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CLINICOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL STUDY OF PRECURSOR LESIONS OF CARCINOMA BREAST

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ABSTRACT
Purpose: To analyse the clinical and histological features of the precursor lesions, to correlate with radiological findings and to determine the role of immunohistochemistry in the diagnosis of these lesions. Methods: Patients with suspicious breast lesions in radio imaging (BI-RADS III&IV) & radiologically ambiguous cases were directed towards core/excision biopsy. The panel of immunomarkers used were p63, SMMHC, CK 5/6, E-Cadherin, ER, PR and HER2/neu. Results: In the Institute of pathology, 1464 surgical specimens of breast (6.9%) were received during November 2011 to October 2013. Precursor lesions constituted 14.03% of benign lesions. Sclerosing adenosis and usual epithelial hyperplasia was common comprising 60.82% (59 cases). Precursor lesions had a peak incidence in the age group of 31-40 years. Commonest symptom of presentation was lump in the breast (63.92%). Most of the cases belonged to BI-RADS III score (30.86%). IHC done with myoepithelial markers such as p63 and SMMHC showed nuclear and cytoplasmic positivity of myoepithelial cells in all cases of sclerosing adenosis, ductal papilloma and DCIS. CK 5/6 was helpful in confirming cases of usual epithelial hyperplasia. Conclusion: We found that there is no significant difference in the incidence and age group of precursor lesions, as compared to literature. Myoepithelial markers were found to be useful in assessing the benignity of lesions in cases of diagnostic dilemma. ER, PR and HER2/neu expression were higher in pure precursor lesions than precursors associated with malignancy. Patients having lesions with ER expression will be benefited by Tamoxifen therapy and further progression to invasion can be arrested.

KEYWORDS: Cytokeratin 5/6, HER2/neu, Breast Precursor lesions, p63, SMMHC, ER/PR.

INTRODUCTION
In India, Carcinoma Breast is the most common cancer in women. 1,000,000 new cases occur worldwide annually, accounting for 22% of cancers in female population.[1] Precursor lesions of carcinoma breast are heterogeneous proliferations which vary in their architecture and cytology. They fall into 3 major categories with varied premalignant potential. They are Proliferative breast diseases without atypia, Proliferative breast diseases with atypia and in-situ (preinvasive) lesions. Most of these lesions are found in premenopausal women. They have the potential to turn malignant over a period of 10-15 years.

Proliferative breast diseases without atypia include moderate (or) florid ductal hyperplasia of usual type (UDH/UEH), sclerosing adenosis, complex sclerosing lesion/Radial scar, intraductal papilloma, fibroadenoma with complex features and columnar cell lesion. They carry a relative risk of 1.5 to 2 times more, in progression to carcinoma.[2]

Proliferative diseases with atypia include atypical ductal hyperplasia (ADH) and atypical lobular hyperplasia (ALH) with a relative risk of 4-5 times more, for developing Carcinoma.[3]

In-situ (preinvasive) lesions are lobular carcinoma in-situ LCIS) and ductal carcinoma in-situ (DCIS), have a relative risk of 8 to 10 times more, in progression to invasive carcinoma.[4] Sometimes it is difficult to differentiate between premalignant lesions and carcinoma by routine histopathology, due to the overlapping histomorphological features. Immunohistochemistry plays a crucial role in the diagnosis of these lesions. Myoepithelial markers p63, Smooth Muscle Myosin Heavy chain (SMMHC) determine stromal invasion in case of in-situ lesions. They highlight the presence of myoepithelial cells in sclerosing lesions associated with extensive desmoplasia and confirm benignity. They distinguish papilloma from papillary carcinoma.

Cytokeratin 5/6 (CK 5/6) makes distinction between usual ductal hyperplasia from atypical ductal hyperplasia and ductal carcinoma in-situ. E-Cadherin helps in the differentiation of ductal and lobular neoplasia. Estrogen receptor (ER), Progesterone receptor (PR) and Human epidermal growth factor receptor (HER2/neu) have prognostic and therapeutic significance.

In this study an attempt has been made to analyse the clinical and histomorphological features of precursor lesions of carcinoma breast and to determine the role of immunohistochemical markers in the diagnosis of these precursor lesions.

METHODS

The patients attending the surgical outpatient department with the complaint of lump in the breast or nipple discharge were subjected to trucut/excision/incision/wide local excision biopsies or mastectomy based on their clinical presentation and radio imaging. As a routine, all specimens were fixed in 10% formalin. Four micron thick sections of the paraffin tissue blocks of the cases prepared were stained with hematoxylin & eosin and examined. The representative formalin fixed, paraffin embedded tissue samples were subjected to immunohistochemical analysis, with the panel of markers p63, SMMHC, CK5/6, E-cadherin, ER, PR and HER2/neu. The immunohistochemically stained slides were analyzed for the presence of reaction, cellular localization, percentage of cells stained, intensity of staining and pattern of staining. Nuclear scoring was assessed for p63, ER, PR and cytoplasmic membranous positivity was assessed for SMMHC, E-cadherin, HER2/neu and membranous staining for CK 5/6.

