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CORRELATION OF KI67 SCORE WITH OTHER PROGNOSTIC INDICATORS IN CASE OF CARCINOMA BREAST NOT UNDERGOING NEOADJUVANT CHEMOTHERAPY

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ABSTRACT

Aims and objectives: This study was conducted with the aim to calculate Ki67 score of all cases of breast cancer not undergoing neoadjuvant chemotherapy and correlate the score with other prognostic factors. **Material and Methods:** In 30 patients cases of carcinoma breast with biopsy diagnosed invasive carcinoma of breast at any age. **Results and Analysis:** The Ki67 score were analyzed and correlated with other prognostic indicators using standard statistical methods. The parameters namely TNM Clinical Staging, tumour size and HER-2/neu had a strong positive statistical correlation. ER, PR status had shown a statistically significant negative correlation value. The expression of Ki67 in breast cancer tissues with age and lymphovascular invasion had no significant statistical correlation. The histological grade of tumour was found to be correlated with expression of Ki67 in a positive way. **Conclusion**: Ki67 score can be used for prognostication and planning of treatment in patients with carcinoma breast because of its cost effectiveness and easy availability.

KEYWORDS: Ki-67, prognostic, molecular subtypes, breast cancer.

INTRODUCTION

Breast cancer over the centuries has remained a dogma to the patient and an enigma to the oncologists. Since the Halstedian days of disfiguring extirpative surgery till the modern day approach of conservative surgery, management of breast cancer has seen many changes. Epithelial malignancy of the breast is the most common cause of cancer in women accounting for about one-third of all cancers. The tumour is highly heterogeneous with wide range of clinical, pathological and biological characteristics, treatment modalities include treatment of local disease with surgery or radiation therapy or both and treatment of systemic disease with cytotoxic chemotherapy, endocrine therapy, biological therapy or a combination of these,[1] the need for selection of various local and systemic therapy is based on several prognostic and predictive indicators.

A prognostic factor is any measurement available at or before the time of surgery that correlates with disease free or overall survival in the absence of systemic adjuvant therapies and, as a result, is able to correlate with natural history of disease.[2] The biomarkers and bio targets for breast cancer include proliferation indices like PCNA and Ki67, indices of apoptosis such as bcl2 and bax: bcl2 ratio, indices of angiogenesis such as VEGF, growth factor and growth receptors HER-2/neu, COX2 enzymes, cyclins, cyclin dependent kinases etc.

Ki67 is a nuclear non histone protein and is a proliferation biomarker with both prognostic and predictive value in breast cancer. High Ki67 score indicates good response to chemotherapy but means poor prognosis. Ki67, a marker of cell proliferation, is expressed in all phases of the cell cycle, except G0.[3] This protein is localized in the nucleus[4] and its expression is often quantified in terms of percentage of positive nuclei. This is usually a semi quantitative estimate determined by pathologists who count as many as 1000 nuclei to determine this proportion. The threshold for differentiating high and low Ki67 score by the literature varies widely from 1% to 28.6%.[5]

The study attempts to evaluate Ki67 score and compare it with other prognostic indicators, helping in predicting recurrences and directing treatment of breast cancer cases.

AIMS AND OBJECTIVE

1. To calculate Ki67 score of all cases of breast cancer not undergoing neo-adjuvant chemotherapy (NACT).
2. To make a comparison with other prognostic indicators.
3. To study the tumour biology with respect to-

a) Bloom Richardson Scoring
b) Lympho-vascular invasion,
c) Receptor Status.

4. To correlate the above findings with Ki67 score

MATERIALS AND METHODS

1. **Study area**:- Department of General Surgery &Department of Pathology Medical College & Hospital, Kolkata.
2. **Study Population**: Patients of any age coming to Medical College surgery outdoor department with biopsy proven carcinoma breast at any stage.
3. **Study Period**: January 2015 to June 2016.
4. **Sample Size**: Selection of 30 patients with carcinoma breast planned for surgery will be done from patients attending Medical College Kolkata. Two days in a week will be spent by the investigator for the study.
5. **Sample Design**: All patients of biopsy proven carcinoma breast, attending General Surgery outdoor in Medical College Kolkata.

