± SD	Median	Minimum	Maximum
Length (in cm)	4.94 ± 1.56	5.00	1.00	12.0
Width (in cm)	4.03 ± 1.65	3.00	2.00	8.0

The mean size of the breast lump among the study population was found to be 4.94 ± 1.56 x 4.03 ± 1.65. (Table 1)

Table 2
Descriptive Analysis of Lymphadenopathy in the Study Population (N=98)

Lymphadenopathy	Frequency	Percentages
Axilla		
Single	42	42.86%
Multiple	6	6.12%
No axillary lymphadenopathy	50	51.02%
Consistency(N=48)		
FIRM	2	4.08%
HARD	46	95.92%
Fixity(N=48)		
Fixed	5	10.20%
Mobile	43	89.80%

Among the study population, 48 had axillary lymphadenopathy. 42(42.86%) of them had single palpable lymph node, 6(6.12%) had multiple palpable lymph nodes. Out of 48 participants, 46(95.92%) were hard in consistency, 5(10.20%) were fixed and 43(89.80%) were mobile. (Table 2)

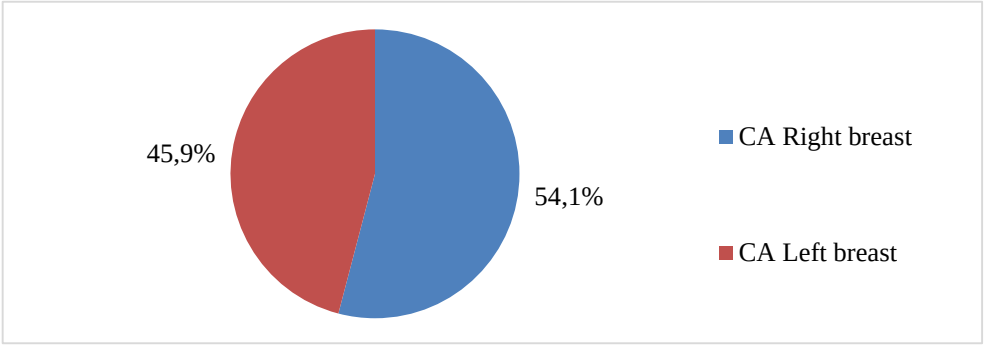

Figure 3. Descriptive Analysis of Diagnosis in the Study Population (N=98)

Out of 98 participants, 53(54.08%) participants were diagnosed with carcinoma right beast and 45(45.92%) with carcinoma left breast. (Figure 3)

Table 3
Descriptive Analysis of Staging in the Study Population (N=98)

Staging	Frequency	Percentages
I	0	0%
IIA	27	27.55%
IIB	15	15.31%
IIIA	13	13.27%
IIIB	43	43.88%
IV	0	0%

On staging the disease among the study population, most of them diagnosed with breast cancer were of stage IIIB (43.88%) followed by IIA(27.55%) (Table 3).

Table 4
Descriptive Analysis of Surgery Done in the Study Population (N=98)

Surgery Done	Frequency	Percentages
Left breast conservative surgery	20	20.41%
Left modified radical mastectomy	25	25.51%
Right breast conservative surgery	20	20.41%
Right modified radical mastectomy	33	33.67%

All the 98 subjects in the study population were managed surgically. 60% of them underwent modified radical mastectomy while 40% of the subjects underwent breast conservation surgery. (Table 4)

Table 5
Comparison of Mean Size of Lump with Ki 67 Index (N=98)

Parameter	KI 67 classification (Mean± SD)		P value
	<20 (N=29)	≥20 (N=69)	
Length (in cm)	5.17 ± 1.42	4.84 ± 1.61	0.339
Width (in cm)	4.24 ± 1.68	3.94 ± 1.63	0.414

The mean <20 ki67 classification in size of length was 5.17 ± 1.42cm and the ≥20 ki67 in length was .84 ± 1.61 cm, the association between two groups was statistically not significant (P value 0.339). The mean <20 ki67 classification in size of width was 4.24 ± 1.68 cm and the ≥20 ki67 in length was 3.94 ± 1.63cm, the association between two groups was statistically not significant (P value 0.414). (Table 5)