RESULTS

During the period of 24 months from November 2011 to October 2013, the number of breast specimens received in the Institute of Pathology, were 1464 constituting 6.9 % of a total of 21,306 surgical pathology specimens. Among these breast specimens, 691 cases were benign (47.19%), 568 were malignant (38.80%), 176 were non-neoplastic (12.02%) and 29 cases were non representative (1.98%). The number of precursor lesions were 97 (14.03%), out of a total of 691 benign cases. Sclerosing adenosis and usual epithelial hyperplasia (UEH), either alone or in combination was the commonest proliferative lesion detected (Table 1).

Lump in breast was the commonest symptom among patients with precursor lesions in our study. Precursor lesions had a peak incidence in the age group of 31-40 years (36.08%). The youngest age of presentation was 13 years and oldest age of presentation was 75 years. DCIS was the commonest lesion in the older age group of more than 60 years and sclerosing adenosis was the commonest lesion in youngest age group. Males represented 3.09% of cases. The cases reported in males were 2 cases of sclerosing adenosis and one case of low grade DCIS. Distribution of cases, according to symptoms are given in Table - 2

Most of the cases belonged to BI-RADS III (30.86%), followed by BI-RADS II (29.62%) and then BI-RADS IV (19.75%). The commonest group of lesion in BI-RADS IV was combined sclerosing adenosis with usual epithelial hyperplasia and DCIS was the commonest lesion in BI-RADS V category.

Predominant side of occurrence of precursor lesion was left breast (53.61%). Most of the lesions (68.42%) had the size range of 2-4 cm and 17.11% had more than 4-6 cm size and none of the cases were more than 6 cm in size.

Among the 97 cases, 32 (32.99%) had double or triple lesions. The commonest combination found was sclerosing adenosis with usual epithelial hyperplasia.

IHC done with myoepithelial markers with p63 and SMMHC showed nuclear and cytoplasmic positivity of myoepithelial cells in all cases of sclerosing adenosis, ductal papilloma and DCIS.

IHC done with CK5/6 in 12 cases of UDH showed strong membranous staining in 50-90% of hyperplasic cells in UEH foci in all the cases. In 25% of cases in addition to UDH, ADH foci were also seen. CK5/6 expression studied in 6 cases of ADH showed complete lack of CK5/6 expression in 66.67% of cases, confirming the diagnosis. 16.67% of cases were reinterpreted as UDH after IHC study and 16.67% showed combined presence of UDH and ADH.

Two cases of lobular hyperplasia (100%) and 3 cases of lobular carcinoma in-situ showed lack of E-cadherin expression (100%). One case having histological resemblance to LCIS, showed strong staining pattern with E- cadherin and was later categorised as DCIS.

Considering ER expression, contiguous pattern of ER positivity was seen in 60% of cases and scattered positivity in 40% of cases. Among the precursor lesions there was over expression of ER in 50% of ADH, 40% of low grade DCIS and 60% of LCIS. There was no ER expression in UDH and high grade DCIS.

HER2 /neu expression was similar in all precursor lesions, with 40% of cases showing positivity, except papilloma which lacked HER2/neu expression. HER2 /neu expression was more in high grade DCIS (75%) than low grade DCIS (40%).

There was a direct association between ER and PR expression.

Table 1: Distribution of cases based on HPE diagnosis

Lesions	Total No cases	Percentage
Sclerosing adenosis	24	24.74%
Usual epithelial hyperplasia	22	22.68%
Sclerosing adenosis with UEH	13	13.40%
Atypical ductal hyperplasia	14	14.43%
UEH with ADH	2	2.06%
Papillary lesions Solitary Papilloma 2 Multiple papillomatosis 9	11	11.34%
Ductal carcinoma in situ Low grade 8 Both low and high grade 1	9	9.28%
Lobular hyperplasia	2	2.06%

Table 2: Distribution of cases according to symptoms

Symptoms	No of cases	Percentage
Lump	62	63.92%
Pain	8	8.25%
Lump, pain	19	19.59%
Discharge	6	6.19%
Lump, discharge	2	2.06%

Table 3: Comparison of DCIS Classification based on IHC

Type	Luminal A ER+, HER2/neu -ve	Luminal B ER +, HER2/neu +ve	HER2 type ER –ve, HER2/neu +ve	Basal type ER,PR, HER2/neu –ve
Pure DCIS	20%	20%	40%	20%
DCIS with invasive carcinoma	33.33%	8.3%	16.67%	25%
Total in current study	53.33%	28.3%	56.67%	45%
Livasy CA, et al	61%	9%	16%	19%

