Original Research Paper

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Pathology

STUDY OF EXPRESSION OF KI-67 IN INFILTRATING DUCTAL CARCINOMA IN A TERTIARY CARE HEALTH CARE CENTER & ITS CORRELATION WITH CLINICOPATHOLOGICAL PARAMETERS.

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ABSTRACT **BACKGROUND -** Ki-67 antigen is a well known marker of proliferating cells. The current study is undertaken to evaluate the proliferating activity of tumour cells by immunohistochemical analysis of Ki-67 expression and to correlate it with clinico-pathological parameters.

MATERIALS & METHODS: The present prospective study was carried out in the Department of Pathology, Silchar Medical College & Hospital, Silchar from June 2016 to May 2017. A total of 46 mastectomy specimens with or without axillary lymph nodes and diagnosed on histopathological examination as invasive ductal carcinoma were included in the study. The results of the immunohistochemical staining for Ki-67 were evaluated.

RESULTS- The range of Ki-67 expression was from 1%-70%.Out of the total 46 cases, 47.8% had low expression.28.3% cases showed moderate expression and 23.9% cases showed high expression. A statistically significant (p-value<0.05) correlation was seen between Ki-67 expression and various clinicopathological parameters like histological grade, lymph node status, tumour size & menopausal status.

KEYWORDS : Invasive Ductal Carcinoma, Ki-67.

INTRODUCTION :

Breast cancer is the most common female cancer in the world which represents a quarter of all cancers. Breast cancer is the fifth cause of death from cancer overall and it has been seen as the most frequent cause of female cancer death in less developed regions (14.3% of total)[1].

In India, the burden of breast cancer is high and increasing, particularly in the urban areas[2]. The incidence of breast cancer is presumed to increase from 153,297 cases to 235,490 cases during 2011 to 2026[3].

Invasive carcinomas are a heterogeneous group of tumors differentiated into histological subtypes. Among these, infiltrating ductal carcinoma is the most common subtype comprising of 70–80% of all invasive lesions[4]. Due to the fact that breast carcinomas present with a wide spectrum of clinical behaviour and morphologic patterns, several attempts have been made to establish reliable and reproducible prognostic parameters. Histological grading has been found to be an important prognostic indicator that can foretell overall survival for breast carcinoma.

The Nottingham modification of the Bloom- Richardson grading system is the most widely accepted method of tumour grading for breast carcinoma.[5] The breast carcinoma grading was introduced by Greenough in 1925 classifying breast carcinoma into three grades of malignancy[6]. In 1957, Bloom and Richardson[7] put forward a simplified system utilizing only three of Greenough's variables: gland-formation (tubularity), degree of variation in nuclear shape and size (pleomorphism), and hyperchromatic and mitotic figures as an estimate of proliferation. Despite the lack of specific criteria for quantifying variables which were measured, the grading system was effective for prognosis. A correlation of final disease outcome with this grading system has been shown consistently over the years.[8-10]

Ki-67 antigen is a well known marker of proliferating cells[11]. Ki-67 by identifies the actively proliferating cells reflecting the percentage of dividing cells. A correlation has been seen between the positivity of the cells for Ki-67 and the degree of differentiation[12]. The use of Ki-67 as a prognostic and predictive marker in breast cancer has been widely investigated. The assessment of proliferation has been taken into consideration as one of the major factors for deciding treatment in patients of breast carcinoma, in addition to the conventional histopathological parameters. Many studies have shown good correlation between grade and proliferative markers[13].

With this perspective, the current study is undertaken to evaluate the proliferating activity of tumour cells by immunohistochemical analysis of Ki-67 expression and to correlate it with the histological grading according to Nottingham Modification of the Bloom - Richardson grading system in cases of invasive ductal carcinoma of the breast.

AIMS & OBJECTIVES :

1. To study the expression of Ki-67 in the cases of Invasive Ductal Carcinoma of the breast.
2. To correlate the Ki-67 expression with clinicopathological parameters.

MATERIALS & METHODS :

The present study was a prospective study carried out in the Department of Pathology, Silchar Medical College & Hospital, Silchar from June 2016 to May 2017.

