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KI-67 Index as a Prognostic Factor in Correlation with Other Clinico-Pathological Factors in Breast Cancer – An Institutional Experience

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ABSTRACT

Introduction: Breast cancer is a complex aggressive disease with different subtypes due to multiple biomolecular interactions (genetic heterogeneity) and demonstrates the extensive variation in patients clinical feature based on the ethnic group that makes the diagnosis and treatment challenging. Numerous breast cancer markers had been established like Progesterone Receptor (PR), Estrogen Receptors (ER), Human Epidermal growth factor Receptor (HER2neu) as well as clinical-pathological factors such as the stage of the disease, extent of axillary lymph node involvement, tumour size, histological grade, a mitotic rate, patient age, menopausal status etc. Ki-67, cyclin D1, cyclin E, and ERβ had been advocated to exhibit both the predictive and prognostic value in breast cancer patients.

Objectives: The objective of the current study is to evaluate the relevance and correlation of Ki-67 index in relation with other clinicopathological factors that determine the prognosis in Carcinoma breast and thereby, defining the staining thresholds and cut-off of Ki-67 immunohistochemistry.

Methods: A consecutive number of 200 patients who had undergone modified radical mastectomy were included in this study. The postoperative specimen was for the assessment of histopathology diagnosis and the tumour size, nodal status, histological grading, tumour pathological type, mitotic rate, and lymphovascular invasion apart from Estrogen Receptor status, Progesterone Receptor status, Human Epidermal growth factor Receptor 2neu status and Ki-67 protein expression. Patient's clinical data were collected prospectively. SPSS software version 19 was used for statistical analysis. One way Anova and chi-square analysis was applied to assess the relation of Ki-67 index score with other prognostic factors.

Results: Ki-67 protein expression showed no correlation with age (irrespective of age classification), menopausal status, histological subtypes, lymphovascular invasion, mitotic rate, ER and HER2neu, but still the levels of Ki-67 protein increases with ER negativity ($p>0.05$). The overall histological grading showed statistical significance between grade I Vs III and grade II Vs III which reflects the linear relationship whereas chi-square analysis did not show. Progesterone Receptor showed an inverse relationship with Ki-67 expression ($p=0.0005$) and whereas, tumour size and nodal involvement revealed a linear relationship($p=0.02$).

Conclusion: Hence, the study had lucidly established the fact that Ki-67 index is superior to the mitotic rate and can be routinely employed for predicting the prognosis in breast cancer patients. The current paper recommends the use of the staining thresholds and cut-offs of Ki-67 immunohistochemistry used in the current study for future studies since all the patients expressed Ki-67 protein.

Key Words: Carcinoma breast, Correlation, Ki-67 index, Prognostic factors, Tumour size

INTRODUCTION

Breast cancer is a complex aggressive disease with different subtypes due to multiple biomolecular interactions (genetic heterogeneity) and demonstrates the extensive variation in patients clinical feature based on an ethnic group that makes the diagnosis and treatment challenging.[1] Carcinoma breast constitutes about 22% of women malignancy and it is the

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second commonest malignancy in females.[2,3] Among the different types of cancers, worldwide data suggested that breast cancer in females occupies 25% which is nearly a quarter with a projected 1.67 million new cases every year. According to GLOBOCAN(The Global Cancer Observatory) 2018 report, the incidence of breast cancer is 11.6%, of which the mortality is documented to be 6.6% in females globally. At the same time, age-standardized risk and cumulative risk (age up to 75 yrs in %) are found to be 46.3 and 5.03 respectively.[4]In India, breast cancer ranks first with an incidence of age-adjusted rate and mortality to be 25.8 and 12.7 per 100,000 women.[5] A recent Indian study had revealed that age-adjusted incidence of breast cancer in Chennai (37.9 per 100,000 women) was higher next to Delhi (41 per 100,000 women) when compared over the other states and also showed that the incidence is escalating annually.[6]

