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Study of Ki-67 Immunostaining in Breast Carcinoma and it's Correlation with Grade of Tumor and Lymph Node

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ABSTRACT

Background: Breast cancer is the commonest cancer in women worldwide and represents a disease with wide spectrum particularly in terms of tumour histology, biology, prognosis and response to therapy. Breast cancer is characterized by cellular heterogeneity.

Methods: The study was conducted in the Department of Pathology, GSVM Medical College, Kanpur and associated hospital of Kanpur from January 2018 to September 2019 on patients of breast carcinoma. These cases were selected from the outpatient and inpatient of LLR Hospital, Kanpur, India. In the present study, we had taken a total of 80 cases of invasive breast carcinoma as well as Histomorphology, Ki-67 immunostaining and it's a correlation with lymph node status and tumour grade were studied.

Results: This study leads us to conclude that the peak incidence of invasive breast carcinoma was reported in the age group of 41 to 50 years (with mean age 47.32 year and the standard deviation was 11.63). In the present study, the most common histological type was invasive ductal carcinoma, not otherwise specified (NOS). In our study, 66.25% of cases had metastatic lymph nodes and half of them were having more than 4 metastatic lymph node.

In the grade-II of Bloom Richardson Grading system, there were 40% cases and in grade-III there were 35% of cases and grade-I has shown 25% of cases. A significant association was found between the grade of the tumor and the lymph node positivity.

Conclusion: The present study concluded a positive correlation between the grade of tumour and Ki-67 immunohistochemistry, which was used as a prognostic and predictive parameter.

Key-words: Bloom Richardson's grading, Breast carcinoma, Histomorphology, Immunostaining, Invasive ductal carcinoma, Ki-67

INTRODUCTION

Breast cancer is known to be a heterogeneous disease. In addition to the conventional histopathological parameters, the assessment of proliferation is one of the major factors for the treatment decisions in breast cancer patients [1]. The incidence of breast cancer is low in India but rising. Breast cancer is the commonest cancer of urban Indian women and the second commonest in rural women.

Owing to the lack of awareness of this disease and in the absence of a breast cancer screening program, the majority of breast cancers are diagnosed at a relatively advanced stage. The quality of care available for breast cancer patients varies widely according to where the patient is treated [2].

Premalignant breast lesions are relatively common but only a small proportion appear to progress to invasive breast cancer. Most breast cancer occurs in women and the number of cases is 100 times higher in women than that in men. Breast cancer can commonly metastasize to distant organs such as the bone, liver, lung and brain. The widely used screening method is mammography for the detection of breast cancer and it reduces the mortality effectively [3].

The mortality rate of breast cancer decreases due to the

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widespread early screenings and advanced medical therapies. Recently biological therapies have been proved to be beneficial for breast cancer [4]. Ki-67 antibody reacts with a nuclear non-histone protein present in all active phases of the cell cycle except the G0 phase [5]. Ki-67 is used as a marker of cell proliferation and used to stratify patients into good and poor prognostic categories [6]. Those tumors, which are Ki-67 positive, are more active in growth, more aggressive in the invasion, and more metastatic [7].

MATERIALS AND METHODS

This study was done in the Department of Pathology, G.S.V.M. Medical College Kanpur, India from January 2018 to August 2019. Total of 80 female patients were included in the study. We included in our study histomorphology and lymph node status in breast carcinomas, and Ki-67 immunostaining in all these breast carcinomas and its prognostic importance. Tumor mass was subjected to Immunohistochemistry (IHC). The above study was approved by the Ethical Committee and informed consent was obtained from the patients before the study. The modified Bloom-Richardson Histopathological grading [8] was used in this present study.

Inclusion criteria

1. Mastectomy specimens of clinically/cytologically diagnosed breast malignancy in the female of all age group
2. Patients, who gave written informed consent

Exclusion criteria

1. Patients with metastatic malignancy of the breast
2. Patients already treated for contra-lateral breast cancer

RESULTS

Table 1 shows that a total of 80 cases of infiltrating breast carcinoma were analyzed in this study and 47.50% cases belonged to the age group of 41 to 50 years and after that 17.5 % cases belonged to 51 to 60 years and also in 61 to 70 years. Mean age was 47.32 and the standard deviation was 11.63.