A total of 46 mastectomy specimens with or without axillary lymph nodes received in the section of histopathology and diagnosed on histopathological examination as invasive ductal carcinoma were included in the study. Patient's demographic profile and relevant clinical data including age, menopausal status, side of breast involved were obtained from the requisition form and noted in the proforma enclosed.

Female cases diagnosed histopathologically as invasive ductal carcinoma of the breast were included in the study. . Male sex and patients who have received or were receiving pre-operative radiotherapy or chemotherapy or hormonal therapy were excluded from the study.

Histological diagnosis was done according to WHO classification of breast tumours (2012).Grading of tumour was done according to Nottingham Modification of Bloom-Richardson System.

IMMUNOHISTOCHEMICALANALYSIS:

Immunohistochemical staining was done in those diagnosed cases of invasive ductal carcinoma at the Department of Pathology, Silchar Medical College and Hospital, Silchar and the results of the immunohistochemical staining were evaluated.

The immunohistochemical analysis of Ki-67 was conducted following recommendations from the International Ki-67 in Breast Cancer Working Group[14]. The immunohistochemical staining was evaluated in a quantitative and visual way under light microscope in high power fields (40x). To calculate the Ki-67 **percentage score or proliferative index**, the percentage of positively- stained cells, within the total number of malignant cells, were counted from the whole tissue

sections including the hot spots. 'Positive staining' was regarded as the appearance of nuclear staining, of any intensity, within the tumor cells. The scoring was performed by counting 1000 invasive tumor cells in fields that are seen to be representative on an initial overview of the whole section. Hot spots are defined as areas in which Ki 67 staining is particularly prevalent.

The distribution of Ki-67 immunostaining levels were divided into 3 categories- <10% (LOW), 10-25% (MODERATE), >25%(HIGH)[15].

STATISTICAL ANALYSIS-All data were collected, compiled and subjected to suitable statistical analysis. For statistical analysis chi-square test were used. A p-value of less than 0.05 was considered statistically significant.

RESULTS & OBSERVATIONS :

The present study entitled "The study of Ki-67 expression in Invasive Ductal carcinoma of the breast and its correlation with Nottingham modification of the Bloom -Richardson grading system at Silchar Medical College and Hospital" was conducted in the Department of Pathology, Silchar Medical College & Hospital during 1 year study period from June,2016 to May,2017.A total of 46 cases of Invasive Ductal Carcinoma of breast were studied. All the cases were Ductal Carcinoma NST. A detailed study of histopathological features, tumour grading and immunohistochemical evaluation of Ki67 expression and correlation with the grading and other clinico-histopathological parameters was carried out.

In the present study, Ki-67 expression has been categorised as <10% (LOW), 10-25% (MODERATE), >25% (HIGH).

The age ranged from 30-76 years. The mean age was 48±11.84 years. In the present study, range of tumour size was 1cm to 13cm. **Majority of the cases i.e. 27 cases (58.7%) had tumour size between 2-5 cm**. Amongst the total cases, **there was no lymph node involvement in 28 (60.9%) cases.** Out of 46 cases, majority of the **cases were grade 2 tumours.**

A statistically significant correlation was found between Ki67 expression and histological grade, lymph node status, tumour size and menopausal status (p-value<0.05).

Table 1: Age Distribution Of Cases

AGE (IN YRS)	NO. OF CASES	PERCENTAGE
≤40	13	28.2%
41-50	18	39.1%
51-60	8	17.4%
61-70	5	10.9%
>70	2	4.4%
TOTAL	46	100%

Table 2.distribution Of Tumour Size

TUMOUR SIZE(cm)	NO.OF CASES	PERCENTAGE
< 2	7	15.2%
2-5	27	58.7%
>5	12	26.1%
TOTAL	46	100%

Table 3.distribution Of Lymph Node Involvement

No. of lymph nodes involved	No.of cases	Percentage
No lymph node	28	60.9%
1-3 lymph nodes	11	23.9%
>3 lymph nodes	07	15.2%
Total	46	100%