SCLEROSING ADENOSIS

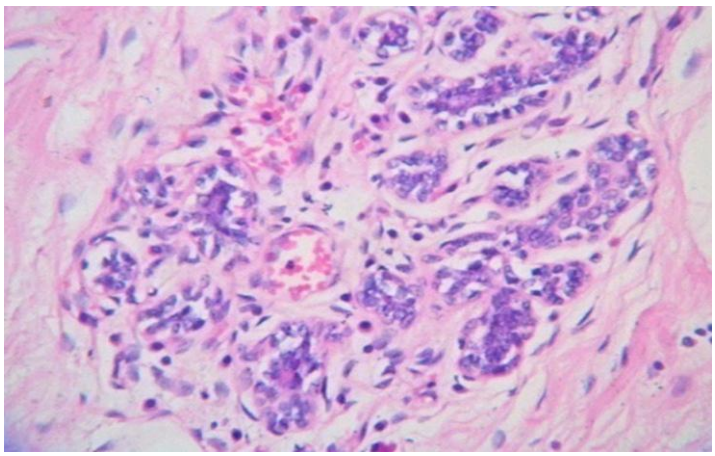

Fig.1.A: H&E stain, 10x. Lobulo-centric configuration with distorted glands in fibrotic stroma.

SCLEROSING ADENOSIS

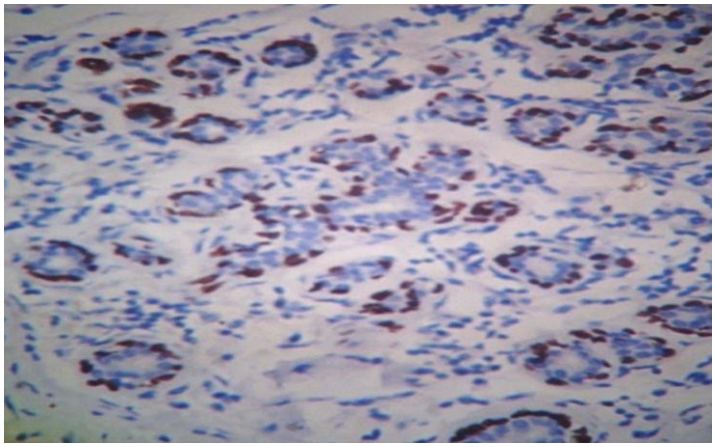

Fig.1.B: p63, 10x. Nuclear staining of myoepithelial cells.

USUAL EPITHELIAL HYPERPLASIA

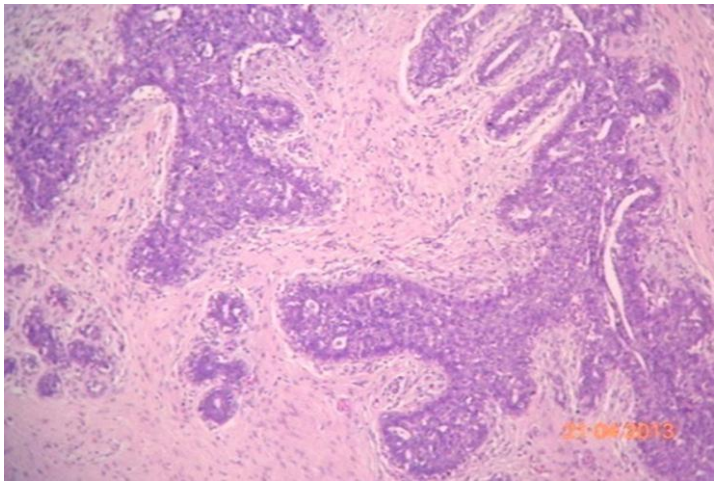

Fig.2.A: H&E stain, 10x. Florid proliferation of duct epithelial cells obliterating the lumens of the ducts.

USUAL EPITHELIAL HYPERPLASIA

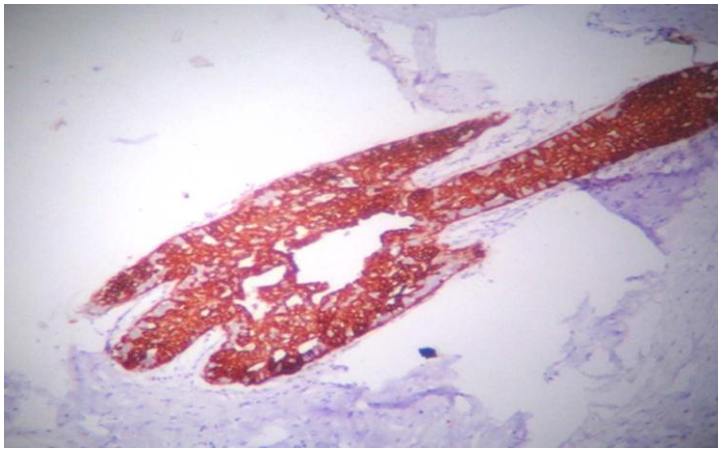

Fig.2.B: CK 5/6 , 10x. Diffuse pattern of 3+ staining in 100 % of hyperplastic cells in both luminal and basal pattern.

