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Original Research Article

Clinicopathological Study and Immunohistochemical Expression of Ki-67 Among Breast Tumors in A Tertiary Care Centre

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Conflict of interest: Nil

Abstract

Introduction: Breast, an anatomical site is constantly under the varying influence of sex hormones and it is one of the frequent sites of neoplasms in the human body. Breast cancer is the leading cause of death in women aged 15–54 years worldwide. Apart from clinical parameters, molecular markers like ER, PR, HER 2 neu and Ki-67 which is a cell proliferative index (PI) are targets or indicators of highly effective therapies against invasive breast cancer. Accurate assessment is essential and mandatory.

Aim: The present study was intended to know the frequency of occurrence of various breast tumors in relevance with the age and their histopathological features. The Ki-67 (PI) was calculated for various breast tumors.

Methodology: An observational study was conducted by light microscopy and immunohistochemical examination of 340 lumpectomy and mastectomy specimens at the Department of Pathology over a period of 2 years between July 2017 – June 2019.Statistical evaluation was done using unpaired t-test.

Results: Benign tumors are more common than malignant tumors accounting for 61% and 33% cases respectively. Most common age group for benign tumors is 21-30 years whereas for malignant tumors the age range is 51-60 years. Most common benign tumor is fibroadenoma (FA) followed by benign phyllodes tumor. The precursor lesions that encountered are atypical ductal hyperplasia (ADH) and ductal carcinoma in situ (DCIS). The common histological type of breast carcinoma was invasive breast carcinoma –no special type (NST) accounting for 90.2% cases. The mean Ki-67 PI of conventional fibroadenoma and fibroadenoma with focal phyllodes like areas is lower than cellular fibroadenoma and benign phyllodes tumor. The mean Ki-67 PI of ADH was lower than DCIS. There is increase in the mean Ki-67 PI with increase in the grade of the tumor. The overall mean Ki-67 PI of benign tumors are lower than precursor lesions and malignant tumor.

Conclusion: In the present study evaluation of Ki-67 helped us to know the variability in the proliferation rates among various breast tumors. Thus, analysis of Ki-67 expression may be useful in clinical practice and when used along with hormone receptor status.

Keywords: Breast tumors, Ki-67 proliferative index, invasive breast carcinoma no special type, Immunohistochemistry.

Introduction

The breast is distinguished from other organs by three important characteristics. First, its major function is to provide for the nutritional support and survival of another individual, the infant. Second, it undergoes dynamic structural changes throughout life. Thirdly major hormonal changes occur during adolescent age and reproductive ages, which produce alterations in mammary tissue and this directly or indirectly affects the disease patterns.

Breast tumors constitute most important lesions of the breast. These include tumors arising from glandular structures, stratified squamous epithelium and mesenchymal connective tissue[1,2,3]. Benign tumors of the breast [4,5,6] are more common than malignant tumors and they are completely curable. But these are overshadowed by magnitude of the problems of malignant tumors of breast.

Breast carcinoma is the most common malignant tumor and the second most common cause of carcinoma death in women, with more than 1.7 million cases occurring worldwide annually[7,8].

The usual treatment for carcinoma is surgery by modified radical mastectomy. Prognostic information is important in counselling patients about the likely outcome of their disease and planning further management. Apart from clinical parameters like age, menopausal status, and disease presentation, important prognostic indicators in histopathology are tumor size and extent, histological type, grade, and lymph node status. In addition, there are other factors which not only are predictive of outcome, but also direct therapies against particular molecular targets. Some of these factors are: ER, PR, HER2/neu & Proliferative rate. In addition to mitotic counts as part of histological grading, proliferation can be measured by IHC detection of cellular proteins produced during cell cycle e.g. Ki-67. Carcinomas with high proliferation rates have a poor prognosis but may respond better to chemotherapy[7].

An attempt has been made to study the various morphological types of benign, precursor and malignant tumors, to evaluate the Ki-67 expression as a proliferation marker among fibroepithelial tumors, precursor lesions and malignant tumors, and to study Ki-67 expression in benign, borderline, and malignant phyllodes tumors. Also, effort has been done to correlate Ki-67 expression with age, histological grade, and lymph node status of invasive breast carcinoma- no special type. (NST)

Materials and Methods:

This is a hospital based observational study performed on specimens received to Department of Pathology, for a period of 2 years from July 2017 to June 2019. For specimen fixation 10% formal saline was used. Clinical data was recorded as per the protocol. Specimens were thoroughly grossed as per standard protocols and representative sections were routinely processed and stained with H&E.

