"IMMUNOHISTOCHEMICAL EXPRESSION OF p53 & Ki-67 IN BENIGN, PROLIFERATIVE AND MALIGNANT BREAST DISEASE"

Thesis submitted in partial fulfillment of the requirements for the degree of

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CERTIFICATE

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ABBREVIATIONS

ADH	–	Atypical Ductal Hyperplasia
BBD	–	Benign Breast Disease
CAT	–	Category
DAB	–	Diamino benzedine
DCIS	–	Ductal Carcinom in-situ
DNA	–	Deoxyribonucleic acid
EDTA	–	Ethylene Diamine Tetraacetic Acid
EGFR	–	Epidermal Growth Factor Receptor
ELISA	–	Enzyme Linked Immunosorbant Assay
ER	–	Estrogen Receptor
FCC	–	Fibrocystic changes
H & E	–	Haematoxylin and Eosin
HRM	–	High Resolution Melting
IBC	–	Invasive Breast Cancer
IDC	–	Intraductal Carcinoma
IHC	–	Immunohistochemistry
ILC	–	Invasive Lobular Carcinoma
LCIS	–	Lobular Carcinoma In Situ
LN	–	Lobular neoplasia
MRM	–	Modified Radical Mastectomy
NAC	–	Neoadjuvant Chemotherapy
NBSS	–	National Breast Screening Service
pCR	–	Pathological Complete Response
pNR	–	Pathological No Response
pPR	–	Pathological Partial Remission
PR	–	Progesterone Receptor
TBS	–	Tris Buffer Saline
TDLU	–	Terminal Duct Lobular Unit

INTRODUCTION

Breast diseases are heterogenous, benign are more common as compared to malignant. Breast cancer remains the most commonly diagnosed malignancy among females after cervical cancer & detected in 20/1, 00,000 women.[1] It is one of the leading causes of morbidity and mortality in women.[2] This increased risk is associated with proliferative & atypical lesions.[3]

Benign lesions have assumed increasing importance in recent years because of the public awareness and theses are a notable risk factor for progression to malignant lesions which can develop in either breast. BBDs constitute a spectrum of lesions ranging from inflammatory, epithelial and stromal proliferations to various neoplasms.[4]

With the advancement of diagnostic modalities numerous efforts have been put in understanding the pathogenesis of developing carcinoma development. Models of breast carcinogenesis suggest that atypical hyperplasia occupies a place between benign and malignant disease. It contains some but not all the requisite features of cancer and thus considered to be premalignant.[4]

In the multistep progression in pathogenesis of breast cancer from benign to malignant, successive changes have been perceived which finally end up with development of malignancy. These are simple hyperplasia with and without out atypia, in-situ carcinoma and ultimately leads to invasive carcinoma. These sequence of events suggest that invasive carcinomas were in fact precursor benign lesions to start with.[5] As compared to non prolifertive one, proliferative have greater risk (two to four times) of developing breast carcinoma.[6]

The elevated risk of developing carcinoma associated with benign lesions was found for both ipsi-lateral and contra-lateral breasts. Various studies done previously concluded that atypical cases of breast diseases were associated with increased risk for future breast cancer.[2]

During the last few decades, IHC has become an integral part of pathology. Although H & E stain remains the fundamental basis for diagnostic pathology of the breast, IHC stains provide useful and vital information.[7]

There is a growing list of available antibodies or antigen retrieval techniques, which all contribute to the broader utility of IHC for solving diagnostic problems or for determining prognosis and response to therapy in breast pathology.[8]

Ki-67, a non-histone protein, involved in the early steps of RNA synthesis and it is a predictive and prognostic marker in cancers and has been extensively study.[8] Ki-67 recognizes a nuclear antigen present in proliferating cells.[9] Ki-67 expression increases progressively across the continuum from benign breast disease, to ductal carcinoma in situ (DCIS), to invasive breast cancer.[10-12] In invasive breast cancer, higher Ki-67 levels have been shown to correlate with worse clinical outcome in numerous studies.[13-14] In DCIS, higher Ki-67 is associated with higher grade lesions.[15]

The p53 gene is located on chromosome 17 which found to encodes a 375 amino acid that prevents propagation of genetically modified cells.[16] Wild-type p53 is a tumour suppressor protein and it plays an essential role in regulating genomic stability by controlling the cell cycle and inducing apoptosis when cell damage cannot be repaired.[17-19]In normal cells, p53 has a very short half-life.[20-21] IHC can be used, as wild-type p53 protein is rapidly degraded, while TP53 mutations are often associated with the production of a stable protein. [22]

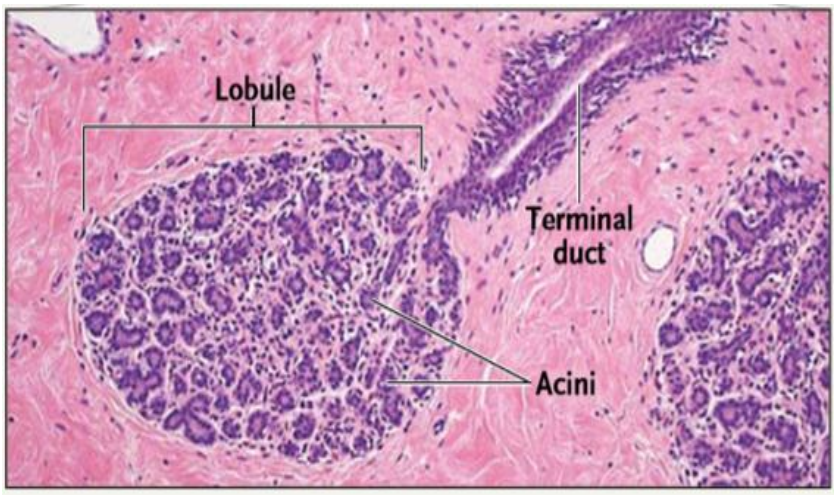
Mutations in the p53 tumor suppressor gene and accumulation of its protein in benign lesions are related to breast carcinogenesis pathogenesis. However, few studies have prospectively investigated the association of p53 immunopositivity and p53 alterations among benign breast disease in relation to the subsequent risk of invasive breast cancer.[25]

Hence identifying such predominantly occurring lesions adjacent to malignancy and studying of Ki-67 proliferative index (MIB-1 index) and p53 status in such lesions could substantiate their possible identity as premalignant lesion in that particular case. The present study aims at identifying high risk lesions occurring adjacent to malignancy and confirming their risk status by Ki-67 index and p53 status.

REVIEW OF LITERATURE

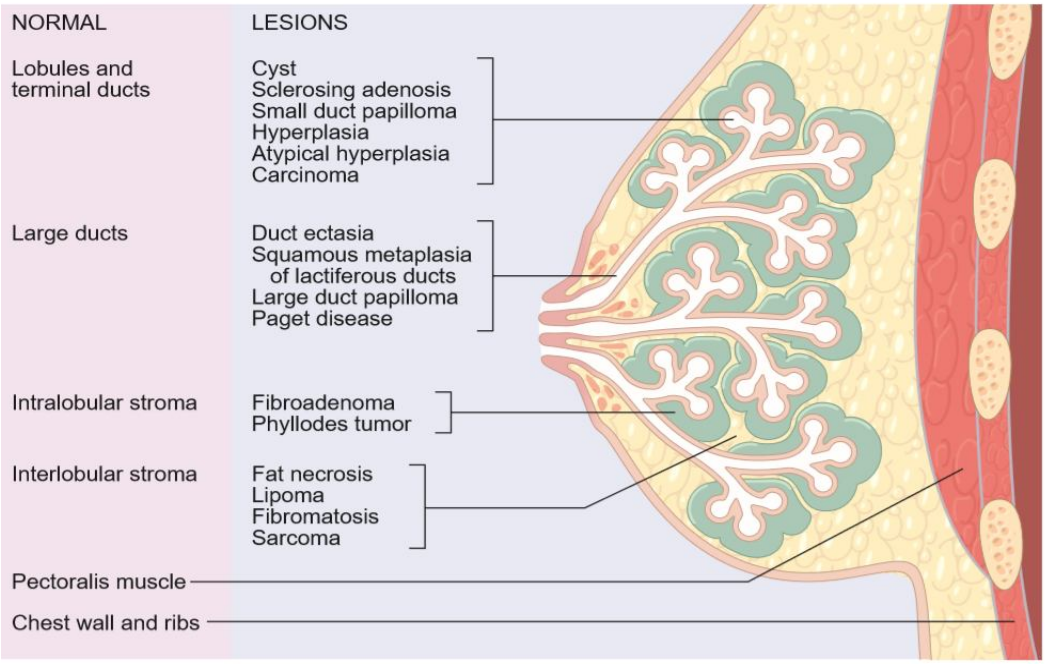
The breast or mammary gland is a modified sweat gland covered by skin and rests on the pectoralis muscle, from which it is separated by a fascia.[26] The breast can be divided into four regions : (a) skin, nipple, subareolar tissues (b) subcutaneous region (c) parenchyma (d) retromammmary region.[27] There are about 15–25 lobes of parenchymatous elements associated with each of the lactiferous ducts which drain into the nipple.[28] The lobules drains into ductules and ducts, these in turn drains into the collecting ducts that open onto the surface of the nipple. Just below the nipple, the ducts are expanded to form lactiferous sinuses. The epithelium throughout the duct system is bilayered, consisting of an inner epithelilal layer, it is cuboidal or columnar and an outer myoepithelial layer. The terminal ductal lobular unit (TDLU) is the physiologically active area of the breast and site of origin of most pathologic lesions.[26]

Figure 1 : Terminal ductal lobular unit (TDLU)[26]



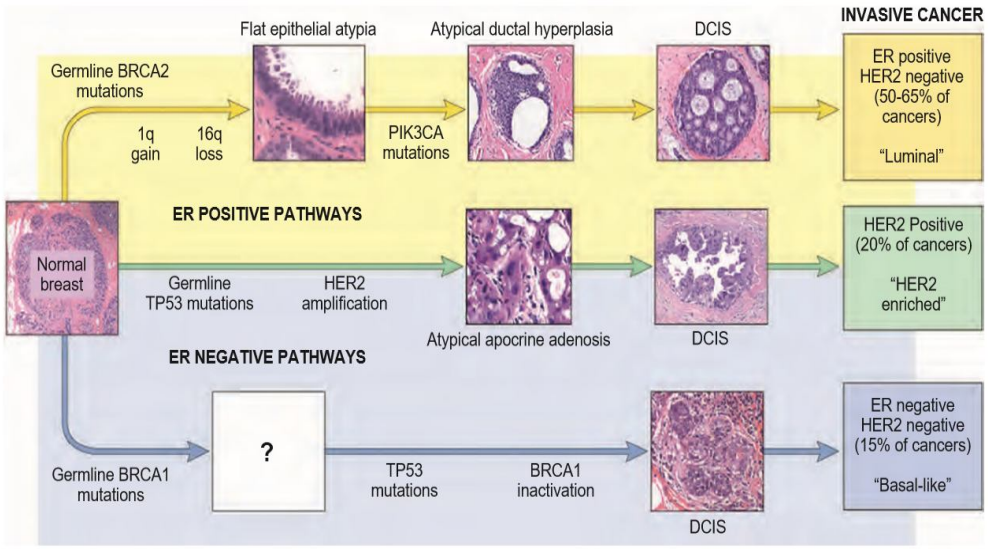
Breast diseases are divided into benign and malignant. Benign epithelial lesions are classified into three groups non-proliferative breast diseases, proliferative breast disease without atypia and proliferative breast disease with atypia. Malignant breast diseases include carcinoma breast.[29]

Figure 2 :Anatomic site of various breast lesions[29]



In the natural history of breast cancer, sequential changes in the breast tissue have been observed which finally end up with development of malignancy. These events are simple hyperplasia without atypia, hyperplasia with atypia, in situ carcinoma. These sequences of events suggests that some of the invasive carcinomas were in fact precursor benign lesions to start with.[5]

Figure 3 : Pathogenesis of breast carcinoma[29]



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.[30]

Fibrocystic changes (FCCs) - They constitute benign disorder of the breast. Such changes generally affect premenopausal women between 20 and 50 years of age. . FCCs may be multifocal and bilateral. The most common presenting symptoms are breast pain and tender nodularities in breasts and estrogen predominance over progesterone play an important role in its pathogenesis.[31]

Fibroadenoma- It is the most common lesion of the breast. Its peak incidence is between the ages of 15 and 35 years. Fibroadenoma (benign tumor) is also thought to represent a group of hyperplastic breast lobules called "aberrations of normal development and involution. It presents as a highly mobile, firm, non-tender, and often palpable breast mass. Although most frequently unilateral, in 20% of cases, multiple lesions occur in the same breast or bilaterally. It develops from the special stroma of the lobule. Macroscopically, the lesion is a well-circumscribed, firm mass, <3 cm in diameter, the cut surface of which appears lobulated and bulging. If the tumor assumes massive proportions (>10 cm), more commonly observed in female adolescents, it is called "giant fibroadenoma." Microscopically, consists of proliferation of epithelial and mesenchymal elements. The stroma proliferates around tubular glands (pericanalicular growth) or compressed cleft-like ducts (intracanalicular growth). Often both types of growth are seen in the same lesion.[31]

Usual ductal hyperplasia- This lesion is characterized by a solid or fenestrated proliferation of epithelial cells that often show streaming growth, particularly in the centre of involved spaces. It is characterized by a cohesive proliferation of benign epithelial cells that display a haphazard orientation with respect to one another. The presence of secondary lumina or fenestrations is characteristic of this lesion. The lumina are often peripherally located and tend to be slit like, as opposed to the very rounded, punched-out lumina seen in ADH and low-grade ductal carcinoma in situ (DCIS).[34]

Phyllodes tumor (Benign)- It is a fibroepithelial tumor of the breast with a spectrum of changes. Benign phyllodes tumor is usually difficult to differentiate from fibroadenoma. hypercellular stroma with cytologic atypia, increased mitoses, and infiltrative margins of the lesion are the most reliable discriminators to separate lesions with recurrence and malignant behavior. Approximately 50% of fibroadenomas contain other proliferative changes of breast, such as sclerosing adenosis, adenosis, and duct epithelial hyperplasia. Fibroadenomas that contain these elements are called complex fibroadenomas. Simple fibroadenomas are not associated with any increased risk for subsequent breast cancer. However, women with complex fibroadenomas may have a slightly higher risk for subsequent cancer.[31]

Atypical ductal hyperplasia- Atypical ductal hyperplasia (ADH) is a proliferation of monomorphic, evenly placed epithelial cells involving terminal-duct lobular units (TDLUs). ADH is characterized by a proliferation within TDLUs of a monomorphic population of epithelial cells that are evenly placed and lack the streaming, swirling, and overlapping of the cells that define UDH. The cell borders are distinct. The proliferation may be solid with or without subtle microacini, cribriform with round,

"punched out" spaces surrounded by polarized epithelial cells, or micropapillary with epithelial projections that are typically narrower at the base than the apex.[34] ALH confers a 3 fold elevated risk for the development of infiltrating breast cancer. In ADH , the presence and role of p53 mutations is is still an open field; p53 mutations were initially not documented; then studies pointing to p53 mutations appeared.[32]

Ductal carcinoma in situ- Proliferation of pleomorphic epithelial cells within the thick-walled ducts of the breast. There is no light microscopic evidence of invasion through the basement membrane into the surrounding stroma. Such lesion is known as ductal carcinoma in-situ (DCIS). Several morphologic patterns of DCIS are recognized, the most common of which are comedo, cribriform, papillary, solid and micropapillary. DCIS- Comedo is diagnosed when atleast one duct is filled and expanded by large, markedly atypical cells and has abundant central luminal necrosis.[33]

Invasive ductal carcinoma breast: It is also known as infiltrating ductal carcinoma (IDC). It is the most common form of breast cancer. These are adenocarcinomas that fail to exhibit sufficient characteristics to warrant their classification in one of the special type.(10) IDC starts in breast milk-ducts and invades the surrounding breast stroma.[23]

Invasive lobular carcinoma- An invasive carcinoma composed of non cohesive cells individually dispersed or arranged in a single-file linear pattern in a fibrous stroma. It is usually associated with lobular carcinoma in situ (LCIS). The proliferation rate, measured by MIB1/Ki67 labelling, is generally low in ILC, although higher in the variants.[34]

Mucinous carcinoma- Another special-type breast cancer, accounts for 2% of all invasive breast cancers and typically presents in the elderly population as a bulky tumor. Lymph node metastases occur in 33% of cases.[31]

Metaplastic carcinoma- Metaplastic carcinoma encompasses a group of neoplasms characterized by differentiation of the neoplastic epithelium into squamous cells and/ or mesenchymal-looking elements, including but not restricted to spindle, chondroid, osseous, and rhabdomyoid cells. These neoplasms may be either entirely composed of metaplastic elements, or a complex admixture of carcinoma and metaplastic areas.[34]

Papillary carcinoma- It is a special-type cancer of the breast that accounts for 2% of all invasive breast cancers. It generally presents in the seventh decade of life. Typically, papillary carcinomas are small and rarely attain a size of 3 cm in diameter. It shows a low frequency of axillary lymph node metastases.[31]

Immunohistochemistry (IHC) has an expanding role in the diagnosis and management of mammary disease.[3] The heterogeneity of immunostaining patterns in the subcategories of benign and proliferative breast disease reinforces that measurement of proliferative activity and may provide valuable information in malignant transformation of these lesions.[35] Studying of Ki-67 proliferative index (MIB-1 index) and P53 in breast lesions could substantiate their possible identity as premalignant lesion in that particular case.[30] The most common immunohistochemical breast cancer prognostic markers are Ki-67 and p53.[8] p53, tumor suppressor gene is located on the short arm of chromosome 17.[36] The p53 gene product is a multifunctional transcription factor that is involved in regulating cell cycle arrest and apoptosis, facilitating DNA repair and promoting chromosomal stability.[37] Mutated p53 protein

tends to have an increased half-life and can then be detected using specific antibodies in tissue and cells. A positive immunohistochemical result with p53 antibodies can then be considered an expression of a mutant p53 gene. Mutations in the p53 gene appear to be the most common genetic change in cancer.[36] p53 mutations and p53 protein accumulation have also been detected in benign breast disease. These observations suggest that p53 changes can occur before the development of breast cancer raising the possibility that such changes might be related to the risk of breast cancer development.[37] Overexpression of p53 protein is associated with a poor prognosis.[36] p53 can also be a predictive marker through identifying the most likely patients to respond to chemotherapy. Immunohistochemical detection of the p53 protein can now be done using antibodies, the most used one being CM1, PAb1801, DO1 and DO7.[11]

Ki-67 is a nuclear protein found in the G1-phase of cell cycle and it is considered a useful marker of cell proliferation. Many studies have found a link between the percentage of positive Ki-67 cells and the clinical evolution. These studies suggest that the measuring of Ki-67 expression can be useful in stratifying patients into two categories, good prognosis and bad prognosis . The Ki-67 antigen is a useful non-histonic protein, is expressed in all active phases of the cell cycle (Ki-67 is not expressed in the G0 phase). An increase in Ki-67-expression indicates an increase in mitotic cell activity and proliferation.[11] Expression of the Ki-67 protein (pKi67) is associated with the proliferative activity of intrinsic cell populations in malignant tumors, allowing it to be used as a marker of tumor aggressiveness.[37] Imbalance in the normal regulation of cell proliferation is a defining feature of the cancer phenotype. Ki-67 expression increases progressively across the continuum from

benign breast disease, to ductal carcinoma in situ (DCIS), to invasive breast cancer . In invasive breast cancer, higher Ki-67 levels have been shown to correlate with worse clinical outcome in numerous studies. In DCIS, higher Ki-67 is associated with higher grade lesions.[38-39]

Pavelic ZP et al[40] in 1992 studied c-myc, c-erbB-2, and Ki-67 expression in normal breast tissue and in invasive and noninvasive breast carcinoma. They examined 11 normal breast tissues and 42 invasive and 14 non invasive breast carcinomas for expression of IHC. The c-myc product was detected in all breast carcinoma specimens and in 7 of 11 normal breast tissues. Membrane staining of the c-erbB-2 protein was demonstrated in 29% (4 of 14) of noninvasive ductal carcinomas and in 45% (19 of 42) of invasive breast carcinomas. None of the 11 normal breast tissue samples was positive. The mean value of Ki-67-positive cells was 0.91 Â±0.31% for normal breast tissue, 4.57 Â±1.36% for noninvasive ductal carcinoma, and 12.76 Â±2.18% for invasive breast cancer.