ATYPICAL DUCTAL HYPERPLASIA

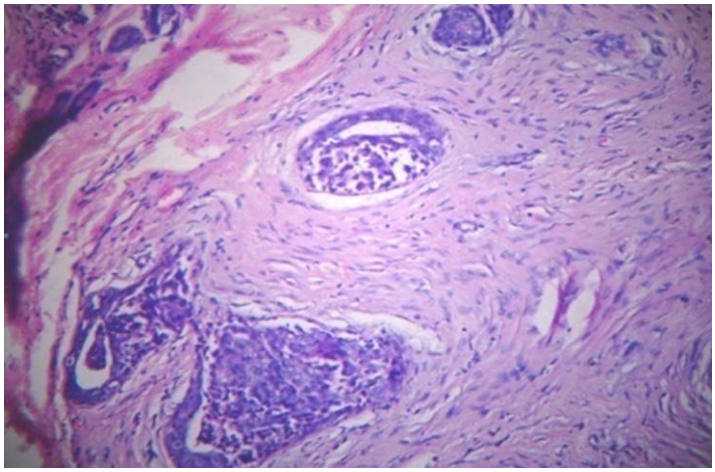

Fig.3.A: H & E stain, 10x. Proliferation of monotonous population of round to oval cells with hyperchromatic nuclei in an area less than 2 mm

ADH

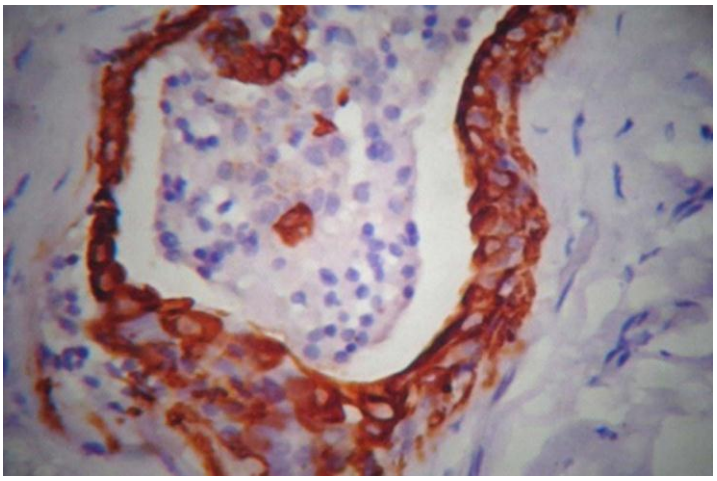

Fig.3.B: CK 5/6, 40x. Positive staining of basal cells and negative staining of luminal cells.

LOW GRADE DCIS

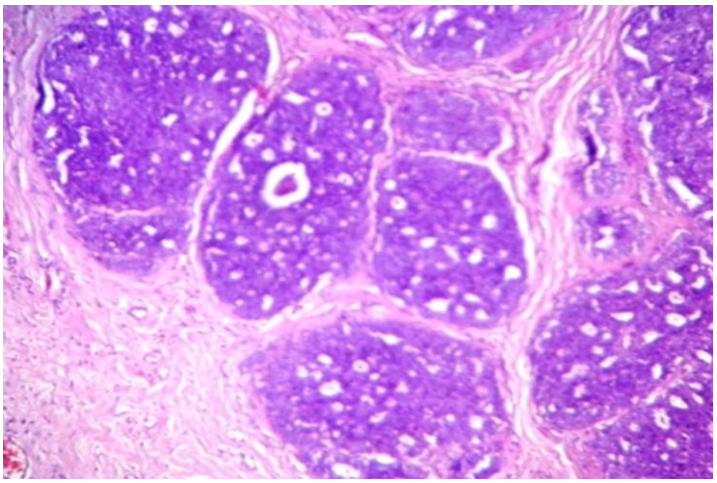

Fig.4.A: H&E stain, 10x. Cribriform pattern of DCIS with intact basement membrane.