Discussion

A total of 98 patients with a mean age of 53.61 ± 12.48 years ranging from 30 to 80 years, out of which 54.08% with right breast lump and 45.92% with left breast lump were included in the final analysis. Our study is a cohort of females with biopsy proven breast carcinoma patients. Soliman et al. had a similar age group in their study with a mean age of 54.6±12 years ranging from 31 to 88 years.[15]

The patients were aged between 20-75 years, with a mean age of 47.41± 11.36 years in Nigam et al.'s study. [16]. In our study group, majority of them had stage IIIB carcinoma at 43.88%, followed by stage IIA at 27.55%, 15.31% stage IIB, 13.27% stage IIIA. Approximately 42% of the patients were grade 2, and95% of the cases displayed tumor size of more than 2 cm in Soliman et al.'s study. [15] The most frequent stage of presentation was IIA (31.7%), followed by IIB and IIIB at 26.8% each, while stages IIIA (9.8%) and IIIC (4.9%) were under 10% in Madhushanker et al.'s study. [17]

The appropriate cut-off point is still a matter of debate among oncologists. Hence, the most suitable cut-off point for Ki-67 in clinical practice is widely investigated. [18] Cases with >=20% positive nuclei were classified as high Ki-67 expression, and those with < 20% were classified as low Ki-67expression in our study. At this cut off value, 70.40% had ≥20 Ki-67 & 29.59% had <20 Ki-67 in our study. Liang et al. chose the median value of 15% for Ki-67 as the threshold. [19] In their study, Kermani et al. found that 53 percent of tumours were Ki-67 positive, with > 1% tumour nuclei stained, and 24 percent had tumours with more than 15% Ki-67 expression. [20]

Axillary nodal metastasis was one of the most important prognostic factors. The survival rate is determined by the number of lymph nodes involved, fixity, and the presence of extranodal extension. Duration of lump, mensural history, physical characteristics of the affected breast, physical characteristics of the lump, size of the lump, staging and nodal status showed no significant association with the Ki-67 expression. Kermani et al. discovered no correlation between Ki-67 expression and age, tumour size, or grade, but a marginally significant correlation between nodal status and Ki 67 expression. [21]

At a cut off value of Ki-67 ≥ 20, Ragab et al. reported as the tumor size increased, nodal affection increased and with advanced grade, Ki-67 expression showed higher values in their study. [22] In accordance with our study, Molina R et al. found no significant relationship between Ki-67 levels and menopausal status (P = 0.53), lymph node status, metastasis, or tumour size, but their findings revealed that Ki-67 levels were associated with BC stage (P = 0.03), higher levels of Ki-67 was found in more invasive tumours. [23] In a prospective observational study, Madhushankar et al. observed that a high Ki-67 index (≥20%) significantly correlated with younger age demonstrating more aggressive tumor and has poor prognosis. They also found a positive relationship between lymph nodes involvement, histological grade, and the mean level of Ki67 expression. [17]

Conclusion

Ki-67 is a nuclear antigen, which exists in proliferative cells. In our study Age, duration of lump, menstrual history, physical characteristics of the effected breast, physical characteristics of the lump, size of the lump, staging and lymph node status had no significant association with the Ki-67 expression. Based on Chi square test, our study demonstrated a significant association between expression of estrogen & progesterone receptor with Ki-67.