Classification of Breast tumors was done as per WHO 2012 classification. For grading of malignant tumors Modified Bloom Richardson grading was used. Ki-67 immunohistochemistry was done using poly excel HRP/DAB detection system proliferative index was calculated by number of positive nuclei per1000 cells counted in hot spot areas, under 400x and

mean calculated. Statistical analysis was done using SPSS version17. The data for Ki-67 proliferative index was expressed as mean standard deviation. Unpaired test was applied to find the statistical difference of means between benign precursor and malignant lesions.

The analysis of variance (ANOVA) evaluated the relationship between the mean score of Ki-67 as a continuous variable with clinicopathologic factors. P value <0.05 was considered statistically significant.

We have included all excisional biopsies and mastectomy specimens received during the study period and excluded all inadequate and improperly fixed specimens, non-neoplastic, inflammatory, and recurrent lesions, male breast lesions, Post chemotherapy and post radiotherapy cases & trucut biopsies.

Observations And Results:

In our study, benign breast tumors were common accounting for 61.47% followed by malignant tumors about 33.8% (Table 1).

Table1: Spectrum Of Breast Tumors (N= 340)

Histological Type	No. of cases(n=340)	Percentage (%)
Benign	209	61.47%
Precursor lesions and borderline lesions	16	4.05%
Malignant	115	33.8%

Most of the tumors are seen between the age groups of 21-30 years (26.17%) followed by 31-40 years (23.2%). The most common age rage for benign tumors is between 21-30 years and malignant tumors are most common between the age groups of 51-60 years**.**

Among the benign tumors most common histological type is fibroadenoma accounting for 92.8% followed by phyllodes tumor (4.30%), tubular adenoma (1.91%) and lactating adenoma (0.95%). The fibroadenomas are subtyped into conventional, cellular, and focal phyllodes like areas of which conventional fibroadenomas predominate accounting for 92.78%.

Among the phyllodes tumors most common is benign phyllodes accounting for 69.2% followed by borderline (15.38%) and malignant phyllodes tumor (15.38%). The most common histological type of breast carcinoma was Invasive carcinoma no special type (NST), 102 (90.26%) patients out of total 113 had IDC NST type followed by invasive lobular carcinoma (4.42%). The remaining are medullary and mucinous carcinoma equally distributed.

Out of 113 invasive carcinomas, (63 patients out of 113) 55.75% presented with lymph node metastasis. 64.60% (73 out of 113 patients) had tumor size between 2-5cm. Whereas 18.58% had tumor size<2cm and 7.96% had tumor size>5cm. 63(55,75%) out of 113 patients had lymph vascular invasion.

Table 2: Ki-67 Proliferative indices in Fibroepithelial Tumors

Histological Type		No. Of Cases (N=38)	Ki-67 Stroma (Mean±SD)	Pvalue	Ki-67 Epithelium (Mean±SD)	P value
FIBROADENOMA	Conventional FA	11	0.64 ±0.50	Not Significant <0.05	3.82 ±0.75	Not Significant
	FA With Focal Phyllodes Like Areas	5	0.80 ±0.84		3.80 ±0.45	
	Cellular FA	9	4.22±0.67		4.11 ±0.93	
PHYLLODES	Benign	9	3.56±0.53	<0.05	4.22 ±0.83	Not Significant
	Borderline	2	6.13±1.32		4.11±0.10	
	Malignant	2	15.32±0.84		4.12±0.88	

The mean stromal Ki-67 proliferative index of conventional fibroadenomas (figure - 1,2) and fibroadenomas with phyllodes like areas (figure - 5,6) lower than the cellular fibroadenoma (figure - 3,4) and benign phyllodes tumor and the P value is significant (0.005). The mean stromal Ki-67 proliferative index of benign phyllodes tumor (figure - 7,8) is lower than malignant phyllodes (figure - 9,10) tumor and the P value is significant (0.002).