LOW GRADE– DCIS

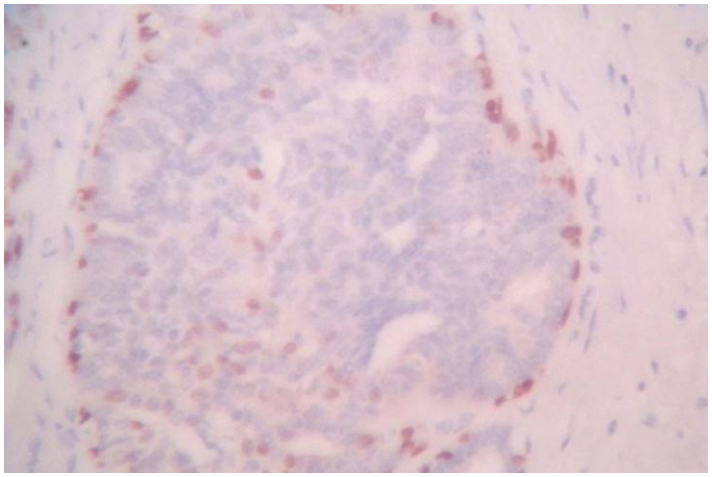

Fig.4.B: p63, 10x. Dot like staining pattern

LOBULAR CARCINIMA IN-SITU

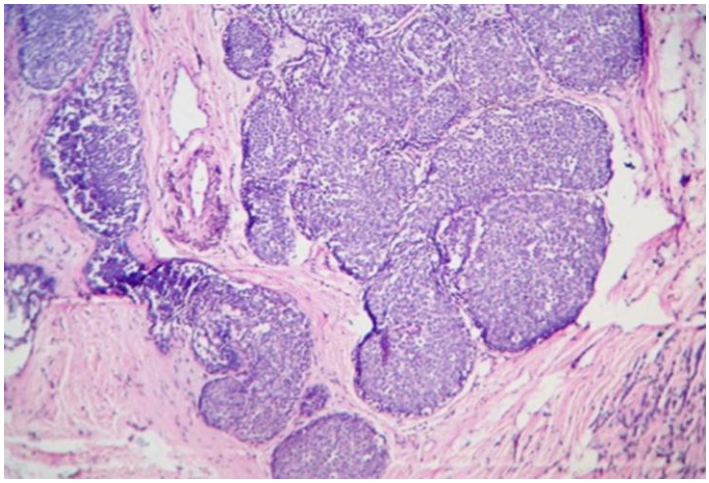

Fig.5.A: H&E stain, 4x. All acini are filled and distended by monomorphic population of cells

