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Original Article

Pathology Section

Comparative Analysis of Ki-67 in Different Scoring Patterns and its Association with other Prognostic Markers of Breast Carcinoma

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ABSTRACT

Introduction: Immunohistochemical evaluation of Ki-67 is widely used for estimation of tumour proliferation in breast cancer. Till date, no specific method or a cut-off point for Ki-67 exists.

Aim: To perform a comparative analysis between different scoring patterns and mean Ki-67 value and association of mean Ki-67 with other prognostic markers like tumour size, lymph node status, tumour stage and grade and different molecular subtypes of breast cancer.

Materials and Methods: A cross-sectional study was conducted at Dr. D.Y. Patil Medical College, Hospital and Research Centre, Pune, Maharashtra, India, between August 2019 to August 2021. Total of 50 new diagnosed cases of breast cancer were studied for the histologic type, grade and stage of the tumour. Immunohistochemistry for Oestrogen Receptor (ER), Progesterone Receptor (PR), Human Epidermal Growth Factor Receptor 2 (HER2) and Ki-67 was performed. Association of Ki-67 with other prognostic markers like tumour size, lymph node status, tumour stage and grade and different molecular subtypes of breast cancer was evaluated by expressing Ki-67 as a continuous variable (mean±SD) and also by dividing Ki-67 into different scoring patterns (I: ≤14%, >14%, II: ≤15%, 16-30%, >30% and III: <20%, 20-50%, ≥50%). Statistical tests like Kruskal-Wallis test (mean Ki-67 with tumour size, tumour grade and molecular subtypes), Mann-Whitney Rank Sum test (mean Ki-67 with lymph node status) and one way Analysis of Variance (ANOVA) (mean Ki-67 with staging) respectively.

Results: Out of 50 patients, 40 (80%) were older than 50-year-old. Twenty six (52%) cases affected the left breast. A total of 49 (98%) were diagnosed as Invasive Ductal Carcinoma (IDC). Among them 26 (52%) cases were of grade III and 25 (50%) cases were of Luminal A. Mean Ki-67 and molecular subtypes of breast cancer had statistically significant association (p=0.002). No association was found between mean Ki-67 and tumour size (p=0.608), lymph node status (p=0.506) stage (p=0.979) and grade (p=0.095) of the tumour. Although scoring pattern I and III had no remarkable findings. Notably, scoring pattern II showed higher tumour sizes, lymph node positivity, higher stage and grade and basal-like tumours demonstrated a higher Ki-67 index.

Conclusion: Evaluation of Ki-67 as a continuous variable yielded significant association with other prognostic markers of breast cancer. There was no single "best" scoring pattern identified. A direct association of Ki-67 was found with molecular subtypes of breast cancer.

Keywords: Breast neoplasms, Immunohistochemistry, Molecular subtypes, Prognosis

INTRODUCTION

According to GLOBOCAN 2020 (Global Cancer Incidence, Mortality and Prevalence), female breast carcinoma has emerged as the most common cancer found worldwide. It accounted for 2.3 million new cases (11.7 %) of the total cancer cases across the globe in 2020 [1].

Breast cancer can be classified according to the histopathological type {World Health Organisation (WHO) 2019} [2], tumour grade (Elston Ellis modification of Scarff-Bloom-Richardson grading system) [3], tumour stage {American Joint Committee on Cancer (AJCC) 8th edition} [4] and different molecular subtypes of breast cancer (Luminal A, Luminal B, HER2 rich and Basal-like/Triple Negative) [5].

The proliferative potential of any malignancy can be estimated using various methods like the historically used method of counting mitotic figures, incorporation of labelled nucleotides into DNA, flow cytometric assessment of S phase as well as by Immunohistochemical (IHC) identification of markers like Proliferating Cell Nuclear Antigen (PCNA), cyclin E and Ki-67 antigen [6,7].

The Ki-67 antigen is also known as MKI67 or MIB-1 [6,8]. It is a non histone protein [8]. It was first described by Gerdes J et al., in 1980's while raising mouse monoclonal antibodies to the nuclei of Hodgkin's disease cell line. Ki-67 is present within the nucleus in all active phases of cell cycle, except the Go phase that is the resting phase [9].

