Original Research Article

Ki 67 expression in breast carcinoma – Its relationship with age of the patients and pathological stage of the disease

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Received: 22-11-2021 / Revised: 25-12-2021 / Accepted: 14-01-2022

Abstract

Background: Breast carcinoma is a heterogeneous tumor with different histological subtypes and classifications. The role of proliferative molecule Ki67 in breast carcinoma is both as a prognostic and theranostic marker. However it lacks analytical validity and not recommended for routine use. In the recently concluded Galen's conference, Ki 67 cut off of 30% was recommended to determine the chemotherapy requirement. Ki 67 working group recommends 5% Ki 67 as negative and 30% Ki 67 index as significant.This study was done to analyze the ki67 expression in different age groups and different TNM stages. **Materials and Methods:** During our one year study period, the received breast specimen cases were analyzed by our panelists. Ki67 Immunohistochemistry was done and on which manual scoring was done. Age wise incidence of breast carcinoma, correlation between ki67 and age and relationship between ki67 and various stages were tabulated. p value was calculated to find out if there is any significance between the variants analyzed. **Results**: Out of 113 cases received, on ninety cases Ki67 Immunohistochemistry was done and rest was excluded. Mean age incidence was 51.8 years. High Ki 67 proliferating index was seen in younger age group, and in those with higher disease stage. No significant p value was reached when Ki 67 indexes was correlated with age or with stage of disease in our study. **Conclusion:** Proliferation marker Ki 67 though lack analytical validation, is still recommended for deciding chemotherapy for breast carcinoma cases. Despite the availability of automated count, manual method is followed in many centers. This study was done to underline the fact that consensus should be reached for uniform assessment of Ki 67 so that the dilemma among pathologists is meted out. The grey zone ki67 index level between 6-30% should be addressed as well for better clarity.

Keywords: Proliferation marker, chemotherapy, immunohistochemistry, scoring, analytical validation.

Introduction

The incidence of breast carcinoma is highest among all carcinomas. 281,550 new cases were reported so far in the year 2021 according to SEER statistics. It is a heterogeneous disease with different histological subtypes and different types of classifications. Common classification methods include immunohistochemistry based and molecular genetics based. In both these classification proliferation marker has a significant role as a prognostic and theranostic tool[1]. Gerdes et al were the one who identified Ki67, which is a nuclear protein in Hodgkins lymphoma descended cell line[2]. It is a marker of proliferating cells and in normal breast it is not seen in ER positive cells and expressed in <3% of ER negative cells[3]. It is used to differentiate Luminal A molecular type of breast carcinoma from type B. But the exact cut off of Ki 67 to differentiate between Luminal type A and B is still under debate[4,5]. In recently released CAP protocol routine ki67 is not recommended. At 2021 St. Galen's conference, Ki67 is recommended in breast carcinoma cases only when chemotherapy is considered. According to the panelists, if Ki-67 ≤5% in ER-positive HER2-negative T1–T2 N0–N1 breast cancer, it would not warrant chemotherapy, whereas if Ki-67 ≥30% it would justify chemotherapy. Ki-67 testing was recommended by majority of the panelist during or two weeks after neoadjuvant endocrine therapy (NET) to assess response to NET and to estimate prognosis in women with ER-positive HER2-negative ductal breast cancer.

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But the significance of grey zone ki67 index level between 6 to 29% is still unaddressed. There is no standard operating procedure for Ki 67 and it is not routinely done in manylaboratories. In developed countries, multigene profiling is being done on breast carcinoma cases to assess the proliferation. Immunohistochemistry for Ki67 is still being done on selected cases in developing countries as it is cost effective. This study is done in Madurai Medical college hospital on breast carcinoma cases for a study period of one year to assess ki67 index in breast carcinoma cases in relation with age and TNM stages and to see if there is any significant relation between the two by calculating the p value.

