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Ki-67 and It's Correlation Between The Clinical And Histological Parameters In Breast Cancer Patients - An Institutional Study

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Compliance with Ethical Standards

Conflict of Interest

Each author certifies that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangements, etc.) that might pose a conflict of interest in connection with the submitted article

Research involving human participants and/or animals

Enclosed article is a case report and did not involve experimentation on humans or animals

Informed consent

Informed consent was obtained from the subject.

ABSTRACT

Breast cancer is a major health issue and yet there lacks studies which show the correlation between Ki-67 as a prognostic marker and the variables of breast cancer.

Aim: to correlate Ki-67 index with clinical and histological parameters of breast cancer. Methods and materials: it is a cross-sectional study done on 86 cases of cancer breast who presented to the institute SBMCH, Chennai in one and a half years duration. Age, tumor size, site , menopausal status, lymph node involvement ,histological type and grade, Ki-67 index were determined and correlated in these patients.

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Results: the mean age was 52 years, and most of them (62.8%) were post-menopausal. The tumors were more common on the left breast compared to right. Size of 2-5cm were more prevalent and more cases had nodal involvement in our study. Out of the histological types Invasive ductal was most common followed by Papillary carcinoma and Invasive lobular carcinoma. In our study Ki-67 did not show any significant correlation with the age(p=0.427), gender(p=0.447),side of the tumor(p=0.619), menopausal status(p=0.121), pathological size of the tumor(p=0.169),nodal involvement(p=0.568) or histological type of the tumor(p=0.373). However a really significant correlation was observed between grade of the tumor(p<0.001).

Concluding that in our study there was a significant correlation between ki-67 and tumor grade which is an established prognostic marker, and hence it plays a vital role in deciding the prognosis of breast cancer.

Key words- Breast Cancer, Ki 67, clinical histopathology.

1. INTRODUCTION

The most common cancer found in females worldwide is breast cancer, representing nearly a quarter (25%) of all cancers with an estimated 1.67 million new cancer cases diagnosed in 2012. Women from less developed regions (883,000 cases) have slightly more number of cases compared to more developed (794,000) regions. [1]

In India, although the age-adjusted breast cancer incidence rate is lower (25.8 per 100,000) than in the United Kingdom (95 per 100,000), mortality is at par with the United Kingdom (12.7 vs 17.1 per 100,000).[2] Incidence and cancer-related morbidity and mortality are significantly increased in the Indian subcontinent as described in global and Indian studies.[3-7] Recently, cervical cancer was Indian women's most common cancer, but now the prevalence of breast cancer has increased cervical cancer and is the leading cause of cancer death, while cervical cancer remains the most common in rural India.[8]

Nevertheless, the use of surgical adjuvant drugs has made significant improvements in both disease-free survival (DFS) and overall survival (OS).[9] Proliferation inhibitors have been widely tested in recent decades as prognostic measures for breast cancer. Nevertheless, certain histological features (e.g. tumor volume, histologic level, nodal status and lympho-vascular intrusion, hormone receptor status, HER-2 status and age) are the only prognostic indicators used in medical decision making. [10-11]

Ki-67 is present in all proliferating cells and its role as a marker of proliferation is of great interest.[12] The Ki-67 antibody reacts with a 395 KD nuclear non-histone protein in all active phases of the cell cycle except in the G0 phase.[13] MIB-1 is a monoclonal antibody against recombinant parts of the Ki-67 antigen; there is a good correlation between Ki-67 and MIB-1.[13] Gene array strategies have recently revealed the function of the Ki-67 gene in several ' proliferation signatures ', demonstrating that a group of genes with increased patterns of expression are associated with tumor cell proliferation levels as assessed by the Ki-67 labeling index.[14,15] Furthermore, Ki-67 is one of Oncotype DXTM assay's 21 prospectively chosen genes used to assess the likelihood of recurrence in a node-negative, tamoxifen-treated BC

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population engaged in the B-14 National Surgical Adjuvant Breast and Bowel Project (NSABP B-14), as well to predict the magnitude of chemotherapy's benefit in women with node-negative, estrogen receptor (ER)-positive breast cancer enrolled in the NSABP B20 trial.[16]

Ki-67 is a nuclear protein widely used to identify and measure proliferating cells.[18]

AIMS AND OBJECTIVES

In our study we try to find the correlation between Ki-67 and clinical parameters of cancer breast specimens.