Eriksson ET et al[41] in 1994 studied immunohistochemical expression of the cellular phosphoprotein p53 in archival, formalin-fixed, and paraffin-embedded surgical breast tissue specimens (543 patients). They included five samples of normal resting breast parenchyma, 35 benign lesions including benign tumors, 54 hyperplastic lesions with and without atypia, 109 carcinomas in situ, and 340 invasive adenocarcinomas. They found mutant p53 protein expression was absent in normal resting parenchyma and in benign lesions, including benign tumors and epithelial hyperplasias. In invasive carcinomas p53 expression was absent in well differentiated neoplasms. In contrast, 58 of 158 (37%) poorly differentiated invasive carcinomas immunoreacted. Intraductal carcinomas of comedo type and poorly differentiated

invasive carcinomas of comedo type expressed the mutaut p53 protein in seven of 18 cases (39%) and in 14 of 22 cases (64%), respectively. However, they concluded immunohistochemically detectable accumulation of mutant p53 protein cannot be observed before the carcinoma in situ phase.

Schmitt FC et al[42] in 1995 studied immunohistochemical analysis of the p53 gene protein and cytometric assessment of nuclear DNA in a series of 51 cases of intraductal breast proliferation. The study included 22 cases of intraductal hyperplasia without atypia, 6 cases of intraductal hyperplasia with atypia, and 23 cases of pure intraductal carcinoma. Expression of p53 protein was detected in one case of intraductal hyperplasia without atypia (4.5 per cent), one case of intraductal hyperplasia with atypia (16.6 per cent) and six cases of intraductal carcinoma (26.0 per cent). No significant correlation was observed between p53 expression and histological subtype of intraductal carcinoma. The results suggested that some of the changes observed in invasive breast carcinoma, such as p53 expression and aneuploidy, were already present in breast intraductal proliferation, especially in areas with atypia and in intraductal carcinoma.

Done SJ et al[43] in 1998 studied p53 Mutations in Mammary Ductal Carcinoma in Situ but not in Epithelial hyperplasias. They included eight cases with associated ductal carcinoma in situ (DCIS), and in total, 27 distinct tissue samples. In all 27 samples, the identical p53 mutation was identified in the DCIS as was present in the invasive carcinoma. In contrast, no p53 mutations were identified in any of the 21 microdissected foci of epithelial hyperplasia analyzed, including one sample with atypia. They concluded that p53 mutations commonly occur early in breast neoplasia, usually at the stage of DCIS, but are not often identified in foci of hyperplasia.

Allered D.C et al[15] studied biomarkers in benign breast diseases and showed the vast majority (96.7%) of women with p53-positive lesions did not develop cancer within the time frame of the study, and the vast majority (86%) of women who developed cancer did not have p53-positive benign disease.

Rohan TE et al[44] 1998 showed p53 protein accumulation, but not cerbB-2 protein overexpression, appears to be associated with an increased risk of progression to breast cancer in women with benign breast disease. They conducted case–control study nested within the cohort of 4888 women in the National Breast Screening Study (NBSS) who were diagnosed with benign breast disease during active follow-up. Case subjects were the women who subsequently developed breast cancer (ductal carcinoma in situ [DCIS] or invasive carcinoma). Accumulation of p53 protein was associated with an increased risk of progression to breast cancer.

Kandal R et al[45] in 2000 demonstrated that p53 protein accumulation detected by immunohistochemistry in normal or benign breast tissue was associated with a 2.5-fold increase in the risk of subsequent breast cancer. In this study, we investigated whether p53 gene mutations were present in the 29 p53 immunopositive normal or benign breast tissue samples and in 15 p53 immunonegative normal or benign breast tissue samples selected randomly from the original study. Sixteen (59.2%) of the 27 immunopositive breast tissue samples and 4 (26.7%) of the 15 immunonegative samples had p53 sequence changes. There was no obvious association between the occurrence of these alterations and any specific histopathologic features.

Chan YJ et al[46] in 2004 performed immunohistochemical analyses using monoclonal antibody to label p53 protein and another monoclonal antibody MIB-1 to label Ki-67

antigen on the tissue sections of 63 phyllodes tumor(PT) from 56 patients. The percentages of positive staining tumor cells were compared with the tumor gradings and clinical outcomes. The p53 protein expression showed a significant difference between benign and malignant lesions. Within the group of benign lesions, 5 out of 50 (10%) tumors had p53 expression > 10%, whereas nine out of 13 (69%) malignant tumors revealed p53 expression > 10% ($p < 0.005$). The Ki-67 antigen was also well correlated with tumor grading. Eleven out of 13 (85%) malignant tumors but only 8 out of 50 (16%) benign tumors showed Ki-67 antigen increased > 10% ($p < 0.005$). Three patients progressed from benign to malignant tumors. All the first and recurrent tumors in these 3 patient showed Ki-67 > 10%.

Mylonas I et al[47] in 2004 studied Expression of Her2/neu, Steroid Receptors (ER and PR), Ki67 and p53 in Invasive mammary ductal carcinoma associated with ductal carcinoma in Situ (DCIS) Versus invasive breast cancer alone.They examined 130 cases of Infiltrating ductal carcinoma and 36 cases of infilterating ductal carcinoma / ductal carcinoma in situ by immunohistochemistry. They found Her2/neu amplification in 49.6% of IDC compared to 31% of IDC/DCIS ($p<0.05$). ER expression showed no statistical differences between IDC and IDC/DCIS. The PR expression was demonstrated in 71% of IDC with significantly lower intensity than IDC/DCIS ($p<0.05$). The Ki67 expression was significantly higher ($p<0.05$) in IDC cases (64%) versus IDC/DCIS (49.7%). No differences were observed between IDC and IDC/DCIS for p53 expression. They concluded that DCIS might be a malignant preform and the interaction with neoplastic tissue could result in an aggressive type of invasive tumor.

Skerlev M.S et al[48] in 2005 conducted a study to assess the expression of protein products of c-myc, erbB-2, p53, nm23-H1 gene in benign and malignant breast lesions, to estimate their possible coexpresssion and to correlate the results of immunohistochemical analysis with various clinicopathological parameters. They found expression of erbB-2 and p53 in malignant breast diseases was 27% and 34% respectively while these protein were also expressed in benign lesions;7.8% of benign lesions were positive for erbB-2 protein and 19.6% for p53 protein. The expression of nm23-H1 is similar in benign as well as malignant lesions.

They concluded some changes found in the malignant breast tumors such as the presence of mutated p53 protein nad the expression of erbB-2 protein may be found in benign lesions as well.

Yonameri et al[49] in 2006 evaluated the immunohistochemical expression of the epidermal growth receptor(EGFR), HER2/neu, CD117/c-kit, p53 & MIB-1 and also analyzed correlations between the immunohistochemical findings and the clinical outcome.They found none of the phylloides tumor was positive for HER2/neu or CD117/c-kit. Positive staining for p53 in 10 Phylloides tumors(24%), and the median MIB-1 index was 10%. Both p53 expression and the MIB-1 index, but not the expression status of EGFR, were significantly corelated with the recurrence free and overall survival. He concluded p53 expression status and MIB-1 index may be significant prognostic factors in patients with phyllodes tumors, and careful postoperative follow-up may be important in those cases showing positive expression of p53 and/or MIB-1 index.

Rohan TE et al[50] evaluated 104 cases (sections of paraffin-embedded benign breast tissue) and 385 controls in 2006. Out of all total 26 cases and 92 controls showed exonic changes. In conclusion , the results of this study suggest that p53 changes detected in normal or benign breast tissue are associated with increased risk of subsequent breast cancer.

Park D et al[51] in 2007 showed proliferative activity of tumour cells assessed by immunohistochemical Ki-67 expression is one of several prognostic indicators in breast cancer. There was a statistically significant up-regulationof Ki-67 protein in the metastatic deposit compared to where the primary tumor was found . A low Ki-67 index in both the primary and the metastatic tumors was a favorable prognostic factor.

Randae KJ et al[52] in 2009 included 63 untreated female patients with IDC and 32 female patients with fibroadenoma and studied expression of Survivin and mutant p53 using immunohistochemical staining method. In fibroadenoma, 53% of patients expressed Survivin and 13% of patients expressed p53 protein. Statistically significant increase in Survivin and p53 protein expression was observed in carcinoma cases. p53 expression showed negative correlation with both ER and PR status. They concluded increased expression of Survivin and p53 in IDC patients and correlation with hormone receptors suggest that Survivin and p53 along with hormone receptors status are likely to contribute significantly to apoptosis resistance and may serve as therapeutic target that could increase the effectiveness of conventional breast cancer therapy.

Plesan DM et al[11] evaluated total 562 cases of mammary cancer in 2010. Of all 100 cases were of Invasive Mammary Carcinoma. Out of 100, 42 cases with invasive mammary carcinoma were positive for p53. In conclusion, the mutations of p53 was associated with a more aggressive behavior and with a lower survival rate. In mammary carcinoma, Ki-67 can be useful in stratifying patients into two categories , good and bad prognosis.

Santisteban M et al[53] evaluated immunohistochemical expression of Ki-67 in 192 cases of atypical hyperplasia in breast in 2010. Also evaluated risk of breast cancer within 10 year and after 10 year of atypia biopsy. Out of all 32 women developed breast cancer over a median of 14.6 years. 30% (58 cases) of the atypias had $\geq$2% cells staining for Ki67. In these women, the risk of breast cancer within 10 years after atypia was increased but not in those with <2% staining. Specifically, the cumulative incidence for breast cancer at 10 years was 14% in the high Ki67 vs. 3% in the low Ki67 group. Ki-67 appears to be a time varying biomarker that may help to better stratify risk in women with atypia.

Mao X et al[54] in 2010 studied 140 cases for p53 mutations in non-invasive breast lesions, including UDH, ADH and DCIS, by high-resolution melting (HRM), followed by DNA sequence analysis and also studied 240 non-invasive breast lesions, which were subjected to the immunohistochemical staining of p53 protein. p53 protein expression was detected in none of the UDH, 14.6% of the ADH and 31.4% of the DCIS samples. Statistically, p53 mutation and protein accumulation gradually increased from UDH to ADH and to DCIS ($P<0.05$). There was a significantly positive association between p53 mutations and expression in these samples. p53 mutations and accumulation occur in non-invasive breast lesions,

including ADH and DCIS, and may represent early events in breast carcinogenesis .

Kabat CG et al[25] evaluated 497 breast cancers cases and 471 controls in 2011. In conclusion, the findings from this study suggested that the combined assessment of p53 overexpression and mutations in women with normal or benign breast tissue may identify a subgroup at increased risk of developing invasive breast cancer.

Kucuk U et al[55] evaluated 26 cases of benign and malignant phyllodes tumor in 2013. Of all 17 cases were benign and nine were malignant phyllodes tumor. In the benign group, the p53 positivity was <20% in 15 cases and 21-42% in two cases. In the malignant group, the p53 positivity was >41% in five cases, 21-40% in three cases and <20% in one case. p53 expression was statistically significantly higher in the malignant tumors than in the benign ones. All the benign tumors showed Ki-67 positivity less than 10% of the stromal cells. But, in the stroma of the malignant tumors, Ki-67 was <10% in four cases and greater than or equal to10% in five cases.

Sathyalakshmi R et al[30] evaluated 694 cases of breast lesions in 2014. Of all 482 cases were of benign breast lesions and 212 cases were of malignant breast lesions. A total of 20 cases were selected for immunohistochemical studies - five cases were of non-proliferative lesions , eight cases were of proliferative lesions without atypia and seven cases were of atypia . Ki-67 positivity was found in seven cases of proliferative lesions with atypia and eight cases of proliferative lesions without atypia. The proliferative index values was very high in case of lesions belonging to Atypical ductal hyperplasia and DCIS. In normal breast Ki-67 was expressed at a very low level (<3% of cells). In this study seven cases of proliferative lesions with atypia and eight cases of proliferative lesions without atypia showed high proliferative index,

four out of five cases in the benign non-proliferative lesion category showed low proliferation rate and one case showed high proliferative index.

Shokouh TZ et al[56] evaluated 566 cases of breast cancers in 2014. The correlation coefficient between both Ki-67 index and p53 mutation and the size of tumor and age was calculated. Correlation coefficient between age and Ki-67 expression was significant, whereas the correlation with p53 mutations was not significant.

Hartmann L.C et al[57] in 2015 performed a study with a median of 12 years follow up which showed that only a minority of women(143 among 698; 20%) with atypical hyperplasia eventually progressed to malignancy even without any preventive strategies. The authors concluded that atypical hyperplasia confers an absolutely risk of subsequent breast cancer of 30% at 25 years of follow up.

R.P Tania et al[9] evaluated 50 cases of breast lesions in 2016. Of all cases 20 were benign , 20 were malignant and 10 were normal (control). Expression of p53 positivity was noted in five benign cases and 12 malignant cases. Results of this study showed that p53 over expression was significant in all grades and stages of breast cancer. p53 correlated well with the grade and stage of tumor indicating that p53 positive tumors were biologically aggressive and were associated with poor prognosis.

Rachna et al[35] evaluated 15 patients each of benign, proliferative and invasive breast disease. The mean ER+/Ki-67+ in benign, proliferative and invasive tumors was 0.81, 0.87 and 1.42 respectively. In benign, proliferative and malignant breast lesion the percentage of Ki-67+ cells ranged from 13.8% to 30% , 4-34.5% , 4-34.5%

respectively.

Muhammas E.M.S et al[58] 2012 conducted a study for the immunohistochemical profile of p53 in breast carcinoma and also assessed its prognostic value in relation to clinico-pathological prognostic factors of breast carcinoma. They included 45 specimens of breast carcinoma. p53 was weakly expressed in 11% of areas of benign breast disease. P53 was negative in all cases of low grade ductal carcinoma in situ (DCIS), positive in 2/3 of intermediate grade DCIS, and positive in all cases of high grade DCIS. All grade I invasive breast carcinoma (IBC) were negative for p53, 50% of grade II and 91% of grade III IBC were positive for p53. p53 expression increased significantly with increased tumor grade of IBC ($p<0.006$), lymphovascular invasion ($p<0.003$) and lymphocytic infiltration ($p<0.004$). They concluded P53 is an indicator for poor prognosis in breast cancer being positively correlated to tumor grade, presence of lymphovascular invasion.

Posso M et al[6] in 2017 conducted a nested case–control study. Women with breast cancer and prior BBDs (86 cases) were matched to women with prior BBDs who were free from breast cancer (172 controls). ER, PR, and Ki67 expression were obtained from BBDs' specimens. Women with >90% of ER expression had a higher risk of breast cancer than women with ≤70% of ER expression. Similarly, women with >80% of PR expression had a higher risk of breast cancer than women with ≤40% of PR expression. Women with proliferative disease and ≥1% of Ki67 expression had a nonsignificantly increased risk of breast cancer than women with <1% of Ki67 expression. A high expression of ER and PR in BBD is associated with an increased risk of subsequent breast cancer. In proliferative disease, high Ki67 expression may also have an increased risk.

Acs B et al[59] in 2017 studied Ki-67 as a controversial predictive and prognostic marker in breast cancer patients treated with neoadjuvant chemotherapy. One hundred twenty patients diagnosed with invasive breast cancer and treated with neoadjuvant chemotherapy (NAC) between 2002 and 2013 were retrospectively recruited to this study. Twenty three out of 120 patients (19.2%) achieved pathologic complete remission (pCR), whereas partial remission (pPR) and no response (pNR) to neoadjuvant chemotherapy (NAC) was detected in 60.8% and 20.0%, respectively.

Ragab H.M et al[60] in 2018 they studied assessment of Ki-67 as a potential biomarker in patients with breast cancer. This study included 92 patients with developed non metastatic breast cancer and 10 women had benign breast tumor served as Positive controls while 10 healthy woman served as negative controls.They measured the serum level by ELISA technique and tissue expression of Ki-67 by immunohistochemical technique. They concluded that tissue Ki-67 expression may add prognostic information.

AIMS AND OBJECTIVES

1. To evaluate the expression of p53 and Ki-67 in benign epithelial lesion, proliferative breast disease without atypia , proliferative breast disease with atypia and carcinoma breast.

2. To correlate p53 and Ki-67 expression with histopathologic subtypes of breast disease.

MATERIAL AND METHODS

Study Design

The study was based on breast specimens including lumpectomy and modified radical mastectomy specimens received in the Department of Pathology, SGT Hospital, FMHS, Gurugram referred by the Department of Surgery. Total of 50 cases of breast lesions were studied.

Study period

This was a prospective study for one year based upon cases presented during May 2017- June 2018.

Hematoxylin & Eosin staining was done on paraffin sections of breast specimens including lumpectomy and modified radical mastectomy and cases were divided into the following categories:- Benign epithelial lesion, Proliferative breast disease without atypia, Proliferative breast disease with atypia, and Carcinoma breast. Immunohistochemical staining for p53 and Ki-67 was done on paraffin sections as per standard procedure.

Inclusion Criteria

All types of breast specimens including lumpectomy and modified radical mastectomy were included in the present study.

Exclusion Criteria

Inflammatory breast lesions and metastatic breast were excluded from the study.

Methods

The following staining procedure was adapted:-

Staining procedure :- Hematoxylin & Eosin

1. Put slides on hot plate and then deparaffinize the sections with xylene 2 changes.
2. Take sections through descending levels of alcohol 90%, 80%, 70% to water for 30-60 seconds each.
3. Wash in tap water and rinse in distilled water.
4. Stain with Harris's hematoxylin for 10-15 minutes.
5. Wash in running tap water.
6. Differentiate in 0.5% HCl for 5-10 seconds.
7. Wash in water.
8. Blue in ammonia water, followed by 5 min tap water wash.
9. Counter stain with 1% Eosin Y for 2-4 minutes.
10. Dehydrate through ascending levels of alcohol 70%, 80%, 95%.
11. Clear in xylene. 3 changes
12. Mount in DPX.

IMMUNOHISTOCHEMICAL STAINING FOR p53:-

IHC was performed by peroxidase-antiperoxidase method in the following manner.

1. Mount 3-4 μm sections on slides coated with suitable tissue adhesive.
2. Deparaffinise in xylene and rehydrate through graded alcohols.
3. Wash slides in running tap water.
4. Antigen retrieval using Citrate or Tris EDTA (Ethylene Diamine Tetraacetic Acid) done in pressure cooker or microwave.
5. Sections rinse in Tris Buffer Saline (TBS) and excess TBS drained off.
6. Endogenous peroxidase activity is blocked using peroxidase block for 20 minutes.
7. Sections wash in TBS for 5 minutes.
8. Incubate with protein block for 5 minutes.
9. Wash in TBS.
10. Optimally diluted primary antibody applied for 60 minutes. (Anti-p53 monoclonal antibody)
11. Wash in TBS.

12. Incubate with a post primary block for 30 minutes.
13. Wash in TBS.
14. Incubate with polymer for 30 minutes.
15. Wash in TBS.
16. Incubate in DAB (Diamino Benzidine) solution for 10 minutes.
17. Rinse slides then rinse in TBS and transfer to running water.
18. Counterstain with hematoxylin.
19. Dehydrate in graded alcohols and xylene.
20. Clearing and mounting is done in DPX mountant.

IMMUNOHISTOCHEMICAL STAINING FOR Ki-67 :-

1. Mount 3-4 µm sections on slides coated with suitable tissue adhesive.
2. Deparaffinise in xylene and rehydrate through graded alcohols.
3. Wash slides in running tap water.
4. Antigen retrieval using Citrate or Tris EDTA (Ethylene Diamine Tetraacetic Acid) done in pressure cooker or microwave.
5. Sections rinse in Tris Buffer Saline (TBS) and excess TBS drained off.
6. Endogenous peroxidase activity is blocked using peroxidase block for 20 minutes.
7. Sections wash in TBS for 5 minutes.
8. Incubate with protein block for 5 minutes.
9. Wash in TBS.
10. Optimally diluted primary antibody applied for 60 minutes. (Nuclear antibody MIB-1)
11. Wash in TBS.
12. Incubate with a post primary block for 30 minutes.
13. Wash in TBS.
14. Incubate with polymer for 30 minutes.
15. Wash in TBS.
16. Incubate in DAB (DiaminoBenzidine) solution for 10 minutes.
17. Rinse slides then rinse in TBS and transfer to running water.