Inclusion Criteria

Patients of any age coming to medical college Kolkata with core needle /fine needle aspiration biopsy proven carcinoma breast of any stage.

Exclusion Criteria

a. Patients operated in other centres
b. Patients with any co-existing second malignancy.
c. History of neo adjuvant therapy in a case of carcinoma breast.
d. Male carcinoma breast

6. Study Tools

a) History
b) Clinical examination
c) Investigations: Haematological, USG, Mammography, FNAC, Ki67 score.
d) Histopathological Examination: Tumour size, Grade, Lymph node, ER, PR status, HER-2/neu status.
e) Predetermined specific proforma, charts for patient particulars and tables.
f) Patient consent form.

7. Study Techniques

Patients attending Department of General Surgery, Medical College, Kolkata with biopsy proven carcinoma breast, Their breast tissues were obtained using mastectomy specimen, the tissues analyzed through histopathological examination and Ki67 score calculated, along with other prognostic indicators like ER/ PR/ HER-2/neu status.

8. Plan for analysis of data

With use of suitable statistical methods, the results will be directed to establish specific objectives of the study.

METHODOLOGY

a. Patients to be evaluated by detailed history and clinical examination followed by clinical staging.
b. Operative notes to be recorded.
c. Post-operative detailed histopathological examination of mastectomy specimen using formalin-fixed, paraffin-embedded, haematoxylin and eosin stained slides representing invasive component of tumour to evaluate tumour size, histological grade and lymph node.
d. Detailed histopathological report to be recorded with regard to ER/ PR/ HER-2/neu status.
e. ASSESSMENT OF KI-67 -The Ki-67 percentage score is defined as the percentage of positively stained nucleus of tumour cells (plus mitotic figures) among the total no of malignant cells assessed[4,12] (at least 1000 cells were counted with a minimum of 500 cells). A cut off point of 20% was defined according to the experience of different pathologists as well as national and international recommendations at present.[4,7,8,9] Scoring is conducted considering the whole tumour section and not only limiting to the hotspots of the carcinoma or to the tumour edge. Internal positive controls include: mitotic figures, normal ducts, and lymphocytes as well as to a lesser extent, endothelial cells and stromal cells serve for this purpose. Primary antibodies are used for Ki67 scoring.
f. Correlation to be done between age, tumour size, clinical staging, receptor status, molecular classification, histological grade, and LVI with Ki67 score.

RESULTS AND ANALYSIS

In this study, 30 female patients suffering from breast cancer and fulfilling the selection criteria of this study were examined.

1. Age

a. The Average age was 51 and range of age of the study population was 40 years.
b. Maximum cases n=12 (40 %) were in the age group of 41-46 years. Among the rest, n=10 (33.33%) were in the age group of 53-58 years, n=3 (10%) were in the age group 59-64 years, n=2 (6.67%) were in 65-70 years age group, n=2 (6.67%) in 47-45 years and 1

case (3.33%) in 29-34 years. Overall n=14 (46.67%) were in the ≤ 50 years age group and n=16 (53.33%) were in the > 50 years age group.[12]

2. Religion

In our study, 23 patients were Hindus (76.66%) and 7 patients were Muslims (23.33%). According to 2011 Census in India, the distribution of population by religion is 80.5% Hindus and 13.4% Muslims hence; the religion distribution in our study can be explained by general distribution in the population and is not specific to Breast Cancer.

3. Laterality

In our study, 16 patients (53.33%) had right sided and 14 patients (46.67%) had left sided breast cancer. However, according to literature, many studies have shown that unilateral Breast cancer is more frequent in the left breast than in the right.[12]

4. Affected Quadrant

In our study, maximum i.e. 15 patients (50%) had cancer in upper outer (UO) quadrant of breast which was consistent with literature. It is considered to be a reflection of the greater amount of breast tissue in this quadrant. Another hypothesis is that underarm cosmetics cause breast cancer in upper outer quadrant.[13]

5. Tumour Size (T Stage)

The mean size of the breast tumour was 3.833 cm, Standard Deviation 1.22, minimum size 2 cm and maximum size 7 cm with a range of 5 cm. The variance was 1.4885. Median size of the tumour was 3.25 cm. The maximum no. of cases i.e. 18 cases (60 %) presented with a tumour size in the range ≥2.5 cm - ≤4.5 cm (T2 Stage). 1 case (3.33%) presented with a tumour size of 2 cm (T1 Stage). Remaining 9 cases (30%) presented with a tumour >5 cm. - ≤7 cm (T3 Stage), 2 cases (6.67%) presented with a tumour size 3 cm but with skin involvement (T4b stage).