Considering the elevating yearly incidence, mortality, different subtypes and the challenges faced in diagnosis and treatment strategy of breast cancer patients, identification of factors that possess both the predictive and prognostic values in breast cancer management becomes essential. Numerous breast cancer markers had been established like Progesterone Receptor (PR), Estrogen Receptors (ER), Human Epidermal growth factor Receptor (HER2neu) as well as clinical-pathological factors such as the stage of the disease, extent of axillary lymph node involvement, tumour size, histological grade, a mitotic rate, patient age, menopausal status etc. Ki-67, cyclin D1, cyclin E, and ERβ had been advocated to exhibit both the predictive and prognostic value in breast cancer patients. Gerdes et al (1983) had described Ki-67 (395kDa) as a master switch controller of cellular proliferation and found to have the most appropriate and superior correlation with histological parameter and proliferating (mitotic) activity by the previous literatures.[7,8]There exist a controversy regarding the staining thresholds and cut-offs of Ki-67 immunohistochemistry (not uniformly standardized) since different criteria have been employed by different studied authors.[9-12] Also, few numbers of literature are available among the Indian population regarding Ki-67 status. Hence, the objective of the current study is to evaluate the relevance of Ki-67 index in relation with other clinicopathological factors that determine the prognosis in Carcinoma breast as well as to correlate Ki-67 protein expression with patient's age, Menopausal Status, Tumour grade, Pathological type, Mitotic rate, Tumour size (T), Nodal status (N), Lymphovascular invasion, ER/PR status, HER2neu receptor status and thereby, defining the staining thresholds and cut-off of Ki-67 immunohistochemistry.

MATERIALS AND METHODS

Patient selection

The present study was conducted after the approval of the Institutional Ethics Committee (IEC) of the hospital. A consecutive number of 200 patients who had undergone modified radical mastectomy for early-stage primary breast carcinoma in our hospital from January 2016 to 2019 were included in this study after obtaining their informed consent. Patients with metastatic breast cancer or presented with a previous history of neo-adjuvant chemotherapy, or breast surgery outside our hospital including male breast carcinoma were excluded from the study. The postoperative specimen was sent for routine hematoxylin and eosin stains for the assessment of histopathology diagnosis and the tumour size, nodal status, histological grading, pathological type of the tumour, mitotic rate, and lymphovascular invasion apart from ER status, PR status, HER2neu status and Ki-67 protein expression. Immunohistochemistry protocol for the analyses of ER, PR, HER2neu and Ki-67 status was performed according to the protocol.[13] Ki-67 protein expression levels were classified based more than 20%, between 10 to 20%, between 1% to 10% and 0% staining as high, borderline, low and negative respectively. Patient's clinical data were collected prospectively. The ethical clearance number: CSP – MED/15/OCT/25/89

Statistical analysis

SPSS software was used for the statistical analysis of the clinical data. One way Anova was applied to assess the relation of Ki-67 index score with other prognostic factors including patient's age, menopausal status, grade of the tumour, pathological type, mitotic rate, tumour size (T), nodal status (N), lymphovascular invasion, ER/PR status and HER2neu receptor status. Correlations of Ki-67 index score level with clinicopathological parameters were determined using chi-square analysis. All the data were expressed as mean ± Standard Deviation (SD) or frequencies by per cent. The relationship between the prognostic factors and the Ki-67 index score was considered statistically significant only if the p-value is less than 0.05.

RESULTS

Patient characteristics

The median age of the study population at the time of presentation was 53 years, ranging from 27 to 84 years. There was no difference in frequency distribution observed concerning menopausal status among the studied populations (50% in each group). Majority of the patients had tumour size ranging from 2cm to 5cm (T2) followed by less than 2cm (T1) and larger than 5 cm (T3). According to the histological analysis, the predominate percentage was observed in well-differentiated carcinoma (grade II). Invasive Ductal Carcinoma (IDC) was the commonest pathological diagnosis and the least miscellaneous groups comprise of Invasive Papillary Carcinoma (IPC), Papillary Carcinoma Variant (PCV), Squamous Cell