18. Counterstain with hematoxylin.

19. Dehydrate in graded alcohols and xylene.

20. Clearing and mounting is done in DPX mountant.

p53 expression –

Nuclear staining was considered positive (dark brown precipitate).

500 cells were counted.

p53 expression[24]

Negative	% of stained cells less than 10%
Positive	% of stained cells more than 10%

Scoring system for positive cases[9]-

0	<5% of the cells revealed positivity for the marker
1+	6-10% positive tumor cells
2+	11-25 % positive tumor cells
3+	26-50% positive tumor cells
4+	>51% positive tumor cells

Ki-67 expression –

Nuclear staining was considered as positive staining (dark brown precipitate).

500 cells were counted.

Scoring system [24]

Negative	% of stained cells will be less than or equal to 2%
1+	% of stained cells will be between 2 to 25%
2+	% of stained cells will be between 26 to 50%
3+	% of stained cells will be between 51 to 75%
4+	% of stained cells will be between 76 to 100%

STATISTICAL ANALYSIS

Categorical variables were presented in number and percentage (%) and continuous variables were presented as mean ± SD and median. Data was tested by Kolmogorov-Smirnov test.

Statistical tests were applied as follows –

1. Quantitative variables were compared using ANOVA (as the data sets were normally distributed) between the four categories.
2. Qualitative variables were correlated using Chi-Square test.
3. Inter rater kappa agreement was used to find out strength of association between Ki67 and p53 expression.

A p value of less than 0.05 was considered as statistically significant difference.

Descriptive statistics was analyzed with SPSS version 21.0 software.

OBSRVATIONS AND RESULTS

The present study was conducted on breast lumpectomy and MRM specimens received in the Department of Pathology, SGT Medical College and Hospital, Gurugram, Haryana. A total of 50 cases were studied, 30 cases were benign and 20 were malignant. Sections were stained by H&E. Histological diagnosis was made and cases were categorized under four categories :- CAT A (Benign breast lesions), CAT B (Proliferative breast lesions without atypia), CAT C (Proliferative breast lesions with atypia) and CAT D (Carcinoma breast). IHC staining of Ki-67 and p53 was prformed on all cases.

Table 1.1 : Distribution of breast lesions according to age

AGE	Frequency	Percentage
1) <=20	2	4.00%
2) 21-30	14	28.00%
3) 31-40	15	30.00%
4) 41-50	13	26.00%
5) 51-60	5	10.00%
6) >60	1	2.00%
Total	50	100.00%

The age group of the cases ranged from 18 to 70 years with the mean age of 38.2 years. The highest incidence was seen in 31 to 40 years of age group 15(30%).

Table 1.2 : Category wise distribution of breast lesions according to age

	CAT				
	A	B	C	D	P value
AGE					
Sample size	16	8	5	21	
Mean ± Stdev	26.12 ± 6.17	35.88 ± 12.47	37.8 ± 4.15	48.43 ± 7.74	<.0001
Median	26	35	40	50	
Min-Max	18-40	23-56	32-42	37-70	
Inter quartile Range	21 - 30	24 – 45	34.250 - 40.500	41.500 - 51.250	

Table 2 : Age distribution of cases in different categories

		CAT				Total	P value
		A	B	C	D		
Age distribution	1) <=20	2 (12.50%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	2 (4.00%)	0.0002
	2) 21-30	11 (68.75%)	3 (37.50%)	0 (0.00%)	0 (0.00%)	14 (28.00%)	
	3) 31-40	3 (18.75%)	3 (37.50%)	4 (80.00%)	5 (23.81%)	15 (30.00%)	
	4) 41-50	0 (0.00%)	1 (12.50%)	1 (20.00%)	11 (52.38%)	13 (26.00%)	
	5) 51-60	0 (0.00%)	1 (12.50%)	0 (0.00%)	4 (19.05%)	5 (10.00%)	
	6) >60	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (4.76%)	1 (2.00%)	
Total		16 (100.00%)	8 (100.00%)	5 (100.00%)	21 (100.00%)	50 (100.00%)	

Out of 16 cases in category A, highest frequency 11(68.75%) was seen in age group 21 to 30 years. In CAT B 3(37.50%) each were seen in age group 21-30 and 31-40 years whereas in CAT D 11(52.38%), highest frequency was noted in age group 41 to 50 years.

Table 3 : Type of specimen

Type of specimen		
	Frequency	Percentage
Lumpectomy	30	60.00%
MRM	20	40.00%
Total	50	100.00%

Out of 50 analyzed cases, 30(60%) were breast lumpectomy specimens and 20(40%) were modified radical mastectomy(MRM).

Table 4 : Distribution of cases according to side in breast lesions

		CAT				Total	P value
		A	B	C	D		
Side	Bilateral	1 (6.25%)	1 (12.50%)	0 (0.00%)	0 (0.00%)	2 (4.00%)	0.718
	Left	9 (56.25%)	3 (37.50%)	2 (40.00%)	11 (52.38%)	25 (50.00%)	
	Right	6 (37.50%)	4 (50.00%)	3 (60.00%)	10 (47.62%)	23 (46.00%)	
Total		16 (100.00%)	8 (100.00%)	5 (100.00%)	21 (100.00%)	50 (100.00%)	

In CAT A and D the number of cases on left side were 9 (56.25%) and 11(52.38%) respectively; whereas in CAT B 4(50%) and 3(60%) C, more number of cases were seen on the right side.

Table 5 : Distribution of site in breast lesions in various categories

SITE	CAT A	CAT B	CAT C	CAT D
UPPER OUTER	4(23.53%)	4(44.44%)	3(60%)	9(42.86%)
UPPER INNER	4(23.53%)	2(22.22%)	0(0%)	1(4.76%)
LOWER OUTER	6(35.29%)	1(11.12%)	2(40%)	7(33.33%)
LOWER INNER	3(17.65%)	2(22.22%)	0(0%)	4(19.05%)
Total	17(100%)	9(100%)	5(100%)	21(100%)

In CAT A 6(35.29%) maximum number of cases were in lower outer quadrant whereas in CAT B 4 (44.44%), CAT C 3(60%) & CAT D 9 (42.86%) maximum number of cases were reported in upper outer quadrant.

Table 6 : Histopathological diagnosis of breast lesions

		No. of cases
HISTOPATHOLOGICAL DIAGNOSIS	FIBROADENOMA	12(24%)
	FIBROADENOMA WITH FIBRCYS CHANGE	2(4%)
	BENIGN PHYLLODES	2(4%)
	FIBROADENOMA WITH EPI HYP	4(8%)
	UDH	4(8%)
	ADH	5(10%)
	DCIS	1(2%)
	IDC	17(34%)
	METAPLASTIC CA BREAST	1(2%)
	MUCINOUS CARCINOMA	1(2%)
	PHYLLODES TUMOR(M)	1(2%)
	TOTAL	50(100%)

- Fibroadenoma with fibrcys change – Fibroadenoma with fibrocystic change
- Fibroadenoma with epi hyp – Fibroadenoma with epithelial hyperplasia

Out of 50 analyzed cases, 30 (60%) were reported as benign breast lesions whereas 20 (40%) cases were from malignant.

Table 7 : Cellular and Nuclear Pleomorphism in CAT D

	Present	Absent
CELLULAR PLEOMORPHISM	21(100%)	0(0%)
NUCLEAR PLEOMORPHISM	21(100%)	0(0%)

All cases in CAT D 21(100%) were characterized by cellular and nuclear pleomorphism.

Table 8 : Ki-67 expression in Categories A, B, C & D

		CAT				Total	P value
		A(n=16)	B(n=8)	C(n=5)	D(n=21)		
Ki-67 expression	Present	4 (25.00%)	2 (25.00%)	3 (60.00%)	19 (90.48%)	28 (56.00%)	0.0002
	Absent	12 (75.00%)	6 (75.00%)	2 (40.00%)	2 (9.52%)	22 (44.00%)	
Total		16 (100.00%)	8 (100.00%)	5 (100.00%)	21 (100.00%)	50 (100.00%)	

In CAT A and B, 4 (25%) and 2 (25%) cases showed positivity for Ki-67 whereas in CAT C and D, 3 (60%) and 19(90.48%) cases were positive.

Table 9 : Co-relation of Ki-67 grading in Categories A, B, C & D

		CAT				P value
		A(n=16)	B(n=8)	C(n=5)	D(n=21)	
Ki-67 expression Grading	1+	4 (25%)	2 (25%)	0 (0.00%)	1 (4.76%)	0.004
	2+	0 (0.00%)	0 (0.00%)	1 (20%)	2 (9.52%)	
	3+	0 (0.00%)	0 (0.00%)	1 (20%)	8 (38.09%)	
	4+	0 (0.00%)	0 (0.00%)	1 (20%)	8 (38.09%)	

Ki-67 grading in breast lesions ranged from 1+ to 4+, benign to proliferative to malignant in ascending order. In CAT A(4) & B(2) , 25% of cases showed positivity of grade 1+. In CAT C (3 cases) heterogenous pattern of Ki-67 expression was seen 1(20%) case of each grade were positive ranging from 2+ to 4+ positivity . In CAT D equal number of cases 8(38.09% each) showed immunopositivity for 3+ and 4+ grading.

Table 10 : Co-relation of Ki-67 grading in CAT D

CAT D (n=21)		Ki-67 expression Grading					Total	P value
		0	1+	2+	3+	4+		
IDC (BRG)	Grade I	0 (0.00%)	1 (4.76%)	1 (4.76%)	0 (0.00%)	0 (0.00%)	2 (9.52%)	0.071
	Grade II	0 (0.00%)	0 (0.00%)	1 (4.76%)	3 (14.29%)	1 (4.76%)	5 (23.81%)	
	Grade III	1 (4.76%)	0 (0.00%)	0 (0.00%)	4 (19.05%)	5 (23.81%)	10 (47.62%)	
	Other cases	1 (4.76%)	0 (0.00%)	0 (0.00%)	1 (4.76%)	2 (9.52%)	4 (19.05%)	
Total		2 (9.52%)	1 (4.76%)	2 (9.52%)	8 (38.10%)	8 (38.10%)	21 (100.00%)	

BRG – Bloom Richardson Grading

In present study IDC & other cases of CAT D showed a variable pattern of Ki-67 expression ranging from 1+ to 4+. As the grade increases in IDC , the immunopositivity grading also increases. In Grade III maximum number of cases 5(23.81%) showed 4+ grading whereas one case of IDC is negative for Ki-67.

Table 11 : p53 expression in categories A, B, C & D

		CAT				Total	P value
		A	B	C	D		
p53 expression	Present	4 (25.00%)	2 (25.00%)	3 (60.00%)	19 (90.48%)	28 (56.00%)	0.0002
	Absent	12 (75.00%)	6 (75.00%)	2 (40.00%)	2 (9.52%)	22 (44.00%)	
Total		16 (100.00%)	8 (100.00%)	5 (100.00%)	21 (100.00%)	50 (100.00%)	

In CAT A(4) & B(2) 25% cases showed positivity for p53 expression. In CAT C 3(60%) & D 19(90.48%) showed positivity.

Table 12: Co-relation of grading of p53 expression in categories A, B, C & D

		CAT				P value
		A(n=16)	B(n=8)	C(n=5)	D(n=21)	
p53 expression Grading	1+	3 (18.75%)	2 (25%)	0 (0.00%)	0 (0.00%)	0.002
	2+	1 (6.25%)	0 (0.00%)	0 (0.00%)	5 (23.80%)	
	3+	0 (0.00%)	0 (0.00%)	2 (40%)	6 (28.57%)	
	4+	0 (0.00%)	0 (0.00%)	1 (20%)	8 (38.09%)	
Total		4 (25%)	2 (25%)	3 (60%)	19 (90.46)	

p53 grading ranged from 1+ to 4+ from benign to proliferative to malignant in ascending order. In CAT A 3(18.75) & B 2(25%) maximum number of cases showed positivity belonging to grade 1+ whereas CAT C showed 3+ grading in maximum number of cases 2(40%) & in CAT D maximum cases 8(38.09) showed 4+ positivity.

Table 13 : Co-relation of p53 grading in IDC

		p53 expression Grading				Total	P value
		0	2+	3+	4+		
IDC (BRG)	Grade1	0 (0.00%)	2 (9.52%)	0 (0.00%)	0 (0.00%)	2 (9.52%)	0.110
	Grade 2	0 (0.00%)	1 (4.76%)	3 (14.29%)	1 (4.76%)	5 (23.81%)	
	Grade 3	1 (4.76%)	2 (9.52%)	1 (4.76%)	6 (28.57%)	10 (47.62%)	
	Other	1 (4.76%)	0 (0.00%)	2 (9.52%)	1 (4.76%)	4 (19.05%)	
Total		2 (9.52%)	5 (23.81%)	6 (28.57%)	8 (38.10%)	21 (100.00%)	

In present study IDC & other cases of CAT D showed a variable pattern of p53 expression ranging from 2+ to 4+. As the Grade increases in IDC, the immunopositivity grading also increases. In Grade III IDC maximum number of cases 6(28.57%) showed 4+ grading whereas one case of IDC was negative for p53.

Table 14 : Ki-67 and p53 expression in categories A, B , C & D

CATEGORY	Ki-67 & p53 EXPRESSION					
	POSITIVE FOR BOTH(n=28)	NEGATIVE FOR BOTH(n=22)	POSITIVE		NEGATIVE	
			P53	Ki-67	P53	Ki-67
CAT A	4(14.26%)	12(54.55%)				
CAT B	2(7.14%)	6(27.27%)				
CAT C	3(10.71%)	2(9.09%)				
CAT D	18(64.29)	1(4.55%)	1			1

Out of 50 cases, 28 cases showed positivity for Ki-67 & p53 expression. In CAT A 4(14.26%) & CAT B 2(7.14%) cases were positive for both whereas in CAT C 3(10.71%) & in CAT D 18(64.29%) showed immunopositivity for both wheras there was one case (mucinous carcinoma) negative for Ki-67 & positive for p53 whereas there was another case (metaplastic carcinoma) which showed negative expression for p53 & immunopositivity for Ki-67.

Table 15 : Comparison of Ki-67 and p53 expression in different categories

		p53 expression		Total	P value	Kappa
		Absent	Present			
Ki-67 expression	Absent	21 (42.00%)	1 (2.00%)	22 (44.00%)	<.0001	0.919
	Present	1 (2.00%)	27 (54.00%)	28 (56.00%)		
Total		22 (44.00%)	28 (56.00%)	50 (100.00%)		

Value of ***K***	Strength of agreement
< 0.20	Poor
0.21 - 0.40	Fair
0.41 - 0.60	Moderate
0.61 - 0.80	Good
0.81 - 1.00	Very good

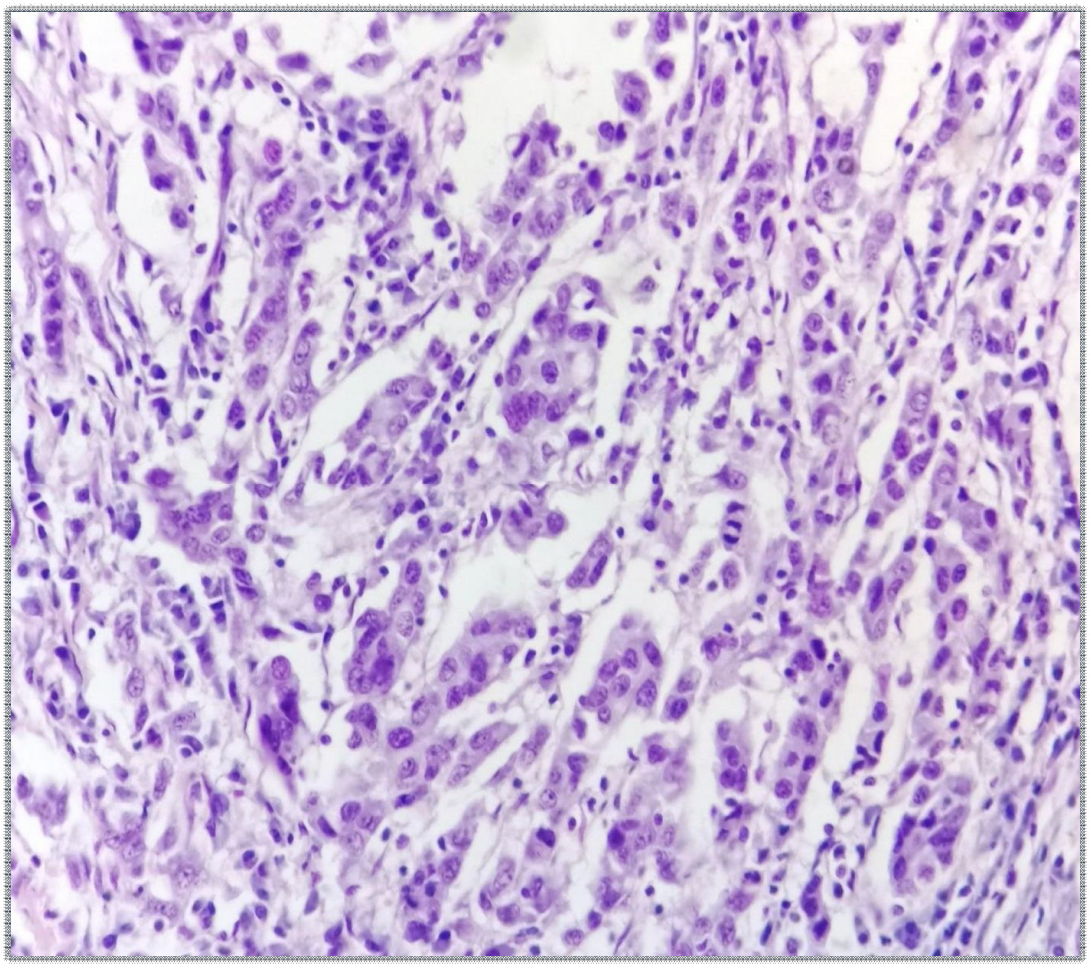


Figure 4 : Infiltrating ductal carcinoma, Grade I (H & E, 400x)

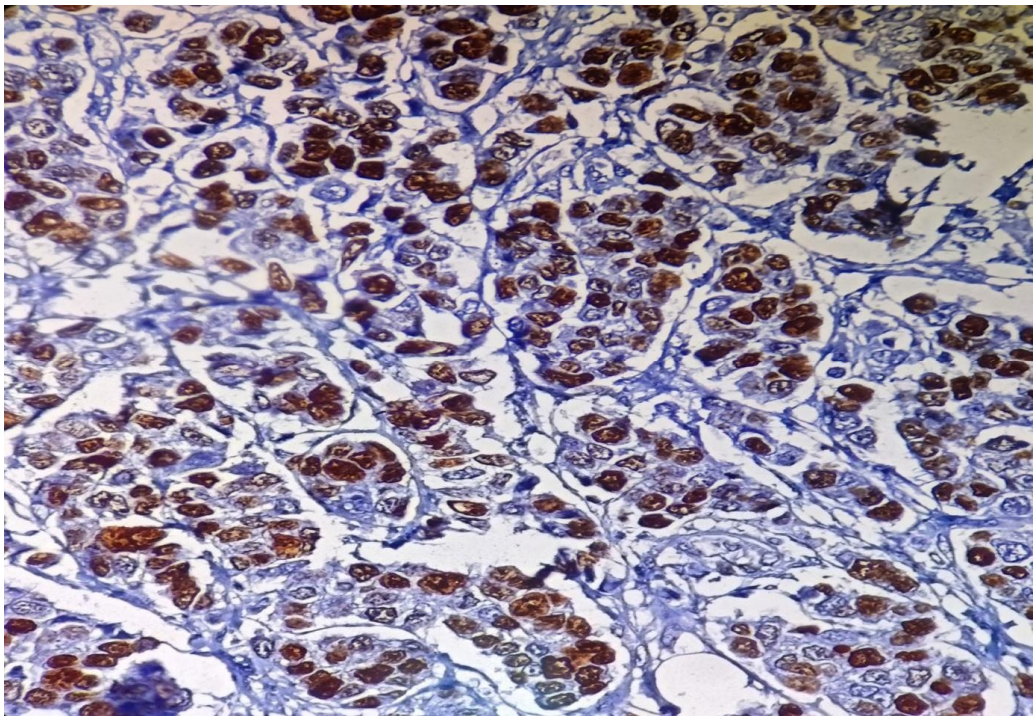


Figure 5 :- Ki-67 expression in IDC, Grade I (IHC, 400x)

