EVALUATION OF P53, HOX D10 AND E CADHERIN STATUS IN BREAST CANCER AND CORRELATION WITH HISTOLOGICAL GRADE

Dissertation submitted in partial fulfilment
of the requirements for the degree of

M.D. (PATHOLOGY)

BRANCH - III

INSTITUTE OF PATHOLOGY AND ELECTRON MICROSCOPY,
MADRAS MEDICAL COLLEGE,
CHENNAI – 600 003.

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

APRIL 2013

CERTIFICATE

This is to certify that this Dissertation entitled **"EVALUATION OF P53, HOX D10 AND E CADHERIN STATUS IN BREAST CANCER AND CORRELATION WITH HISTOLOGICAL GRADE"** is the bonafide original work of **Dr.S.PREETHI,** in partial fulfillment of the requirement for M.D., (Branch III) in Pathology examination of the Tamilnadu Dr.M.G.R Medical University to be held in April 2013.

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DECLARATION

I **Dr.S.PREETHI,** solemnly declare that the dissertation titled **"EVALUATION OF P53, HOX D10 AND E CADHERIN STATUS IN BREAST CANCER AND CORRELATION WITH HISTOLOGICAL GRADE"** is the bonafide work done by me at Institute of Pathology, Madras Medical College under the expert guidance and supervision of **Prof.Dr.P.KARKUZHALI, M.D.,** Professor and Director of Institute of Pathology and Electron Microscopy, Madras Medical College. The dissertation is submitted to the Tamilnadu Dr.M.G.R Medical University towards partial fulfillment of requirement for the award of M.D., Degree (Branch III) in Pathology.

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Dr. S. Preethi
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Madras Medical College, Chennai-3,

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The Institutional Ethics Committee of Madras Medical College reviewed and discussed your application for approval of the proposal entitled " Evaluation of p 53 , HOX D 10 and E cadherin status in breast cancer and correlation with histological type and grade" No. 16012011.

The following members of Ethics Committee were present in the meeting held on 28.01.2011 conducted at Madras Medical College, Chennai -3.

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EVALUATION OF P53, HOX D10 AND E CADHERIN STATUS IN BREAST CANCER AND CORRELATION WITH HISTOLOGICAL GRADE Dissertation submitted in partial fulfilment of the requirements for the degree of M.D. (PATHOLOGY) BRANCH - III INSTITUTE OF PATHOLOGY AND ELECTRON MICROSCOPY, MADRAS MEDICAL COLLEGE, CHENNAI – 600 003. THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY CHENNAI APRIL 2013 INTRODUCTION Breast carcinoma is the most common cause of cancer related mortality in urban Indian women overtaking cervical cancer. The incidence is 30 – 33 per 1,00,000 women in urban India and it is the second commonest cause in rural women.1There is a gradual rise in the incidence of breast carcinoma worldwide. It...

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Words are not enough to thank my family for their understanding, moral support and encouragement.

ABBREVIATIONS

ER	:	Estrogen Receptor
PR	:	Progesterone Receptor
HER 2 NEU	:	Human epidermal growth factor receptor 2
EGFR	:	Epidermal growth factor receptor
HoxD10	:	Homeobox D10
CDH1	:	Cadherin-1
P53	:	Protein 53
DNA	:	De oxy ribonucleic acid
IHC	:	Immunohistochemistry
PCR	:	Polymerase chain reaction
RT PCR	:	Reverse Transcriptase Polymerase Chain Reaction
IDC NOS	:	Invasive ductal carcinoma not otherwise specified
ICMR	:	Indian Council of Medical Research
WHO	:	World Health Organisation
FISH	:	Fluorescent in situ hybridization
cDNA	:	Complementary deoxy ribonucleic acid
mRNA	:	Messenger ribonucleic acid
ACTB1	:	Actin beta 1
CT	:	Threshold cycle
ΔΔCT	:	Delta delta threshold cycle
N	:	Number of cases
SD	:	Standard deviation
EC	:	E Cadherin
HMW CK	:	High molecular weight cytokeratin
DCIS	:	Ductal carcinoma in situ
GCDFP	:	Gross cystic disease fluid protein

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INTRODUCTION

Breast carcinoma is the most common cause of cancer related mortality in urban Indian women overtaking cervical cancer. The incidence is 30 – 33 per 1,00,000 women in urban India and it is the second commonest cause in rural women.[1]There is a gradual rise in the incidence of breast carcinoma worldwide. It accounts for about 23% of all cancers in women.[2] Early detection and treatment can certainly reduce the mortality rates.

Breast cancers exhibit widely varying behaviour with regard to the likelihood of recurrence, metastasis and response to therapy.Study of tumour molecular characteristics has enhanced our understanding of both the risk of breast cancer recurrence and the response to therapy. Hundreds of putative markers have been identified by immunohistochemistry or bioassays and thus serve as an important prognostic and predictive factor.

Some of the genes implicated in breast cancer progression and evaluated in this study include the p53, E Cadherin and the HoxD10 gene.

One of the most commonly mutated genes in human cancer is p53.[3]Alteration of this tumour suppressor gene is a critical step in the development of cancers.[4] The gene is present on chromosome 17p, and produces a nuclear phosphoprotein. The protein functions as a

transcription factor and regulates entry into S phase of the cell cycle.[5,6] The p53 protein also influences the occurrence of apoptosis in tumour cells.[7] Research has shown p53 gene mutation in breast cancers is associated with worse prognosis.[8]IHC detects mutant p53 protein in the cells as a result of conformational changes in the polypeptide which results in increased stability.[9]

E Cadherin (EC) is a calcium regulated adhesion molecule present in most normal epithelial cells and is a classic tumour suppressor gene.[10] The EC gene, CDH1 is located on chromosome 16q22.1.2.[11]It helps in formation of glands, epithelial polarization and stratification.[12]Loss of EC results in dedifferentiation and invasion in carcinomas.[13] While majority of the infiltrating lobular carcinomas show a complete loss of EC expression, ductal carcinomas show heterogeneous loss of EC expression, due to epigenetic transcriptional downregulation.[14] Studies show CDH1 under expression to be associated with histological type, higher tumour grade, stage and nodal status.[15]

The Hox gene network is essential for spatio temporal cell localisation and for cell to cell signal decoding so as to attain phenotype cell identity.[16] The thoracic Hox genes are involved in breast organogenesis,[17] whereas the cervical and lumbo-sacral Hox genes are involved in progression of breast cancer.[18] The genes indicated in breast

cancer progression include HoxD10, B13, A11 in lumbo sacral part and HoxB2, D3, D4 in cervical part.[19]

In this study of 60 cases of invasive ductal carcinoma NOS, an attempt is made to evaluate the p53 status by IHC, the HoxD10 and E Cadherin status by PCR and to correlate them with histological grade and other prognostic factors.

AIMS AND OBJECTIVES

1. To identify the relative frequency and distribution of breast carcinoma in the population.

2. To study the histomorphological features of breast carcinoma including grade, lymph node status, lymphovascular invasion, lymphocytic response and necrosis.

3. To study the immunohistochemical expression of p53 in invasive carcinoma breast.

4. To study the E Cadherin and HoxD10 gene expression by PCR in invasive carcinoma breast.

5. To assess the correlation between p53 status, E Cadherin and HoxD10 gene expression and with other known prognostic factors like histological grade, tumour size, axillary node status, tumour necrosis, lymphocytic response, lymphatic and vascular invasion by tumour.

REVIEW OF LITERATURE

Invasive breast carcinomas are a group of malignant epithelial tumours which show invasion of the adjacent tissues, with an increased tendency for distant metastasis.[20]Breast carcinoma is one of the cancers commonly described in ancient documents due to its visibility. The Edwin Smith Papyrus gives the oldest description of breast cancer, discovered in Egypt and dating back to1500 BC.[21] First documented case of breast cancer was described by Imhotep in 2650 BC.

Leonides (30 AD) compared cancers to crabs, due to the tenacious adherence to the surrounding tissues. In 1874, Paget described the changes in the nipple that preceded breast cancer and it continues to bear his name.[22]

Radical mastectomy was first performed by William Stewart Halsted in 1882.[23] X-rays were discovered by Wilhelm Conrad Röntgen in1895 and it forms the basis for mammogram and radiotherapy.[24]

In 1925, Greenhough was the first to evaluate grading system for breast cancer.[25] In 1928, Scarff et al proposed tubule formation, nuclear pleomorphism and hyperchromasia as criteria to grade breast cancers. In 1957,Bloom and Richardson proposed the numeric scoring system based on tubule formation, nuclear pleomorphism and mitosis for grading

adapted by WHO.[26] In 1983, Bloodgood et al recognized ductal carcinoma in situ where neoplastic cells are limited within the terminal duct lobular unit.[27] Early 1990, Nottingham modification of Bloom Richardson grading system was adapted by WHO.[28]

EPIDEMIOLOGY

According to the 2001-03 ICMR report, breast cancer constitutes about 25% of the total cancers among Indian women.[29] Breast cancer is the most common cancer in metropolitan cities and the second most common in rural Indian women after cervical cancer.

In India, the crude incidence rate of breast carcinoma is 85/100,000 women/ year.[30] The death per incident ratio is highest in India, with 50%, compared to 30% in China and 18% in the US.[31]

The annual age-adjusted rate is 30 to 33 per 100,000 in urban women and 8.6 per 100,000 in rural women.[32]

India is rapidly stepping towards industrialization resulting in lifestyle changes. This probably contributes to the increase in breast cancer incidence in our country.

The presenting symptoms include breast lump, nipple discharge, retraction or eczema. Screening for breast abnormalities are done by the

triple assessment which includes clinical examination, imaging and tissue sampling.

RISK FACTORS

The risk factors strongly associated with breast cancers include early menarche, late menopause, nulliparity, older age at first child birth, sedentary life style with high caloric diet, obesity, use of exogenous oestrogens and positive family history in a first degree relative.[33]

ETIOLOGY

The main etiological factors of breast carcinoma include hormone excess and genetics.

Oestrogen and breast cancer

The main function of oestrogen is stimulation of cell growth and proliferation by acting via oestrogen receptor (ER) as a transcriptional activator.[34] However, this process is slow. Recently, a non genomic pathway has been demonstrated which does not involve ER, but acts through a G-protein coupled receptor, GPR30. This results in activation of metalloproteinases and cleavage of heparan-bound EGF (epidermal growth factor). The released EGF then acts on its receptor, EGFR and stimulates cell proliferation.[35] The existence of this pathway indicates

that drugs acting only through ER may not be enough to inhibit tumour growth.

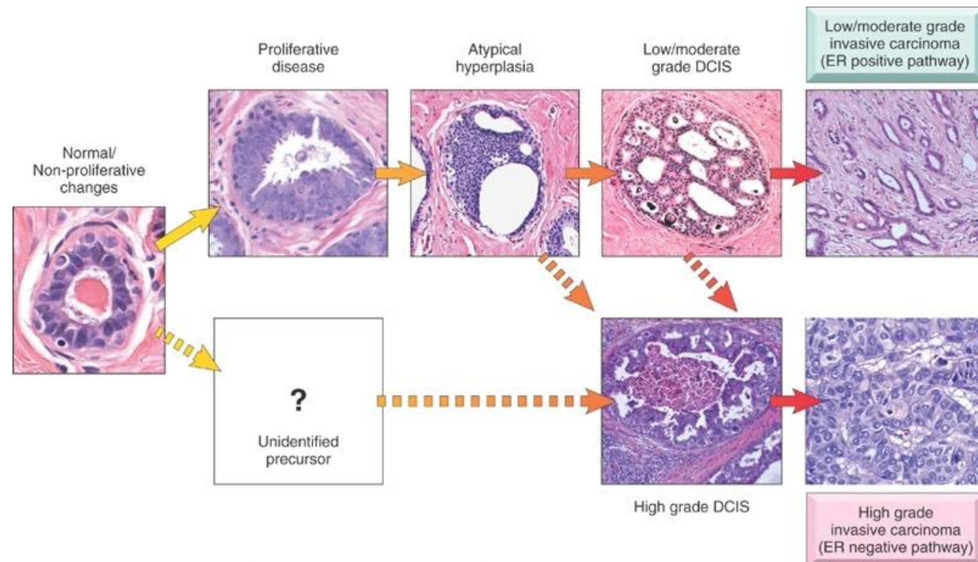


Fig.1: Proposed precursor-carcinoma sequences in breast cancer

Genes involved in Breast Cancer

About 5% to 10% of breast cancers are thought to be hereditary. The genes which increase the risk of developing breast cancer include the BRCA1, BRCA2, PTEN,CDH1, STK11 and the TP53 gene.

BRCA1 and BRCA2 are the major genes involved in hereditary breast cancer. The BRCA1 gene is present on chromosome 17q and its product is responsible for DNA repair. Women with these mutations have an increased risk of developing breast cancer, ovarian cancer and pancreatic cancer.[36,37] BRCA2 gene mutations are associated with high risk of developing carcinoma of the male breast, cancers of the pancreas, prostate and cutaneous melanoma.[38,39]

Invasive ductal carcinoma is a group of breast carcinoma in which the stromal invasion of malignant cells is evident beyond the epithelial component. Current histomorphological subtyping of breast carcinoma is based on World Health Organisation classification (Annexure II).

INVASIVE DUCTAL CARCINOMA NOT OTHERWISE SPECIFIED (IDC NOS)

This is the most common type of breast cancer, accounting for 75-80% of the breast cancers.[40] These tumours elicit a marked fibroblastic stromal reaction and produce a firm palpable mass. It may produce dimpling of the skin due to traction on the suspensory ligaments. Grossly, the tumour is ill defined, firm, with a yellow grey cut surface, with radiating trabeculae into the surrounding parenchyma, resulting in a stellate appearance.

Histologically, the tumour cells are arranged in a variety of patterns such as acinar configurations, cords and broad sheets of cells, with surrounding dense stroma. The tumours show a wide range of differentiation with poorly differentiated tumours showing solid sheets of pleomorphic cells. These tumours are graded using Nottingham modification of Scarff Bloom Richardson system (Annexure III).

INVASIVE LOBULAR CARCINOMA

Lobular carcinoma is the second commonest type of breast cancer accounting for 10% of the cases. The tumour is more often bilateral and multicentric.

The amount of stromal reaction varies from scanty to dense desmoplasia and therefore it may present as a discrete mass or diffuse indurated area.

The tumour cells are small, uniform and bland looking, often arranged in Indian file pattern or may form concentric arrays around ducts resulting in targetoid pattern. 10% of cases show mixed features of invasive ductal and lobular carcinomas.

It is characterised by the presence of HMW keratin, lack of accumulation of p53, and most importantly decrease or absence of E-cadherin.[41,42,43] To these, p120 catenin has been recently added, supported by the claim that lobular carcinoma shows a characteristic cytoplasmic staining pattern with this marker.[44]

MEDULLARY CARCINOMA

It is common in patients under 50 years of age, particularly in BRCA1 mutations carriers.[45] Grossly, the tumour is well circumscribed. Its cut surface is homogeneous, solid and grey with occasional foci of

necrosis. Microscopy shows solid sheets of large pleomorphic cells with prominent nucleoli, forming a syncytium. The tumour has scant fibrous stroma and frequent mitotic figures. Numerous lymphocytes surround the sheets of tumour cells with most of them being cytotoxic T lymphocytes.[46] They typically express CK7, often express vimentin, S-100 and P53, but not CK20.[47] They are almost invariably negative for hormone receptors as well as c-erbB-2 ('triple negative' phenotype).

MUCINOUS CARCINOMA

It commonly occurs in postmenopausal women. Grossly, it is well circumscribed, with a glistening jelly like mass held together by delicate septa. Microscopically, the tumour cells form small clusters and appear to float in a sea of mucin. These clusters may be solid, exhibit acinar formations or form micropapillary structures.[48] Histochemically, the mucins secreted by this tumour are distinct O-acylated forms of sialomucins.[49] Immunohistochemically, there is strong MUC2 cytoplasmic immunoreactivity and decreased MUC1 immunoreactivity compared with ductal carcinoma NOS.[50] Hormone receptors are always positive, while c-erbB-2 is almost always negative.

TUBULAR CARCINOMA

It commonly occurs in patients around 50 years of age. Grossly, it is characteristically small, measuring about 1cm with poorly

circumscribed margins and hard consistency. Microscopically, it is characterised by the haphazard arrangement of irregular and angulated glands in a desmoplastic stroma with the lining cells being small and regular. Low-grade DCIS and flat epithelial atypia are thought to be precursor lesions of tubular carcinoma.[51,52]

CRIBRIFORM CARCINOMA

These tumours accounts for 0.8 to 3.5% of breast carcinomas. Histologically, more than 90% of tumour shows cells arranged in islands in which well-defined spaces are formed by arches of cells resulting in a sieve like or cribriform pattern.

INVASIVE PAPILLARY CARCINOMA

These tumours accounts for less than 1 to 2 % of breast carcinoma. Fischer et al first reported that invasive papillary carcinoma is grossly circumscribed. Microscopically, the cells are arranged as delicate or blunt papillae with amphophilic cytoplasm.

INVASIVE MICROPAPILLARY CARCINOMA

The tumour is composed of small clusters of cells lying within clear stromal spaces resembling dilated vascular channels. They account for less than 2% of the breast cancers.

APOCRINE CARCINOMA

It accounts for 1- 4% of the breast cancers.>90% of the tumour is composed of apocrine cells.[53] There are 2 types of apocrine cells – Type A cells have abundant eosinophilic granular cytoplasm and Type B cells have clear, foamy cytoplasm. It is typically positive for GCDFP-15 and negative for bcl2 protein, ER and PR.

METAPLASTIC CARCINOMA

It account for less than 1% of the breast cancers. The neoplasm is heterogeneous,showing intimate admixture of adenocarcinoma with areas of spindle, squamous or mesenchymal differentiation ranging from chondroid and osseous differentiation to frank sarcoma.Grossly, they present as well delineated, firm, pearly white glistening mass.

NEUROENDOCRINE CARCINOMA

They constitute about 5% of all breast carcinomas. It comprises carcinoid tumours, large cell neuroendocrine carcinomas and small cell carcinomas. Microscopically, the neoplastic cells are small, arranged in solid nests separated by fibrous stroma. Ribbons and rosette like formations may be seen. Mitoses are generally rare. They express neuroendocrine markers in more than 50% of the tumour cells, and this feature helps to distinguish them from breast carcinoma with focal endocrine differantiation.[54]

PROGNOSTIC FACTORS

Patient's age: Women younger than 50 years of age have the best prognosis. Relative survival declines after 50 years.

Size: The tumour size shows a good correlation with the nodal status and survival rate.[55,56]

Cytoarchitectural type: There is no significant prognostic difference between ordinary invasive ductal and invasive lobular carcinoma.[57] Morphologic variants of invasive ductal carcinoma with a more favourable prognosis are medullary carcinoma, mucinous carcinoma, tubular carcinoma, cribriform carcinoma, papillary carcinoma, adenoid cystic carcinoma and secretory carcinoma.[58] Squamous cell carcinoma, metaplastic carcinoma, carcinomas with neuroendocrine features, and signet ring cell carcinoma behave in an aggressive way.[59]

Microscopic grade: Tumours are graded based on Nottingham modification of the Scarff Bloom–Richardson system (Annexure III). Ellis et al reported this grading system to have excellent correlation with patients' survival and rate of metastasis.[28]

Axillary lymph node metastases: This is an important prognostic factor. There is a marked difference in survival between patients with positive and negative nodes and the survival rate also varies depending on the level of axillary node involved, their absolute number,[60] the amount of

tumour cells in the node,[61] the presence or absence of extranodal spread and the presence or absence of tumour cells in the efferent vessels.

Other factors reported to have poor prognosis include tumour necrosis, lymphocytic infiltration and skin infiltration. After the advent of immunohistochemistry and polymerase chain reaction, more molecular biomarkers play significant predictive as well as prognostic role in breast cancer management.

IMMUNOHISTOCHEMISTRY (IHC)

IHC is a molecular technique which was first described by Dr.Albert Coons in 1941. The original method consisted of an antibody developed in rabbits and then tagged with a fluorescent probe. It was mixed with tissue sections and examined using a fluorescent microscope after a period of incubation. Since then, numerous advancements have been made.[62] The most commonly used techniques are the peroxidase-antiperoxidase immune complex method developed by Sternberger in 1970 and the biotin-avidin immunoenzymatic technique developed by Heitzman and Richards in 1974.[63,64]

USES OF IMMUNOHISTOCHEMISTRY IN BREAST PATHOLOGY[65,66]

1. The use of myoepithelial markers to assess stromal invasion.

2. E Cadherin to differentiate between ductal and lobular carcinoma.

3. High molecular weight cytokeratins to distinguish between usual ductal hyperplasia and ductal carcinoma in situ.

4. To find the site of origin in metastatic cancers.

5. Cytokeratin stain to detect sentinel lymph nodes metastasis.

6. Assessment of Estrogen and Progesterone receptor status & HER2neu overexpression using specific antibodies to receptor proteins.

7. Evaluation of spindle cell lesions to distinguish metaplastic carcinoma from mesenchymal lesions.

ANTIGEN RETRIEVAL

Shi et al in 1991developed the antigen retrieval technique, in which he used a heating method at high temperatures to bring out the antigenicity of the tissue which had been masked by formalin fixation.

Antigen retrieval can be done by heat induced epitope retrieval or proteolytic induced epitope retrieval.

HEAT INDUCED EPITOPE RETRIEVAL

The technique involves application of heat for varying period of time to the tissue sections in the retrieval solution. This results in

breakdown of protein cross-links formed by formalin fixation and recovers the tissue antigenicity.[67]

Some of the commonly used heating devices are the microwave oven, pressure cooker, steamer, autoclave and water bath. Heating is usually done for about 20 minutes followed by 20 minutes of cooling. The retrieval solution commonly used is the Citrate buffer with pH 6.0. Other retrieval solutions include the TRIS-EDTA with pH9.0 and EDTA with pH8.0.

PROTEOLYTIC INDUCED EPITOPE RETRIEVAL [68]

Proteases like proteinase K, trypsin, chymotrypsin and pepsin are used to restore the tissue antigenicity. However, this technique can destroy some epitopes and alter the tissue morphology. Therefore the optimal concentration of the enzyme and incubation time needs to be validated.

TARGET ANTIGEN DETECTION METHODS

After addition of specific antibodies to the antigens, next step is to visualize the antigen antibody reaction complex. The methods employed are the direct and the indirect methods.

The direct method is a one step staining procedure in which a labelled antibody directly reacts with the antigen in the tissue sections.

Most commonly used labels are fluorochrome, horse radish peroxidase and alkaline phosphatase. Although this method is simple, rapid, and uses only one antibody, the sensitivity is lower. This is because signal amplification is less, and therefore it is not as commonly used when compared to the indirect methods.