Carcinoma (SCC), Invasive Medullary Carcinoma (IMC) with one patient each. All the patients had non-metastatic status. Almost all the patients had a nearly similar frequency of proliferation (mitotic rate) but still, moderate (grade II) was prominent followed by rapid (grade III) and low (grade I). A maximum number of patients had the absence of lymph node involvement and lymphovascular invasion. ER, PR, HER2neu positivity was documented in the 52%, 42% and 30% of the population accordingly. The Ki-67 index score levels according to the Ki-67 protein staining cells, patients had high levels in 68% followed by low levels in 18% and then, borderline levels in 14% among the studied population. The mean percentage of Ki-67 protein expression was found to be 36.8 ± 22.9 (range from 2 to 80%) of the complete cases. Patient's detailed information's is presented in Table 1. The median age of the study population at the time of presentation was 53 years, most of the patients had tumour size ranging from 2cm to 5cm (T2), Grade II was the most common tumour grade. Invasive Ductal Carcinoma (IDC) was the commonest pathological diagnosis. A maximum number of patients had the absence of lymph node involvement and lymphovascular invasion. ER, PR, HER2neu positivity was documented in the 52%, 42% and 30% of the population accordingly. The mean percentage of Ki-67 protein expression was found to be 36.8 ± 22.9.

Table 1: Demographic clinical features of the study population (n=200)

S. No	Parameters	Status	Mean ±SD/ Number (%)
1	Age (yrs)		54.3±11.9
2	Menopausal status	Premenopausal	100 (50%)
		Postmenopausal	100 (50%)
3	Tumour size(T)	T1	32 (16%)
		T2	144 (72%)
		T3	24 (12%)
4	Histological grade	Grade I	44 (22%)
		Grade II	124 (62%)
		Grade III	32 (16%)
5	Pathological type	Invasive Ductal Carcinoma	148 (74%)
		Ductal Carcinoma in situ	28 (14%)
		Invasive Lobular Carcinoma	8 (4%)
		Miscellaneous	16 (8%)
6	Mitotic rate	Grade I	60 (30%)
		Grade II	72 (36%)
		Grade III	68 (34%)
7	Nodal status	No	80 (40%)
		N1	56 (28%)
		N2	44 (22%)
		N3	20 (10%)
7	Lymphovascular Invasion	Present	80 (40%)
		Absent	120 (60%)
8	ER	Positive	104 (52%)
		Negative	96 (48%)
9	PR	Positive	84 (42%)
		Negative	116 (58%)
10	HER2NEU	Positive	60 (30%)
		Negative	140 (70%)
11	Ki-67 Index Score	High	136 (68%)
		Low	36 (18%)
		Borderline	28 (14%)
12	Ki-67 Index Score (%)	Mean	36.8 ± 22.9

Ki-67 protein expression correlation with clinical characteristics

To identify the significance of Ki-67 protein expression percentage with demographic and clinic-pathological parameters, the prognostic factors were divided into subcategories as shown in Table 2. On association analysis through student t-pair test, it was observed that the subcategories within age, menopausal status, histological tumour type, mitotic rate, lymphovascular invasion, ER, HER2neu concerning Ki-67 index score level of the total studies cases do not exhibit any correlation. In converse, tumour size, histological grade, nodal involvement and PR showed a positive correlation. The comparison within the PR status showed a statistically high significant correlation concerning the percentage of Ki-67 protein expression than the other parameter. The statistically significant association was found only between T1 and T2 (tumour size), grade I and grade III, and grade II and grade III (histological grade) and N0 with N2 and N3 (Nodal involvement) when compared against the respective percentage of Ki-67 score index.

Table 2: Correlation of Ki-67 Index score percentage with other prognostic factors