LCIS

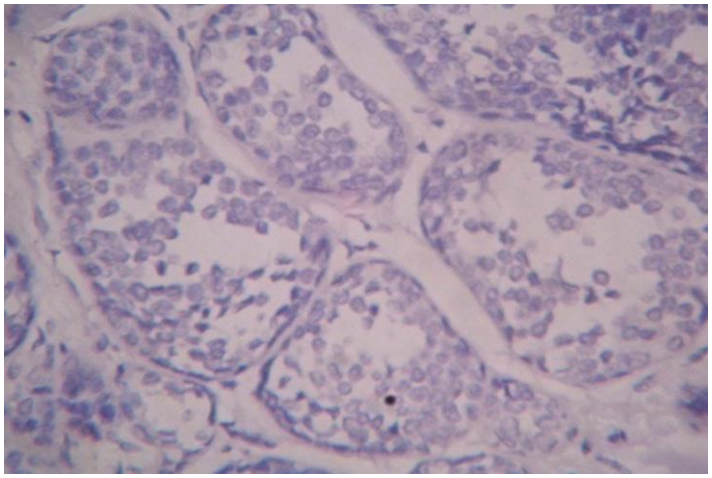

Fig.5.B. 10x. Lack of E-cadherin expression

UDH

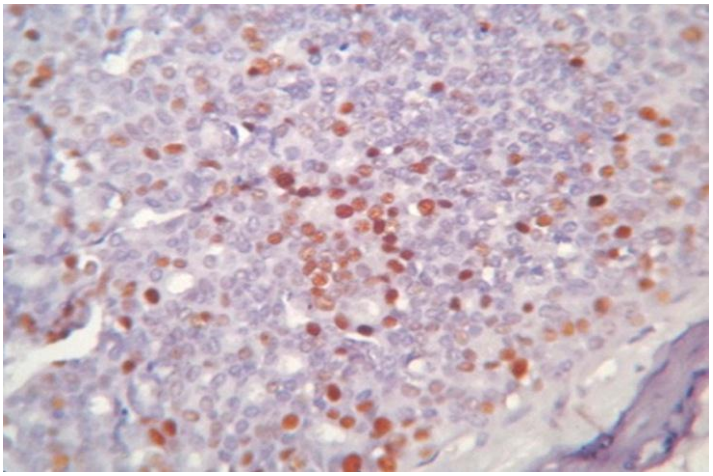

Fig.6. 10x. Scattered positivity of ER expression

ADH

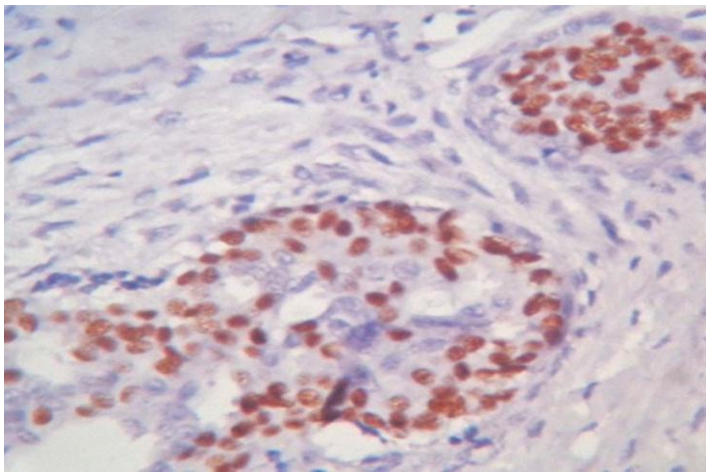

Fig.7. 40x. Contiguous positivity of ER expression

DCIS

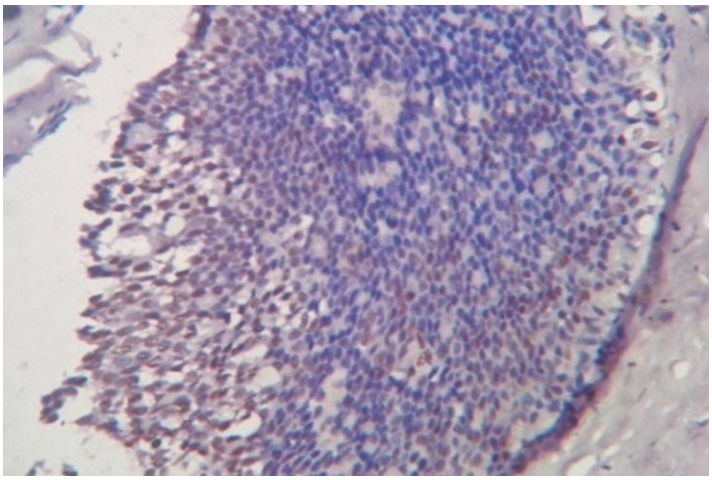

Fig.8, 10x. 1+2 positivity of PR in low grade DCIS

DCIS

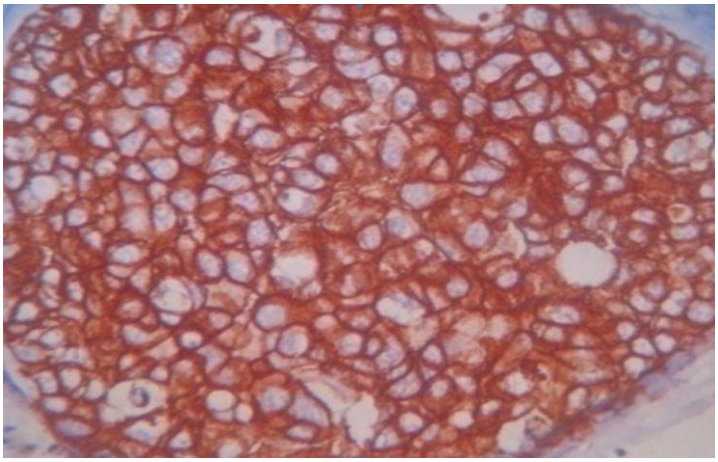

Fig.9, 40x. HER/2Neu expression-3+ score in high grade DCIS

DISCUSSION

In the present study, the clinical and histomorphological features of 97 precursor lesions were evaluated. Immunohistochemical analysis was done for 40 cases using myoepithelial & epithelial markers (p63, SMMHC, CK 5/6, E-Cadherin, ER, PR and HER2/neu). ER, PR and HER2/neu expression were also studied in 20 cases (18.18%) of precursor lesions associated with malignancy and were compared with that of pure precursor lesions.

In our study, lump was the commonest presenting symptom constituting approximately 63%, followed by lump and pain constituting approximately 20%. The finding was similar to the study by Ayoade BA et al.[5]

Sclerosing adenosis: Microscopically sclerosing adenosis is characterised by lobulo-centric pattern with distorted glands and tubules surrounded by myoepithelial cells in a sclerotic stroma. Immunohistochemical markers p63 and SMMHC highlighted the myoepithelial cells confirming their benignity.

In this study, the incidence of sclerosing adenosis was 24.74 % of precursor lesions and 3.47 % of all benign breast lesions, which is comparable to the study by Roy A Jenson et al, which had the incidence of 5.3% of benign breast lesions.[6] About 50% of sclerosing adenosis were categorised under BI-RADS III, whereas Fusan Taskin et al, have reported an incidence of 40% in the BIRADS III category.[7] Association of sclerosing adenosis with DCIS, UDH and apocrine metaplasia was seen in our study which was similar to the study by Kutsikoseo et al.[7] Immunohistochemical markers p63 and SMMHC highlighted the myoepithelial cells, aiding us to confirm the diagnosis.

Usual ductal hyperplasia-moderate to florid

Microscopic characters are proliferation of cytologically benign epithelial cells filling and distending the lumen of the duct forming slit like secondary lumens, the nuclei differing in size, shape and orientation.

In our current study, the incidence of usual epithelial hyperplasia was 22.68%. According to Ian O Ellis et al, the incidence was 25%.[8] The UDH occurred predominantly in the age group of 41 to 50 years (31.82%). Literature states that commonest age for UDH is 45 years. In the study by Georgiana Luminita et al, combined UDH and ADH were seen in 4.10% of cases which is concordant with our study.[9]

CK 5/6 expression was studied in 12 cases of UDH. All the 12 cases showed strong membranous staining in 50-90% of hyperplastic cells in UDH foci. Mommers EC et al, have reported similar results, in which 50-80% of hyperplastic cells showed strong staining foci for CK 5/6.[10] In the present study, among the 3 out of the 12 cases (25%), there was complete lack of expression of CK 5/6, adjacent to hyperplastic foci, which indicated the presence of ADH foci, in addition to UDH foci.