Table 1: Distribution of cases based on age group

S. No.	Age group	No. of cases (n=80)	Percentage (%)
1	0–30 yrs	03	3.75
2	31–40 yrs	10	12.5
3	41–50 yrs	38	47.5
4	51–60 yrs	14	17.5
5	61–70 yrs	14	17.5
6	> 70 yrs	01	1.25

Table 2 shows that 60 (78%) cases were diagnosed as invasive ductal carcinoma, 4 cases (5%) of invasive lobular carcinoma, and 2 cases (2.50%) of invasive papillary carcinoma. Total 7 cases (8.75%) of medullary carcinoma, 5 cases (6.25%) of mucinous carcinoma and 2 cases (2.50%) of tubular carcinoma were found in our study.

Table 2: Distribution of cases based on histological types

S. No.	Histological types	No. of cases (n=80)	Percentage (%)
1.	Invasive ductal carcinoma	60	78
2.	Invasive lobular carcinoma	04	05
3.	Invasive papillary carcinoma	02	2.50
4.	Medullary carcinoma	07	8.75
5.	Mucinous carcinoma	05	6.25
6.	Tubular carcinoma	02	2.50

Table 3 shows that in Bloom Richardson grading, 40% of cancer belongs to grade II. Total 35% of cases belong to grade III and 25% of cases belong to grade I.

Table 3: Distribution of cases based on histological grades (Bloom Richardson grade)

S. No.	Histological Grades	No. of cases (n=80)	Percentage (%)
1	BRG-I	20	25
2	BRG-II	32	40
3	BRG-III	28	35

BRG= Bloom Richardson grade

Table 4 shows that Tumor size had 2 to5 cm in 49 cases (61.25%) had tumor size between 2 to 5 cm, 22 cases (27.50%) had tumor size more than 5 cm and 9 cases (11.25%) had tumor size between 1 to 2 cm.

Table 4: Distribution of cases based on tumor size

S. No.	Tumor size (cm)	No. of cases (n=80)	Percentage (%)
1	< 2	09	11.25
2	2–5	49	61.25
3	>5	22	27.50

In Table 5, out of 80 cases of invasive breast carcinoma, a total of 53 cases [27 positive (1-3 nodes) and 26 positives (4 or more)] were lymph node-positive found in our study.

Table 5: Distribution of cases based on lymph node status

| S. No.	Lymph node status	No. of cases (n=80)	Percentage (%)
1	Not identified	17	22.97
2	Negative (0 node)	10	13.51
3	Positive (1-3 nodes)	27	32.43
4	Positive (4 or more)	26	31.08

In Table 6, total of 17 cases showed low Ki-67 immunostaining, 25 cases show high Ki-67 immunostaining whereas, 38 cases show very high Ki-67 immunostaining.

Table 6: Distribution of cases based on Ki-67 immunostaining

S. No.	Ki-67 immunostaining	No. of cases (n=80)	Percentage (%)
1	Low (<10%)	17	21.75
2	High (10–15%)	25	31.25
3	Very high (>15%)	38	47.50
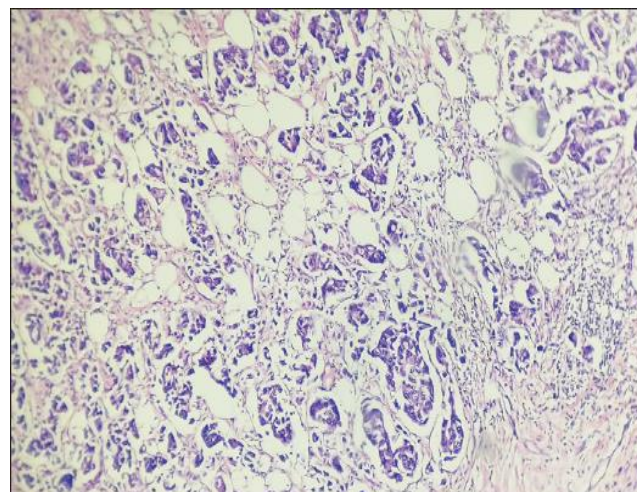
A. (H&E, 10x)
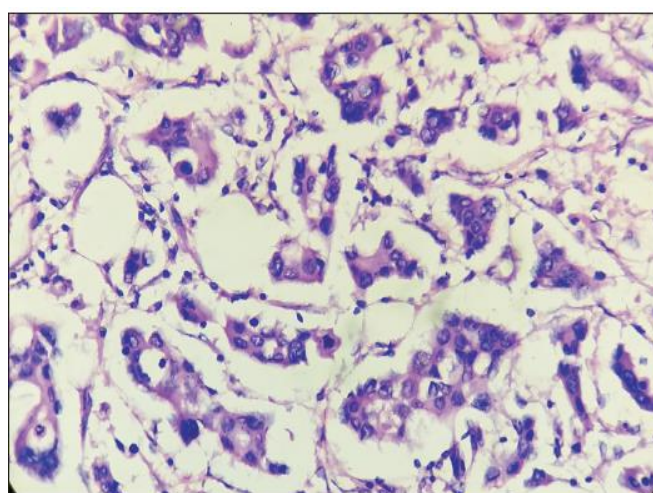
B. (H&E, 40x)