S. No.	Parameters	Category	No	Ki-67 score (%)	95% confidence interval for mean		p value
				Mean ± SD	Upper limit	Lower Limit	
	Age (yrs)	≤55	120	40.7 ± 21.6	4	80	0.15
		≥ 55	80	31.1 ± 24.1	2	80	
	Menopausal status	Pre	100	42.0 ± 23.5	4	80	0.11
		Post	100	31.6 ± 21.8	2	80	
	Histologic tumour type	Invasive Ductal Carcioma	148	36.1 ± 21.9	2	80	
		Ductal Carcinoma In Situ	28	36.1 ± 24.0	20	80	0.99
		Miscellaneous	24	42.3 ± 30.2	4	80	
	Tumour size (T) cm	T1(<2)	32	22.5 ± 22.4	4	70	
		T2 (2-5)	144	39.9 ± 20.9	4	80	0.04*
		T3 (>5)	24	37.7 ± 30.7	2	80	NS
	Histological grade	Grade I	44	12.2 ± 8.9	4	30	Vs Grade III 0.0001*
		Grade II	124	39.9 ± 19.6	2	80	0.02*
		Grade III	32	58.8 ± 18.9	40	80	
	Mitotic rate	Grade I	60	13.7 ± 10.3	2	30	
		Grade II	72	41 ± 19.1	20	80	0.07
		Grade III	68	52.8 ± 18.1	28	80	
	Nodal involvement	No	80	25.6 ± 21.2	2	70	Vs N2 0.02* Vs N3 0.006*
		N1	56	39.6 ± 23.1	4	80	
		N2	44	45 ± 20.1	20	80	0.5
		N3	20	56 ± 15.2	40	80	
	Lymphovascular Invasion	Present	80	42.7 ± 19.2	20	80	0.13
		Absent	120	32.9 ± 24.5	2	80	
	ER	Positive	104	24.8 ± 19.7	2	80	3.2
		Negative	96	49.8 ± 18.8	23	80	
	PR	Positive	84	23.4 ± 16.9	2	60	0.0001*
		Negative	116	46.6 ± 21.9	4	80	
	HER2neu	Positive	60	37.3 ± 20.8	4	80	0.91
		Negative	140	36.6 ± 23.9	2	80	
	Ki-67 Index Score	High	136	48.4 ± 17.9	23	80	
		Low	36	6.2 ± 3.1	2	10	NS
		Borderline	28	20	20	20	

UL-Upper Level; LL-Lower Level; Miscellaneous-Invasive Papillary Carcinoma, Papillary Carcinoma Variant, Squamous Cell Carcinoma, Invasive Medullary Carcinoma and Invasive Lobular Carcinoma; Vs-Versus; NS-Non significant;*- represents the statistical significance.

Correlation of Ki-67 scoring with clinical characteristics

Ki-67 protein expression scoring levels were assessed for correlation with the prognostic factors. In similar to the findings of Table 2, the correlation analysis (chi-square) of Table 3 also further provided the profound findings of tumour size, nodal involvement and PR status positively related with different Ki-67 score levels with statistical significance except for histological grade. Concerning the histological tumour type, Invasive Ductal Carcinoma and ductal carcinoma in-situ combined (60%) showed the higher level of expression than the rest of the pathology but statistical significance was not identified within the groups. Table 3 showed a linear relationship when compared the tumour size with ki-67 proteins levels. As the tumour grade increases, Ki-67 protein expressions also raised and in converse, when the tumour grade decreases, the respective expressions levels also minimized (p=0.02). When the nodal system involvement advances, Ki-67 protein expressions levels also increased and had decreased expression, when the nodal involvement is less (0.02) in similar tumour grade findings. There was no statistical significance difference observed either between the presence and absence or the positivity and negativity of the lymphovascular invasion or ER and HER2neu respectively concerning the either high or low or borderline levels of Ki-67 protein expression. The majority of the patients who had shown negative results for ER, PR and HER2neu demonstrated increased levels of Ki-67 proteins. The higher level of statistical significance was noted between the positivity and negativity of PR with the Ki-67 score index levels among the studied cases.