There are innumerable studies in literature on the correlation of Ki-67 Proliferation Index (PI) in breast cancer with other prognostic markers like tumour size, lymph node status, tumour stage and grade and different molecular subtypes. The results of each study were found to differ when compared with other well established prognostic markers of breast carcinoma. Multiple studies evaluating Ki-67 as continuous variable and categorising Ki-67 PI into different scoring patterns/cut-off points have been performed [7,10-13]. However, standardisation of a definite method or an accepted cut-off point distinguishing Ki-67 into a low or a high PI is still a matter of debate [7,8,10]. The aim of the present study was to analyse the effectiveness of Ki-67 as a prognostic indicator, to establish an association between Ki-67 with other prognostic markers of breast cancer by evaluating Ki-67 both as continuous (mean±SD) and categorisation into different scoring patterns. A comparative analysis was also performed of the different scoring patterns of Ki-67 used in the present study.

MATERIALS AND METHODS

A cross-sectional study was carried out in the Department of Pathology, Dr. D.Y. Patil Medical College, Hospital and Research

Centre, Pune, Maharashtra, India, between August 2019 to August 2021. Institutional Ethical Committee clearance was obtained before the start of the study (I.E.S.C/280/2019).

Inclusion criteria: All female patients diagnosed with breast carcinoma were included in the study.

Exclusion criteria: Male breast, inadequate sample for histopathology and inconclusive IHC results due to technical errors of any of the molecular markers- ER, PR, HER2 and Ki-67 were excluded from the study.

Sample size calculation: The study included 50 cases of breast cancer which were diagnosed as invasive breast carcinoma on the basis of histology. Sample size was calculated through convenient sampling method. There were total 55 cases collected over a period of two years. Five cases were lost due to lack of follow-up or were discarded from the study due to insufficient data.

Study Procedure

Relevant demographic and clinical presentation data was obtained. The breast tissue obtained in the form of Modified Radical Mastectomy (MRM), an excision and or a tru-cut biopsy was fixed in 10% of buffered formalin (formalin buffered to pH 7.0-7.4) for 12-24 hours. Grossing of the specimen was done according to the standard protocols followed by the department [14]. The tissue was processed, and the sections were stained with Haematoxylin and Eosin (H&E). The H&E sections were studied under a light microscope for the histologic type (World Health Organisation, WHO 2019) [2], histologic grade (Elston Ellis modification of Scarff-Bloom-Richardson grading system) [3] and pathologic stage classification (AJCC 8th edition) [4] of the breast tumour.

Immunohistochemical Staining

A representative block with maximum tumour tissue and adjacent normal breast tissue was selected for IHC. Four serial sections were obtained on Poly-L-lysine coated slide. These sections were used for IHC staining of ER, PR, HER2 and Ki-67.

For IHC all the reagents and staining materials were brought down to room temperature. The sections were incubated in a peroxide block for 5-10 minutes at room temperature followed by washing with SuperSensitiveTM Wash Buffer. Antigen retrieval was performed at a pH of 6.0 by microwave technique. PowerBlock, a proteinaceous blocking reagent was used to prevent non specific binding of antibodies. The slides were further incubated with primary antibodies for 60 minutes. The sections were counter stained using haematoxylin, dehydrated and cleared using ethyl alcohol and xylene respectively. The primary antibodies used for ER was clone SP1 (Thermoscientific), for PR was clone SP2 (Thermoscientific), for HER2 was clone SP3 (Thermoscientific), and Ki-67 was a rabbit monoclonal antibody, Clone SP6 (Epredia) [15].

According to the guidelines recommended by American Society of Clinical Oncology (ASCO) and College of American Pathologists (CAP), Allred system of scoring was used for the reporting of ER and PR receptors [16].

Allred Score= Proportion Score (PS) + Intensity Score (IS)

The PS is the number of cells that are stained and IS is the intensity of staining that is pale or dark. The two scores were added together for the final score that is the Allred score. A total score of 0-2 was considered negative and a score of 3-8 was considered positive [16].