In the indirect method, first layer is formed by an unlabelled primary antibody which binds to the target antigen. Then, a second layer is formed by using a labelled secondary antibody that reacts with the primary antibody. This technique is more sensitive than the direct method because of better signal amplification. This is due to the binding of several secondary antibodies with conjugated fluorochrome to each primary antibody. Another advantage with this method is that it uses only a small number of secondary antibodies.[69]

TP53

It was first discovered by Lane et al in1979 and it has been found to be one of the most commonly mutated gene in human cancers (Hollstein, 1991). The TP53 gene is present on chromosome 17p.[70] It produces a 393 amino acid phosphoprotein, which is normally present at a low level in the cells.[71]

The p53 gene functions as a tumour suppressor and inhibits cell proliferation and plays a role in inducing cellular differentiation and

apoptosis. Wild-type p53 blocks the cell cycle near the GI/S phase.[72] IHC detects nuclear accumulation of the protein, occurring due to conformational changes, resulting in a prolonged half-life.[73,74] The p53 mutation rate in breast tumours is 20-40%.[75] p53 over expression in tumours are associated with higher histological grade, increased mitotic activity, aggressive behaviour and hence a worse prognosis.[76]

POLYMERASE CHAIN REACTION (PCR)

PCR is a technique in molecular biology which involves repeated cycles of DNA denaturation, annealing with primer, followed by extension with DNA polymerase resulting in the amplification of a piece of DNA, generating millions of copies of a particular DNA sequence.

The invention of PCR in 1983 is credited to Kary Mullis.[77] The DNA polymerases initially used were unable to withstand high temperatures.[78] So the earlier methods for DNA replication were very inefficient, requiring large amounts of DNA polymerase and constant handling throughout the entire process.

In 1988, Saiki et al proposed the use of Taq polymerase, a DNA polymerase obtained from the thermophilic bacterium, Thermus Aquaticus. This resulted in dramatic improvements of the PCR method. It is stable at high temperatures and remains active even after DNA denaturation. Thus, it obviates the need to add a new DNA polymerase

after each cycle and hence an automated thermocycler-based process could be used for DNA amplification.[79,80] This technique showed increased specificity but with 40% of amplified DNA fragments showing altered base due to absence of proof reading activity. In 1996, Cline J et al developed an alternative heat stable DNA polymerase derived from Pyrococcus furiosus and Thermococcus Litoralis with 3` to 5` exonuclease activity. This technique showed only 3.5% of DNA with altered base. In 1992, Higuchi et al first documented real time PCR that enabled the quantification of gene expression and DNA copy measurements.[81]

PCR requires the following components: template, primer, deoxyribonucleotides, DNA polymerase, buffer solution, magnesium and potassium ions.[82] Also, part of the target DNA sequence needs to be known so as to design an appropriate primer. The first step is denaturation, where the target DNA is heated above 90°C (194°F). This procedure results in separation of the two strands of DNA. Each strand can now function as a template. The second step is annealing of the primers with their respective complementary sequence on each template and is carried out at 50°C (122° F). In the third step, primer extension is done using DNA polymerase and provided nucleotides. Hence, the numbers of DNA molecules double at the end of each cycle.[83]

Reverse transcription-PCR (RT-PCR) is a sensitive method for detecting mRNA expression levels. RT-PCR consists of two steps: the RT reaction and PCR amplification. First, RNA is reverse transcribed into cDNA using a reverse transcriptase. The resulting cDNA can then be used as templates and PCR amplification is done using specific primers. There is also a one step RT-PCR in which all the reagents are added in one tube prior to starting the process. Though it is simple, convenient, with minimal risk of contamination, the cDNA produced cannot be repeatedly used as in two step RT-PCR.[84]

In 1988, Haqqi et al. developed nested PCR. Its purpose is to decrease the contamination in products due to the amplification of unwanted primer binding sites. The procedure uses two sets of primers in two successive runs of PCR. The second set amplifies a target within the product of the first run. The basic idea is that if the wrong locus was amplified, there is a very low probability that it would also be amplified a second time by the second set of primers.

In 1988, Chamberlain et al. described multiplex PCR where different target sequence can be amplified simultaneously in a single reaction using different primers. Competitive PCR is a process of co amplification in same reaction tube of 2 different templates of equal length and with same primer binding sequences.

Real time PCR involves amplification and simultaneous quantification of a targeted DNA sequence. The quantity could either be an absolute number of copies or a relative amount when normalized to the DNA input.

A key feature of this procedure is that the amplified DNA can be detected as the reaction proceeds in real time. Whereas, in standard PCR, the reaction product is detected at the end. Products in real-time PCR are detected by: (1) non-specific fluorescent dyes which integrate with any double stranded DNA and (2) sequence specific DNA probes consisting of fluorescent labelled oligonucleotides that permits detection only after binding of the probe with its complementary DNA sequence.[85]

Frequently, real-time PCR and reverse transcription are combined in order to quantify mRNA and non-coding RNA in tissues.

Advantages of PCR include high speed, ease of use, high sensitivity and its ability to amplify the desired target DNA sequence.

A limitation of PCR is that only 0.1- 5 kb size DNA sequences can be amplified. The amplification product levels off due to finite enzyme.

ROLE OF HOX D10 GENE IN BREAST CANCER

HOXD10 is a sequence-specific transcription factor which regulates the developmental system and provides cells with specific

positional identities on the anterior-posterior axis. It is a member of the Abd-B homeobox family and is present on chromosome 2. It encodes a nuclear protein which functions as a sequence specific transcription factor which is essential for limb development and differentiation.

Apart from its role in embryogenesis, Hox genes are also active in adult cells where they regulate the genes which are involved in cellular proliferation and also cell-cell and cell-extracellular matrix signalling. It is widely established that several Hox genes show an altered pattern of gene expression in certain malignancies like leukemia and solid neoplasms such as breast, endometrium, brain, colon, prostate, lung, and kidney. Research has shown that loss of various Hox genes is often associated with the development of tumour.

Breast cancer is characterised by a progressive loss of epithelial cell polarity, growth control, macrophage infiltration and increased angiogenesis. While HoxD10 gene shows high expression in normal breast epithelial and endothelial cells, invasive breast carcinomas show progressive loss of HoxD10 in both breast epithelial and endothelial cells. Studies have shown restoration of HoxD10 gene in metastatic breast cancer cells, results in growth arrest and cell polarisation. Also, restoration of HoxD10 in angiogenic endothelium blocks angiogenesis.[86] HoxD10 also suppresses the chemokine expression in tumours which evokes an inflammatory reaction. Thus, HoxD10 is a potent tumour

suppressor gene which directly impacts the epithelial cells and inhibits angiogenesis and inflammation, thus stabilizing the tumour microenvironment.[87]

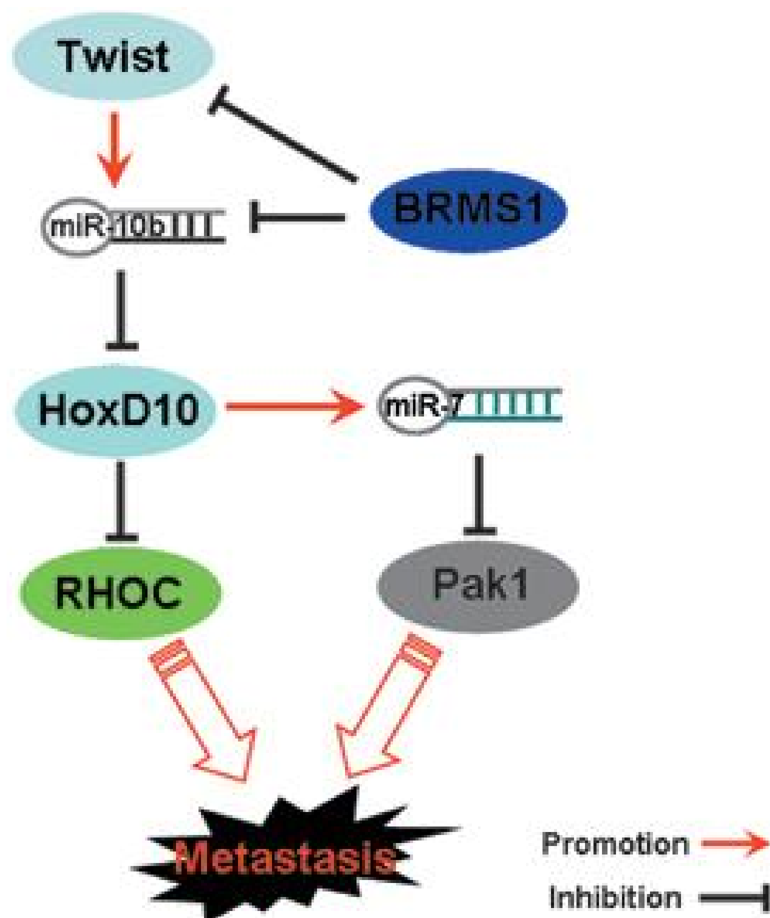


Fig.2: Regulation of tumor metastasis by HoxD10

Breast cancer metastasis suppressor-1 (BRMS1) regulates Twist expression. Elevated expression of the transcription factor Twist in breast cancer cells induces the transcription of miR-10b which suppresses the synthesis of the HOXD10 protein, a negative regulator of breast cancer progression, permitting expression of pro-metastatic gene products, RhoC and p21-activated kinase1(Pak1). This in turn favours cancer cell migration, invasion, and metastasis.

ROLE OF E CADHERIN GENE IN BREAST CANCER :

E Cadherin is a tumour suppressor gene present on chromosome 16. Most epithelial tissues show cell surface expression of E Cadherin (Takeichi, 1990). It is considered to be a key molecule in the formation of intercellular junctions and for establishing the cell polarization (Gumbiner et al,1988). The cytoplasmic tail of E-cadherin is linked to the actin cytoskeleton via catenins (Cowin, 1994) and the extracellular domain is involved in mediating cell–cell adhesion (Shapiro et al, 1995).

E Cadherin under expression leads to invasion in breast cancer (Siitonen et al, 1996), cancer progression and metastasis. E Cadherin activation can cause growth retardation of cancer cells (Navarro et al, 1991; St Croix et al, 1998)

Partial or complete loss of E Cadherin expression leads to dedifferentiation, invasion, higher tumour grade, metastasis and worse prognosis in breast cancer.[88]

MATERIALS AND METHODS

This study is a prospective and retrospective descriptive study of invasive breast cancers conducted in the Institute of Pathology, Madras Medical College and Government hospital, Chennai during the period between Jan 2010 to Feb 2012.

Source of data

The invasive ductal carcinoma cases reported in mastectomy specimen received in the Institute of Pathology, Madras Medical College between Jan 2010 to Feb 2012 from the Department of Surgery, Oncology, Plastic surgery and Geriatrics, Government General Hospital. A total of 274 mastectomy specimens (simple, modified radical or radical mastectomy) were received during this period.

Inclusion criteria

All the invasive ductal carcinoma cases reported in mastectomy specimens irrespective of the age and sex were included for the study.

Exclusion criteria

- Non neoplastic lesions and benign tumors of breast.

- Ductal carcinoma breast reported in incision/excision biopsy and completion mastectomy specimens.

- Cases with inadequate material from the tumor for doing both immunohistochemistry and polymerase chain reaction were not included in the study.

METHOD OF DATA COLLECTION

Detailed history of the cases regarding age, sex, menstrual history, side of the breast, type of procedure, history of neo adjuvant therapy, details of gross characteristics such as tumour size, nodal status details were obtained for all the 274 mastectomy cases reported during the period from surgical pathology records. Freshly cut, hematoxylin & eosin stained 4 µ thick sections of the paraffin tissue blocks of mastectomy specimens were reviewed and graded using the Nottingham modification of the Scarff Bloom Richardson Grading system (Annexure III) and they were further evaluated for the presence of necrosis, lymphocytic response and lymphovascular invasion by tumour. 20 cases of each grade from Invasive ductal carcinoma NOS subtype were randomly selected from the total cases and their representative formalin fixed paraffin embedded tissue samples were subjected to p53 immunohistochemical analysis and to HoxD10 & E Cadherin gene analysis (using fresh tissue) by PCR. Due to economic constraints, CDH1 gene analysis was done only for 25 cases. The results were recorded with photographs.

IMMUNOHISTOCHEMICAL EVALUATION

Immuohistochemical analysis of p53 was done in paraffin embedded tissue samples using Supersentive polymer HRP system based on non biotin polymeric technology.

Antigen	**Vendor**	**Species**	**Dilution**	**Positive control**
P53	BIOGENEX	mouse	Ready to use	Breast

4 μ thick sections from selected formalin fixed paraffin embedded tissue samples were transferred onto gelatin coated slides. Heat induced antigen retrieval was done. The p53 antigen is bound with mouse monoclonal antibody (Biogenex) and then detected by the addition of secondary antibody conjugated with horse radish peroxidase-polymer and Diaminobenzidine substrate. The step by step procedure of Immunohistochemistry is given in Annexure IV.

INTERPRETATION & SCORING SYSTEM

p53 positivity can be seen in the nucleus of tumour cells. An estimation of >10% nuclei distinctly stained with anti p53 antibody was taken as positive.[89]

REAL TIME POLYMERASE CHAIN REACTION FOR HOXD10

ISOLATION OF TOTAL RNA

Sections of 10μ thickness were collected from all the formalin fixed paraffin embedded tissue samples. Total RNA from the samples were purified with RNeasy kit (Qiagen) and stored in collection tubes at -20ºC to -70ºC. (Step by step procedure given in Annexure V). The concentration of total RNA isolated from each sample was determined by measuring the absorbance at 260nm in a spectrophotometer. The volume of each sample containing 5ng of total RNA was calculated.

REAL TIME PCR AMPLIFICATION

The real time one step polymerase chain reaction is carried out with Rotor Gene Q system using Rotor gene SYBR green RT PCR kit. Normal breast tissues obtained from autopsy material were used as controls to study the relative gene expression in breast cancer. β actin 1 (ACTB1) is used as house keeping gene in this study. Samples were run in duplicates for both HoxD10 and ACTB1 gene.

To check the efficiency of the RT PCR a standard curve with log concentration obtained by dilution of control sample was generated for both HoxD10 and ACTB1 gene simultaneously. The concentration dilutions were 100ng, 10ng and 1 ng respectively.

HoxD10 gene and β actin gene were amplified using the extracted RNA as templates and the following forward and reverse primers.

HoxD10 forward (5′CCCTTACACCAAGCACCAAACG) primers,
HoxD10 reverse (5′CTCGGATCCTGGCCTCACATC) primers,
β actin forward (5'CCCCTGGCCAAGGTCATCCATGACAACTTT-3') primers&
β actin reverse (5'GGCCATGAGGTCCACCACCCTGTTGCTGTA-3') primers.

The PCR was carried out as follows,

- Thaw 2x Rotor gene SYBR green RT PCR master mix, RNA template, primer and RNAse free water to prevent premature complementary DNA synthesis.

- The reaction mix was prepared in PCR tubes as follows

    - 2xRotor gene SYBR green RT PCR master mix - 12.5 ml
    - Rotor gene RT mix - 0.25 ml
    - Primer for HoxD10/β actin - 2.5 ml
    - Template RNA of each sample with concentration of 5 ng
    - The total reaction volume was made into 25 µl with RNAse free water.

- The PCR tubes were placed in Rotor Gene cycler and the cycling conditions were programmed as follows

STEP	TEMPERATURE	TIME
Reverse transcription	55ºC	10 minutes
PCR Initial activation step	95ºC	5 minutes
One step PCR cycling x 40 cycles		
Denaturation	95ºC	5 seconds
Combined annealing/extension	60ºC	10 Seconds

- Melting curve analysis was performed at the end of reactions with temperature range of 55ºC to 95ºC to check the specificity of the reaction.

RELATIVE GENE EXPRESSION ANALYSIS

The HoxD10 RNA levels were calculated in the breast cancer samples and normal breast tissue in a relative quantification approach by using a reference gene ACTB1. Relative expression was calculated by deriving the delta delta CT values for HoxD10 with reference to ACTB1 gene expression. These values were generated automatically by Rotor Gene Q 2 Plex series software version 1.74. Relative concentration of HoxD10 RNA in tumour samples to that of control samples was calculated as a linear value from the equation $2^{-\Delta\Delta CT}$. These values were subjected to statistical analysis.

POLYMERASE CHAIN REACTION FOR E CADHERIN

SAMPLE COLLECTION

A small piece of the tumour tissue and the adjacent normal tissue were collected in sterilized vials containing saline.

DNA EXTRACTION

- DNA was extracted by phenol- chloroform method.
- The tissue was homogenized with lysis buffer.
- The digested mixture was then incubated with proteinase K enzyme. The mixture was then centrifuged.
- This was followed by phenol treatment. The mixture was again centrifuged.
- The aqueous layer was collected and treated with 10M Nacl. The mixture was again centrifuged and the supernatant was treated with ethanol and the DNA precipitated by centrifugation.

AMPLIFICATION OF E-CADHERINS

Primers used for PCR amplification of E-cadherin exons were as per the sequences described by G.Berx et al,1995.The amplification for each amplicon was optimized for annealing temperature and for MgCl2 concentration.The amplified PCR product was then subjected to agarose gel electrophoresis.

MUTATIONAL SCREENING

The amplified E-cadherin gene was then sequenced and compared with the sequence of DNA isolated from normal tissue for the presence of mutation.

STATISTICAL ANALYSIS

The statistical analysis was done using the statistical package for social science software version 11.5. Correlation between P53& E Cadherin expression and clinicopathological prognostic factors such as tumour size, nodal status, necrosis and lymphovascular invasion was analysed using Pearson chi square test.

The efficiency of the real time PCR experiment derived from standard curve was within a range of 0.82 and 0.86 for HoxD10 and ACTB1 reference genes respectively elucidating the validity of experiment. Standard curve from first set was used for the other set runs subsequently. Melt curve analysis showed minimized range of primer dimers with peaks at 75ºC.

The expression of HoxD10 gene was non parametric, so the correlation with other prognostic factors was done using Mann Whitney U test, student t test and Kruskal-Wallis Test.

OBSERVATION AND RESULTS

In the study period of 26 months from Jan 2010 to Feb 2012, a total of 21,566 specimens were received in the Institute of Pathology, Madras Medical College for histological examination.

Total numbers of breast specimens received were 1,301 cases, of these breast tumours accounted for 912 cases with a percentage of 4.23% of all cases (both incisional biopsies and mastectomy specimens).

The relative frequency of breast cancers among the specimen received was 2.04%.

The total number of non neoplastic, benign and malignant cases were 389, 472 and 440 respectively. Thus the distribution of non neoplastic breast lesions were 29.90%, benign tumours were 36.28% and of malignant tumours were 33.82%.

Out of a total of 440 breast cancer cases, only 274 cases constituted mastectomy specimens.

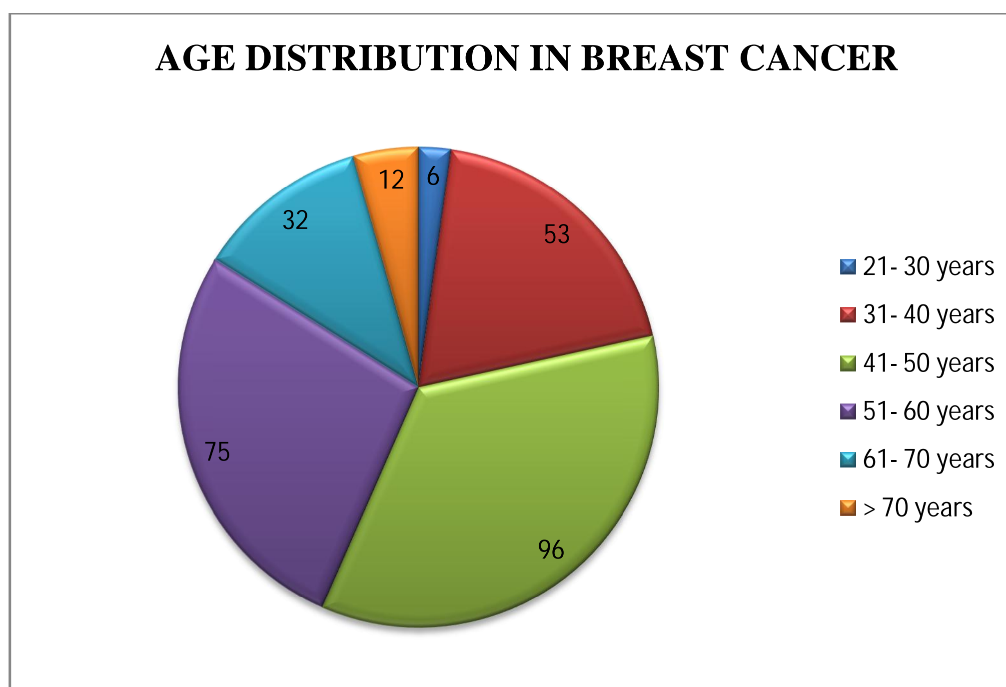
The age wise distribution of these 274 cases is given below (Table 1 and Chart 1)

Table 1: Age wise distribution of Breast Cancers

AGE GROUP	NUMBER OF CANCERS	PERCENTAGE
21- 30 years	6	2.20 %
31-40 years	53	19.3 %
41- 50 years	96	35 %
51- 60 years	75	27.4 %
61- 70 years	32	11.7 %
>70 years	12	4.4 %
Total cases	274	100 %

Breast cancers had the highest incidence in the 41-50 year age group.

Chart 1



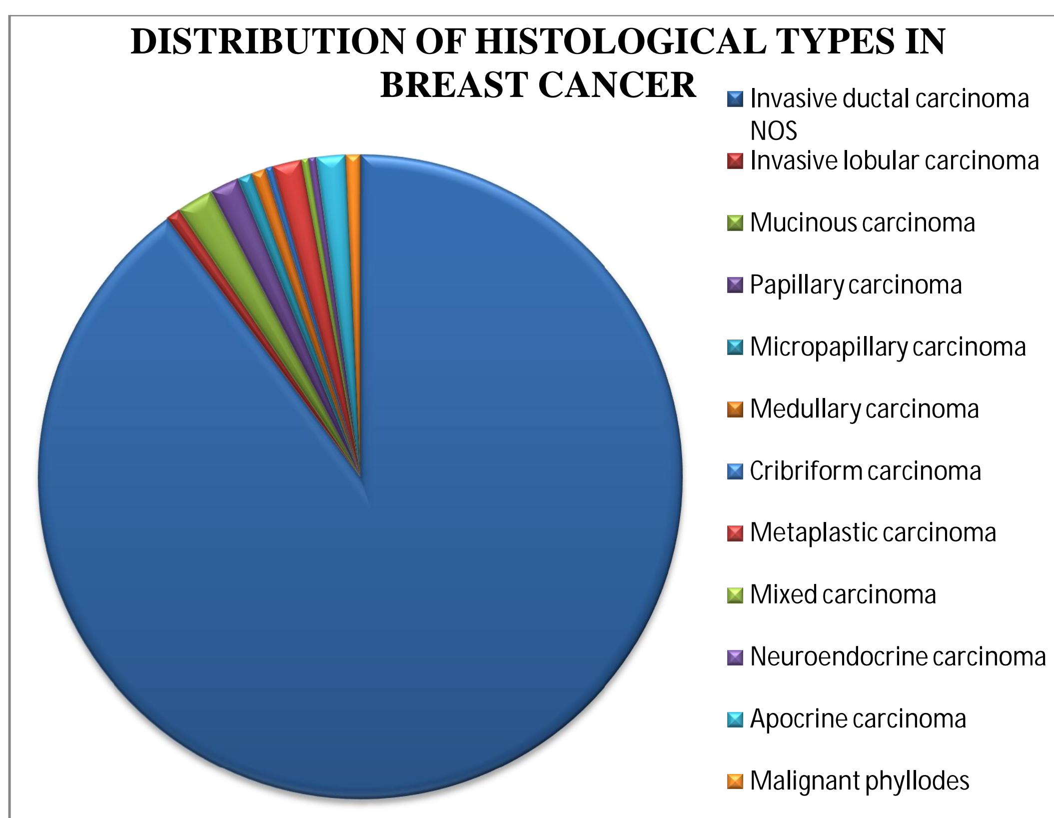
The youngest age of presentation of breast cancer is at 25 years in this study.