To correlate the expression of Ki-67 with the grade[NOTTINGHAM SYSTEM] and histo-pathology of the breast cancer specimen

2. MATERIALS AND METHODS

- The study is a Cross sectional study, which involves all subjects with breast cancer, who were operated in SBMCH,Chennai between the period of APRIL 2018– OCT 2019.
- Duration of study – 1 1/2 yrs
- Sample size - 86
- Sample includes all post MRM specimen's done in the dept of general surgery and sent for histopathology to the dept of pathology .
- Prior to surgery baseline characters of the patient was assessed like age ,gender, menopausal status, size of the tumor , lymph node involvement and distant metastasis, by clinical and radiological investigations.
- Only the patients who were subjected to MRM were included and the pathological specimen was analysed for tumor grade, size and lympho-vascular invasion.
- Histopathology grading is done based on NOTTINGHAM SYSTEM
- The Ki-67 percentage score is defined as the percentage of pos itively stained tumor cells among the total number of malignant cells assessed. To ensure quality assurance of the staining, positive control tissues are compared.
- The results are correlated with the clinical parameters of the patient, histopathological grade of the tumor

STATISTICAL ANALYSIS

Data was entered into Microsoft Excel spreadsheet and was checked for any discrepancies. Summarized data was presented using Tables and Graphs. The data was analysed by SPSS (21.0 version). Shapiro Wilk test was used to check which all variables were following normal distribution. Data was found to be normally distributed (p-value was more than 0.05). Therefore, bivariate analyses were performed using the parametric tests i.e Independent test (for comparing two groups). Chi square test was used for frequency analysis. Level of statistical significance was set at p-value less than 0.05

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3. RESULTS

A total of 110 patients were included in the study, out of which 10 patients were lost in follow up after biopsy. 10 cases were sent for neo-adjuvant chemotherapy outside. 2 patients were cases of cystosarcoma phyllodes and 2 cases refused surgery.

- In our study , we found that the mean age of the patients who took part in the study was 52.65 years and the range was around 25-82 years.
- The menopausal status was found to be ,62.8% of the breast cancer cases were postmenopausal and rest 37.2% were premenopausal , thus showing that incidence of breast cancer was more in post-menopausal group in our study.
- This study also shows that left sided was more commonly afftected with breast cancer than right . 46 patients [53.5%] had cancer on the left breast compared to 39 patients [45.3%] who had it on the right , one patient presented with bilateral cancer [1.2%].
- Distribution according to the pathological size of the tumor revealed that 2-5cm was most common accounting for 72.1% (62 patients) and second most common size was <2cm which was 25.6% (22 patients) , followed by >5cm which was 2.3%(2 patients).
- The nodal involvement in the current study was found to be high, around 77.9%(67 cases) of the cases had pathological node involvement compared to only 22.1%(19 cases) who were node negative.
- Grade of the tumor was assessed based on the Nottingham scoring system[33,34], and the common grade of tumors in this study was grade 2, with 38 cases (44.2%) followed by grade 3, which was 25cases (29.1 %) and then grade 1 with 23 cases (26.7%).
- The histological subtypes which were seen were Invasive Ductal carcinoma - NOS (73 cases) , Invasive Lobular carcinoma (4 cases) , Papillary carcinoma (5 cases) , Metaplastic carcinoma (3 cases) and Mucinous carcinoma (1 case).
- In the present study the range of Ki-67 index was from 2% to 80% with a mean of 28.06%.
- The number of cases in the low ki-67 / <15% group to be 46 cases (53.5%), and those in the high Ki-67 / >15% group was found to be around 40 cases (46.5%).
- In our study Ki-67 did not show any significant correlation with the age($p=0.427$), gender($p=0.447$),side of the tumor($p=0.619$), menopausal status($p=0.121$), pathological size of the tumor($p=0.169$),nodal involvement($p=0.568$) or histological type of the tumor($p=0.373$).
- Significant correlation was observed between Ki-67 and the grade of the tumor($p<0.0001$)

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[insert TABLE 1]

TABLE 1-correlation between ki-67 and variables of patient with cancer breast

VARIABLES	KI-67<15%	KI-67>15%	p value
AGE			
<50yrs	30.4	45.0	0.121 NS
>50yrs	69.6	55.0	
SEX			
MALE	2.2	5.0	0.447 NS
FEMALE	97.8	95.0	
MENOPAUSAL			
PRE-	30.4	45.0	0.121 NS
POST-	69.6	55.0	
SITE			
BILATERAL	2.2	0.0	0.619 NS
LEFT	54.3	52.5	
RIGHT	43.5	47.5	
SIZE			
<2cm	17.4	35.0	0.169 NS
2-5cm	80.4	62.5	
>5cm	2.2	2.5	
LYMPH NODE			
YES	78.3	77.5	0.568 NS
NO	21.7	22.5	
TYPE			
DUCTAL	82.6	87.5	0.373 NS
OTHER	17.4	12.5	
GRADE			
1	34.8	17.5	<0.001 S
2	60.9	25.0	
3	4.3	57.5	

4. DISCUSSION

In the present study 86 modified radical mastectomy specimens, were received for breast cancer were evaluated by light microscopy to determine the histologic type and ki-67

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percentage score was calculated. Correlation between ki-67 and clinical and histological parameters were done.