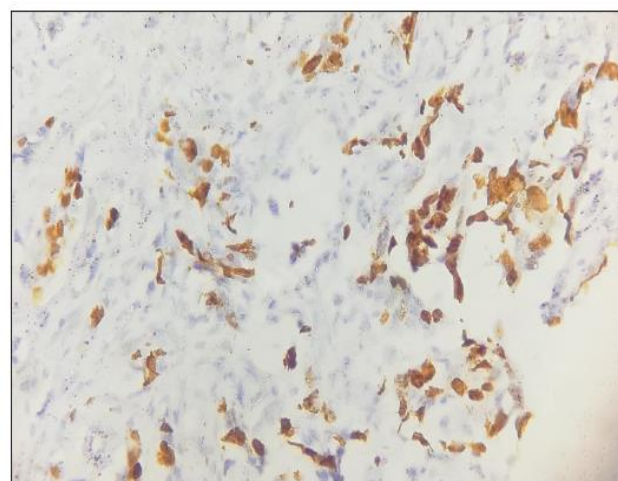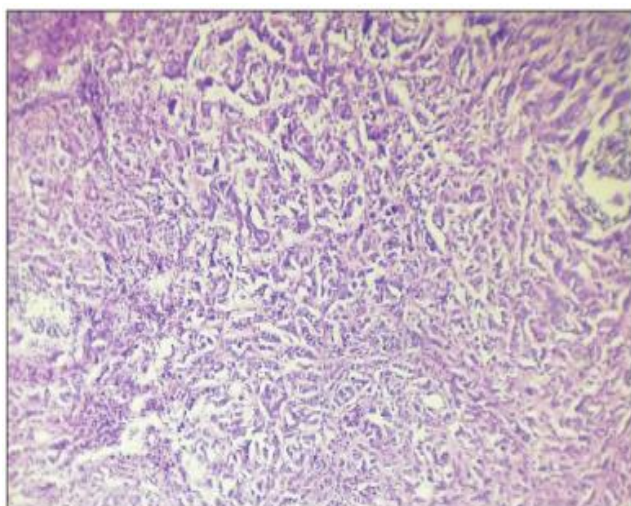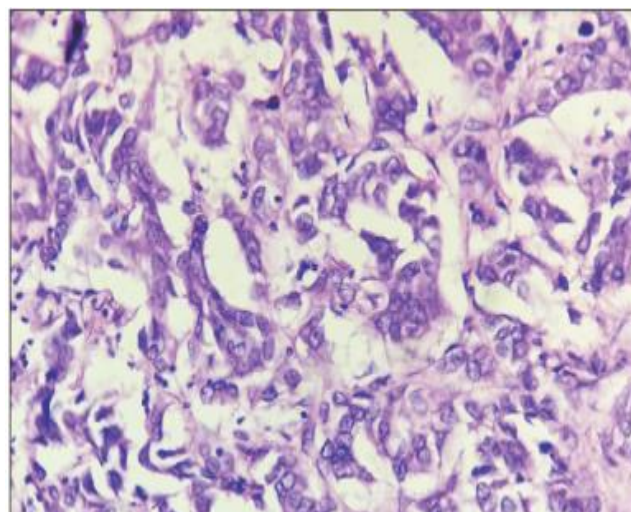**Fig. 1 (A & B):** Infiltrating ductal carcinoma, grade-1

Fig. 2: Infiltrating ductal carcinoma, grade-1 [Immunohistochemical staining of Ki-67 positivity showing low (<10%) proliferative activity (40x)]