Table 4.distribution Of Histological Grade

GRADE	NO. OF CASES	PERCENTAGE
GRADE 1	09	19.6%
GRADE 2	26	56.5%
GRADE 3	11	23.9%
TOTAL	46	100%

Table 5.distribution Of Expression Of Ki67

Ki67 expression	No. of cases	Percentage
<10% (Low)	13	28.3%
10%-25% (Moderate)	22	47.8%
>25% (High)	11	23.9%
TOTAL	46	100%

Table 6. Distribution Of Menstrual Status

MENOPAUSAL STATUS	NO. OF CASES	PERCENTAGE
PRE MENOPAUSAL	26	56.5
POST MENOPAUSAL	20	43.5
TOTAL	46	100

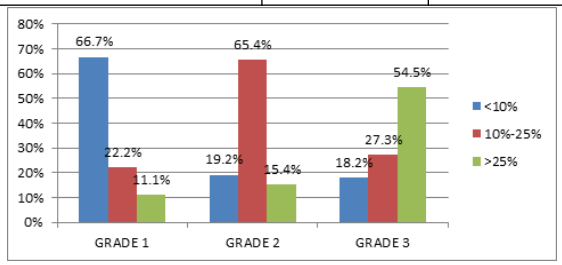

Chart 1.correlation Of Ki-67 Expression With Histological Grade

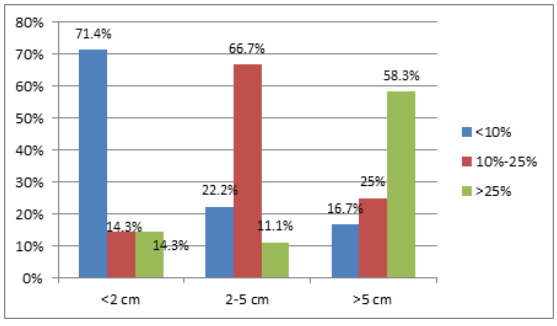

Chart 2.correlation Of Ki-67 Expression With Tumour Size

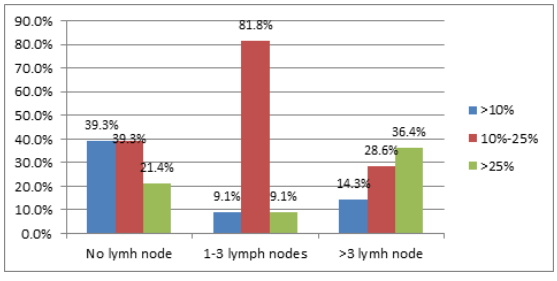

Chart 3.correlation Of Ki-67 Expression With Lymph Node Status