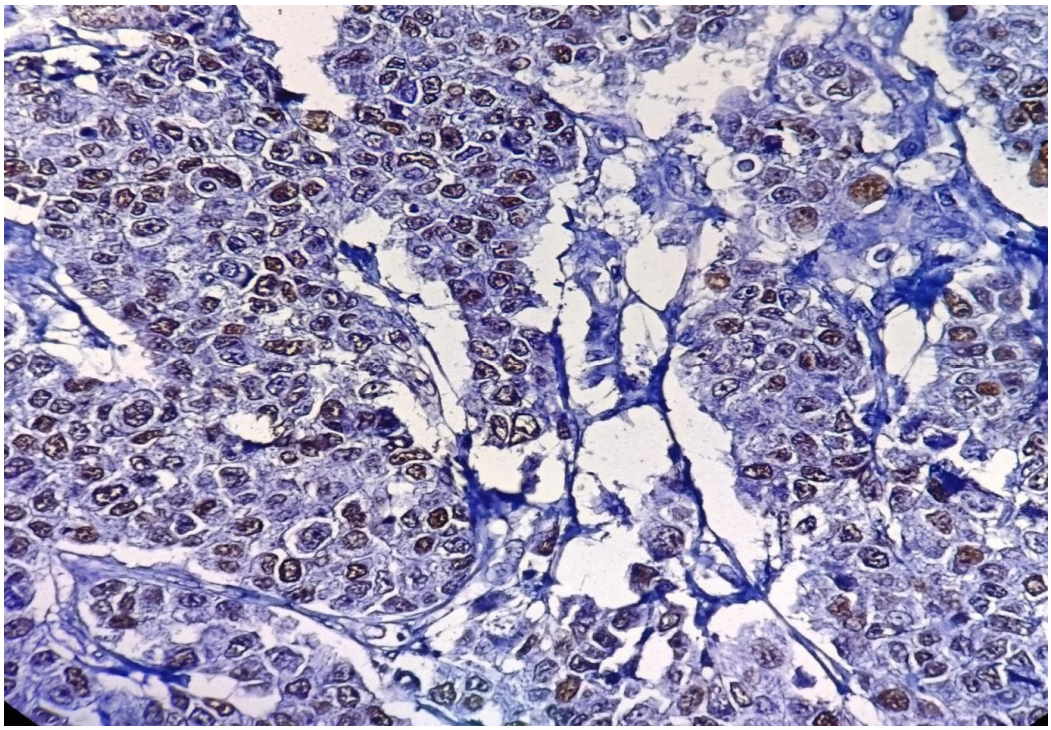


Figure 6 :- p53 expression in IDC, Grade I (IHC, 400x)

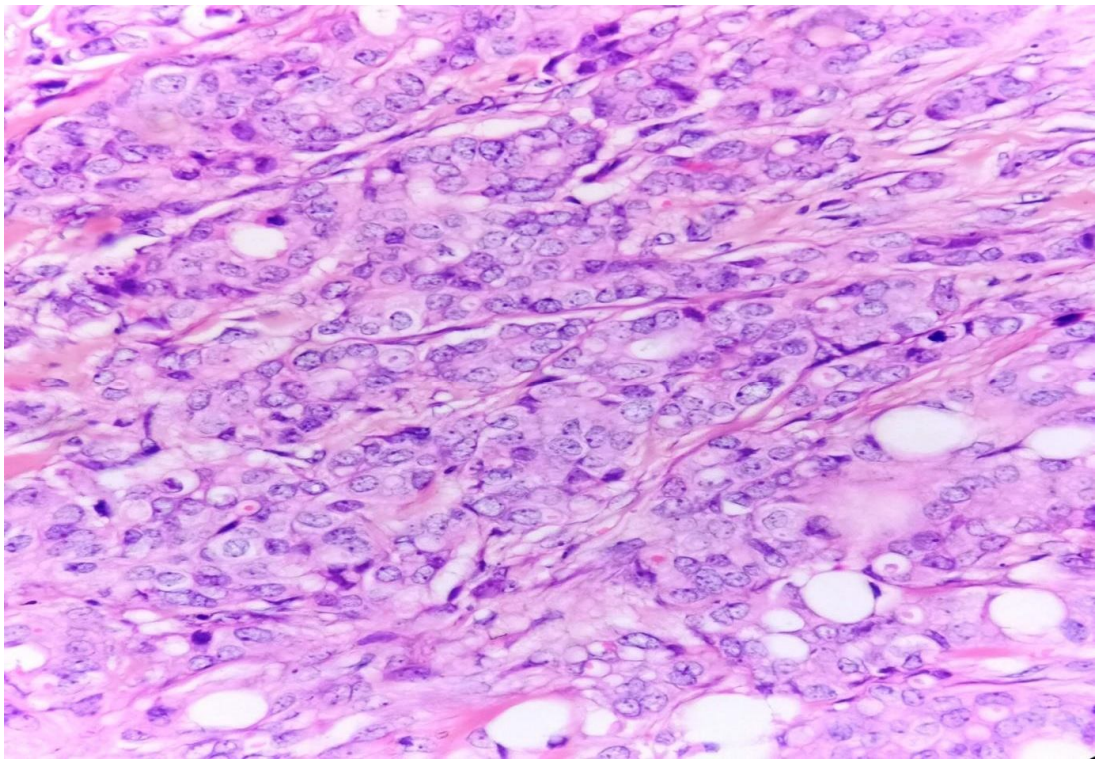


Figure 7:- Infiltrating ductal carcinoma, Grade II (H & E, 400x)

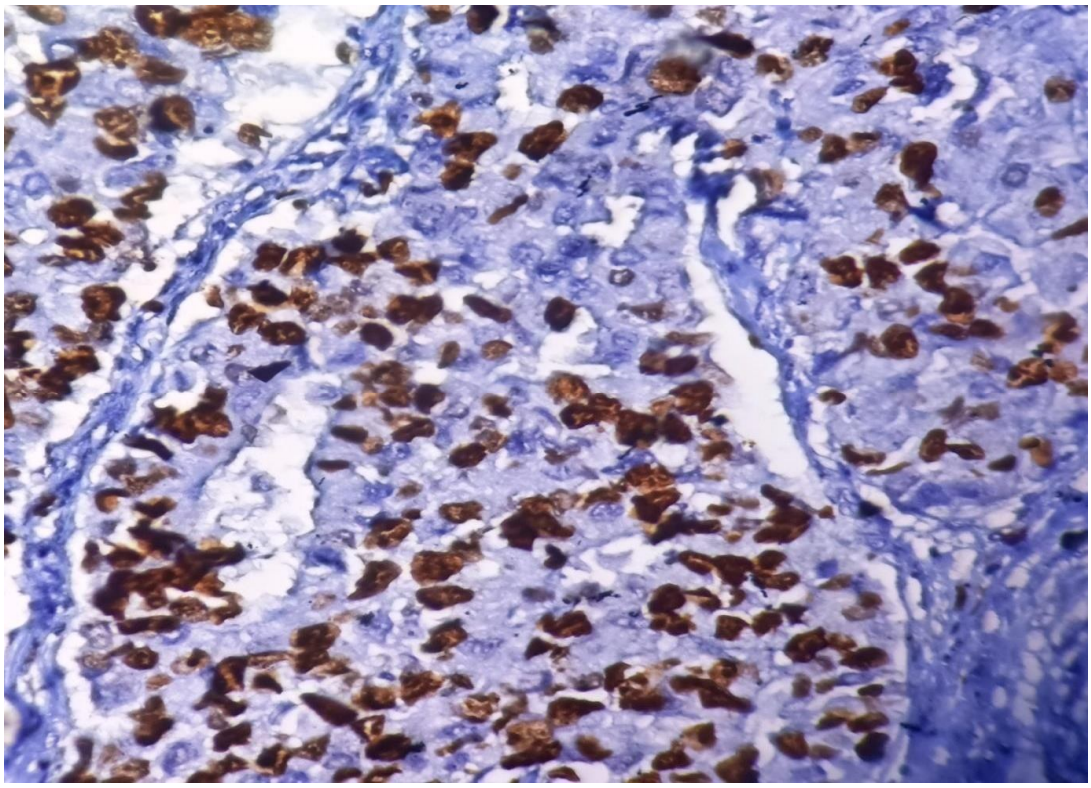


Figure 8 :- Ki-67 expression in IDC, Grade II (H & E, 400x)

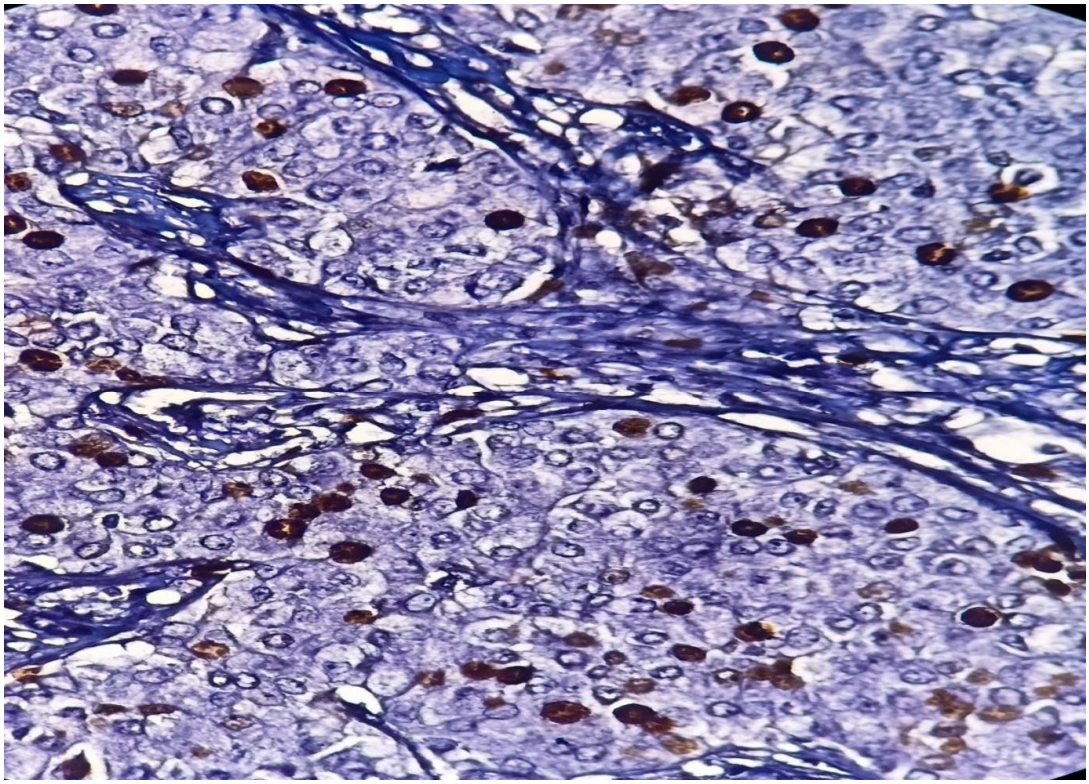


Figure 9 :- p53 expression in IDC, Grade II (H & E, 400x)

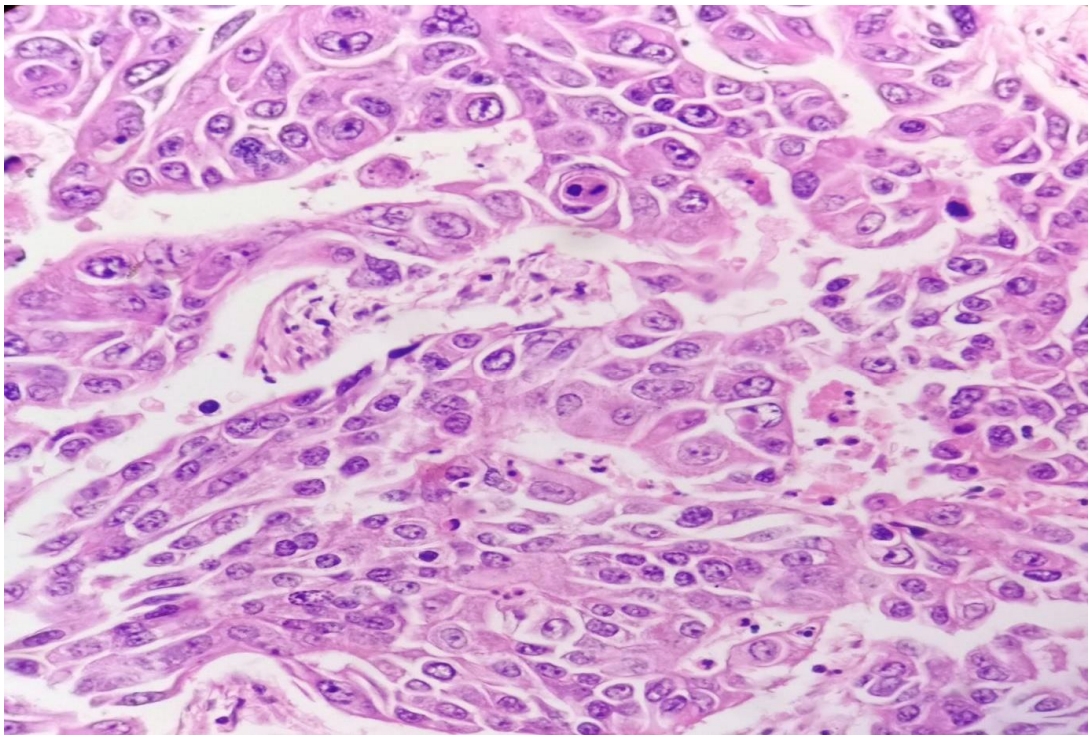


Figure 10 :- Infiltrating ductal carcinoma, Grade III (H & E, 400x)

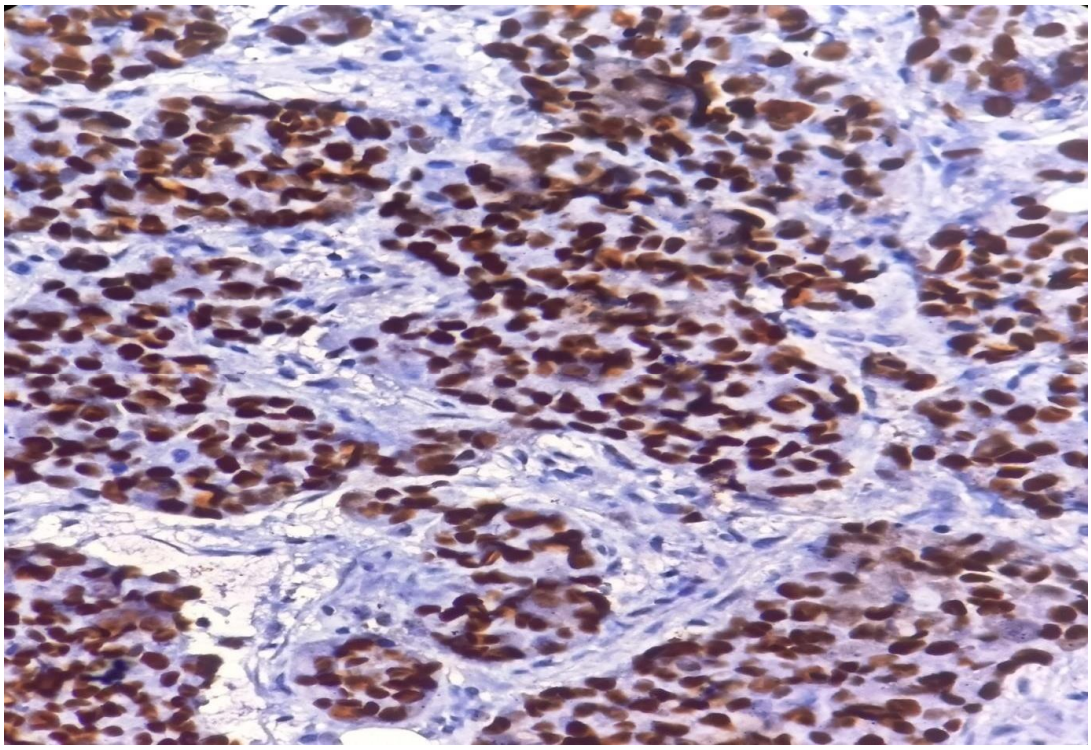


Figure 11 :- Ki-67 expression in IDC, Grade III (IHC, 400x)

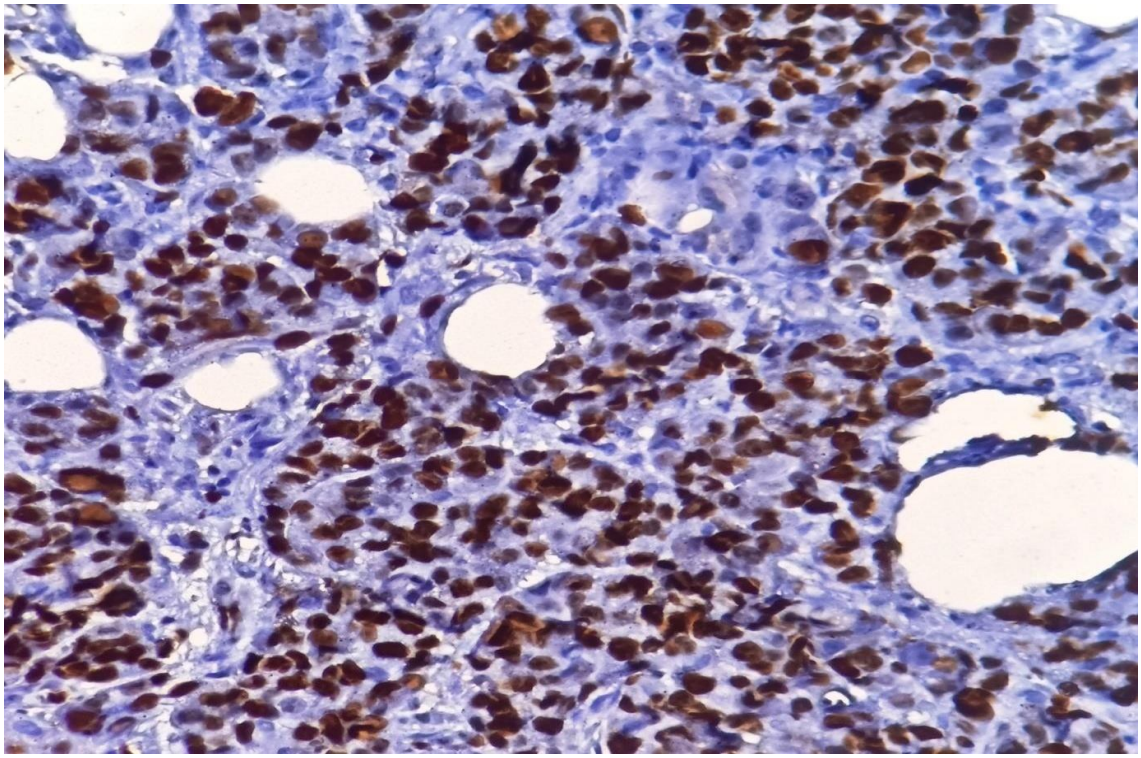


Figure 12 :- p53 expression in IDC, Grade III (IHC, 400x)

