Retrospective study of infiltrating lobular carcinoma of breast with assessment of utility of p120 catenin and E-cadherin double immunostaining in the diagnosis.

A DISSERTATION SUBMITTED IN PART FULFILLMENT OFTHE

REGULATION FOR THE AWARD OF THE DEGREE OFM.D.

PATHOLOGY BRANCH III.

THE TAMIL NADU DR. M.G.R. UNIVERSITY

CHENNAI, TAMIL NADU

MAY-2018

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CERTIFICATE

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CERTIFICATE – II

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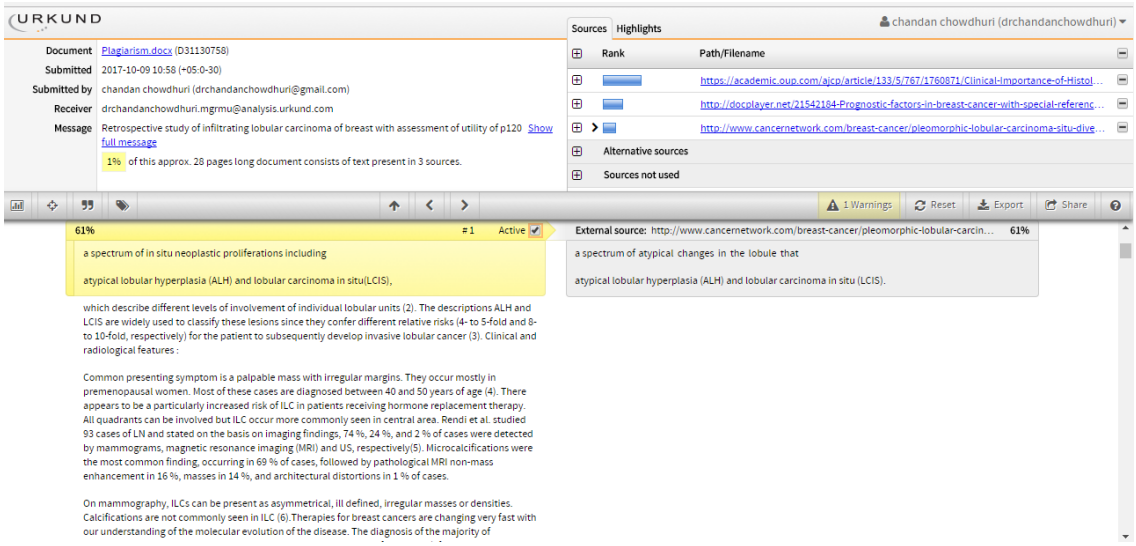
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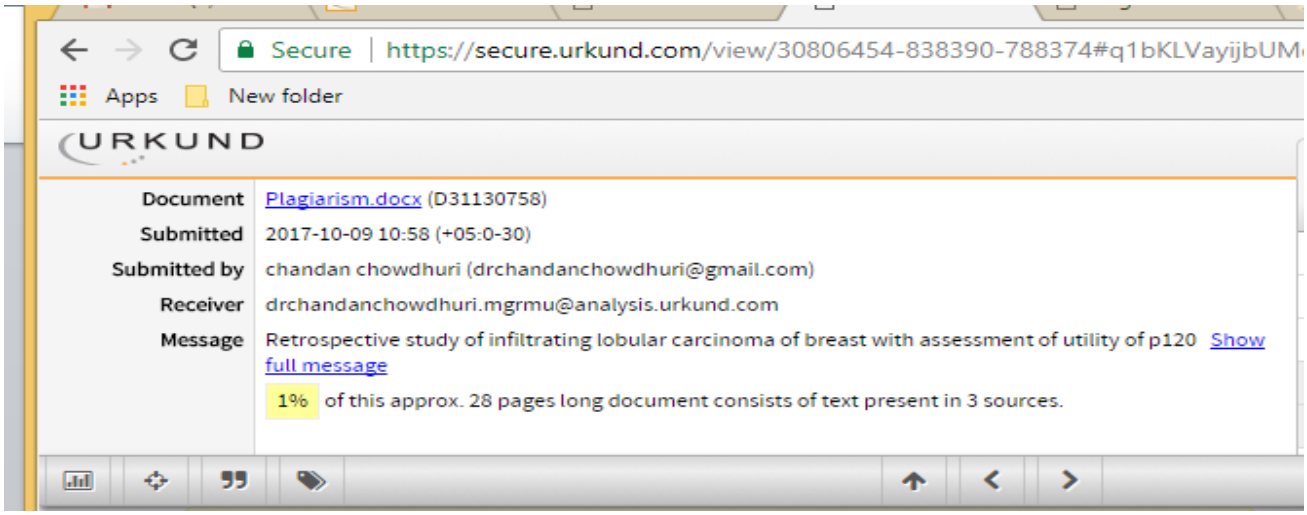
which describe different levels of involvement of individual lobular units (2). The descriptions ALH and LCIS are widely used to classify these lesions since they confer different relative risks (4- to 5-fold and 8- to 10-fold, respectively) for the patient to subsequently develop invasive lobular cancer (3). Clinical and radiological features :
Common presenting symptom is a palpable mass with irregular margins. They occur mostly in premenopausal women. Most of these cases are diagnosed between 40 and 50 years of age (4). There appears to be a particularly increased risk of ILC in patients receiving hormone replacement therapy. All quadrants can be involved but ILC occur more commonly seen in central area. Rendi et al. studied 93 cases of LN and stated on the basis on imaging findings, 74 %, 24 %, and 2 % of cases were detected by mammograms, magnetic resonance imaging (MRI) and US, respectively(5). Microcalcifications were the most common finding, occurring in 69 % of cases, followed by pathological MRI non-mass enhancement in 16 %, masses in 14 %, and architectural distortions in 1 % of cases.
On mammography, ILCs can be present as asymmetrical, ill defined, irregular masses or densities. Calcifications are not commonly seen in ILC (6).Therapies for breast cancers are changing very fast with our understanding of the molecular evolution of the disease. The diagnosis of the majority of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ACKNOWLEDGEMENT

I express my sincere thanks to Dr. Marie Therese Manipadam, my guide and mentor for her constant support and her patient and calm demeanor that helped me stay focused throughout. I wish to express my gratitude to Mrs Hepsy, department of biostatistics for her help in data analysis. Last, but not the least, I dedicate this work to all the patients, without whose clinical material, this would not have been a reality.

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INTRODUCTION

Invasive lobular carcinoma(ILC) represents 5% to 15% of invasive breast carcinomas(1). It is a distinct entity that is genetically and biologically different from invasive ductal carcinoma(IDC). It is the second most common type of breast carcinoma(2). The term lobular carcinoma was introduced by Foote and Stewart in their seminal articles where they described the characteristic loss of cellular cohesion, bland cytologic features and diffuse infiltration(3). The loss of cellular cohesion described by Foote and Stewart has been shown in a molecular level to reflect inactivation of E-cadherin gene(3–5). Morphological features along with immunohistochemical (IHC) staining for E-cadherin is routinely used to differentiate ductal carcinoma from lobular carcinoma. Circumferential diffuse membranous staining of E-cadherin is the typical pattern in ductal carcinoma, whereas lobular cancers lack or show decreased E-cadherin expression. However, aberrant E-cadherin reactions have been described in breast carcinoma and include retained weak or partial incomplete thin membrane reaction in lobular-type tumours and reduced membrane reaction in ductal-type tumours(6,7). This was seen in upto 15-20% of cases in published literature(7) . The reduction of E-cadherin stain in some high-grade infiltrating duct carcinoma and retained weak E-cadherin staining in some lobular neoplasia raise questions about using E-cadherin stain to distinguish lobular from ductal lesions. The distinction is especially important in the in- situ stage. Lobular carcinoma in situ diagnosed by core or excisional biopsy requires no further surgery, whereas duct carcinoma necessitates complete surgical excision and possible additional radiotherapy(8). Other immunostains that aid in the differential is p120 catenin. Ductal carcinoma cells display membranous staining of p120 catenin whereas

lobular carcinoma cells show diffuse cytoplasmic staining of p120 catenin(9). Recent studies have shown that double labelling for E-cadherin and p120 catenin enhances the diagnostic accuracy. In this study we plan to retrospectively study the clinicopathologic features of lobular carcinoma cases diagnosed in our department. In addition, we plan to assess the utility of p120 and E-cadherin double immunostaining in diagnosing lobular carcinoma of the breast and compare it with E-cadherin immunostaining .

AIMS

Aim of this study is to evaluate the clinicopathologic features of lobular carcinoma cases diagnosed in our department from January 2012 to September 2016. We also aim to compare the utility of double immunostaining (p120/E-cadherin) with single immunolabelling with E-cadherin in diagnosis of Lobular carcinomas.

OBJECTIVES

1. To do a detailed clinical and histomorphological study of lobular carcinoma cases diagnosed in our department from January 2012 to September 2016 and to further subclassify the different variants of lobular carcinoma.

2. To study the utility of p120 catenin/ E-cadherin double labelling immunohistochemistry in diagnosing in-situ and invasive lobular carcinoma and compare it with E-cadherin immunohistochemistry when used as a single antibody.

REVIEW OF LITERATURE

Invasive Lobular carcinoma represents 5% to 15% of invasive breast carcinomas (1, 2). The incidence of ILC has increased relative to IDC (1). The tumour was first described by Foote and Stewart in 1941(10).The term 'lobular neoplasia' was first introduced to encompass a spectrum of *in situ* neoplastic proliferations including atypical lobular hyperplasia (ALH) and lobular carcinoma *in situ*(LCIS), which describe different levels of involvement of individual lobular units(11). The descriptions ALH and LCIS are widely used to classify these lesions since they confer different relative risks (4- to 5-fold and 8- to 10-fold, respectively) for the patient to subsequently develop invasive lobular cancer(12).

Clinical and radiological features :

Common presenting symptom is a palpable mass with irregular margins. They occur mostly in premenopausal women. Most of these cases are diagnosed between 40 and 50 years of age(13). There appears to be a particularly increased risk of ILC in patients receiving hormone replacement therapy. All quadrants can be involved but ILC occur more commonly seen in central area. Rendi et al. studied 93 cases of LN and stated on the basis on imaging findings, 74 %, 24 %, and 2 % of cases were detected by mammograms, magnetic resonance imaging (MRI) and US, respectively(5). Microcalcifications were the most common finding, occurring in 69 % of cases, followed by pathological MRI non-mass enhancement in 16 %, masses in 14 %, and architectural distortions in 1 % of cases.

On mammography, ILCs can be present as asymmetrical, ill defined, irregular masses or densities. Calcifications are not commonly seen in ILC(2).Therapies for breast

cancers are changing very fast with our understanding of the molecular evolution of the disease. The diagnosis of the majority of neoplastic breast lesions by light microscopy is a relatively straight-forward task for the surgical pathologist, therapeutic demands are resulting in increasing demands on the pathologist to correctly diagnose the specific types of breast neoplasia so that appropriate risk assessment and prognostic and predictive tests will be performed and appropriate therapies instituted. The broad categories of ductal and lobular carcinomas of the breast represent the majority of important breast lesions, but there are marked differences in morphology, molecular alterations, and treatment strategies for each category and within each category. The diagnostic discrimination between ductal and lobular carcinomas is important in several circumstances for therapeutic purposes. Invasive ductal carcinoma is composed of monoclonal proliferation of cohesive epithelial cells, which usually cause mammary ductal expansion. The IDC tumour cells range from low to high grade and are cohesive with preservation of membranous E-cadherin expression. The Invasive lobular carcinoma cells are usually uniform and dyscohesive with low nuclear grade and scant cytoplasm (7, 8). ILC lack membranous E-cadherin immuno-positivity. Distinguishing lobular from ductal neoplasia is a task for which reproducibility data shows a k value that is moderate at best(6,14). A diagnosis of invasive lobular carcinoma on breast core biopsy may, depending on patient suitability for breast conserving therapy, necessitate magnetic resonance imaging of the breast to determine the extent of disease for purposes of obtaining negative margins for breast conserving therapy(1,8). Lobular carcinomas tend to have a poorer response to primary chemotherapy compared with ductal carcinomas.

Although there seemingly is a plethora of antibodies in the diagnostic armamentarium of the surgical pathologist, less than a handful of antibodies are useful in identifying breast carcinomas in metastatic sites.

Histopathology

Atypical lobular hyperplasia/ lobular carcinoma in situ

Both ALH and LCIS can be defined by using the criteria given by Page and colleagues by a population of cells that are small, round, monomorphic, dyscohesive with an increased nuclear to cytoplasmic ratio; ALH is diagnosed when <50% of the acini in the affected terminal duct lobular unit are involved by the lobular proliferation and these cells do not completely occlude the lumen or produce marked distension of the acini(14). LCIS is diagnosed when >50% of the acini in the affected terminal duct lobular unit are completely filled and distended by the cellular proliferation(9). ALH and LCIS often coexist, and it can be difficult to differentiate ALH from LCIS. Intracytoplasmic vacuoles are often present in both ALH and LCIS and can be a prominent feature. Some of the cells have clear vacuoles, known as intracytoplasmic lumina or magenta bodies(15). When these magenta bodies are found in a Fine needle aspiration(FNA) biopsy they are suggestive of lobular lesion. Mitotic figures and necrosis is very rare. Pagetoid spread which is another characteristic feature is also commonly seen. Occasionally in some cases cloverleaf pattern which is ductal involvement by lobular cells also can be seen. Sometimes LCIS can colonize pre-existing breast lesions such as sclerosingadenosis, radial sclerosing lesions, papillomas, fibroadenomas, and collagenous spherulosis which leads to confusion.

Variants of LCIS:

Several variants of LCIS have been recognized. These include pleomorphic LCIS, pleomorphic apocrine LCIS, LCIS with comedo necrosis and carcinoma in situ with mixed ductal and lobular features. Clear cell and signet ring cell variants of LCIS have also been described.

Classic LCIS:

It is a distension of lobules with a monomorphic proliferation of discohesive epithelium exhibiting round shape, centrally placed nuclei and mild nuclear atypia and enlargement. Cellular discohesion refers to the loss of normal adherence between cells. This is manifest as a rim of space in between individual LCIS tumor cells(16). This is a major feature of lobular differentiation.

Intracytoplasmic vacuoles or a signet ring appearance may be present. There may be a distinct thin pink rim that outlines the intracytoplasmic vacuoles; and the vacuoles may contain a dot-like material, producing a targetoid appearance. Vacuoles and signet ring formation can be helpful clues of lobular differentiation since these are not usual features of ductal lesions. LCIS is frequently a lobulocentric process but it can extending to ducts or, as discussed later, it can be a purely ductocentric process. At low magnification, lobulocentric LCIS presents as a cluster of enlarged acini filled and expanded by tumour cells, much like a cluster of grapes. Ductocentric LCIS may present in a number of different growth patterns. In longitudinal section, minimal involvement of a duct may present with a pagetoid pattern alternatively, florid

involvement may fill and expand the duct.In cross-section, minimal involvement of a duct mayproduce bulbous outpouchings of the duct that resemble a cloverleaf.

Pleomorphic LCIS :

It was described as a distinct entity by Eusebi et al in 1992.Sneige et aldescribed in detail the morphological features of pleomorphic LCIS not associated with invasive lobular carcinoma(10). The cytological appearances of these cells are quite different to those of classic LCIS. Although the cells appear dyscohesive as in classic LCIS, they show a higher degree of nuclear pleomorphism and usually have abundant cytoplasm. Their cytoplasm may appear eosinophilic and finely granular, giving the cells an apocrine appearance (pleomorphic apocrine LCIS). Central comedo necrosis and calcifications are quite commonly associated with this lesion and can be confused with comedo ductal carcinoma in situ (DCIS).

LCIS with Comedo Necrosis:

Lobular carcinoma with comedo necrosis has recently been described. Ductocentric LCIS may cause marked expansion and filling of the duct accompanied by central necrosis(11,12). The degree of central necrosis may range from focal to extensive. Calcification may also be associated with necrosis. This pattern can mimic comedo-type DCIS. Diagnosis of LCIS might not be considered if the characteristic pattern of lobulocentric LCIS is not present. One clue to lobular differentiation is discordance between the degree of nuclear atypia and presence of necrosis. Most comedo-type

DCIS exhibit tmoderate or severe nuclear atypia and pleomorphism, whereas LCIS with necrosis exhibits monomorphic nuclei with mild atypia. LCIS with necrosis has to be distinguished from DCIS(13,14). Some in situ lesions show features that resemble both LCIS and DCIS. These lesions are composed of small, monotonous cells typical of LCIS, but appear more cohesive(20). These lesions may resemble DCIS with the formation of microacinar-like structures but display loss of cohesion typical of LCIS.

Mixed Ductal and Lobular:

These cases often are comprised of small, monotonous cells typical of LCIS, but appear more cohesive(17). They resemble DCIS architecturally with the formation of microacinar-like structures but display loss of cohesion typical of LCIS(18).

These tumours are hypocellular and composed of small to medium sized dyscohesive cells, which are individually dispersed in a fibrous connective tissue or arranged in single-file or linear cords. Classic ILCs invade the breast parenchyma without destruction of residual ductal-lobular structures, often with limited host reaction. Desmoplasia or lymphocytic reaction are not common features. The infiltrating cells frequently present a concentric pattern around normal ducts, a feature known as targetoid growth pattern. Cytologically the cells have round or notched ovoid nuclei with low grade nuclear atypia and a thin rim or slightly more abundant cytoplasm. Intracytoplasmic vacuolations are characteristic of lobular cells, sometimes leading to a signet-ring cell appearance. Mitotic figures are not readily found.

Histological variants of ILC:

ILCs have been classified according to structural features in histological subtypes named trabecular, alveolar and solid variants. These structural variants indicating would have distinct prognostic implications, with the solid variant indicating a worse prognosis. The trabecular growth pattern is characterized by invasive tumours similar to classic ILCs but composed of broader bands of cells instead of single file cell pattern(3). In the alveolar pattern the tumour cells are arranged in globular aggregates of atleast 20 cells separated by thin bands of fibrous stroma. The solid pattern is characterized by large sheets of uniform cells with lobular morphology with little intervening stroma. The homogeneity of the tumour cell population, the cellular dyscohesivenessand the presence of intracytoplasmiclumina are useful diagnostic clues. The 'classic' variant of ILC have a lower incidence of axillary lymph node metastases (P = .0005)(23).This variant is more pleomorphic and having a higher mitotic rate. Different growth patterns can occur in same tumour, the tumour should be classified as an ILC of mixed type. Histological grade has an independent significant impact on the prognosis of ILC, and it should be taken into consideration when planning the postoperative treatment in this group of patients(19).

Tubulolobular variant:

It is a unique type of invasive breast carcinoma which shows both ductal and tubular differentiation. Fisher et al. came to a conclusion that this lesion represents a tubular variant oflobular invasive carcinoma in 1977(20). Tubulo-lobular carcinoma is a rare

variant of invasive breast cancer and displays small, round tubules and cords of small cells arranged in diffuse and targetoid pattern. These tubules typically lack apical snouts and are smaller and less angulated than those seen in tubular carcinoma. The overall infiltrative pattern is highly reminiscent of classical ILC. The neoplastic cells are uniform with small rounded nuclei and inconspicuous nucleoli.

Signet ring cell variant:

This is a mucin rich aggressive carcinoma, which shows a prevalence of 2 to 4.5%. It has a high risk for metastatic spread. To call it as signet ring cell carcinoma >20% of the tumour cells should show signet ring morphology(21). These cells are arranged in loose or dyscohesive clusters with a variable size-small to intermediate, with large eccentrically placed nuclei with abundant cytoplasmic mucin(22). There is moderate anisonucleosis with moderate to marked hyperchromasia. Occasional cytoplasmic vacuolation can be seen. The targetoid pattern of classical invasive lobular carcinoma and Indian file pattern may also be seen here.

Histiocytoid variant:

These tumours composed of sheets of pale histiocyte-like cells with abundant, finely vacuolated to granular cytoplasm and inconspicuous cell membranes interspersed in the breast parenchyma. Often the term ‘ground-glass’ has been used to describe the cytoplasm(17). Nuclei are centrally or eccentrically placed which is generally dark to vesicular(18). Nucleoli can be indistinct in nature. Mitoses are generally sparse. Nuclear pleomorphism is mild to moderate, with mostly grade 1 to 2 nuclei as described(23). Cytoplasmic vacuoles can rarely be seen. The cells are disposed

individually or in loose aggregates, without obvious glandular differentiation.Sometimes cells can be arranged in a ‘targetoid’ pattern.(3, 6, 9,22). Accompanying lobular neoplasia (atypical lobular hyperplasia or lobular carcinoma in situ) may be seen sometimes.

Pleomorphic variant:

This variant is characterized by dissociated cells arranged in a loosely cohesive pattern(24). Occasionaly it displays intracytoplasmic mucin vacuoles (targetoid mucin). Cells display a higher degree of cellular pleomorphism and brisk mitotic activity and nuclear hyperchromasia, nuclear membrane irregularities and prominent nucleoli(25). The presence of abundant eosinophilic cytoplasm which suggests apocrine differentiation confirmed by positivity with gross cystic disease fluid protein 15 (GCDFP-15), a marker of apocrine differentiation(26).Pleomorphic lobular carcinomas were frequently ER- and PR-positive, E-cadherin-negative and occasionally HER2- and p53-positive(27).The smears are cellular and the cells are arranged predominantly in a dyshesive pattern, occasionally forming a few, small aggregates(26). Tumour cells display significant nuclear pleomorphism, prominent nucleoli, and abnormal chromatin distribution. Cytoplasm is abundant, pale to eosinophilic, and can be granular or vacuolated. Although the pleomorphic subtype is of biological interest, it does not appear to be of prognostic value(28).

The development of breast cancer is a multistep process which originates from terminal duct lobular units (TDLUs) and progressing towards invasive cancer(20,35). The four precursor lesions are atypical ductal hyperplasia (ADH), atypical lobular

hyperplasia (ALH), lobular carcinoma in situ (LCIS), and ductal carcinoma in situ (DCIS)(29). The low grade tumors include tubular, cribriform, lobular and tubulolobular carcinoma (TLC) allof which have a favorable prognosis, possibly a consequence of fewer genetic changes(26). They frequently show recurrent loss of chromosome 16q and gain of 1q, positive expression for estrogen receptor (ER) and glandular differentiation. The high grade malignancies of the breast shows a greater degree of nuclear atypia and are frequently associated with negative for hormonal receptors. These tumours generally carry complex genetic alterations such as loss of 8p,11q,13q and gain of 1q, 5p and 17q. Sometimes amplification of 6q22 and 8q22 are also seen(30). There is frequent coexistence of columnar cell lesions with low grade DCIS with some overlapping features. These findings suggest that columnar cell lesions are potential precursors for low grade DCIS and invasive carcinoma(22). Loss of chromosomal material of 16 q is seen in well and intermediately differentiated DCIS. This shows LCIS and well differentiated DCIS have common evolutionary pathways(31).

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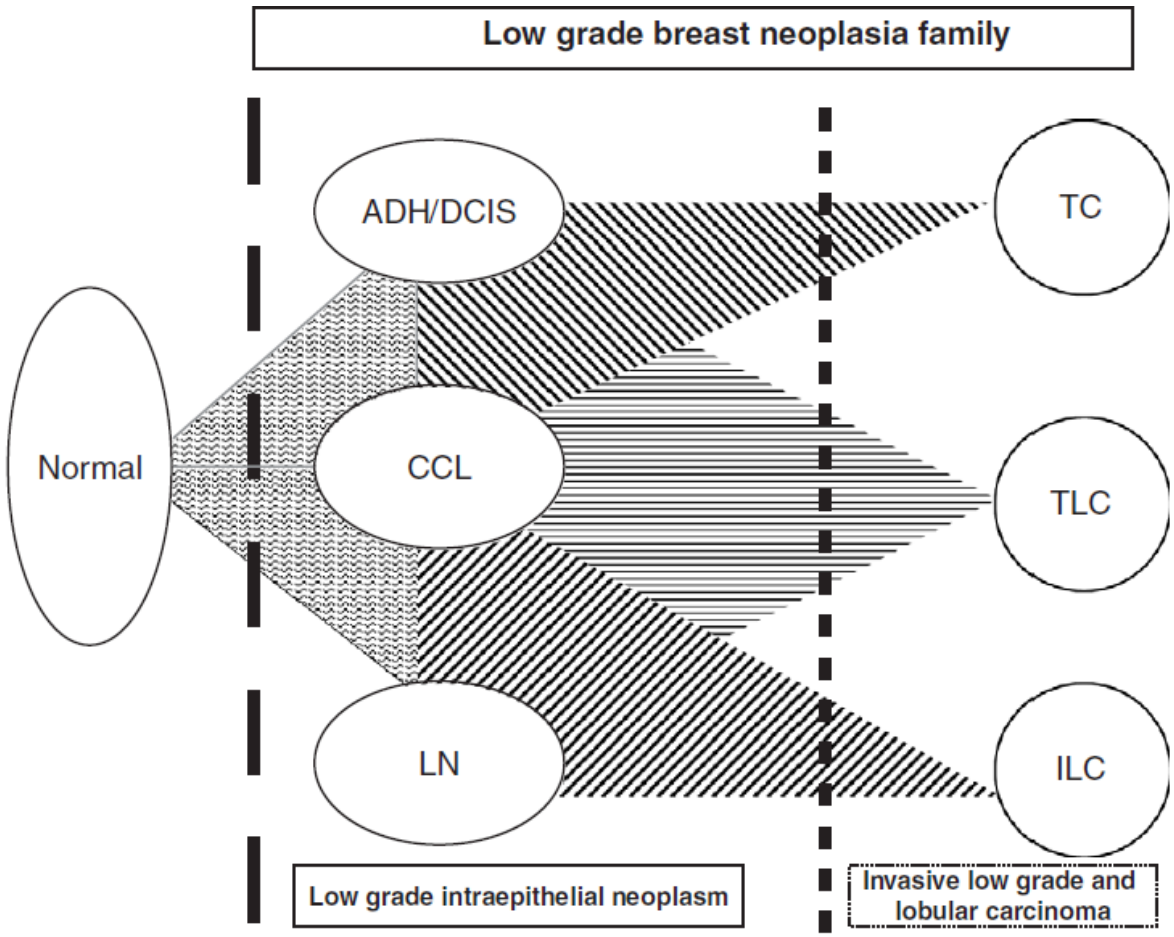


Figure 1: Evolutionary pathways of low grade breast neoplasia

. [taken fromAbdel-Fatahet al,(22)]

Molecular basis for the morphological characteristics of lobular carcinoma:

Molecular studies have been instrumental in highlighting the role of E-cadherin inactivation in the development of lobular lesions. E-cadherin, the protein product of the CDH1 gene (16q22.1), is a member of the calcium-dependent adhesion (ie, cadherin) family of transmembrane proteins, whose expression is found in epithelial cells(32). E-cadherin is an adherens-type of junctions and that forms homodimers in a calcium-dependent manner with other E-cadherin molecules of the adjacent cells(See

Figure-1). E-cadherin is composed of an extracellular, a transmembrane, and an intracytoplasmic domain. This intracytoplasmic domain is the juxtamembrane domain, which binds p120 catenin and a catenin-binding domain, which interacts directly with beta-catenin.