HER2 staining (ASCO and CAP guidelines) was considered positive (Score 3+) when complete, intense, circumferential membrane staining was seen in >10% of invasive tumours cells and it was negative (0 or 1+) when no or incomplete, faint membrane staining was seen in >10% of invasive tumour cells [17]. In the present study, molecular subtyping of breast tumours was done according to [Table/Fig-1] [13].

Molecular subtype	ER status	PR status	HER2 status
Luminal A	Positive	Positive	Negative
Luminal B	Positive	Positive	Positive
HER2 enriched	Negative	Negative	Positive
Basal-like	Negative	Negative	Negative

[Table/Fig-1]: Molecular subtyping of breast cancer [13].

Ki-67 score or PI was evaluated as percentage of positively stained cells against the total number of tumour cells scored (0-100%). For each case a minimum of 500 cells were counted under high power. Areas with highest concentration of positively stained Ki-67 cells (tumour edge or hot spots) were selected [18].

Ki-67 (PI)=No of positive cells/Total no of cells×100 [18].

For each case, IHC reporting of ER, PR, HER2 and Ki-67 was done by two individual authors (MN and AB). It was primarily done by MN using the above said methods and guidelines. These results were re-checked and confirmed by AB.

Ki-67 was evaluated as a continuous variable (Mean±Standard Deviation) to establish a relationship with other prognostic markers of breast cancer using different statistical tests. Ki-67 was also categorised into different scoring patterns. The three scoring patterns used in the present study were:

- Scoring Pattern I: ≤14%, >14% (Low, High) [11]
- Scoring Pattern II: ≤15%, 16-30%, >30% (Low, Intermediate, High) [10,12]
- Scoring Pattern III: <20%, 20-50%, ≥50% (Low, Intermediate, High) [13]

STATISTICAL ANALYSIS

Ki-67 was analysed as a continuous variable by expressing it in terms of Mean±SD. An association between the mean difference of Ki-67 index and other prognostic markers of breast cancer was established using different statistical tests like Kruskal-Wallis test (tumour size, tumour grade and molecular subtypes), Mann-Whitney Rank Sum test (lymph node status) and ANOVA (staging) respectively. A p-value <0.05 was regarded as significant.

RESULTS

From the overall of 50 patients with confirmed breast carcinoma, 40 (80%) cases were older than 50 years of age. Twenty six (52%) of the cases affected the left breast. A total of 49 (98%) cases were diagnosed as IDC. The frequency of grade III was the highest 26 (52%). The frequency of Luminal A, Luminal B, Her2 enriched and basal-like tumours were 25 (50%),10 (20%), 7 (14%) and 8 (16%) respectively [Table/Fig-2].

Characteristics	Number of cases (N=50)	Percentage (%)
Age (years)		
≤50	10	20
>50	40	80
Tumour laterality		
Left side	26	52
Right side	24	48
Histopathological type		
Invasive Ductal Carcinoma (IDC)	49	98
Invasive Lobular Carcinoma (ILC)	1	2
Tumour grade		
Grade I	6	12
Grade II	18	36
Grade III	26	52

Molecular subtype		
Luminal A	25	50
Luminal B	10	20
HER2 enriched	7	14
Basal-like	8	16

[Table/Fig-2]: Distribution of clinicopathological characteristic of breast cancer amongst study cases (N=50).

To establish an association between Ki-67 and various other prognostic markers, Ki-67 was analysed both as a continuous variable (mean±SD) and by using different scoring patterns for categorising Ki-67 into a low or a high PI. These findings are shown in [Table/Fig-3-6].

Breast parameters	Ki-67 (mean±SD)	Median (IQR)	Total (n)	p-value
Tumour size				
T1	28.71 (25.16)	16	7	Kruskal-Wallis Test p-value=0.608
T2	28.25 (21.22)	28	32	
T3	36 (24.17)	34	11	
Lymph node status				
Absent	27.93 (22.18)	23.5	28	Mann-Whitney Rank Sum Test, p-value=0.506
Present	32.68 (22.40)	31	22	
Tumour stage				
Stage I	28.71 (25.16)	-	7	ANOVA, F=0.02, df (2,47) p-value=0.979
Stage II	29.93 (22.43)	-	28	
Stage III	30.80 (21.87)	-	15	
Tumour grade				
Grade I	30.17 (23.61)	33.5	6	Kruskal-Wallis Test, p-value=0.095
Grade II	21.89 (22.23)	15	18	
Grade III	35.62 (20.89)	34.5	26	
Molecular subtype				
Luminal A	20.08 (17.90)	16	25	Kruskal-Wallis Test, p-value=0.002
Luminal B	30 (20.81)	35	10	
HER2 enriched	34.86 (22.75)	40	7	
Basal-like	56.88 (11.99)	60	8	

[Table/Fig-3]: Descriptive statistics of Ki-67 (mean±SD) with other prognostic markers of breast tumours (n=50).