Compared to the other recent studies which correlated ki-67 with prognostic parameters our sample size was 86 cases, whereas Gül Kanyılmaz et al [19] included 258 patients over a period of 2010 -2017. Nguiessan Alphonse Aman et al [20] included 125 patients over a period of 8 months from Oct 2014 to June 2015. Atif Ali Hashmi et al[21] studied 1951 cases of breast cancer from period of Jan 2011 till DEC 2016. Li Ding et al [22] studied 260 breast cancer patients retrospectively. Seyed-Hamid Madani et al [23] studied 260 breast cancer patients retrospectively.

In our study , we found that the mean age of the patients who took part in the study was 52.65 years and the range was around 25-82 years. Compared to the other studies , Nguiessan Alphonse Aman et al [20] had a mean age of 47.7 years and range of 29-83 years.

Seyed-Hamid Madani et al[23] had a mean age of 47.6 years with a range of 24-84 years.

Taghipour Zahir Shokouh et al [24] had a mean age of 50 years with an age range of 17 to 98 years. In the study by REIKI NISHIMURA et al [25] the mean age of the patients was 51.6 years with a range of 25–95 years.

The menopausal status was found to be ,62.8% of the breast cancer cases were postmenopausal and rest 37.2% were premenopausal , thus showing that incidence of breast cancer was more in post-menopausal group in our study. In the study by Gül Kanyılmaz et al [19] 39% were premenopausal, 9% were perimenopausal and 52% were postmenopausal. The study by Nguiessan Alphonse Aman et al [20] among the 125 patients, 59.2% were premenopausal compared to 40.8% of postmenopausal. The study by REIKI NISHIMURA et al [25] showed contradictory results where 53% were in the premenopausal group and 47% in the postmenopausal group.

This study also shows that left sided was more commonly afftected with breast cancer than right . 46 patients [53.5%] had cancer on the left breast compared to 39 patients [45.3%] who had it on the right , one patient presented with bilateral cancer [1.2%]. But in the study by Li Ding et al [22] 124 cases had tumor on the left and 132 had it on the right , with 2 cases bilateral.

Distribution according to the pathological size of the tumor revealed that 2-5cm was most common accounting for 72.1% (62 patients) and second most common size was <2cm which was 25.6% (22 patients) , followed by >5cm which was 2.3%(2 patients). Indicating an earlier detection of the cancer due to the advancements in diagnosis and screening. This was in correlation to the study by Gül Kanyılmaz et al [19] ,where the common pathological tumor size was pT2 (2-5cm) accounting to 58%. Also in the study by Seyed-Hamid Madani et al[23] the most tumors were in the size group of >2cm which accounted for around 207 cases (85.2%) and the remaining in the <2cm group ,36 cases (14.8). Similarly in the study by JINZHONG SUN et al [26] the most common pathological tumor size noted was pT2 (2-5cm) reporting around 824 cases (65%) , followed by pT1 (<2cm) reporting around 295 cases (23%), and then pT3 (>5cm) which had around 140 cases (11%).

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The nodal involvement in the current study was found to be high, around 77.9%(67 cases) of the cases had pathological node involvement compared to only 22.1%(19 cases) who were node negative. This shows that most cases metastasize early to the lymph nodes. This is in consensus Seyed-Hamid Madani et al[23] study where out of 242 patients ,155 had lymph node involvement (64%) and 87 (36%) were node negative. Another study by REIKI NISHIMURA et al[25] showed node involvement in more cases ,426 cases(64%) compared to node negative which was only 220 cases (33%). Contradictory to the findings a study by JINZHONG SUN et al[26] demonstrated more node negative cases compared to node positive, which was 673(53%) node negative and 586 (47 %) node positive

Grade of the tumor was assessed based on the Nottingham scoring system[27,28], and the common grade of tumors in this study was grade 2 ,with 38 cases (44.2%) followed by grade 3, which was 25cases (29.1 %) and then grade 1 with 23 cases (26.7%). Other studies Gül Kanyılmaz et al [19] had more cases in grade 2 (65%) followed by grade 3 (24%) and then grade 1 (11%). Similarly in Nguiessan Alphonse Aman et al [20] there were more cases in Grade 2 (69 cases) followed by grade 3(26 cases) and grade 1 (18 cases). Which leads to inference that grade 2 tumors are apparently more common.