Beta-catenin in turn binds to alpha-catenin, a-actinin, and vinculin, which establish a direct link between the cadherin-catenin complex and the actin cytoskeleton(33). The catenins are normally located at the junction of the cytoplasm and internal aspect of the cell membrane where they link E-cadherin with the actin cytoskeleton(34). In normal breast tissue the E-cadherin is located on the membrane and the catenins are located at the inner membrane area. Thus normal breast duct epithelial cells and IDC show diffuse and strong membranous stain for both E-cadherin and p120 catenin. In ILC, on the other hand, there is loss of membranous E-cadherin and the inner membranous distribution of p120 catenin, which gives the dyscohesive morphology of ILC. The loss of membranous distribution of both E-cadherin and p120 catenin are concurrent. The loss of E-cadherin in ILC also results in the loss of α-, β- and γ-catenins, and p120-catenin becomes up-regulated and re-localised to the cytoplasm.Increased cytoplasmic p120 catenin activates a series of cytoplasmic Rho-GTPases resulting in increased cell motility(7–11). IHC analysis of epithelial cells lacking E-cadherin reveals cytoplasmic rather than membrane localization of p120 catenin(9). Neoplastic lobular proliferations (in situ and invasive; classic and pleomorphic types) characteristically show loss or marked down-regulation of the transmembrane protein E-cadherin in approximately 85% of cases, whereas luminal

epithelial cells and most ductal proliferations (ADH, DCIS, and IDC) exhibit positive staining by immunohistochemistry(9).

p120 Catenin:

p120ctn, initially described in association with E-cadherin as a tyrosine kinase substrate was localized to chromosome band 11q11. It was originally identified as an Src-tyrosine kinase substrate(9). The α and β catenins are complexed with the carboxy-terminal cytoplasmic tail of E-cadherin and the p120ctn is anchored to E-cadherin in a juxta-membranous site. p120 is not just a static structural component of the junctional complex, but rather p120 is actively involved in the status of cell motility, E-cadherin trafficking, E-cadherin turnover, promotion of cell junction formation and regulation of the actin cytoskeleton(30). p120ctn is detectable in the cell membranes of a wide variety of epithelial and nonepithelial tissues by immunohistochemistry, but predominates in virtually all types of epithelia. Like beta-catenin and plakoglobin these proteins are involved in mediating cell-cell adhesion(35).In mammalian cells, the presence of p120ctn promotes the assembly of the junctional complex with E-cadherin within the cell membrane. Overexpression of p120 recruits endogenous kinesin to N-cadherin thus dissociates the interaction between N-cadherin and p120 that leads to a delayed accumulation of N-cadherin at cell-cell contacts during calcium-initiated junction reassembly(36). E-cadherin is necessary and sufficient for recruitment and stabilization of p120ctn. Uncoupling of P120ctn and E-cadherin results in disruption of the tight junction and restoration of

coupling restores the tight junction(37). p120ctn that is bound to E-cadherin exists in equilibrium with a small cytoplasmic pool of p120ctn. When E-cadherin is absent, the cytoplasmic pool of p120ctn increases(38), which in turn sets into motion a chemical cascade that results in increased cell motility. Specifically, increased cytoplasmic P120ctn as occurs in the absence of membrane E-cadherin causes a decrease in the cytoplasmic activity of GTPases(39), RhoA, and increases cytoplasmic activity of Cdc42 and Rac1, all of which work to increase cell motility. The morphology correlates with dispersed dyscohesive cellular anatomy of invasive lobular carcinoma.

Sarrio et al has proven that p120ctn had a cytoplasmic localization in casesofinvasive lobular carcinoma(40), whereas ductal carcinomas showed reduced membrane p120ctn but no appreciable cytoplasmic p120ctn accumulation(39).

Mastracci et al1 demonstrated cytoplasmic relocation of p120ctn in early lobular neoplasia and invasive lobular carcinoma(37).

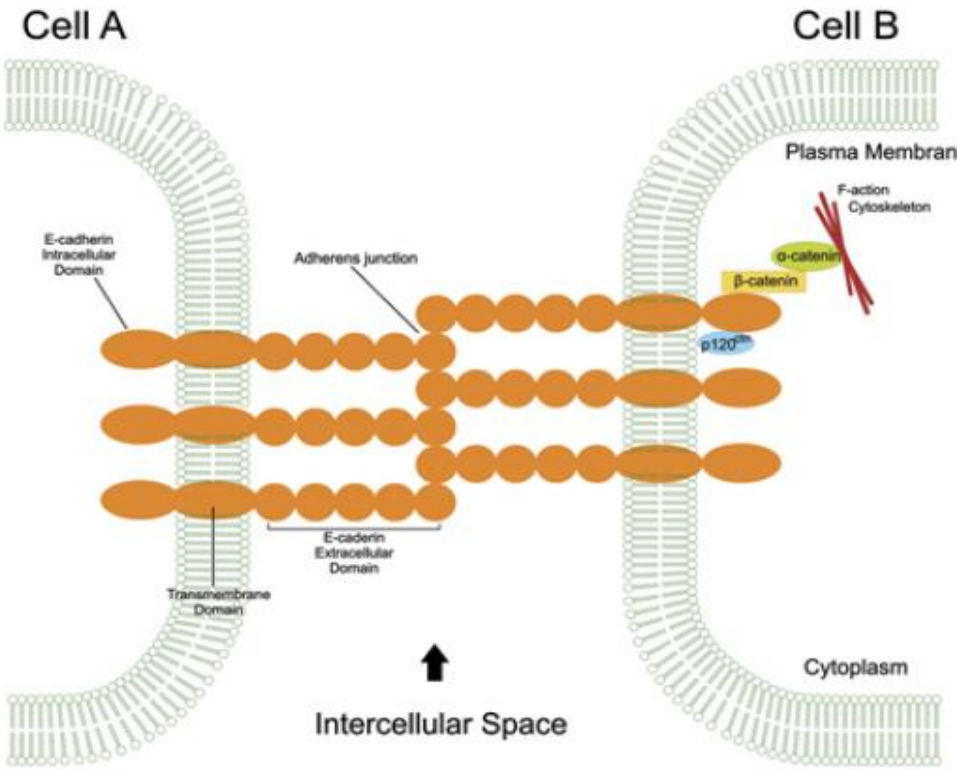


Figure 2: E-cadherin mediated cell-cell adhesion.

E-cadherin homodimers expressed on the plasma membrane of adjacent cells interact in a zipper-like manner.

The intracellular adhesion Complex, which consists of alpha catenin, beta catenin, gamma catenin and p120, links E-cadherin homodimers to the actin cytoskeleton [taken from Rafael de Deus Moura et al (41).

E-cadherin:

ILCs are characterized by the loss of membranous E-cadherin expression(42). E-cadherin membranous positivity does not preclude the diagnosis of a lobular carcinoma. E-cadherin function in lobular breast cancer can be disrupted due to

germline mutations and epigenetic silencing by DNA methylation(43). In case of E-cadherin downregulation, α- and β-catenins are rapidly degraded via an adenomatous polyposis coli–dependent mechanism that ultimately targets β-catenin for destruction by the proteasome. Some LCIS and ILCs may display aberrant E-cadherin membranous expression. E-cadherin inactivation or downregulation occurs through a combination of genetic, epigenetic, and transcriptional mechanisms(44). Loss of chromosome 16q is usually accompanied by truncating mutations or gene promoter methylation leading to biallelic inactivation of the gene and loss of protein expression. Recent massively parallel sequencing studies of invasive breast cancers have confirmed a statistically significant association between lobular carcinomas and CDH1 gene mutations. Identical CDH1 truncating mutations have been found in LCIS and associated ILC supporting the role of LCIS as a precursor for ILC. The loss of protein expression accompanied by E-cadherin DNA alterations in lobular carcinoma in situ(4).

Gene expression profiling in lobular carcinoma:

LNs and ILCs are more likely to be diploid than ductal tumours (45). Indeed, chromosomal and array-based comparative genomic hybridisation (aCGH) analyses have defined, on a gross scale, the genomic profile of lobular carcinomas - in short, they harbour fewer chromosomal changes than ductal carcinomas and are generally less complex. Genomic losses, such as at 16p, 16q, 17p and 22q, and gains at 6q were detected in LN by chromosomal CGH (30). The key alterations identified more recently by aCGH in classic LCIS, florid/extensive LCIS and PLCIS are 1q gain and

16q loss, with increased genomic complexity observed in the latter two groups of lesions, including loss of 8p, 11q and 17p and amplifications at 11q13 (*CCND1*) and 17q12 (*ERBB2*) . Like their pre-invasive counterparts and ER-positive IC-NST, both classic and pleomorphic ILC exhibit a high frequency of gain of chromosome 1q and loss of 16q (46–48), and it has been reported that all ILCs lose at least part of 16q (48). Other recurrent alterations include losses at 8p23-p21, 11q14.1-q25, and 13q, gains of 8q and 16p, and high-level amplifications at 1q32, 8p12-p11.2, and 11q13(49). Although some candidate genes in the various regions have been postulated (for example, *FGFR1* in 8p12-p11.2 and *CCND1* in 11q13 (50), no definitive data confirming the drivers contained in these various regions have been reported specifically for lobular breast cancer. This is likely a result of the complexity of the chromosomal changes and the context-dependent nature of some of these alterations. Numerous candidate oncogenes have been identified in these regions but not specifically for lobular tumours - for example, *ZNF703* gene amplification at 8p12 specifies luminal B breast cancer (51). As mentioned above, PLC contains a similar profile of chromosomal change, although there is increased complexity and additional amplifications are present - 8q24 (*MYC*), 17q12 (*ERBB2*/Her2) and 20q13, which are usually considered to be archetypal changes of high-grade ductal tumours (52). Some attempts have been made to classify tumour genome profiles based on genomic architecture as either simple, complex-firestorm or complex-sawtooth. The genomes of both classic and pleomorphic ILC are generally classified as simple (in that they frequently harbour 1q gain and 16q loss and few other alterations) or complex-firestorm (relating to the additional presence of complex, high-level amplifications at

the stated loci). It is conceivable that those ILCs that are classed as complex-firestorm have a worse prognosis, though this has yet to be explored.

Pitfalls in the diagnosis of LCIS:

Co-existence of DCIS and pleomorphic LCIS in the same slide or even in the same duct may occur. Again, if two morphologic patterns of in situ carcinoma are present and one appears to be pleomorphic LCIS, E-cadherin is advised to rule out the possibility that the other pattern is DCIS. LCIS involving collagenous spherulosismay mimic cribriform DCIS. The key thing is to recognize the features of collagenous spherulosis (pink collagenous or blue mucinous spherules surrounded by the thin waxy pink myoepithelial cell cytoplasm and nucleus); this will allow for excluding consideration of DCIS(53). When LCIS involves sclerosing adenosis, it can mimic invasive cancer. Thismay occur in two settings: First, if the sclerosing adenosis is extensive and already on its own resembles infiltrative growth, the superimposed LCIS may contribute further to the mimicry of invasive cancer. Second, if the LCIS is florid within the sclerosing adenosis, the LCIS- expanded acini/ducts may appear to coalesce and give the appearance of a solid sheet of invasive carcinoma. Recognition of sclerosing adenosis in the periphery of such lesions should be a clue to exercise caution and consider myoepithelial immunostaining before diagnosing invasion.

Pitfalls in the diagnosis of ILC:

The most common differential diagnosis of ILC is invasive ductal carcinoma with lobular features (IDC-L). This type of IDC with lobular features has similar architecture, histology, and hormone receptor profile. Clues to the diagnosis of IDC-L are slightly larger tumor cells and small nests. However, IDC-L often has infiltrative growth mimicking ILC. Both these entities can be differentiated via E-cadherin expression. Whereas IDC-L is positive for E-cadherin, ILC typically shows loss of E-cadherin expression. Other rare entities that differ in treatment but morphologically resemble ILC and may involve the breast include metastases of signet ring carcinomas from other primary sites, sclerosing epithelioid fibrosarcoma, myeloid sarcoma, mucosa-associated lymphoid tissue lymphoma, and plasma cell dyscrasias.

Diagnosis of lobular carcinomas and role of immunohistochemistry:

Diagnosis of lobular carcinoma is made on the basis of characteristic histologic features of a dyscohesive infiltrate of mostly small tumour cells. Loss of e-cadherin immunostaining has been traditionally used in aiding the diagnosis. However, recent studies have shown that up to 15% of ILCs retained membranous E-cadherin expression (9). In such cases, p120 catenin immunostain will help. The expression pattern of p120 catenin can be used to differentiate lobular from ductal lesions(54). p120 catenin show membranous localization by immunohistochemistry in normal luminal epithelial cells and most ductal proliferations. In lobular lesions, p120 catenin displays a cytoplasmic localization(55). In a study by Rakha et al, all cases of lobular carcinoma including the cases which showed aberrant membranous expression of e-

cadherin, showed strong cytoplasmic positivity for p120 catenin indicating the usefulness of the immunostain in the diagnosis of lobular cancers (11).The cytoplasmic expression of p120 catenin indicates a dysfunctional e-cadherin molecule, even if it is expressed by immunohistochemistry (11, 12). Dabbs et al found strong cytoplasmic staining for p120 catenin in 100% of their ILC cases and 0% of their IDC cases (5). Brandt et al found strong cytoplasmic staining for p120 catenin in 100% cases of LCIS and 88.7% of their ILC cases and weak cytoplasmic staining in remaining 11.3% cases. There was no cytoplasmic staining in ductal carcinoma cases (13). About 10% of the IDC cases in the study however showed weak membrane staining for p120 catenin, unlike the strong membrane p120 catenin staining seen in majority of the IDCs. Brandt et al concluded that both e- cadherin and p120 catenin are equally effective when used alone (13). However, utilisation of both markers has been considered to be the best diagnostic strategy for lobular carcinoma. (12)

Recent literature shows studies where double labeling techniques have been used for p120 catenin and E-cadherin (12). Double labelling can be applied in daily practice on paraffin-embedded tissue and is especially useful in small biopsies with small foci of CIS lesions and it showed 100% concordance with single antibody immunostaining using either E-cadherin or p120 catenin antibody. These studies also found that this double labelling may enhance accuracy and confidence in the differential diagnoses(10,12). Because of the small tissue volume, and even smaller volume of lesions picked up on screening, the number of IHC stains which can run a biopsy may be limited. To solve this problem, they developed a cocktail immunostainingcomposed of two primary antibodies raised against E-cadherin and

p120 catenin from rabbit and mouse, respectively. The two primary antibodies were then detected using secondary antibodies conjugated with different colors of chromogen(58). Thus only one slide is required for the double staining with E-cadherin and p120 catenin.

Significance of diagnosis of in situ lobular carcinoma :

The distinction of lobular carcinoma in situ from ductal carcinoma in situ has important therapeutic implications(53). Patients with the former are closely followed up without intervention, whereas, in latter case, complete eradication of the lesion is important (11,2).According to current guidelines, when LCIS is diagnosed on a core biopsy, a multi-disciplinary approach with careful clinicoradiological and pathological correlation is warranted to exclude any associated high risk lesion. Not all cases of LCIS diagnosed on a core biopsy need surgical excision. The same applies for LCIS diagnosed on an excision specimen, even when the lobular neoplasia involves the margins. A close mammographic follow up is recommended. Since some of LCISaremorefrequentlyassociated with adjacent invasive lobular carcinoma, such as the pleomorphic variantorcomedo-typeLCIS, excision appearswarranted when these variants are found in a core biopsy(59). Similarly, LCIS presenting with a mammographicstructural abnormalityshould be excised to exclude invasive carcinoma. LCIS is associated with increased risk of subsequent invasive lobular carcinoma, with equal predisposition in either breast. The minimum risk of developing ILC after LCIS is 7.1% at 10 year(60).So excisional biopsy of lobular carcinoma in situ, atypical lobular hyperplasia or lobular neoplasia only when it is associated with a

synchronous mass lesion or there is a discordance between radiologic findings and pathology(61).

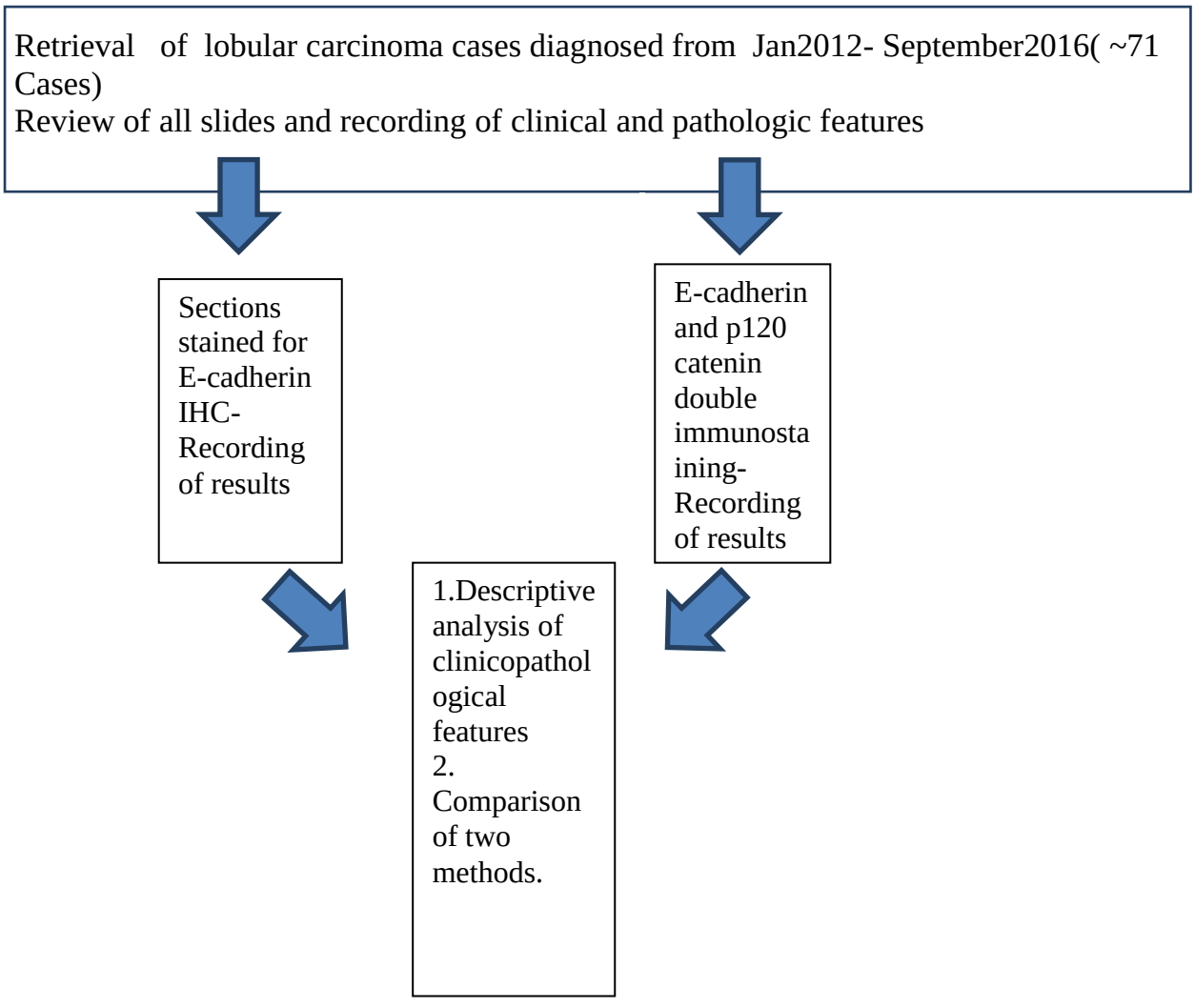
Clinical significance of diagnosis of invasive lobular carcinoma:

ILC tends to be more often bilateral and multicentric when compared to IDC. The variant is also known to metastasise to unusual sites like peritoneum, leptomeninges and visceral organs in the gastrointestinal and gynaecologic tracts (12). Metastases were found more frequently in ILC than in IDC in the bone (P=0.02) and/or in various other sites (peritoneum, ovary, digestive tract, skin) (P<0.001)(62).In terms of tumor size, 14% of ILCs were found to exceed 5 cm as compared with only 9.1% of IDCs(45). In a study done by KalnishaNaidoo et al, showed that 93% of ILC diagnosed in core biopsies show complete agreement between core and excision and hence MRIs is not necessarily performed.(60)

MATERIALS AND METHODOLOGY

Materials and Methods:

This was a retrospective study done on cases diagnosed as lobular carcinoma (both in situ and invasive) over the period from January 2012 to September 2016. The archived slides and blocks were retrieved and slides were reviewed and pathologic features recorded. Clinical details were obtained from electronic database. The study was carried out in Christian Medical College, Vellore in the department of General pathology. Invasive lobular breast cancers diagnosed in the department during the period January 2012 to September 2016 were retrieved using key word search of the electronic pathology database. Biopsies without sufficient material for evaluation were excluded from the study.



The clinical details were recorded from the electronic medical records and case sheets. The haematoxylin and eosin stained slides were reviewed and histopathological details were recorded. E-cadherin slides where available were reviewed. Few cases, where it was not available, immunostaining for E-cadherin was done on freshly cut sections and staining graded(intensity, pattern and proportion of positive tumour cells). Freshly cut sections were stained for double immunolabelling by E-cadherin and p120 markers and staining graded (intensity, pattern and proportion of positive tumour cells). Utility of both the staining methods was compared.

We had 71 cases from 62 patients, 9 of whom had bilateral tumours.

Of the 71 tumours, 38 had only core biopsies, 25 had core biopsy and resections(20 were mastectomy, 5 were WLE), 8 had only slide and block which were submitted for review. Of the resections 10 were prechemo and 15 were post chemo.

Clinical features recorded were laterality of the tumour, bilaterality and multicentricity if present and whether or not neo adjuvant therapy was administered.

Gross features were noted from the Pathology electronic database and included size, consistency and colour of the tumour.

Histopathological features:

Detailed histopathological study was done on 71 cases by evaluating the archival Hematoxylin and Eosin stained slides. The findings were noted in the proforma with relevant patient details. For those cases in which slides were not available, fresh sections were taken from the block. The slides were initially reviewed for histologic parameters by CC, and then further reviewed by both CC and MTM together on a double-header microscope. This combined review helped in eliminating observer bias. The following were the histological parameters evaluated.The observations were recorded in a Microsoft Excel worksheet.

Patterns: Under low power, we looked for various patterns of ILC.

The patterns recorded were single cell, Indian file pattern, nests and targetoid pattern. Among these patterns many were in combinations of various patterns.

These combinations were noted.

Dyscohesiveness: The characteristic feature of ILC is lack of cohesion. This is defined as separately lying tumour cells. This feature was observed under high power.

Mitosis: Mitosis is an infrequent finding in case of ILC. All the slides were scanned for Mitosis under high power objective.

Lymphocytic reaction: Lymphocytic reaction is one of the good prognostic factors. The presence or absence of lymphocytic reaction around tumour cells were noted.

Intracytoplasmic lumen: One of the characteristic feature of the ILC is intracytoplasmic lumen. Its presence or absence were noted.

Signet ring cell: Signet ring cells are a common feature for ILC. These cells have bland eccentrically placed nuclei, inconspicuous nucleoli and abundant amount of vacuolated cytoplasm. The presence or absence of signet ring cell morphology among tumour cells were noted.

Tubule formation: Tubule formation is rarely seen in ILC. Tubules are structures formed by malignant ductal epithelial cells with polarised nuclei surrounding a central lumen.

Nuclear pleomorphism: It is defined as variability in shape and size of the cells. It is a feature of all the malignant neoplasms. Nuclear pleomorphism was graded 1-3 depending on the variability and size and shape of nuclei, presence of prominent nucleoli.

Histological grading: Histological grading was done according to modified Bloom Richardson grading (see appendix).

Desmoplasia: This term denotes growth of fibrous connective tissue around a tumour. Tumour desmoplasiawas seen in many of our cases and it was noted.

Variants of ILC: The ILC variant in each case was noted and was categorised under one of the following : Classical, tubulolobular, solid, pleomorphic and histiocytoid .

LCIS: Presence or absence of associated Lobular carcinoma in situ was noted. Pagetoid spread: Presence or absence of Pagetoid spread of LCIS component was noted. Pagetoid spread in LCIS is defined as malignant cells extending along the ducts between intact overlying epithelium and underlying basement membrane.

Elastosis: It is one of the degenerative changes in the dermal connective tissue with increased amounts of elastotic material, usually in periductal or perivascular location or around tumour cell clusters. This finding is common in ILC.

Adjacent breast changes: Adjacent breast tissue was examined for pre neoplastic and non-neoplastic alterations including ductal carcinoma in situ, Atypical lobular hyperplasia, usual ductal hyperplasia, intraductal papillomatosis, Columnar cell changes and flat epithelial atypia. Presence or absence of calcification was also noted.

Lymphovascular invasion: Presence or absence of lymphovascular invasion was looked for in the periphery of the tumour and the presence or absence was noted.

Lymph nodes: Total number of excised lymph nodes and number of positive lymph nodes were noted.

Immunohistochemistry for E- cadherin: E-cadherin is regularly used to distinguish between ILC and Invasive ductal carcinoma. Absence of E-cadherin staining is a diagnostic feature of ILC. However, aberrant positivity for E-cadherin has been reported in ILC. Cases with aberrant positivity were recorded. The aberrant positivity was classified into one of the following staining patterns: discontinuous membrane staining, continuous membrane staining, dot like positivity and cytoplasmic positivity. E-cadherin slides were available in 50 of the 71 cases. Percentage of positive cells , intensity and pattern of staining was recorded. Percentage of positive cells was rounded off to the closest multiple of 10.

Double immunostaining for E-cadherin/p120was carried out on the blocks (37cases) according to standard procedure (appendix). Double immunostainingwas done on biopsies/ tissues taken prior to neoadjuvant therapy to avoid any treatment effects on staining.

p120: Intensity and localisation of staining and proportion of positive cells were recorded for p120. Intensity of p120 was graded as mild, moderate and marked and given the score as 1, 2 and 3. Localisation of the staining recorded as cytoplasmic and membranous. Proportion of positive cells (was recorded as percentage of total number of cells) and was rounded off to the closest multiple of 10. A total score combining proportion score and intensity score was given for p120 immunostaining akin to the Allred score for hormone receptor assessment (See appendix). Internal controls (normal ducts) were assessed for membrane staining for p120.

E-cadherin: Staining intensity, pattern and percentage of positive cells for e-cadherin in the double immunostaining method was also recorded as mentioned earlier.

Utility of both staining methods were compared and the concordance between two methods were noted.

Statistical methods

Sample size: The aim of the study was to compare between p120 & E-cadherin double immunohistochemical staining with E-cadherin immunostaining on all cases of Invasive lobular carcinomas between the period of Jan 2012 to September 2016. The difference in the sensitivity of the tests is approximately 10% in published literature. Ideal sample size was calculated as 73 with an alpha error of 5%. The sample size was calculated using n Master software version 2.0. However, due to non-availability of paraffin blocks and insufficiency of tissue in the blocks, double immunostaining could be done only on 37 cases.