Scoring pattern I (≤14, >14%)			
Breast parameters	Low Ki-67 n (%)	High Ki-67 n (%)	Total
Tumour size			
T1	2 (28.5%)	5 (71.4%)	7
T2	11 (34.37%)	21 (65.62%)	32
T3	3 (27.27%)	8 (72.72%)	11
Lymph node status			
Absent	10 (35.71%)	18 (64.28%)	28
Present	6 (27.27%)	16 (72.72%)	22
Tumour stage			
Stage I	2 (28.5%)	5 (71.4%)	7
Stage II	10 (27.77%)	18 (64.28%)	28
Stage III	4 (26.66%)	11 (73.33%)	15
Tumour grade			
Grade I	2 (33.33%)	4 (66.66%)	6
Grade II	9 (50%)	9 (50%)	18
Grade III	5 (19.23%)	21 (80.7%)	26
Molecular subtype			
Luminal A	11 (44%)	14 (56%)	25
Luminal B	4 (40%)	6 (60%)	10
HER2 enriched	1 (14.28%)	6 (85.71%)	7
Basal-like	0	8 (100%)	8

[Table/Fig-4]: Distribution of cases according to scoring pattern I with other prognostic markers of breast tumours (N=50).

Scoring pattern II (≤15%, 16-30%, >30%)				
Breast parameters	Low Ki-67 n (%)	Intermediate Ki-67 n (%)	High Ki-67 n (%)	Total
Tumour size				
T1	2 (28.5%)	3 (42.8%)	2 (29%)	7
T2	11 (34.37%)	7 (21.87%)	14 (43.75%)	32
T3	4 (36.36%)	2 (18.18%)	5 (45.45%)	11
Lymph node status				
Absent	10 (35.71%)	7 (25%)	11 (39.28%)	28
Present	7 (31.81%)	5 (22.72%)	10 (45.45%)	22
Tumour stage				
Stage I	2 (28.5%)	3 (42.8%)	2 (28.5%)	7
Stage II	10 (35.71%)	4 (14.28%)	14 (50%)	28
Stage III	5 (33.33%)	5 (33%)	5 (33%)	15
Tumour grade				
Grade I	2 (33.33%)	1 (16.66%)	3 (50%)	6
Grade II	9 (50%)	5 (27.77%)	4 (22.22%)	18
Grade III	6 (23.07%)	6 (23.07%)	14 (53.84%)	26
Molecular subtype				
Luminal A	11 (44%)	10 (40%)	4 (16%)	25
Luminal B	4 (40%)	0	6 (60%)	10
HER2 enriched	1 (14.28%)	2 (28.5%)	4 (57.14%)	7
Basal-like	1 (12.5%)	0	7 (87.5%)	8

[Table/Fig-5]: Distribution of cases according to scoring pattern II with other prognostic markers of breast tumours (N=50).