A. (H&E, 10x)

B. (H&E, 40x)

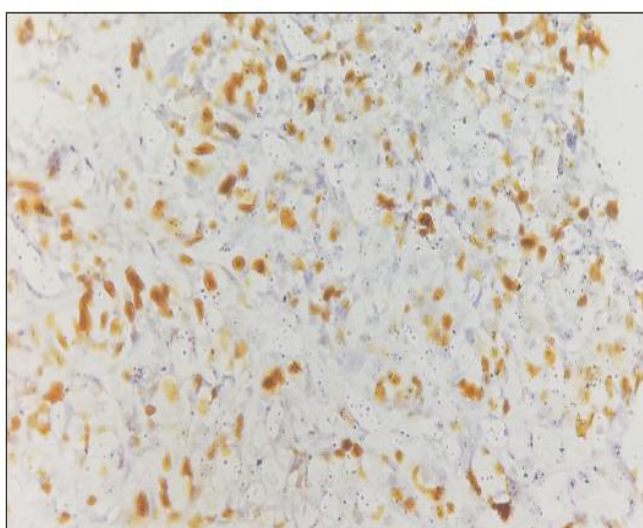**Fig. 3:** (A&B): Infiltrating ductal carcinoma (NOS), Grade 2

Fig 4: Infiltrating ductal carcinoma, grade-2 [Immunohistochemical staining of Ki-67 showing high (10-15%) proliferative activity (40x)]
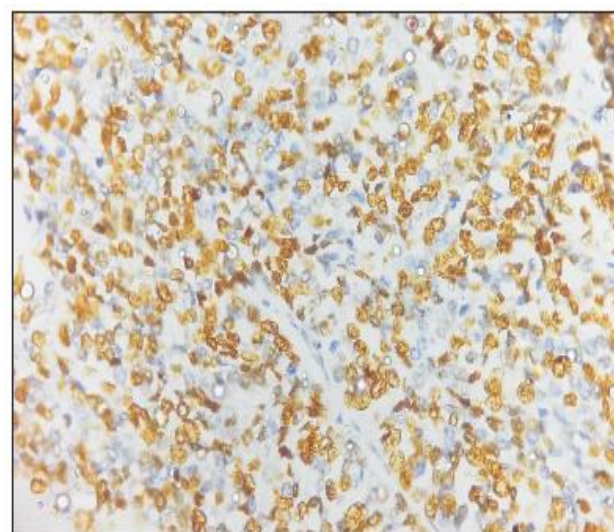

Fig 6: Infiltrating ductal carcinoma (NOS), grade-3 (40x) [Immunohistochemical staining of Ki-67 showing very high (>15%) proliferative activity]
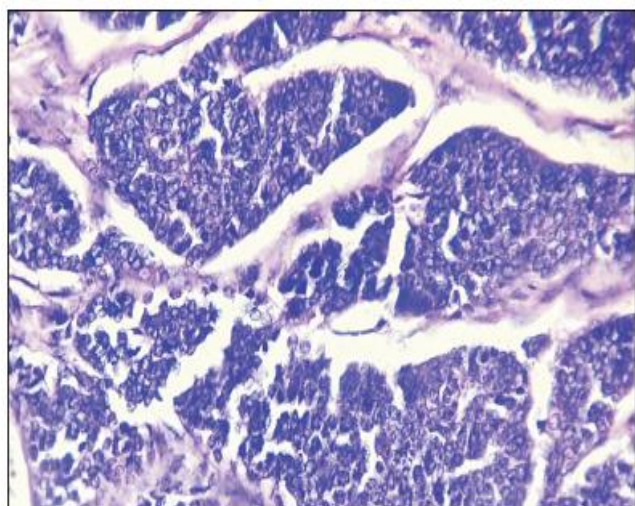

Fig 5: Infiltrating ductal carcinoma (NOS), grade-3 (H&E, 40x)

In Table 7, in grade I, out of 20 cases, maximum 9 numbers of cases had low Ki-67 immunostaining, 8 cases had high and 3 cases had very high Ki-67 immunostaining. In grade II, out of 32 cases, maximum 15 numbers of cases showed very high, 13 cases had high Ki-67 immunostaining and 4 cases shown low Ki-67 immunostaining. In grade III, out of 28 cases, maximum 20 number of cases shown very high Ki-67 immunostaining and 4 cases belonged to each low and high Ki-67 immunostaining.

Table 7: Correlation between grade of tumor and Ki-67 immunostaining

Grade of tumor	No. of cases (n=80)	No. of low Ki-67 immunostaining cases (n=17)	No. of high Ki-67 immunostaining cases (n=25)	No. of very high Ki-67 immunostaining cases (n=38)	Significance
I	20	09	08	03	Chi square (X^2) =
II	32	04	13	15	18.89
III	28	04	04	20	p value= 0.008

Chi-square statistics was used and the value of chi-square was 18.89 and the p-value was 0.008. It showed that there was a significant association between the grade of tumor and Ki-67 immunostaining.