Categorical variables were summarised as frequencies and percentages. Quantitative variables were summarised as mean and standard deviation for normally distributed variable or median and IQR for skewed variables. The Chi square test was used to compare the proportions in the two categorical variables and the Independent t-test was used to compare the means between two groups. Diagnostic accuracy were given with 95% confidence interval. For all the analysis, 5% level of significance will be considered to be significant. All the statistical analysis were done using stata/ic v.13.

RESULTS

The study included 71 tumours from 62 patients, 9 of whom had bilateral tumours. The cases were diagnosed between January 2012 and September 2016.Histomorphological and clinical details were studied in 71 cases. 50 cases had E-cadherin immunostaining done. Double immunostaining for E- cadherin/ p 120 was done only on 37 cases due to non availablity of tissue blocks or insufficient material remaining in the paraffin blocks.

Nature of specimen: Of the 71 tumours, 38 had only core biopsies, 25 had core biopsy and resections(20 were mastectomy, 5 were WLE), 8 had only slide and block which were submitted for review. Of the resections 10 were pre-chemotherapy and 15 were post chemotherapy.

Of the 71 tumours, 70 cases were invasive lobular carcinoma. One case had only lobular carcinoma in situ arising in a benign phyllodes tumour.

Gender: All tumours were in female patients. There were no cases of lobular carcinoma of male breast.

Side of the breast: In this study we had 28 left sided and 34 right sided breast tumours. Nine patients had bilateral tumours.

Age: Of the 62patients, one patient's age was not known. The mean age of the remaining 61 patients was 51.96years with a standard deviation of 11.95 years. The age ranged from 20-85 years with a median of 49.0years .66 % of patients cases had age more than 40 years.

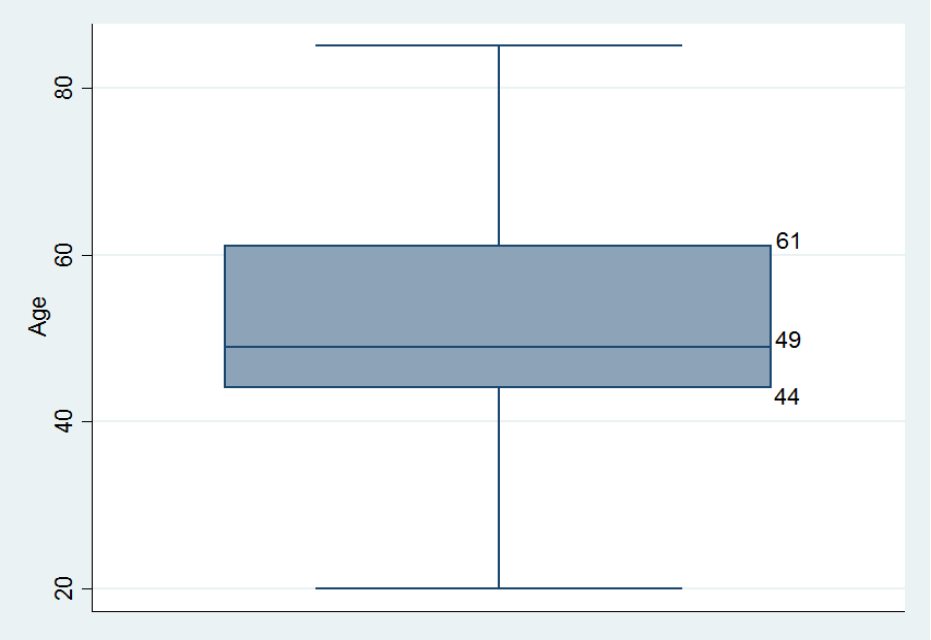


Figure 3: Box plot showing age distribution

Bilaterality: Invasive lobular carcinoma has the predilection to involve bilateral breasts. In our study of 62 patients, 9 had bilateral tumours (12.68 %).

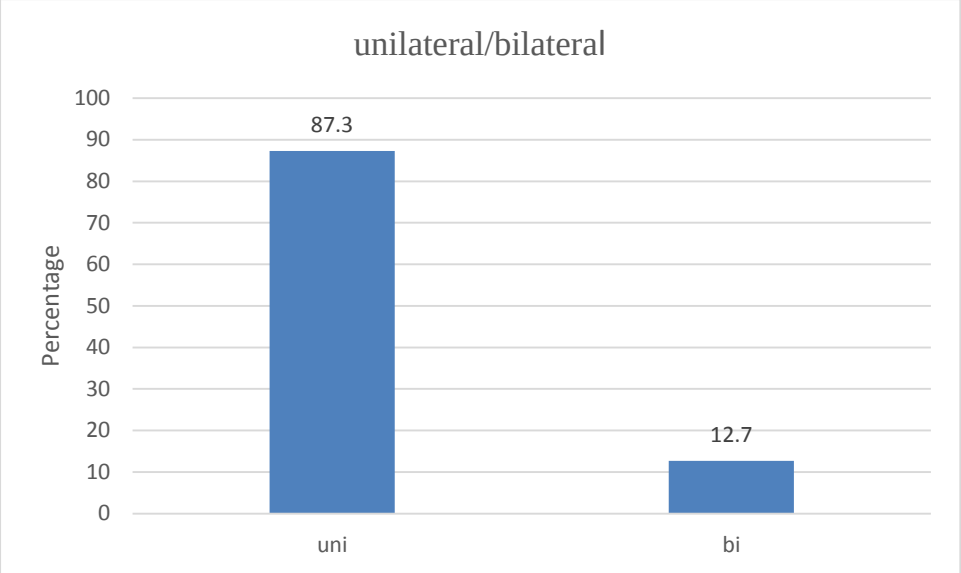


Figure 4: Frequency of bilateralityin invasive lobular carcinoma

Unicentric/Multicentric: There were no cases with multicentric tumour in our study.

Size : Mean size of the tumours were 3.47cm with a median of 3.50 and standard deviation of 1.64cm .

Gross features: The cut surface of the tumours were predominantly grey-white and the tumours had a firm consistency.

Histomorphology of lobular carcinoma

Pattern of arrangement of tumour cells: The most common pattern observed in our study was single cells, seen in 67 of the 70 tumours. The second most common was Indian file pattern which was observed in 65 tumours. The third common pattern was nests, seen in 40 cases (57%). (See fig.3) (See fig. 4).

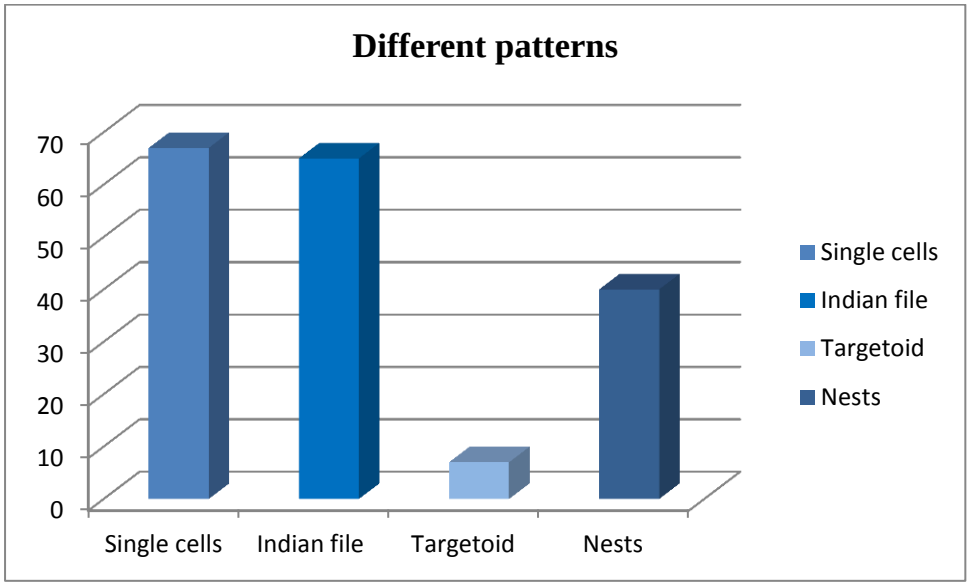


Figure 3: Common patterns seen in invasive lobular carcinoma.Y axis denotes number of cases.

Various combinations of multiple patterns were seen in every case. The most common combination was Indian file, single cells and nests. The next most common combination was Indian file and single cells and the third most common was Targetoid,Indian file and single cells. (See fig.6) In 2 cases, pattern could not be studied due to paucity of tissue.

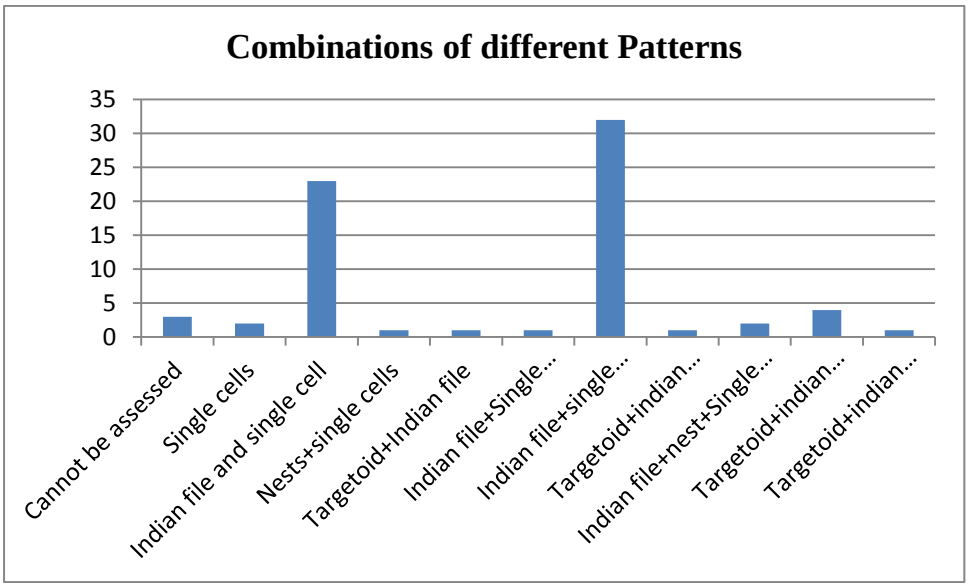


Figure 4 Combinations of multiple architectural patterns. Y-axis denotes number of cases.

Nuclear pleomorphism: Nuclear pleomorphism was noted in 68 of71 cases. In 3 cases, pleomorphism was not assessed due to paucity of tissues. 19 cases showed mildpleomorphism(nuclear score 1), 47 cases showedmoderatepleomorphism(nuclear score 2) and two cases showed marked nuclear pleomorphism(nuclear score 3).

Tubules: One case had score 1 for tubules, 10 cases(14.28%) had score 2 and rest of 57 cases had been given score 3.

Mitosis: We had 63 cases which had a score of 1 for mitosis, 2 cases had given a score of 2 and 3 cases had given score 3.

Table 1: **Summary of the scores histological grading :**

	Score 1	**Score 2**	**Score 3**
Nuclear pleomorphism	19	47	2
Tubules	1	10	57
Mitosis	63	2	3

Cumulative score according to Modified Bloom Richardson grading system

5 cases had a cumulative score of 5, 58 cases had a cumulative score of 6, 3 cases had cumulative score of 7 and 2 cases had a cumulative score of 8.

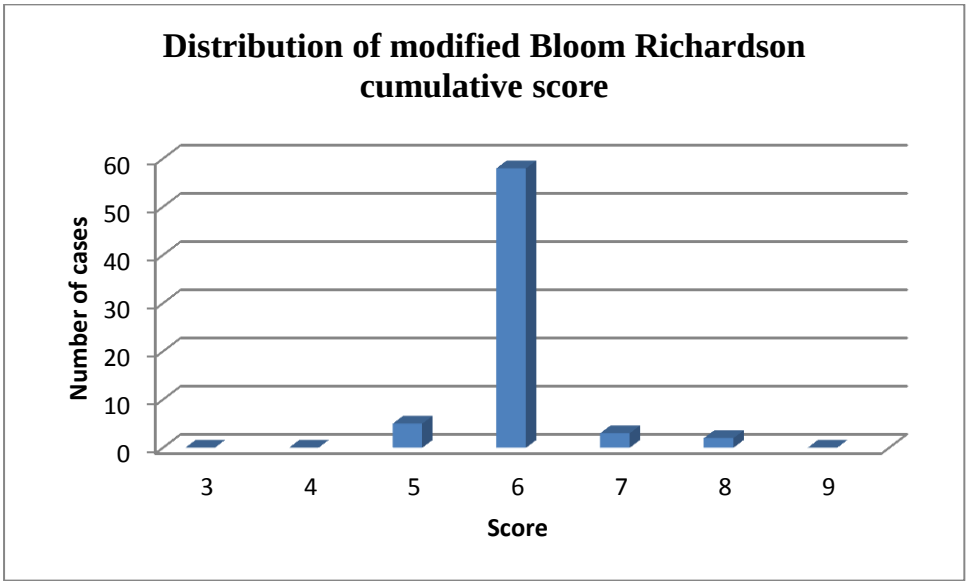


Figure 5: Distribution of modified Bloom Richardson cumulative score

Histopathological grading: According toScarff- Bloom-Richardson system(See Appendix),8.82% of the cases were grade 1, 88.24% were grade 2 and 2.94% cases were grade 3.

Intratumoral lymphocytic infiltrates: Lymphocytic reaction is commonly found in ILC. 59 of 71 cases showedlymphocytic reaction.

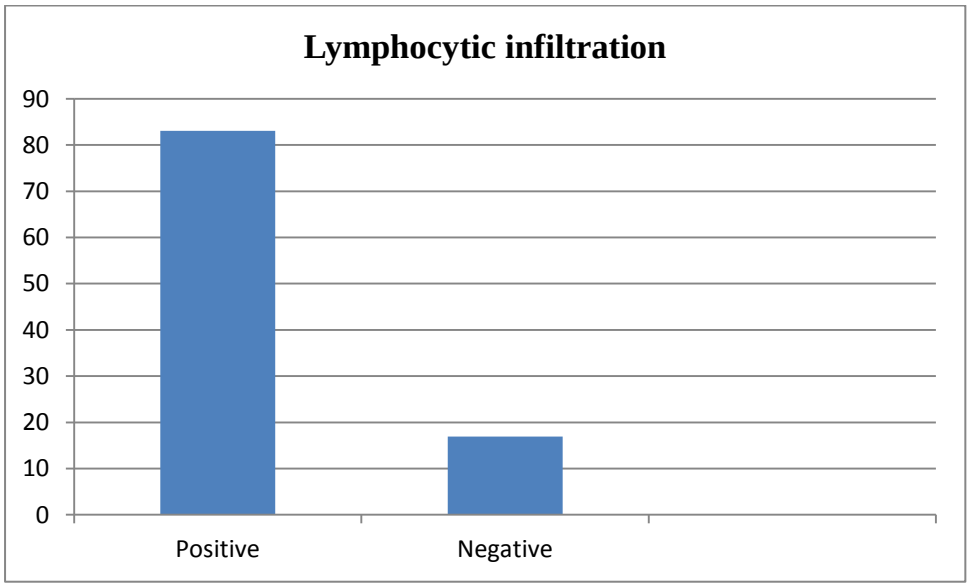


Figure 6:Frequency of Intratumoral lymphocytic infiltrates

Desmoplasia: Tumour desmoplasia was present in 68 of our cases.

Elastosis: We had 51(71.83%) which show elastosis.

Histological variants of ILC.: Subtyping was done in 69 cases. 64 cases were categorised as Classical variant (92.75%), 3 as pleomorphic(4.34%), 1 as tubulolobular variant (1.43%) and 1 as histiocytoid(1.43%).

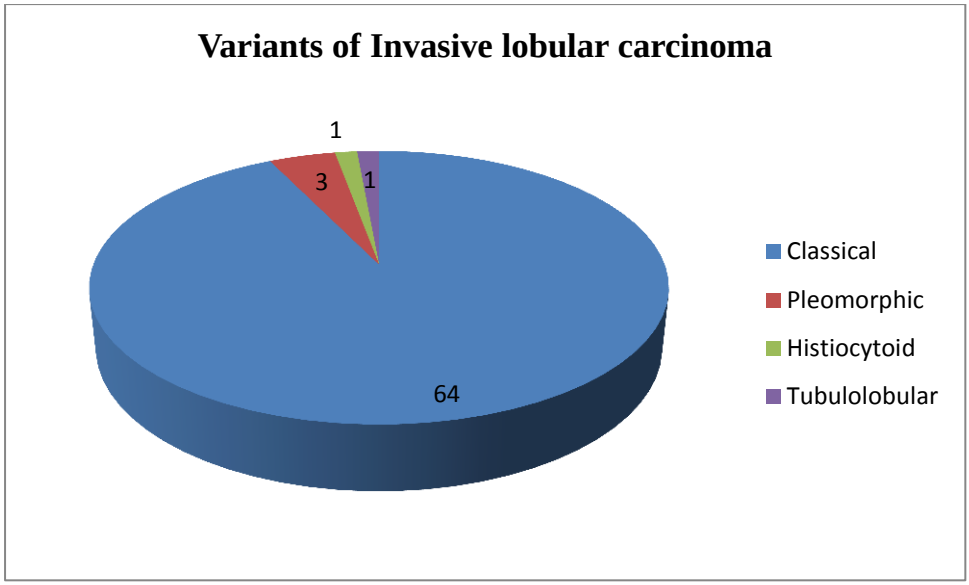


Figure 7: Variants of Invasive lobular carcinoma

LCIS: Associated LCIS was seen in 33 of our cases. In one case LCIS was seen involving sclerosingadenosis. One tumour had only LCIS without an invasive component, arising in phyllodes tumour.

Lymph nodes: 25 resection cases had axillary lymph node dissection,of this, 19 cases had positive lymphnodes. The distribution of number of positive lymph nodes is shown in figure 10. We had two cases which had 11 positive lymph nodes.

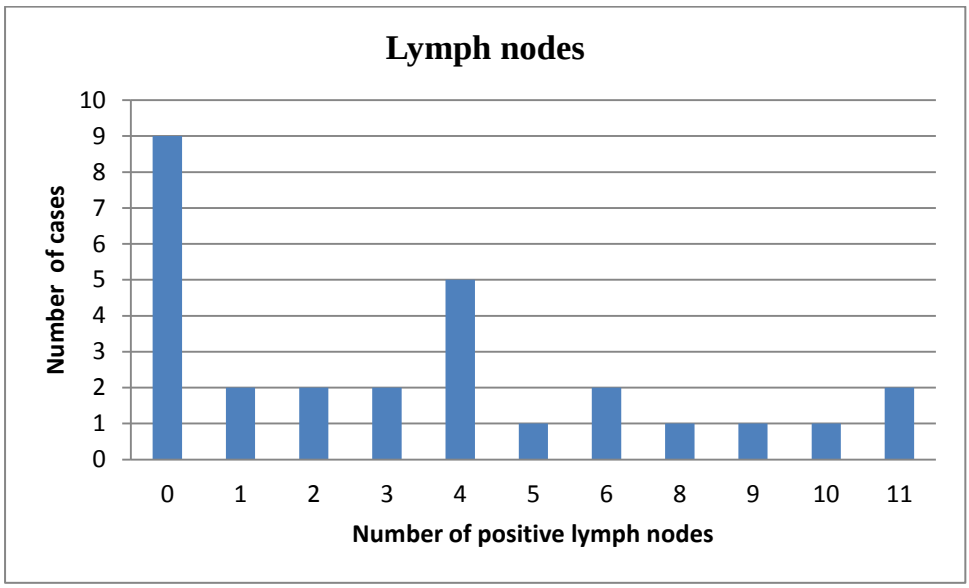


Figure 8: Number of Positive Lymph nodes

Proliferative changes in adjacent breast: Non-neoplastic alterations in adjacent breast seen included usual ductal hyperplasia, intraductal papillomatosis, columnar cell changes and occasional calcification. See table 1.

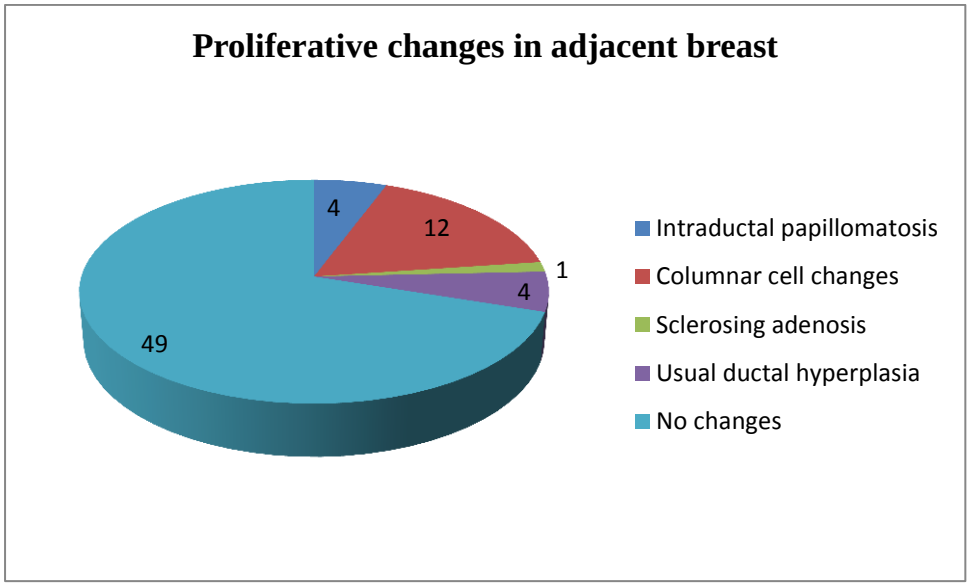


Figure 9: Proliferative changes in adjacent breast

Lymphovascular invasion: We had 28 cases(39.44) which showed lymphovascularinvasion , all of which were histologic grade 2.

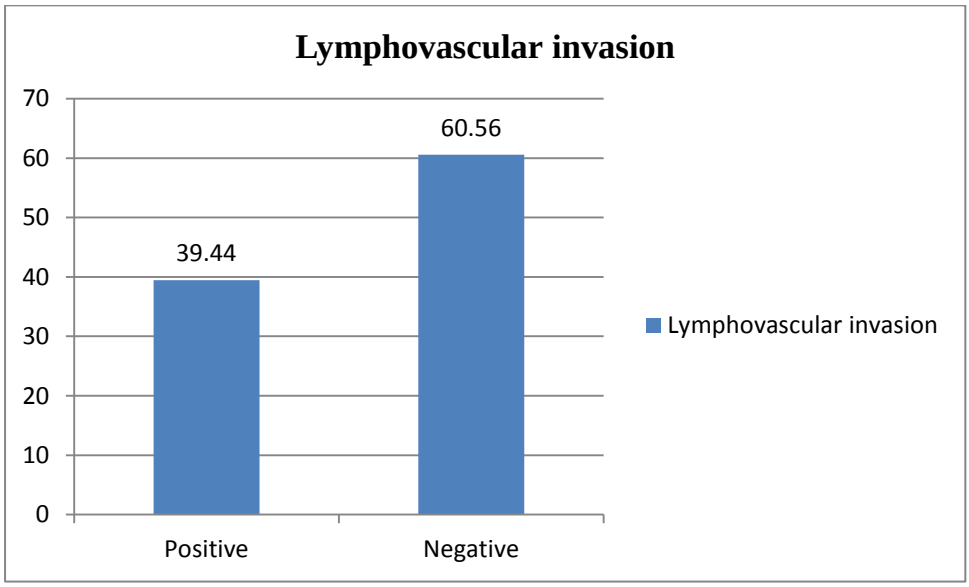


Figure 10 : Frequency of lymphovascular invasion

Perineural invasion: 5 of our cases showed perineuralinvasion , all of which belonged to histologic grade 2.

Skeletal muscle invasion: We had 4 cases which showed skeletal muscle invasion.

Table 2: Summary of histological findings

Histological Findings	Percentage
ILC Variant	
Classical	92.75%
Pleomorphic	4.34%
Histiocytoid	1.43%
Tubulolobular	1.43%
Patterns	
Single cells	95.71%
Indian file	92.86%
Nests	57.14%
Targetoid	10%
LCIS	28.57%
Proliferative changes in adjacent breast	
Intraductalpapillomatosis	5.63%
Columnar cell changes	16.90%
Ductal hyperplasia	5.63%

Table 3 :Summary of histological findings

Histological features	Numbers and percentage
Desmoplasia	68(95.77%)
Elastosis	51 (71.83%)
Calcification	5 (7.14%)
Skeletal muscle invasion	4(5.71%)
Perineural invasion	5(7.14%)
Lymphovascular invasion	28(39.44%)

Immunohistochemistry: Immunohistochemistryfor E-cadherin alone was available for 50 cases. Freshly cut sections were stained for double immunolabelling by E-cadherin and p120 IHC markers on 37 cases. The intensity, pattern and percentage of positive tumour cells was recorded in both methods. Utility of both the staining methods was compared. 15 cases had both E- cadherin staining and double immunostaining for E- cadherin/p 120.

Results of Immunostaining for E-cadherin:

E-cadherin immunostaining was available in 50 cases . 15 of the 50 cases (30 %) showed E cadherin positivity in the ILC component . 9 of these cases showed cytoplasmic staining, 4 showed only incomplete membranous staining and 1 case showed both incomplete membranous staining with cytoplasmic staining and one case showed both of the above staining patterns with dot like positivity. Pattern of aberrant staining and percentage of positive cells is given in Table 4.

In the 50 cases where E-cadherin was done, LCIS was present in 10 cases,4 of which showed positivity for E-cadherin.

