

Immunohistochemical studies (ER & Ki-67) in Proliferative breast lesions adjacent to malignancy

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Abstract: Breast carcinoma is the second most frequently occurring malignant tumor and the leading cause of cancer death in women. Breast carcinomas usually arise in a multistep fashion from intermediary lesions to invasive cancer. The presence of different lesions within the same breast adjacent to malignancy is a phenomenon that can prove the multistep progression to malignancy (1,3). The aim of this study was to identify the predominantly occurring lesion adjacent to malignancy and study the histological appearance, Proliferative Ki-67 index (MIB-1 index) and Estrogen receptor (ER) status in such lesions and substantiate their possible identity as a premalignant lesion in that particular case. Adjacent lesions were categorized as non proliferative lesions, proliferative lesions with and without atypia and DCIS. It was observed that the predominant lesions occurring adjacent to malignancy were proliferative lesions with and without atypia. ER and Ki-67 values were high in those cases with adjacent lesions with atypia. The high positivity of ER and Ki 67 in the adjacent proliferative lesions with atypia hence proves that these could have been the precursors which had progressed to malignancy in these cases.

Hence finding of lesions in benign breast biopsies showing a high proliferative rate and ER positivity necessitates regular follow up of these patients and helps in identifying early progression of these lesions to malignancy. Initiating therapy to these patients at an earlier stage can reduce the incidence of breast cancer.

Key Words: Breast carcinoma, proliferative breast lesions adjacent to malignancy, Estrogen Receptor status, Proliferative index

I. Introduction

Breast carcinoma is the second most commonly occurring solid epithelial tumors in women next to cancer cervix. In our Department of Pathology, Stanley Medical College we received a total of 18,968 cases for histopathological examination during Jan 2006 to Sep 2008 and 694 of those cases were breast lesions. Benign breast lesions constituted about 482 cases and about 212 cases were malignant.

Breast carcinomas usually arise in a multistep fashion from intermediary lesions to invasive cancer (1,3). The identification of premalignant lesions hence play a very important role in preventing progression to malignancy.

The presence of different lesions within the same breast adjacent to malignancy is a usual phenomenon proving the multistep progression to malignancy (1,3). Hence identifying such predominantly occurring lesions adjacent to malignancy and studying of Ki-67 proliferative index (MIB-1 index) and Estrogen receptor (ER) status in such lesions could substantiate their possible identity as premalignant lesion in that particular case.

This work aims at histologically identifying such high risk lesions occurring adjacent to malignancy and confirming their risk status by MIB 1 index and ER status. The association between ER positivity and Ki 67 index has also been studied in this work to establish that they correlate proportionately. The study tries to establish the lesions which have the potential for progression to overt malignancy, thereby indicating early identification and appropriate treatment to reduce the incidence of breast malignancies.

II. Materials And Methods

The total breast specimens received during the study period of two and a half years from 2006 July to 2008 December was 694, out of which benign lesions were 482 and malignant lesions constituted 212. 50 cases of Radical and simple mastectomy proved histologically malignant were selected at random for this study. Sampling was done from all four quadrants surrounding the malignant lesion and the tissues were subjected to routine processing and stained with Hematoxylin & Eosin. Slides from the nearest point where tumor infiltration was not seen were studied and the lesions were classified morphologically (Table: 1).

Based on the predominant lesions present the lesions were classified as

mild - <25%,

moderate - 26-50%, and

florid - >50%

20 cases representing lesions from all the above categories were selected from the total of 50 cases for study of ER and Ki-67 status.

Ki67

MIB 1 labelling indices were estimated as a percentage of positively stained nuclei of atleast 100 cells counted in three or more fields of a given lesion or foci at a magnification of 400x . The cut off value to define High Ki 67 proliferation index was set at 30% (3).

RESULTS

- Category A – Benign non proliferative lesions – 19 cases
- Category B – Proliferative lesions without atypia – 22 cases
- Category C – Proliferative lesions with atypia and in situ changes – 9 cases

Most frequently (about 60%) occurring lesions adjacent to malignancy was usual ductal hyperplasia (Table 3)

III. Ihc Results

A total of 20 cases were selected for immunohistochemical studies -5 cases which showed non-proliferative lesions , 8 cases which showed proliferative lesions without atypia and 7 cases which showed atypia .

ER VALUES

ER positivity was found in 9 cases out of 20. Proliferative lesions with atypia including in situ lesion showed increased ER (Fig 4) and majority of the proliferative lesions without atypia showed ER negativity and few showed ER positivity (Fig 3).

Ki-67 VALUES

Ki-67 positivity was found in 7 cases of proliferative lesions with atypia and 8 cases of proliferative lesions without atypia (Fig 5). The proliferative index values are very high in case of lesions belonging to Atypical ductal hyperplasia (Fig 6) and DCIS.