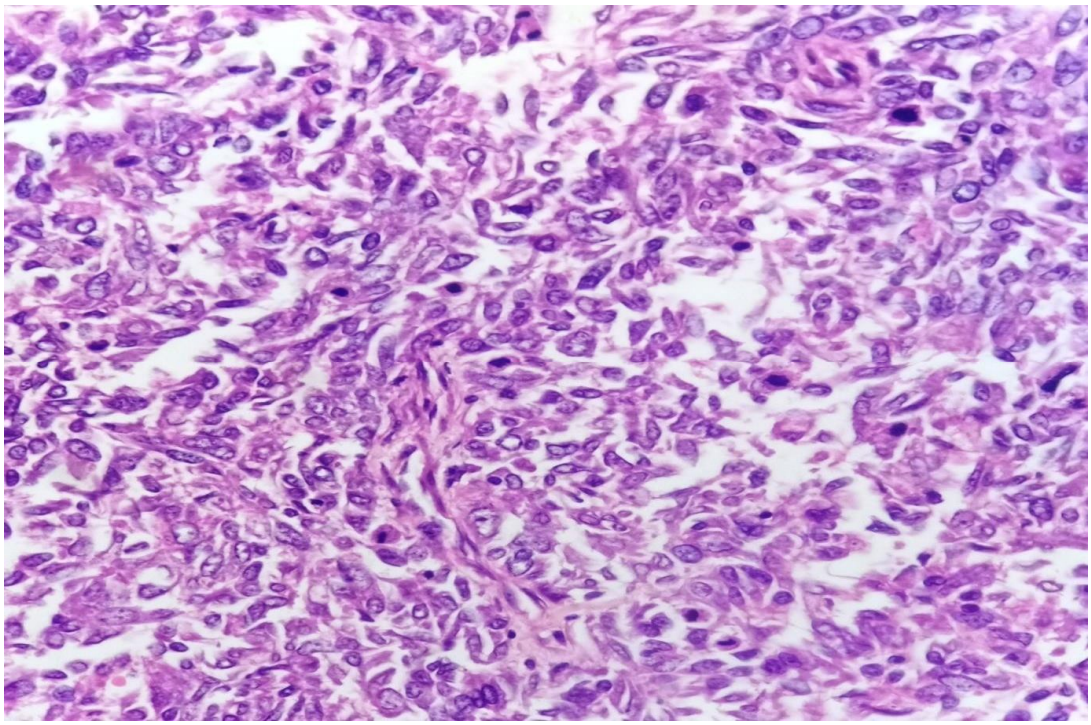


Figure 13 :- Metaplastic breast carcinoma (H & E,400x)

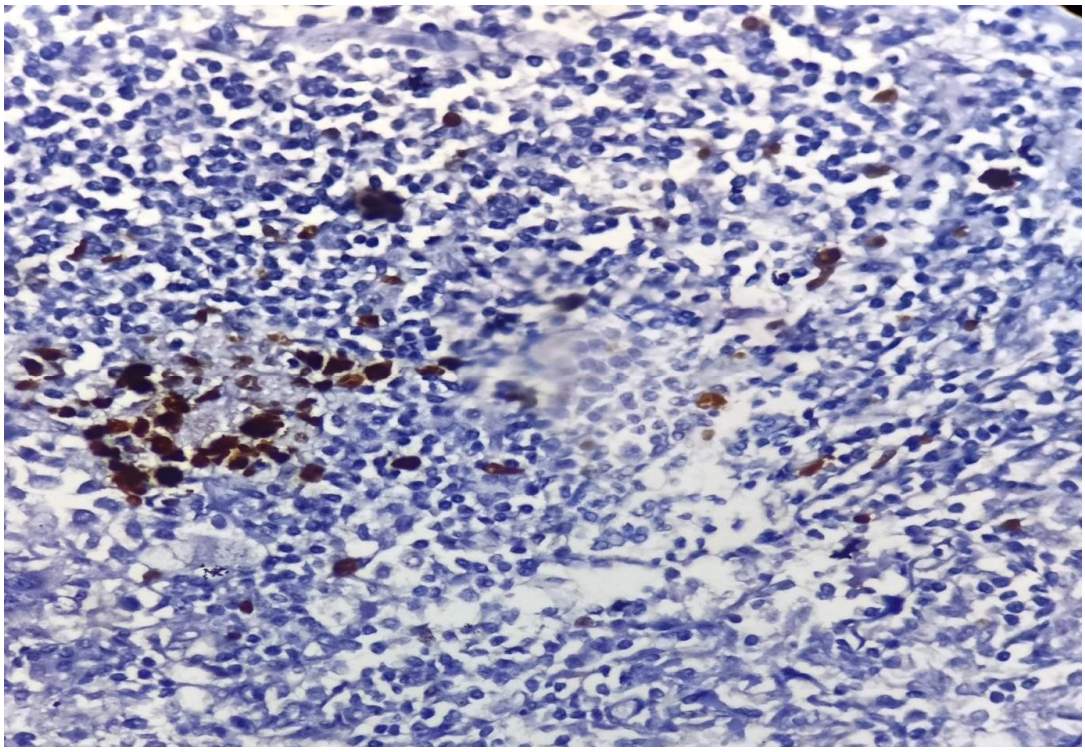


Figure 14:- Ki-67 expression in metaplastic breast carcinoma (IHC, 400x)

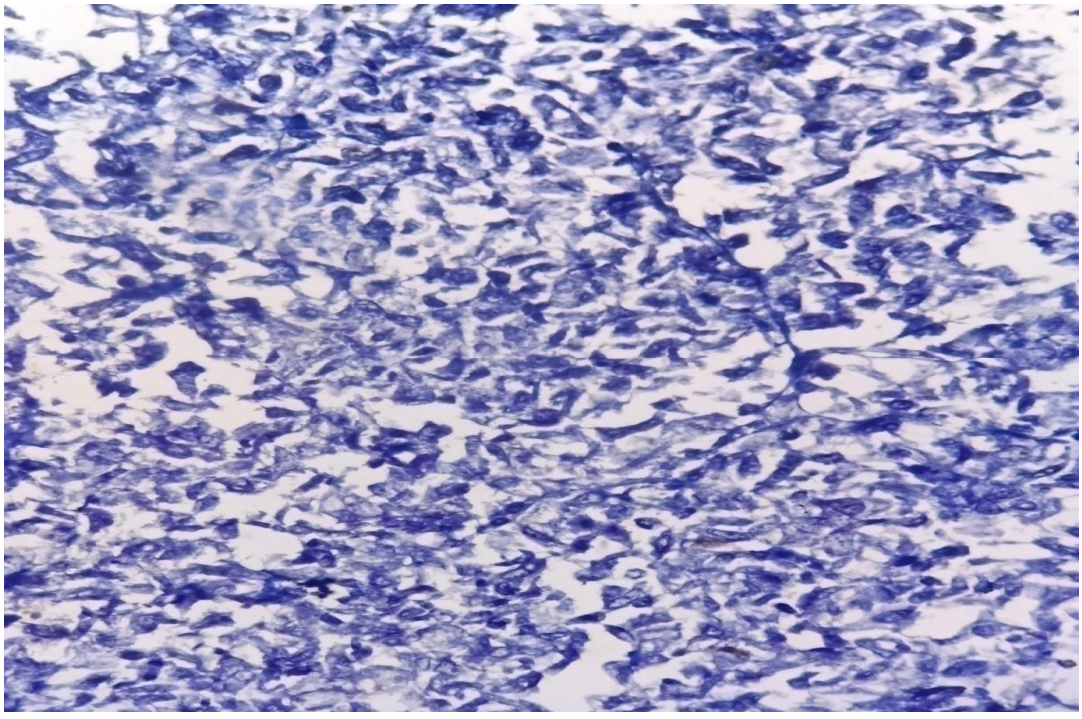


Figure 15 :- Negative expression of p53 in metaplastic breast carcinoma (IHC, 400x)

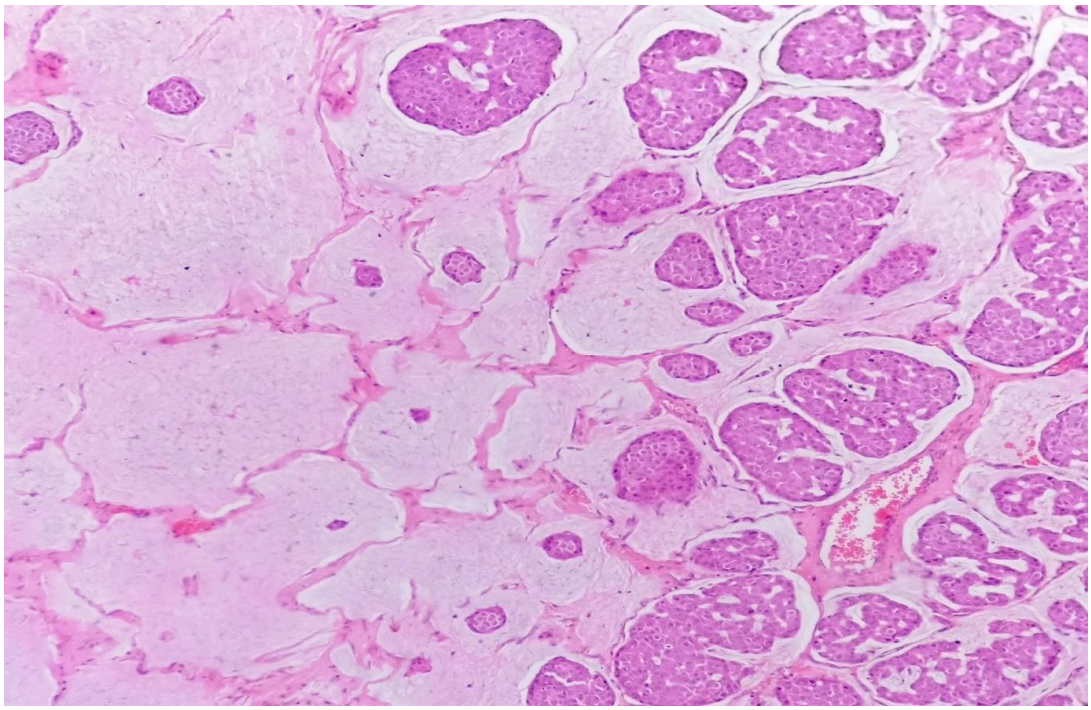


Figure 16 :- Mucinous breast carcinoma (H & E, 100x)

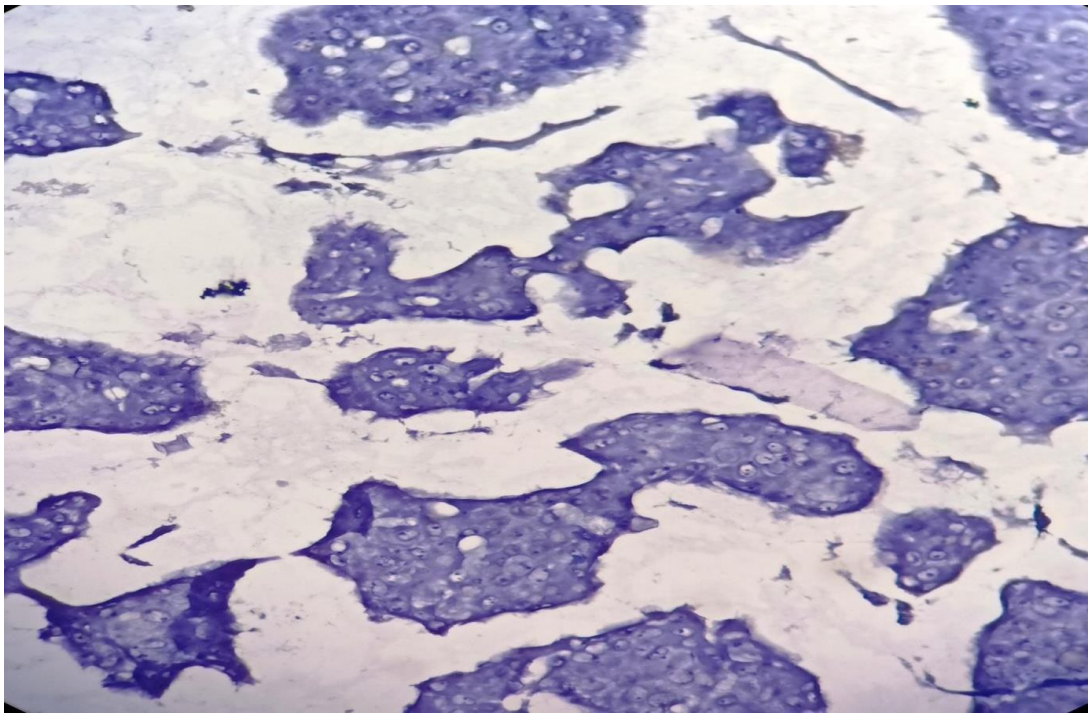


Figure 17 :- Negative Ki-67 expression in mucinous breast carcinoma (H & E, 400x)

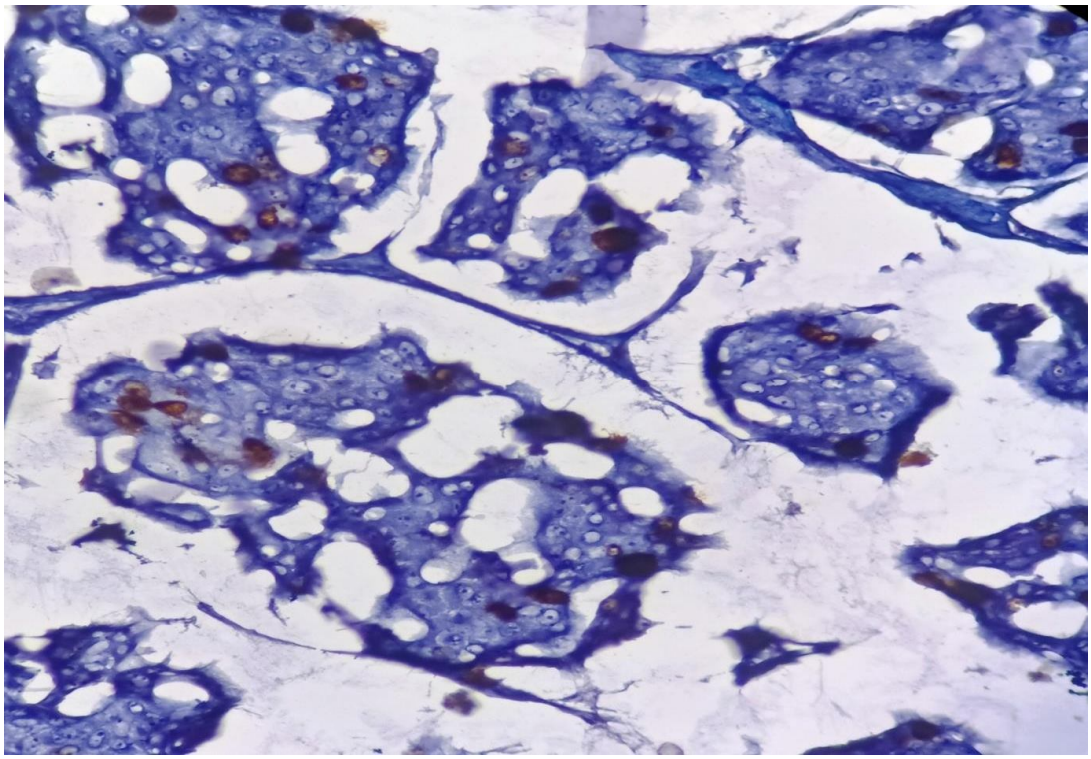


Figure 18 :- p53 expression in mucinous carcinoma breast (IHC, 400x)

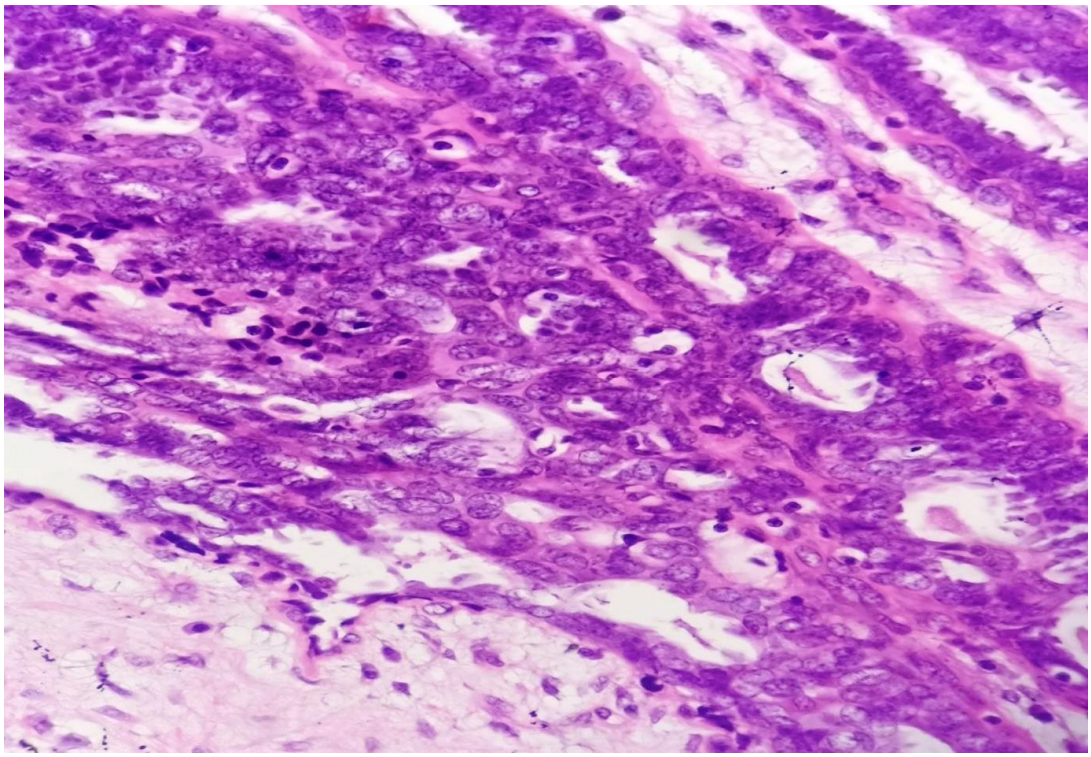


Figure 19 :- Atypical ductal hyperplasia (H & E, 400x)

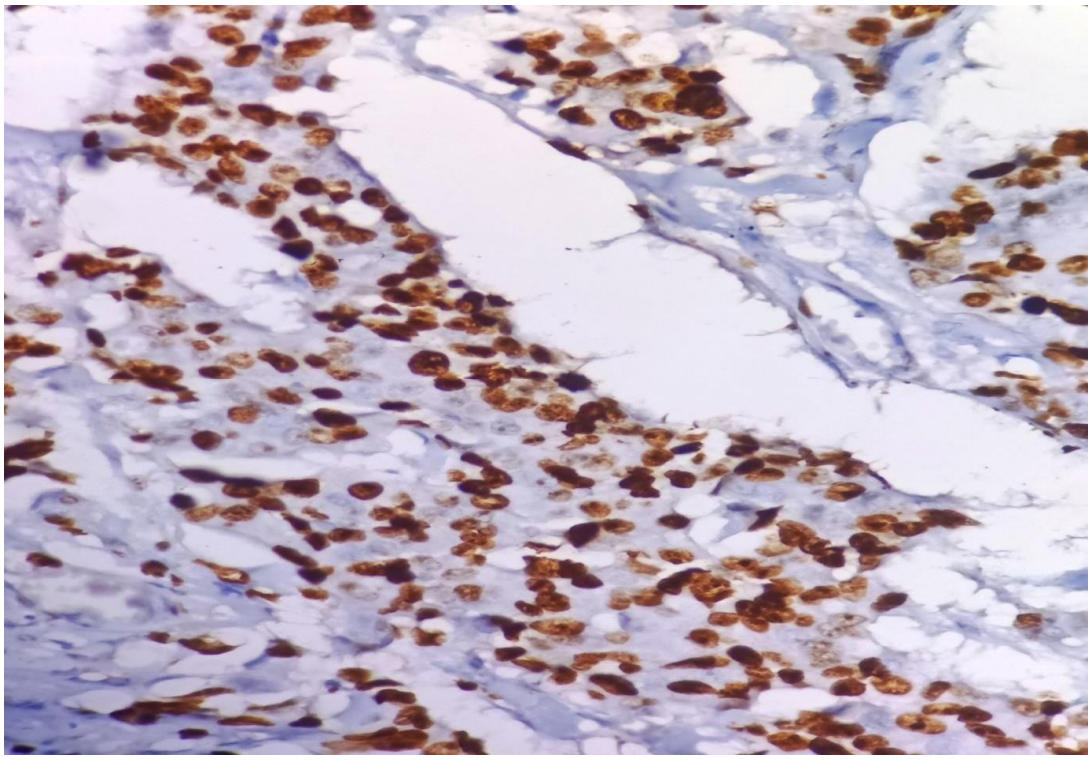


Figure 20 :- Ki-67 expression ADH (IHC, 400x)