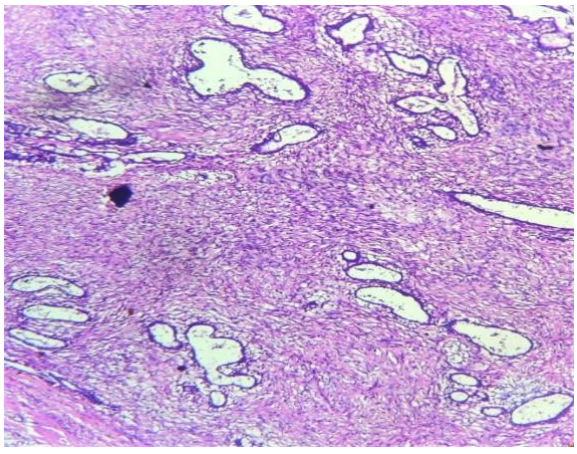

Figure 1: Photomicrograph of conventional peri canalicular FA showing glands and stroma, with regular round to oval glands (H&E,100x).

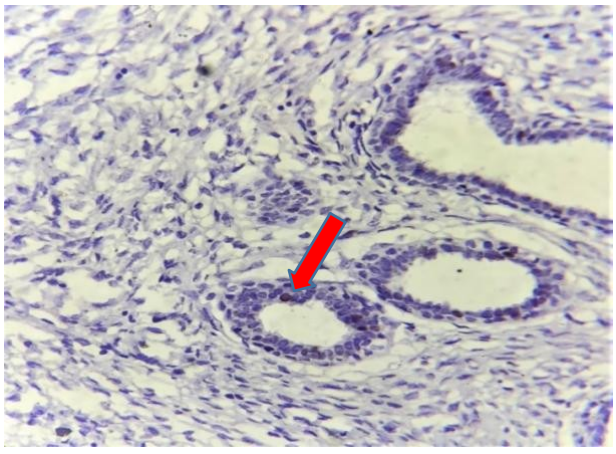

Figure 2: Ki-67IHCimage of Conventional FA a) glands-4% b) stroma- <1% (IHC,400x).

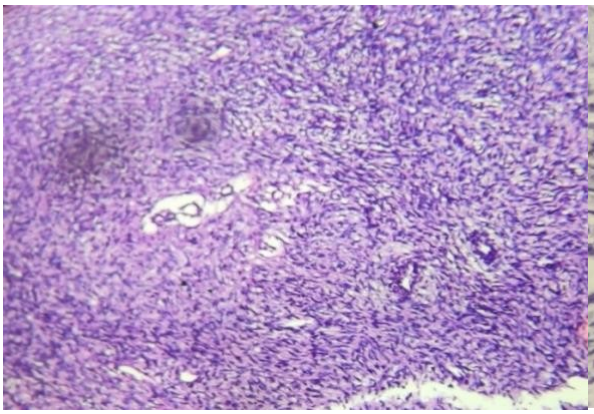

Figure 3: Photomicrograph of Cellular Fibroadenoma showing stromal overgrowth (H&E,100x)

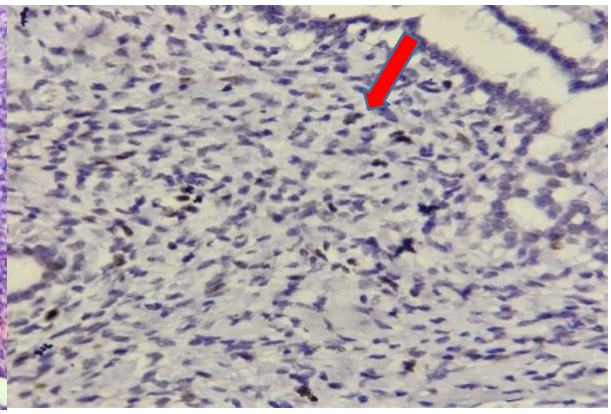

Figure 4: Ki-67 IHC image of Cellular FA a) glands-4% b) stroma–4% (IHC,400x).

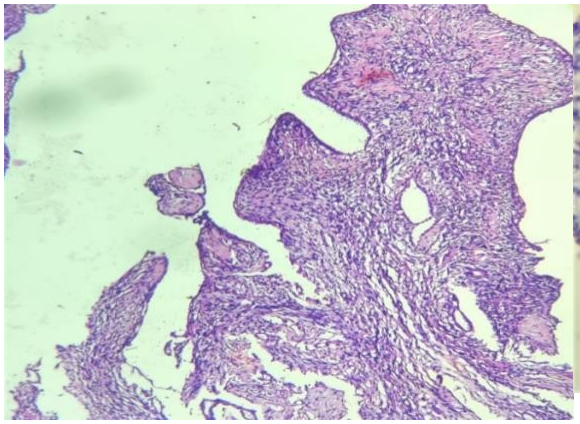

Figure 5: Photomicrograph of FA with focal Phyllodes like areas (H&E,100x).