The distribution of histological subtypes of breast carcinoma is shown in Table 2 & Chart 2.

Table 2: Distributions of Histological Subtypes of Breast Cancers

S. NO	HISTOLOGICAL SUBTYPES	NO.OF CASES	PERCENTAGE
1	Invasive ductal carcinoma NOS	246	89.78 %
2	Invasive lobular carcinoma	2	0.73 %
3	Mucinous carcinoma	5	1.83 %
4	Papillary carcinoma	4	1.46 %
5	Micropapillary carcinoma	2	0.73 %
6	Medullary carcinoma	2	0.73 %
7	Cribriform carcinoma	1	0.37 %
8	Metaplastic carcinoma	4	1.46 %
9	Mixed carcinoma	1	0.37 %
10	Neuroendocrine carcinoma	1	0.37 %
11	Apocrine carcinoma	4	1.46 %
12	Malignant phyllodes	2	0.73 %
	Total number of cases	274	100 %

Chart 2



Among the 274 cases, 272 (99.3%) cases were reported in females and 2(0.7%) cases were reported in male breast. (Table 3 & Chart 3)

Table 3: Sex Distribution in Invasive Ductal Carcinoma

SEX	TOTAL NO. OF CASES	PERCENTAGE
Male	2	0.7 %
Female	272	99.3 %
Total	274	100 %

132 cases of Invasive ductal carcinoma were reported in left breast, 141 cases were reported in right breast and 1 case had cancer in both the breasts. (Table 4 and Chart 4)

Table 4: Distribution of side of involvement in Breast

SIDE	NO. OF CASES	PERCENTAGE
Left	132	48.1 %
Right	141	51.5 %
Both breasts	1	0.4 %
Total	274	100 %

CHART 3

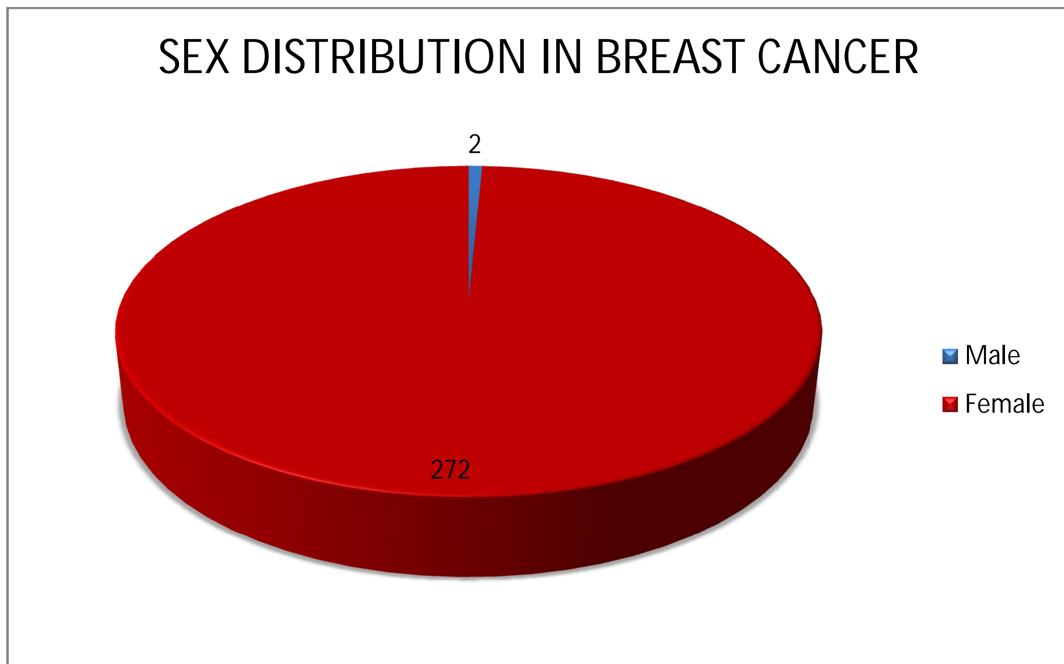
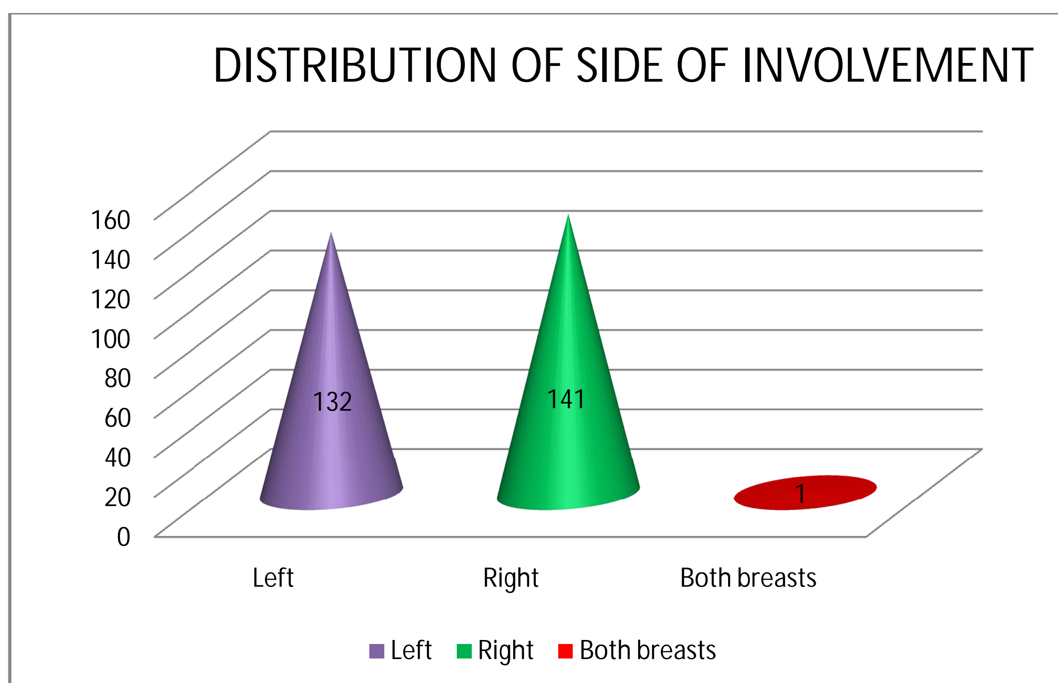


CHART 4



20 cases (7.3%) had tumour less than 2 cm in size, 189 cases (69%) were of 2 to 5 cm in size and 65 cases (23.7%) were more than 5 cm in size. (Table 5 & Chart 5).

Table 5: Distribution of size in Invasive Ductal Carcinoma

SIZE OF TUMOUR	NO. OF CASES	PERCENTAGE
<2cm(T1)	20	7.3 %
2-5cm(T2)	189	69 %
>5cm(T3)	65	23.7 %

There were 245 Invasive ductal carcinoma NOS type breast cancers in the study sample which were graded according to modified Scarff Bloom Richardson grading system out of which 77 cases(31.43%) were in grade I, 128 cases (52.24%) were in grade II and 40 cases (16.33%) were in grade III. (Table 6 & Chart 6)

Table 6: Distribution of Histological Grade in Invasive Ductal Carcinoma NOS Type

GRADE	NO. OF CASES	PERCENTAGE
Grade 1	77	31.43 %
Grade 2	128	52.24 %
Grade 3	40	16.33 %
TOTAL	245	100 %

CHART 5

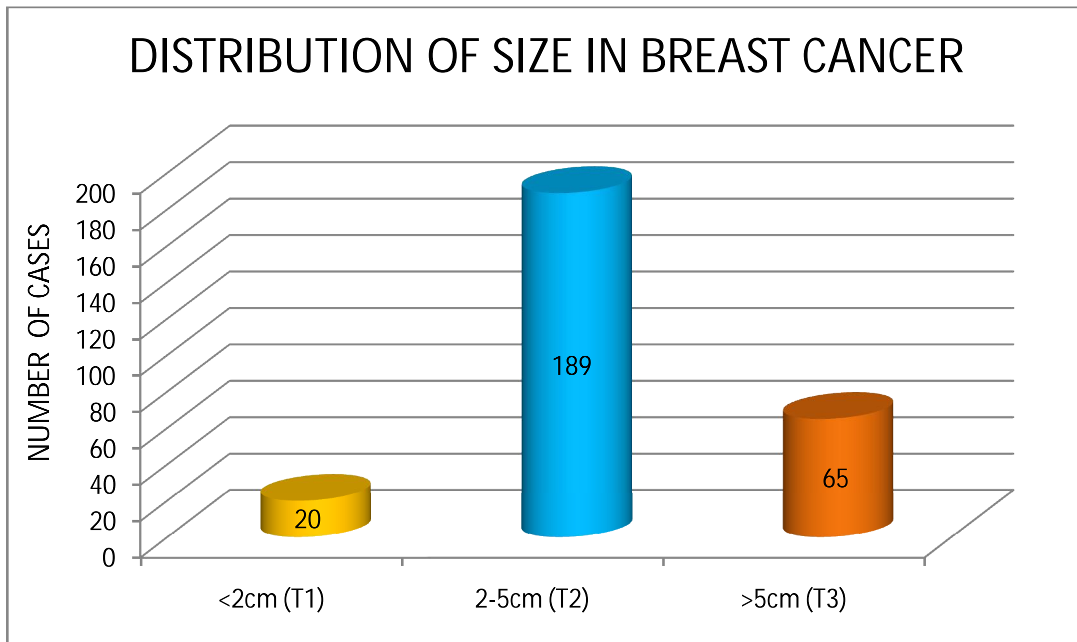
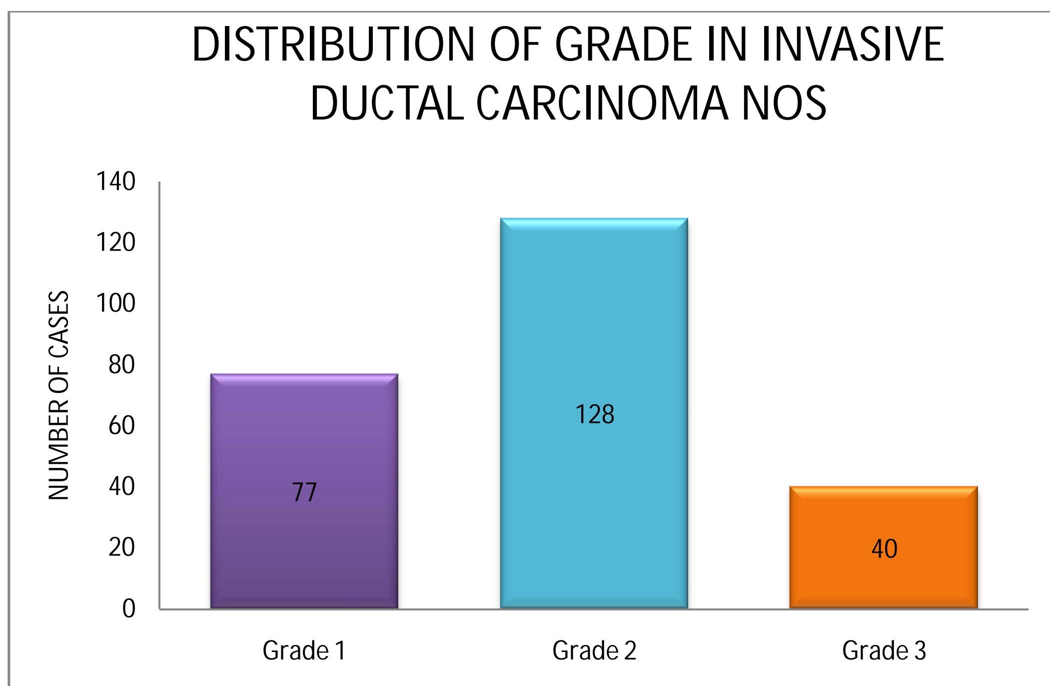


CHART 6



76 cases (27.7%) had up to 3 nodes with metastatic ductal carcinomatous deposit, 56 cases (20.4%) had 4 to 10 involved nodes, 12 cases (4.4%) had more than 10 involved nodes, while 130 cases (47.4%) had no lymph node involvement (Table 7 & Chart 7).

Table 7: Distribution of Lymph Node Metastasis in Breast Cancers

LYMPH NODE STATUS	NO OF CASES	PERCENTAGE
Negative	130	47.4 %
1-3 positive nodes	76	27.7 %
4-10 positive nodes	56	20.4 %
>10 positive nodes	12	4.4 %
Total	274	100 %

182 cases (66.4%) had lymphatic invasion as against 92 cases (33.6%) without lymphatic invasion (Table 8 & Chart 8).

Table 8: Distribution of lymphatic invasion in Invasive Ductal Carcinoma Breast

LYMPHATIC INVASION	NO OF CASES	PERCENTAGE
Present	182	66.4 %
Absent	92	33.6 %
Total	274	100 %

CHART 7

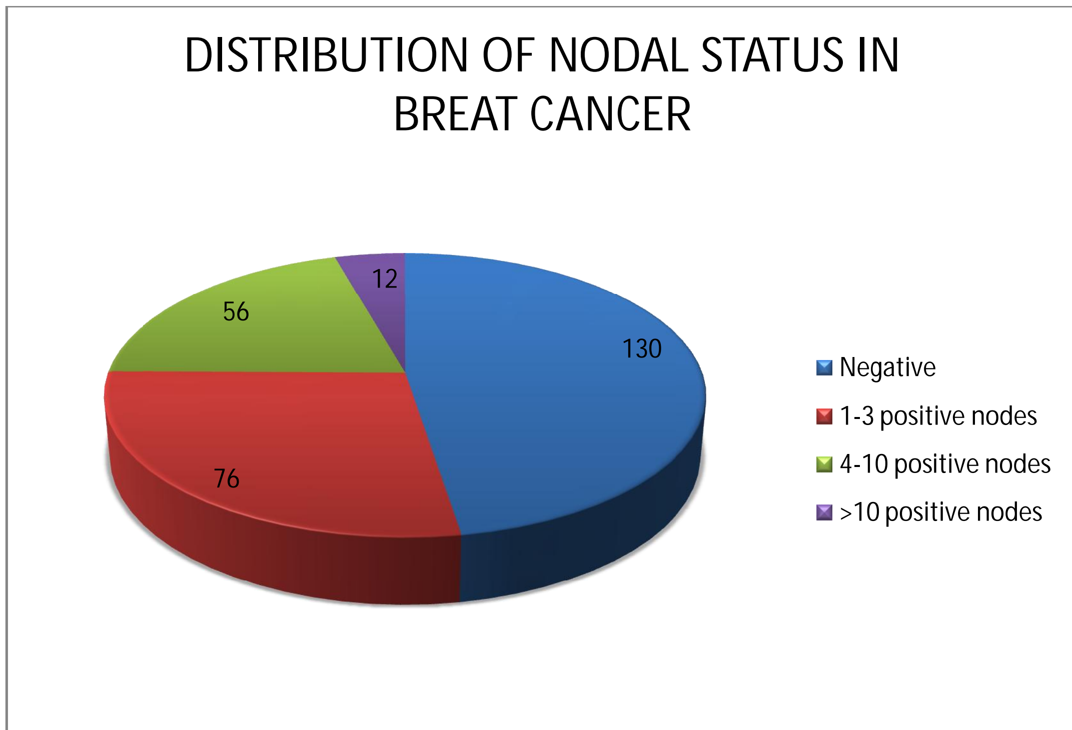
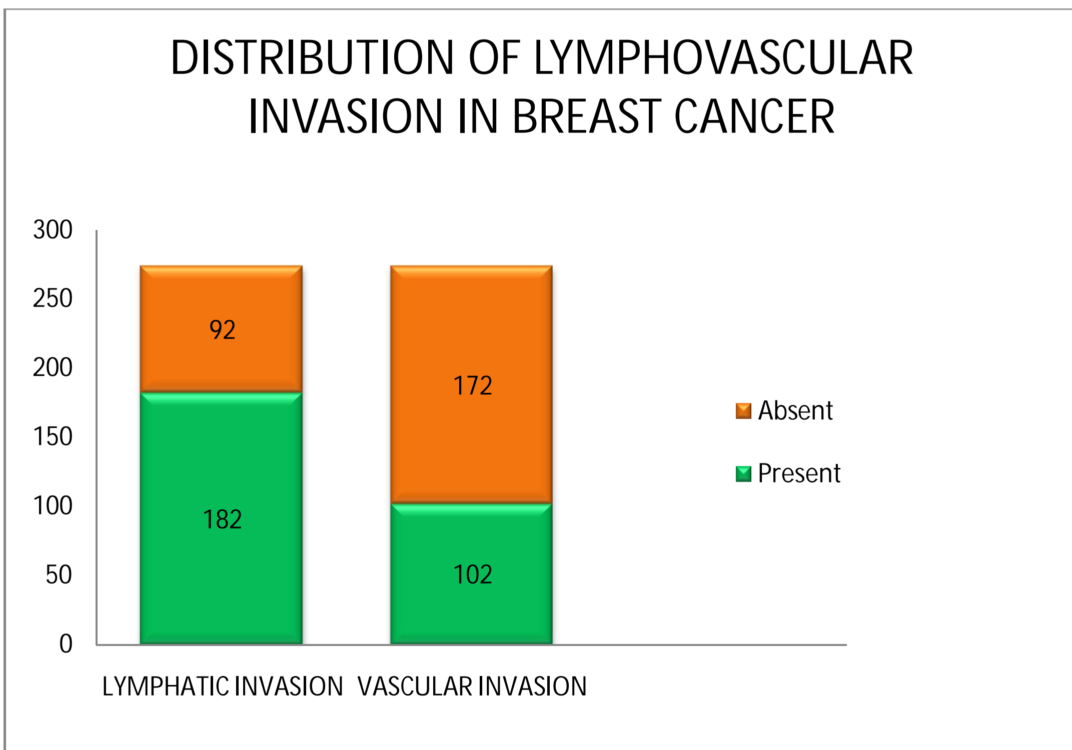


CHART 8



102 cases (37.2%) showed vascular invasion while 172 cases (78%) cases had no vascular invasion. (Table 9& Chart 8)

Table 9: Distribution of vascular invasion in Invasive Ductal Carcinoma Breast

VASCULAR INVASION	NO OF CASES	PERCENTAGE
Present	102	37.2 %
Absent	172	62.8 %
Total	274	100 %

19 % of the cases had skin infiltration (Table 10), 58.4% of the cases had lymphocytic infiltration (Table 11)& 27.4% of the cases had necrosis(Table 12)as shown in Chart 9.