The histological subtypes which were seen were Invasive Ductal carcinoma -NOS (73 cases) ,Invasive Lobular carcinoma (4 cases) , Papillary carcinoma (5 cases) , Metaplastic carcinoma (3 cases) and Mucinous carcinoma (1 case). In other studies aswell , Nguiessan Alphonse Aman et al [20] , Li Ding et al [22] , Taghipour Zahir Shokouh et al [24] incidence of infiltrating ductal carcinoma was significantly higher, and Mucinous carcinoma was least prevalent. In study by Atif Ali Hashmi et al [21] the most prevalent was Invasive Ductal carcinoma and the least was adenoid cystic carcinoma.

In our study Ki-67 values were acquired as the percentage of positively marking malignant cells using the anti-human Ki-67 monoclonal antibody MIB1 which is one of the most commonly used antibodies and considered as the “gold standard”[29]

The 2011 St Gallen Expert Panel set the cut of for ki-67 index at ≥14% distinguished luminal B from luminal A tumors in BC molecular subtyping[30]

A Ki-67 cut-off point of 15 % was defined according to the experience of different pathologists as well as national and international recommendations at present [,30,31,32,33,34].

In the present study the range of Ki-67 index was from 2% to 80% with a mean of 28.06%. In the study by Gül Kanyılmaz et al [19] mean ki-67 was 30% ,with a range of 0% to 95%. The study by Nguiessan Alphonse Aman et al [20] had a mean ki-67 score of 56.0%.

We have found that the number of cases in the low ki-67 / <15% group to be 46 cases (53.5%), and those in the high Ki-67 / >15% group was found to be around 40 cases (46.5%). In the study by Wang B et al [35] Ki67 index was set at 14% as the boundary to divide the patients into two subgroups, 263 cases (28.6%) were ≤ 14%, and 655 cases (71.4%) were >14% . Similarly in the study by Nguiessan Alphonse Aman et al [20] index cut off was set at

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14% and those with >15% (high ki-67) were 106 patients (84.8%) and those with ki-67 <14% were 19 cases (15.2%).

The correlation of Ki-67 with each clinical and histopathologic parameter was studied and assessed for a correlation.

In our study Ki-67 did not show any significant correlation with the age(p=0.427), gender(p=0.447),side of the tumor(p=0.619), menopausal status(p=0.121), pathological size of the tumor(p=0.169),nodal involvement(p=0.568) or histological type of the tumor(p=0.373).

Significant correlation was observed between Ki-67 and the grade of the tumor(p<0.001). This correlation between the grade and Ki-67, helps reinforce the similar behaviour between the two parameters, both are associated with proliferation.

The Histological grade assessment is a combination of nuclear grade, tubes formation and mitosis. Both Mitotic index and Ki67 are cell proliferation markers, however Ki67 is expressed in all cell cycle phases except in G0 (or resting stage) and therefore would be a superior prognostic marker.[35]

Our results that Ki-67 positive is associated with the development of carcinoma to a higher histological stage are consistent with previous studies and improve the accuracy of this new biomarker[33] as a predictor of the outcome of the prognosis for breast cancer

There are studies which show Ki-67 significantly correlated with younger age. A strong correlation has been found between the percentage of cells positive for Ki-67 and age in certain studies.[36][37] However our study did not show any correlation with age or menopausal status with the Ki-67 index.

There are studies which show a correlation of Ki-67 index with the tumor size and node involvement.[19][26][[38][39] But our study did not show any significant correlation of Ki67 and the tumor size and nodal involvement.

The probable cause for the difference in results of the various studies is the type of division of Ki-67. In many studies the ki-67 has been divided into groups of <15% ,16-30%,>30%.[20] or into quartiles of <15%,15-24%,25-44%,>44% [21] or using cut off points like <20%, >=20%[23] , <14%, >=14%[26] and <15%,>=15%[40], similar to our study. Therefore for a good result in correlation between Ki-67 and other factors of breast cancer ,we need to standardise the Ki-67 numbers and make it a constant throughout.

5. CONCLUSION

Ki-67 is a controversial marker and there has always been a debate regarding its use as a prognostic marker and in routine Immuno-histochemistry. Its role as a proliferative marker is well established, and still studies are trying to establish it as routine marker in assessment of cancer breast.

We inferred that out of the cases of cancer breast which presented to our institute the mean age was 52 years, and most of them (62.8%) were post-menopausal. The tumors were more

common on the left breast compared to right. Size of 2-5cm were more prevalent and more cases had nodal involvement in our study.

Out of the histological types Invasive ductal was most common followed by Papillary carcinoma and Invasive lobular carcinoma.

There was no significant correlation between ki-67 and the clinical parameters like Age, menopausal state, size, nodal involvement.

However a really significant correlation was observed between grade of the tumor, showing its vital role as a prognostic variable in breast cancer patients.

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