6. Clinical Staging

All the cases were clinically staged and the results were T2N0M0 (n=11, 36.67%); T2N1Mo (n=6, 20%); T3N0M0 (n=5, 16.67%); T3N1M0 (n=4, 13.37%); n=4, 13.32% 1 each were in T1N0M0, T2N2M0, T4bN1M0 and T4bN2M0 respectively.

7. Histopathological Grade and Lymphovascular Invasion

All tumours were graded histologically as per Scarff-Bloom Richardson (SBR) classification. Out of 30 patients, Grade I (n=13, 43.33%), Grade II (n=6, 20%) and Grade III (n=3, 36.67).

Lymphovascular invasion, as seen by histopathological examination of specimen was present in 12 cases (40%) and absent in 18 cases (60%). The observed difference between the sample mean was not convincing enough to say that the Ki67 status differed significantly. Its presence determined poorer prognosis and indicated risks of micrometastasis.[14]

8. Receptor Status (ER, PR, and HER-2/neu)

Estrogen Receptor status was collected for 30 cases and 19 patients (63.33%) were ER positive whereas 11 patients (36.67%) were ER negative. Progesterone Receptor status was collected for 30 cases. Out of 30 patients, 15 patients (50%) were PR positive whereas 15 patients (50%) were PR negative.ER and PR receptors predict response to hormone therapy in breast cancer and are independent prognostic factors as well. ER negative, PR negative and triple negative breast cancers carry a poorer prognosis and are not amenable to hormone therapy.

Out of 30 patients, 13 patients (43.33%) were HER-2/neu positive whereas 17 patients (56.67%) were HER-2/neu negative. Over expression of HER-2/neu is prognostic marker of tumour aggressiveness and responsiveness to adjuvant therapy. HER-2/neu oncogene over expression is higher (46.37%) among Indian patients in comparison to 25–30% shown in most Western literature-consistent with finding in our study.[15]

9) Lymph node status

Out of 30 patients, 17 patients (56.67%) were no, 11 patients (36.67%) were N1 and 2 patients (6.67%) were N2. The standard deviation 7.549 and sample variance 57 were observed.

10) Ki67 receptor status

Out of 30 patients, 19 patients (63.33%) were in ≤ 20% and 11 patients (36.67%) were in >20% status. The mean percentage of status 19.567, standard deviation 1.2795 and sample variance 1.637 had been found. The minimum and maximum Ki67 score was 5% and 50% respectively. The coefficient of dispersion 68.22 and mean deviation 10.84 had also been found.

11) Histopathology of breast mass

Out of 30 patients, 25 patients (83.33%) were diagnosed with IDC and 5 patients (16.67%) were diagnosed with ILC.

12) Molecular classification

Out of 30 patients, 19 patients (63.33%) were in ≤ 20% and 11 patients (36.67%) were in >20% status. Further, 9 patients in Luminal A, 10 patients in Luminal B were found under low ≤ 20% category. However, 8 patients in Basal like and 3 patients in HER-2/neu type were found high >20% category. The HER-2/neu type had highest mean value of Ki67 expression followed by Basal Like group, Luminal A and Luminal B (in descending order) and difference of mean was found to be statistically significant by ANOVA test.

13) Correlation of Ki67 With AGE, ER, PR Status

The overall 14 cases (46.67%) were in ≤ 50 years age group and 16 cases (53.33%) being of 50 years of age or older. We noted an overall 63.33% of ER and 50% of PR positivity. The prevalence of hormone receptor positive breast cancer in Asian Countries has been found to be lower than the Western world. Western world have reported 70-80% ER and 60-70% PR expression in the case of invasive ductal carcinoma respectively [16,17,18,19] The overall 19 cases under various clinical staging had ≤ 20% Ki67 expression whereas 11 cases of clinical staging was observed under > 20% Ki67 expression.