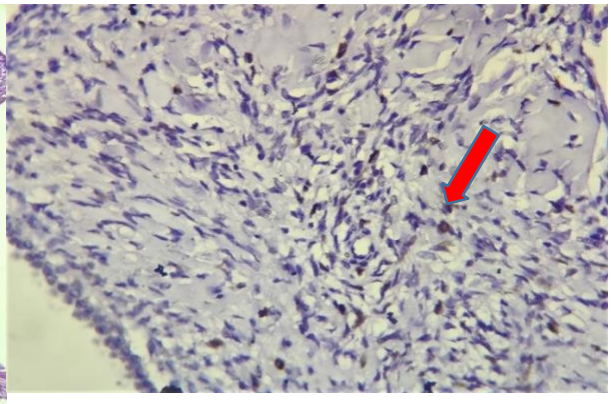

Figure 6: Ki-67 IHC image of FA with focal Phyllodes like areas a) glands - 2% b) stroma -2% (IHC,400x).

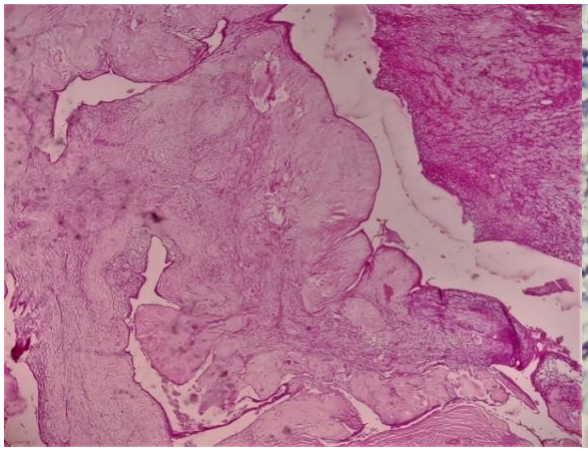

Figure 7: Photomicrograph of Benign Phyllodes tumor with stromal hypercellularity and cleft like spaces (H&E,100x).

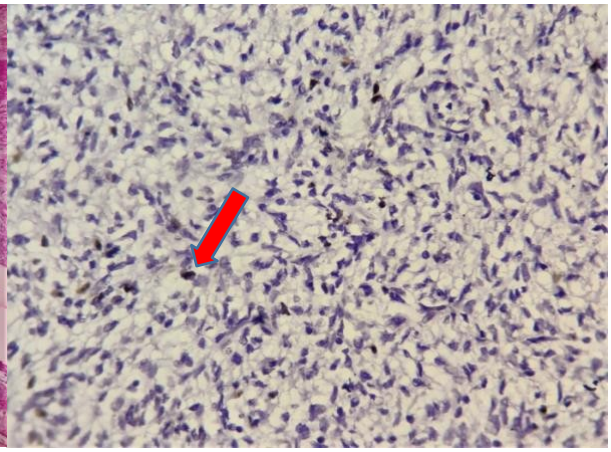

Figure 8: Ki-67 IHC image of Benign Phyllodes tumor a) glands-4% b) stroma- 5%(IHC,400x).

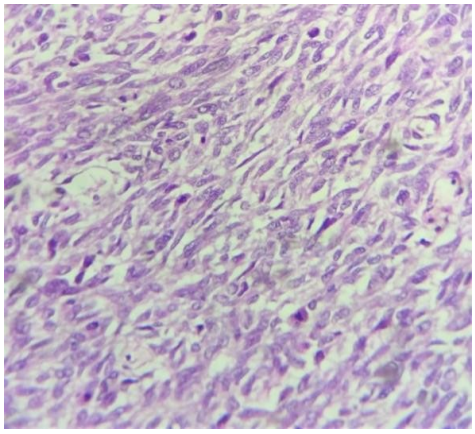

Figure 9: Photomicrograph of Malignant Phyllodes tumor showing stromal hypercellularity with atypia. A typical mitosis seen (H&E,400x)