Materials and Methods

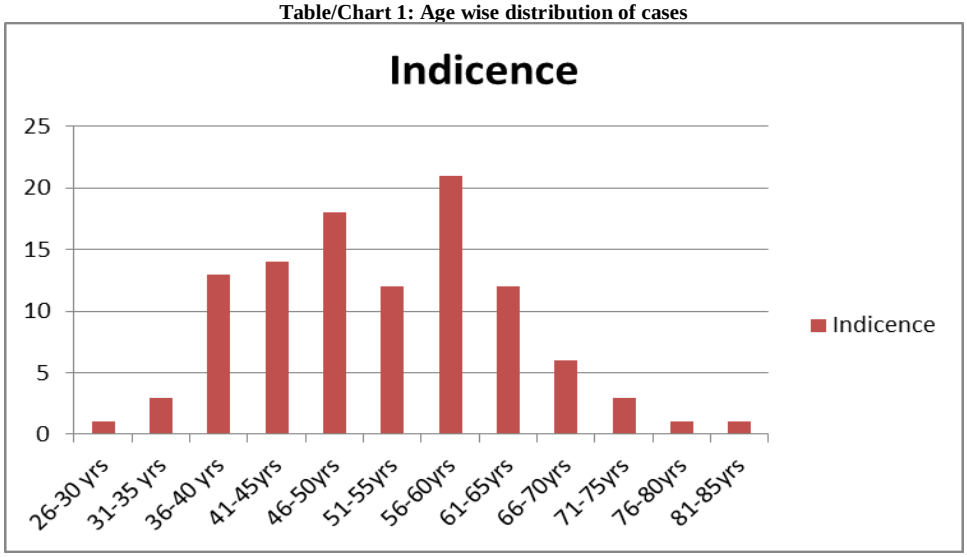
This study was done in our referral hospital for the period of one year. Breast carcinoma cases that were sent to our department were included in our study. The slides were analyzed and the tumor was histologically classified. The carcinoma was staged based on eight AJCC TNM staging. Age wise stratification was made. Age and stage of the disease was correlated. Ki67 immunohistochemistry was done on the slides which had tumor tissue.Tissues that were not well processed or where IHC staining was not clear enough were not included in our study. Only those slides with clear nuclear markings were taken as positive.Ki67 index was counted at the hotspots and in invasive edges. 1000 cells were counted by our panelists and the index was arrived. The cut off of ki67 less than 30% and more than 30% is used as this value is used to determine the need for chemotherapy according to 2021 Galen's conference recommendation. According to international working group on Ki 67 for prognostic significance, Ki 67 cut off of <5% and > 30 % are recommended, <5% taken as negative and >30% as a significant score. Based on that

we took <5% and >30% cut off for Ki67 in our study.The stage of the carcinoma and age of the patient was correlated with Ki67 to see if there is any significance between the two.Fisher's test of significance was used to arrive at two tailed p value.

Results

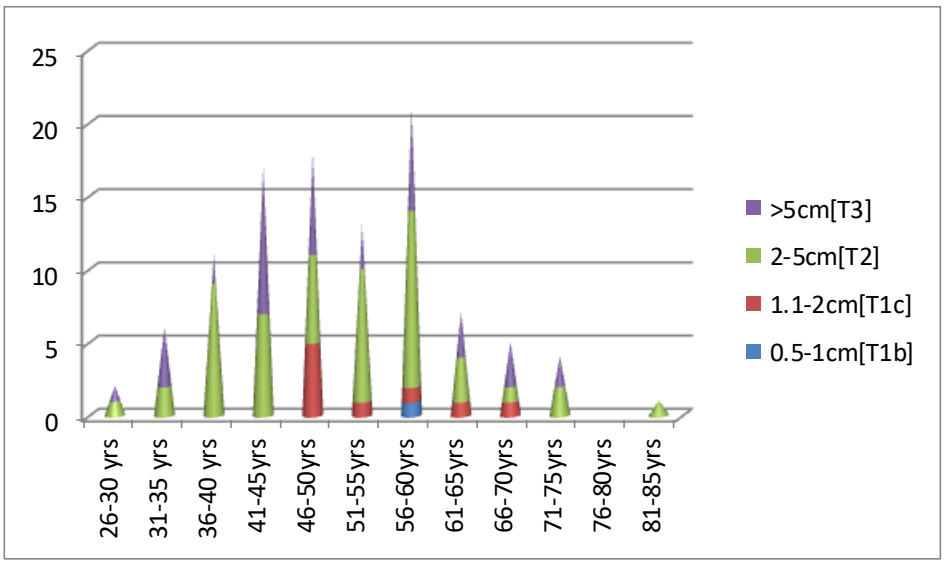
During our study period, we received 131 breast specimens. Out of that, ninety nine cases were picked up to assess the ki67 values. Almost all the cases we studied were infiltrating ductal carcinoma except one which was mucinous adenocarcinoma. The mean age was 51.8 years with age range of 30 to 84 years [Table 1].