Atypical Ductal hyperplasia

Microscopically ADH is characterised by monotonous population of small to medium sized round to polygonal cells with hyperchromatic nuclei, distributed evenly forming regular punched out spaces. The entire lesion should measure less than 2mm in maximum dimension or occupy less than 2 duct spaces.

The incidence of ADH in our study was 14.43% of precursor lesions and 2.02 % of benign breast biopsies. Peng Sul et al, has reported an incidence of 16.09% of precursor lesions, and a study by Stamper PC et al, have shown an incidence of 4% of benign breast biopsies.[11,12]

ADH was found predominantly in the mean age group of 44 yrs. Page DL et al, have reported a mean age of 46 years for ADH.[13] Majority of ADH cases in our study belonged to BI-RADS III category (33.33%), followed by BI-RADS IV category (25%). But, in a study by Noursneige et al, 97.6% of cases were BI-RADS IV and 2.4% were BIRADS I.[14] In our study, one case of ADH

interpreted in trucut biopsy was later diagnosed as DCIS after excision biopsy, done after 20 days (7.1%). Another case diagnosed as ADH, after modified radical mastectomy was found to have invasive ductal carcinoma (7.1%). In the study by. Michael J et al, after diagnosis of ADH by core needle biopsy, 17.9% showed adjacent DCIS in the subsequent excision biopsy.[15] In another study by Rohit K Jain et al, 2 out of 137cases (1.46%) were reinterpreted as DCIS and 7 out of 137 cases (5.1 %) were reinterpreted as usual epithelial hyperplasia.[16] Hence excision is a must following the diagnosis of ADH.

We studied CK 5/6 expression in six cases of ADH. Four cases (66.67%) showed complete lack of CK 5/6 expression, confirming the diagnosis of ADH. In the rest of the two cases, one case (16.67%) showed strong staining for CK5/6 in 70-80% of hyperplastic cells, indicating that hyperplasia was usual epithelial type. The other case revealed CK5/6 positive as well as negative foci indicating the combined presence of UDH and ADH. In the study by Rutika Mehta et al, among cases diagnosed as ADH, 8% were found to be usual epithelial hyperplasia after immunohistochemical study.[16] In our study, 16.66% cases turned out to be UDH. This signifies the diagnostic value of CK5/6.

DCIS

Microscopically DCIS is characterised by cytological features of ADH but more than 2 mm in size or occupies more than 2 duct spaces.

Incidence of pure ductal carcinoma in-situ without invasive component in the present study was 9.28%, with low grade DCIS constituting 88.89% and both low grade and high grade DCIS constituting 11.11%. Dutch comprehensive centre in the year 2007 has reported an incidence of 8.3%.[17] Average age group of DCIS in our study was 50.66 years and a study by Kerlikowske et al, has shown the mean age to be 50-59 years.[18] In the current study most of the cases were BI-RADS V and III, each constituting 33.33%.

Out of nine cases of DCIS diagnosed, four belonged to solid, cribriform type (44.44%) and the Papillary-cribriform, cribriform-micropapillary, solid-papillary, solid and papillary pure forms constituted one each. In the study by Malone et al and others, solid and cribriform type was the commonest one as in our study.[19, 20] In the current study incidence of DCIS with adjacent invasive carcinoma was 54.55%. In the study by Georgiana Luminita Fota et al, DCIS was associated with invasive ductal carcinoma in 79.36% of cases.[9]

Study done with p63 and SMMHC showed discontinuous pattern of nuclear and cytoplasmic staining of myoepithelial cells in all the 4 cases studied. Maha M et al, reported similar pattern of staining in all the 12 cases of DCIS studied.[21] Among the cases of Pure DCIS, 40% showed ER expression, 40% had PR expression and these 40% did not show HER2/neu over expression. Another 40% of cases showed HER2/neu over expression and they were negative for ER, PR expression.

In our study, HER2/neu type was most common, followed by Luminal A type, while in the study by Livasy et al, Luminal A type was the commonest one. In both studies Luminal B type was the least common.[22] (Table.3).

Papilloma

Microscopically papillomas are characterised by central fibro vascular core lined by cuboidal to columnar cells surrounded by myoepithelial layer. Multiple inter anastomosing branching papillae of five and more in number constitutes multiple papillomas.

In our study, the incidence of papilloma was 11.34% of precursor lesions, 1.59% of benign breast lesions and 0.07% of all breast specimens. According to WHO, the incidence of papilloma was 10% of all benign breast biopsy specimens. In our study 25% presented as solitary papilloma and 75% as multiple papillomata. ADH and DCIS were seen in association with multiple papillomatosis, as has been reported by Jacob Schachter et al.[23] In this study, 70% of papillomas were diagnosed as BI-RADS II category. In the study by Xin Li et al, 98.6% of papillomas belonged to BI-RADS IV category.