References

1. Momenimovahed Z, Salehiniya H. Epidemiological characteristics of and risk factors for breast cancer in the world. Breast cancer [Internet]. 2019 Apr 10;11:151–64. Available from: https://pubmed.ncbi.nlm.nih.gov/31040712
2. Zendehdel M, Niakan B, Keshtkar A, Rafiei E, Salamat F. Subtypes of Benign Breast Disease as a Risk Factor for Breast Cancer: A Systematic Review and Meta-Analysis Protocol. Iran J Med Sci. 2018 Jan;43(1):1–8.
3. Mavaddat N, Pharoah PDP, Michailidou K, Tyrer J, Brook MN, Bolla MK, et al. Prediction of breast cancer risk based on profiling with common genetic variants. J Natl Cancer Inst. 2015;107(5):djv036.
4. https://www.who.int/acessed on 6-7-21.
5. Leong AS, Zhuang Z. The changing role of pathology in breast cancer diagnosis and treatment. Pathobiology. 2011;78(2):99–114.
6. Doval DC, Sharma A, Sinha R, Kumar K, Dewan AK, Chaturvedi H, et al. Immunohistochemical Profile of Breast Cancer Patients at a Tertiary Care Hospital in New Delhi, India. Asian Pac J Cancer Prev. 2015;16(12):4959–64.
7. Kermani TA, Kermani IA, Faham Z, Dolatkhah R. Ki-67 status in patients with primary breast cancer and its relationship with other prognostic factors. Biomed Res Ther. 2019;6(2):2986–91.
8. Lu X, Gu Y, Ding Y, Song W, Mao J, Tan J, et al. Correlation of ER, PgR, HER-2/neu, p53, and VEGF with clinical characteristics and prognosis in Chinese women with invasive breast cancer. Vol. 14, The breast journal. United States; 2008. p. 308–10.
9. Altintas S, Lambein K, Huizing MT, Braems G, Asjoe FT, Hellemans H, et al. Prognostic significance of oncogenic markers in ductal carcinoma in situ of the breast: a clinicopathologic study. Breast J. 2009;15(2):120–32.
10. Baek J-M, Chae B-J, Song B-J, Jung S-S. The potential role of estrogen receptor β2 in breast cancer. Int J Surg. 2015 Feb;14:17–22.
11. Kontzoglou K, Palla V, Karaolanis G, Karaiskos I, Alexiou I, Pateras I, et al. Correlation between Ki67 and breast cancer prognosis. Oncology. 2013;84(4):219–25.
12. Juríková M, Danihel Ľ, Polák Š, Varga I. Ki67, PCNA, and MCM proteins: Markers of proliferation in the diagnosis of breast cancer. Acta Histochem. 2016 Jun;118(5):544–52.
13. Şahin S, Işık Gönül İ, Çakır A, Seçkin S, Uluoğlu Ö. Clinicopathological Significance of the Proliferation Markers Ki67, RacGAP1, and Topoisomerase 2 Alpha in Breast Cancer. Int J Surg Pathol [Internet]. 2016 Jun 9;24(7):607–13. Available from: https://doi.org/10.1177/1066896916653211
14. Ellis MJ, Suman VJ, Hoog J, Goncalves R, Sanati S, Creighton CJ, et al. Ki67 Proliferation Index as a Tool for Chemotherapy Decisions During and After Neoadjuvant Aromatase Inhibitor Treatment of Breast Cancer: Results From the American College of Surgeons Oncology Group Z1031 Trial (Alliance). J Clin Oncol Off J Am Soc Clin Oncol. 2017 Apr;35(10):1061–9.
15. Soliman NA, Yussif SM. Ki-67 as a prognostic marker according to breast cancer molecular subtype. Cancer Biol Med. 2016;13(4):496–504.
16. Nigam JS, Kumar T, Bharti S, Sinha R, Bhadani PP. Association of Ki-67 With Clinicopathological Factors in Breast Cancer. Cureus. 2021;13(6).
17. Madhushankar L, KA S, Reddy G. Analysis of Relationship between Carcinoma Breast and Expression of Ki67-A Prospective Study.

18. Petrelli F, Viale G, Cabiddu M, Barni S. Prognostic value of different cut-off levels of Ki-67 in breast cancer: a systematic review and meta-analysis of 64,196 patients. Breast Cancer Res Treat. 2015;153(3):477–91.
19. Liang Q, Ma D, Gao R-F, Yu K-D. 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):1–9.
20. Kermani TA, Kermani IA, Faham Z, Dolatkhah R. Ki-67 status in patients with primary breast cancer and its relationship with other prognostic factors. Biomed Res Ther. 2019;6(2):2986–91.
21. Min K-W, Kim D-H, Do S-I, Pyo J-S, Chae SW, Sohn JH, et al. High Ki67/BCL2 index is associated with worse outcome in early stage breast cancer. Postgrad Med J. 2016;92(1094):707–14.
22. Ragab HM, Samy N, Afify M, Abd El Maksoud N, Shaaban HM. Assessment of Ki-67 as a potential biomarker in patients with breast cancer. J Genet Eng Biotechnol. 2018;16(2):479–84.
23. Molina R, Barak V, van Dalen A, Duffy MJ, Einarsson R, Gion M, et al. Tumor markers in breast cancer- European Group on Tumor Markers recommendations. Tumour Biol J Int Soc Oncodevelopmental Biol Med. 2005;26(6):281–93.