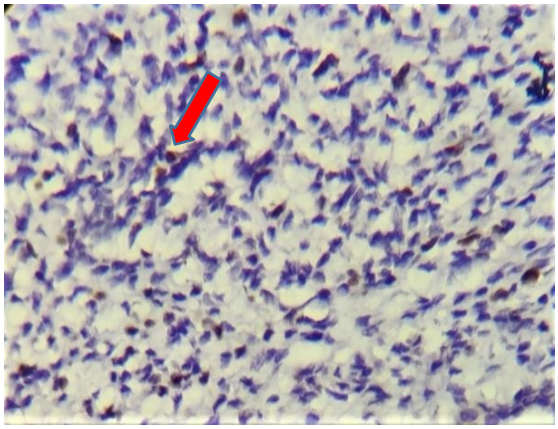

Figure 10: Ki-67 IHC image Malignant Phyllodes tumor, stroma-15%(IHC,400x)

The Ki-67 expression in Atypical ductal hyperplasia (figure - 11,12) is lower than ductal carcinoma in situ (13,14). The mean Ki-67 PI of ADH is 6.92% whereas the mean Ki-67 PI of DCIS is 13.6% and the P value is significant (0.002).

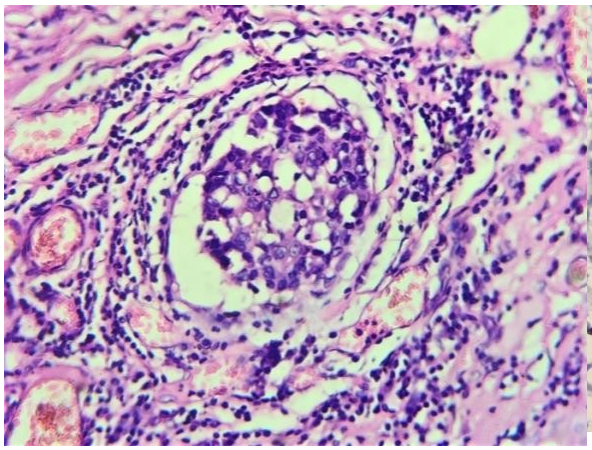

Figure 11: Photomicrograph of ADH showing monomorphic cells and cribriform spaces(H&E,400x)

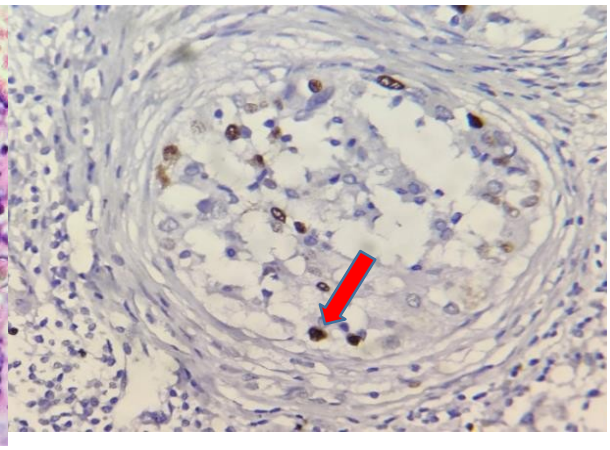

Figure Fig 12: Ki-67 IHC image of ADH showing 6% positivity, (IHC,400x)

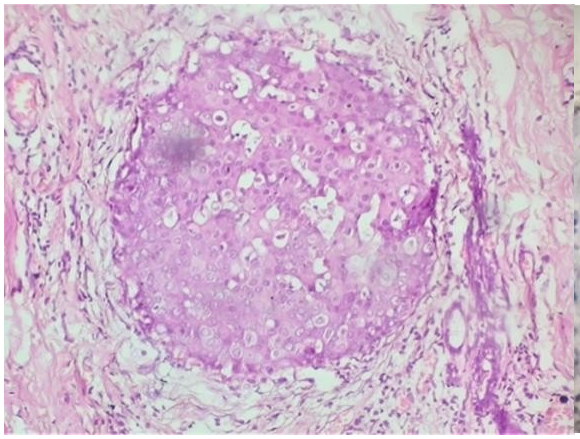

Figure 13: Photomicrograph of DCIS with monomorphic cells almost filling the duct space. (H&E,400x)

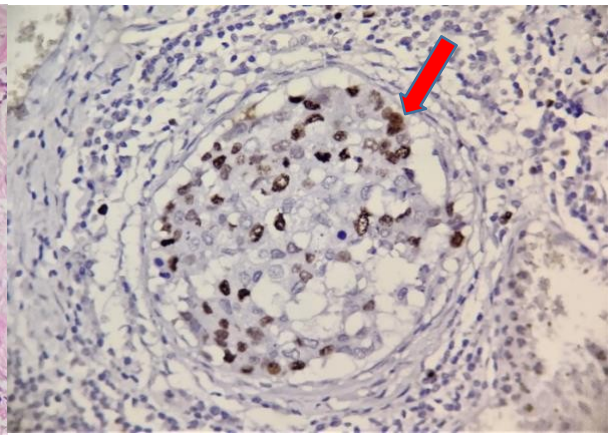

Figure 14: Ki-67 IHC image of DCISshowing15% positivity (IHC,400x)