Diffuse continuous staining of peripheral basal layer of papilloma with SMMHC and irregularly spaced nuclear staining of p63 were seen in all the five cases of papilloma, distinguishing them from malignant papillary lesions. Since p63 alone causes discontinuous nuclear staining, significance of combined use with SMMHC was highlighted. A similar observation was reported by Cheryl B et al who studied 27 cases of papilloma and all showed the presence of myoepithelial cells.[24, 25]

LOBULAR NEOPLASIA

Histologically, Atypical Lobular Hyperplasia is characterised by monomorphic evenly placed dyscohesive cells with pale cytoplasm and round to oval eccentric nuclei having intracytoplasmic lumens occupying less than one half of acini, whereas, in LCIS more than half of the acini should be filled and distended by monomorphic population of small cells with thin rim of cytoplasm.

In our study, we had two cases of atypical lobular hyperplasias, among the pure precursor lesions accounting for 0.3 % of benign breast biopsies and 2.6 % of all precursor lesions. This was similar to the incidence quoted by Frykberg et al, i.e., 0.5 to 4 %.[26] We had three cases of lobular carcinoma in-situ, associated with malignancies (0.52%), among which two cases were seen with lobular carcinoma and one case with combined ductal and lobular carcinoma. Mean age in our study was 46.2 years.

Immunohistochemical study showed lack of expression of E-Cadherin in all the five cases, confirming the diagnosis. In the study by Pezza JA et al, E-Cadherin showed strong membranous staining in all cases of DCIS, while occasional cases of LCIS also showed expression but weak and cytoplasmic staining pattern.[27]

In the present study, one case histologically resembled LCIS, but, lacked E-cadherin expression and had strong membranous staining pattern of E-Cadherin, and, was later categorised under low grade DCIS. 60 % of LCIS had ER over expression and 33.33% showed HER2/neu over expression.

Significance of ER, PR and HER 2/neu expression in premalignant lesions: According to the literature, there will be an increase in ER expression with increase in age, in normal breast. But, in our study, there was no increase in ER expression with increasing age (more ER expression under 40 years of age). This breakdown in normal age regulatory mechanism of ER positive cell numbers may indicate an early precursor change. This was similar to the study by Shoker BS et al, who stated that the escape from normal age related mechanism for ER positivity occurred at ADH stage.[28]

In normal breast epithelium, ER positive cells are distributed singly and surrounded by ER negative cells. The precursor lesions, however, show ER positivity in contiguous cells in contrast to normal breast epithelium. Lawson et al reported that ER positivity in contiguous pattern in premalignant lesions had more risk for progression to invasion. PR expression was parallel to ER expression in our study and in study by Lawson et al.[29] He also stated that ER expression in the adjacent normal acini had more risk than the positivity within the lesion. In our study, contiguous pattern of ER positivity was seen in 60% of cases and scattered positivity in 40% of cases. Jarvis et al stated that contiguous expression could represent a precancerous stage.[28] Among the precursor lesions, ER expression was higher in ADH, low grade DCIS and LCIS which was similar to Prosser J et al study. UDH and High grade DCIS did not show ER expression in our study, and they showed very low level of expression in Prosser J et al study.[30]

HER2/neu expression was higher in pure precursors than precursors associated with malignancy in our study. ER, PR expressions were more in pure precursors than the precursors associated with malignancy. This was similar to the study by Shoker et al, who found out that increased ER expression would start at the ADH stage and the expression would get, down regulated during progression to invasion.[28]

To summarise, the incidence of precursor lesions in our study was 14.04% and most of the precursor lesions in our study occurred at an earlier age group (31-40 yrs), than malignant lesions (35-50 years), both features coinciding with several other studies. Sclerosing adenosis and usual epithelial hyperplasia, constituted the bulk of the lesions amounting to 60.82% (59 cases). Commonest symptom of presentation was lump in the breast (63.92%).

Majority of precursor lesions were diagnosed as BI-RADS III in this study, but in many other studies, most of the precursor lesions were diagnosed as BI-RADS IV.

IHC done with myoepithelial markers with p63 and SMMHC showed nuclear and cytoplasmic positivity of myoepithelial cells in all cases of sclerosing adenosis, ductal papilloma and DCIS. These myoepithelial markers proved their utility in confirming the diagnosis of benignity of the lesions. This study also highlights the value of CK 5/6, in the diagnostic dilemma, between usual ductal hyperplasia and atypical ductal hyperplasia. This study also implies the necessity of excision following the diagnosis of atypical ductal hyperplasia on core needle biopsy, since adjacent invasive/in-situ lesions have been diagnosed in the subsequent excision. As per literature, patients with contiguous pattern of ER expression have increased risk of malignancy. ER, PR expression was more in pure precursor lesions, than in precursor lesions associated with malignancy. These patients can be treated with tamoxifen and further progression to invasion can be arrested, thereby reducing the morbidity and mortality.

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