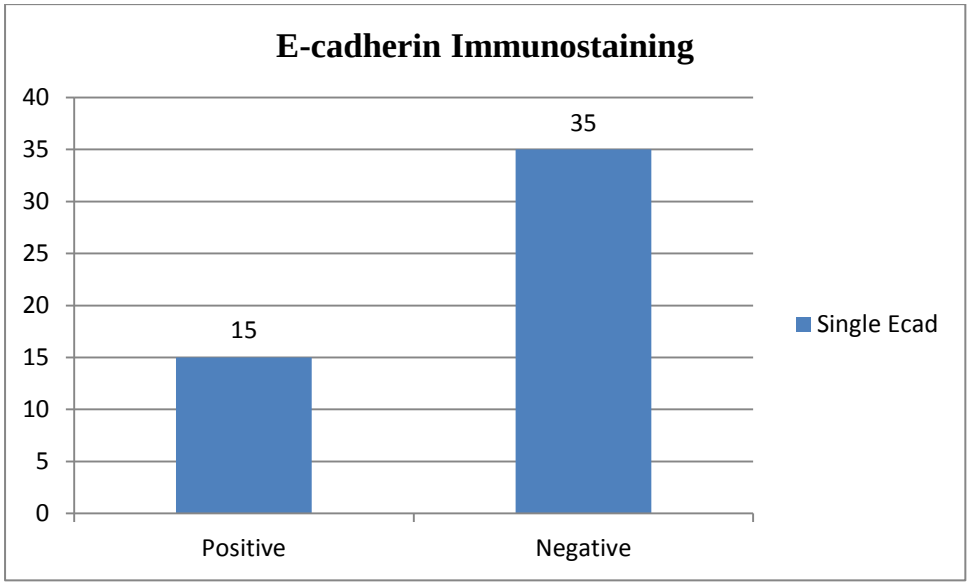


Figure 11: E-cadherin immunohistochemistry in invasive lobular carcinoma

Clinical significance of E-cadherin positivity:

We have compared age, percentage of lymphovascular invasion, grading of tumours and number of positive lymph nodes with E-cadherin positive and negative cases. E-cadherin positivity is not correlated with any of the above mentioned variables (Refer to table no 4)

Table 4: Summary of staining characteristics of E-cadherin in cases of E-cadherin positive invasive lobular carcinoma(Single antibody staining)

Serial no of cases	Percentage positive cells	Pattern
1.	50	Cytoplasmic Incomplete membranous
2.	10	Cytoplasmic
3.	60	Cytoplasmic
4	100	Cytoplasmic Dot like Incomplete Membranous
5.	60	Incomplete Membranous
6.	10	Cytoplasmic
7.	50	Incomplete Membranous
8.	5	Cytoplasmic
9.	70	Cytoplasmic
10.	50	Incomplete Membranous
11.	60	Cytoplasmic
12.	20	Cytoplasmic
13.	5	Cytoplasmic
14.	5	Cytoplasmic
15.	20	Incomplete Membranous

Table 5: Comparison between E-cadherin positive and negative cases

	E-Cadherin Positive	E-Cadherin Negative	p.value
Age	57.46 years	52.37 years	0.096
Lympovascular invasion	33.33%	51.4%	0.239
Grade 2	100%	94.29%	0.502
Lymph node positive	40%	42.86%	0.875

Double Immunostaining for E-cadherin/ p120:

Double immunostaining was done in 37 cases. 13 of these showed positive staining for E- cadherin. Six cases had cytoplasmic staining, 7 cases had incomplete membranous pattern of staining. Two of the E-cadherin positive cases >66% of the cells stained positive, two cases had 33-66% of cells positive and rest of the nine cases had 1-10% cells positive.

In all 37 cases of ILC (100%) in which double immunostaining was done, cytoplasmic staining for p120 was demonstrated in the tumour cells. 13 cases showed weak cytoplasmic staining (1+), 16 of the cases showed moderate intensity (2+) of staining, 8 showed strong (3+)cytoplasmic staining. Semiquantitative estimation of p120 of the invasive lobular carcinoma was done based on a system akin to Allred scoring system, adding proportion and intensity score of the cytoplasmic p 120 staining . (See appendix for scoring system).

In the thirty seven cases with double immunostaining, 16 had an LCIS component, and all of them (100%) were positive for p120 staining. 5 of these cases had strong(3+) cytoplasmic positivity, 7 had moderate (2+) cytoplasmic positivity and 4 cases had mild positivity (1+). The LCIS component in all these cases had a proportion score of 5(>66% of the cells stained positive). All 37 cases showed membrane staining for p120 and E-cadherin in normal ducts, which served as internal controls.

Correlation between the percentage of positive cells and intensity of p120: The following graph depicts the correlation between the percentage positive cells and intensity of grading. Thirteen cases had intensity of one. Of these 13 cases, 8 were given proportion score of 5. 16 of the cases with moderate intensity(2+) had proportion score of 5.

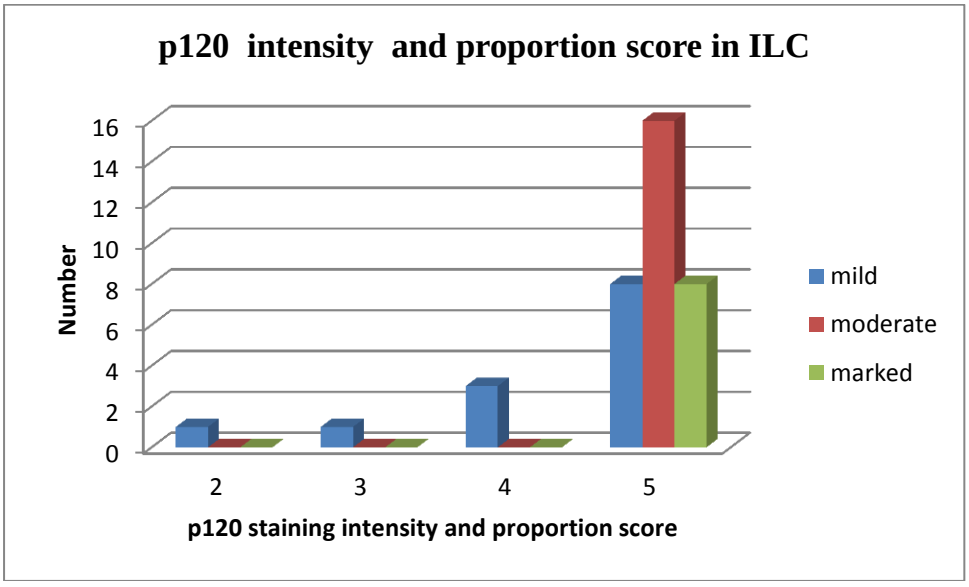


Figure 12: p120 - Intensity and proportion score in Invasive Lobular Carcinoma

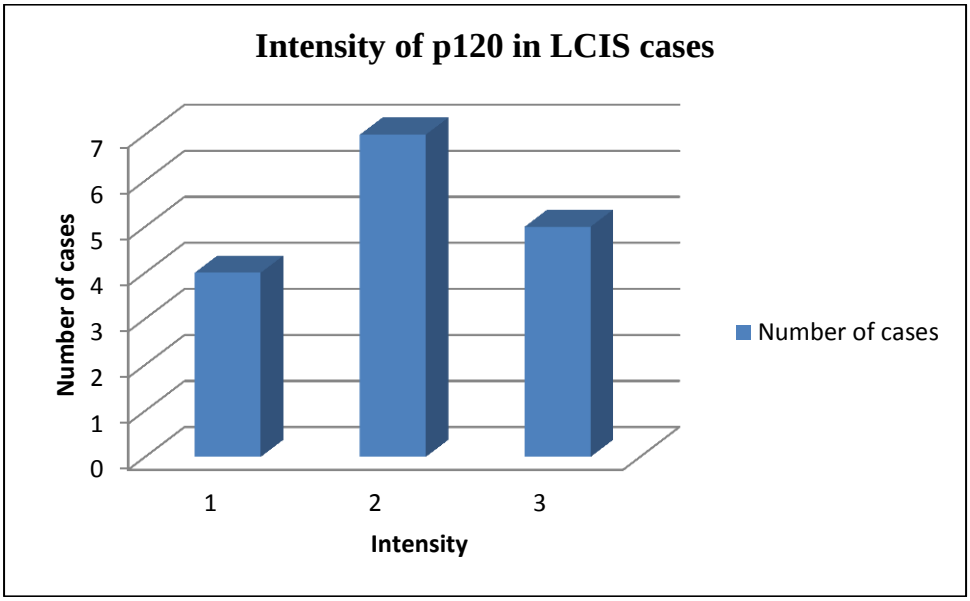


Figure 13: Distribution of intensity of p120 staining in LCIS

Table 6: Summary of intensity score and proportion score of p120 in in Invasive lobular carcinoma

	Proportion score 2	Proportion score 3	Proportion score 4	Proportion score 5
Number of cases with intensity 1	1	1	3	8
Number of cases with intensity 2	----	----	----	16
Number of cases with intensity 3	----	----	----	8

Table 7:Summary of staining characteristics of double immunostaining for E-Cadherin/ p120 in E- cadherin positive invasive lobular carcinoma

Case	Intensity	pattern	Percentage of positive cells	p120 percentage positive cells	p120 Intensity
1	1	Incomplete membranous	20	90	1
2	2	Cytoplasmic	80	90	2
3	1	Cytoplasmic	10	90	2
4	1	Cytoplasmic	5	100	3
5	1	Incomplete membranous	10	100	2
6	1	Incomplete membranous	5	80	2
7	2	Incomplete membranous	60	100	2
8	1	Incomplete membranous	40	100	2
9	1	Incomplete membranous	70	100	2
10	1	Incomplete membranous	5	100	2
11	1	Cytoplasmic	10	50	1
12	1	Cytoplasmic	5	100	3
13	1	Cytoplasmic	5	100	3

Images

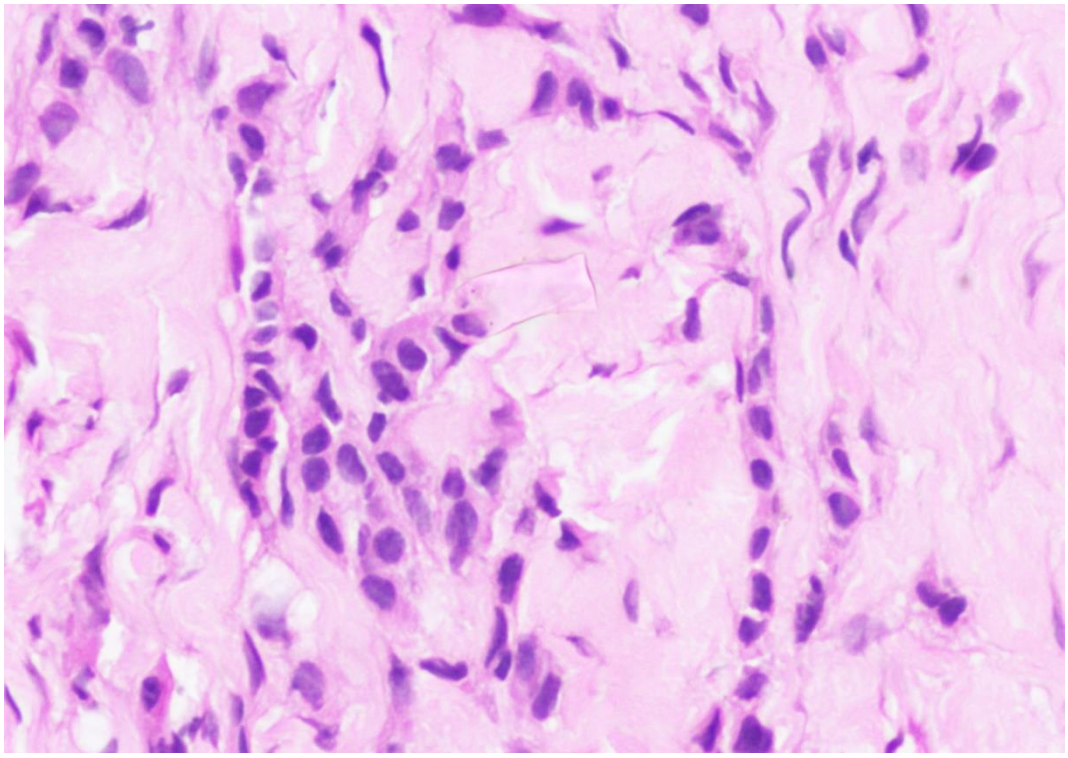


Figure 14: Tumour cells arranged in Indian file pattern in invasive lobular carcinoma (H&E,200x)

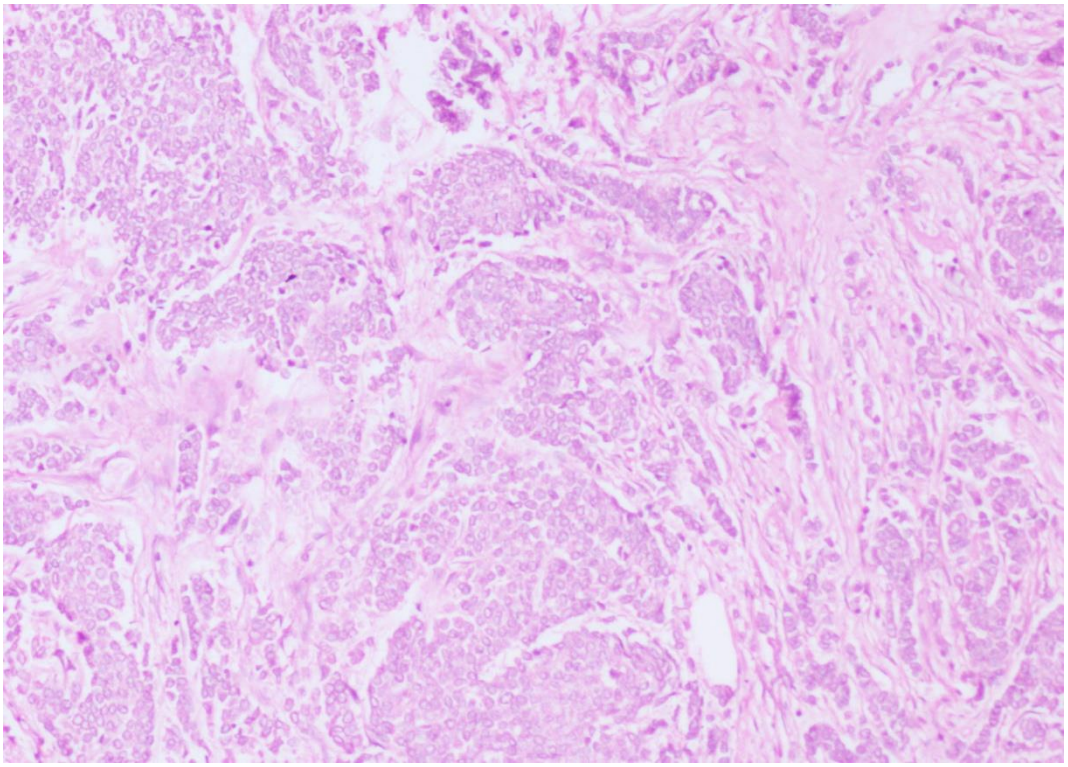


Figure 15 : Tumour cells arranged in nests in invasive lobular carcinoma(H&E, 100x)

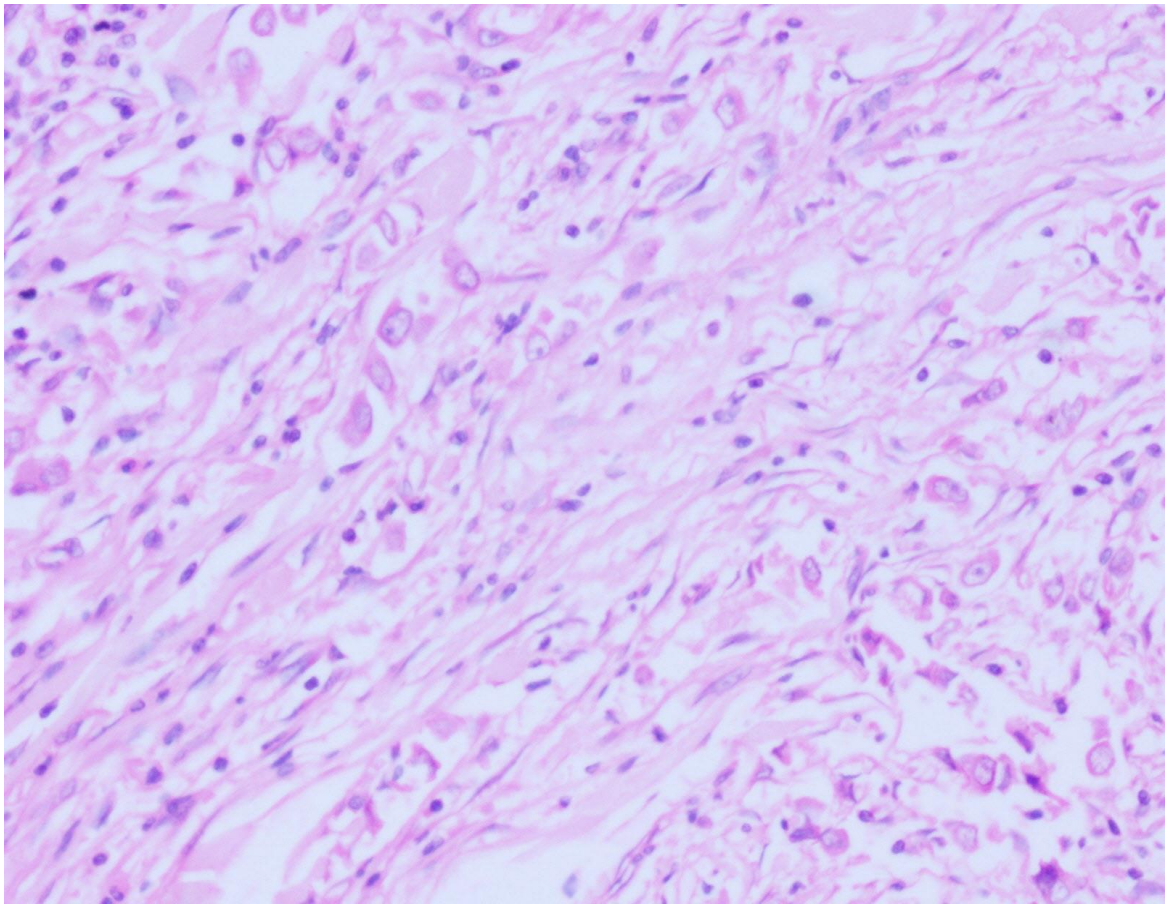


Figure 16: Tumour cells are arranged in single cells in invasive lobular carcinoma (H&E, 400x)

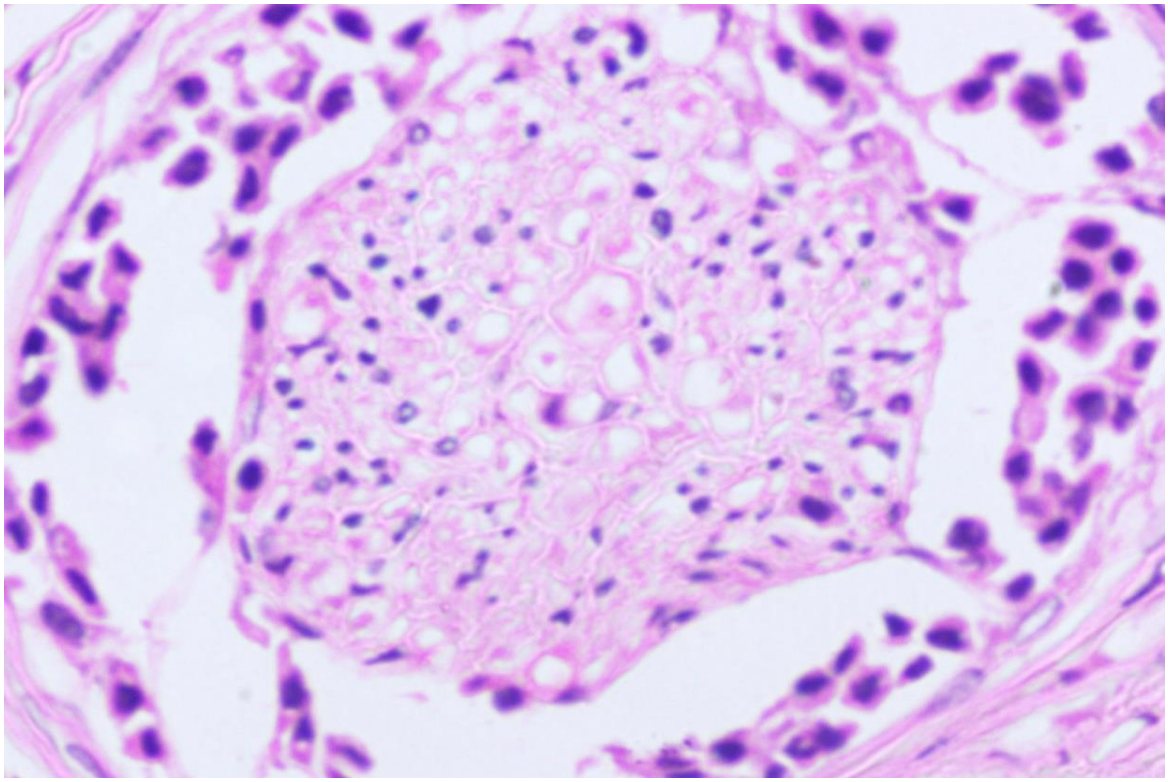


Figure 17: Perineural invasion(H&E, 400x)

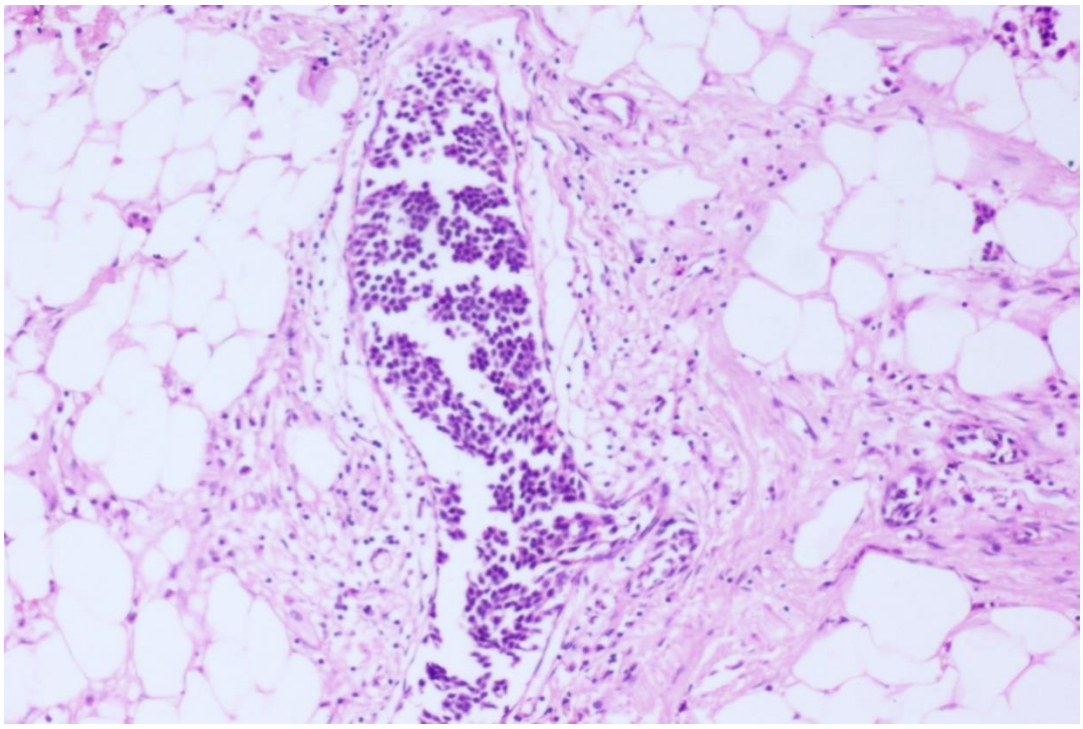


Figure 18:Lymphovascular Invasion (H&E,100x)

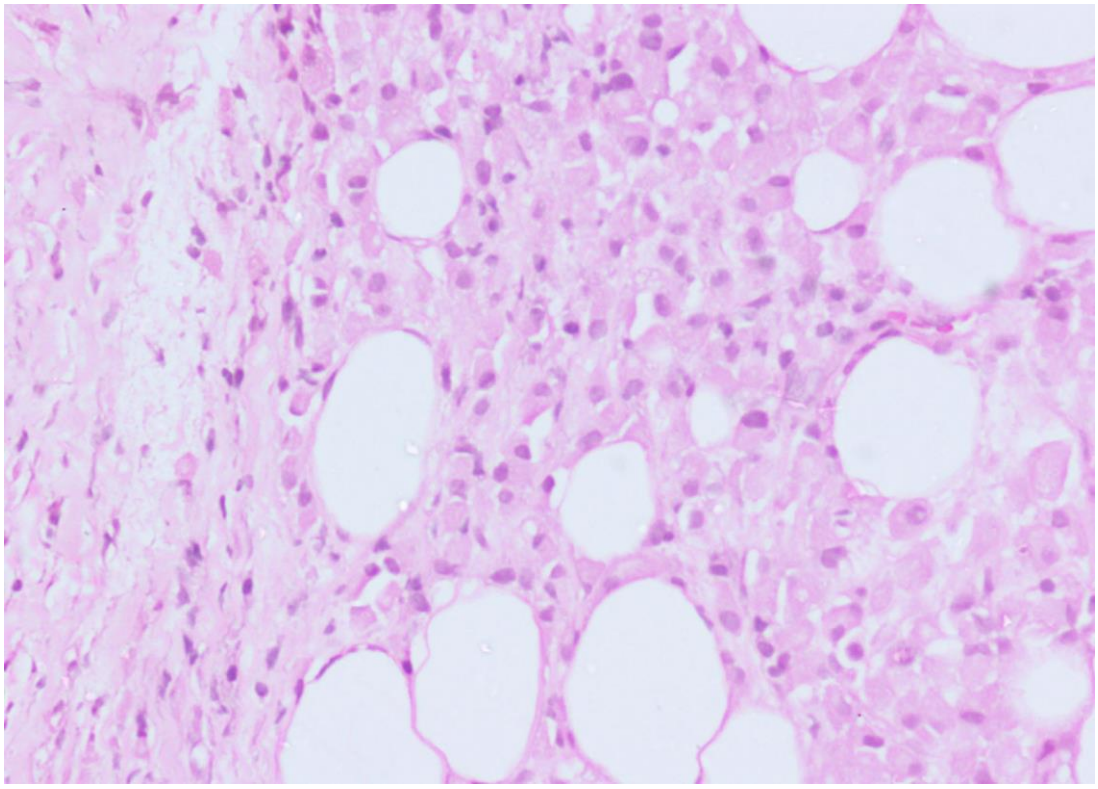


Figure 19: Invasive lobular carcinoma, histiocytoid variant(H&E, 200x)