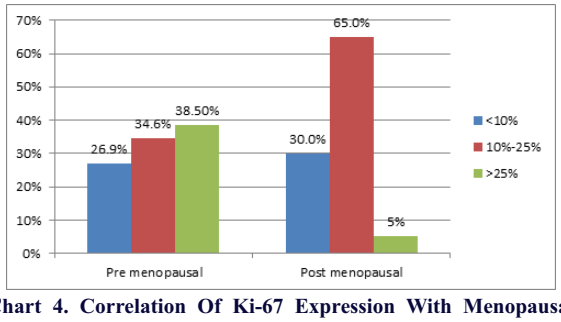

Chart 4. Correlation Of Ki-67 Expression With Menopausal Status

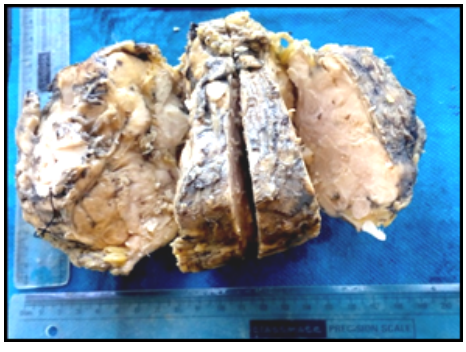

Fig 1: Gross picture IDC NST with a grey-white tumour

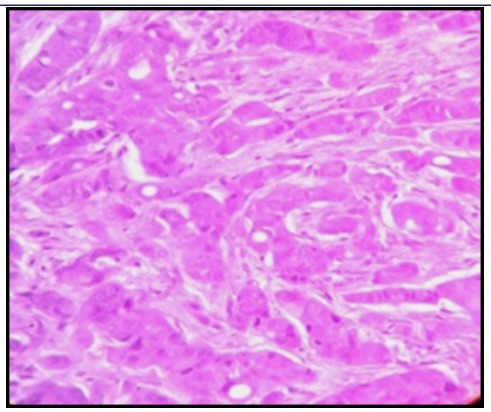

Fig 2: IDC NST Grade 1 (H&E, 400x)

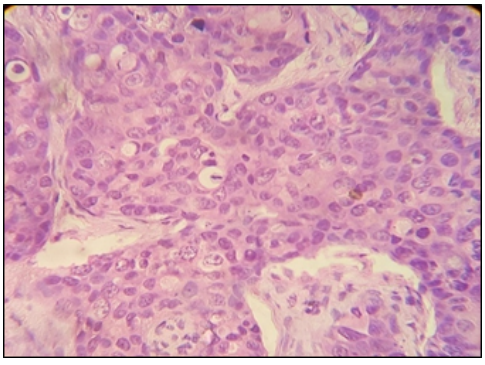

Fig 3: IDC NST Grade 2 (H&E, 400x)

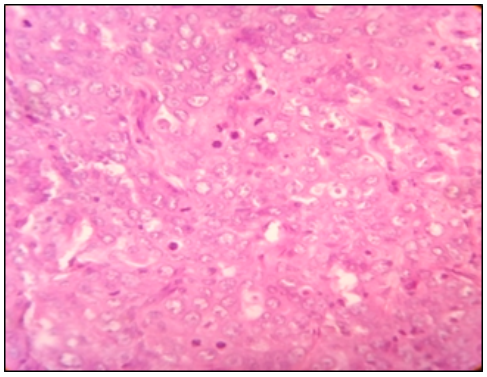

Fig 4: IDC NST Grade 3 (H&E, 400x)

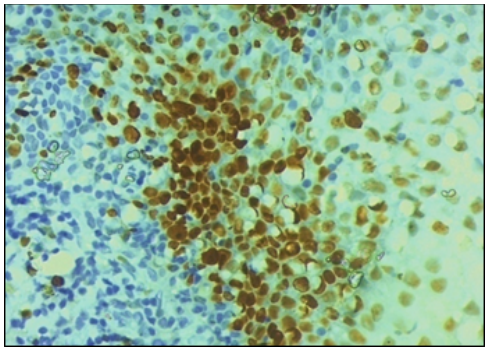

Fig 5 : Ki-67 expression in control (Human Tonsil Tissue) (IHC, 400x)

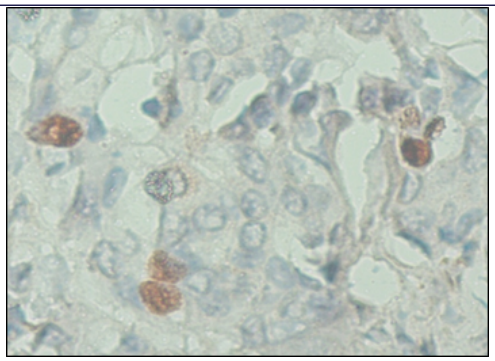

Fig 6 : Ki-67 positivity in IDC NST (<10%) (IHC, 400x)

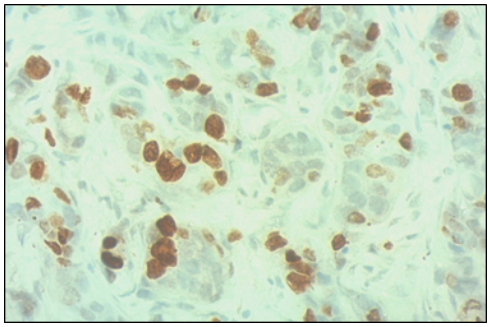

Fig 7 : Ki-67 positivity in IDC NST (10%-25%) (IHC, 400x)