Table 3: Comparison of Mean Ki-67 PI Among Fibroepithelial Tumors, Precursor Lesions And Malignant Tumors

Breast Tumors	Ki-67 PI (mean ± SD)	P-value
Fibroepithelial tumors	4 ±0.76	< 0.005
Precursor lesions	11.71±3.63	
Malignant lesions	27.41 ±10.86	

In the present study the overall mean Ki-67 PI of benign tumors is less than the precursor lesions which is further lower than malignant tumors and the P value is significant (< 0.005) (Table 3). As the grade and tumor size increases Ki-67 increased (Figure 15 to Figure 20).(Table 4)

Table 4: Comparison Of Ki-67 Proliferative Indices With Various Parameters Of Invasive Carcinoma Nst Type

Parameters	No. of cases(n=48)	Ki-67 PI(Mean±SD)	P value
Menopausal status			
Premenopausal	15	29.00±11.76	Not significant
Postmenopausal	33	21.33 ±6.75	
Tumor grade			
Grade1	16	17.19 ±2.29	<0.001
Grade2	16	24.69 ±5.51	
Grade3	16	40.38 ±6.60	
Tumor size			<0.001
<2cm	7	18.00 ±4.80	
5cm	33	18.43 ±4.47	
>5cm	8	36.71 ±10.32	
Lymph node metastasis			
Negative	21	21.67 ±8.65	<0.002
Positive	27	29.76 ±9.80	
Lymphovascular invasion			
Absent	21	20.95 ±7.43	<0.002
Present	27	29.43 ±10.12	

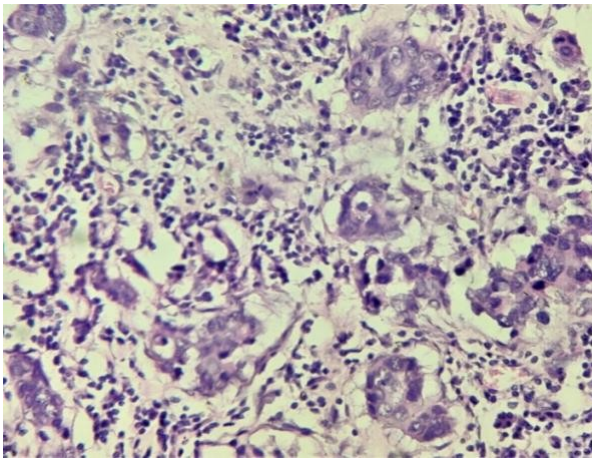

Figure 15: Photomicrograph of Grade 1 Invasive Breast Carcinoma showing pleomorphic cells arranged in tubular pattern. (H&E,400x)

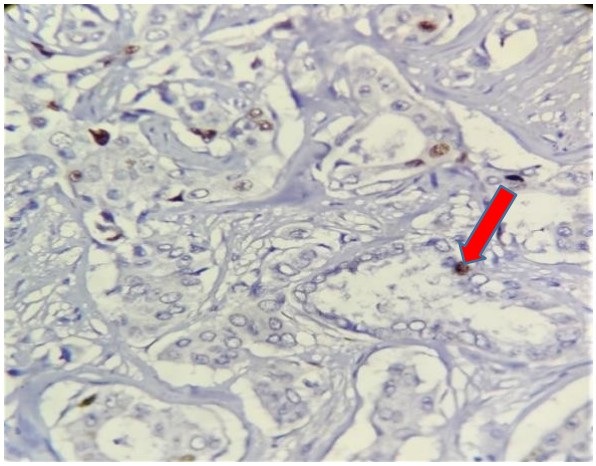

Figure 16: Ki-67 IHC image of Grade 1IDC-NST showing 21% positivity (IHC,400x)