Table 10: Distribution of skin infiltration in Invasive Ductal Carcinoma Breast

SKIN INFILTRATION	NO OF CASES	PERCENTAGE
Present	52	19 %
Absent	222	81 %
Total	274	100 %

Table 11: Distribution of lymphocytic infiltration in Invasive Ductal Carcinoma Breast

LYMPHOCYTIC INFILTRATION	NO OF CASES	PERCENTAGE
Present	160	58.4 %
Absent	114	41.6 %
Total	274	100 %

Table 12: Distribution of necrosis in Breast Cancer

NECROSIS	NO OF CASES	PERCENTAGE
Present	75	27.4 %
Absent	199	72.6 %
Total	274	100 %

RESULTS OF IMMUNOHISTOCHEMICAL AND MOLECULAR STUDIES

In this study, 71.7% expressed positive reaction for P53 (Table 13 & Chart 10)

Table 13: Distribution of P53 expression in Invasive Ductal Carcinoma NOS

PARAMETER	POSTIVE	NEGATIVE
P 53	43(71.7%)	17(28.3%)

CHART 9

DISTRIBUTION OF SKIN INFILTRATION, LYMPHOCYTIC INFILTRATION AND NECROSIS IN BREAST CANCER

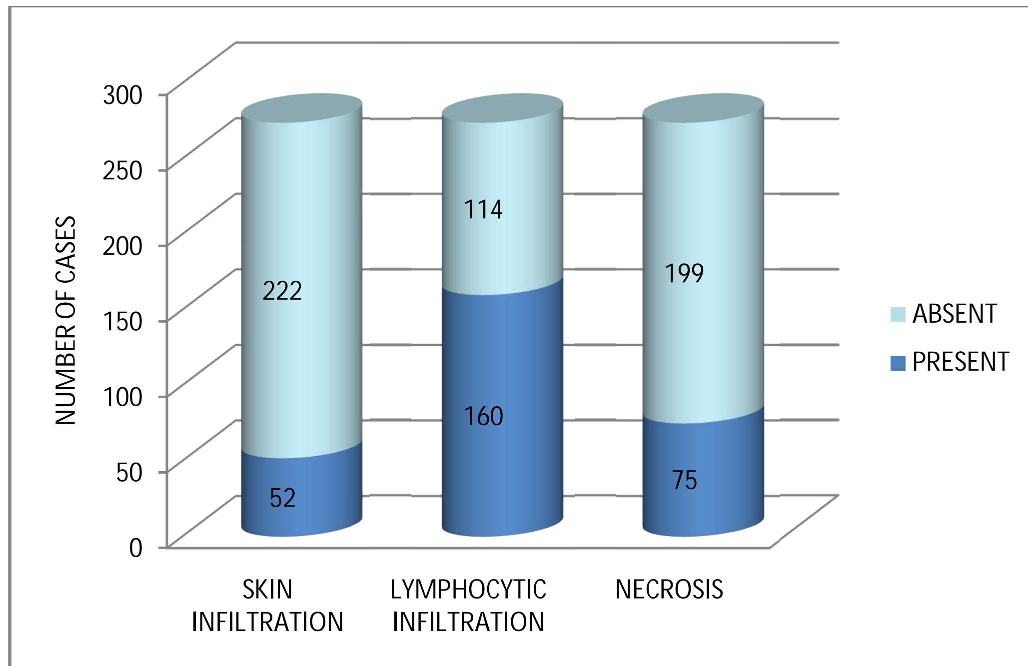
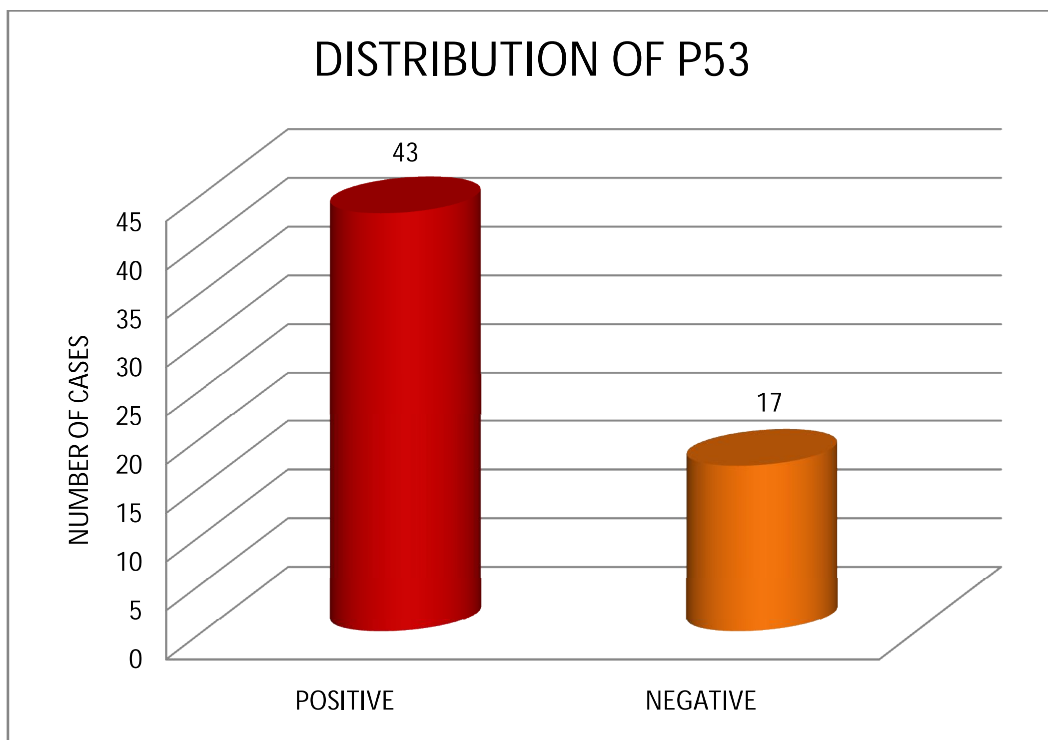


CHART 10



CORRELATION OF P53 WITH OTHER PROGNOSTIC FACTORS

P53 over expression was noted in 80% of premenopausal women and 63.33% of postmenopausal women. The correlation between menstrual status and p53 over expression was not significant. (Table 14& Chart 11)

Table 14: Correlation of menstrual status with P53 expression

Menstrual status	P53 positive (%)	P53 negative (%)	Total	Pearson chi square test
Premenopausal	24(80%)	6(20%)	30	P=0.15
Postmenopausal	19(63.33%)	11(36.67%)	30	

P53 expression was noted in 75% of T1 size tumors, 69.05% of T2 size tumors and 78.57% of T3 size tumors. No significant correlation was found between the tumor size and P53 over expression (Table 15& Chart 12).

Table 15: Correlation of tumor size and P53 expression

Average size	P53 positive (%)	P53 negative (%)	Total	Pearson chi square test
<2 cm (T1)	3(75%)	1(25%)	4	P=0.78
2 -5 cm (T2)	29(69.05%)	13(30.95%)	42	
>5 cm (T3)	11(78.57%)	3(21.43%)	14	

CHART 11

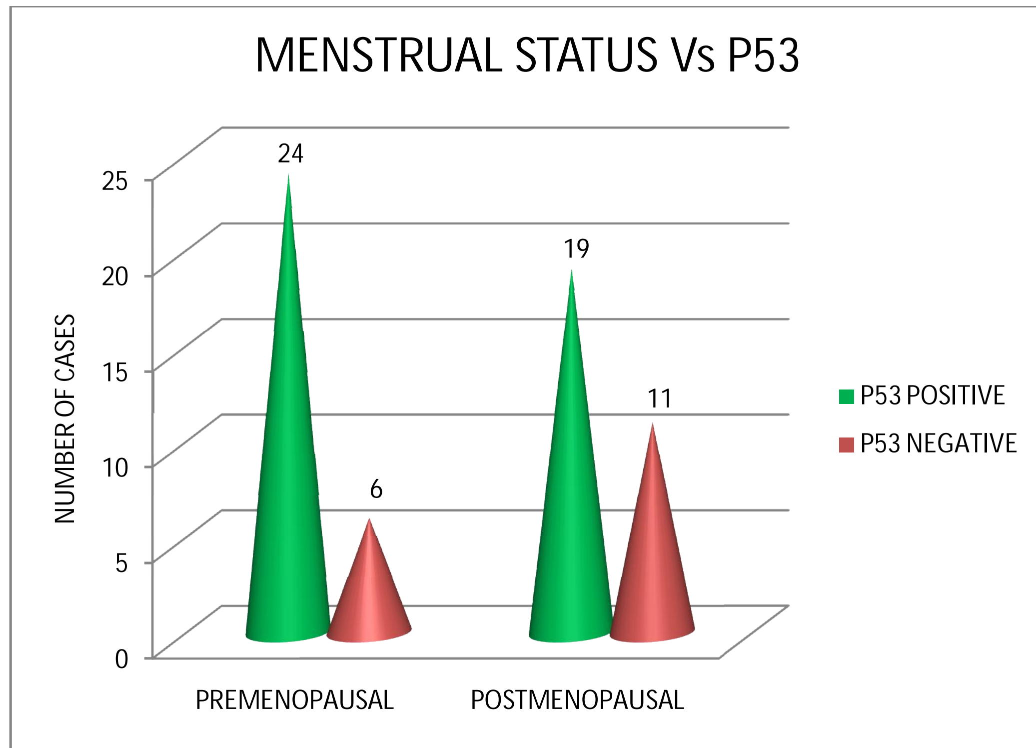
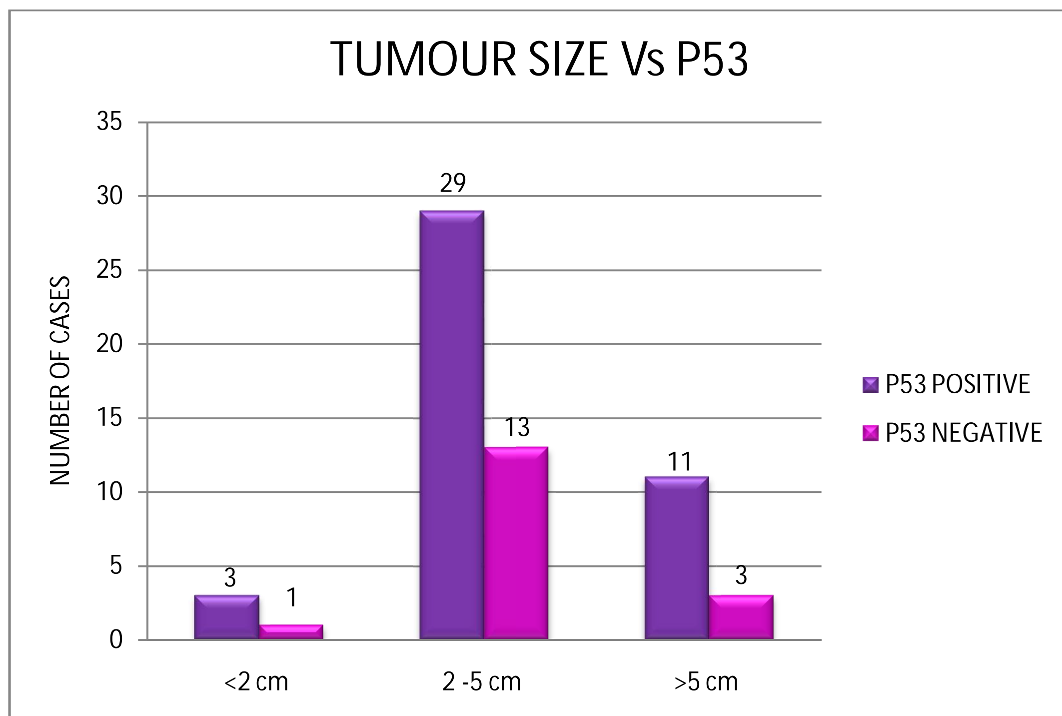


CHART 12



P53 expression was noted in 68.75% of nodal metastasis positive group as against 75% of nodal metastasis negative group. The correlation between nodal metastasis and P53 over expression was not significant. (Table 16 & Chart 13)

Table 16: Correlation of nodal metastasis and P53 expression

Nodal metastasis	**P53 positive (%)**	**P53 negative (%)**	**Total**	**Pearson chi square test**
Present	22(68.75%)	10(31.25%)	32	P=0.65
Absent	21(75%)	7(25%)	28	

65% of grade 1, 75% of grade 2 and 75% of grade 3 tumors were found to be positive for P53 expression (Table 17& Chart 14).Thus, there was an increase in the P53 expression with increasing grade of breast cancer and the association was statistically significant(p=0.025).

Table 17: Correlation of grade and P53 expression

Grade	**P53 positive (%)**	**P53 negative (%)**	**Pearson chi square test**
Grade 1	13(65%)	7(35%)	P = 0.18
Grade 2	15(75%)	5(25%)	P = 0.025
Grade 3	15(75%)	5(25%)	P = 0.025

CHART 13

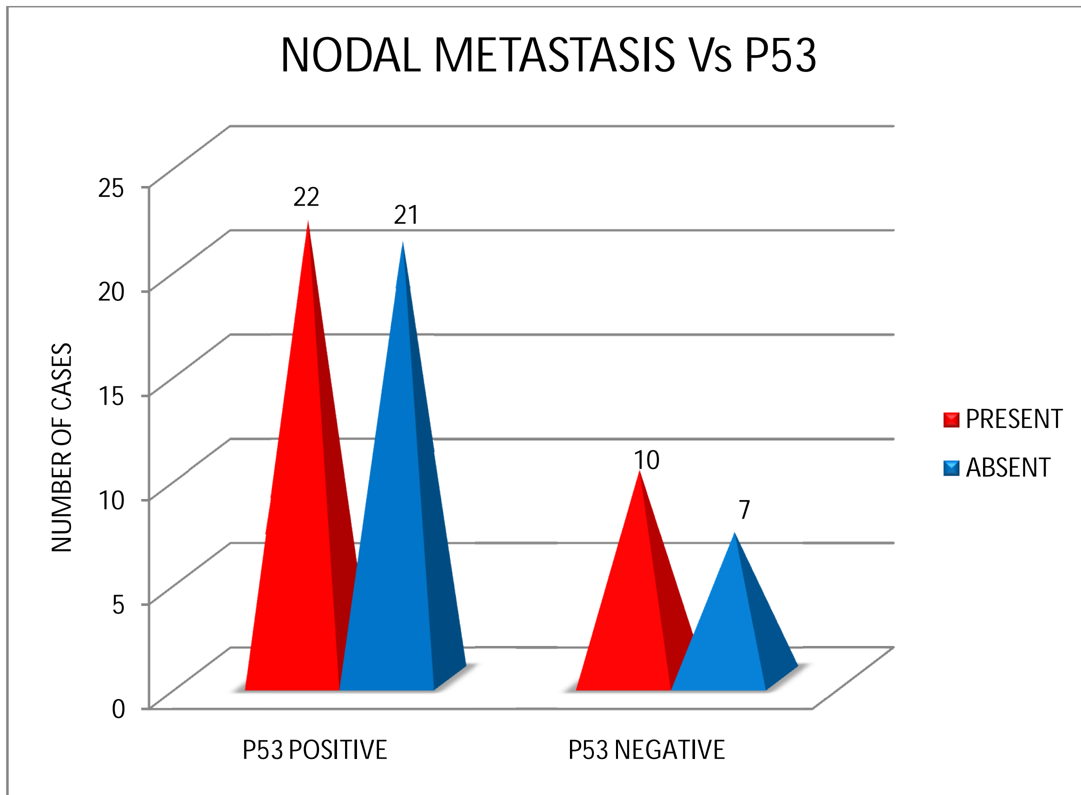


CHART 14

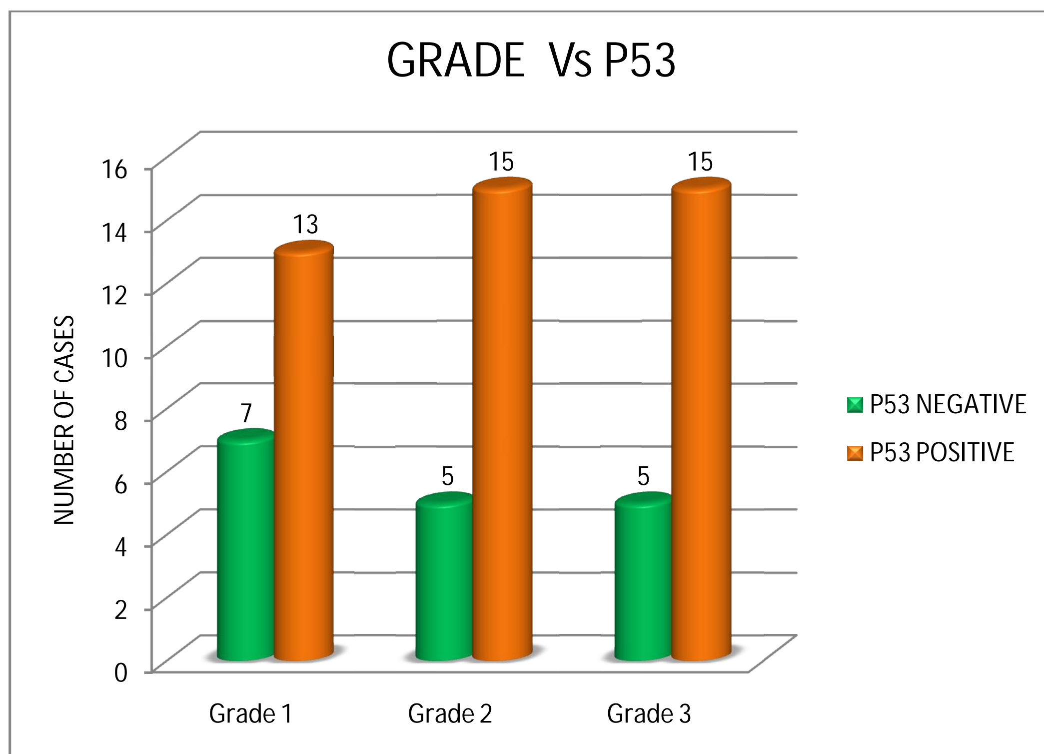


Table 18: Correlation of P53 with other histological prognostic factors

Patient characteristics		P53		Pearson chi square test
		Negative	Positive	
Skin infiltration	Present	4	9	P=0.82
	Absent	13	34	
Lymphatic invasion	Present	13	29	P=0.49
	Absent	4	14	
Vascular invasion	Present	7	22	P=0.48
	Absent	10	21	
Lymphocytic infiltration	Present	8	34	P=0.15
	Absent	9	9	
Necrosis	Present	7	11	P=0.23
	Absent	10	32	

No significant correlation was noted between p53 expression and other prognostic factors such as skin infiltration, lymphatic invasion, vascular invasion, lymphocytic infiltration and necrosis as shown in table 18.

EVALUATION OF E CADHERIN GENE MUTATION

Mutation of E Cadherin gene, CDH1was analyzed for 25 cases of invasive ductal carcinoma NOS by PCR and 60% of the cases showed mutation (Table 19 & Chart 15).

Table 19: Distribution of CDH1 gene mutation in Invasive Ductal Carcinoma NOS

PARAMETER	MUTATION POSITIVE	MUTATION NEGATIVE
CDH1	15(60%)	10(40%)

CORRELATION OF CDH1 GENE MUTATION WITH OTHER KNOWN PROGNOSTIC FACTORS

CDH1 gene mutation was noted in 56.25 % of premenopausal women and 66.67% of postmenopausal women. There was no significant correlation between the menstrual status and CDH1 gene mutation. (Table 20 & Chart 16)

Table 20: Correlation of menstrual status and CDH1 gene mutation

Menstrual status	CDH1 mutation positive (%)	CDH1 mutation negative (%)	Total	Pearson chi square test
Premenopausal	9(56.25%)	7(43.75%)	16	P=0.61
Postmenopausal	6(66.67%)	3(33.33%)	9	

CHART 15

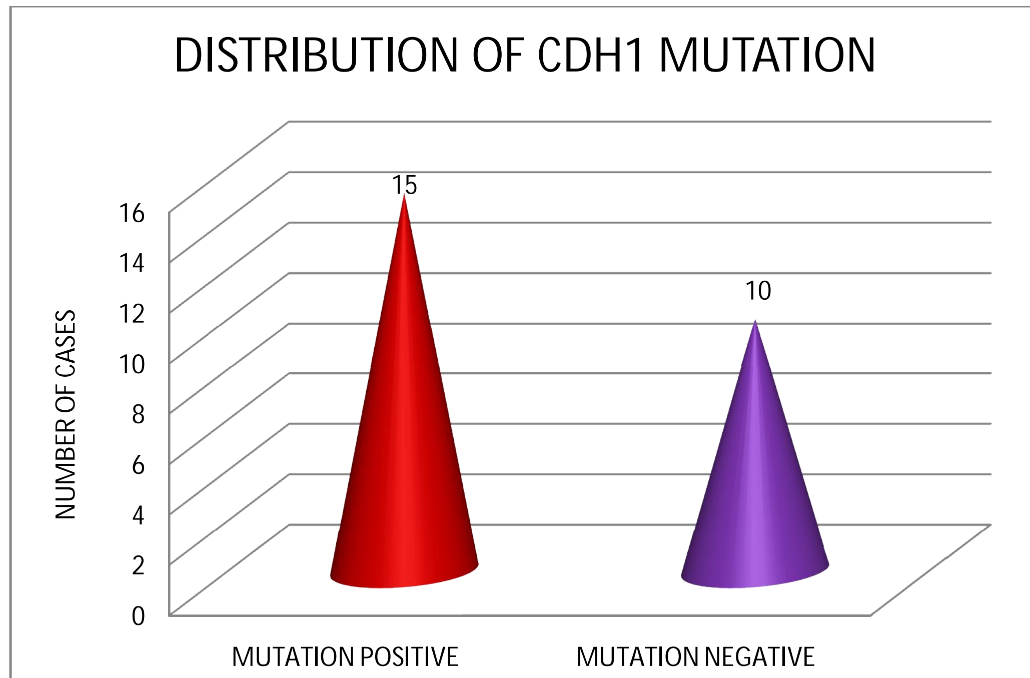
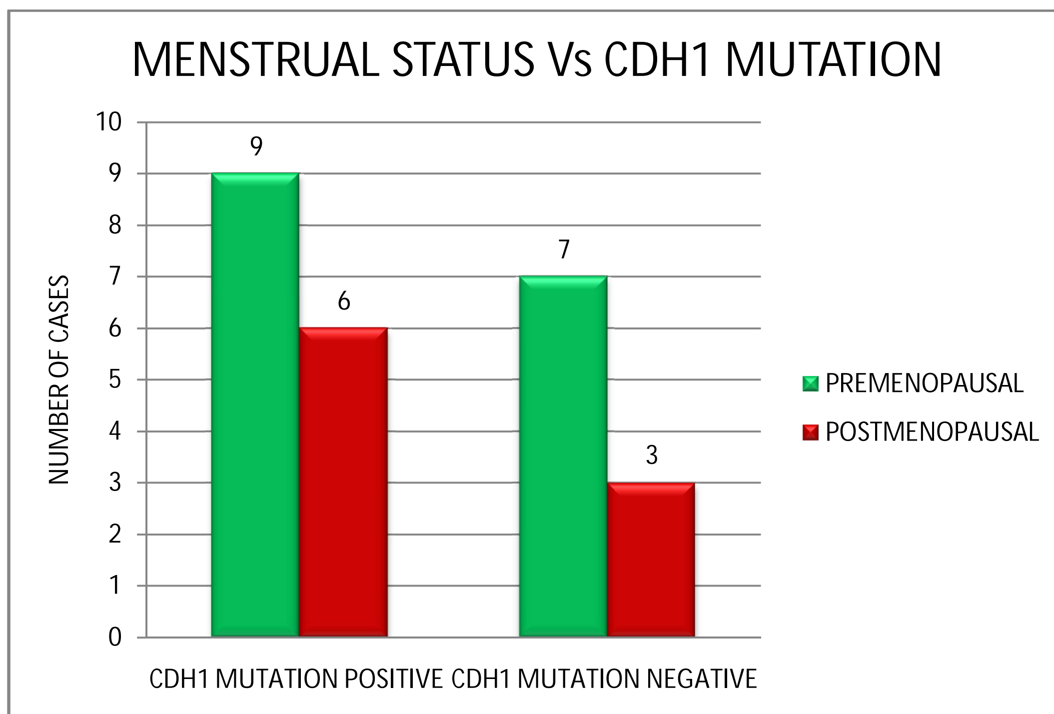


CHART 16



CDH1gene mutation was noted in 50% of T1 size tumors, 52.95% of T2 size tumors and 83.33 % of T3 size tumours. There was no significant correlation between the tumour size and CDH1gene mutation. (Table 21 & Chart 17)

Table 21: Correlation of tumour size and CDH1 gene mutation

Average size	CDH1 mutation positive (%)	CDH1 mutation negative (%)	Total	Pearson chi square test
<2 cm (T1)	1(50%)	1(50%)	2	P=0.40
2 -5 cm (T2)	9(52.95%)	8(47.05%)	17	
>5 cm (T3)	5(83.33%)	1(16.67%)	6	

CDH1 gene mutation was noted in 66.67% of nodal metastasis positive group as against 50% of nodal metastasis negative group. Thus, there was an increase in the CDH1 gene mutation among the nodal metastasis positive group but no significant correlation was found in statistical analysis. (Table 22 & Chart 18)

Table 22: Correlation of nodal metastasis and CDH1 gene mutation

Nodal metastasis	CDH1 mutation positive (%)	CDH1 mutation negative (%)	Total	Pearson chi square test
Present	10(66.67%)	5(33.33%)	15	P=0.49
Absent	5(50%)	5(50%)	10	