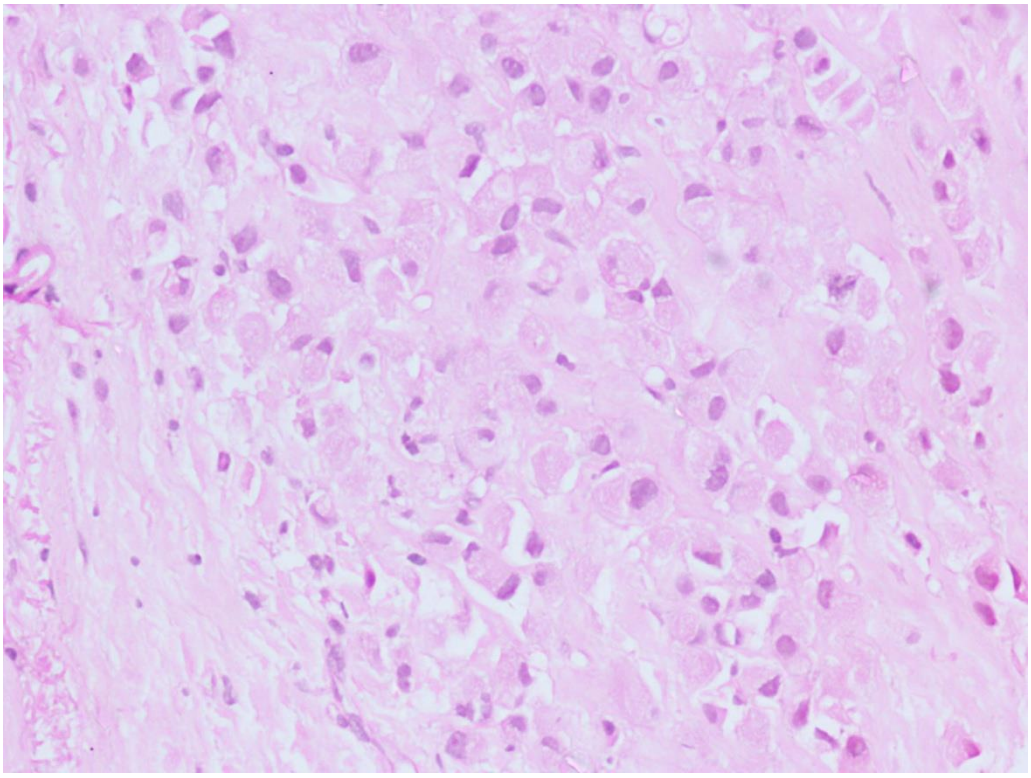


Figure 20 Invasive lobular carcinoma histiocytoid variant(H&E, 400x)

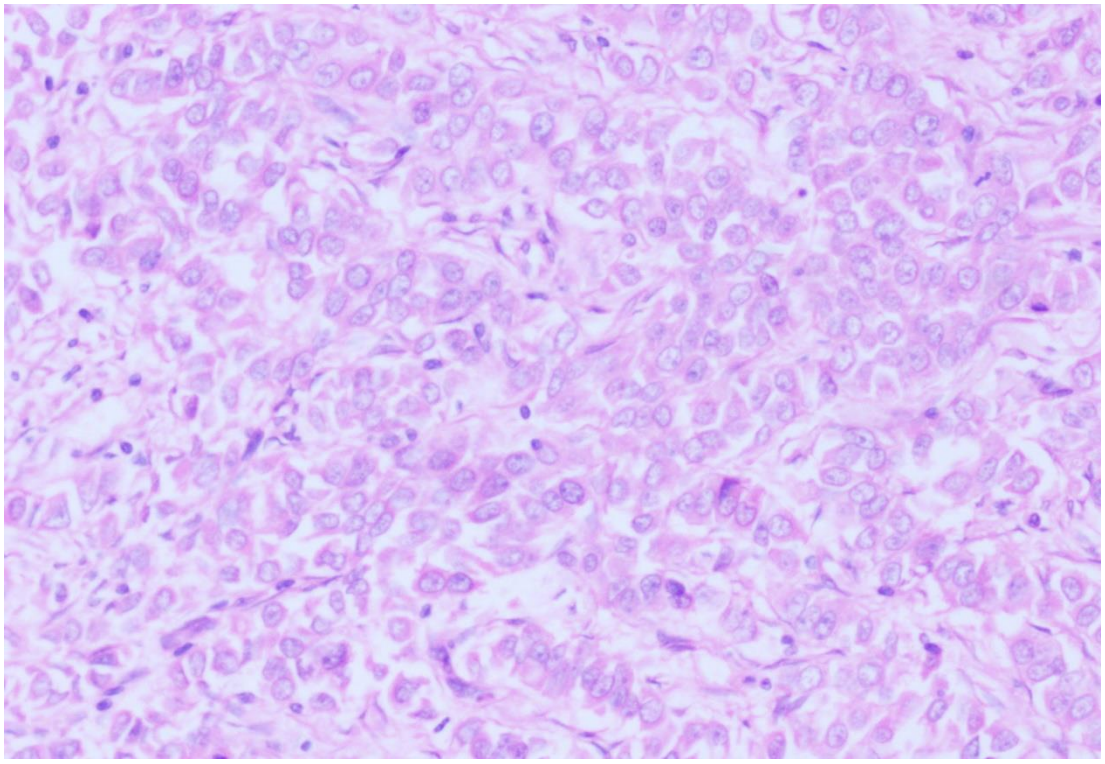


Figure 21: Invasive lobular carcinoma pleomorphic variant(H&E, 200x)

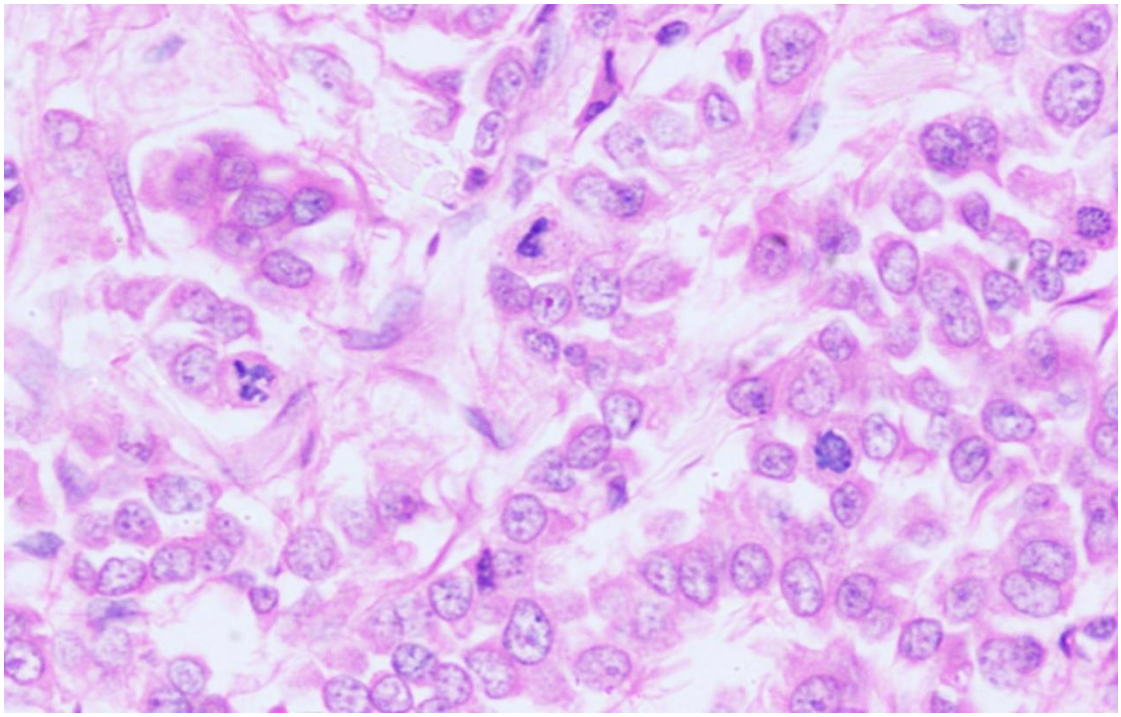


Figure 22: Invasive lobular carcinoma Pleomorphic variant(H&E, 400x)

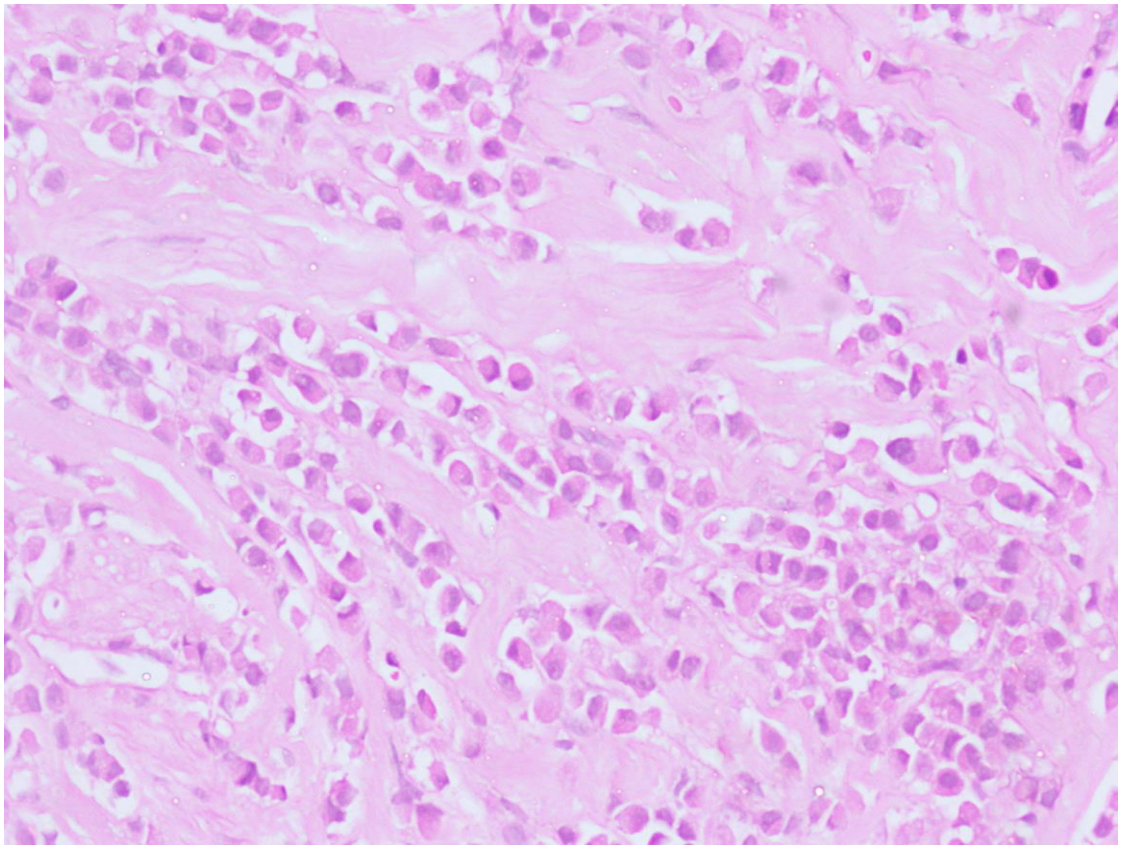


Figure 23 : Signet ring cells in invasive lobular carcinoma(H&E, 200x)

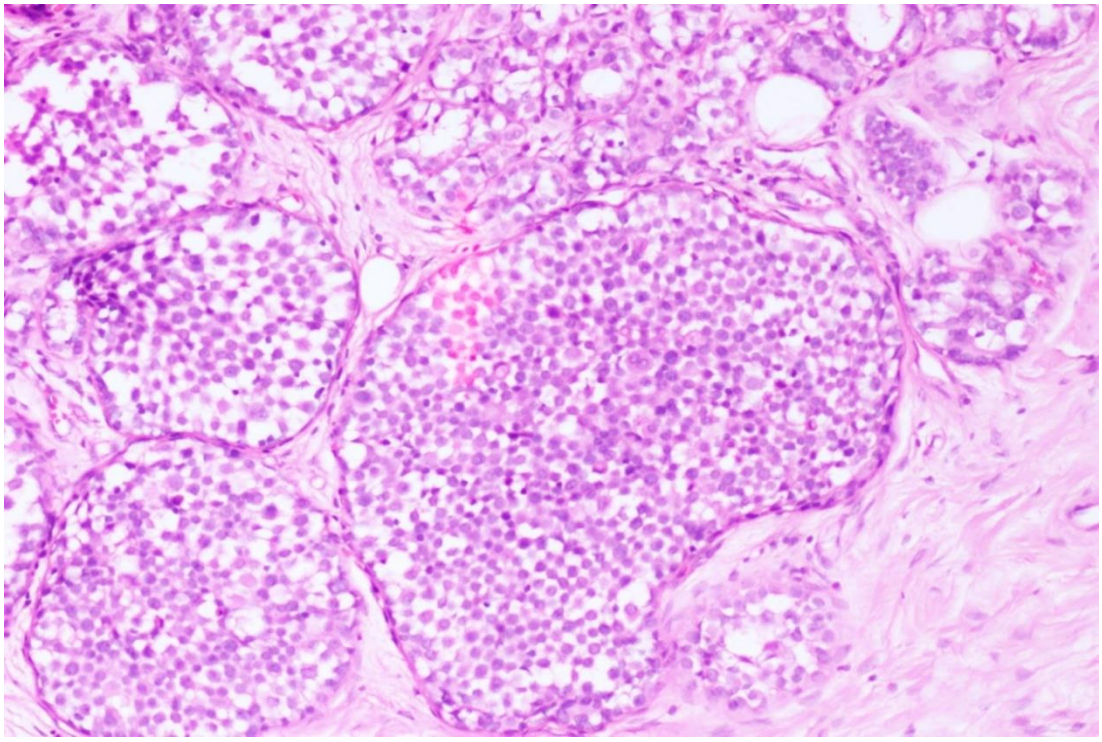


Figure 24: Lobular carcinoma in situ showing dyscohesivenessof the tumour cells(H&E at 200x)

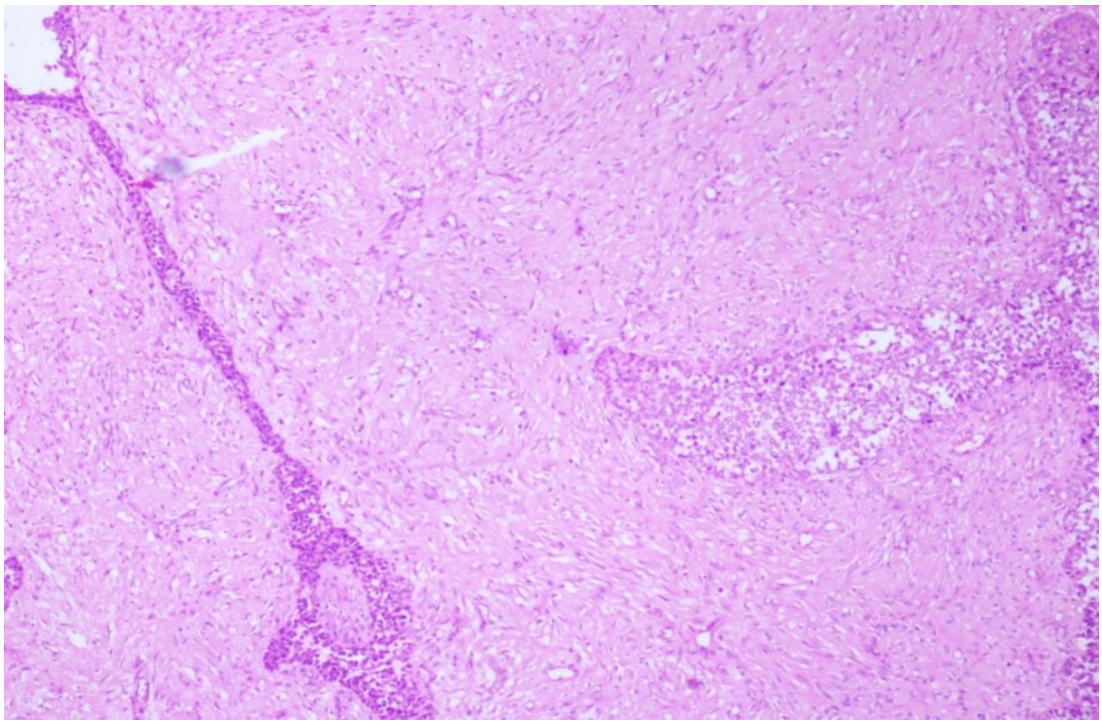


Figure 25: Phyllodes tumour involved by lobular carcinoma in situ(H&E at 100x)

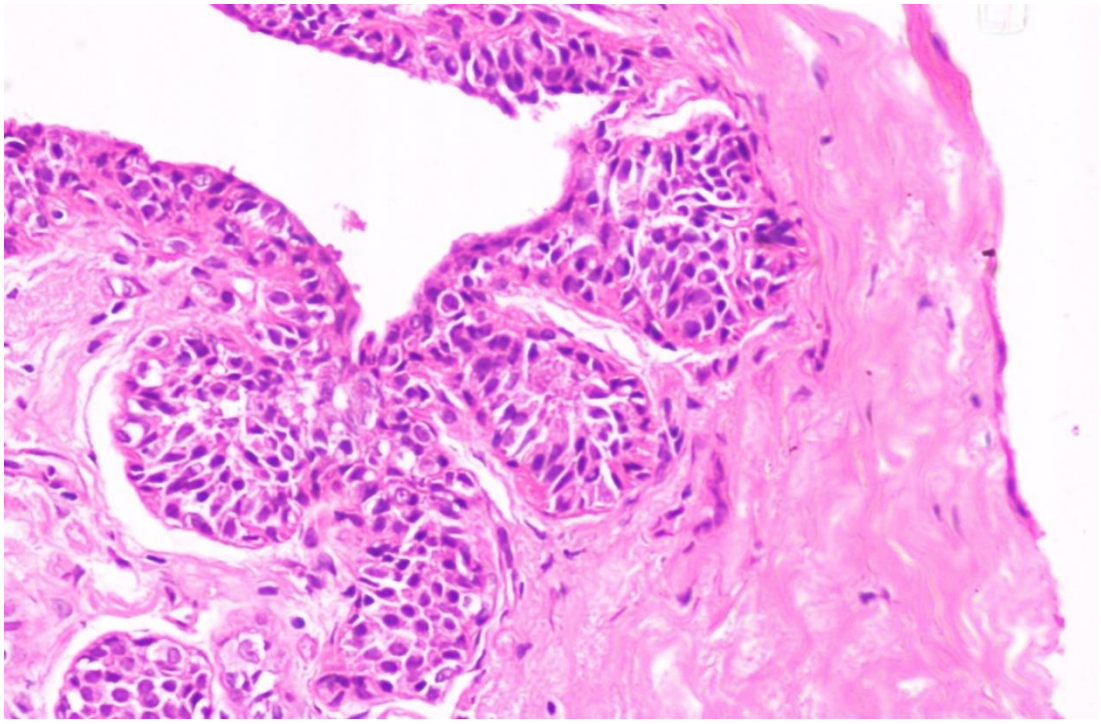


Figure 26: Cloverleaf pattern in in situ lobular carcinoma(H&E, 200x)

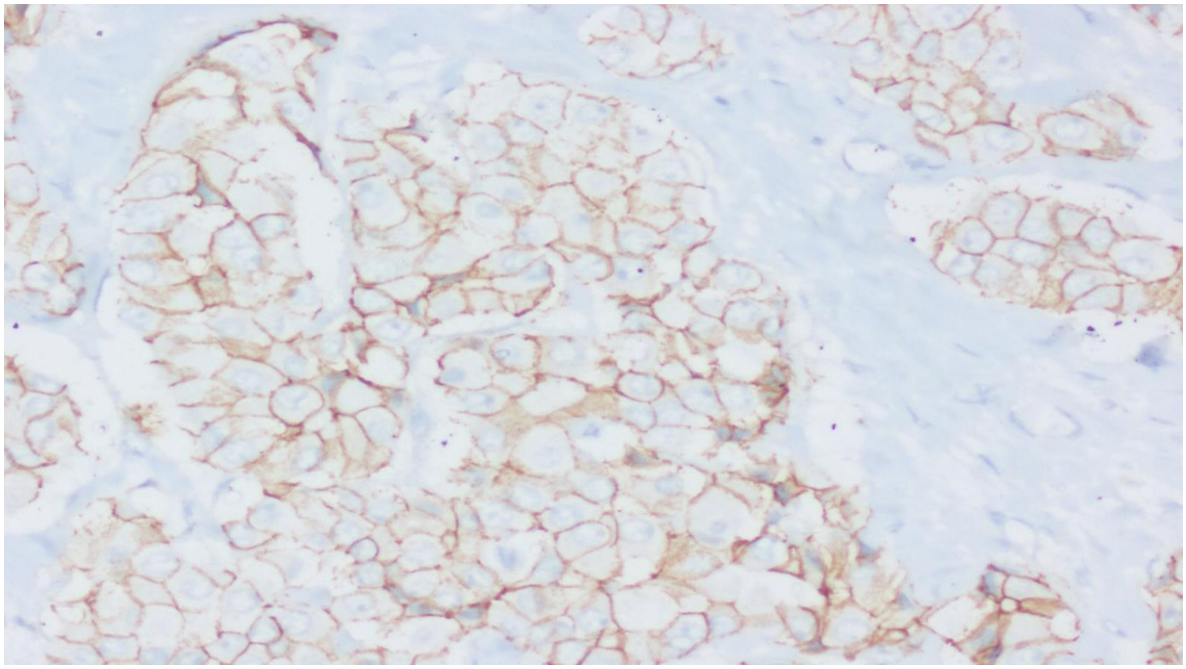


Figure 27: Incomplete membranous pattern of E-cadherin in Invasive lobular carcinoma(E-cadherin, 400x)

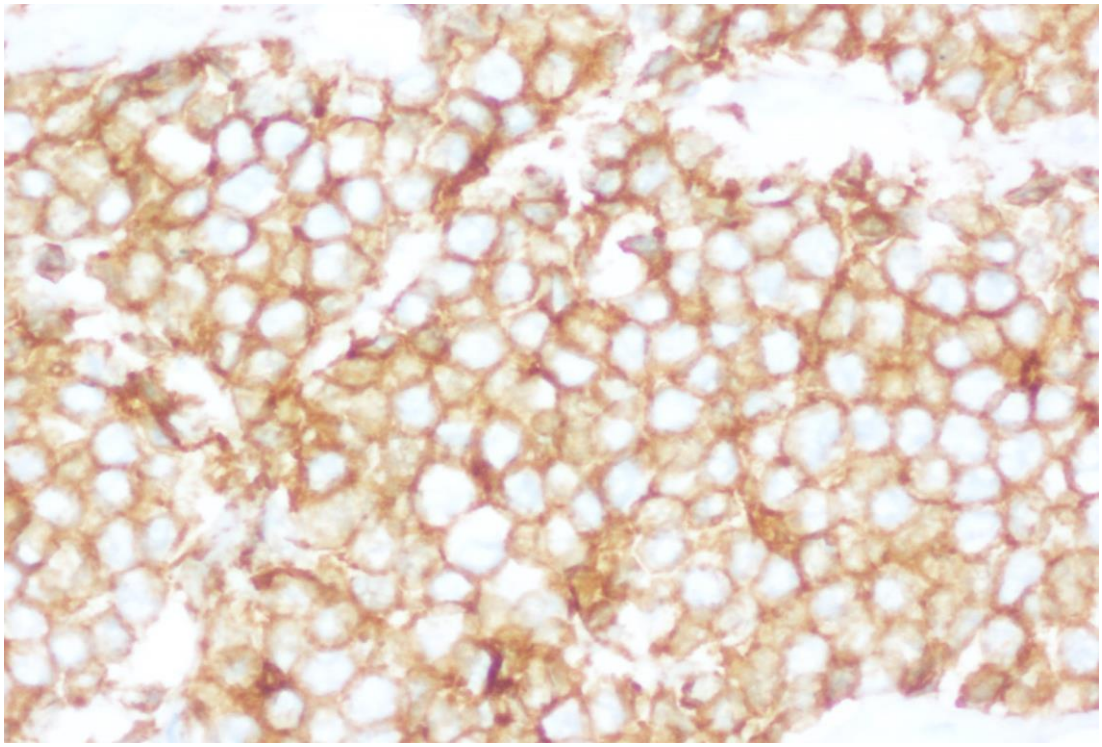


Figure 28: Granular cytoplasmic pattern in E-cadherin Immunostaining, 400x

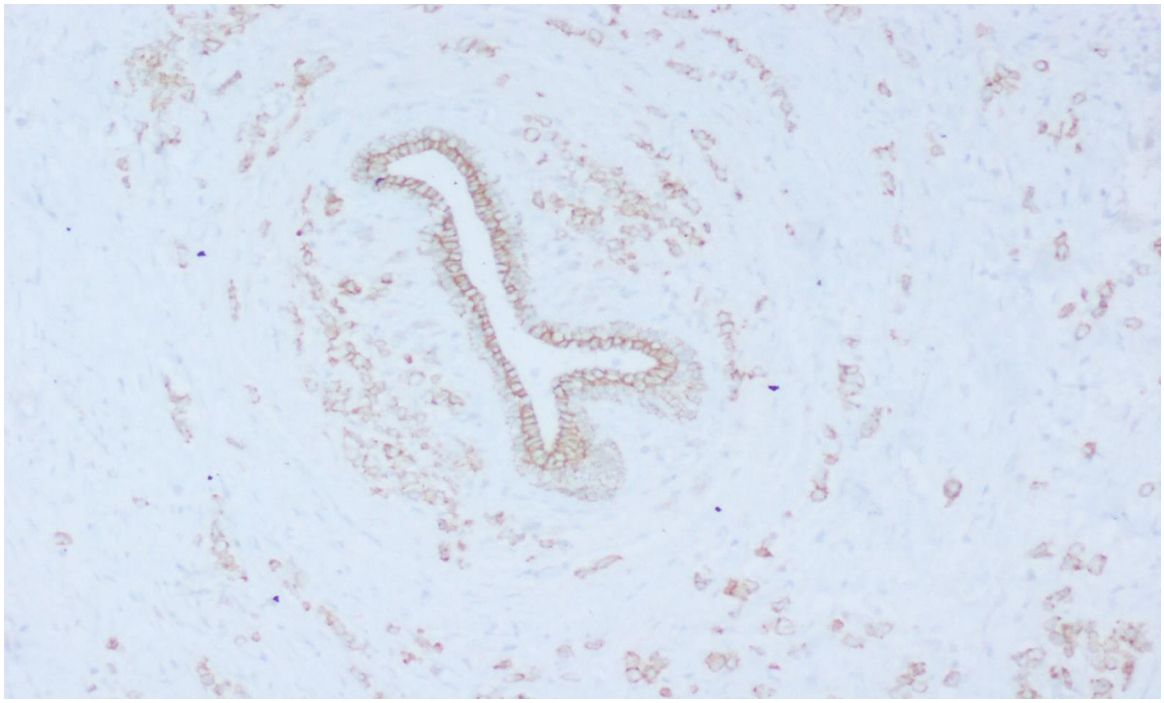


Figure 29: Aberrant E-cadherin positivity in Invasive lobular carcinoma cells, targetoidpattern(E-cadherin at 200x)