Scoring pattern III (<20%, 20-50%, ≥50%)				
Breast parameters	Low Ki-67 n (%)	Intermediate Ki-67 n (%)	High Ki-67 n (%)	Total
Tumour size				
T1	4 (57.14%)	1 (14.28%)	2 (28.5%)	7
T2	13 (40.62%)	13 (40.62%)	6 (18.75%)	32
T3	5 (45.45%)	2 (18.18%)	4 (36.36%)	11
Lymph node status				
Absent	13 (46.42%)	9 (32.14%)	6 (21%)	28
Present	9 (40.90%)	7 (32%)	6 (27%)	22
Tumour stage				
Stage I	4 (57.14%)	1 (14.28%)	2 (28.5%)	7
Stage II	11 (39.28%)	11 (39.28%)	6 (21.42%)	28
Stage III	7 (46.66%)	4 (26.66%)	4 (26.66%)	15
Tumour grade				
Grade I	2 (33.33%)	2 (33%)	2 (33%)	6
Grade II	13 (72.22%)	2 (11.11%)	3 (16.66%)	18
Grade III	7 (26.92%)	12 (46.15%)	7 (26.92%)	26
Molecular subtype				
Luminal A	15 (60%)	7 (28%)	3 (12%)	25
Luminal B	4 (40%)	5 (50%)	1 (10%)	10
HER2 enriched	3 (42.85%)	2 (28.5%)	2 (28.5%)	7
Basal-like	0	2 (25%)	6 (75%)	8

[Table/Fig-6]: Distribution of cases according to scoring pattern III with other prognostic markers of breast tumours (N=50).

The mean values of Ki-67 varied with increasing tumour size. The mean Ki-67 was higher in presence of lymph node involvement. The mean difference in Ki-67 values showed a surge through the progressive stages of the tumour. It did not show any significant

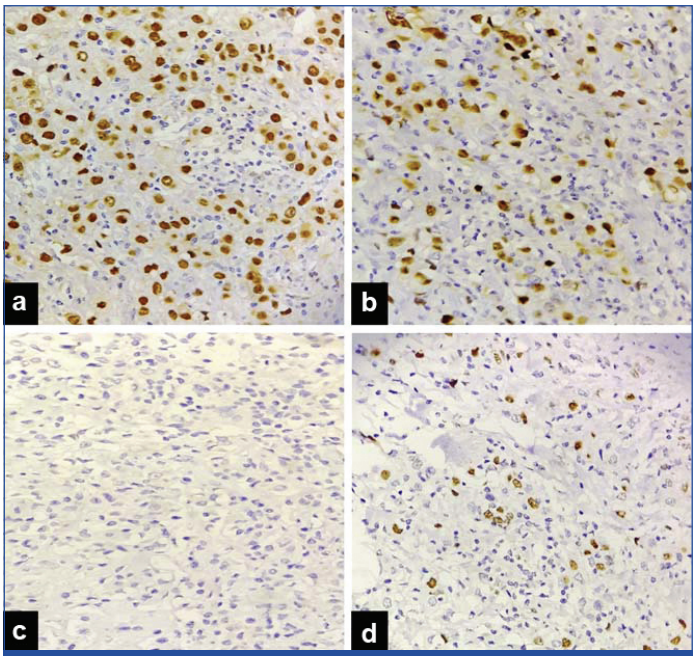
change with different grades of tumour. A statistically significant relationship between Ki-67 and these parameters was not established. The molecular subtyping of the tumours revealed that the mean value of Ki-67 was highest in basal-like tumours indicating the aggressive nature of these tumours. This was statistically significant with p-value=0.002 (p-value <0.05) [Table/Fig-3].

In scoring pattern I, with low (≤14%) and high (>14%) index categorisation of Ki-67, 5 (71.4%) of T1, 21 (65.62%) of T2 and 8 (72.72%) of T3) cases, 18 (64.28%) and 16 (72.72%) cases of lymph node negativity and positivity, 5 (71.4%), 18 (64.28%) and 11 (73.33%) cases of stage I, II and III respectively and 4 (66.66%), 9 (50%), and 21 (80.7%) cases of grade I, II and III, respectively belonged to higher Ki-67 index. Similarly, 14 (56%), 6 (60%) and 6 (85.71%) of Luminal A, B and HER2 had a higher Ki-67 PI. However, the percentages were significantly higher in basal-like tumours 8 (100%) [Table/Fig-4].