Table/Chart 1: Age wise distribution of cases



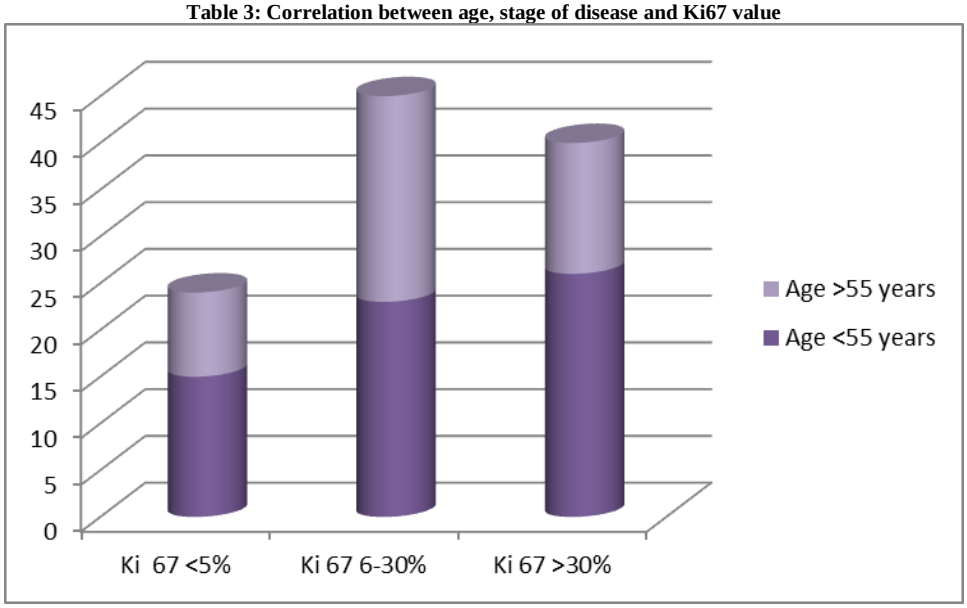
Pathological stage 2 and Stage 3 disease was most commonly seen in those who were less than 55 years of age [Table 2].

Table/Chart 2: Relationship between age and stage of the disease



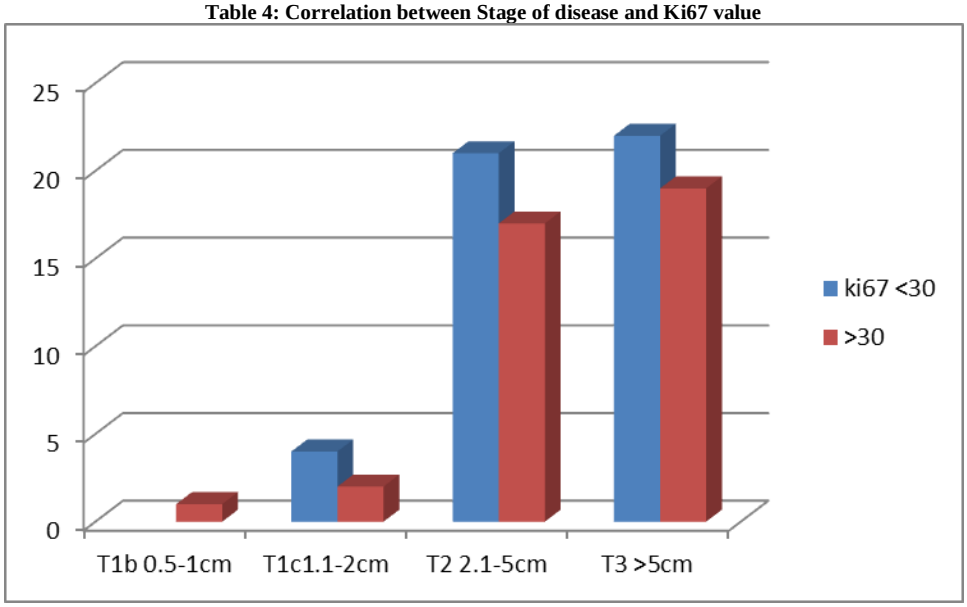
When age of the patient was correlated with high and low Ki67 values, higher index was seen in those who were less than 55 year old [Table 3].

Table 3: Correlation between age, stage of disease and Ki67 value



High proliferation index was noted both inT2 and T3 stages [Table 3].

Table 4: Correlation between Stage of disease and Ki67 value



When p value was obtained to find out the significance between Ki67 and age, the two-tailed P value was 1.0000. Likewise we tried to obtain p value between Ki67 and different stages of the disease. Ki67 values were compared between Stage T1c and stage 2 .The two-tailed P value was 0.6843, hence the association was not statistically significant. Ki 67 values were compared between Stage T1c and stage 3, the two-tailed P value was0.6780. Hence it was not considered to be statistically significant.