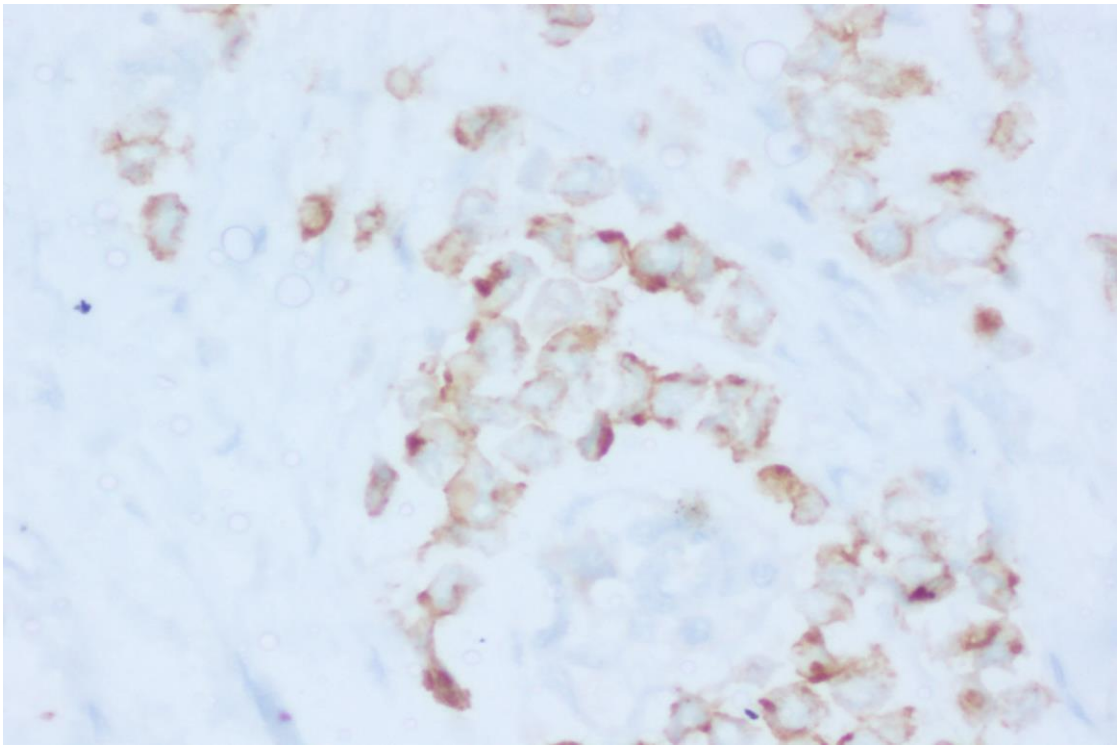


Figure 30: Aberrant E-cadherin positivity - cytoplasmic dot like pattern (E-cadherin at 400x)

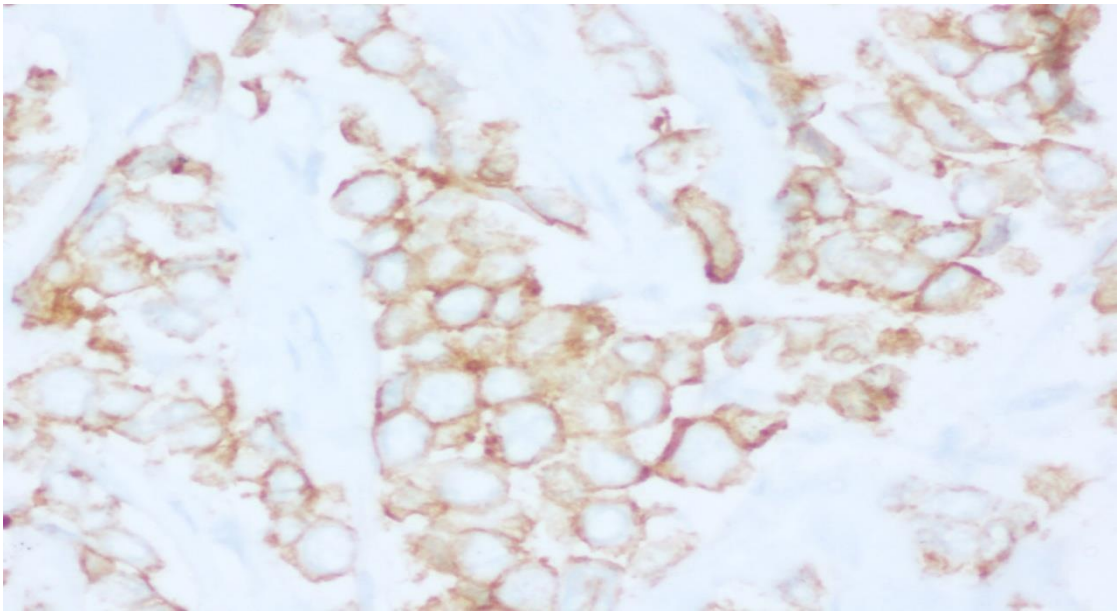


Figure 31: Aberrant E-cadherin positivity- granular cytoplasmic and Incomplete membranous pattern(400x)

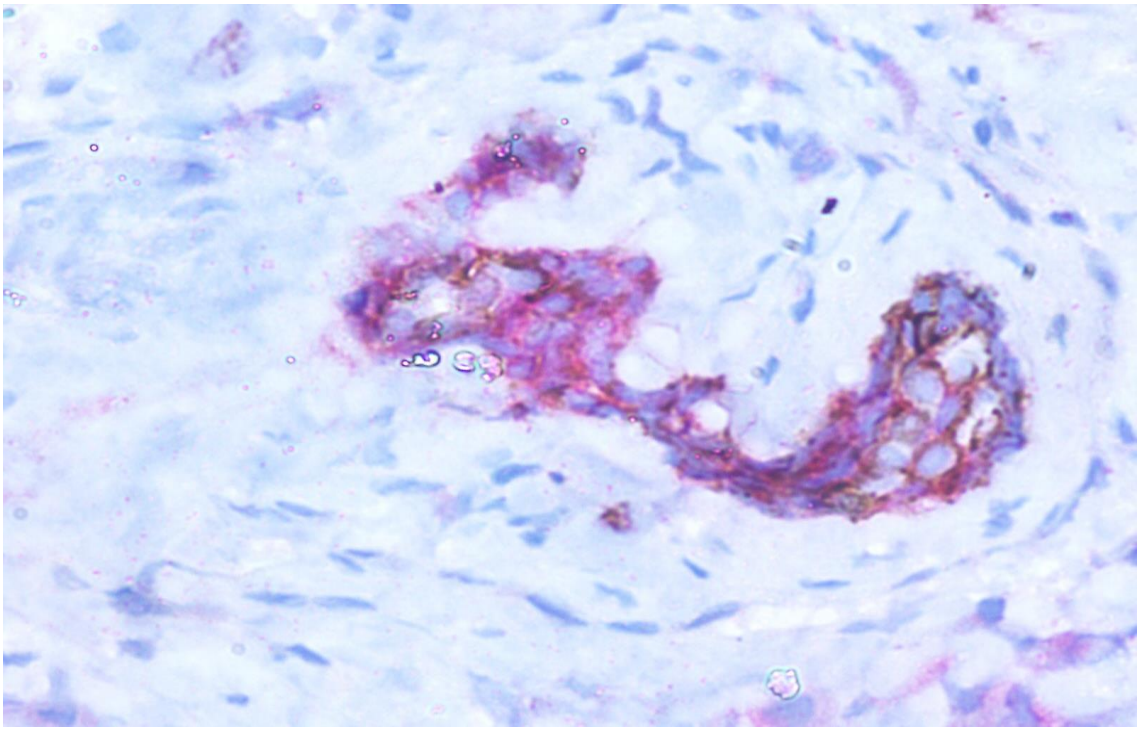


Figure 32: Internal control for Double immunostaining for p120/E-cadherin – normal ducts with membrane staining for p120 and E-cadherin. (200x)

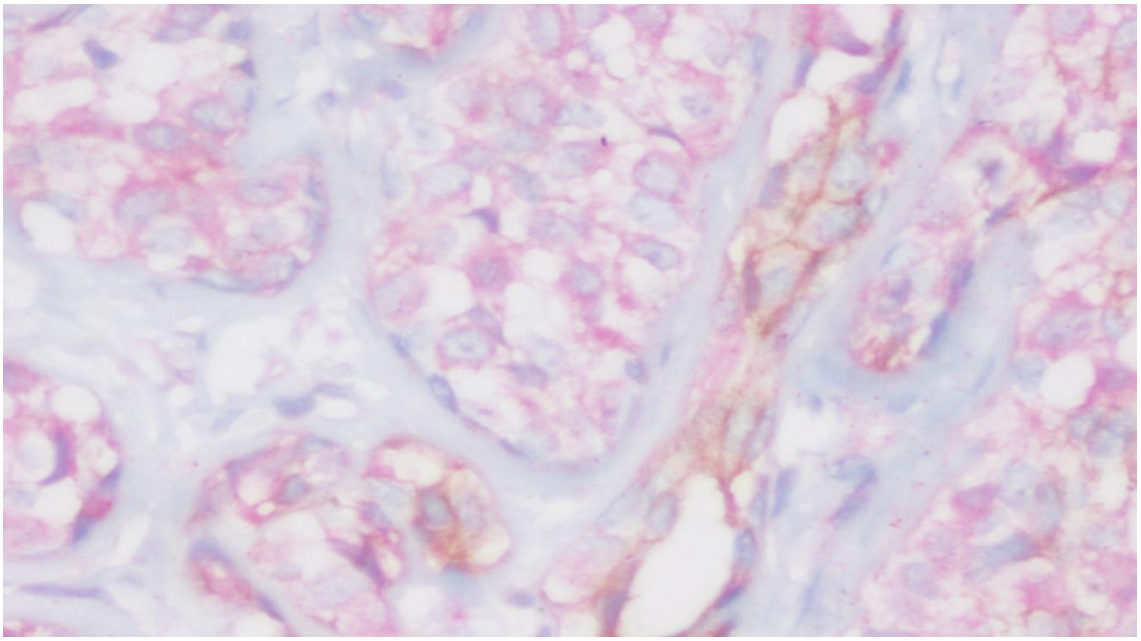


Figure 33: Cytoplasmic weak (1+) staining for p120 and incomplete membrane staining for E-cadherin (Double immunostaining for p120/ E-cadherin, 400x).

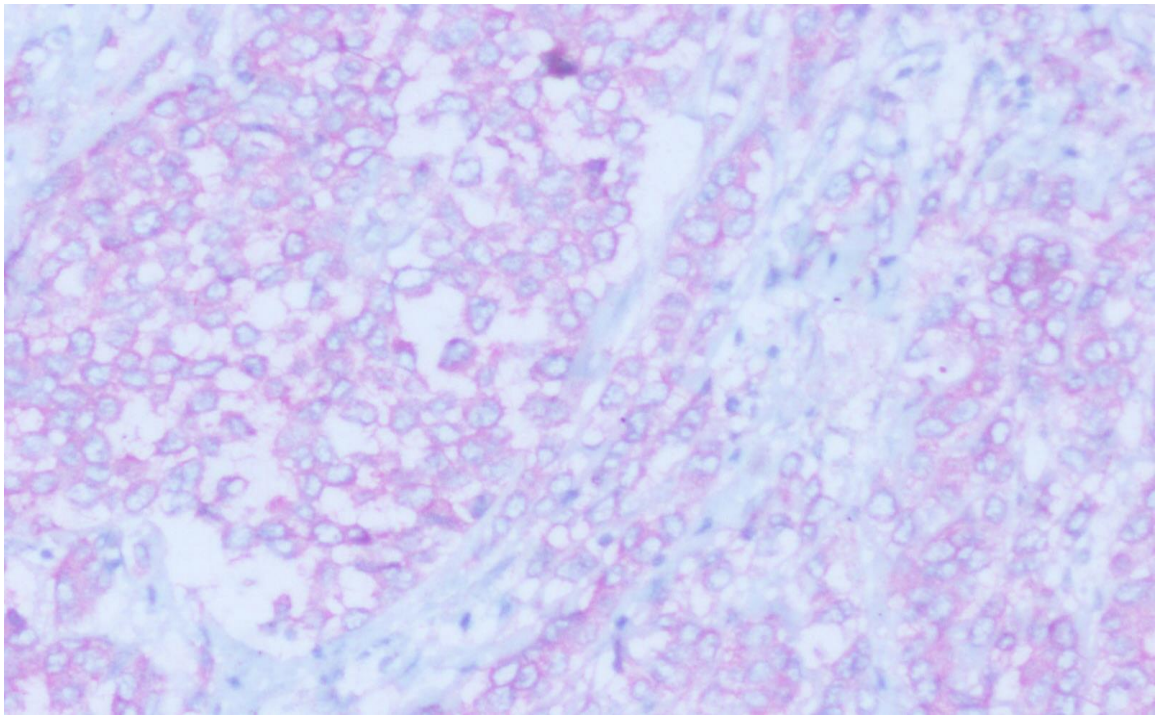


Figure 34: Moderate (2+) cytoplasmic staining for p120 and negativity for E-cadherin (Double immunostaining, 400x)

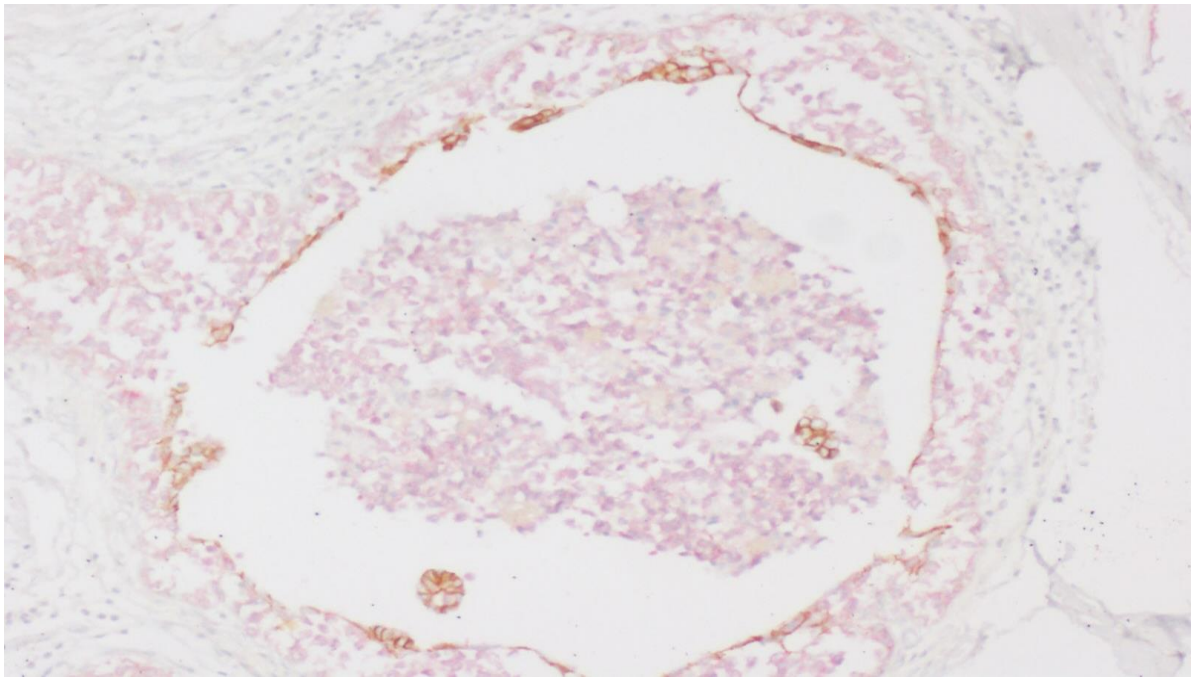


Figure 35: Pagetoid spread of in situ lobular carcinoma with remnant ductal epithelium positive for E-cadherin and lobular carcinoma in situ cells staining weak cytoplasmic p120. (Double immunostaining,100x)

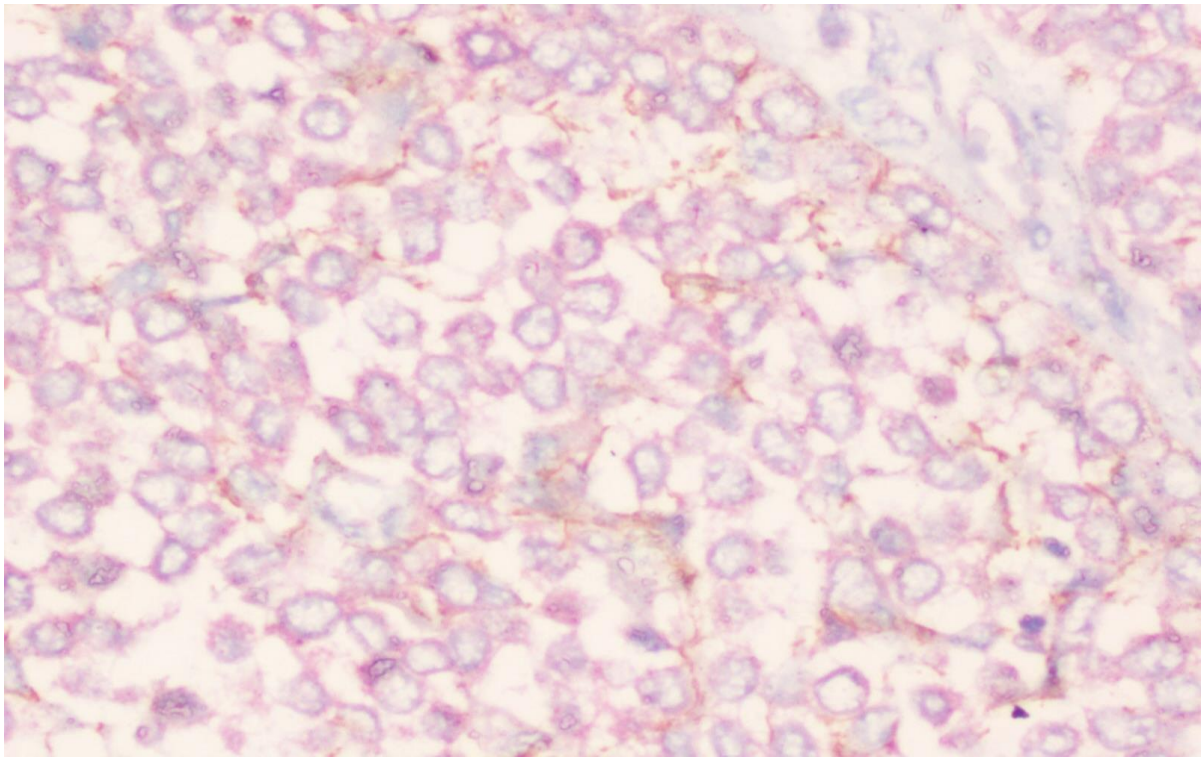


Figure 36:Weak(1+) Cytoplasmic staining for p120 and aberrant incomplete membrane staining for E-cadherin (Double immunostaining, 400x)

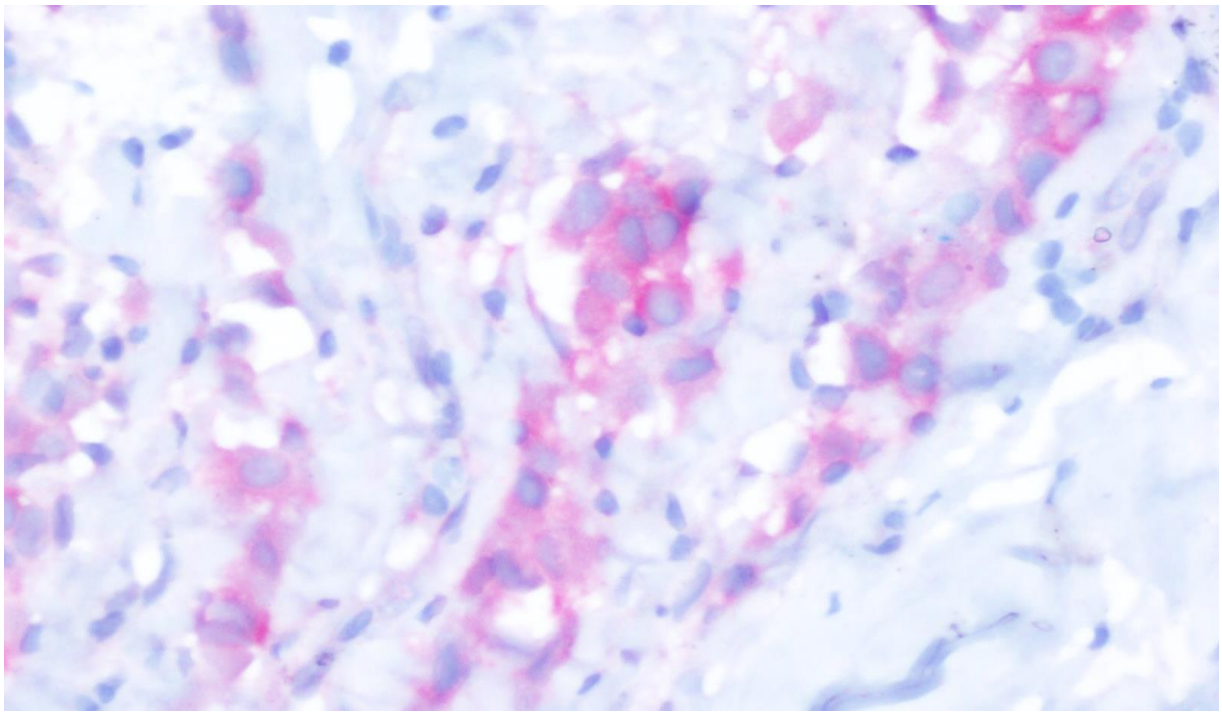


Figure 37: Strong (3+) cytoplasmic staining for P120 and negative E-cadherin (Double immunostaining, 400x)

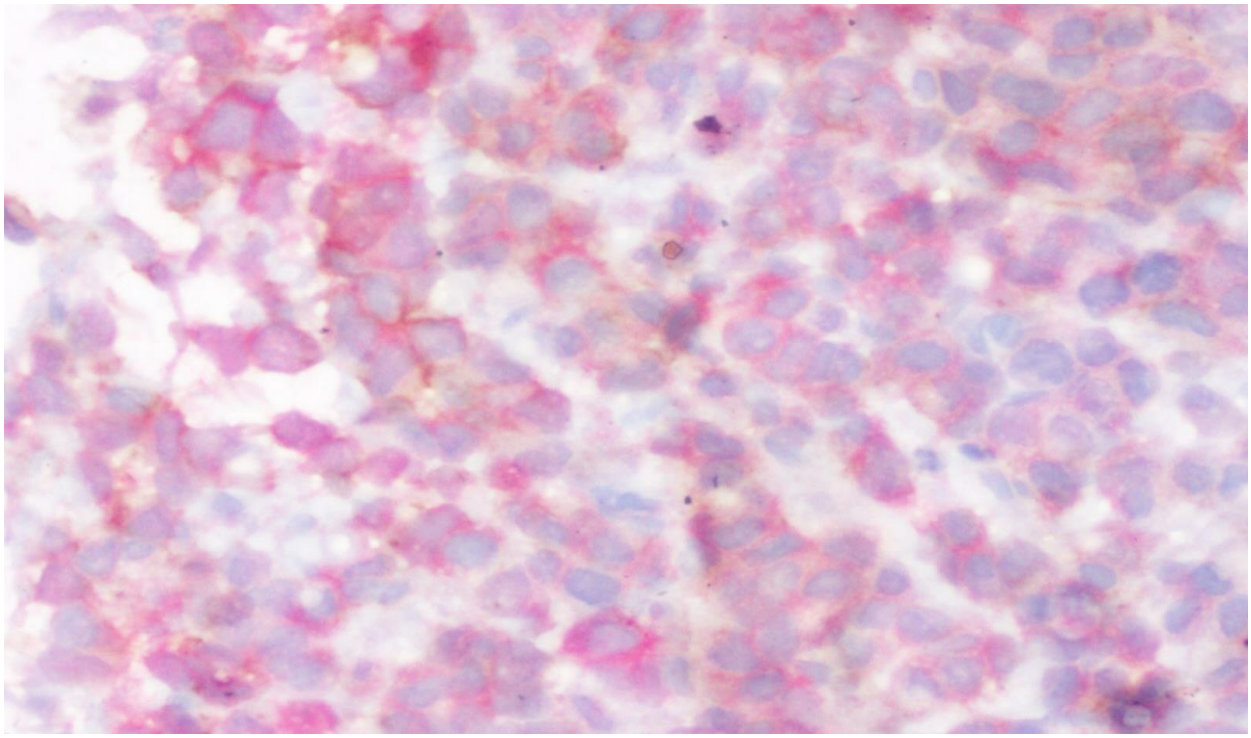


Figure 38: Incomplete membrane staining for E-cadherin and cytoplasmic p120 staining in invasive lobular carcinoma cells (Double immunostaining, 400x)

DISCUSSION

Invasive lobular carcinoma represents 5-15% of all types of breast carcinomas(1). It is the second most common type of breast carcinoma(2). It is important to make an accurate diagnosis of lobular carcinoma because it changes the prognosis and treatment of the patient.

Identification of lobular carcinoma in situ may necessitate a patient into high risk follow up, whereas in case of ductal carcinoma in situ excision with proper margin and radiation therapy is treatment of choice(8). Mastectomy is preferred over lumpectomy in case of ILC than Invasive ductal carcinoma(63). E-cadherin immunostaining has traditionally been used to distinguish between ductal or Lobular carcinoma.

There are several drawbacks of using a single immunohistochemical stain like absence of the single targeted antigen in the tumour cells, false negative results due to other factors like, quality of antibody, detection kit, procedural, technical and human errors(64). Alternate antigens have been explored to identify these cases. Immunostaining for p120 catenin is one method. Cytoplasmic location of p120 staining has been observed in lobular neoplasia(8). Double immunostaining with p 120 catenin and E-cadherin has also been employed to increase the accuracy of diagnosis (64).

This 10 year retrospective study has been done to assess in detail the clinicopathologic features of lobular carcinoma in our setting. We also aimed to assess the diagnostic utility of double immunostaining for p120 and e cadherin in a subset of these cases.

CLINICAL FEATURES

Age: The mean age affected by invasive lobular carcinoma is between 57-65 years(1). This age group is slightly older than the patients with Invasive ductal carcinoma. In our study the mean age was 51.96 years. There was no significant correlation found between tumour size and age of the patient.

Risk factor: It can be due to increased consumption of alcohol(65) . But in case of ductal carcinoma alcohol is not a risk factor(66).

Bilaterality: Invasive lobular carcinoma has the propensity towards bilateral presentation in almost 5-19% cases(67,68). There is some evidence that incidence of bilateral tumours are higher in classical variant of ILC than in other variants of ILC. In our study there were 12.68% cases which had bilateral presentation. In this study women presented with ill-defined palpable mass as mentioned in other various studies. The rate of multifocality and bilaterality in lobular carcinomas are 50% and 30% respectively but in our study we did not find any multifocal cases(69).

Types of specimens:

We had 71 cases from 62 patients, 9 of whom had bilateral tumours.Of these 71 tumours, 38 had only core biopsies, 25 had core biopsy and resections(20 were mastectomy, 5 were WLE), 8 had only slide and block review (diagnosed outside). Of the resections, 10 were pre chemo and 15 were post chemo.

Gross Features: In the 25 resection specimens, the gross features of the tumour were noted. Majority of the tumours are grey white in colour with a firm cut surface(97.9%). The gross features of invasive lobular carcinoma have been variously described as numerous, hard nodules that can be felt as grains of sand in the breast parenchyma and as very ill circumscribed tumour with a diffuse growth pattern. Hence the difficulty in accurately measuring the tumour size (70). The mean size of the tumour in the current study was 3.47cm. There was no correlation between the tumour size and grade of the tumour in the current study. In a study by O'Malley et al there was a positive correlation between the size and the grade of the tumour(71).