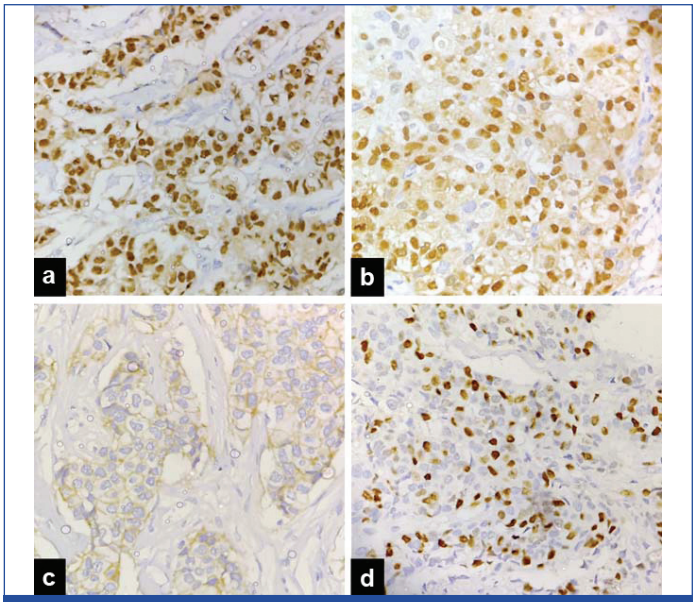
According to scoring pattern II, Ki-67 was divided into low (≤15%), intermediate (16-30%) and high (>30%) indices. Five (45.45%) of the T3, 10 (45.45%) of lymph node positive tumours and 14 (53.84%) of grade III showed a higher PI of Ki-67. However, for stage III, equal number of cases 5 (33%) belonged to low, intermediate and high Ki-67 index category. A higher Ki-67 index was found in 7 (88%) of basal-like subtype of breast [Table/Fig-5].

In scoring pattern III (<20%, 20-50%, ≥50%), 4 (57.14%) of T1 and 5 (45.45%) of T3 cases belonged to low Ki-67 PI category, whereas equal number of cases 13 (40.62%) of T2 had a low and intermediate Ki-67 index. Thirteen (46%) and 9 (40.90%) cases of lymph node negativity and positivity showed a low Ki-67 PI. Four (57.14%) and 7 (46.66%) cases of stage I and III had low Ki-67 index and equal number of cases 11 (39.28%) of stage III had a low and intermediate Ki-67 index. In regards to grade, equal number of cases 2 (33.33%) belonged to low, intermediate and high Ki-67 index. Thirteen (72.22%) of grade II had low Ki-67 PI and 12 (46.15%) of grade III had higher Ki-67 index. Similarly, 15 (60%) of Luminal A and 3 (42.85%) of HER2 had a low Ki-67 PI whereas, 5 (50%) of Luminal B had an intermediate index. However, 6 (75%) of basal-like tumours had a higher Ki-67 PI [Table/Fig-6].

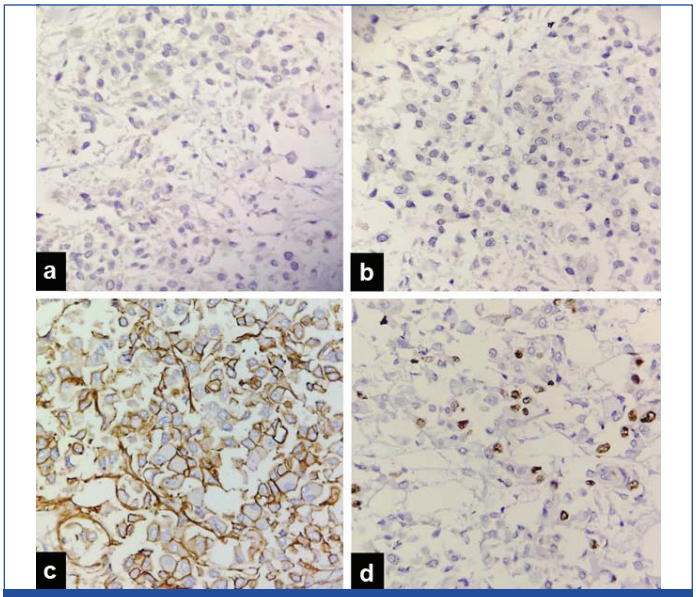
Photomicrographs demonstrating the Ki-67 index value in different molecular subtypes of breast cancer have been depicted in [Table/Fig-7-10].