CHART 17

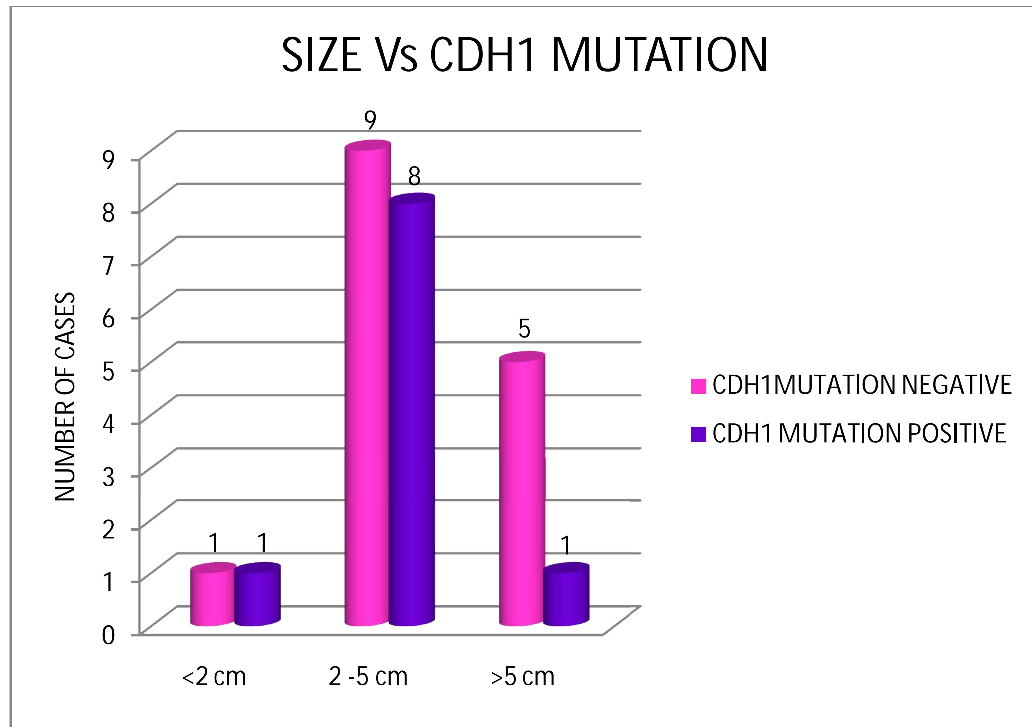
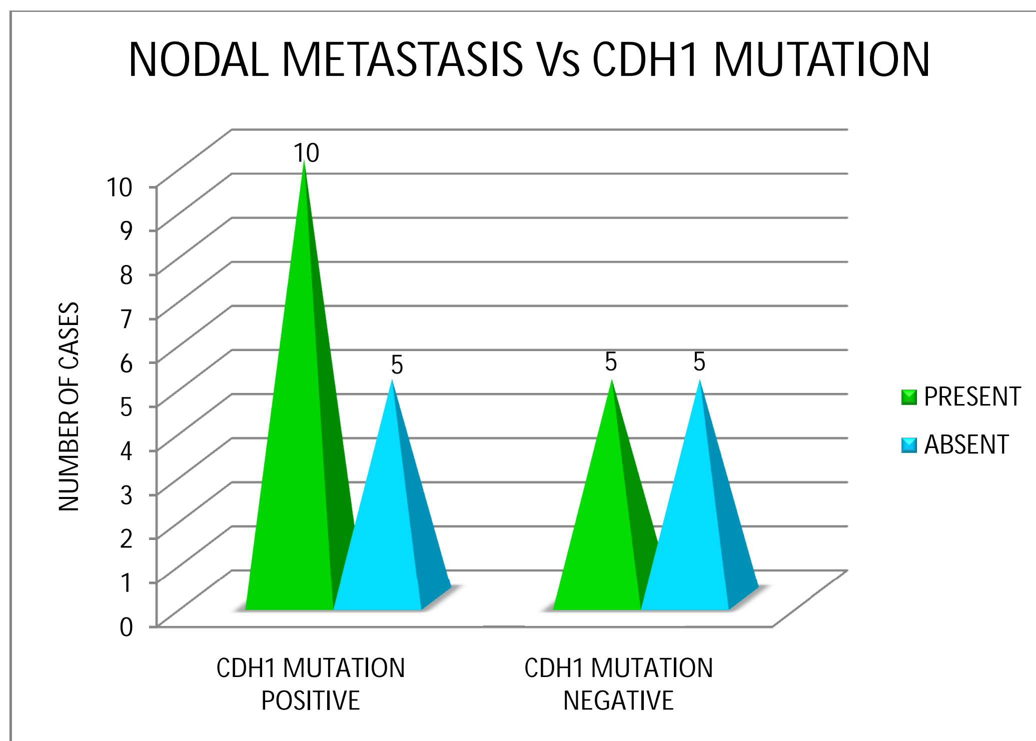


CHART 18



50% of grade I, 53.3% of grade II and 83.3% of grade III tumours were found to be positive for CDH1 gene mutation (Table 23 & Chart 19). Thus, there was an increase in the mutation in higher grade tumours but the association was not found to be statistically significant.

Table 23: Correlation of grade and CDH1 gene mutation

Grade	CDH1 mutation positive (%)	CDH1 mutation negative (%)	Pearson chi square test
Grade 1	2(50%)	2(50%)	P=0.40
Grade 2	8(53.3%)	7(46.67%)	
Grade 3	5(83.3%)	1(16.67%)	

CHART 19

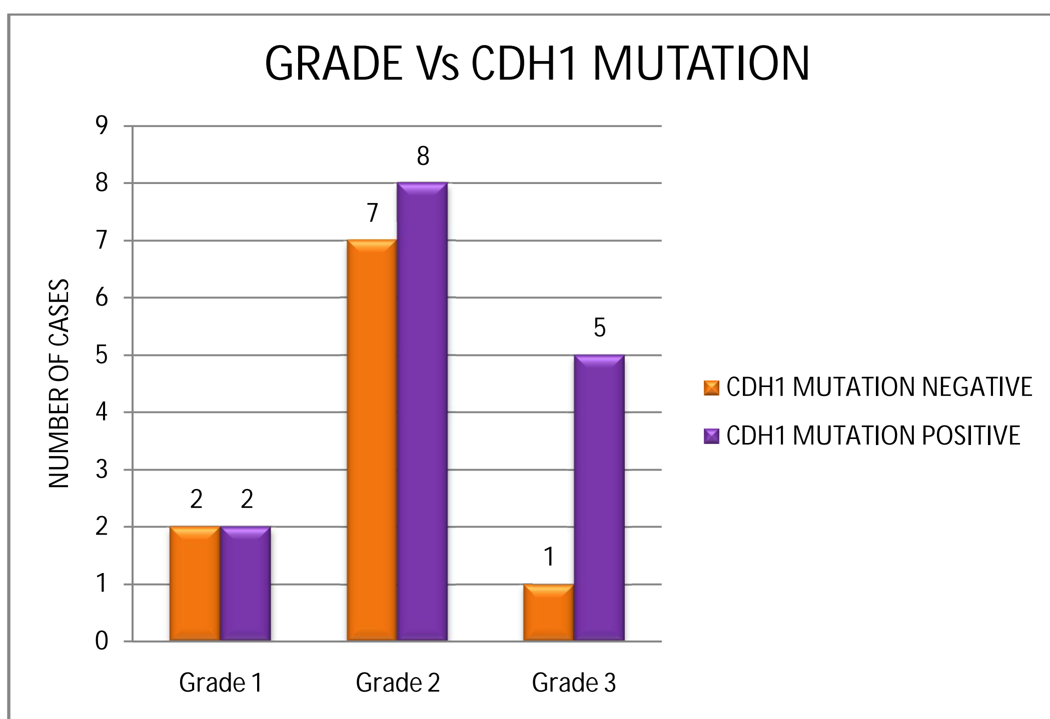


Table 24: Correlation of CDH1 gene mutation with other histological prognostic factors

Patient characteristics		CDH1 Mutation		Pearson chi square test
		Negative	Positive	
Skin infiltration	Present	2	3	P=1.00
	Absent	8	12	
Lymphatic invasion	Present	8	12	P=1.00
	Absent	2	3	
Vascular invasion	Present	7	10	P=0.86
	Absent	3	5	
Lymphocytic infiltration	Present	10	12	P=0.13
	Absent	0	3	
Necrosis	Present	1	3	P=0.50
	Absent	9	12	

There was no statistically significant association noted between CDH1 gene mutation and other prognostic factors such as skin infiltration, lymphatic invasion, vascular invasion, lymphocytic infiltration and necrosis as shown in Table 24.

CORRELATION OF HOX D10 GENE EXPRESSION WITH OTHER KNOWN PROGNOSTIC FACTORS

HoxD10 gene was downregulated in 46.67% of the cases. Table 25 and Chart 20 shows increased mean relative concentration of HoxD10 gene in premenopausal women as opposed to lesser relative concentration in postmenopausal women. The correlation was not found to be statistically significant.

Table 25: Correlation of menstrual status and HoxD10 gene expression

Menstrual status	N	Mean relative concentration of HoxD10 mRNA	Standard Deviation	T Test
Premenopausal	30	3.28	4.19	P=0.87
Postmenopausal	30	3.08	5.67	

The mean relative concentration of HoxD10 mRNA was higher in small size tumors when compared to large size tumors. The correlation was not found to be statistically significant (Table 26 & Chart 21).

Table 26: Correlation of tumor size and HoxD10 gene expression

Size	N	Mean relative concentration of HoxD10 mRNA	Standard Deviation	Kruskal Wallis test
<2 cm	4	2.538	2.927	P=0.145
2 to 5 cm	42	3.780	5.552	
>5 cm	14	1.596	2.813	

CHART 20

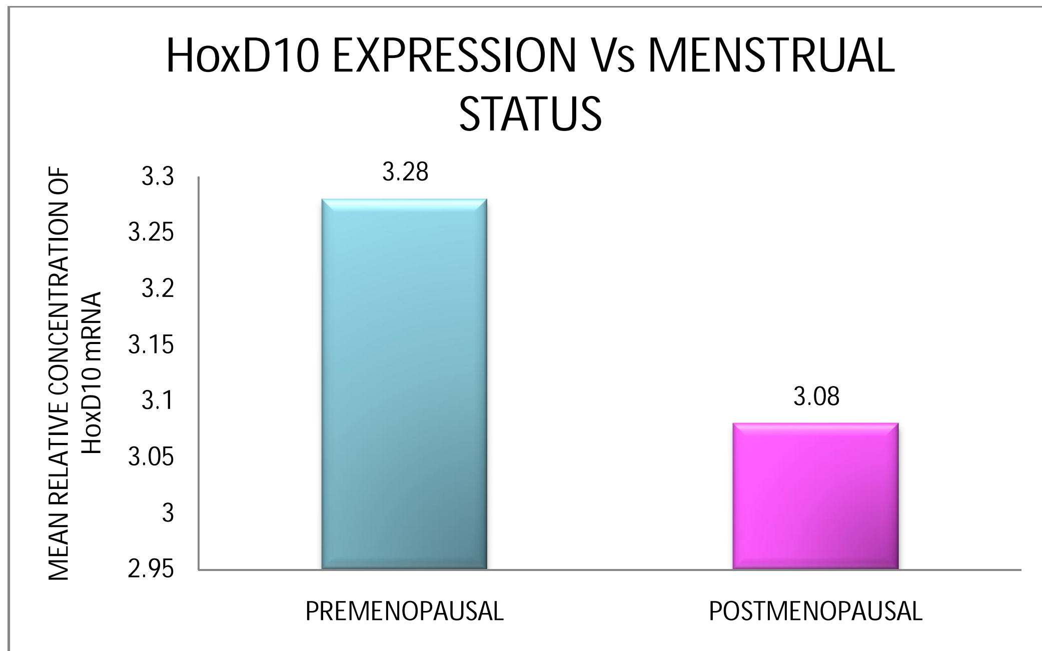
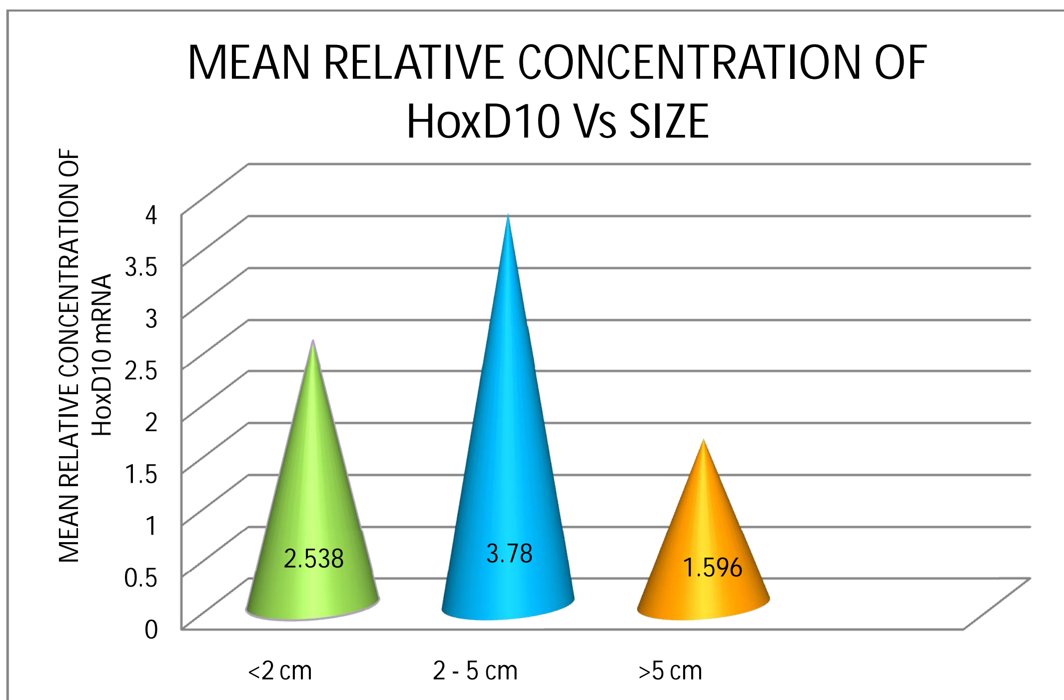


CHART 21



The relative expression of HoxD10 mRNA was higher in patients without axillary node metastasis but the correlation was not statistically significant (Table 27& Chart 22).

Table 27: Correlation of nodal metastasis and HoxD10 gene expression

<table>
<tr><th>Lymph node metastasis</th><th>N</th><th>Mean relative concentration of HoxD10 mRNA</th><th>Standard Deviation</th><th>Mann whitney U test</th></tr>
<tr><td>Absent</td><td>28</td><td>3.491</td><td>4.953</td><td rowspan="2">P=0.415</td></tr>
<tr><td>Present</td><td>32</td><td>2.923</td><td>5.007</td></tr>
</table>

HoxD10 mRNA was found to be markedly downregulated in grade 3 tumors when compared to the other grades and the correlation was statistically significant. (P=0.00) (Table 28& Chart 23)

Table 28: Correlation of grade and HoxD10 gene expression

<table>
<tr><th>Grade</th><th>N</th><th>Mean relative concentration of HoxD10 mRNA</th><th>SD</th><th>Kruskal Wallis test</th></tr>
<tr><td>1</td><td>20</td><td>3.368</td><td>5.697</td><td rowspan="3">P=0.00</td></tr>
<tr><td>2</td><td>20</td><td>6.141</td><td>4.898</td></tr>
<tr><td>3</td><td>20</td><td>0.054</td><td>0.058</td></tr>
</table>

CHART 22

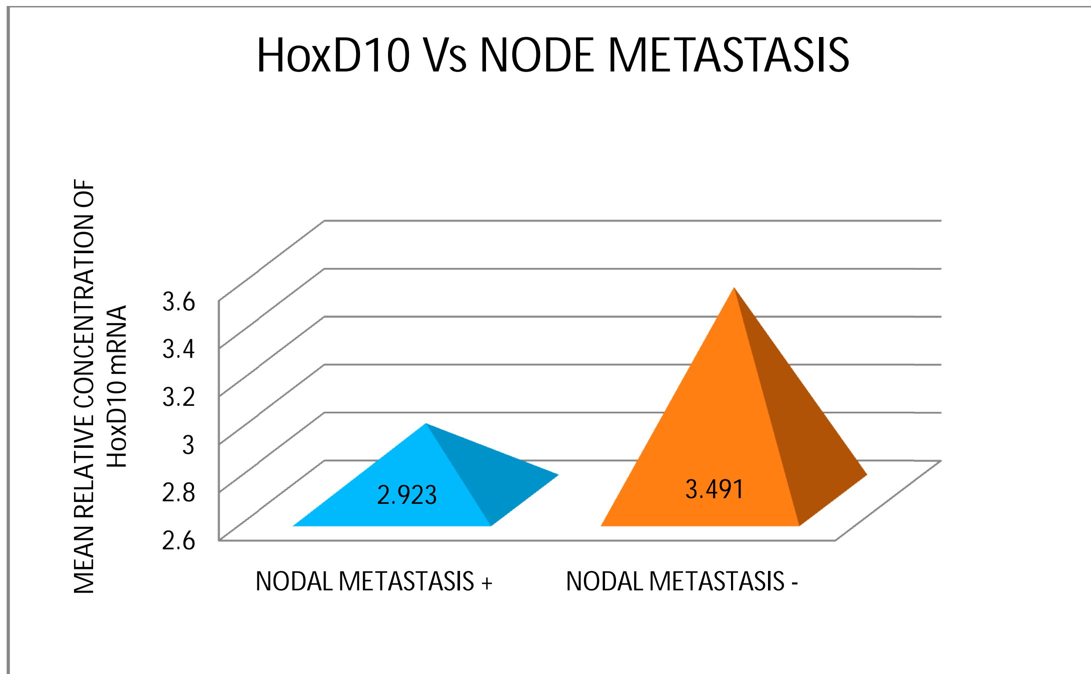
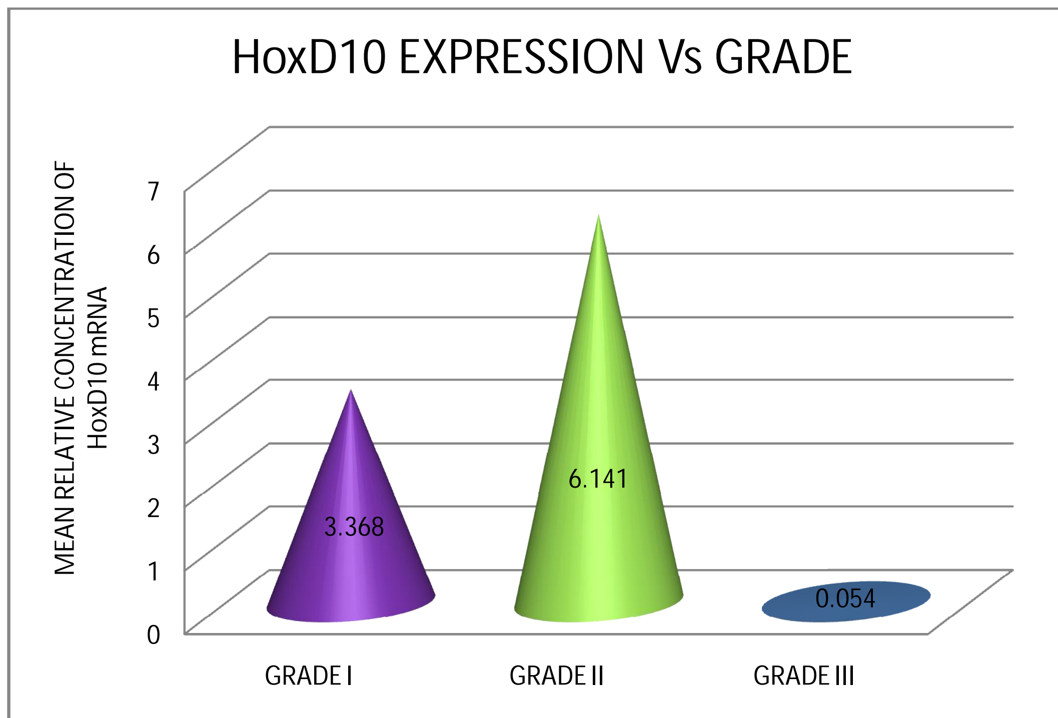


CHART 23



No significant association was present between the HoxD10 gene concentration and other prognostic factors such as skin infiltration, lymphatic invasion, vascular invasion, lymphocytic infiltration and necrosis (Table 29).

Table 29: Correlation of HoxD10 and other histological prognostic factors

Tumour characteristics		N	Mean	SD	Mann whitney U test
Skin infiltration	Absent	47	3.24	4.77	P=0.26
	Present	13	2.97	5.72	
Lymphatic invasion	Absent	18	3.98	5.45	P=0.24
	Present	42	2.84	4.74	
Vascular invasion	Absent	31	3.01	4.37	P=0.67
	Present	29	3.36	5.56	
Lymphocytic infiltration	Absent	18	2.86	4.39	P=0.94
	Present	42	3.32	5.21	
Necrosis	Absent	42	3.36	5.02	P=0.37
	Present	18	2.77	4.87	

CORRELATION BETWEEN P53 STATUS, CDH1 GENE MUTATION & HOXD10 GENE EXPRESSION

The mean relative concentration of HoxD10 mRNA was higher in P53 negative group when compared to P53 positive group (table 30& Chart 24). However, the correlation was not statistically significant.

Table 30: Correlation between P53 and HoxD10 gene expression

P53	N	Mean relative concentration of HoxD10 mRNA	SD	Mann whitney U test
Positive	43	3.10	4.95	P=0.49
Negative	17	3.39	5.07	

CDH1 gene mutation was found in 63.64% of p53 positive cases and 33.33% of p53 negative cases (table 31& Chart 25). There was no statistically significant association noted between CDH1 gene mutation and p53 expression.