In our study we had 38 core biopsies, 25 had core biopsy and resections(20 were mastectomy, 5 were WLE), 8 had only slide and block review (diagnosed outside). Of the resections 10 were pre chemo and 15 were post chemo.

MICROSCOPIC FEATURES

Architecture: One of the main characteristics of lobular carcinomas the lack of cohesion of tumour cells. Singly lying cells dispersed in the stroma are often seen. The tumour cells also have a tendency to form a single line of dyscohesive cells, known as Indian file pattern. Often, more than one of these patterns are present in single tumour.67 cases in this study (95.71%) had singly lying cells. 65 cases had Indian file appearance. The single tumour cells sometimes perlocate around normal ductal structures in a concentric manner this is known as targetoid pattern of growth(72). Inflammatory cells around the ducts sometimes mimic a targetoid pattern. This targetoid distribution is not associated with desmoplastic response. In our study, we had 7 cases which had targetoid distribution of tumour cells. There were various combinations of architectural patterns in the study cases. The most common combination of patterns seen was that of Indian files, single cells and nests. The next most common combination was that of Indian file and single cells and the third most common pattern was targetoid pattern, Indian file and single cells.

Cytomorphological characteristics:

The characteristic appearance of lobular carcinoma cells are round to ovoid nuclei, a thin rim of cytoplasm and an occasional intracytoplasmic lumen(65,73,74). In this study, 63 cases (88.73%) showed cells with intracytoplasmiclumina. The common variants of invasive lobular carcinoma are classical, solid variant, pleomorphic variant, tubulolobular variant and mixed. In our study we had 64 cases of classical variant of ILC, 3 cases of pleomorphic variant, 1 tubulolobular and 1 case of

histiocytoid variant. In pleomorphic variant the nuclei is three to four times larger than a normal lymphocyte. Signet ring cells are also a common feature of invasive lobular carcinoma(75). Scattered signet ring cells were seen in 67 cases. In pleomorphic variant there is occasional in situ ductal involvement by tumour cells which is known as 'Clover leaf' appearance(76). In this case study we had two cases where Cloverleaf pattern is noted.

Histological grading:

Nottingham system is followed for histologic grading of ILCs. The relevance of the three tier grading system of Nottingham is a matter of controversy due to rarity of tubule formation in ILC. ILCs receive a grade 3 for tubule formation. Marked nuclear pleomorphism is a rare finding in ILC. Nuclear grade for ILC is commonly 1 or 2. Nuclear pleomorphism is one of the components of grading system but there was no correlation between tumour size and lymph node status in a study by O' Malley et al(71). Mitosis is infrequent in ILCs. In a study of 171 invasive lobular carcinomas byDabbs et al, 20 % of the cases were grade I, 64% grade II and 16% grade III (15). We had 8.82% of grade 1 cases and 88.24% of grade 2 cases. We found only two cases (2.94%) of grade 3 in our study. One case had score 1 for tubules, 10 cases(14.28%) had score 2 and rest of 57 cases had been given score 3.Mitotic activity is infrequently seen in invasive lobular breast carcinoma(65). However, other studies have found that mitotic activity in isolation is highly correlated with prognosis(71). Mitotic activity is the single most important factor which determines the stage and aggressiveness of the Invasive lobular carcinoma. Bane et al has proposed that mitotic

activity is the strongest predictor of survival and it gives more information than the rest of the modified Bloom Richardson criteria(77). In this study we had 63 cases which had a score of 1 for mitosis, 2 cases had given a score of 2 and 3 cases had given score 3.

Lymphocytic reaction:

Lymphocytic reaction is a very occasional finding in case of ILCs. Lymphocytic reaction affects generally the lobules which are infiltrated by the tumour cells and occasionally it can be seen throughout the ducts in the adjacent breast parenchyma(78). However, in this study we had 59 cases (83.10%) cases in which lymphocytic reaction was found in the peritumouralstroma. Any degree of lymphocytic reaction was counted as positive in our study. This could be the reason for the high frequency.

LCIS:

Lobular carcinoma in situ foci commonly accompany classical variant of ILC(79). DisCostanzo et al. in their study found out that LCIS was associated in 65% of 176 cases of classical ILC(79). In our study we found that 33 cases(46.48%) had foci of LCIS. LCIS is diagnosed when >50% of the acini in the terminal duct is replaced by monomorphic cell proliferation(76). Complete absence of lumen is not necessary. The characteristic feature of LCIS is dyscohesiveness of the tumour cells. The tumour cells have bland round to oval nuclei, inconspicuous nucleoli and scant

cytoplasm. Presence of intracytoplasmic lumen is also a wellknowncharacteristicof LCIS. There are various types of LCIS like pleomorphic, pleomorphic apocrine, LCIS with comedo necrosis and mixed type of LCIS(80). It is common to see the extension of this lobular neoplasia in between the myoepithelial cells and ductal cells. This is known as Pagetoid spread. We had 10 cases in which Pagetoid spread was present (14.08%). When LCIS grows in a ductulocentric pattern with minimal bulbous outpouchings, it gives a ‘ Cloverleaf ’ appearance(76). Two of our cases showed a cloverleaf appearance. One of our cases had only LCIS and the in situ component was seen involving a benign phyllodes tumour. The reason behind this is induced by the changes in the stromal component (16). Some other study showed it can be due to growth factors and hormones.

Atypical lobular hyperplasia:

Atypical lobular hyperplasia is diagnosed when <50% of the acini in a lobule are affected by lobular neoplasia. This is again predominantly due to loss of E-cadherin expression. Here cells are monomorphic population of polygonal to cuboidal cells. Cells are loosely cohesive and regularly spaced. Myoepithelial cells are admixed with malignant cells.Peritumoral lymphovascular invasion is reported to be seen in 10% of the cases of invasive lobular carcinoma(70). In this study we had 28 cases (39.44%) in which lymphovascular invasion was seen. Shrinkage artefact in the sections can appear as lymphovascular invasion. Lymphovascular invasion is more common in pleomorphic variant as per study done by Buchanan et al(81). Five of the cases

(7.14%) in our study showed perineural invasion. It is quite rare in cases of lobular carcinoma and has no prognostic significance(82).

Lymph node metastasis: Lymph node metastasis is quite common in ILC. According to one study there are lesser number of lymph node metastasis associated with classical variant of invasive lobular carcinoma when compared to pleomorphic variant of ILC (70). Invasive lobular carcinoma generally spreads through lymphatics or by hematogenous spread. The tumour cells mainly involves the sinuses and sometimes the lymph node parenchyma. It is difficult to detect the metastases when the infiltrate is sparse. Even in cases with extensive lymphnodal metastasis, it can be difficult to differentiate the metastasis from sinus histiocytosis, because of the bland nature of tumour cells. Rarely, the lymph node metastases can be concentrated in the sinusoids of hilum, (83). In this study we had 28 cases, where lymph nodes were excised and 19 of the cases were positive for lymph node metastasis. The growth patterns of lymphnode involvement have been reported to classified as nodular, diffuse and nodular and diffuse(83).In the 19 cases with lymph node metastasis, diffuse pattern was most common followed by nodular pattern.

Stromal reactions:

Stromal desmoplasia was noted in 68 of our cases (95.77%). Elastosis was noted in 51 of our cases(71.83%).

Nonneoplastic/ preneoplastic alterations in adjacent breast:

Columnar cell changes are well known for their associations with lobular neoplasia. It comprise morphological changes in the duct epithelial lining, which gives it as a columnar cell appearance and it involves the terminal duct lobular unit(16). There is variable dilatation of the acini which are lined by columnar epithelial cells. These columnar epithelial cells have often apical snouts. There is very bland nuclei, minimal nuclear pleomorphism, fine chromatin and moderate amount of cytoplasm. Mitosis is very rare.

The histological triad of tubular carcinoma, columnar cell lesions and lobular carcinoma is also known as Rosen triad. Loss of chromosomal material on 16q and gains on 1q were detected in lobular neoplasia, similarly to columnar cell lesions, low grade DCIS, tubular carcinoma and ILC. This suggest a common evolutionary pathway for LCIS and ILC(2). Abdel-Fatah et al in their study had 60% of the cases of ILC associated with columnar cell changes(22). In our study we had 12 cases which were showing columnar cell changes (16.90%) but none of the cases showed flat epithelial atypia. Loss of chromosomal material of 16q as a central event in well differentiated and intermediately differentiated DCIS. These results show a high genetic homology between LCIS and well differentiated DCIS(31).

Ductal hyperplasia can be either usual or atypical types. Usual ductal hyperplasia show a streaming pattern of growth particularly in the centre of the involved spaces. In this study we had 4 cases of ductal hyperplasia. We had one case which had coexisting DCIS.

Molecular pathogenesis:

There is much evidence now that suggests that alterations in the E-cadherin-Catenin complex play an important role in tumour behaviour and progression(85). Defects in the several components of the E-cadherin complex result in decreased cell-cell adhesiveness and increased risk of tumour metastasis(85). Invasive lobular carcinoma and Lobular carcinoma in situ(LCIS) shows absence of E-cadherin expression, but in case of Invasive ductal carcinoma, E-cadherin expression is retained. Loss of E-cadherin expression can be due to many factors such as inactivating mutations, loss of heterozygosity and promoter hypermethylation(85). Transcriptional repression through activation of transforming growth factor-beta/SMAD2, this down regulates E-cadherin expression(7). Behind this non functional E-cadherin there are some unknown point mutations also seen(86). Mutations in the E-cadherin have been found in classical and pleomorphic variant of ILC(87). There is a protein truncating mutation in exon 21(66%) of 32 ILCs(5).

E-Cadherin:

E-Cadherin is an epithelium specific molecule which helps in cell to cell adhesion. Generally E-cadherin helps in distinguishing from ILC and IDC. In case of ILC, E-cadherin is absent or shows weak discontinuous staining. In case of IDC, it shows complete membrane staining. Some of the high grade invasive ductal carcinoma cases show reduction in the intensity in staining and some Invasive lobular carcinomas have retained weak positivity. This results in difficulties in diagnosis. The aberrant staining pattern of E-cadherin in ILC can be recognised as partial or weak linear membrane staining, complete membrane staining, dot like staining or cytoplasmic staining(88). This aberrant staining can be due to presence of non functionalEcadherin in the membrane(89).

Qureshi et al reported 10% ILCs to be E-cadherin positive. (3). Frequency of E-cadherin positivity in ILCs range from 2% to 29%.(6). The current study showed an aberrant positivity of 30% (n=15) on E-cadherin staining with single antibody. Of these, 9 cases showed cytoplasmic pattern of staining and 2 had incomplete membrane pattern of staining. Rest of the two cases had multiple patterns and one had all the three patterns like cytoplasmic, dot like and incomplete membranous. One of the cases showed complete membranous positivity.

In literature, different studies have shown variable degrees of aberrant staining of E-cadherin(See table 7). This can relate to the difference in techniques of

immunohistochemistry like sections taken per case, thickness, different clones of antibody used and antigen retrieval system. Goldstein et al in their study of 143 cases of ILC with HECD-1 clone of E-cadherin showed complete absence of staining in 136 of their cases but complete membranous pattern of staining in 7(5%) of their cases. Kowalski et al in their study of 8 cases of ILC showed 100% cytoplasmic positivity with HECD-1 clone of E-cadherin. Most of the other studies had used HECD-1 clone of E-cadherin but the dilutions, antigen retrieval system, buffer ,pH, incubation and linking system were different for each study (88). In our study heat induced antigen retrieval was done for 1 hour with incubation period of 40 minutes. The antibody used was Dako and the clone was NCH38.

Clinical significance of E- cadherin positivity:

In our study, there was no significant correlation between the E-cadherin positive status and the age of presentation, lymphovascular invasion, grade or lymph node metastasis. All 15 of the E -cadherin positive cases were grade 2 tumours.

p120 immunostaining:

Cateninsare proteins involved in the connection between the E-cadherin membrane complex and intracellular actin cytoskeleton. In case of normal breast epithelial cells p120 catenin is present at the cell membrane . If E-cadherin is lost or becomesnon functional, p120 gets upregulated and aggregates in the cytoplasm. p120 exerts oncogenic properties by regulating the Rho GTPases(90). Immunohistochemistry

reveals cytoplasmic localization rather than membrane in p120 staining(80). The sensitivity for p120 in invasive lobular carcinoma is around 95% according Mastaracci et al(91). In our study, sensitivity of p120 was 100%. All our ILC cases stained for p120. More importantly, cytoplasmic staining for p120 was seen in all our cases which were positive for E-cadherin. p120 is localised to cytoplasm in cases of LCIS and metastatic Invasive lobular carcinoma as well(85).Dabbs et al in their study showed that 100% of ILC were positive for p120(92).

Rakha et al found that 84% of ILC cases showed diffuse cytoplasmic positivity for p120 . All these cases showed moderate to strong cytoplasmic positivity. The summary of relevant literature on p120 staining in lobular neoplasia is given in Table 6. These results reflect the transcriptomic analyses of lobular carcinoma that showed downregulation of actin cytoskeleton (93).

Table 8: Relevant studies on p120 staining pattern in lobular neoplasia

Study	Patterns	ALH	LCIS	ILC
Sarrio et al(90)	Normal membrane	-----	-----	9%(6/67)
	Reduced membrane	-----	-----	3%(2/67)
	Cytoplasmic(Weak)	-----	-----	23.9%(16/67)
	Cytoplasmic(Strong)	90%(26/29)	100%	64.1(43/67)
	Cytoplasmic	-----	-----	0%(0/67)
Mastracci et al(91)	Positive	90.9%(10/11)	100%(10/10)	
	Negative	9.1%(1/11)	0%(0/10)	-----
Dabbs et al(9)	Positive	-----	-----	100%(64/64)
	Negative	-----	-----	0%(0/64)
Brandt et al	Strong Cytoplasmic	-----	100%(19/19)	88.7%(39/44)
	Weak cytoplasmic	-----	0%(0/19)	11.3%(5/44)
	Cytoplasmic –	-----	0%(0/19)	0%(0/44)
Current study	Strong Cytoplasmic positivity	-----	31.25%(5/16)	21.62%(8/37)
	Moderate cytoplasmic positivity		43.75%(7/16)	43.24%(16/37)
	Weak cytoplasmic positivity		25%(4/16)	35.13%(13/37)

DOUBLE IMMUNOSTAINING

There are several drawbacks of using a single immunohistochemical stain like absence of the single targeted antigen in the tumour cells, false negative due to other factors, quality of antibody, detection kit, procedural errors technical and human errors. Purpose of this study was to detect the utility of double immunostaining which would be particularly useful to pick up small foci of ILCs in biopsies where scarcity of the tissue has to be taken into consideration. Loss of membranous distribution of both E-cadherin and p120 are concurrent(64).

.In the 37 cases of ILC in which double immunostaining was done, 13 cases showed aberrant positive staining for E-cadherin. Of these, six cases showed cytoplasmic positivity and 7 cases showed incomplete membrane staining. The intensity ranged from weak to moderate. Cytoplasmic staining for p120 was seen in all the 37 cases including the 13 cases which were positive for E-cadherin. Hence, in our study sensitivity of p120 is 100%.

The frequency of aberrant staining for E- cadherin in ILC in our study was 34% on double immunostaining and 30% with E-cadherin done by single antibody staining. This rate is higher than other published studies(4,36,94). This could be due to inclusion of cases with even very focal aberrant staining.

In the study done by Dabbs et al scoring for E-cadherin was given as negative for no immunoreactivity, weak 1+ was given as any percentage of cells, 2+staining in <10%

and 3+ for >10% of cells(40). In our study we have given 1+ score for any percentage of positive cells.In a study done by LiL et al, p120-catenin was diffusely cytoplasmic positive for 66.7% of ILCs(95).Cytoplasmic staining was detected in the absence of E-cadherin expression were observed 55.6%.

All the 37 cases of ILC in which double immunostaining was done showed cytoplasmic staining for p120. For the p120 component of double immunostaining, we used a scoring system akin to Allred scoring system for semiquantitative estimation of the staining. 86.5% of our cases showed strong positivity in case of p120 staining. Of the 37 cases, 16 had an LCIS component, all of which (100%) showed cytoplasmic staining for p120. All the 16 LCIS caseswerepositive(100%) for p120 staining.

There is only one published study in which double immunostaining for p 120/ E-cadherin was done. This study included 12 cases, 100 % of cases showed p 120 cytoplasmic staining and all cases were negative for E-cadherin(64).

Table 9: Comparison of literature on Aberrant E-cadherin immunoreaction in lobular neoplasia

Publication	Cases/ Diagnosis	Complete absence	Complete membrane	Reduced/ Partial	Focal	IHC staining technique
Moll et al	22 ILC	19(86.4%)	-----	------	3(13.6%)	Heat induced antigen retrieval(HIER)Citrate bufferAvidinbiotin complex(ABC)
Goldstein et al(92)	22 LCIS	20(91%)	-----	----	-----	HIER Citrate, pH 6 Horseradish Peroxidase(HRP)
Angeirsson et al(55)	14 ILC	9(64%)	1(7%)	----	4(29%)	HIER (microwave) Citrate, pH 6 HRP
Goldstein et al(93)	82 ILC	73(89.1%)	-----	-----	9(10.9%)	HIER (steamer) EDTA, pH 7.5 EnVision Plus
Kovacs et al(94)	16ILC	13(81%)	----	----	3(19%) Cytoplasmic Staining	HIER(pressure cooker) Citrate, pH 6.5 ABC
Acs et al(95)	42 ILC	41 (98%)	1(2%)	------	-----	
Wahed et al(96)	14 PILC	12(86%)	-----	----	2(14%)	No Ag retrieval ABC
Qureshi et al(3)	49 ILC	5(10%)	-----	-----	------	
Current study	50 ILC	-----	------	6(40%)	9(60%) Cytoplasmic	HIER

Table 10: Comparison of E-cadherin positivity between single immunostaining and double immunostaining.

Serial no of cases	Single E-cadherin and % positive cells	Double E-cadherin and % positive cells
1.	Negative	Negative
2.	Positive (20%)	Negative
3.	Positive (40%)	Negative
4.	Positive (20%)	Positive (10%)
5.	Positive (5%)	Positive (5%)
6.	Negative	Positive (10%)
7.	Positive (40%)	Positive (5%)
8.	Negative	Negative
9.	Negative	Negative
10.	Negative	Negative
11..	Positive (80%)	Positive (60%)
12.	Positive (60%)	Positive (70%)
13.	Positive (50%)	Positive (10%)
14.	Positive (5%)	Positive (5%)
15.	Positive (30%)	Positive (40%)
16.	Positive(60%)	Negative

In our study, there were 16 cases where both double immunostaining for p 120/ E-cadherin and E- cadherin immunostaining by single antibody was done. See Table (8). The concordance between the two methods for E-cadherin immunostainingwas 75% .

Utility of p120/E-cadherin double immunostaining over E-cadherin immunostaining for diagnosis of invasive lobular carcinoma

In our study, cytoplasmic p120 staining was seen in 100% of invasive lobular carcinoma and in situ lobular carcinoma highlighting the high sensitivity of this molecule for diagnosis of lobular neoplasia. The cytoplasmic p120 staining was seen in all cases which showed aberrant E-cadherin positivity, emphasising its usefulness in the diagnosis. Although we did not study p120 staining in invasive ductal carcinoma, all our cases showed membrane staining for p120 in normal ducts which served as internal controls. Double immunostaining for p120/ E-cadherin would be a very useful technique to distinguish lobular neoplasia from ductal carcinomas, especially in small biopsies. This is especially important in cases of in situ neoplastic proliferations where the distinction between lobular and ductal has great relevance in therapeutic decision making.

CONCLUSION

This was a retrospective study on cases diagnosed as lobular carcinoma from January 2012 to September 2016. Clinicopathological and histomorphological features were studied and utility of E-cadherin immunostaining and dual immunostaining with p120/E-cadherin was compared.

1. We studied 71 tumours from 62 patients, 70 were invasive lobular carcinoma. One was a case of lobular carcinoma in situ.
2. The mean age of presentation in our study was 51.96 years and mean tumour size was 3.47cm.
3. The frequency of bilaterality was 12.68%. This is correlating with the literature.
4. 64 cases (92.75%) were classical variant. We had three cases of pleomorphic variant of ILC, one tubulolobular and one histiocytoid variant.
5. Single cell pattern as most common architectural pattern(95.71%) followed by Indian file pattern (92.86%).
6. Invasive lobular carcinoma was associated with LCIS in 46.48% of cases. In one case which had only lobular carcinoma in situ, the in situ neoplasia was seen involving a phyllodes tumour.
7. Lymphovascular invasion was seen in 28 cases(39.44%), all of which were grade 2 tumours.
8. We had one case of coexisting high grade DCIS associated with Invasive lobular carcinoma.

9. Aberrant E-cadherin staining was seen in 30% of invasive lobular carcinoma. We noted different patterns of aberrant staining, cytoplasmic being the most common (60%) followed by incomplete membranous pattern(40%). Two cases show multiple aberrant patterns like dot positivity, incomplete membranous and cytoplasmic staining.

10. In dual immunostaining for p120/E-cadherin, cytoplasmic p120 positivity was seen in all 37 cases in the invasive and in situ lobular carcinoma components resulting in a sensitivity of 100%. Cytoplasmic staining for p120 was seen in all cases which showed aberrant E-cadherin staining, reiterating its utility in diagnosing lobular neoplasia. All 37 cases showed membrane staining for p120 and E-cadherin in normal ducts, which served as internal controls.

11. Double immunostaining for p120/E-cadherin will be especially useful in small biopsies with scanty foci of in situ/ invasive carcinoma to determine its lobular phenotype which will affect management decisions.

12. The concordance rate between single and dual immunostaining for E-cadherin in our study was 75%.

Limitations

Limitations:

1. Immunohistochemistry was not done in all the 71 cases due to non avaialability of paraffin blocks and paucity of tissue in the available blocks. Hence the statistical significance of the results of the immunohistochemical tests could not be calculated.
2. We did not include cases of Invasive ductal carcinoma as control, so the specificity, positive predictive value and negative predictive value of the double immunostaining method could not be calculated. However membrane staining for p120 in normal ducts and lobules showed the utility of cytoplasmic p120 staining in diagnosing neoplastic lobular proliferations.

References

1. Lakhani SR, Ellis IO, Schnitt S. J, et al. WHO Classification of Tumours of the Breast. Lyon: IARC Press; 2012.

2. Oliveira TMG, Elias J, Melo AF, Teixeira SR, Filho SC, Gonçalves LM, et al. Evolving concepts in breast lobular neoplasia and invasive lobular carcinoma, and their impact on imaging methods. Insights Imaging. 2014 Mar 16;5(2):183–94.

3. Qureshi HS, Linden MD, Divine G, Raju UB. E-cadherin status in breast cancer correlates with histologic type but does not correlate with established prognostic parameters. Am J Clin Pathol. 2006 Mar;125(3):377–85.

4. Mastracci TL, Tjan S, Bane AL, O'Malley FP, Andrulis IL. E-cadherin alterations in atypical lobular hyperplasia and lobular carcinoma in situ of the breast. Mod Pathol Off J U S Can Acad Pathol Inc. 2005 Jun;18(6):741–51.

5. Berx G, Cleton-Jansen AM, Strumane K, de Leeuw WJ, Nollet F, van Roy F, et al. E-cadherin is inactivated in a majority of invasive human lobular breast cancers by truncation mutations throughout its extracellular domain. Oncogene. 1996 Nov 7;13(9):1919–25.

6. Asgeirsson KS, Jónasson JG, Tryggvadóttir L, Olafsdóttir K, Sigurgeirsdóttir JR, Ingvarsson S, et al. Altered expression of E-cadherin in breast cancer. patterns, mechanisms and clinical significance. Eur J Cancer Oxf Engl 1990. 2000 Jun;36(9):1098–106.

7. Da Silva L, Parry S, Reid L, Keith P, Waddell N, Kossai M, et al. Aberrant expression of E-cadherin in lobular carcinomas of the breast. Am J Surg Pathol. 2008 May;32(5):773–83.

8. Dabbs, David J., Rohit Bhargava, Mamatha Chivukula. Lobular versus Ductal Breast Neoplasms: The Diagnostic Utility of P120 Catenin. *Am J Surg Pathol.* 31, no. 3 March 2007: 427–37.

9. Golenhofen N, Drenckhahn D. The catenin, p120ctn, is a common membrane-associated protein in various epithelial and non-epithelial cells and tissues. Histochem Cell Biol. 2000 Aug;114(2):147–55.

10. Choi YJ, Pinto MM, Hao L, Riba AK.Interobserver variability and aberrant E-cadherin immunostaining of lobular neoplasia and infiltrating lobular carcinoma. Mod Pathol Off J U S Can Acad Pathol Inc. 2008 Oct;21(10):1224–37.

11. Schnitt SJ LS.WHO Classification of Tumours of the Breast. Lyon [Internet]. 2012. Available from: IARC Press

12. Li CI, Anderson BO, Daling JR, Moe RE. Trends in incidence rates of invasive lobular and ductal breast carcinoma.JAMA. 2003 Mar 19;289(11):1421–4.

13. O'Malley FP. Lobular neoplasia: morphology, biological potential and management in core biopsies. Mod Pathol Off J U S Can Acad Pathol Inc. 2010 May;23 Suppl 2:S14-25.

14. Page DL, Simpson JF. What Is Atypical Lobular Hyperplasia and What Does It Mean for the Patient? J Clin Oncol. 2005 Aug 20;23(24):5432–3.

15. Dabbs DJ. Breast Pathology.Elsevier Health Sciences; 2012.817 p.

16. Harbhajanka, Aparna, Paolo Gattuso et al Prognostic Value of Coexisting Lobular Carcinoma In Situ With Invasive Lobular Carcinoma. *AIMM* 24, no. 10 Dec 2016: 738–43.

17. Ho BC-S, Tan PH. Lobular neoplasia of the breast: 68 years on. Pathology (Phila). 2009 Jan;41(1):28–35.

18. Rakha EA, Gill MS, El-Sayed ME, Khan MM, Hodi Z, Blamey RW, et al. The biological and clinical characteristics of breast carcinoma with mixed ductal and lobular morphology. Breast Cancer Res Treat. 2009 Mar;114(2):243–50.

19. Talman M-LM, Jensen M-B, Rank F. Invasive lobular breast cancer.Prognostic significance of histological malignancy grading. Acta Oncol Stockh Swed. 2007;46(6):803–9.

20. Fisher ER, Gregorio RM, Redmond C, Fisher B. Tubulolobular invasive breast cancer: a variant of lobular invasive cancer. Hum Pathol. 1977 Nov;8(6):679–83.

21. Frost AR, Terahata S, Yeh IT, Siegel RS, Overmoyer B, Silverberg SG. The significance of signet ring cells in infiltrating lobular carcinoma of the breast. Arch Pathol Lab Med. 1995 Jan;119(1):64–8.