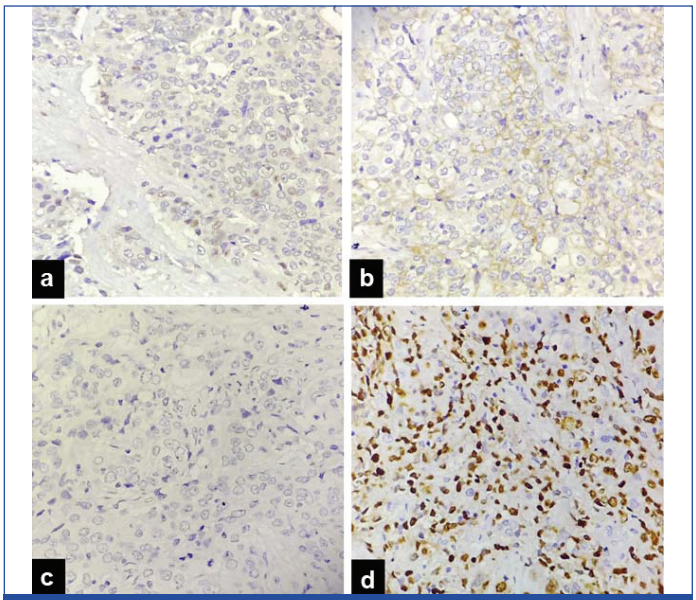
[Table/Fig-7]: Molecular Subtype: Luminal A; a) ER positive (IHC,400x); b) PR positive (IHC,400x); c) HER2 negative (IHC,400x); d) Ki-67:6% of nuclear positivity for Ki-67 in tumour cells (IHC,400x).



[Table/Fig-8]: Molecular Subtype: Luminal B; a) ER positive (IHC,400x); b) PR positive (IHC,400x); c) HER2 positive (IHC,400x); d) Ki-67–38% of nuclear positivity for Ki-67 in tumour cells (IHC,400x).



[Table/Fig-9]: Molecular Subtype: HER 2 Enriched; a) ER negative (IHC,400x); b) PR negative (IHC,400x); c) HER2 positive (IHC,400x); d) Ki-67:18% of nuclear positivity for Ki-67 in tumour cells (IHC,400x).



[Table/Fig-10]: Molecular subtype: Basal-Like; a) ER negative (IHC,400x); b) PR negative (IHC,400x); c) HER2 negative (IHC,400x); d) Ki-67:66% of nuclear positivity for Ki-67 in tumour cells (IHC,400x).

DISCUSSION

Prognostication of breast cancer through evaluation of Ki-67 proliferation marker has been utilised widely over more than a decade now. However, Ki-67 is yet to achieve a standardised cut-off value in order to upsurge its efficacy as a prognostic marker for oncologists and pathologists.

The present cross-sectional study indicated that the expression of Ki-67 index as a continuous variable (mean±SD) yielded better statistical association with respect to other prognostic parameters of breast cancer than the different scoring patterns used. It was also found that the three scoring patterns used in the present study showed varied distribution of Ki-67 with respect to other prognostic markers. Therefore, none could be selected as a single "best" scoring pattern. However, scoring pattern II showed notable findings with respect to other factors affecting prognosis of breast cancer.

It was also noticed that all three scoring patterns showed a higher Ki-67 PI in basal-like breast tumours, signifying its aggressive behaviour and poor outcome. The study also indicated that, irrespective of the method of Ki-67 evaluation, Ki-67 and molecular subtyping can be considered as important prognostic factors in breast cancer.

Soliman NA and Yussif SM reported the mean age of patients in his study was 54.6±12. 94.4%, 42% and 41% of the cases in his study were reported as IDC, grade II tumours and Luminal A tumours respectively [19]. These findings were in accordance with the present analysis. Another study by Amer MH showed a predominance of left-sided breast cancer, with a left to right ratio of 1.1. A relative excess incidence of left to right breast cancer has been well-documented in the literature, with the left to right side ratio ranging from 1.05 to 1.26 [20]. These results were in accordance with the current study.