14) Correlation between Ki67 and other parameters tumour size, LVI, clinical staging and HER-2/NEU.

Table 1: Correlation of ki67 with tumour size.

Analysis	Correlation Value	P-value
Pearson Correlation Coefficient	r= 0.9822	0.0178
T-test two tailed	t-crit= 3.1824	0.01627

Table 2: Correlation of ki67 with values of grade.

Description	Frequency	Mean	STDEV	Sample Variance	Standard error of difference between the Mean	Actual difference of Mean
Grade I & II (Low & Moderate)	19	11.053	4.170	17.386	2.661	23.220
Grade III (High)	11	34.272	8.235	67.818		

Table 3: Correlation of Ki67 with Her-2-neu.

Analysis	Correlation Value	P-value
Two tailed T- Test	t-crit =12.7062	0.0334

This implied that higher Ki67 values were found in HER-2/neu positive tumours with bigger size and advanced stage, thereby showing high Ki67 values in relatively aggressive tumours. The Pearson Correlation Coefficient (t-test) (r= - 0.09662; < 0.03376) and Pearson Correlation Coefficient (Fisher) p< 0.0421 indicated the strength of association between the Ki67 expression and had revealed statistically significant negative correlation with ER, PR positivity.

In case of histological grade, actual difference of mean was more than twice the Standard error of difference of mean and hence statistically significant difference in Ki67 expression existed between high grade tumours and other tumours. ANOVA test showed the F value (56.2975) > F crit (2.975), and since P value (tending to infinity) also showed statistical significance, we rejected the null hypothesis. Therefore, there was evidence that there were differences in the mean across the subgroups of molecular classification. Finally, in case of LVI (t-obs =39.51<4.30=t-cric; p> 0.731) we retained the null hypothesis i.e. the expression of Ki67 was not statistically significantly correlated in LVI of patient. We also retained the null hypothesis in case of age correlation.

DISCUSSION

Among the 30 patients of breast cancer in this study, mean age was 51 years, Standard Deviation 8.8279 of which the most common affected age group was 41-46(40%) & 53-58(33.3%) years of age group.24 patients had malignancy in upper outer quadrant. Median size of the tumours was 3.25 cm, having a range 2 to 5 cm, standard deviation 1.22 and variance 1.4885.

18 cases (60%) were in T2 stage. LVI was absent in 60 % cases. An overall 63.33% of ER and 50% of PR positivity was noted.HER-2/neu was absent in 17 patients (56.67%) and present in 13 patients (43.33%). We had divided Ki67 to two groups: 19 patients (63.33%) had low Ki67 (Ki67 ≤ 20%) and 11 patients (36.67%) with high Ki67 >20%.

With respect to the Histological grade of the tumours, 13 patients (43.33%); 6 patients (20%) and 11 patients (36.67%) were in Grade I, II and III respectively. More

than half of the cases, 36.67% were in T2NOMO & 20% were in T2N1MO stage respectively. Higher mean Ki67 expression value was found in grade III cases compared to grade I & II cases combined and the difference of mean in the 2 groups was statistically significant.

In our study, parameters like tumour size, HER-2/neu, tumour stage were statistically significantly positively correlated with Ki67. The mean values of Ki67 expression in the subgroups namely Luminal A, Luminal B, Basal Like and HER-2/neu type were subjected to ANOVA test. The statistically significant relations such as: HER-2/neu type > Basal Like > Luminal A > Luminal B were derived. However, in case of correlation with LVI and age, we retained the null hypothesis, i.e. expression of Ki67 was not statistically significantly correlated with these parameters.

CONCLUSION

1. Higher ki67 score was found to be associated with larger size of tumours and higher grades of tumours (grade3).
2. Higher ki67 score was found in tumours with her-2-neu types and basal cell types.
3. No correlation of ki67 was found to be associated with age or lymphovascular invasion.

Hence Ki67 score can be used for prognostication and planning of treatment in patients with carcinoma breast .Because of its cost effectiveness and easy availability this biomarker may be used. Repeat studies with higher sample size should be performed to ascertain any correlation of Ki67 score with age of the patient.

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