IV. Discussion

The incidence and mortality due to breast malignancy are high in women. Breast carcinomas arise in a multistep fashion through a series of intermediate lesions to invasive cancer and hence the identification of premalignant lesions involved in the development of breast cancer becomes very essential.

An attempt was made here to ascertain the association between these adjacent changes and the malignant lesion. This study also tried to substantiate that these changes could be the probable premalignant lesion in these cases. The adjacent changes were studied and placed under three categories based on the predominant lesion seen by grading the lesions as mild, moderate and florid.

Category A – Benign non proliferative lesions

Category B – Proliferative lesions without atypia (Fig 1)

Category C – Proliferative lesions with atypia (Fig 2) and in situ changes.

On analyzing the results it was found that the lesion occurring predominantly adjacent to malignancy was Usual Ductal Hyperplasia.

Proliferative index and ER status were also studied in these cases. These studies have recently received increased attention as increased proliferative index signifies the lesion is a fertile soil for future malignancy. Estrogens are mitogenic to breast epithelial cells and the resulting proliferation increases the probability of mistakes made in the DNA replication and setting these mistakes as mutations.

In the current study of Ki 67 positivity was found to be high in categories B and Andreas Ludwig and Bettina et al has showed similar results in their study (Table 5)

Previous literature (4) also cites that high proliferative index in adjacent area suggest an invasive carcinoma in the neighbourhood and hence the results of High Ki 67 in the adjacent lesions suggest that it could have been the premalignant lesion for the current malignancy.

Normal premenopausal terminal duct lobular units contain about 30% of ER positive cells and nearly all cells express very high ER levels in the majority of premalignant breast lesions (5). The results obtained from this study also correlates with the literature stating the high positivity of ER in atypical lesions. Harold J. Burnstein in his study (Table 6) has also showed that ER positivity in DCIS is low in high grade lesions.

The results of this study hence correlates with the literature that the ER negative DCIS are high grade and this was also showed histologically (comedo and solid types of DCIS). Previous studies (6) state that ER positivity and Ki 67 index are high in cases of premalignant lesions of the breast and this fact is proved in our

study. On comparing the cases of ER positivity and High Ki 67 index it was observed that both were directly proportional.

In conclusion this study showed that the predominant lesion found adjacent to malignancy was usual ductal hyperplasia. Ki-67 index were high in adjacent areas showing proliferative lesions with and without atypia. ER values were high in proliferative lesions with atypia and in situ lesions. ER positivity and Ki-67 rate showed a direct proportional increase. This high positivity of ER and Ki-67 in the adjacent proliferative lesions with atypia hence proves that these could have been the precursors which have progressed to malignancy in these cases. Hence finding of these proliferative lesions in benign breast biopsies showing a high proliferative rate and ER positivity necessitates regular follow up of these patients and helps in identifying early progression of these lesions to malignancy and initiating treatment to these patients at an earlier stage and thereby reduce the incidence of breast cancer.

V. Er And Ki-67 Comparison

This study has shown that proliferative lesions with atypia and some DCIS cases which showed ER positivity also showed a high Ki-67 index. Anderson et al in his publication has also stated that the proportion of ER positive / Ki-67 positive proliferating cells is increased in all risk associated lesions of the breast and it increases from normal breast lobules through hyperplasia to insitu cancer and invasive cancer

VI. Ki-67 Proliferation Index

In normal breast Ki-67 is expressed at a very low level (<3% of cells). In our study 7 cases of proliferative lesions with atypia and 8 cases of proliferative lesions without atypia showed high proliferative index, 4 out of 5 cases in the benign non-proliferative lesion category showed low proliferation rate and one case showed high proliferative index. This high value is probably due to the UDH found focally in this case. High proliferative index in adjacent area suggest an invasive carcinoma in the neighbourhood and hence the results of High Ki 67 in the adjacent lesions suggest that it could be the premalignant lesion for the current malignancy. They could have been the fertile soil leading on to malignancy in these cases (4)

VII. Association Between Er And Ki-67

In normal breast tissue ER and the proliferation associated antigen Ki-67 are negatively associated. ER positive cells are non dividing or the receptor is down regulated as cells enter the cycle. This relation is completely or partially lost in many ER positive breast cancer and in in-situ proliferations associated with an increased cancer risk, where co-expression of the two markers is also found.