In a review by Inwald EC et al., a strong association was observed between mean Ki-67 and grade of the tumour. Higher nodal status was associated with higher mean Ki-67. The mean Ki-67 values were lower in ER and PR positive tumours and higher in HER2 positive tumours [7]. Similar findings were reported by Ahmed ST et al., and Haroon S et al., [11,12] Additionally, Ahmed ST et al., revealed that the mean Ki-67 was higher in the triple negative groups with no significant association. No association was seen between Ki-67 and tumour size and lymph node status [11]. Haroon S et al., also exhibited the mean Ki-67 values were significantly higher in T3 tumours [12]. In another study by Awadelkarim, nothing significant could be demonstrated with tumour size, stage and hormone receptors- ER, PR and HER2 [12]. In present analysis, the mean values of Ki-67 had a significant difference between the different tumour sizes (p-value=0.608), lymph node status (p-value=0.506) stages (p-value=0.979) and grades (p-value=0.095) of breast tumour but no statistically significant association was found. However, it was found that Ki-67 statistically correlated with different molecular subtypes of breast cancer (p-value=0.002).

Ahmed ST et al., used a threshold of 14% to categorise patients into low and high Ki-67 PI index. Accordingly, Ki-67 was reported to be significantly associated with tumour grade. Higher PI of Ki-67 was present in HER2 positive and the triple negative breast cancer. No significant association was observed with tumour size and lymph node status [11]. However, in the current study according to scoring pattern I (≤14%, >14%), majority of different tumour sizes, absence or presence of lymph node positivity, different stages and grades of breast tumour belonged to higher Ki-67 index. Majority of the different molecular subtypes had a higher Ki-67 PI, which was significantly higher in basal-like tumours.

Haroon S et al., divided the tumour cases into low (<15%), intermediate (16-30%) and high (>30%) Ki-67 index levels. Significant correlation was found between Ki-67 and tumour grade, HER2neu, PR positivity and lymph node status. High Ki-67 index was seen in T2 and T3 tumours. HER2neu and lymph node status showed direct correlation with Ki-67 and PR status was inversely related. Ki-67 and tumour size and ER status showed no significant association [12]. Similar findings were reported by Abebe E et al., [10]. This was in agreement with the current study, where scoring pattern II (≤15%, 16-30%, >30%) depicted that higher tumour sizes, lymph node positivity, higher stage and grade of the tumour and basal-like breast tumours had a higher Ki-67 PI.

Nishimura R et al., found the median value of Ki-67 to be 20% and divided breast tumours into low (<20%), intermediate (20-50%) and high (≥50%) Ki-67 categories. Accordingly, Luminal A tumour had a low Ki-67 PI. A higher PI was observed in triple negative tumours. Large sized tumours, younger age, lymph nodes positivity, a higher grade, negative ER/PR status and positive HER2 receptor status was significantly associated with high Ki-67 index [13]. However, scoring pattern III (<20%, 20-50%, ≥50%) used in present study, showed that majority of the cases of different tumour sizes, absence or presence of lymph node positivity, different stages and grades of breast tumour belonged either to low or intermediate Ki-67 PI. Maximum cases of basal-like tumours had a higher Ki-67 index.

According to literature, significant difference in Ki-67 PI between biopsies and surgical specimens has been observed due to several factors like poor tissue fixation, poor staining and intratumoural heterogenicity to name a few. Studies also suggest performing immunostaining for Ki-67 PI on biopsies as well as on surgical specimens for verification [21,22].

Limitation(s)

The major limitation of the current study was a small sample size and the various types of breast specimens which were included in the study. However, immunostaining of Ki-67 on biopsy and corresponding surgical specimens for confirmation was not feasible in present study. Some pathological parameters like distant metastasis and overall survival could not be accessed due to lack of follow-up. Intratumoural heterogenicity in terms of Ki-67 expression could also be considered. A distribution error could also be implicated.

CONCLUSION(S)

The present study highlights that the association of Ki-67 with other prognostic markers of breast cancer was established appropriately when evaluated as a continuous variable rather than using different two or three tier scoring patterns. However, amongst the different scoring patterns used here in the present study, scoring pattern II (≤15%, 16-30%, >30%) showed remarkable findings with Ki-67. Moreover, Ki-67 showed a direct relationship with different molecular subtypes of breast tumours, irrespective of the method of Ki-67 evaluation. Thus, suggesting that of all the various prognostic markers of breast cancer, Ki-67 and molecular subtyping could be considered the best.

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