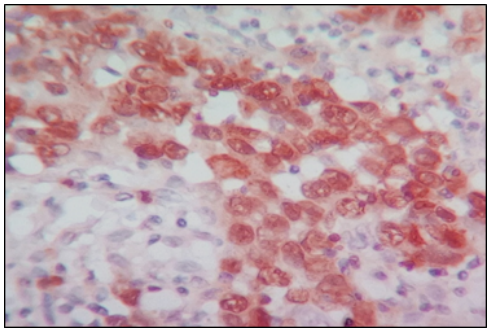

Fig 8 : Ki-67 positivity in IDC NST (>25%) (IHC, 400x)

DISCUSSION :

In the present study, the number of cases less than 50 years of age comprised of 67.3% of total cases and the number of cases above 50 years of age comprised of 32.7% (Table 1) similar to the studies by Madani SH et al[16], Ermiah et al[17],Cho et al[18].

Maximum number of cases in the present study had tumour size more than or equal to 2 cm accounting for 84.8% of total cases and remaining 15.2% cases had size less than 2cm (Table 2) which was in concordance with studies by Madani SH et al[16] & Elkablawy MA et al[19]. Also, 60.9% cases had no lymph node involvement whereas 39.1% cases had lymph node (Table 3) involvement which was similar to studies by Inwald EC et al[20] & Alco G et al[21]. Majority of the cases were premenopausal comprising of 56.5% of the total cases and rest 43.5% cases were postmenopausal which was comparable to study by Ermiah et al[17].

In the present study, 19.6% cases were grade 1 tumour, 56.5% cases were grade 2 and grade 3 comprised of 23.9% of total cases (Table 4) which was comparable to studies by Inwald EC et al[20], Trihia H et al[5] & Madani SH et al[16].

There have been various attempts to set an optimal cut-off value for Ki-67 by previous studies but no standardised cut-off value has

been set. This difference could be attributed to differences of methodology of techniques and to variation in the interpretation in different studies. **There is no existing clear evidence regarding the interpretation and scoring Ki-67 levels.** In the present study, Ki-67 expression has been categorised as <10%(low), 10-25%(moderate) and >25%(high) similar to previous studies done by **Alco G et al[21], Tawfik O et al[15] and Inwald EC et al[20]**.

28.3% of total cases in this study showed <10% expression and 73.1% of total cases showed expression above 10% .Also,23.9% of total cases showed >25% expression (Table 5). This was in concordance with studies by **Alco G et al[21], Tawfik O et al[15] and Inwald EC et al[20]**.

A statistically significant correlation (p<0.05) was found between KI-67 expression and histological grading (Chart 1) which was comparable to studies by Trihia H et al[5], Alco G et al[21] & Nishimura R et al[23]. A higher tumor grade was found to be the most important poor prognostic parameter. Among the histopathological parameters, grading was strongly correlated with Ki-67 expression in all the studies above with which the present study was concordant i.e. a higher grade was associated with a high Ki-67 levels.

Similarly, the Ki-67 expression was found to be strongly correlated (p<0.05) with both lymph node status & menopausal status (Chart 3 &4) similar to studies by Inwald EC et al[20] & Ermiah E et al[17].

A statistically significant correlation(p-value<0.05) was found between KI-67 expression and tumour size of the cases (Chart 2) which was found to be in concordance with studies by Trihia H et al[5] & Nishimura R et al[23].

CONCLUSION :

The rate of tumour proliferation plays an important role in determining the clinical course in invasive breast carcinoma. A strong correlation exists between increased proliferation and poor prognosis.Ki-67 is a well known marker of proliferation that is an easy and reliable means of assessing the growth fraction. Thus it can be used as a valuable tool to determine tumour proliferation.

The parameters like histological grade, tumour size and lymph node status guide the prognosis and management of breast carcinoma. A correlation has been found between Ki-67 expression and tumour grade, tumour size and lymph node status. Thus, Ki-67 proliferative index can prove valuable in making decision regarding patient therapy.

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