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TABLE: 1

Category A Non proliferative(fibrocystic) changes	Cyst formation
	Apocrine changes
	Hyalinization and calcification
	Mild epithelial hyperplasia
Category B Proliferative lesions without atypia	Fibroadenoma
	Intraductal papilloma and papillomatosis
	Microglandular adenosis
	Blunt duct adenosis
	Radial scar/complex sclerosing lesions
	Sclerosing adenosis
Category C Proliferative lesions with atypia In situ lesions	Moderate and florid hyperplasia
	Atypical ductal hyperplasia
	Atypical lobular hyperplasia
	Ductal carcinoma in situ
	Lobular carcinoma in situ

TABLE 2 ER ASSAY METHOD Quick score (Barnes et al 1995)

- Assigning value to both intensity and proportion of positivity .

Score for intensity

no staining	<u>0</u>
weak staining	<u>1</u>
moderate staining	<u>2</u>
strong staining	<u>3</u>

Score for proportion of positivity

no staining	<u>0</u>
<1% of nuclei staining	<u>1</u>
1%-10% of nuclei staining	<u>2</u>
11%-33% of nuclei staining	<u>3</u>
34%-66% of nuclei staining	<u>4</u>
67%-100% of nuclei staining	<u>5</u>

The scores were summed to give a maximum of 8.

Scores 2 or less were regarded as ER negative

TABLE 3 Proliferative Breast Lesions n 50

Proliferative breast lesions	Mild	Moderate	Florid	Total
Usual ductal hyperplasia	15	8	7	30
Atypical ductal hyperplasia	5	3	1	9
In situ lesions	9	1	1	11

TABLE 4

Type of lesion	No of cases	Proliferative index
Proliferative lesions with atypia	7	High
Proliferative lesions without atypia	8	High
Benign non-proliferative lesions	4/5	High

TABLE No : 5

Diagnosis	Mean(%) (min – max)	Median (%)	Standard deviation(%)
Normal breast	1.83(0-11.93)	0.99	+/-2.644
Fibrocystic lesion	0.37(0-4.76)	0	+/-0.99
Adenosis	3.23(0-17.60)	1.96	+/-3.39
UDH	2.41(0-20.64)	0.99	+/-4.02
DCIS (non high grade)	8.40(0-20.00)	7.83	+/-5.32