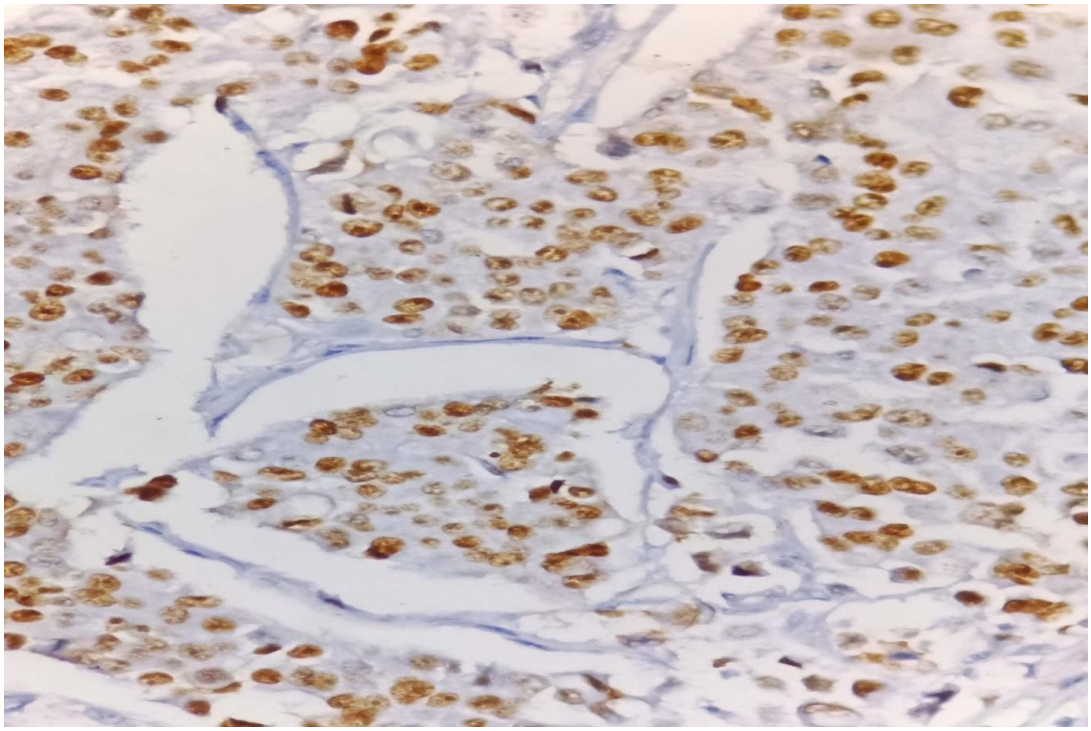


Figure 21:- p53 expression in ADH(IHC, 400x)

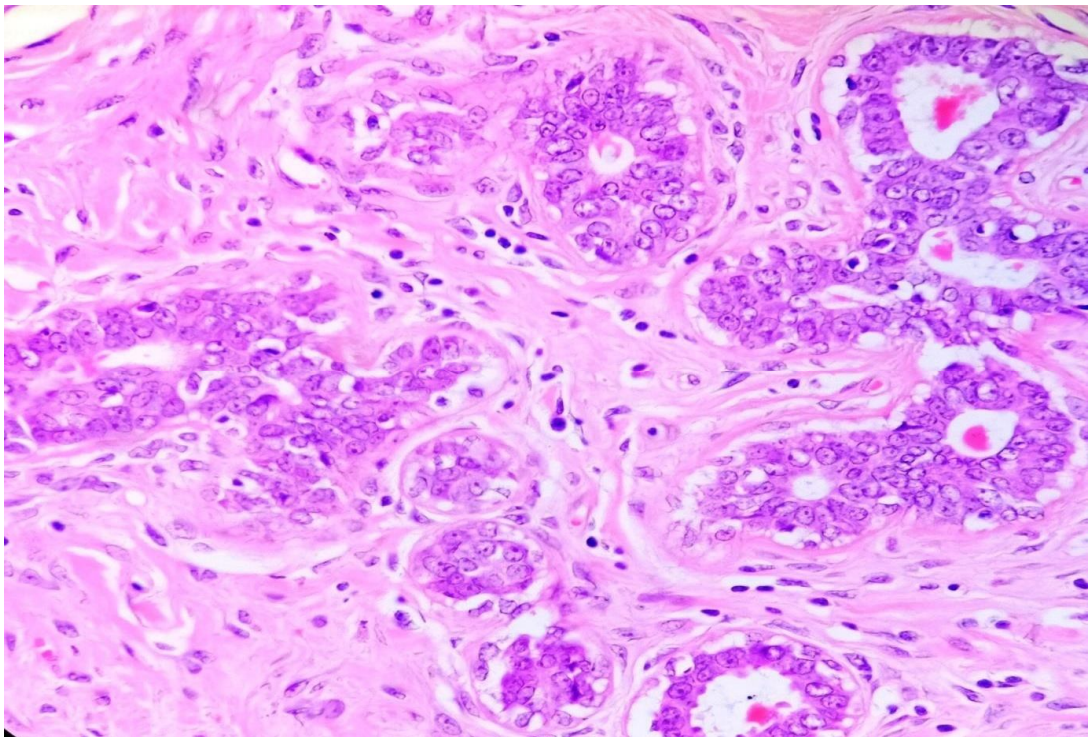


Figure 22 : Usual ductal hyperplasia (H & E, 400x)

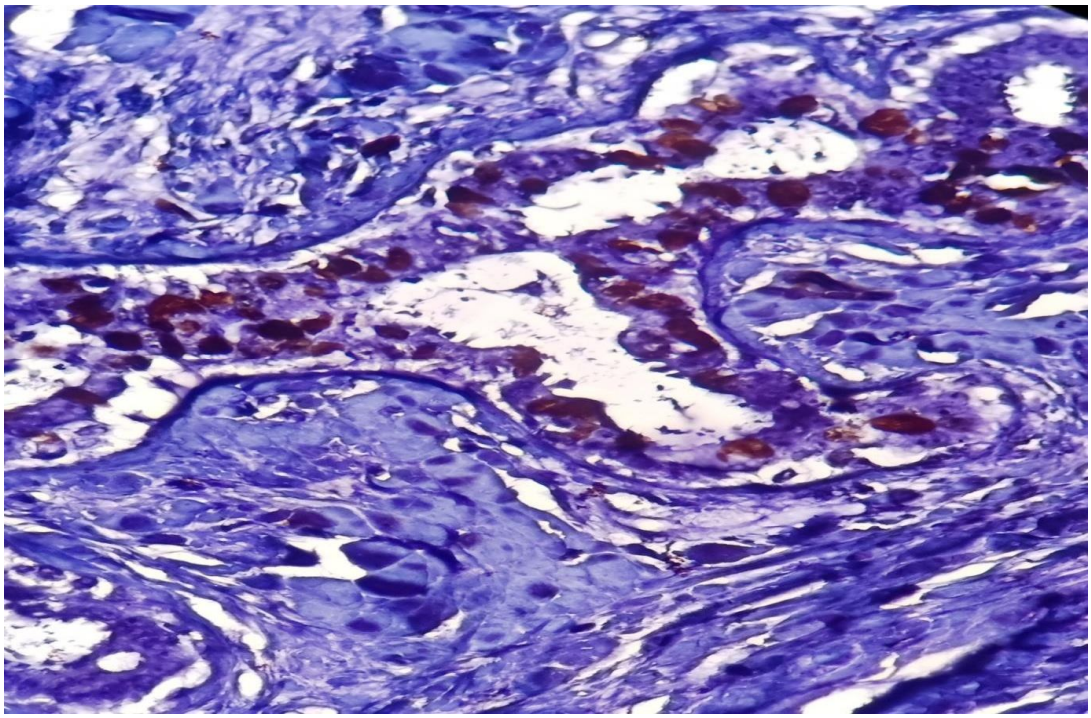


Figure 23 :- p53 expression in UDH (IHC, (400x)

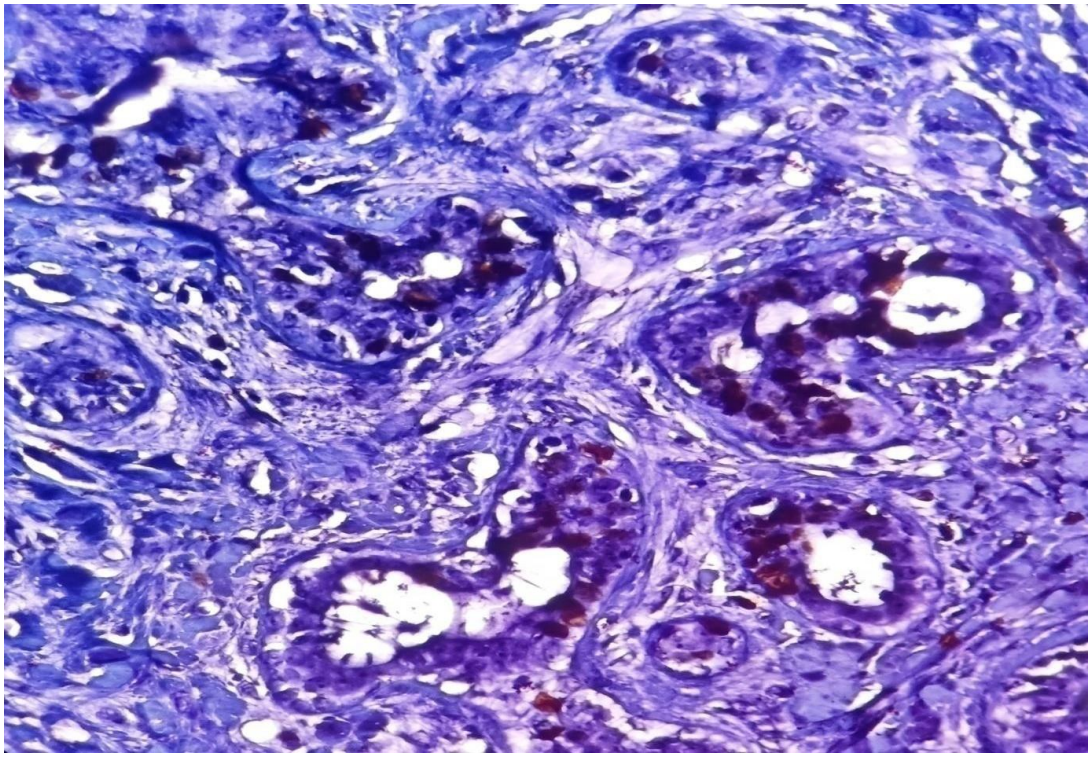


Figure 24: Ki-67 expression in UDH (IHC,400x)

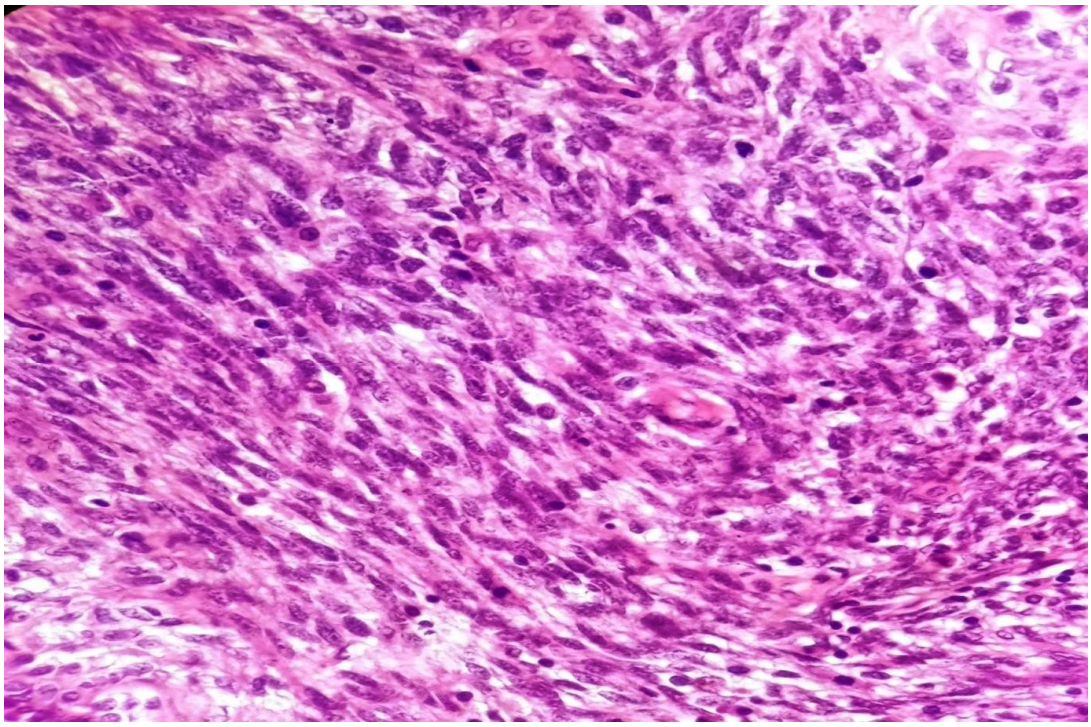


Figure 25 :- Malignant phyllodes tumor (H & E, 400x)

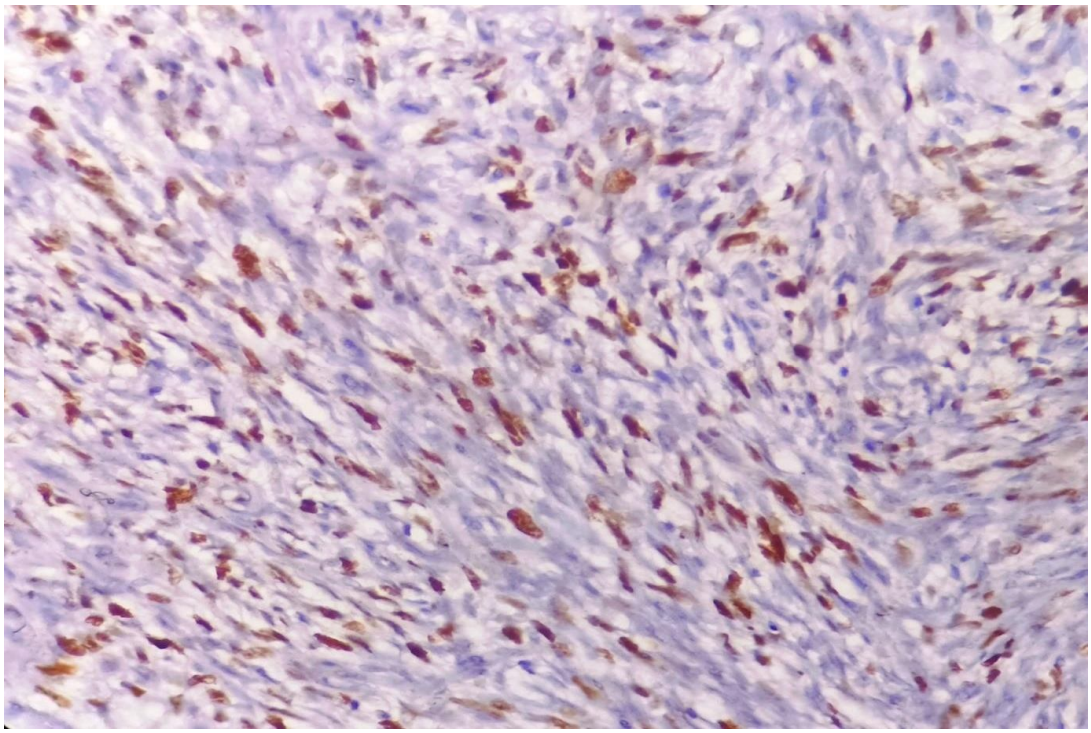


Figure 26 :- Ki-67 expression in malignant phyllodes tumor (IHC, 400x)

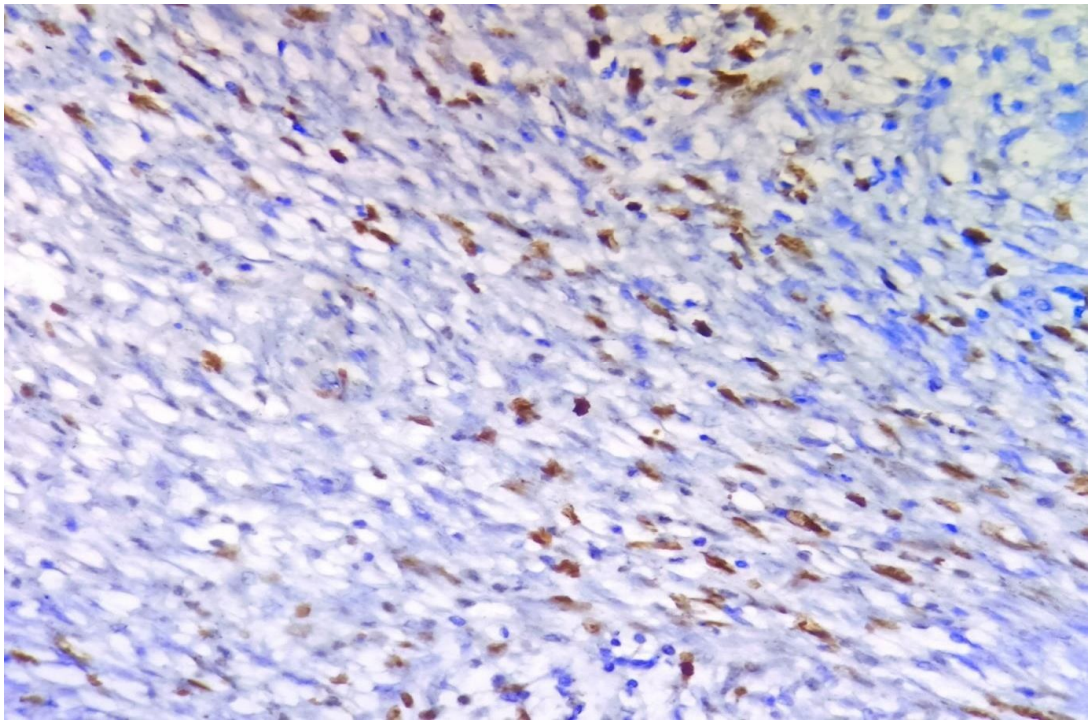


Figure 27:- p53 expression in malignant phyllodes tumor (IHC, 400x)

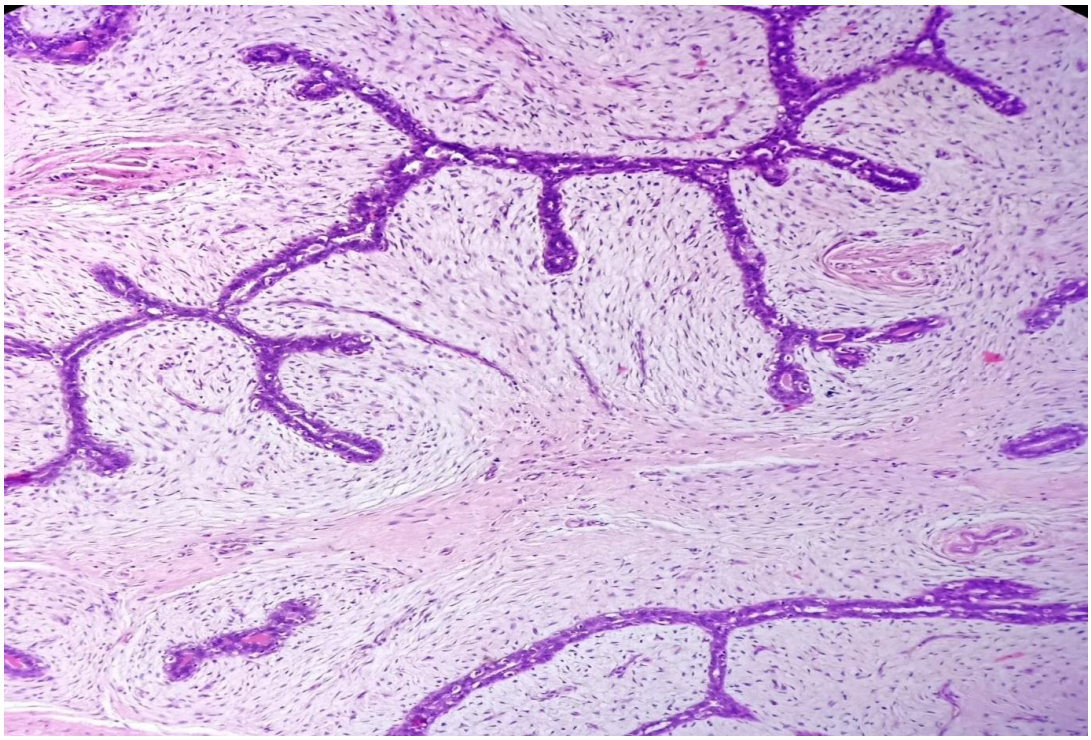


Figure 28 :- Fibroadenoma (H & E, 100x)