Discussion

Ki67, a proliferative marker is widely used in breast carcinoma. It is expressed all phases of proliferating cycle except G_o phase. Expression of Ki67 is associated with survival and outcome of breast carcinoma cases[6]. There are lots of controversies regarding cut off value of Ki67 with recommendations ranging from 5 to 34[7]. According to Meermira et al, median of Ki67 value obtained in the laboratory can be used to calculate the cut off of that laboratory[8]. Despite several issues in the methodology and validation of ki67 testing, several studies on ki67 confers worst prognosis in both node positive and node negative patients with high KI67 value[9]. In a RNA signature based study by Milde-Langosch K, et al, two other proliferation markers TOP2A and RacGAP1were studied along with ki67[10]. They concluded that in luminal carcinomas, RacGAP1 was superior in predicting recurrence.In triple-negative tumors, Ki67 was a significant marker, whereas none showed prognostic impact in Her2-positive cases. They also found that in untreated patients, all three were significant and independent prognostic markers. In the cases treated with chemotherapy and in those treated with endocrine therapy, only RacGAP1 retained significance.

In a resource poor setting like our hospital Ki67 immunohistochemistry and visual count of the score is still followed. No consensus was reached regarding the site at which the score should be done. Ki67 workinggroup recommends that 500 or 1000 cells are counted either in the invasive edge, hotspots or whole

section[11]. Quinci Romero et al in their study recommend step wise calculation of Ki 67. They claim that if followed this reduce the counting time[12]. According to their study, 50 cells in the hot spot is counted, if 0-2 cells are positive, the sample is considered as Ki67-negative. If 19-50 cells are positive, it is taken as Ki67-positive. If the number of positive cells is in the 3-18 range, another 10 cells are counted. This goes on in 10 cell increment and they have formulated a table for the same. Even after counting 400 cells, if results are not reached then it is taken as equivocal. Ki67 labeling index is another method used to calculate Ki67. In this method 500 cells are counted in the invasive edge and from that the percentage of cells with nuclear ki67 positivity is calculated which is time consuming[11]. There is yet another method called Eye 10 method, where hot spot was identified by using ×4 objective. After that using ×10 and ×20 objective fields, percentages of Ki67-positive cells in 10% intervals are assessed at a glance and scoring is made as less than 10%, 10%, 20%, 30% and so on[13]. Hida AI, et al in their study has recommended a method called Eye5 where hotspots are marked and then ki67 is read from those marked areas. They say that marked areas increase the reproducibility among the pathologists[14]. Automated counting is another candidate for assessingKi 67 values. Differentiating invasive cancer cells from non-invasive region or from benign cells is not an easy job for those softwares[14]. And it is not affordable for developing and under developed nation. Even though there are lot of studies and recommendations there is no set rules for assessment of Ki67 and concern for analytical validity of the test still exists.

Invasive ductal carcinoma was the diagnosis of all the cases we studied and it was in correlation with Erić I etal's study group[15]. Different studies have confirmed that invasive ductal carcinoma as one of the most frequently seen carcinoma in women who were under 40 years of age whereas in our study group maximum number of cases were seen in those over 55 years of age[20]. Mean age group who had breast cancer was 51.8 years in our study group whereas in Inwald EC et al's study group the mean age was 63 years[16]. Median age of breast cancer in Hosseini MS, et al' study was 49 years[17]. The peak age incidence of breast carcinoma was between 40 and 50 years in Asian countries, whereas in the Western countries it was between 60 and 70 years[18]. Women over 50 years of age accounted for approximately 78% of new breast cancer cases in the United States according to DeSantis C, et al[19]. When stage of disease were compared with age, higher stage was seen in younger patients.Young age is associated with a poor prognosis due to the presence more invasive disease among this population[21].

When proliferation index Ki67 was compared with age of the patients high index was seen in those who were less than 55 year old which was in correlation with Eric L et al's study group[15]. When ki67 index was compared with the stage of disease we found that it was higher in higher stage. As such younger patients had high proliferating index, present with high grade tumors, and with multicentric tumor and aggressive disease course according to Eric L et al[15]. Even though no significant p value was obtained in our study, younger individuals with breast carcinoma had higher Ki-67 values according to Eric L et al's study which was supportive to our study findings[15]. Higher proportion of BRCA1 and BRCA2 mutations seen in young patients are the reason for aggressive course, higher histological grade, higher proliferation rate and ER negativity[22]. p53 mutation, c-erbB-2 over expression and tumor proliferation markers are the reason for increase in local recurrence and more aggressive tumor course according to Dubsky et al[23]. Increase in ki67 expression might be the marker for BRCA mutations in younger individuals.

Future recommendation

More studies are needed for exact recommendation and for analytical assessment of Ki67 in breast carcinoma cases. The significance of Ki 67 level between 6- 30% is unknown and should be addressed for better clarity.

Conclusion

Ki 67 a proliferative marker which is used to classify the breast carcinoma into Luminal A and B type is still not analytically validated. Highly proliferating tumor is common among young females and it is seen in higher stage disease. Recent Galen conference recommends the use of ki67 only for those who need chemotherapy.

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Conflict of Interest: Nil Source of support: Nil