TABLE : 6

ER status	Tumor grade		
	1	2	3
Positive	87%	85%	61%
Negative	13%	15%	39%

PICTURES

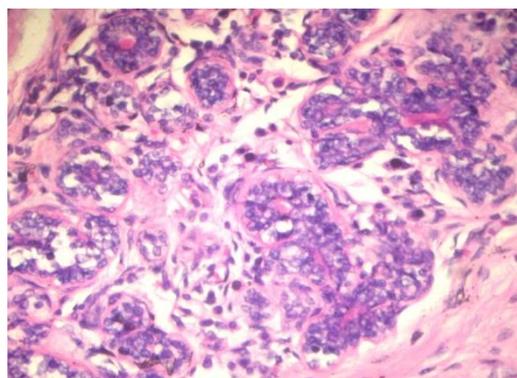


Figure 1: Moderate epitheliosis

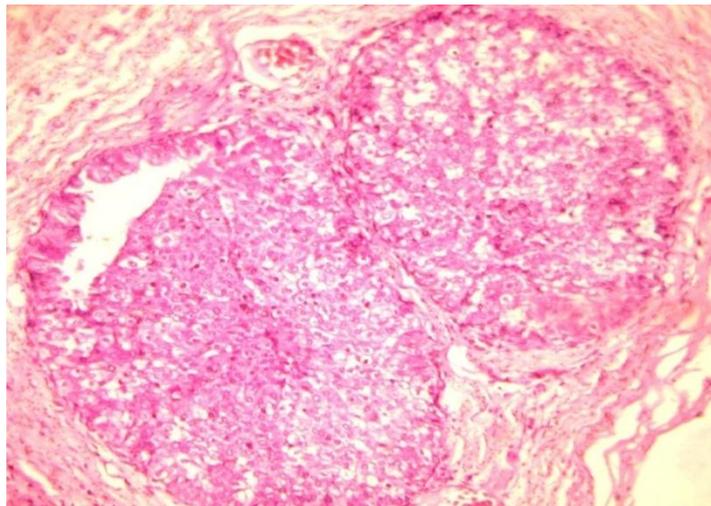


Figure 2: Atypical ductal hyperplasia

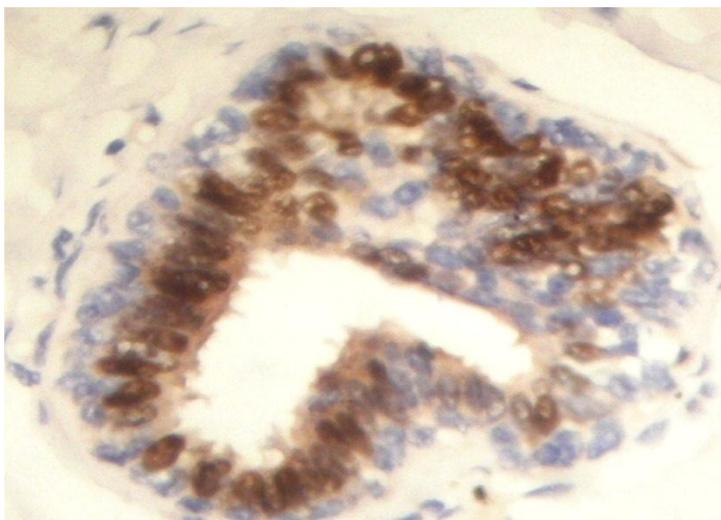


Figure 3: ER positivity in Moderate epitheliosis

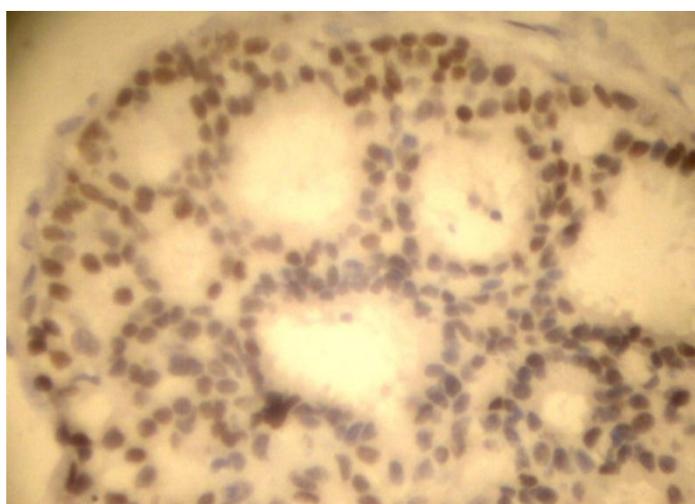


Figure 4: ER positivity in ADH

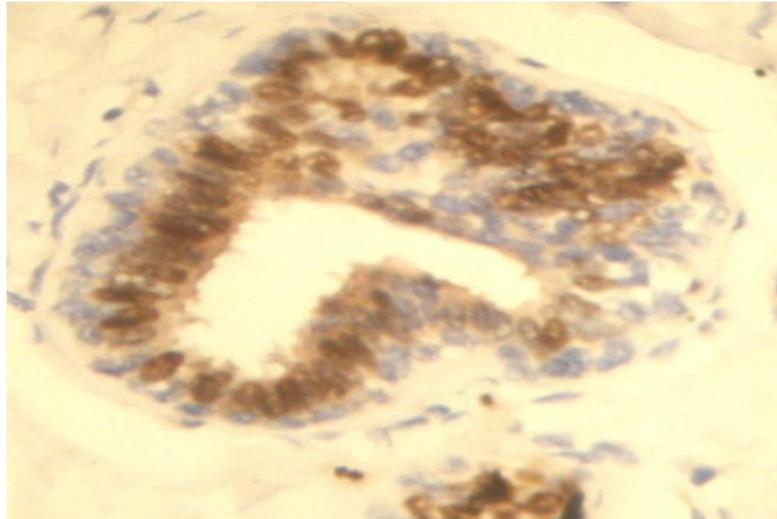


Figure 5: Ki 67 positivity in moderate epitheliosis

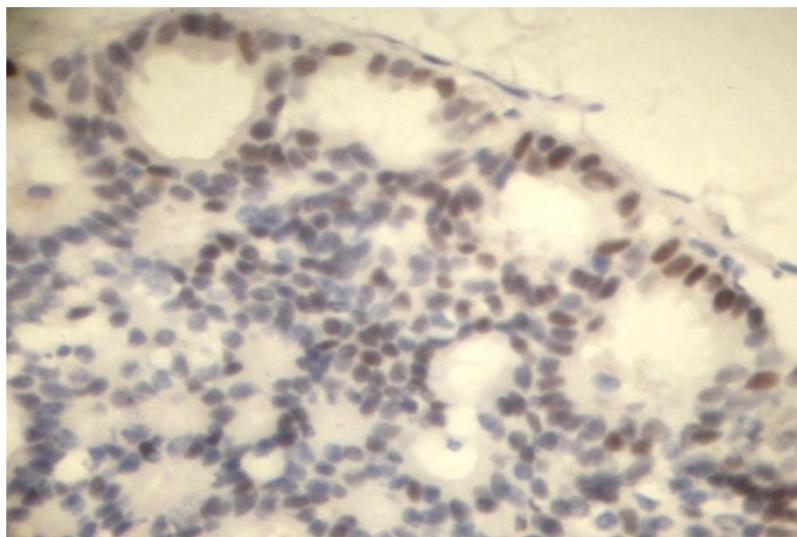


Figure 6: Ki 67 positivity in ADH