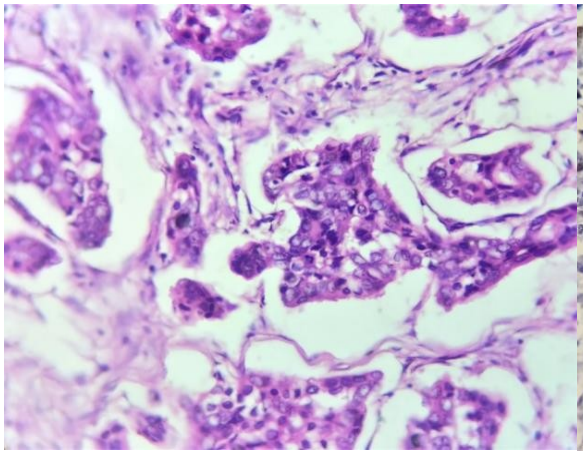

Figure 17: Photomicrograph of Grade 2 invasive Breast Carcinoma showing pleomorphic cells arranged in nests and tubules. (H&E,400x)

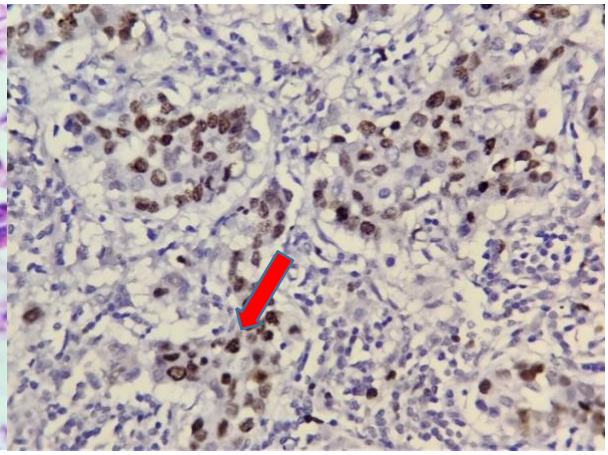

Figure 18: Ki-67 IHC image of Grade 2 IDC-NST showing 39% positivity (IHC,400x)

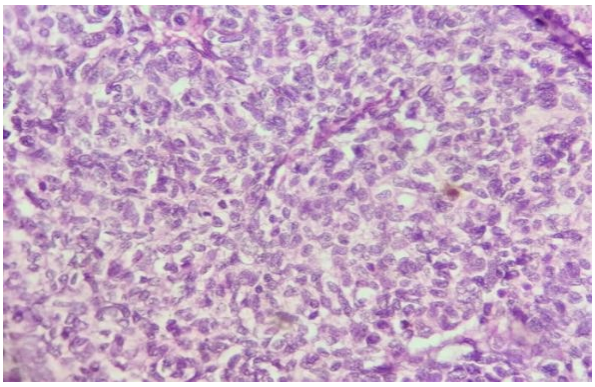

Fig 19: Photomicrograph of Grade 3 invasive Breast Carcinoma showing pleomorphic cells arranged in sheets (H&E,400x).

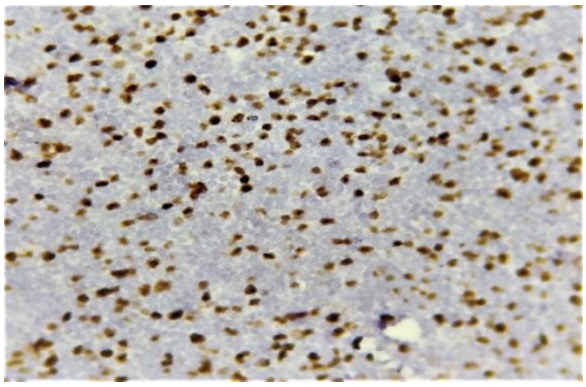

Fig 20: Ki-67 IHC image of Grade 3 IDC-NST showing 48% positivity (IHC,400x)

Discussion

Breast pathology encompasses a wide range of benign, precursor and malignant lesions.[1] Even though benign tumors outnumber the carcinomas in most of the cases, incidence of carcinoma in female breast is so common that every lump in the breast is to be regarded as a possible carcinoma until proven otherwise. Ki-67 is a nuclear antigen, which exists in proliferative cells. Several studies have shown that the immune response of Ki-67 is closely associated with the cell cycle. Uncontrolled proliferation and increased expression of Ki-67 is a key characteristic of malignant tumors and, therefore, tumor proliferation is one of the major factors associated with prognosis.

Fibroepithelial tumors:

In the present study fibroadenoma is the most common tumor and it is the most common benign tumor accounting for 92.8%. Stone et al. [8] reported 71.1% of the lumps as Fibroadenomas. The present study correlated well with the study done by Stone et al. [8].