Table 31: Correlation of P53 and CDH1 gene mutation

	CDH1 mutation positive(%)	CDH1 mutation negative(%)	Pearson chi square test
P53 positive	14(63.64%)	8(36.36%)	P=0.31
P53 negative	1(33.33%)	2(66.67%)	

CHART 24

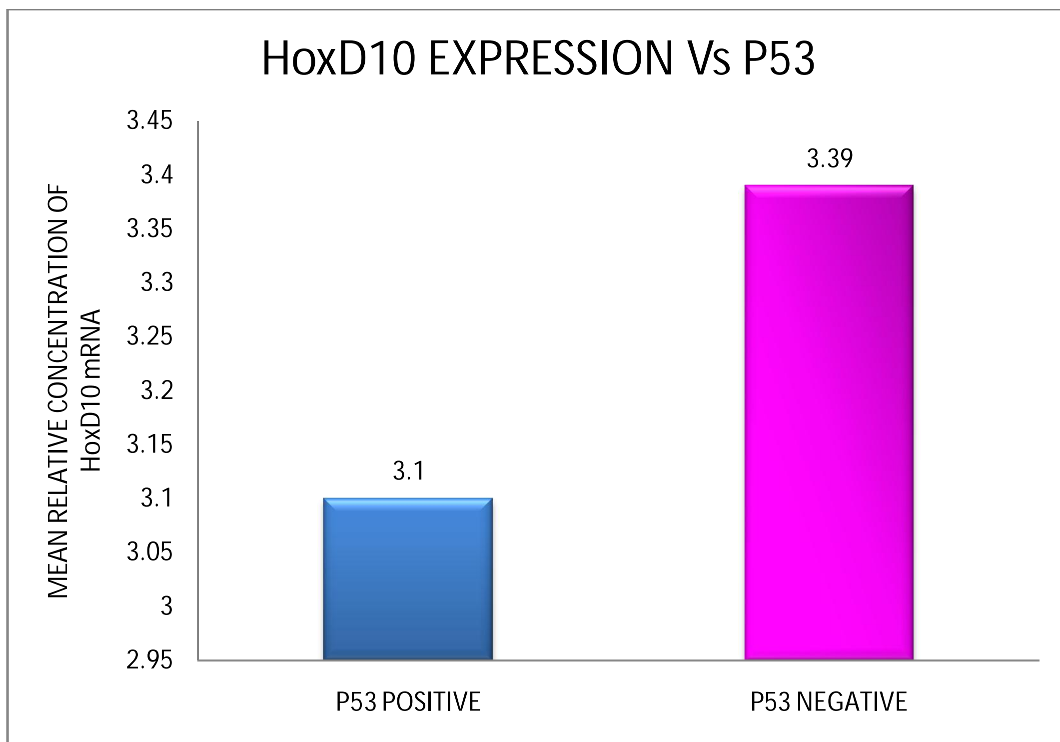
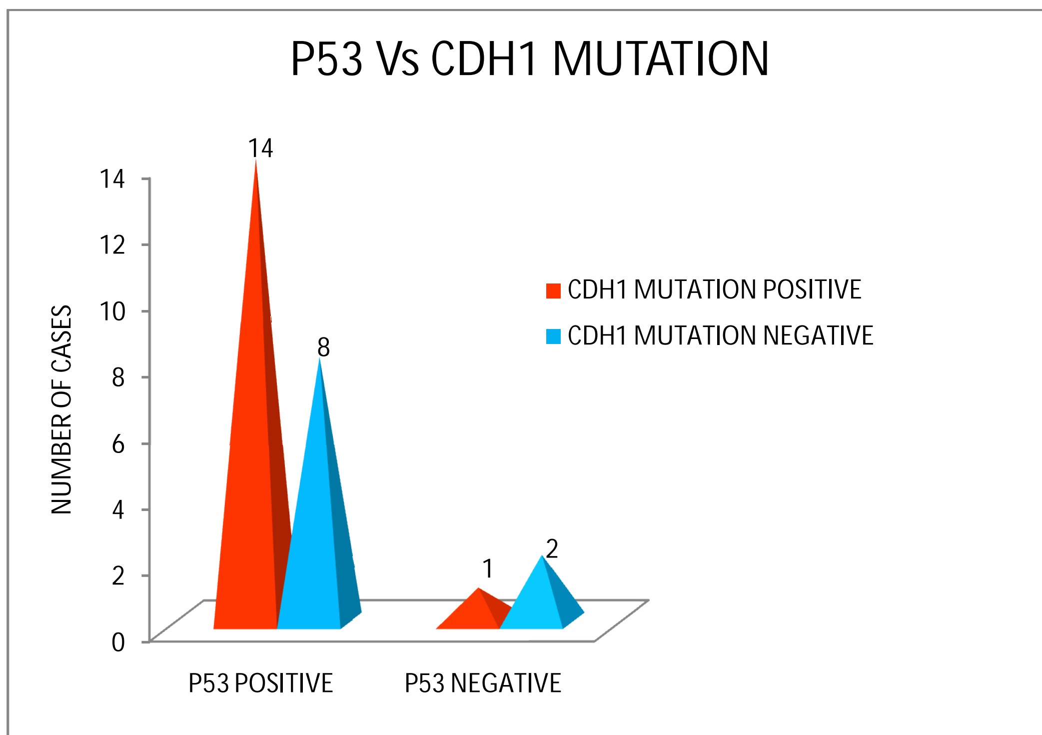


CHART 25

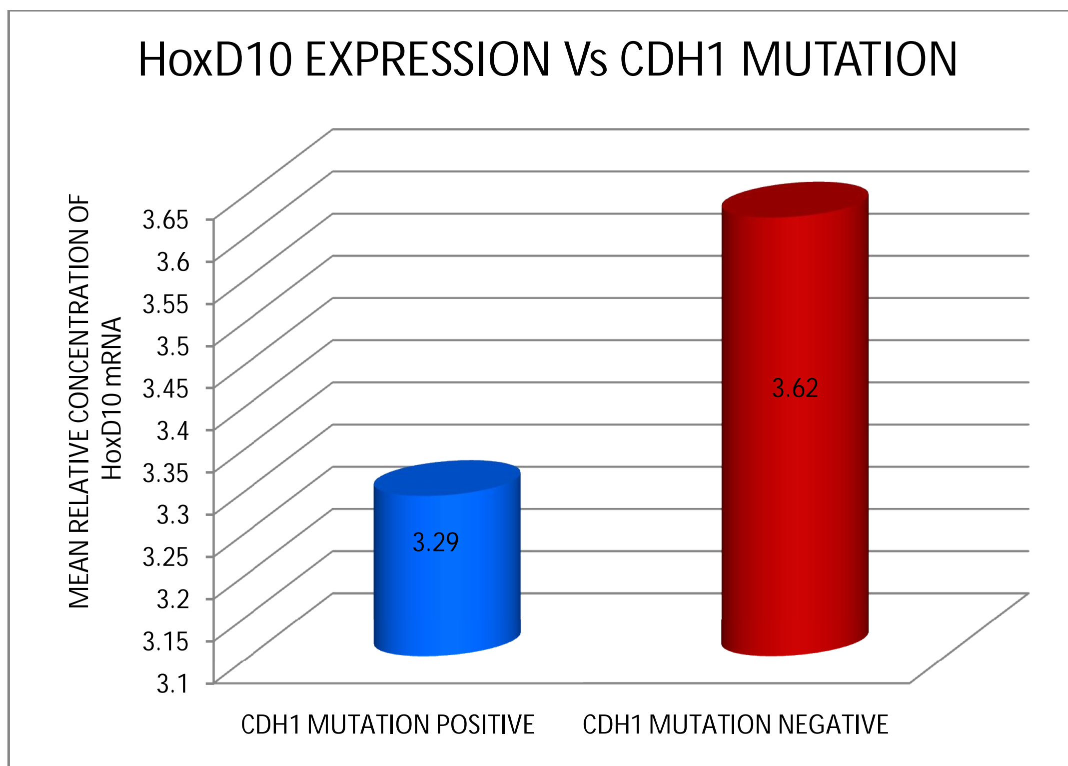


The mean relative concentration of HoxD10 mRNA was higher in the CDHI gene mutation negative group (table 32& Chart 26). The correlation was not statistically significant.

Table 32: Correlation between HoxD10 gene expression and CDH1 gene mutation

CDHI mutation	N	Mean relative concentration of HoxD10 mRNA	SD	Mann whitney U test
Positive	15	3.29	3.75	P=0.64
Negative	10	3.62	4.39	

CHART 26



DUCTAL CARCINOMA BREAST

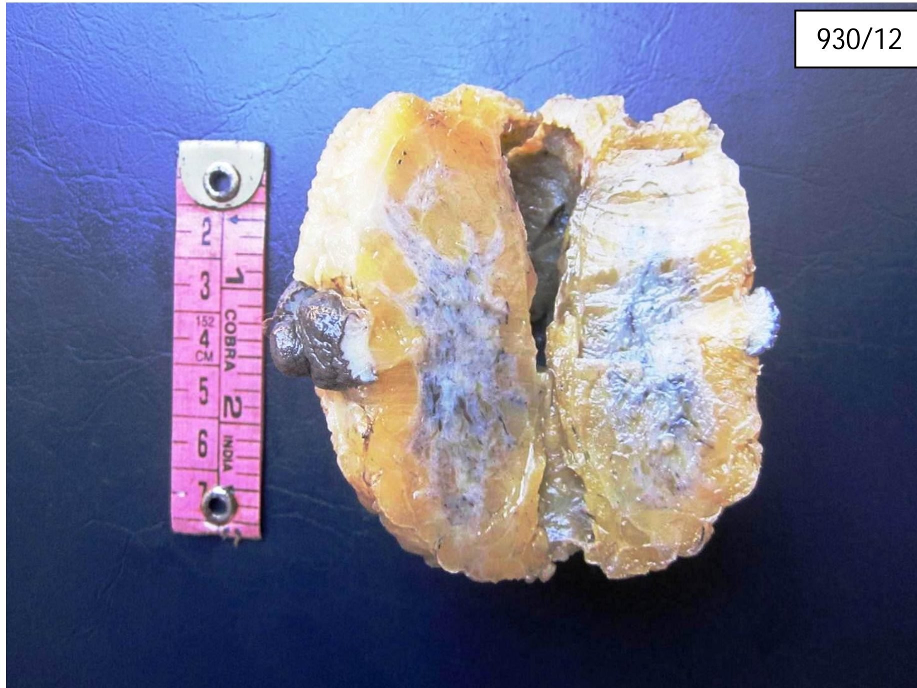


Figure 3: Grey white firm mass with irregular margins

MUCINOUS CARCINOMA

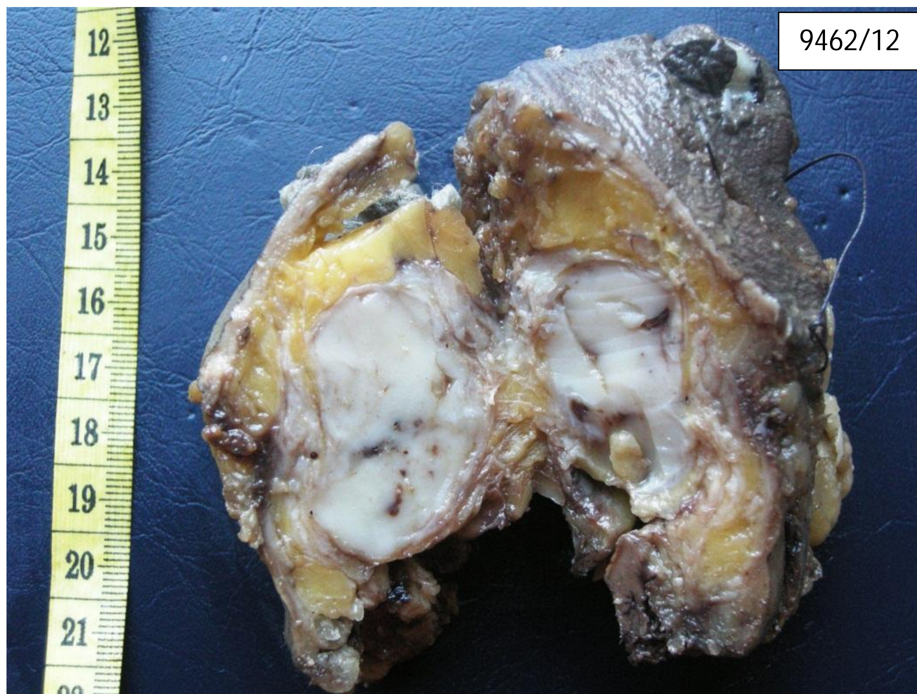


Figure 4: Well circumscribed glistening gelatinous mass

PAPILLARY CARCINOMA

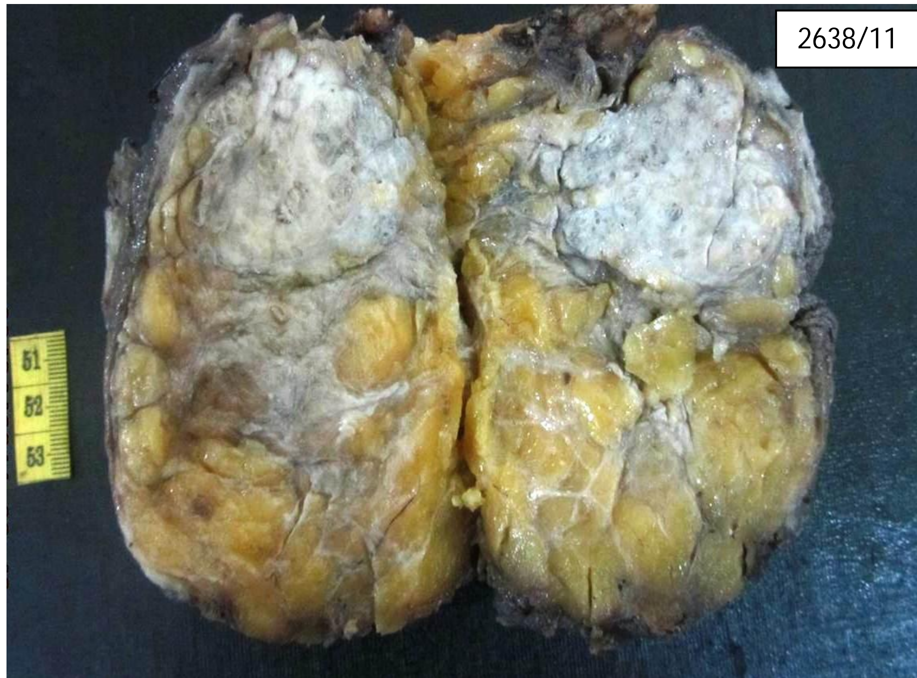


Figure 5: Well circumscribed grey white mass with granular surface

APOCRINE CARCINOMA

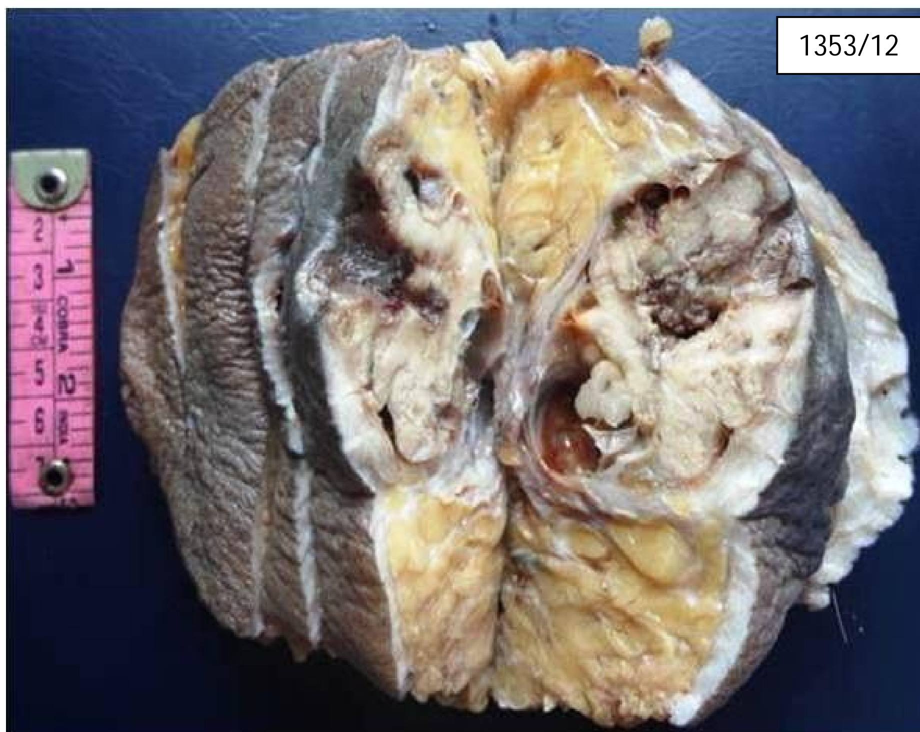


Figure 6: Well circumscribed grey white mass with cystic degeneration and hemorrhage

MEDULLARY CARCINOMA

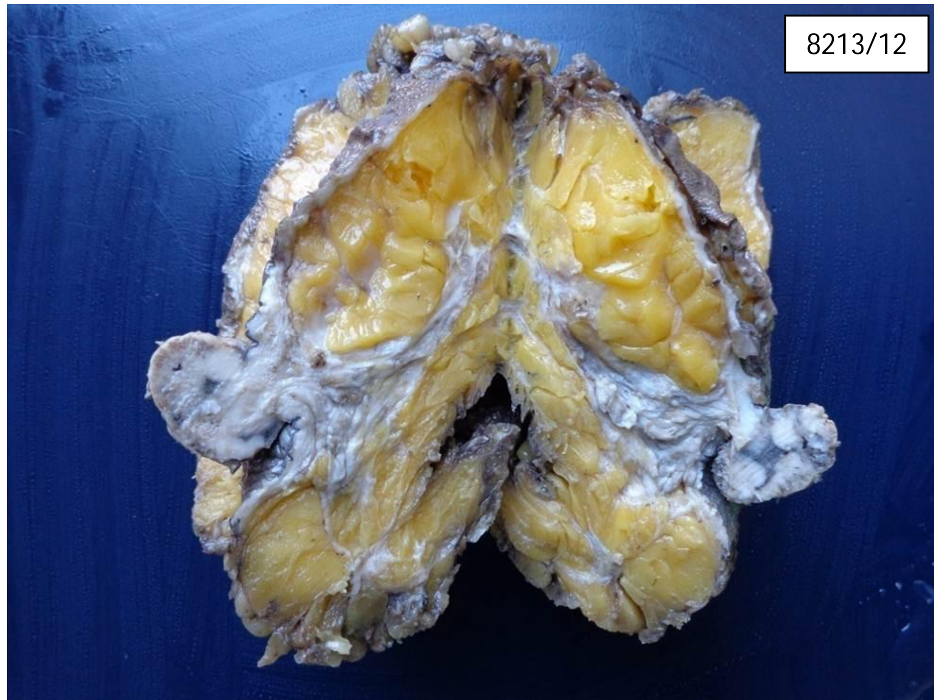


Figure 7: Well circumscribed grey white fleshy mass

METAPLASTIC CARCINOMA

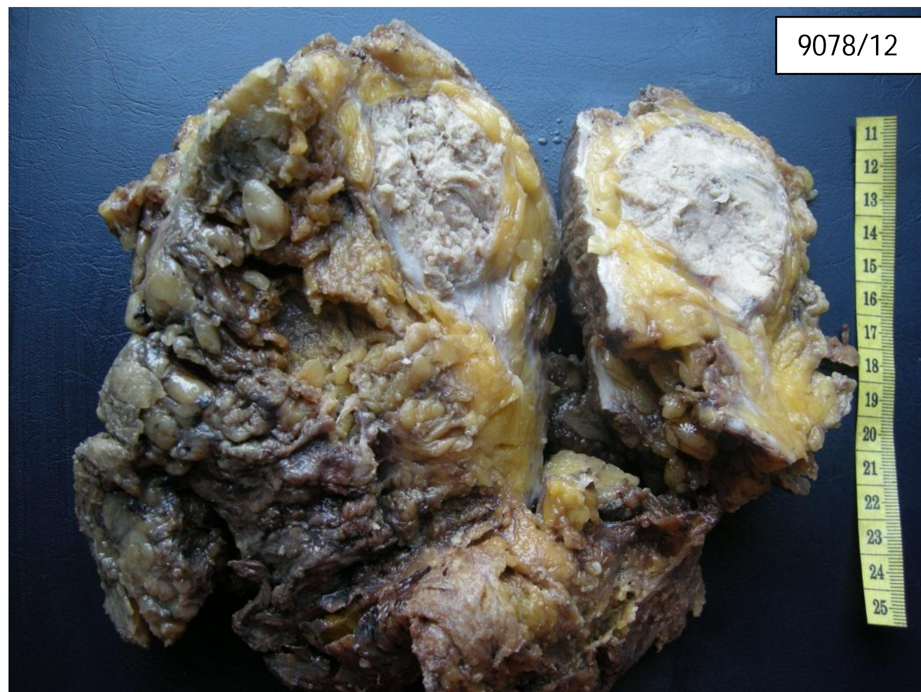


Figure 8: well circumscribed grey white firm mass

INVASIVE DUCTAL CARCINOMA NOS - GRADE 1

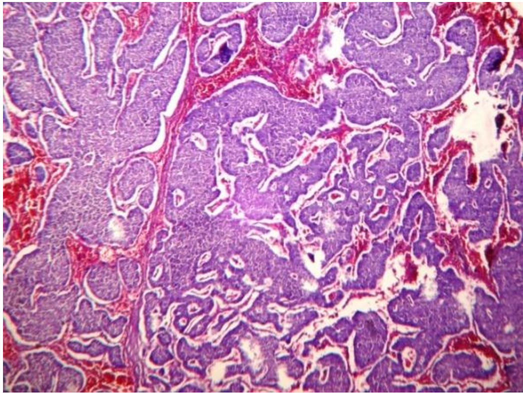


Figure 9: Invasive ductal carcinoma NOS tubule formations >75% tumor cells (40X) HPE 7495/11

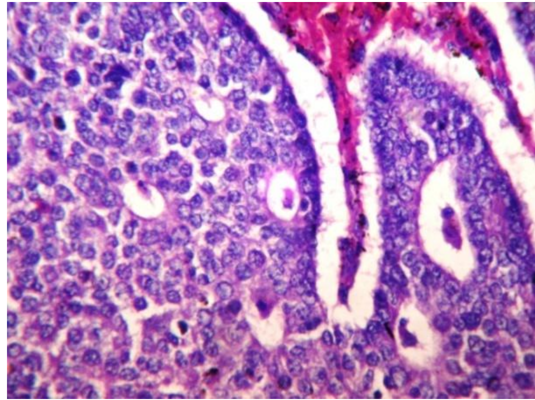


Figure 10: Malignant ductal epithelial cells with mild nuclear pleomorphism & low mitosis HPE 7495/11 (400X)

INVASIVE DUCTAL CARCINOMA NOS GRADE 2

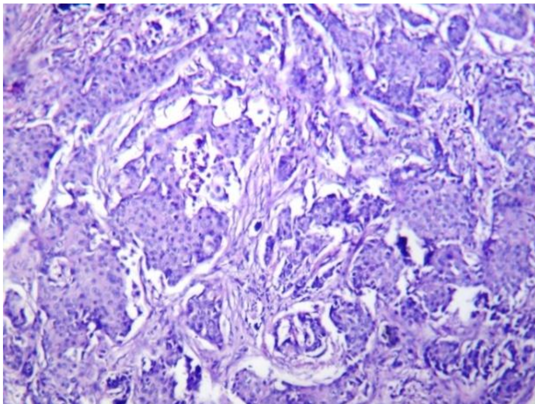


Figure 11: Sheets of malignant ductal epithelial cells, 30% tubule formation HPE 9438/12(100X)