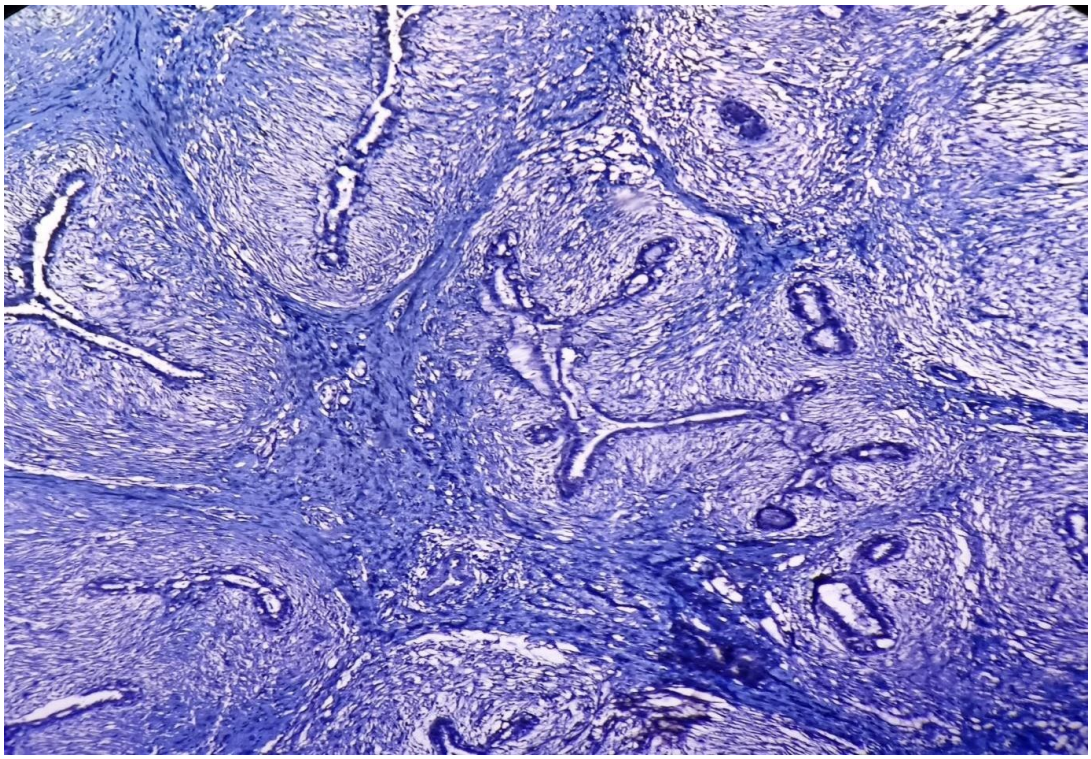


Figure 29 :- Negative Ki-67 expression in fibroadenoma (IHC, 100x)

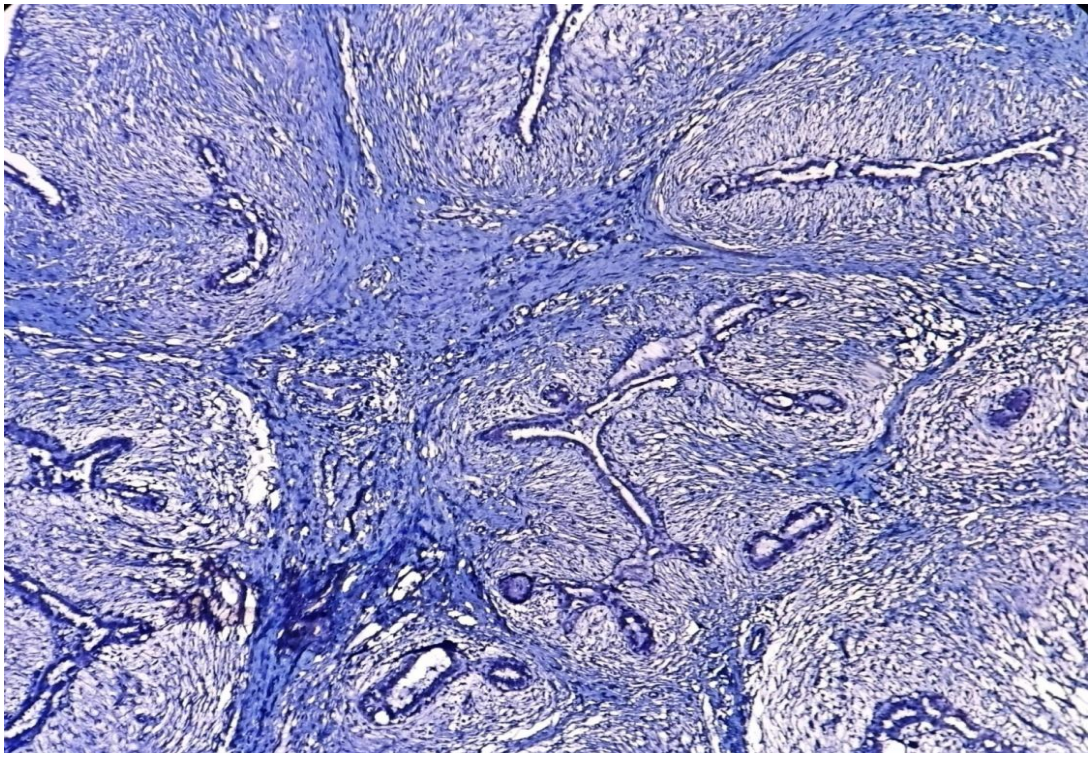


Figure 30:- Negative p53 expression in fibroadenoma (IHC, 400x)

DISCUSSION

Breast diseases are showing a rising trend worldwide. There is a wide variation in the spectrum of breast diseases in various countries or ethnic groups.[62] Invasive breast cancers (IBCs) appear to develop over long periods of time from pre-existing benign lesions. Among many only a few appear to have significant premalignant potential.[15] Precursors and pre-invasive lesions, which include atypical ductal hyperplasia (ADH), ductal carcinoma in situ (DCIS), represent a heterogeneous entity.[32] Examination of the routine hematoxylin-eosin (H&E) stained sections is the gold standard for the diagnosis of breast specimens.[94] But nowadays use of molecular markers is the common clinical practice and it seems to have promising role for the diagnosis and prognosis.[32]

Immunohistochemistry (IHC) is used to characterize intracellular proteins in various tissues.

Breast tumours are classified on the basis of Ki-67 labelling index as low, intermediate, and highly proliferating. With regard to the molecular breast cancers, high Ki-67 proliferation index can be used to classify triple negative breast cancer into subtypes with different prognosis or responses to treatment.[8]

The stage of initiation of the p53 mutation has been the subject of debate. Majority of studies done previously on p53 alterations in breast cancer have been limited to the isolated cases of ductal carcinoma in situ and IDC whereas few studies concluded that p53 mutation appears to occur at the stage of ADH during breast cancer carcinogenesis.[62]

The present study was undertaken to ascertain the association between the adjacent changes and the malignant lesion, thus these adjacent changes were studied and placed under three categories (CAT A, B & C). In the present study, 50 cases were included with detailed history and histopathological examination with IHC expression of p53 and Ki-67.

Age Group Comparision :

In present study, the age of the cases ranged from 18 to 70 years with the mean age of the patient being 38.2 years and the highest frequency 15(30%) was seen in 31 - 40 years of age group. These findings are in concoderence with the study done by **Thakral A et al[61]** and **Geetanjali et al.[64] Thakral et al[61]** reported that majority of the patients were within 13 to 85 years of age with a mean age of 40.5 years. **Geetanjali et al[64]** observed that cases in the study ranged between 10 to 60 years of age.

In present study, among sixteen benign (CAT A) cases highest frequency 11(68.57%) was seen in age group 21- 30 years. Out of thirteen cases of proliferative breast lesions (CAT B & C) highest frequency 7(53.84%) was seen in age group 31-40 years. In twenty one cases of malignant breast lesions (CAT D) highest frequency 11(52.38) was seen in age group 41-50 years. These findings are in concordance with the studies done by **Pudale et al[65]**, **Thakral et al[61], Kapoor et al[31], Geetanjali et al[64], Patil et al[63]** and **Hatim et al.[71]**

Patil et al[63] revealed that most of the cases (62) of benign breast lesions were in the age group of 21-30 years (38.7%). **Thakral et al[61]** analyzed that most of the benign breast diseases occurred in the age group of 21-30 years, whereas the most common age group facing the malignant breast lesion was 41-50 years. **Kapoor et al[31]** reported

that fibroadenoma was in benign category during 12-40 years of age. **Geetanjali et al[64]** observed that the peak age of occurrence was found to be in the 2nd and 3rd decades and malignant was found to be in the 4th and 6th decades. Higher incidence of fibroadenoma in 2nd to 3rd decade was also reported by **Hatim et al[71]** and **Pudale et al.[65]**

Side :

In the present study, 25(50 %) of the breast lesions were on the left side, 23(46%) were on the right side and 2(4%) cases were found involving both the breast. In CAT A, left side 9(56.25%) accounted for maximum number of cases whereas proliferative breast lesions (CAT B & C) constituted maximum number of cases on right side 7(53.84%) of breast. In CAT D maximum number of cases were reported on left side 11(52.38%) of breast.

These findings are in concordance with the findings of **Kapoor et al[31]**, **Geethamala et al[76]** and **Takalkar et al.[77]** **Kapoor et al[31]** observed right sided breast involvement in 49.88 %, while 38.37 % had left breast involvement among benign and malignant lesions respectively. Bilateral involvement was seen in 11.73 % patients. It has been observed in the past that breast carcinomas were more common in the left breast than the right (**Azizun-Nisa et al[75], 2008**). The possible explanations was that the left breast being more bulky and having a larger volume of breast tissue comparatively. However, side of the breast involved has no clinical significance (**Sandhu et al[73], 2010; Ambroise et al[74], 2011**). **Geethamala et al[76]** (2015) also found marginally more cases on left side than right with a single case of bilateral breast carcinoma. **Takalkar et al[77]** (2016) observed 50.77% cases on the left side and 49.23% on the right side.

But **Shrivastava et al[78]** (2016) found breast carcinoma to be more common on right side (55.7%). **Patil et al[63]** observed that 50.9% lesions on right side of breast and 43.3% on left and bilateral fibroadenoma were seen in 5.6% cases.

Site :

In the present study, 21(42%) cases of breast lesions were found in upper outer quadrant. In CAT A, maximum number of lesions were seen in lower outer quadrant 6(35.29%). In CAT B 4(44.44%) and in CAT C 3(60%) and in CAT D (7(50%) highest number of cases were located in upper outer quadrant. The upper outer quadrant, is the most common site for carcinoma breast as per standard textbook of surgery (**Sainsbury et al[93]**, **2008**). These findings are in concordance with the study done **by Mudhoulkar et al.[66]**

Mudhoulkar et al[66] observed that the maximum number of benign breast neoplasm cases were seen in upper outer quadrant (47%). This was followed by upper inner quadrant (16%) and lower outer quadrant (16%).

Histopathological Diagnosis :

In present study benign breast diseases 30(60%) were more commonly reported as compared to malignant breast lesions 20(40%). These findings are in concordance with the studies done by **Kapoor et al[31], Geetanjali et al[64], Patil et al[63]** and **Nandem et al.[72]**

Kapoor et al[31] observed that benign cases 342(77.20 %) out of 443 were more as compared to malignant 101(22.79 %) in his study. **Geetanjali et al[64]** analyzed 99 cases, in which benign lesions accounted for 74.75% whereas malignant case constituted 25.25% . Similar findings were observed by **Patil et al[63]** and **Nandem et**

al[72] that benign breast diseases outnumbered malignant cases in their studies. **Thakral et al**[61] show variation as compared to most studies, out of the 340 cases, 186 were malignant (54.71%). This variation in literature was observed as the patients admitted were referred for malignancy in a tertiary health-care centre.

In the present study, amongst CAT A, fibroadenoma 12 (75%) accounted for maximum number of cases, followed by fibroadenoma with fibrocystic change and benign phyllodes 2(12.5%). In proliferative breast lesion without atypia (usual ductal hyperplasia and fibroadenoma with epithelial hyperplasia) constitute equal number of cases 4(50%). In proliferative breast lesion with atypia (CAT C) constituted 5(100%) of atypical ductal hyperplasia. In malignant breast lesions, IDC (17 out of 20) accounted for maximum number of cases (85%) followed by ductal carcinoma in situ, metaplastic carcinoma, mucinous carcinoma & phyllodes, 1(5%) case each. These findings are in concordance with the studies done by **Thakral et al**[61]**, Kapoor et al**[31]**, Geetanjali et al**[64]**, Patil et al**[63] and **Nandem et al.**[72]

Thakral et al[61] observed that the most common benign lesion was found to be fibroadenoma whereas in malignant cases, infiltrating ductal carcinoma accounted for maximum number of cases. **Kapoor et al**[31] analyzed common benign breast diseases seen in their setup was fibroadenoma whereas among all malignant breast lesion were IDC. **Patil et al**[63] also observed that fibroadenoma accounted for highest number of cases (65.7%) followed by fibrocystic disease (10%) and benign phyllodes tumour (5.6 %) cases of all benign lesions.

Similar findings were reported by **Geetanjali et al**[64] and **Nandem et al.**[72] Various past studies conducted by **Mansoor et al**[79] **in 2001, Shanthi et al**[80] **in 2011, Aslam et al**[81] **in 2013** and **Rahman et al**[82] **in 2014** concluded that fibroadenoma and IDC was

the most common benign breast lesion and breast carcinoma respectively.

Expression of p53 in breast lesions :

p53 protein expression is a nuclear marker. In present study 50 cases were studied, p53 positivity was seen in 28 cases; maximum number of positive cases were found in CAT D 19(90.48%). In CAT A and B p53 positivity was seen in 4(25%) and 2(25%) of cases respectively. In CAT C 3(60%) cases showed p53 positivity. The percentage of cells varied from 7% to 93% in different breast diseases These finding are in concordance with the study done by **Rohan et al[44,50], Kalogoraki et al[83]** and **Kandel et al.[45]**

Rohan et al[44,50] conducted two studies in 1998 and 2006 and found that patients with benign breast lesions have slightly elevated levels of p53 increased relative risk (two to three fold) of developing IBC. **Kalogoraki et al[83]** observed p53 nuclear expression in fiboradenomas(25%), ADH(20%) and a statistically significant difference between p53 expression of breast carcinomas, fibroadenomas, ADH was found. **Kandel et al[45]** concluded that 16(59.2%) out of 27 cases of normal and benign breast disease cases were p53 immunopositive and 4(26.7%) out of 15 cases were p53 immunonegative but had p53 sequence changes. Study done by **Berardo et al[67]** reported conflicting results that there was no evidence of p53 mutations in normal or benign breast epithelium. Similar findings were observed by **Younes et al.[68]**

In present study CAT D 19(90.48%) out of 21 cases showed positivity for p53. Among 17 IDC, maximum cases (10) were of grade 3 (BLOOM RICHARDSON GRADING). Out of 10 cases of IDC, 9 cases showed positivity for p53 with 6(28.57%) showing 4+ positivity and 2(9.52%) 2+ grading. These finding are in concordance with the study done by **Kang et al[62], Muhammad et al[58]**, **Shoukouh et**

al[56], and Plesan et al[11] and **Chan et al.[46]**

Kang et al[62] observed that two out of seven cases of ADHs harbor p53 DNA alteration, the same mutations were observed in the adjacent non invasive and invasive lesions but not in the normal lobules. They concluded that p53 mutation occurs not only at the DCIS but also at the ADH stage during the tumorigenesis of breast cancer. **Muhammad et al[58]** studied 45 specimens of breast carcinoma, p53 was negative in all cases of low grade ductal carcinoma in situ (DCIS), positive in 2/3 of intermediate grade DCIS, and positive in all cases of high grade DCIS. All grade I invasive breast carcinoma (IBC) were negative for p53, 50% of grade II and 91% of grade III IBC were positive for p53. p53 expression increased significantly with increased tumor grade of IBC. p53 was weakly expressed in 11% of areas of benign breast disease. They concluded p53 was an indicator of poor prognosis in breast cancer being positively correlated to tumor grade. **Shoukouh et al[56]** concluded that p53 mutation increased significantly with the grade of the breast tumour (IDC). **Kim et al[84]** also concluded similar findings. **Plesan et al[11]** analyzed 100 cases of invasive mammary carcinoma, the invasive ductal carcinomas were p53-positive in 40 cases (44.44%) of all invasive ductal carcinoma cases. The cases that had the overexpression of the p53 had a high histological degree (G3), and only 12 cases had a low histological degree (G1 and G2). **Chan et al[46]** analyzed 50 benign and 13 malignant phyllodes tumors and found 5 out of 50 showed P53 positivity whereas 9 out of 13 malignant cases showed p53 positivity. p53 expression in phyllodes tumors is correlated with histological grading.

These findings are in concordance with the studies done by **Feakins et al[69], Kim et al[84]**and **Kleer et al.[85]**

Studies conducted by **Done et al[43], Tripathy et al[89], Malley et al[88], Rajan et al[86]** and **Bartek et al[87]** were in agreement that p53 mutations and p53 protein accumulation from in 13% to 70% in invasive intraductal carcinomas (DCIS). Similar finding was reported in benign breast disease and in normal appearing breast tissue. **Zangouri et al[32]** on the contrary concluded that p53 was immunopositivity was seen in ADH and DCIS and Infiltrating breast cancer whereas it was totally absent in epithelial hyperplasia without atypia.

Many studies were conducted and they concluded that p53 mutation and p53 protien accumulation together was assosciated with greater risk of developing breast cancer as compared to p53 overexpression and mutation.[42,45,68,95-98]

The present study also suggested that p53 changes can occur before the development of breast cancer.

Expression of Ki-67 in breast lesions :

Ki-67 protein expression is nuclear marker. In present study, 50 cases were studied, Ki-67 positivity was seen in 28 cases, with maximum number of cases were found in CAT D. In CAT A(4) and B(2) Ki-67 positivity was seen in 25% cases whereas in CAT C positivity was seen in 3(60%) cases. In CAT D 19(90.48%) out of 21 cases showed positivity for Ki-67. The percentage of cells varied from 6 to 90% among breast lesions. Out of 17 cases of IDC, 16 cases showed positivity for Ki-67 with 7(33.33%) showing 3+ positivity followed by 4+ grading in 6(28.57%).

These finding are in concordance with the study done by **Satyalakshmi et al[30], Santisteban et al[53], Yonomori et al[49], Ragab et al[60], Shoukouh et al[56], Plesan et al[11], Shoker et al[70], Rachna et al[35], Chan et al[46]**, **Mylonasi et al[47], Zangouri et al[32]**

and **Oh et al[92]**.

Satyalakshmi et al[30] showed that Ki-67 positivity was present in 7 cases of proliferative lesions with atypia and 8 cases of proliferative lesions without atypia, the proliferative index values were very high in case of lesions belonging to ADH and DCIS. High Ki-67 in the adjacent lesions suggested that it could be the premalignant lesion for the current malignancy. **Santisteban et al[53]** done a study and concluded that atypical cases with a higher proliferation index had an increased short-term (within 10 years) risk of breast cancer. In the high Ki67 group, 89% of the breast cancers occurred in the first 10 years; in the low Ki67 group, 83% of the breast cancers occurred after 10 years. **Yonomori et al[49]** included 41 patients with Phyllodes tumor (20 benign, 5 boderline and 16 malignant). Ki-67 positivity was seen in 10 cases and the median MIB-1 index was 10%. **Ragab et al[60]** analyzed 92 patients of breast cancer and observed Ki-67 expresssion was more frequently associated with grade of tumor, as it had a close association with proliferation. As the grade of tumor increases, the Ki-67 positivity also increases. Similar findings were observed by **Spyratos et al[90], Inwald et al[91] and Shoukouh et al[56].** They showed significant relationship between Ki-67 and tumour grade, as the tumor with high grades have higher level of cell proliferation.