Fibroadenomas with hypercellular stroma and those with focal phyllodes like areas are often difficult to distinguish from benign PT, and there are some PT with foci that are indistinguishable from FA. Fibroadenomas with hypercellular stroma may be considered to be cellular variant fibroadenoma, and these cases have stromal cellularity values that are equivalent to PT. It is important to discriminate between PT and FA because the malignant potential of PT is higher than that of FA.

In our study, we did not find any statistically significant difference in the proliferative activity between cellular FA and benign phyllodes tumor and also between conventional fibroadenoma and fibroadenoma with focal phyllodes like areas. But there was statistically significant difference in the Ki-67 PI between conventional fibroadenoma and fibroadenoma with focal phyllodes like areas from cellular fibroadenoma and benign phyllodes tumor which correlated with the study done by Umekitha et al. [13]. In contrast Kocova et al. [11] found that there was a statistically significant difference in MIB1 indices of benign PT and all subtypes of FAs.

Phyllodes Tumor (PT):

In the present study, among 13 cases of phyllodes tumor encountered, 9 were benign, 2 were borderline and 2 were malignant which means majority were benign phyllodes tumor which is comparable with the study done by Rajan

PB et al [10] and Moffat CJC et al. [9].

In our study, we investigated the role of Ki-67 in the grading of PT. Ki-67 expression increased with increase in the grade of tumor in phyllodes tumors which was well correlated with the studies done by Kucuk et. al. [12], Kocova et al. [11] and Umekitha et al. [13] Ki-67 expression in the epithelial component did not show a significant difference between the subgroups in the present study which was also well correlated with the studies done by Kucuk et al. [12] and Kocova et al.[11]

Precursor Lesions:

In a study done by Zhou et al. [31], on 56 cases of precursor lesions 4 cases are of ADH and 26 cases are of DCIS. The mean Ki-67 proliferative value of ADH is 7.2% and of DCIS is 11.3%. Hence there is increase in the Ki-67 proliferation with increase in the histological grade of precursor lesions.

The present study also showed increase in the proliferative index from ADH to DCIS.

Malignant Tumors:

In the present study IDC (NST) is the most common histological type, which was correlated with other studies like Priti Lal et al. [14] & Mehrdal Nadiji et al. [18]. Majority of the malignant tumors occurred in the left breast, which is comparable with the study done by Samir S et al. [16]. In the present work lymph node metastasis was seen in 63 out of 113 cases accounting for 55.7%, correlated with study done by Lakshmi K.B. Mudduwa[15].

In the current work Grade 2 tumors predominated, followed by Grade 1 and 3 which was correlated with other studies like TB Pathak et al. [19] & Adedayo A. et al. [17] but did not correlate with the study done by Lakshmi K.B. Mudduwa[15] where grade 3 tumors predominate over grade-1 and grade-2 tumors. Lymphovascular invasion was noted in a greater number of cases in the present study when compared to other studies Zafrani B et al. [32] Peiro G et al. [33].

In this study Ki-67 expression and its association with tumor size, lymph node stage and Modified Bloom Richardson grading was statistically significant with P value <0.005. Similar findings were noted in Mohammad A et al.[21], Shervin et al.[22], Ayodeji et al.[23] Ki-67 expression in our study was not significantly associated with age which is similar to many studies in literature, while others have demonstrated strong associations of Ki-67 with all clinicopathologic parameters in a large study population (Inwald et al.[24] Park et al.[25], Wang et al.[26]).

The more the tumor grade increases, the higher the Ki-67. Our result corroborates with several studies like Haroon et al. [20], Inwald et al. [24], Sun et al. [27], Puay-Hoontan et al. [28]; Offersen BV et al. [30] and Fitzgibbons PL et al. [29]. Tumors with high Ki-67 scores are clinically more aggressive.

Due to the limited period study, we could not follow up all the patients as it is a hospital-based study and sample selected is not representative of general population. Ki-67 IHC has to be combined with ER, PR and Her 2 neu expression for better results.

Conclusion

To conclude, evaluation of Ki-67 proliferative index in various breast tumors i.e. fibroepithelial tumors, precursor lesions and malignant tumors helped us to know the variability in the proliferation rates and a significant correlation was identified between Ki-67and tumor size, lymph node status, tumor grade in invasive breast carcinoma, which indicates that Ki-67 presents an important biomarker. Thus, analysis of Ki-67 expression may be useful in clinical practice and when used along with hormone receptor status may present an option for the personalized treatment of Breast cancer patients.

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