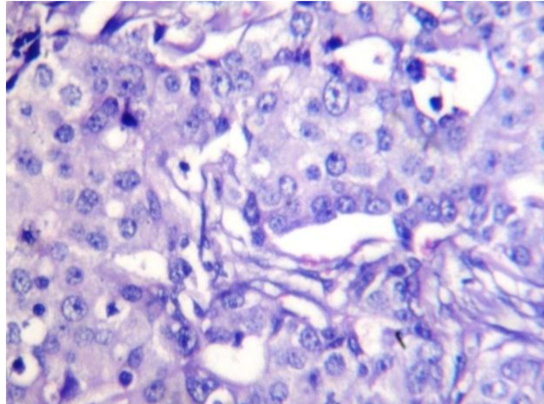


Figure 12: Malignant ductal epithelial cells in sheets, 30% tubules and mild nuclear pleomorphism HPE 9438/12(400X)

INVASIVE DUCTAL CARCINOMA NOS - GRADE 3

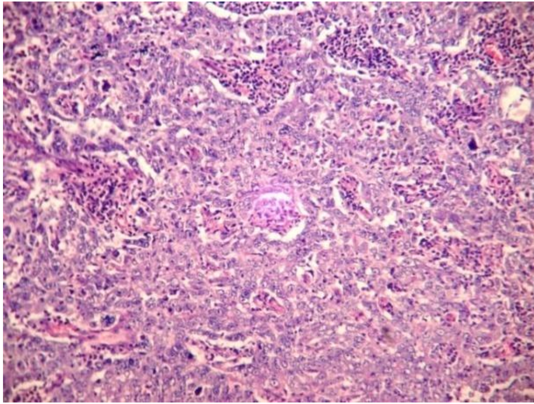


Figure 13: Malignant ductal epithelial cells in sheets HPE 948/12 (100X)

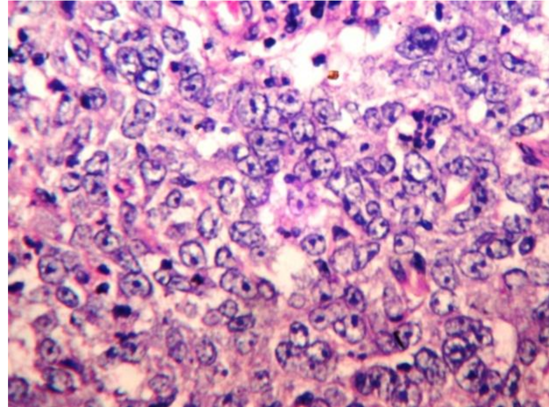


Figure 14: Malignant ductal epithelial cells with no tubules, marked nuclear pleomorphism, increased mitosis HPE 948/12(400X)

MUCINOUS CARCINOMA

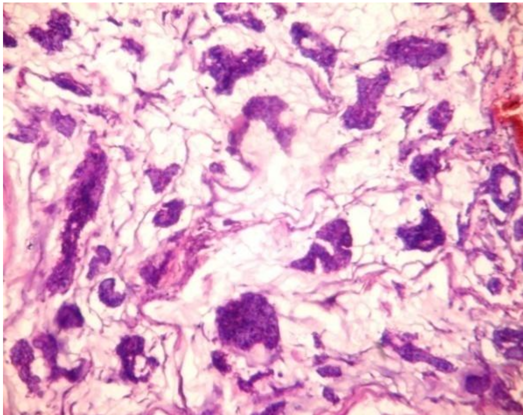


Figure 15: Tumor nests floating in mucin HPE 7764/11(100X)

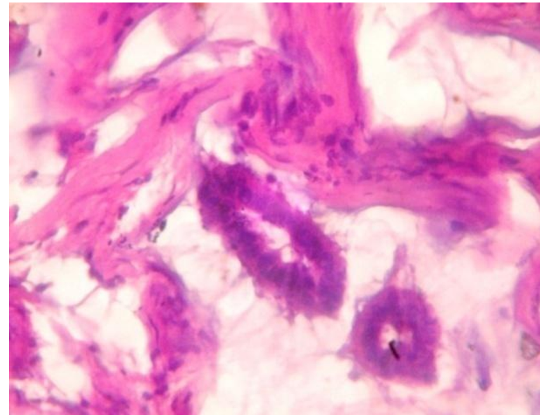


Figure 16: Malignant ductal epithelial cells with mild nuclear pleomorphism and no mitosis HPE 7764/11 (400X)

LOBULAR CARCINOMA

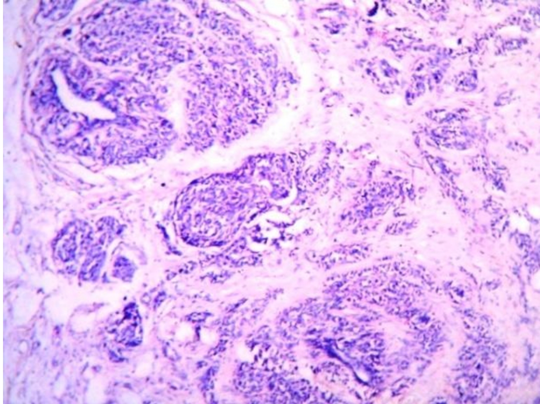


Figure 17: Tumor cells arranged in lobular pattern with pagetoid spread around ductal elements HPE 291/10 (100X)

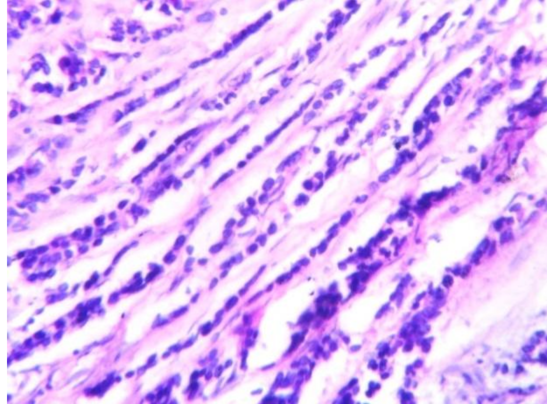


Figure 18: Tumor cells arranged in singles in Indian file pattern HPE 291/10 (400X)

MEDULLARY CARCINOMA

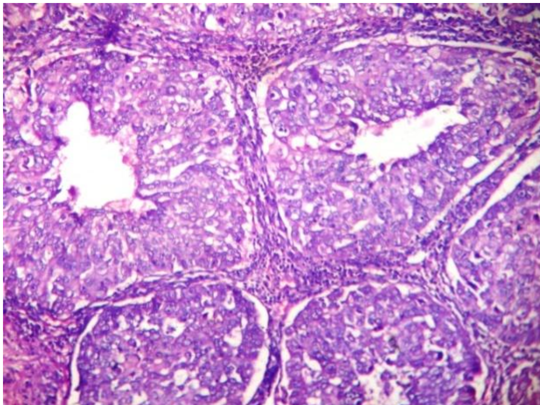


Figure 19: Nodular arrangement of tumor cells with lymphoplasmacytic infiltrate in periphery HPE 8213/12 (100X)

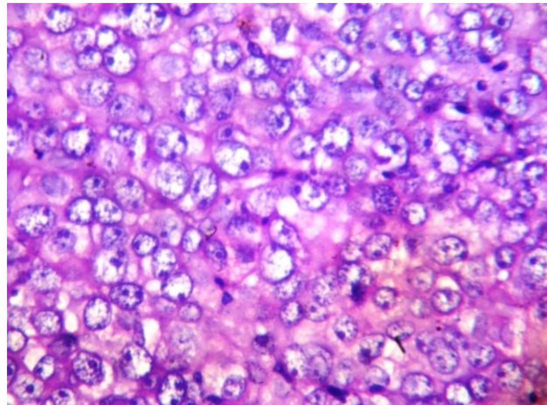


Figure 20: Tumor cells in syncytial pattern with marked nuclear pleomorphism and prominent nucleoli HPE 8213/12 (400X)

PAPILLARY CARCINOMA

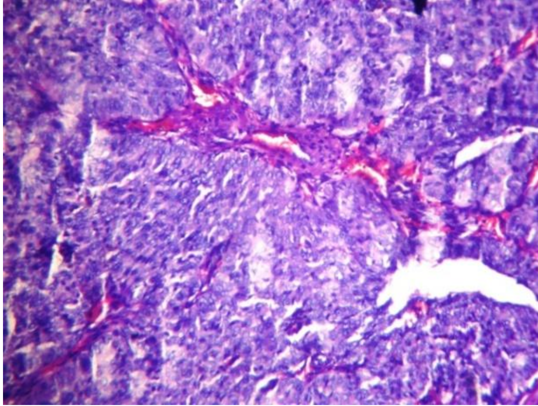


Figure 21: Tumor cells in papillary pattern HPE 9053/12 (100X)

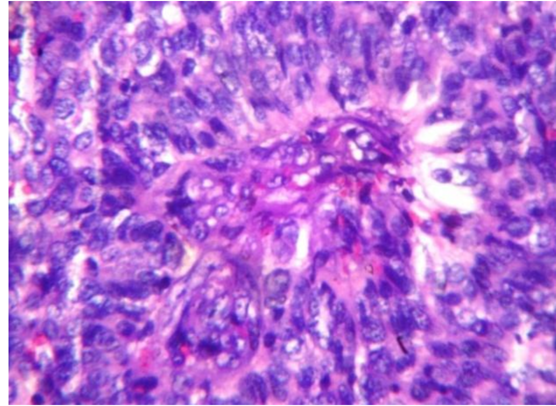


Figure 22: Tumor cells in delicate papillary pattern HPE 9053/12 (400X)

INVASIVE CRIBRIFORM CARCINOMA

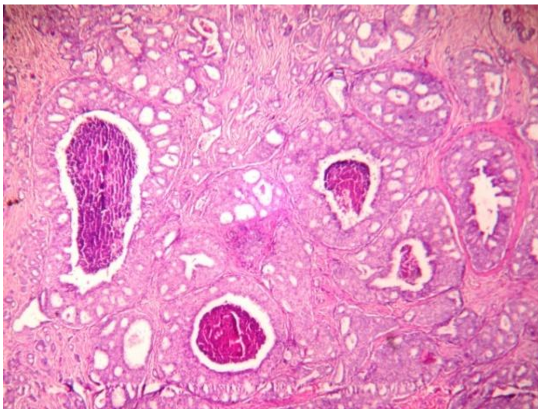


Figure 23: Tumor cells in cribriform pattern HPE 2375/12 (40X)

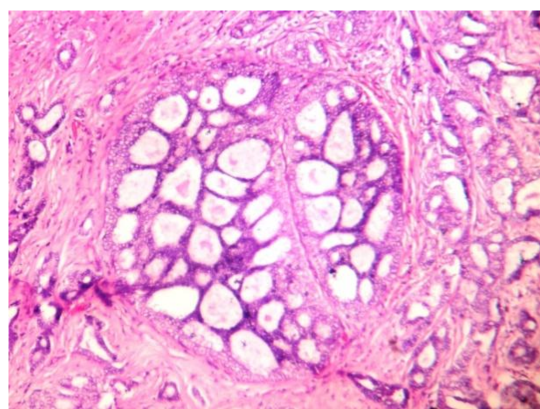


Figure 24: Malignant cells in cribriform pattern with infiltration HPE 2375/12 (400X)

INVASIVE MICROPAPILLARY CARCINOMA

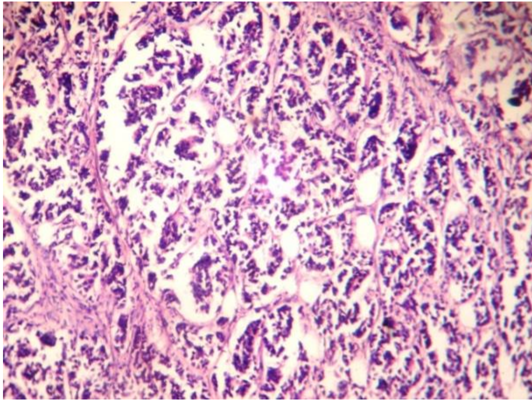


Figure25: Tumour cells in micropapillary pattern with infiltration HPE 2683/10 (100x)

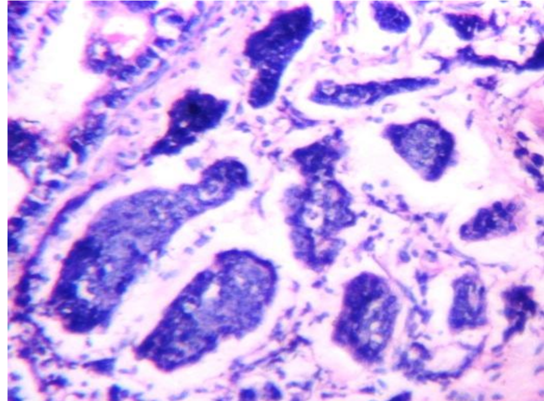


Figure 26: Clusters of tumour cells lying within clear stromal spaces HPE 2683/10 (400X)

APOCRINE CARCINOMA

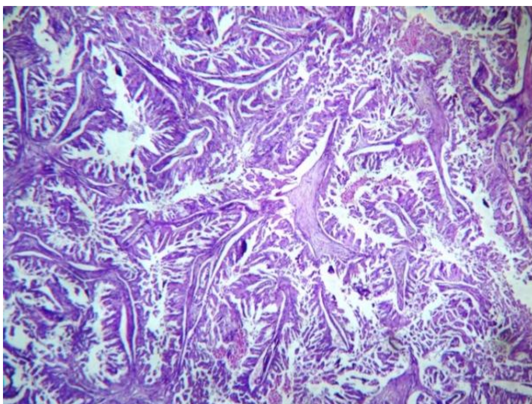


Figure 27: Apocrine cells in papillary pattern HPE 6973/11(100X)

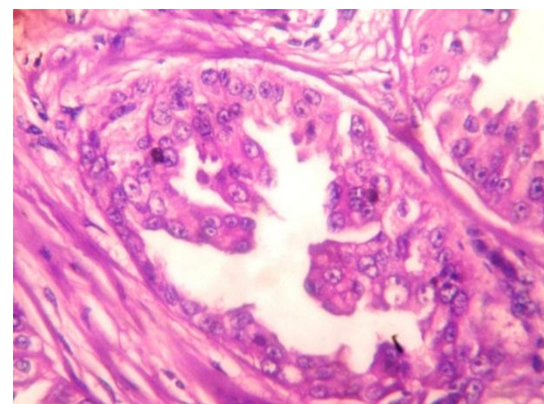


Figure 28: Apocrine cells with abundant granular eosinophilic cytoplasm (400X) HPE 6973/11

METAPLASTIC CARCINOMA WITH SQUAMOUS DIFFERENTIATION

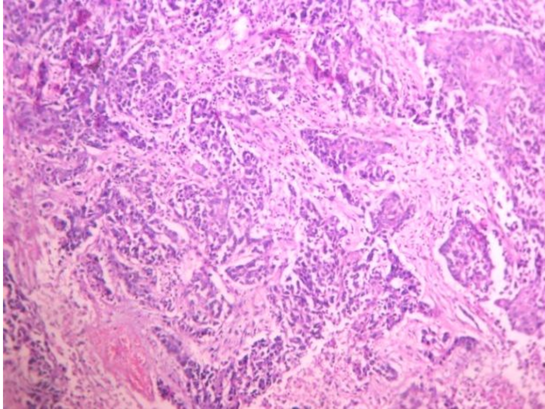


Figure 29: Nests of tumor cells with squamous cell differentiation HPE 5538/12 (100X)

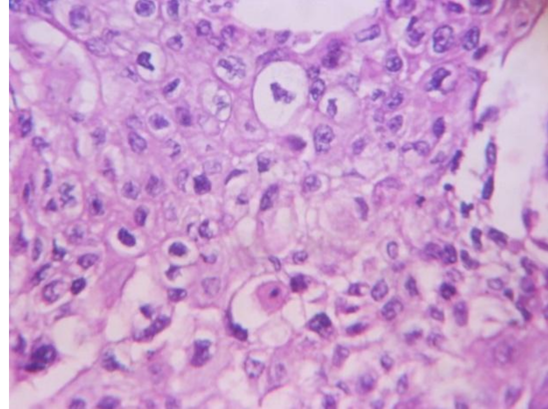


Figure 30: Squamous cell nest in between the tumour cells HPE 5538/12 (400X)

NEUROENDOCRINE CARCINOMA

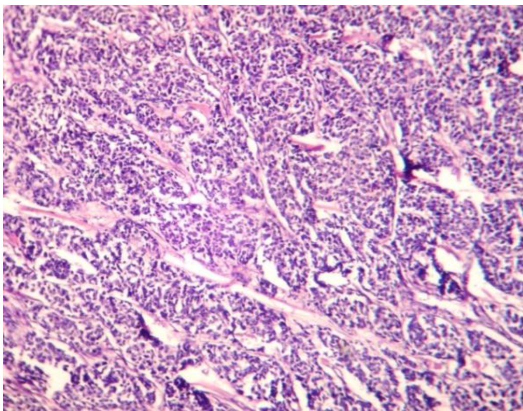


Figure 31: Tumor cells in nests separated by fibrovascular septa HPE 1164/12

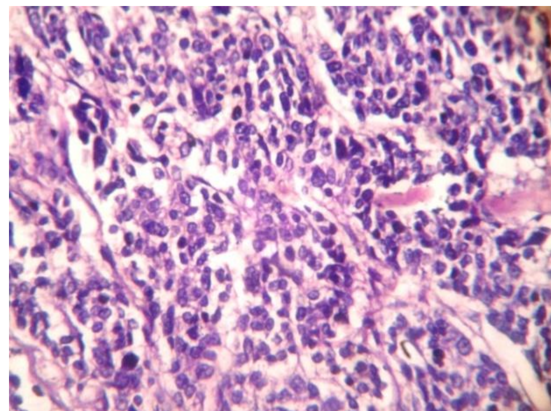


Figure 32: Uniform oval shaped tumor cells with salt and pepper chromatin HPE 1164/12

OTHER PROGNOSTIC FACTORS

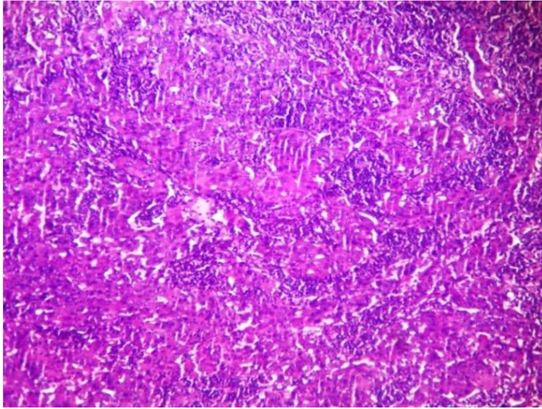


Figure 33:Metastatic deposit in node (100X) HPE 9235/12

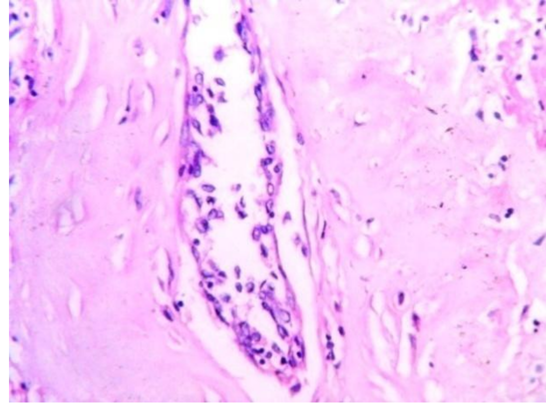


Figure 34: Lymphatic invasion HPE 5791/11

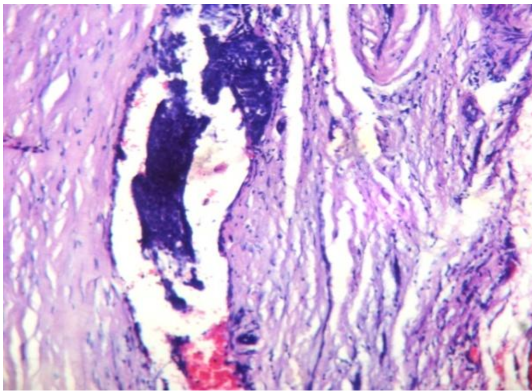


Figure 35: Vascular invasion (400X) HPE 9053/12

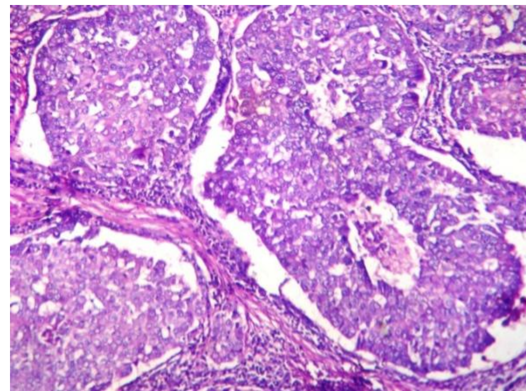


Figure 36: Lymphocytic infiltration (100X) HPE 8213/12

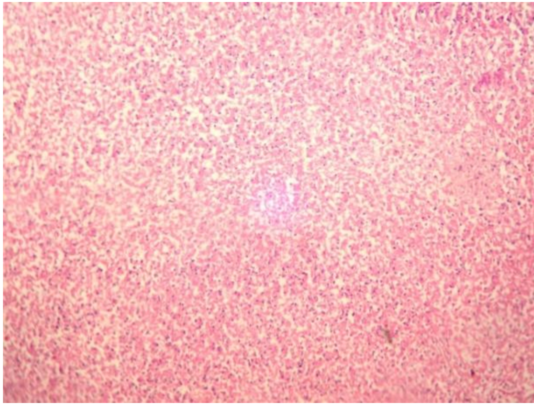


Figure 37: Necrosis : HPE 9413/10 (100X)

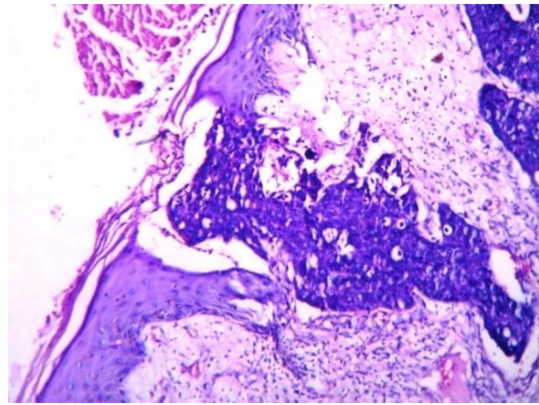


Figure 38: Skin infiltration: HPE 1947/11 (100X)

P53

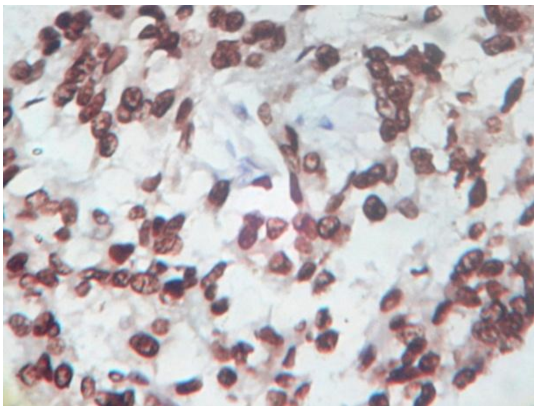


Figure 39: Invasive ductal carcinoma NOS Positive nuclear staining with p53 antibody HPE: 4511/11

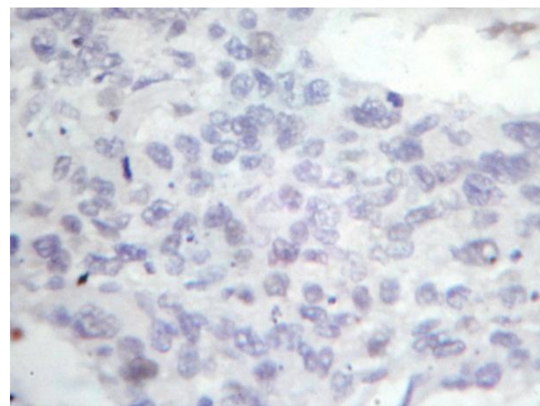


Figure 40: Invasive ductal carcinoma NOS negative for p53 HPE: 4555/11

DISCUSSION

Breast carcinoma is the most common cause of cancer related mortality in urban Indian women and the second commonest cause in rural women[1].There is a gradual increase in the breast cancer incidence worldwide. It accounts for about 23% of all cancers in women[2].Early detection and treatment can certainly reduce the mortality rates.