In Table 8, total 53 of cases were lymph-node positive in our study and out of total 53 cases, 9 cases shown low Ki-67 immunostaining, 16 cases shown high Ki-67 immunostaining and 28 cases showed very high Ki-67 immunostaining.

The Chi-square statistics was applied and it shows the value of chi square was 9.48 and the p-value was less than 0.05 so there was a significant association between Ki-67 immunostaining and lymph-node positivity.

Table 8: Correlation between Ki-67 immunostaining and lymph node positive cases

Ki-67 immunostaining	Low (<10%)	High (10-15%)	Very High (>15%)	Significance
No. of lymph node positive cases (total 53 cases)	09	16	28	Chi square (X^2) =9.48 p value less than 0.05

DISCUSSION

This study shows that peak incidence of breast cancer was in the 5th decade, followed by 6th decade and 7th decade, which was similar to previous, studies i.e. Tiwari *et al*. [9]; Nabi *et al*. [10]; and Nggada *et al*. [11]. All of the cases were females, no male case identified, which was similar with another previous study that done by Shet *et al*. [12]; Godwin *et al*. [13]; and Yip [14]. In this study, out of the total 80 cases, 25 were pre-menopausal and rest 55(68.75%) were postmenopausal; this result was similar with other previous studies Surakasula *et al*. [15]; Sebastiani *et al*. [16]; and Hall *et al*. [17]. In this study, the most common histological type was invasive ductal carcinoma (NOS) comprised of 75% of total cases. Out of the total 80% cases, 40% of cases belonged to grade II of Bloom Richardson grading, while 35% and 25% of cases were under grade III and grade I, respectively. This result was similar to other previous studies done by Ayad *et al*. [18]; Soliman *et al*. [19]; Rakha *et al*. [20]; Pierga *et al*. [21]; and Aman *et al*. [22]. In our study, 49 (61.25%) cases have tumor-size 2 to 5 cm, 22(27.50%) cases have tumor, size >5 cm, 9 (11.25%) cases have tumor-size less than 2 cm. Another study also recorded similarly with our study with the majority of patients presenting with tumor-size of 2 to 5 cm by Pinto *et al*. [23]; Cheang *et al*. [24].

In our study, 66.25% of cases have metastatic lymph nodes and half of them are having more than 4 metastatic lymph node, similar studies were observed in the previously published other studies [9,25].

In our study, Grade- 1 consists of total 20 cases out of which the maximum number of cases [9 cases, (45%)] belonged to low Ki-67 immunostaining. Grade-2 comprises of total 32 cases out of which majority of cases had high and very high Ki-67 immunostaining and Grade-3 consists of 28 cases, out of which, maximum cases [20 cases, (71.4%)] had very high Ki-67 immunostaining. There was a significant association between the grade of

tumor and Ki-67 immunostaining (P-value <0.05). Our study was shown that the incidence of lymph node metastasis was higher in high Ki-67 positive groups than low Ki-67 positive groups. Other studies also recorded similar findings by Ragab *et al.* [26]; Madani *et al.* [27]; and Jain *et al.* [28].

CONCLUSIONS

Initially, breast cancer was considered as a single disease; but recent advances in gene expression and genomic profiling have revealed that breast cancer is a collection of diseases expressing distinct anatomical features, responses to treatment and survival. Ki-67 protein is a marker for proliferation. This nuclear protein is expressed in proliferating cells during G1 through M phase but is not detected in resting cells. The Ki-67 immunoreactivity detected by immunohistochemistry was the most reliable indicators of the proliferative activity of cancer cells. It was considered as a biomarker for prognosis and sensitivity of cancer cells to endocrine therapy or chemotherapy.

Breast cancer patients will also be treated by focusing on specific oncogenic pathways, which are activated in a particular tumor rather than the location of the tumor or histological type.

CONTRIBUTION OF AUTHORS

Research concept- Dr. Mahendra Singh, Dr. Swetlana Sachan

Research design- Dr. Swetlana Sachan

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Data collection- Dr. Swetlana Sachan

Data analysis- Dr. Swetlana Sachan

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Final approval- Dr. Mahendra Singh, Dr. Swetlana Sachan

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