Table 3: Association of Ki-67 score index level with other prognostic factors

S. No.	Parameters	Category (n)	Ki-67 score level			p-value
			High (%)	Low (%)	Borderline (%)	
1	Age (yrs)	≤55 (120)	93 (46)	12 (6)	16 (8)	0.17
		≥ 55 (80)	44 (22)	24 (12)	12 (6)	
2	Menopausal status	Pre (100)	76 (38)	12(6)	12(6)	0.44
		Post (100)	60 (30)	24 (12)	16 (8)	
3	Histologic tumour type	Invasive Ductal Carcioma (148)	104 (52)	28 (14)	16 (8)	
		Ductal Carcinoma In Situ (28)	16 (8)	0(0)	12 (6)	0.11
		Miscellaneous (24)	16 (8)	8 (4)	0 (0)	
4	Tumour size (T) cm	T1(32)	8 (4)	16 (8)	8 (4)	
		T2 (144)	112 (56)	12 (6)	20 (10)	0.02*
		T3 (24)	16 (8)	8 (4)	0 (0)	
5	Histological grade	Grade I (44)	4 (2)	28 (14)	12 (6)	
		Grade II (124)	100 (50)	8 (4)	16 (8)	2.4
		Grade III (32)	32 (16)	0(0)	0(0)	
6	Mitotic rate	Grade I (60)	12 (6)	36 (18)	12(6)	
		Grade II (72)	56 (28)	0(0)	16 (8)	
		Grade III (68)	68 (34)	0(0)	0(0)	1.5
7	Nodal involvement	No (80)	32 (16)	32 (16)	16 (8)	
		N1 (56)	44 (22)	4 (2)	8 (4)	0.02*
		N2(44)	40 (20)	0 (0)	4 (2)	
		N3(20)	20 (10)	0 (0)	0 (0)	
8	Lymphovascular Invasion	Present (80)	68 (34)	0 (0)	12 (6)	0.13
		Absent (120)	68 (34)	36 (18)	16 (8)	
9	ER	Positive (104)	40 (20)	36 (18)	28 (14)	1.9
		Negative (96)	96 (48)	0(0)	0(0)	
10	PR	Positive (84)	32 (16)	28 (14)	24 (12)	0.0005*
		Negative (116)	104 (52)	8 (4)	4 (2)	
11	HER2neu	Positive (60)	52 (26)	4 (2)	8 (2)	0.18
		Negative (140)	84 (42)	32 (16)	24 (12)	

DISCUSSION

Cellular proliferation is the hallmark in any cancer and hence, regular examination of cell proliferation is suggested in the pathological assessment especially in invasive carcinoma breast, which in turn accounts for the traditional scoring of mitotic activity (simple and commonly employed tool). Hence, cell proliferation is an essential part of histological grading and well known as the prognostic marker. The major disadvantage of mitotic index assessment is that it is time-consuming and the results are non-reproducible but still are employed in clinical practice.[13,14] Therefore, investigation on more sensitive tumour biomarker has led to the identification of several markers related to the patient and the tumour has been established in predicting the risk of mortality, recurrence and metastasis in breast malignancy. These factors are the number of positive axillary lymph nodes, size of the tumour, histological grade of the tumour, lymphovascular invasion, hormone receptor (ER/PR) positivity and Her2neu gene amplification.[15] Recently, one such tumour proliferation biomarker known as Ki-67 protein (proliferating cell nuclear antigen) is being established as a prognostic and predictive indicator in patients with breast cancer and possesses strong association with cellular proliferation.[16, 17]

Ki-67 protein (non-histone) is expressed during all the phases of the cell cycle (late G1, S, G2 and M phase) except at early G1 and G0 (quiescent cells) phases, and are detected during M phase at the chromosomal surface which makes it unique and more specific biomarker.[18,19] The expressions of Ki-67 protein is up-regulated in rapidly dividing cells and hence, the cells stains more positive. The role of Ki-67 has been well analyzed in various research studies for its prognostic value and reliability in breast carcinoma, cervical cancer, sarcomas, neurological malignancies, bronchogenic carcinoma and prostatic cancer.[20-24] Many investigating modalities have used Ki-67 as a successful diagnostic marker.[25-28] Positive immunostaining for the Ki-67 expression has been accepted as an investigative tool and positive staining ranging from 10 to 14% has been defined as high risk.[29-31] The 2009 Saint Gallen consensus declared that the patients with higher levels of Ki-67 nuclear protein expression required additional chemotherapy and hormonal therapy for all ER PR positive breast malignancies.[32]