Plesan et al[11] observed Ki-67 positivity in all cases that were studied. Tumors with a high grading (G3) always had a high Ki67 index as compared with the tumors with a low grading. **Shoker et al[70]** demonstrated Ki-67 expression in normal breast tissue, proliferative breast disease without atypia, atypical hyperplasias, in situ neoplasia & invasive cancer. Its expression showed variation in above categories. 3% Ki-67 expression was noted in normal breast epithelia. Ki-67 expression is less in hyperplasia without atypia with lower mean percentage than that seen in ADH &

DCIS whereas invasive breast cancers had a high percentage. **Rachna et al**[35] analyzed ER and Ki-67 expression and ration of ER/Ki-67 among benign , proliferative and malignant breast lesions. They observed Ki-67 positivity ranged from 13.8% to 30% , 12-34.5% and 4-34.5% in Benign , Proliferative and Malignant breast lesions respectively. **Chan et al**[46] studied 50 benign and 13 malignant phyllodes tumor and found 8 out of 50 showed Ki-67 >10%. Three benign cases among them progressed to malignant tumors. So they concluded tumors with benign morphology (Ki-67>10%) should be followed properly to avoid progression. **Mylonas et al**[47] concluded that Ki-67 expression was significantly higher in IDC cases (64%) as compared to DCIS(49.17%). In the present study 3 cases of phyllodes were analyzed two benign and one malignant, all were positive for Ki-67. **Zangouri et al**[32] observed that ADH was associated with low Ki-67 expression/bcl-2 positivity and p53 negativity whereas poorly differentiated carcinoma was associated with high Ki-67 expression/bcl-2 negativity within the lobules. **Oh et al**[92] reported that Ki-67 expression was significantly associated with ADH and had four fold higher breast cancer risk.In present study we observed that it had heterogenous pattern of expression among various breast lesions from benign to malignant, and its expression was associated with common histopathological parameters, especially grading and survival.

SUMMARY

This is a prospective study for a period of one year conducted at SGT Medical College and Hospital, Gurugram, Haryana. The present study aimed to evaluate the expression of Ki-67 and p53 among breast diseases and their relation with histopathological profile. It was conducted on breast lumpectomy and MRM breast specimens received in the Department of Pathology and H&E staining was done routinely and further representative sections were stained for IHC markers Ki-67 and p53.

The key features of present study :-

1. Total 50 cases (benign, proliferative and malignant) were studied out of which 30 cases were benign and 20 were malignant.
2. The age of the patients was from 18 to 70 years.
3. The maximum number of cases were seen in the age group of 31 to 40 years.
4. Amongst the benign cases fibroadenoma was the most common finding.
5. Amongst the malignant cases infiltrating ductal carcinoma constituted the most common lesion.
6. Ki-67 and p53 had heterogenous pattern of expression in breast lesions ranging from benign to proliferative to malignant with ascending pattern of grading of cells and correspondingly the number of positive cases.
7. The Ki-67 expression was positive in 28 cases, out of which 10 were benign and 18 were malignant.

8. The Ki-67 expression was seen in increasing grade ranging from 1+ in benign and proliferative lesions without atypia to 4+ in proliferative with atypia and malignant breast lesions with maximum expression seen in the malignant one.
9. The p53 immunopositivity was seen in 28 cases, out of which 10 were benign and 18 were malignant.
10. The p53 expression in breast lesions was seen in increasing grade ranging from 1+ to 2+ in benign breast lesions and proliferative breast lesions without atypia to 4+ in proliferative with atypia and malignant cases.

CONCLUSION

In conclusion, the breast lesions are biologically and clinically very heterogenous with different histomorphological patterns. The immunoexpression of Ki-67 and p53 helps in understanding the prolifertive process of breast lesions and their positivity in lumpectomy specimens can give positive predictions about further breast disease process combined with genetic mutations studies in the patient. Dual marker study is more sensitive and specific for getting towards a more conclusive diagnostic as well as providing the treating clinician with better prognostic overview of the case. Thus the study helps in stratifying high risk patients who might benefit from closer monitoring and follow-up over a longer period of time.

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CASE PROFORMA

Case No:-
CR/OPD/Ward No.:-
Histopathology No.:-
Age :-
Sex :-
Laterality :-
Type of Specimen received:-
Pathological Findings:-

Gross Examination

Microscopic Examination (H&E)

Immunohistochemistry:-

p53 expression

Negative	% of stained cells less than 10%
Positive	% of stained cells more than 10 %

Ki-67 expression

Negative	% of stained cells less than or equal to 2%
1+	% of stained cells between 2 to 25%.
2+	% of stained cells between 26 to 50%.
3+	% of stained cells between 51 to 75%.
4+	% of stained cells between 76 to 100%.

	p53+		Ki-67+	
	-ve	**+ve**	**-ve**	**+ve**
Benign epithelial lesion				
Proliferative breast disease without atypia				
Proliferative breast disease with atypia				
Carcinoma breast				

S.NO	Code	AGE	TOS	SD	OSC	ND	SITE	TUMOR SIZE	C/S	NEC	MP	EH	SH	AC	PATT	PATT (%)	CP	NP	NPG	MIT	NECRO	DCC	HD	HD	BRG	CAT	NO. TUM CELLS	Positive/ Negative	Grading	NO. TUM CELL	Positive/ Negative	Grading
1	AB1	32	1	1	0	2	3	2 X 2	Grey white	2	1	2	2	1	0	0	0	0	0	0	0	0	Fibro	0	0	1		0	0		0	0
2	AB2	21	1	1	0	2	4	2 X 1	Firm, GW , Slit like spaces	2	1	2	2	0	0	0	0	0	0	0	0	0	Fibro	0	0	1		0	0		0	0
3	AB3	50	2	1	1	1	2	4 x 3 x2	GW irregular growth	1	0	0	0	0	0	0	1	1	0	Absent	1	0	DCIS	0	0	4	275	1	3	325	1	4
4	AB4	23	1	3	0	2	1,4	2 X 1.5, 2 X 1	Grey white	2	1	1	2	0	0	0	0	0	0	0	0	0	Fibro with epi hyp	0	0	2		0	0		0	0
5	AB5	42	2	1	0	2	1	2 X 2	Necrotic growth	1	0	0	0	0	1	< 10%	1	1	Marked	8/10 HPF	1	2	IDC	0	3	4	300	1	3	100	1	2
6	AB6	33	1	1	0	2	3	4.5 X 3.5 X 3	slit like spaces & mucoid fluid	2	1	2	2	0	0	0	0	0	0	0	0	0	Fibro	0	0	1		0	0		0	0
7	AB7	24	1	2	0	2	1	3 X 2.5 X1	Firm , GW, Slit like spaces	2	1	1	2	0	0	0	0	0	0	0	0	0	Fibro with epi hyp	0	0	2		0	0		0	0
8	AB8	18	1	1	0	2	3	5.5 X 2.5 X 1.5	GW multiple nodules, SLS	2	3	1	1	0	0	0	0	0	0	0	0	0	Bening Phyll	0	0	1	60	1	1	35	1	1
9	AB9	50	2	2	1	1	3	3.5 X 2.5 X 2	Irregular GW growth	2	0	0	0	0	1	< 10%	1	1	MOD	6/10 HPF	2	2	IDC	0	2	4	175	1	2	110	1	2
10	AB10	21	1	2	0	1	1	1 X 1	Grey white, slit like spaces	2	1	2	2	0	0	0	0	0	0	0	0	0	Fibro	0	0	1		0	0		0	0
11	AB11	32	1	1	0	2	3	3 X 1	Firm, GW growth	1	0	1	2	2	0	0	0	0	0	0	0	0	ADH	0	0	3	150	1	2	200	1	3
12	AB12	21	1	1	0	2	1	3 X 2 X 1.5	GW , Slit like spaces	2	1	2	2	0	0	0	0	0	0	0	0	0	Fibro	0	0	1		0	0		0	0
13	AB13	30	1	1	2	2	1	2.5 x 2 x 2	GW , tiny slit like spaces	2	1	2	2	0	0	0	0	0	0	0	0	0	Fibroa	0	0	1		0	0		0	0
14	AB14	45	2	2	0	1	1	3 X 3	Firm , GW , ill defined growth	1	0	0	0	0	1	>75%	1	1	MILD	3/10HPF	1	1	IDC	0	1	4	80	1	1	100	1	2
15	AB15	23	1	1	0	2	2	2.5 x 1 x 1	Multinodular	2	1	2	2	0	0	0	0	0	0	0	0	0	Fibro	0	0	1		0	0		0	0
16	AB16	27	1	2	0	1	3	6 x 5.5 x 3	Circumscribed, GW nodular	2	0	2	1	0	0	0	0	0	0	0	0	0	Phyll tum(B)	0	0	1	60	1	1	40	1	1
17	AB17	40	2	1	2	2	3	4 X 3	Friable GW growth	2	0	0	0	3	2	0	1	1	0	11/10HPF	1	2	Meta Ca Bre	0	0	4	400	1	4		0	0
18	AB18	39	2	2	0	2	3	4.5X2.5X3	GW, irregular,Hard	2	0	0	0	0	1	25%	1	1	MOD	8/10 HPF	2	1	IDC	0	2	4	340	1	3	380	1	4
19	AB19	37	2	1	0	2	4	3 X 2	Firm, GW irregular growth	2	0	0	0	0	1	>10%	1	1	Marked	15/10 HPF	1	1	IDC	Comedo	3	4	425	1	4	440	1	4
20	AB20	32	1	1	0	2	3	3.5X2.5X2	GW,Slit like spaces	2	1	1	2	0	0	0	0	0	0	0	0	0	Fibro with epi hyp	0	0	2		0	0		0	0
21	AB21	18	1	3	0	2	4 , 3	3.5X2.5, 3X2	GW, Firm, slit like spaces	2	1,2	2	2	0	0	0	0	0	0	0	0	0	Fibro	0	0	1	95	1	1	85	1	2

S.NO	Code	AGE	TOS	SD	OSC	ND	SITE	TUMOR SIZE	C/S	NEC	MP	EH	SH	AC	PATT	PATT (%)	CP	NP	NPG	MIT	NECRO	DCC	HD	HD	BRG	CAT	TUM CELL	e/ Negati	Grading	TUM NO.	e/ Negati	Grading
22	AB22	40	1	2	0	2	1	4.5 X 3.5	Hard, GW, irregular	2	0	1	2	0	0	0	0	0	0	0	0	0	ADH	0	0	3		0	0		0	0
23	AB23	50	2	1	0	2	1	5.5 X 4 X 2.5	Hard, immobile, irregular	2	0	0	0	0	1	>75%	1	1	MILD	3/10HPF	2	2	IDC	0	1	4	145	1	2	115	1	2
24	AB24	25	1	2	0	2	1	2X1X1,3X2X2	Growth in outer quadrant	2	1	2	2	1	0	0	0	0	0	0	0	0	Fibro	0	0	1		0	0		0	0
25	AB25	40	1	1	0	2	4	6.5X5X4	GW, Lobulated, slit like spaces	2	0	1	2	0	0	0	0	0	0	0	0	0	UDH	0	0	2	70	1	1	40	1	1
26	AB26	50	2	1	4	2	1	3 X 2	GW, irregular	1	0	0	0	0	1	< 10%	1	1	MOD	15/10 HPF	1	1	IDC	0	3	4	430	1	4	445	1	4
27	AB27	50	2	2	3	2	1	3 X 4	Firm to hard GW growth	1	0	0	0	0	1	< 10%	1	1	Marked	20/10 HPF	1	1	IDC	Comedo	3	4	350	1	3	410	1	4
28	AB28	70	2	2	3	2	3	5.5 X 4 X 3.5	Circumscribed, lob, Varigated	2	0	0	0	0	3	0	2	1	0	4/10 HPF	2	2	Muc car	0	0	4		0	0	210	1	3
29	AB29	55	2	2	1	1	4	4.5 X 4 X 3.5	Hard, immobile, irregular	1	0	0	0	0	1	20%	1	1	MOD	7/10 HPF	2	2	IDC	0	2	4	330	1	3	190	1	3
30	AB30	42	1	2	0	2	1	5.5 X 5 X 2.5	GW,Irregular	2	0	1	2	0	0	0	0	0	0	0	0	0	ADH	0	0	3		0	0		0	0
31	AB31	40	1	1	1	2	1	1.5 X 1	Firm, GW , ill defined growth	2	0	0	0	0	1	20%	1	1	Marked	11/10HPF	2	2	IDC	0	3	4		0	0		0	0
32	AB32	30	1	1	0	2	2	3X2.5X2.5	GW, Slit like spaces	2	1	2	2	0	0	0	0	0	0	0	0	0	Fibroa with fibr cys	0	0	1		0	0		0	0
33	AB33	40	2	1	0	2	4	2 X 1	Firm , GW , ill defined growth	1	0	0	0	0	1	40%	1	1	MOD	6/10 HPF	1	1	IDC	0	2	4	415	1	4	235	1	3
34	AB34	50	1	2	0	2	1	3 X 4	Lobulated	2	1,2	1	2	0	0	0	0	0	0	0	0	0	Fibroa with epi hyp	0	0	2		0	0		0	0
35	AB35	28	1	2	0	2	1	3X2X1, 3X2X2	Grey white, slit like spaces	2	1	2	2	1	0	0	0	0	0	0	0	0	Fibro	0	0	1		0	0		0	0
36	AB36	21	1	2	0	2	4	3 X 2 X 1.5	Grey white, slit like spaces	2	1	2	2	1	0	0	0	0	0	0	0	0	Fibro	0	0	1	30	1	1	35	1	1
37	AB37	38	1	2	0	2	2	4 X 2	Firm, GW growth	1	0	1	2	2	0	0	0	0	0	0	0	0	UDH	0	0	2		0	0		0	0
38	AB38	40	1	2	0	2	2	2.5 X 1.5 X 1.5	Slit like spaces	2	1	0	0	0	0	0	0	0	0	0	0	0	Fibro	0	0	1		0	0		0	0
39	AB39	40	1	1	0	1	3	3 X 2	Firm, GW growth	2	0	1	2	2	0	0	2	2	0	Absent	2	2	ADH	0	0	3	285	1	3	190	1	3
40	AB40	56	1	2	0	2	2	1 X 1	GW	2	0	1	2	0	0	0	0	0	0	0	0	0	UDH	0	0	2		0	0		0	0
41	AB41	58	2	1	0	1	1	2.5 X 2 X2	Hard, immobile, irregular	1	0	0	0	0	1	< 10%	1	1	Marked	4/10 HPF	1	2	IDC	0	2	4	320	1	3	215	1	3
42	AB42	48	2	2	1	1	1	4.5 X 2.5 X 2	Chalky streaks, irregular , Hard	1	0	0	0	0	1	< 10%	1	1	Marked	11/10HPF	1	1	IDC	Comedo	3	4	445	1	4	450	1	4
43	AB43	50	2	1	1	1	3	3.5 X 2 X 2	Firm , Irregulaar growth	1	0	0	0	0	0	0	1	1	0	16/10HPF	2	2	Phyll tumM)	0	0	4	450	1	4	150	1	3

S.NO	Code	AGE	TOS	SD	OSC	ND	SITE	TUMOR SIZE	C/S	NEC	MP	EH	SH	AC	PATT	PATT (%)	CP	NP	NPG	MIT	NECRO	DCC	HD	HD	BRG	CAT	TUM CELL	Negati e/	Gradi ng	TUM CELL	Negati e/ NO.	Gradi ng
44	AB44	55	2	2	0	1	1	6 X 4 X 3.5	Firm, irregular GW growth	1	0	0	0	0	1	< 10%	1	1	Marked	20/10 HPF	1	1	IDC	0	3	4	455	1	4	465	1	4
45	AB45	24	1	1	0	2	1	3.5 X 2	Firm, GW	2	0	1	2	2	0	0	0	0	0	0	0	0	UDH	0	0	2	45	1	1	40	1	1
46	AB46	48	2	1	1	1	3	3.5 X 2 X 1.5	Firm to hard GW growth	2	0	0	0	0	1	< 10%	1	1	Marked	15/10 HPF	1	1	IDC	0	3	4	430	1	4	225	1	3
47	AB47	30	1	1	0	2	2	2X1.5X1,1.5x1x0.5	Firm,nodular,Gw growth	2	1	2	2	0	0	0	0	0	0	0	0	0	Fibro with fibr cys	0	0	1		0	0		0	0
48	AB48	35	1	2	0	2	1	2.5 X 1.5 X 1.5	Firm, Grey white	2	0	1	0	0	0	0	0	0	0	0	0	0	ADH	0	0	3	420	1	4	440	1	4
49	AB49	55	2	2	0	1	3	4 X 3.5 X 3	Irregular GW growth,Hrad	2	0	0	0	0	0	0	1	1	Marked	7/10 HPF	1	2	IDC	0	3	4	310	1	3	370	1	4
50	AB50	45	2	2	0	1	4	4 X 3 X 2.5	Hard, immobile, irregular	1	0	0	0	0	1	< 10%	1	1	Marked	9/10 HPF	1	2	IDC	0	3	4	300	1	3	100	1	2

KEY TO MASTER CHART

1. **S.No. – serial number**
2. **Code**
3. **Age in years**
4. **TOS – Type of specimen** [1 – Lumpectomy ; 2 - MRM]
5. **Side** [1 – Left ; 2 – Right; 3 - Bilateral]
6. **OSC – overlying skin changes** [0 – absent; 1 – nipple retraction; 2 – tender; 3 – firm to hard,mobile; 4 – firm to hard immobile]
7. **ND – nipple discharge** [1 – present; 2 - absent]
8. **Site** [1 – upper outer; 2 – upperinner; 3 – lower outer; 4 – lower inner]
9. **TS – tumour size**
10. **CS – cut section**
11. **Nec – necrosis** [1 – present; 2 - absent]
12. **MP – morphological pattern** [0 – not present; 1 – intracanalicular; 2 – pricanalicular; 3 – leaf like pattern]
13. **EH – epithelial hyperplasia** [0 – not associated; 1 – present; 2 - absent]
14. **SH – stromal hyperplasia** [0 – not associated; 1 – present; 2 - absent]
15. **AC – associated changes** [0 – not associated; 1 – hyalinized stroma; 2 –absent; 3 – dense lymphocytic infiltrate]
16. **PATT – pattern** [0 – not associated; 1 – tubular pattern; 2 – sheets; 3 – tabecular, nest and cords]
17. **PATT (%) – percentage of tubular pattern**
18. **CP – cell pleomorphism** [0 – not associated; 1 – present; 2 - absent]
19. **NP – nuclear pleomorphism** [0 – not associated; 1 – present; 2 - absent]
20. **NPG - nuclear pleomorphism grading** [0 – not associated; 1 – mild; 2 – moderate; 3 - marked]
21. **MIT – mitosis** [0 – not associated]
22. **NECRO – necrosis** [0 – not associated; 1 – present; 2 - absent]
23. **DCC – DCIS component** [0 – not associated; 1 – present; 2 - absent]
24. **HD – histopathlogical diagnosis**
25. **HDD – histopathological diagnosis**
26. **BRG – Bloom Richardson grading** [0 – not associated; 1 – grade 1; 2 – grade 2; 3 – grade 3]
27. **CAT – category** [1 – CAT A; 2 – CAT B; 3 – CAT C; 4 – CAT D]
28. **Number of tumour cells – Ki-67 expression**
29. **Positive/ Negative – Ki-67 expression** [0 – absent; 1 – prsent]
30. **Grading – Ki-67 expression** [0 – absent; 1 – 1+; 2 – 2+; 3 – 3+; 4 – 4+]
31. **Number of tumour cells – p53 expression**
32. **Positive/ Negative – p53 expression** [0 – absent; 1 – prsent]
33. **Grading – p53 expression** [0 – absent; 1 – 1+; 2 – 2+; 3 – 3+; 4 – 4+]

URKUND

IQAC/D43301196/2018/24

Urkund Analysis Result

Analysed Document:	Dr Sonu Yadav.docx (D43301196)
Submitted:	10/31/2018 6:14:00 AM
Submitted By:	astha.chaudhry@sgtuniversity.org
Significance:	2 %

Sources included in the report:

Thsis final-kriti.docx (D43147596)
https://www.jrmds.in/abstract/histopathological-spectrum-of-benign-breast-lesions-1643.html

Instances where selected sources appear:

8

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