22. Li CI, Daling JR, Malone KE, Bernstein L, Marchbanks PA, Liff JM, et al. Relationship between established breast cancer risk factors and risk of seven different histologic types of invasive breast cancer. Cancer Epidemiol Biomark Prev Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol. 2006 May;15(5):946–54.

23. Tan PH, Harada O, Thike AA, Tse GM-K. Histiocytoid breast carcinoma: an enigmatic lobular entity. J Clin Pathol. 2011 Aug;64(8):654–9.

24. Ilic IR, Djordjevic NP, Randjelovic PJ, Stojanovic NM, Radulovic NS, Ilic RS. Seven-year survey of classical and pleomorphic invasive lobular breast carcinomas in women from southeastern Serbia: Differences in clinicopathological and immunohistochemical features. J BUON Off J Balk Union Oncol. 2016 Oct;21(5):1113–20.

25. Bentz JS, Yassa N, Clayton F. Pleomorphic lobular carcinoma of the breast: clinicopathologic features of 12 cases. Mod Pathol Off J U S Can Acad Pathol Inc. 1998 Sep;11(9):814–22.

26. Moe RE, Anderson BO.Distinctive biology of pleomorphic lobular carcinoma of the breast.J Surg Oncol. 2005 May 1;90(2):47–50.

27. Simpson PT, Reis-Filho JS, Lambros MBK, Jones C, Steele D, Mackay A, et al. Molecular profiling pleomorphic lobular carcinomas of the breast: evidence for a common molecular genetic pathway with classic lobular carcinomas. J Pathol. 2008 Jul;215(3):231–44.

28. Rakha EA, van Deurzen CHM, Paish EC, Macmillan RD, Ellis IO, Lee AHS. Pleomorphic lobular carcinoma of the breast: is it a prognostically significant pathological subtype independent of histological grade? Mod Pathol Off J U S Can Acad Pathol Inc. 2013 Apr;26(4):496–501.

29. Abdel-Fatah TMA, Powe DG, Hodi Z, Lee AHS, Reis-Filho JS, Ellis IO. High frequency of coexistence of columnar cell lesions, lobular neoplasia, and low grade ductal carcinoma in situ with invasive tubular carcinoma and invasive lobular carcinoma.Am J Surg Pathol. 2007 Mar;31(3):417–26.

30. Buerger H, Simon R, Schäfer K-L, Diallo R, Littmann R, Poremba C, et al. Genetic relation of lobular carcinoma in situ, ductal carcinoma in situ, and associated invasive carcinoma of the breast. Mol Pathol. 2000 Jun 1;53(3):118–21.

31. Kiss A, Troyanovsky RB, Troyanovsky SM. p120-catenin is a key component of the cadherin-gamma-secretase supercomplex. Mol Biol Cell. 2008 Oct;19(10):4042–50.

32. Dabbs DJ, Bhargava R, Chivukula M. Lobular versus ductal breast neoplasms: the diagnostic utility of p120 catenin. Am J Surg Pathol. 2007 Mar;31(3):427–37.

33. Shibata T, Kokubu A, Sekine S, Kanai Y, Hirohashi S. Cytoplasmic p120ctn regulates the invasive phenotypes of E-cadherin-deficient breast cancer. Am J Pathol. 2004 Jun;164(6):2269–78.

34. Hatzfeld M. The p120 family of cell adhesion molecules. Eur J Cell Biol. 2005 Mar;84(2–3):205–14.

35. Chen X, Kojima S, Borisy GG, Green KJ. p120 catenin associates with kinesin and facilitates the transport of cadherin-catenin complexes to intercellular junctions. J Cell Biol. 2003 Nov 10;163(3):547–57.

36. Ireton RC, Davis MA, van Hengel J, Mariner DJ, Barnes K, Thoreson MA, et al. A novel role for p120 catenin in E-cadherin function. J Cell Biol. 2002 Nov 11;159(3):465–76.

37. Sarrió D, Pérez-Mies B, Hardisson D, Moreno-Bueno G, Suárez A, Cano A, et al. Cytoplasmic localization of p120ctn and E-cadherin loss characterize lobular breast

carcinoma from preinvasive to metastatic lesions. Oncogene. 2004 Apr 22;23(19):3272–83.

38. Shibata T, Kokubu A, Sekine S, Kanai Y, Hirohashi S. Cytoplasmic p120ctn Regulates the Invasive Phenotypes of E-Cadherin-Deficient Breast Cancer. Am J Pathol. 2004 Jun;164(6):2269–78.

39. Dabbs DJ, Bhargava R, Chivukula M. Lobular versus ductal breast neoplasms: the diagnostic utility of p120 catenin. Am J Surg Pathol. 2007 Mar;31(3):427–37.

40. Carlos E. Bacchi R de D. Immunohistochemistry Applied to the Differential Diagnosis Between Ductal and Lobular Carcinoma of the Breast.

41. Turashvili G, Bouchal J, Burkadze G, Kolár Z. Differentiation of tumours of ductal and lobular origin: I. Proteomics of invasive ductal and lobular breast carcinomas. Biomed Pap Med Fac Univ Palacky Olomouc Czechoslov. 2005 Jun;149(1):57–62.

42. van Roy F, Berx G. The cell-cell adhesion molecule E-cadherin.Cell Mol Life Sci CMLS. 2008 Nov;65(23):3756–88.

43. Thoreson MA, Anastasiadis PZ, Daniel JM, Ireton RC, Wheelock MJ, Johnson KR, et al. Selective uncoupling of p120(ctn) from E-cadherin disrupts strong adhesion. J Cell Biol. 2000 Jan 10;148(1):189–202.

44. Arpino G, Bardou VJ, Clark GM, Elledge RM. Infiltrating lobular carcinoma of the breast: tumor characteristics and clinical outcome. Breast Cancer Res. 2004;6(3):R149–56.

45. Reis-Filho JS, Simpson PT, Turner NC, Lambros MB, Jones C, Mackay A, et al. FGFR1 emerges as a potential therapeutic target for lobular breast carcinomas. Clin Cancer Res Off J Am Assoc Cancer Res. 2006 Nov 15;12(22):6652–62.

46. Roylance R, Gorman P, Papior T, Wan Y-L, Ives M, Watson JE, et al. A comprehensive study of chromosome 16q in invasive ductal and lobular breast carcinoma using array CGH.Oncogene. 2006 Oct 19;25(49):6544–53.

47. Loo LWM, Grove DI, Williams EM, Neal CL, Cousens LA, Schubert EL, et al. Array comparative genomic hybridization analysis of genomic alterations in breast cancer subtypes. Cancer Res. 2004 Dec 1;64(23):8541–9.

48. Mohsin SK, O'Connell P, Allred DC, Libby AL. Biomarker profile and genetic abnormalities in lobular carcinoma in situ. Breast Cancer Res Treat. 2005 Apr;90(3):249–56.

49. Michaut M, Chin S-F, Majewski I, Severson TM, Bismeijer T, de Koning L, et al. Integration of genomic, transcriptomic and proteomic data identifies two biologically distinct subtypes of invasive lobular breast cancer. Sci Rep. 2016 Jan 5;6:18517.

50. Ciriello G, Gatza ML, Beck AH, Wilkerson MD, Rhie SK, Pastore A, et al. Comprehensive Molecular Portraits of Invasive Lobular Breast Cancer. Cell. 2015 Oct 8;163(2):506–19.

51. Calhoun BC, Collie AMB, Lott-Limbach AA, Udoji EN, Sieck LR, Booth CN, et al. Lobular neoplasia diagnosed on breast Core biopsy: frequency of carcinoma on excision and implications for management. Ann Diagn Pathol. 2016 Dec;25:20–5.

52. Adachi Y, Ishiguro J, Kotani H, Hisada T, Ichikawa M, Gondo N, et al. Comparison of clinical outcomes between luminal invasive ductal carcinoma and luminal invasive lobular carcinoma. BMC Cancer [Internet]. 2016 Mar 25 [cited 2017 Jan 3];16. Available from: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4807554/

53. Reynolds AB, Roczniak-Ferguson A. Emerging roles for p120-catenin in cell adhesion and cancer. Oncogene. 2004 Oct 18;23(48):7947–56.

54. Bellovin DI, Bates RC, Muzikansky A, Rimm DL, Mercurio AM. Altered localization of p120 catenin during epithelial to mesenchymal transition of colon carcinoma is prognostic for aggressive disease. Cancer Res. 2005 Dec 1;65(23):10938–45.

55. Dabbs DJ, Schnitt SJ, Geyer FC, Weigelt B, Baehner FL, Decker T, et al. Lobular neoplasia of the breast revisited with emphasis on the role of E-cadherin immunohistochemistry. Am J Surg Pathol. 2013 Jul;37(7):e1-11.

56. Turashvili G, Bouchal J, Ehrmann J, Fridman E, Skarda J, Kolar Z. Novel immunohistochemical markers for the differentiation of lobular and ductal invasive breast carcinomas. Biomed Pap Med Fac Univ Palacký Olomouc Czechoslov. 2007 Jun;151(1):59–64.

57. Li, Xiaoxian, Mary R. Schwartz, Jae Ro, Candice R. Hamilton, Alberto G. et al Diagnostic Utility of E-Cadherin and P120 Catenin Cocktail Immunostain in Distinguishing DCIS from LCIS.*International Journal of Clinical and Experimental Pathology* 7, no. 5 (2014): 2551–57.

58. Naidoo K, Beardsley B, Carder PJ, Deb R, Fish D, Girling A, et al. Accuracy of classification of invasive lobular carcinoma on needle core biopsy of the breast. J Clin Pathol. 2016 Dec;69(12):1122–3.

59. Chuba PJ, Hamre MR, Yap J, Severson RK, Lucas D, Shamsa F, et al. Bilateral risk for subsequent breast cancer after lobular carcinoma-in-situ: analysis of surveillance, epidemiology, and end results data. J Clin Oncol Off J Am Soc Clin Oncol. 2005 Aug 20;23(24):5534–41.

60. Middleton LP, Grant S, Stephens T, Stelling CB, Sneige N, Sahin AA. Lobular carcinoma in situ diagnosed by core needle biopsy: when should it be excised? Mod Pathol Off J U S Can Acad Pathol Inc. 2003 Feb;16(2):120–9.

61. Wachtel MS, Halldorsson A, Dissanaike S. Nottingham grades of lobular carcinoma lack the prognostic implications they bear for ductal carcinoma. J Surg Res. 2011 Mar;166(1):19–27.

62. Yeatman TJ, Cantor AB, Smith TJ, Smith SK, Reintgen DS, Miller MS, et al. Tumor biology of infiltrating lobular carcinoma. Implications for management. Ann Surg. 1995 Oct;222(4):549-559; discussion 559-561.

63. Li X, Schwartz MR, Ro J, Hamilton CR, Ayala AG, Truong LD, et al. Diagnostic utility of E-cadherin and P120 catenin cocktail immunostain in distinguishing DCIS from LCIS. Int J Clin Exp Pathol. 2014;7(5):2551–7.

64. Ferlicot, S., A. Vincent-Salomon, J. Médioni, P. Genin, C. Rosty, B.et al Wide Metastatic Spreading in Infiltrating Lobular Carcinoma of the Breast. *European Journal of Cancer* 40, no. 3 Feb 2004: 336–41.

65. Kabat GC, Kim M, Shikany JM, Rodgers AK, Wactawski-Wende J, Lane D, et al. Alcohol consumption and risk of ductal carcinoma in situ of the breast in a cohort of postmenopausal women. Cancer Epidemiol Biomark Prev Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol. 2010 Aug;19(8):2066–72.

66. Yeatman, T. J., A. B. Cantor, T. J. Smith, S. K. Smith, D. S. Reintgen et al. Tumor Biology of Infiltrating Lobular Carcinoma.Implications for Management. *Annals of Surgery* 222, no. 4 Oct1995: 549-559;
.

67. Rendi MH, Dintzis SM, Lehman CD, Calhoun KE, Allison KH. Lobular in-situ neoplasia on breast core needle biopsy: imaging indication and pathologic extent can identify which patients require excisional biopsy. Ann Surg Oncol. 2012 Mar;19(3):914–21.

68. Lakhani SR, Audretsch W, Cleton-Jensen A-M, Cutuli B, Ellis I, Eusebi V, et al. The management of lobular carcinoma in situ (LCIS). Is LCIS the same as ductal carcinoma in situ (DCIS)? Eur J Cancer Oxf Engl 1990. 2006 Sep;42(14):2205–11.

69. Orvieto E, Maiorano E, Bottiglieri L, Maisonneuve P, Rotmensz N, Galimberti V, et al. Clinicopathologic characteristics of invasive lobular carcinoma of the breast: results of an analysis of 530 cases from a single institution. Cancer. 2008 Oct 1;113(7):1511–20.

70. Bane AL, Tjan S, Parkes RK, Andrulis I, O'Malley FP. Invasive lobular carcinoma: to grade or not to grade. Mod Pathol. 2005 May;18(5):621–8.

71. Li P, Zheng J, Zhang T, Cao R, Chen X, Geng M. Histiocytoid breast carcinoma: a case report showing immunohistochemical profiles. Int J Clin Exp Pathol. 2013;6(11):2609–14.

72. Tsuchiya S. Intracytoplasmic lumina of human breast cancer--a microscopic study and practical application in cytological diagnosis. Acta Pathol Jpn. 1981 Jan;31(1):45–54.

73. Crasta JA, Makhija P, Kumar KR, Sheriff S. Cytological features of lobular carcinoma of breast: how important are the intracytoplasmic lumina? Indian J Pathol Microbiol. 2005 Apr;48(2):170–2.

74. Bezic J, Gugic D. Signet ring lobular carcinoma in situ as a part of the "Rosen Triad" (tubular carcinoma, columnar cell hyperplasia, and lobular carcinoma in situ). Turk Patoloji Derg. 2013;29(2):134–7.

75. O'Malley FP. Lobular neoplasia: morphology, biological potential and management in core biopsies. Mod Pathol Off J U S Can Acad Pathol Inc. 2010 May;23 Suppl 2:S14-25.

76. Simpson JF, Gray R, Dressler LG, Cobau CD, Falkson CI, Gilchrist KW, et al. Prognostic value of histologic grade and proliferative activity in axillary node-positive breast cancer: results from the Eastern Cooperative Oncology Group Companion Study, EST 4189. J Clin Oncol Off J Am Soc Clin Oncol. 2000 May;18(10):2059–69.

77. Chetty R, Butler AE. Lymphocytic mastopathy associated with infiltrating lobular breast carcinoma. J Clin Pathol. 1993 Apr;46(4):376–7.

78. DiCostanzo D, Rosen PP, Gareen I, Franklin S, Lesser M. Prognosis in infiltrating lobular carcinoma. An analysis of "classical" and variant tumors.Am J Surg Pathol. 1990 Jan;14(1):12–23.

79. Correlation of Prognostic Factors of Invasive Lobular Carcinoma with ADC Value of DWI and SUVMax of FDG-PET. - PubMed - NCBI [Internet]. [cited 2017 Jun 14]. Available from: https://www.ncbi.nlm.nih.gov/pubmed/28584792

80. Perineural invasion has no prognostic value in patients with invasive breast carcinoma. - PubMed - NCBI [Internet]. [cited 2017 Sep 28]. Available from: https://www.ncbi.nlm.nih.gov/pubmed/16466917

81. Fernández B, Paish EC, Green AR, Lee AHS, Macmillan RD, Ellis IO, et al. Lymph-node metastases in invasive lobular carcinoma are different from those in ductal carcinoma of the breast. J Clin Pathol. 2011 Nov;64(11):995–1000.

82. Kanai Y, Oda T, Tsuda H, Ochiai A, Hirohashi S. Point mutation of the E-cadherin gene in invasive lobular carcinoma of the breast. Jpn J Cancer Res Gann. 1994 Oct;85(10):1035–9.

83. Huiping C, Sigurgeirsdottir JR, Jonasson JG, Eiriksdottir G, Johannsdottir JT, Egilsson V, et al. Chromosome alterations and E-cadherin gene mutations in human lobular breast cancer. Br J Cancer. 1999 Dec;81(7):1103–10.

84. Shimoyama Y, Nagafuchi A, Fujita S, Gotoh M, Takeichi M, Tsukita S, et al. Cadherin dysfunction in a human cancer cell line: possible involvement of loss of alpha-catenin expression in reduced cell-cell adhesiveness. Cancer Res. 1992 Oct 15;52(20):5770–4.

85. Schackmann RCJ, Tenhagen M, van de Ven RAH, Derksen PWB.p120-catenin in cancer - mechanisms, models and opportunities for intervention. J Cell Sci. 2013 Aug 15;126(Pt 16):3515–25.

86. Mastracci TL, Tjan S, Bane AL, O'Malley FP, Andrulis IL. E-cadherin alterations in atypical lobular hyperplasia and lobular carcinoma in situ of the breast. Mod Pathol Off J U S Can Acad Pathol Inc. 2005 Jun;18(6):741–51.

87. Thoreson, M. A., P. Z. Anastasiadis, J. M. Daniel, R. C. Ireton, M. J. Wheelock, K. R. Johnson, D. K. Hummingbird, and A. B. Reynolds. Selective Uncoupling of P120(Ctn) from

E-Cadherin Disrupts Strong Adhesion.*The Journal of Cell Biology* 148, no. 1 (January 10, 2000): 189–202.

88. Immunohistochemistry applied to the differential diagnosis between ductal and lobular carcinoma of the breast. - PubMed - NCBI [Internet]. [cited 2017 Sep 28]. Available from: https://www.ncbi.nlm.nih.gov/pubmed/22595945

89. Lee, Taeg Ki, Chang Hwan Choi, Youn Jeong Kimet al. Multi-Focal Lobular Carcinoma *In Situ* Arising in Benign Phyllodes Tumor: A Case Report. *Journal of the Korean Society of Radiology* 73, no. 2 (2015): 127.

90. Li L, Bi X, Xu X, Liu X, Shen G, Guo L, et al. [Expression pattern of E-cadherin and p120-catenin in infiltrating lobular carcinoma and ductal carcinoma of the breast and its significance]. Zhonghua Zhong Liu Za Zhi. 2010 Apr;32(4):273–7.

91. Goldstein NS, Bassi D, Watts JC, Layfield LJ, Yaziji H, Gown AM. E-cadherin reactivity of 95 noninvasive ductal and lobular lesions of the breast.Implications for the interpretation of problematic lesions.Am J Clin Pathol. 2001 Apr;115(4):534–42.

92. Goldstein NS, Kestin LL, Vicini FA. Clinicopathologic implications of E-cadherin reactivity in patients with lobular carcinoma in situ of the breast.Cancer. 2001 Aug 15;92(4):738–47.

93. Kovács A, Dhillon J, Walker RA. Expression of P-cadherin, but not E-cadherin or N-cadherin, relates to pathological and functional differentiation of breast carcinomas. Mol Pathol MP. 2003 Dec;56(6):318–22.

94. Acs G, Lawton TJ, Rebbeck TR, LiVolsi VA, Zhang PJ. Differential expression of E-cadherin in lobular and ductal neoplasms of the breast and its biologic and diagnostic implications.Am J Clin Pathol. 2001 Jan;115(1):85–98.

96. Wahed A, Connelly J, Reese T. E-cadherin expression in pleomorphic lobular carcinoma: an aid to differentiation from ductal carcinoma. Ann Diagn Pathol. 2002 Dec;6(6):349–51.

Appendix

Protocol for automated immunostaining:

1. Paraffin embedded tissue sections were cut at 4µ thickness and floated in poly L-lysine coated slides and incubated overnight at 37˚C.
2. These slides were then treated with 4% milk solution for 10 minutes to eliminate the hydrophobic effect and give positive charge to the slides.
3. Then the slide labels were bar coded and the labeled slides were loaded in Ventana Benchmark XT autostainer (a fully automated immunostainer).
4. Individual protocols have been designed in the software attached to the machine for each marker. Specific protocols were selected according to the marker.
5. A standard protocol was used for most of the markers with a minimal variation for few individual markers. The steps included in this protocol were as follows:
    a. Deparaffinization
    b. Liquid coverslip application.
    c. Heat induced antigen retrieval by treating with standard CC1 solution (pH patent with the company) for one hour at 90˚C.
    d. Then the primary antibody(E-cadherin) was added and incubated for 40 minutes @ 37˚C.
    e. Then the secondary antibody (Multimer) was added and incubated for 8 minutes.
    f. Finally the slides were counterstained with Haematoxylin and incubated for 8 minutes, followed by incubation with the bluing reagent for 4 minutes.

    (From antigen retrieval till counterstaining, in between every step the slides were washed with reaction buffer. The whole process is automated).

Then the slides were brought to 80% alcohol (2 changes) to remove the liquid coverslip and then dried and mounted in DPX.

DUAL STAIN

Tissue taken on positively charged slide

↓

Slide baked in hot air oven at 37*Degrees overnight

↓

Then print the dual stain barcode label and stick to slide.

↓

Then load the slide and both the detection kit (DAB & Red)

Steps

Depraffinization – EZ prep

↓

Antigen retrival – CC1 solution either (short, Mild, standard)

↓

Blocking -. UV DAB inhibitor

↓

First Primary added (this would take DAB as chromogen)(E-cadherin)

↓

Then secondary antibody (p120)

↓

DAB chromogen

↓

DAB enhancer

↓

Second Primary antibody added (Red chromogen) (p120)

↓

Then Secondary antibody (p120)

↓

Red chromogen

↓

Counterstain with Haematoxylin

↓

(Wash steps in-between all the steps)

↓

Then slides are rinsed in mild detergent

↓

Alcohol and xylene

↓

Cover slipped with DPX.

1. First added Primary would give brown color DAB
2. Second added Primary would give Red color.

Allred scoring system:

Proportion score	Percentage positive cells	Intensity score	Remarks
0	NIL	0	None
1	1%	1	Weak
2	1-10%	2	Intermediate
3	10-33%	3	Strong
4	33-66%		
5	66-100%		

PROFORMA

Retrospective study of infiltrating lobular carcinoma of breast with assessment of utility of P120 catenin and E-cadherin double immunostaining in the diagnosis.

Serial no: Biopsy no:

Age: Gender: M/F

CLINICAL:

Side of the breast:

Unilateral/Bilateral:

Unifocal/Multifocal:

Prechemo/Postchemo:

Nature of specimen: Core biopsy WLE Resection

GROSS:

Tumour size:

Cut surface: colour consistency

MICRO:

Cohesiveness:

Pattern: Targetoid growth Indian file Nests Single cells

Mitosis: + / -

Lymphocytic reaction: + / -

Intracytoplasmic lumina: + / -

Signet ring cells: + / -

Intracellular mucin: + / -

Nuclear pleomorphism: + / -

Desmoplasia: + / -

ILC variant: Classical Trabecular Solid Alveolar Pleomorphic Tubuloalveolar

Scarff-Bloom Richardson Grade – Nuclear grade score Tubule Pleomorphism

Cumulative score

LCIS: Present /Absent

Adjacent Breast: Elastosis:

Columnar cell change:

Calcification:

Intraductal papillomatosis:

Pagetoid spread:

Total number of lymphnodes:

Number positive:

pTNM staging:

IHC:

E-Cadherin (ILC)

Localisation	Pattern	Intensity	% pos cells

E-cadherin(LCIS)

Localisation	Pattern	Intensity	% pos cells

Internal controls (normal breast ducts)

Double immunostaining e- cadherin and p120 catenin

stain	localisation	pattern	intensity	% pos cells
e-cadherin				
P120 catenin				

Internal controls (normal breast ducts)

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA

Dr. B.J. Prashantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Alfred Job Daniel, D Ortho MS Ortho DNB Ortho.
Chairperson, Research Committee & Principal

Dr. Nihal Thomas,
MD, MNAMS, DNB (Endo), FRACP (Endo), FRCP (Edin), FRCP (Glasg)
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

February 01, 2016

Dr. Chandan Chowdhuri,
Post Graduate Registrar,
Department of Pathology,
Christian Medical College,
Vellore 632 004.

Sub: **Fluid Research grant project NEW PROPOSAL:**
Retrospective study of lobular carcinoma of breast with assessment of utility of p120 catenin and E-cadherin double immunostaining in the diagnosis.
Dr. Chandan Chowdhuri (Emp. No. 20968), Pathology, Dr. Marie Therese Manipadam (Emp. No. 31129), General Pathology, Dr. Raju Titus Chacko, Medical Oncology, Dr. Selvamani, Radiotherapy, Dr. MJ Paul, Endocrine Surgery, Dr. Deepak Abraham, Endocrine Surgery.

Ref: IRB Min No: 9779 [OBSERVE] dated 03.12.2015

Dear Dr. Chandan Chowdhuri,

The Institutional Review Board (Blue, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project titled "Retrospective study of lobular carcinoma of breast with assessment of utility of p120 catenin and E-cadherin double immunostaining in the diagnosis" on December 03rd 2015.

I enclose the following documents:-

1. Institutional Review Board approval 2. Agreement

Could you please sign the agreement and send it to Dr. Nihal Thomas, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

Dr. NIHAL THOMAS
MD.,MNAMS..DNB(Endo),FRACP(Endo),FRCP(Edin),FRCP(Glasg)
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

Cc: Dr. Marie Therese Manipadam, Dept. of Pathology, CMC

Ethics Committee Blue, Office of Research, 1st Floor, Carman Block, Christian Medical College, Vellore, Tamil Nadu 6
Tel: 0416 – 2284294, 2284202 Fax: 0416 – 2262788, 2284481 E-mail: research@cmcvellore.ac.in

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Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

Dr. Jayaprakash Muliyil	BSc, MBBS, MD, MPH, Dr PH (Epid), DMHC	Retired Professor, CMC, Vellore	External, Scientist &Epidem
Mrs. Emily Daniel	MSc Nursing	Professor, Medical Surgica Nursing, CMC, Vellore	Internal, Nurse
Mrs. Sheela Durai	MSc Nursing	Professor, Medical Surgical Nursing, CMC, Vellore	Internal, Nurse
Mr. C. Sampath	BSc, BL	Advocate, Vellore	External, Legal Expert
Dr. Anuradha Rose	MBBS, MD, MHSC (Bioethics)	Associate Professor, Community Health, CMC, Vellore	Internal, Clinician

We approve the project to be conducted as presented.

Kindly provide the total number of patients enrolled in your study and the total number of withdrawals for the study entitled: "Retrospective study of lobular carcinoma of breast with assessment of utility of p120 catenin and E-cadherin double immunostaining in the diagnosis" on a monthly basis. Please send copies of this to the Research Office (research@cmcvellore.ac.in)

Fluid Grant Allocation:
A sum of 1,00,000/- INR (Rupees One Lakh Only) will be granted for 3 years. 50,000/- INR (Rupees Fifty Thousand only) will be granted for 12 months as an Ist Installment. The rest of the 50,000/- INR (Rupees Fifty Thousand only) each will be released at the end of the first year as 2 nd Installment.

Yours sincerely

Dr. NIHAL THOMAS
MD,MNAMS,DNB(Endo),FRACP(Endo),FRCP(Edin),FRCP(Glasg)
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board
IRB Min No: 9779 [OBSERVE] dated 03.12.2015

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