In our study, the protein expression levels of Ki-67 was defined as negative, high, low and borderline only when the positive tumour cells percentage (Ki-67-LI) is 0%,>20%,<10 and 10-20% respectively. All breast cancer patients showed positive results in the present study. In contrast, Bouzubar et al.[10] defined negative tumour cell percentage as less than 5%, while Tan et al[11] considered 0% as negative is similar to our study. Tan et al.[11] and Bouzubar et al[10] defined > 10% and >20% staining as high in dissimilar and similar to our study respectively. To coincide with our classification, Marwah N et al[13] defined high as >20% except <5% and 5-20% as negative and positive accordingly. In the current study, the positive correlation of Ki-67 protein expression was observed with different clinicopathological factors as compared with other studies[10,12]At the same time, our study had recommended the cut-offs of Ki-67 immunohistochemistry to be between 10 to 20% as in disagreement with Cho et al (20%).[33] Hence, we advocate the use of histoimmuno chemistry staining cut-off of Ki-67 used in the current study because all the breast cancer patients showed positive and at least low or minimal expression.

Kermani et al[34] studied 220 patients who had presented with primary breast cancer and evaluated the relationship of Ki-67 immunohistochemistry index expression with age (less than or equal and more than 50 years respectively) and concluded that there exists no association is similar to our study and Li FY et al (age less than or equal to 35 and more than 35).[35]Hence, we conclude that there exists no correlation between the expression of ki-67 protein and the patient's age (irrespective of age classification).

A recent Indian study on 75 patients who had undergone radical or modified radical mastectomy was analyzed for the relationship of Ki-67 index score with the clinicopathological parameters.[13] The study resulted that there was no correlation between the Ki-67-LI index and menopausal status, lymph node involvement and HER2/neu. The same result also showed a direct association with tumour size, and histological grade and whereas, an inverse association with ER and PR status. The current study had shown a similar result with menopausal status, HER2neu, tumour size, histological grade (Table2: Grade I vs III, Grade II vs Grade III for overall Ki-67 protein percentage and not concerning the levels of expression), PR and dissimilar result with lymph nodal involvement and ER. Kermani et al.[34]concluded the relationship between Ki-67 expression, and tumour size, ER in disagreement, and lymph node, PR in agreement concerning our study. Querzoli et al.[12] had shown a direct association of tumour size concerning the expression of Ki-67-LI except few other authors.[36]

Haroon S et al.[37] performed the study on 194 subjects diagnosed with primary breast carcinoma and examined the relationship of Ki-67 with other prognostic factors. The mean age and Ki-67 index were found to be 51.7 years (similar to our study: 54.3 years) and 26.9% (lesser than our study: 36.8%) accordingly. The study had a similar and contrast observation with PR, ER, tumour grade, lymph node status, and with tumour size, HER2neu in comparison to our study.

The present study had shown that higher the histological grading of the tumour, the higher would be the Ki-67 index as similar to Haroon S et al.[37] There was no significant relevance between the pathological subtype and Ki-67score. However, the majority of the patients in our study had the

histological subtype of IDC in 74% of the population among the total population. Similar results were found in a study done by Haroon S et al. [37]

In overall, our study had demonstrated that Ki-67 protein expression did not correlate with age, menopausal status, histological subtypes, lymphovascular invasion, mitotic rate, ER and HER2neu, but still the levels of Ki-67 protein increases with ER negativity. The overall percentage of Ki-67 (t-pair test) in histological grading showed statistical significance between grade I Vs III and grade II Vs III which reflects the linear relationship but chi-square analysis did not show any relevance. PR showed an inverse relationship with Ki-67 expression and whereas, tumour size and nodal involvement revealed a linear relationship.

CONCLUSION

Hence, the current study had lucidly established the fact that Ki-67 index is superior to the mitotic rate and can be routinely employed for predicting the prognosis in breast cancer patients. Also, the present study recommends the use of current staining thresholds and cut-offs of Ki-67 immunohistochemistry applied in the current study since all the patients expressed Ki-67 protein.

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Conflict of Interest: "The authors declares that there is no conflict of interest regarding the publication of this paper."

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Data Availability: The data used to support the findings of this study are available from the corresponding author upon request.

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