In the present study, immunohistochemical evaluation and genetic analysis by PCR were done in 60 invasive ductal carcinoma cases and an attempt has been made to evaluate p53, E Cadherin and HoxD10 status in breast cancers and their correlation with histological grade and other prognostic factors.

Madras Medical College being a tertiary referral centre, the relative frequency of breast cancers among the other surgical cases was 2.04%. Among the entire breast specimens received for histopathological examination, 33.82% of the cases were reported to be malignant.

The age of breast cancer patients ranged from 25 to 85 years with a mean age of 50.7 years. The highest incidence of breast cancer occurred in 41 to 50 year age group. This is in concurrence with the study done by Rajesh Singh Laishram et al.[90]

The most common histological subtype of breast cancer in this study is Invasive ductal carcinoma NOS type. This is similar to the study of Albrektsen et al,[91] Shirley SE et al[92] and AM Dauda et al[93]. The incidence of invasive ductal carcinoma NOS type is higher in Indian population (89.8%) than that of western population accounting for the worse prognosis (Table 33).

Table 33: Comparison of distribution of histological subtypes of breast cancers

Histological subtypes	AM Dauda et al[93]	Shirley SE et al[92]	Albrektsen et al[91]	Current study
Invasive ductal carcinoma NOS	78.8%	69.3%	81.4%	89.8%
Lobular carcinoma	6.7%	5.6%	6.3 %	0.7%
Tubulolobular carcinoma	-	0.5%	-	-
Mixed carcinoma	-	-	-	0.4%
Mucinous carcinoma	2.4%	3.6%	2%	1.8%
Micropapillary carcinoma	-	0.5%	-	0.7%
Microinvasive carcinoma	-	0.3%	-	-
Papillary carcinoma	4.2%	3.5%	-	1.5%
Metaplastic carcinoma	2.4%	1.3	-	1.5%
Tubular carcinoma	-	0.8%	2%	-
Cribriform carcinoma	-	0.1%	-	0.4%
Medullary carcinoma	3.6%	1%	1.1%	0.7%
Apocrine carcinoma	-	-	-	1.5%
Adenoid cystic carcinoma	-	0.1%	-	0.42%
Malignant phyllodes	1.8%	-	0.4%	0.7%
Neuroendocrine carcinoma	-	-	-	0.4%
Inflammatory carcinoma	-	1.4%	-	-
Other specific types	-	-	1.2%	-
Adenocarinomas unspecific	-	5.3%	5.5%	-
DCIS	-	7.1%	-	-
Paget’s disease	-	0.1%	1.5%	-

In the study done by Zeeshan Butt et al, breast cancer incidence was 42.7% in pre-menopausal and 57.3% in post-menopausal women.[94] In the current study, the incidence of breast cancer among pre and post-menopausal women were 56.6% and 43.4% respectively. 0.7% of the total breast cancers were reported in males. 48.5% of the breast cancers were present in left breast, 51.5% in right breast and 1 case was found to have bilateral breast cancer.

Table 34: Comparison of distribution of AJCC staging in breast cancers

Stage	Hong Suk Son et al[95]	Rajesh Singh Laishram et al[90]	Carey et al[96]	Current study
Stage I	24.3	3.37	39	6.93
Stage II	70.3	12.36	51	43.8
Stage III	5.4	76.4	8	48.18
Stage IV	NA	7.87	3	1.09

Most of the cases presented in stage III followed by stage II which was similar to that of Rajesh Singh Laishram et al[90] who studied 142 breast cancers in Manipur and reported the increased incidence of higher stage tumors in their population when compared to the western studies done by Hong Suk Son et al[95] and Carey et al[96] (Table 34).

A higher proportion of T2 sized tumors (59.12 %) were seen (Table 35) similar to the study of Christine L. Carter et al (USA),[97] and Lakmini et al (India).[99]

Table 35: Comparison of size of tumors

Size	Christine L. Carter et al[97]	F S Al-Joudi et al[98]	Lakmini et al[99]	Current study
T1	33.6	3.14	14.5	17.16%
T2	55.4	19.37	74	59.12%
T3	11	77.49	11.5	23.72%

The Grade II tumors were more frequent than other grades of breast cancers. This observation was similar to the study carried out by Qiu J et al[100], Carey et al[96] and GG Van den Eynden et al[101] (Table 36).

Table 36: Comparison of grade of tumor

Grade	Qiu J et al[100]	Carey et al[96]	GG Van den Eynden et al[101]	Current study
Grade I	33.3%	25%	32.63%	28.1%
Grade II	54%	26%	36.84%	46.7%
Grade III	12.7%	49%	30.53%	14.6%

Nodal metastasis was noted in 52.6 % of the cases while it was not seen in 47.4%. This result correlates with the study done by Jun Qiu

et al[100] and SE Shirley et al[92] who reported nodal metastasis in 60.32% and 75.7% of their cases.

66.4% of cases had lymphatic invasion and 37.2% of the cases had vascular invasion which was similar to the observation made by GG Van den Eynden et al[101], who reported 69.5% lymphatic invasion and 37.9% vascular invasion in his study.

There were lymphocytic infiltration in 58.4%, skin infiltration in 19% and necrosis in 27.4% of the cases, in concurrence to the 33% skin infiltration reported in the study conducted by Chanda Bewtra et al[102] and 38.1% necrosis in the study conducted by Gloria Perio et al.[103]

TABLE 37 COMPARISON OF P53 EXPRESSION IN WORLD STATISTICS

	P53 positive (%)
Iwaya K et al[89]	21.4
Hong Suk Song et al[95]	51.6
F S Al-Joudi et al [98]	29.6
Barbareschi M et al[104]	17
Zainab W. Aziz[105]	38.3
M.Etebary et al[106]	40.3
Current study	71.7

CORRELATION OF P53 EXPRESSION WITH OTHER KNOWN CLINICOPATHOLOGICAL PROGNOSTIC FACTORS

Mattia Barbareschi et al (1996) studied 178 breast cancer patients in Italy and found direct statistically significant relationship between p53 expression and lower age, greater tumour size, ductal morphology, higher grade and ER negative status.[104]

M.Etebary et al (2002) studied the P53 expression in 72 Iranian breast cancer patients and reported statistically significant direct association between p53 expression and high tumour grade, age at diagnosis above 45 years. He reported that p53 overexpression can be considered as a marker of increased malignant potential in breast cancers and accounts for poor prognosis.[106]

Hong Suk Song (2006) et al studied p53 expression in 440 Korean breast cancer patients. The p53 overexpression inversely correlated with lymph node metastasis. The tumor size, histological type, grade, hormone receptor status and stage of the tumour were not related to the p53 overexpression.[95]

F S Al-Joudi et al (2008) studied p53 expression in 382 breast cancer patients in Malaysia. P53 expression showed significant association with the age and histological grade of the tumour. No

significant association was noted with nodal status, tumour size, side of the tumour and ER & PR expression.[98]

Zainab W. Aziz et al (2011) studied p53 expression in 60 breast cancer patients in Mosul city.Over expression of P53 showed significant correlation with patient's age, tumour grade, stage, and size, but no correlation was found with menopausal status and axillary lymph node metastasis.[105]

In comparison with the above mentioned studies, the present study showed significant correlation between p53 expression and higher histological grade. The correlation with other clinicopathological variables was not significant.

Table 38: Comparison of CDH1 gene inactivation in world statistics

	CDH1 gene inactivation (%)
Shohreh A. Shargh et al[107]	94
Celebiler Cavusoglu A et al[15]	33.9
Caldeira José Roberto F et al[108]	72
Masaru Shinozaki et al[109]	53
Mozhgan Rasti et al[110]	41
Current study	60

CORRELATION OF E CADHERIN GENE MUTATION WITH OTHER KNOWN CLINICOPATHOLOGICAL PROGNOSTIC FACTORS

C Parker et al (2001) studied E Cadherin expression by IHC in 174 breast cancer patients from the Nottingham breast cancer series. Significant correlation was found between E Cadherin expression and histological type, grade and ER status. No significant association was found with tumour recurrence, distant metastases, lymph node status, vascular invasion, tumour size and survival.[111]

Masaru Shinozaki et al (2005) studied 151 breast cancer patients in California and found that hypermethylation of the CDH1 gene was significantly associated with primary breast tumors demonstrating lymphovascular invasion, infiltrating ductal histology, negativity for the oestrogen receptor and also was frequently associated with sentinel lymph node metastasis.[109]

Anca Botezatu et al (2008) studied 25 breast cancer patients in Romania and found that epigenetic silencing of E Cadherin gene is associated with high mitotic activity, poorer tumour differentiation, and increased tendency for regional lymph node metastases.[112]

Mozhgan Rasti et al (2009) studied 67 Iranian breast cancer patients and found that there was a significant correlation between

hypermethylation of CDH1 locus and tumour size ≥ 5 cm. The association with other clinicopathological parameters like age, histological type, grade, nodal involvement, ER & PR status were not found to be significant.[110]

Celebiler Cavusoglu A et al (2010) studied 62 breast cancer patients from Turkey and found that CDH1 under expression showed significant association with advanced tumour stage, histological type, higher tumour grade and lymph node metastasis.[15]

Shohreh A. Shargh et al (2011) studied 50 breast cancer patients in Iran and found significant association between CDH1 gene mutation and higher tumour grade, stage and tumour metastasis.[107]

In the present study the correlation between CDH1 gene mutation and other clinicopathological parameters were not found to be significant.

CORRELATION OF HOX D10 GENE EXPRESSION WITH OTHER CLINICOPATHOLOGICAL PROGNOSTIC FACTORS

Kokonoe Makiyama et al (2004) studied 18 breast cancer patients in Japan and found that there was a significant down regulation of the HoxD10 gene in cancerous tissue compared to non cancerous tissue. There was no significant correlation between age, menopausal status, tumour size, serum CEA and CA 125 levels.[19]

Meritxell Carrio et al (2005) analysed HoxD10 gene in human breast epithelial cell cultures by PCR. Strong expression of HoxD10 was found in premalignant epithelial cells, but the expression was largely absent in invasive breast carcinomas. They also showed that when HoxD10 was re-introduced in breast cancer cells using retroviral genetic transfer, cell migration was impaired and there was restoration of normal cellular polarisation with acinar morphology.[86]

Li Ma et al (2007) showed by cell culture studies that inhibition of HoxD10 by miR-10b, resulted in increased expression of pro metastatic gene RHOC leading to tumour invasion and metastasis.[113]

Sirigiri Divijendra Natha Reddy et al (2008) demonstrated by cell culture studies using PCR that HoxD10 is a positive regulator of miR-7, the loss of which results in increased Pak1 expression and leads to increased motility, invasiveness, anchorage-independent growth and breast cancer progression from low to highly invasive phenotypes.[87]

Connie Myers et al (2002) studied HoxD10 expression in endothelial cell cultures by PCR. Their study showed that HoxD10 was highly expressed in quiescent endothelial cells, but the expression decreases in angiogenic vessels in the tumour microenvironment and that sustained HoxD10 expression can inhibit angiogenesis in vivo.[114]

SUMMARY

60 breast cancer samples were subjected to immunohistochemistry and polymerase chain reaction to evaluate the p53, HoxD10 and E Cadherin status and the results were correlated with histological grade and other known clinicopathological prognostic factors. The relative frequency of breast carcinoma among other surgical cases of Madras Medical College is 2.04%.

- The non neoplastic breast lesions form 29.9%, benign tumors 36.28% and malignant tumors 33.82%.
- Breast carcinoma had the highest incidence in the 41 to 50 year age group.
- The most common histological subtype was Invasive ductal carcinoma NOS type which constituted 89.8%.
- 69% of the breast cancer presented with T2 size (2 to 5 cm) tumours.
- Grade II was the most common grade observed accounting for 52.24%.
- Nodal metastasis was observed in 52.6 %.

- Lymphatic invasion and vascular invasion were seen in 66.4% and 37.2% respectively.
- Skin infiltration was seen in 19% of the tumours.
- Lymphocytic infiltration was observed in 58.4% of the tumours.
- Necrosis was found in 27.4% of the tumours.
- p53 expression was seen in 71.7% of the tumours.
- p53 over expression showed statistically significant association with high grade tumours.
- An increase in the number of cases with p53 positivity was seen with increasing tumour size and cases with lymphovascular invasion.
- No statistically significant association was found between p53 expression and age, nodal status, skin infiltration, lymphocytic infiltration or necrosis.
- HoxD10 gene was down regulated in 46.67% of the tumours.
- HoxD10 gene down regulation showed statistically significant association with grade III tumours.
- The relative expression of HoxD10 gene was lower among postmenopausal women, tumours with larger size, positive nodal status, skin infiltration, lymphatic infiltration and necrosis.

- No association was found between HoxD10 gene expression and vascular invasion and lymphocytic infiltration.
- CDH1 gene mutation was seen in 60% of the tumours.
- CDH1 gene mutation was more frequent among postmenopausal women, tumours with increasing size, grade, nodal status and lymphovascular invasion. However, the association was not statistically significant.
- No association was found between CDH1 gene mutation and other prognostic factors like skin infiltration, lymphocytic infiltration and necrosis.
- The mean relative concentration of HoxD10 mRNA was lower in the p53 positive group when compared to P53 negative group. However, the correlation was not statistically significant.
- The mean relative concentration of HoxD10 mRNA was lower in the CDHI gene mutation positive group. The correlation was not statistically significant.
- CDH1 gene mutation was found to be more common among the p53 positive cases than the p53 negative cases. But, there was no statistically significant association noted between CDH1 gene mutation and p53 expression.

CONCLUSION

The incidence of Invasive ductal carcinoma NOS was higher in this study. Many of our patients presented in younger age with large sized tumors accounting for aggressive nature of breast cancer in our population.P53 over expression and HoxD10 gene down regulation showed significant association with higher grade tumours. This suggests that p53 and HoxD10 gene play an important tumour suppressor role and the loss of which results in breast cancer progression.

The current study shows that E Cadherin gene mutation occurs with high frequency in invasive ductal carcinoma. Although not statistically significant, E Cadherin gene mutation was found to be more common in higher grade tumours. This could be due to the small size of the study sample and investigation in larger series is essential to evaluate its prognostic value.

In conclusion, molecular analysis of breast carcinoma may serve as an important prognostic tool to predict patient outcome and for the development of targeted therapy in this new era of early cancer detection.

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ANNEXURE – I

PROFORMA

Case number : Name :

HPE number : Age :

IP number : Sex :

Clinical diagnosis : Menstrual status :

Risk factors if any :

Side of breast : Right/Left

Specimen : Simple Mastectomy / Modified radical mastectomy / Radical Mastectomy / Toilet mastectomy / Others

GROSS

Specimen size :

Nipple areola : Skin :

Tumor size : Tumor margin:

Appearance :

Resected margis : Superior : Inferior :

Medial : Lateral :

Posterior :

Associated findings :

Total number of nodes dissected :

Largest node size :

MICROSCOPY

Histological subtype :

Histological score: Nuclear score: Mitotic score:

Modified Scarff Bloom Richardson Grade : I / II / III

Skin : Free / Involved

Nipple & Areola	:	Free / Involved		
Margins	:	Superior :	Free / Involved	
		Inferior :	Free / Involved	
		Medial :	Free / Involved	
		Lateral :	Free / Involved	
		Posterior :	Free / Involved	

Lymphatic invasion: Present / Absent

Vascular invasion : Present / Absent

Lymphocytic infiltration: Present / Absent

Necrosis : Present / Absent

Associated breast lesions :

Total number of nodes dissected:

Number of nodes involved :

P53 status : positive / negative

CDH1 mutation status : positive / negative

Relative expression of Hox D10 in relation to ACTB1 gene:

ANNEXURE II

WHO HISTOLOGICAL CLASSIFICATION OF EPITHELIAL BREAST TUMORS

INVASIVE BREAST CANCERS

Invasive ductal carcinoma not otherwise specified
- Mixed type carcinoma
- Pleomorphic carcinoma
- Carcinoma with osteoclastic type of giant cells
- Carcinoma with choriocarcinomatous features
- Carcinoma with melanotic features

Invasive lobular carcinoma
Tubular carcinoma
Invasive cribriform carcinoma
Medullary carcinoma
Mucinous carcinoma
Cystadenocarcinoma
Signet ring carcinoma
Neuroendocrine tumors
- Solid neuroendocrine carcinoma
- Atypical carcinoid tumor
- Small cell/oat cell carcinoma
- Large cell neuroendocrine carcinoma

Invasive papillary carcinoma
Invasive micropapillary carcinoma
Metaplastic carcinoma
Apocrine carcinoma
Pure epithelial metaplastic carcinoma
- Squamous cell carcinoma
- Adenocarcinoma with spindle cell metaplasia
- Adenosquamous carcinoma
- Mucoepidermoid carcinoma

Mixed epithelial/mesenchymal metaplastic carcinoma
Lipid rich carcinoma
Secretory carcinoma
Oncocytic carcinoma
Adenoid cystic carcinoma
Acinic cell carcinoma
Glycogen rich carcinoma
Sebaceous carcinoma
Inflammatory carcinoma
Intraductal papillary carcinoma
Intracystic papillary carcinoma
Microinvasive carcinoma

NON INVASIVE BREAST CANCERS

Ductal carcinoma in situ
Lobular carcinoma in situ
Atypical papilloma

BENIGN EPITHELIAL TUMORS

Tubular adenoma
Lactating adenoma
Apocrine adenoma
Pleomorphic adenoma
Ductal adenoma
Papilloma

FIBROEPITHELIAL TUMORS

Fibroadenoma
Phyllodes tumor
- Benign
- Borderline
- Malignant

Periductal stromal sarcoma
Mammary hamartoma

INTRADUCTAL PROLIFERATIVE LESIONS

Atypical ductal hyperplasia
Flat epithelial atypia
Usual epithelial hyperplasia

METASTATIC TUMORS

ANNEXURE III

NOTTINGHAM MODIFICATION OF SCARFF BLOOM RICHARDSON GRADING SYSTEM

TUBULE FORMATION	**SCORE**
Tubule formation in >75% of the tumor	1
Tubule formation in 10 to 75% of the tumor	2
Tubule formation in <10 % of the tumor	3
NUCLEAR PLEOMORPHISM	**SCORE**
Minimal variation in size and shape of nuclei	1
Moderate variation in size and shape of nuclei	2
Marked variation in size and shape of the nuclei	3
MITOTIC RATE	**SCORE**
<10 Mitosis per 10 high power field	1
10 to 20 mitosis per 10 high power field	2
>20 mitosis per 10 high power field	3

GRADE	***SCORE***
Grade 1	3,4,5
Grade 2	6,7
Grade 3	8,9

ANNEXURE IV

IMMUNOHISTOCHEMISTRY PROCEDURE

1. 4μ thick sections were cut from formalin fixed paraffin embedded tissue samples and transferred to gelatin-chrome alum coated slides.
2. The slides were incubated at 58°C for overnight.
3. The sections were deparaffinized in xylene for 15 minutes x 2 changes.
4. The sections were dehydrated with absolute alcohol for 5 minutes x 2 changes.
5. The sections were washed in tap water for 10 minutes.
6. The slides were then immersed in distilled water for 5 minutes.
7. Heat induced antigen retrieval was done with microwave oven in appropriate temperature with appropriate buffer for 20 to 25 minutes.
8. The slides were then cooled to room temperature and washed in running tap water for 5 minutes.
9. The slides were then rinsed in distilled water for 5 minutes.
10. Wash with appropriate wash buffer (phosphate buffer) for 5 minutes x 2 changes.
11. Apply peroxidase block over the sections for 10 minutes.
12. Wash the slides in phosphate buffer for 5 minutes x 2 changes.
13. Cover the sections with power block for 15 minutes.
14. The sections were drained (without washing) and appropriate primary antibody was applied over the sections and incubated for 45 minutes.
15. The slides were washed in phosphate buffer for 5 minutes x 2 changes.
16. The slides were covered with Super Enhancer for 30 minutes.
17. The slides were washed in phosphate buffer for 5 minutes x 2 changes.
18. The slides were covered with SS Label for 30 minutes.
19. Wash in phosphate buffer for 5 minutes x 2 changes.
20. DAB substrate was prepared by diluting 1 drop of DAB chromogen to 1 ml of DAB buffer.
21. DAB substrate solution was applied on the sections for 8 minutes.
22. Wash with phosphate buffer solution for 5 minutes x 2 changes.
23. The slides are washed well in running tap water for 5 minutes.
24. The sections were counterstained with Hematoxylin stain for 2 seconds (1 dip).
25. The slides are washed in running tap water for 3 minutes.
26. The slides are air dried, cleared with xylene and mounted with DPX.

ANNEXURE V

RNA EXTRACTION

1. 10 Sections of formalin fixed paraffin embedded tissue samples with 10 μ thickness were collected in micro centrifuge tube.
2. Sections were cleared with 1 ml xylene, vortex, centrifuged for 2 minutes and supernatant was removed.
3. Sections were dehydrated with absolute ethyl alcohol, vortex, centrifuged for 2 minutes and supernatant was removed.
4. The air dried tissue pellet was resuspended in 240 μl Buffer PKD & 10μl proteinase K mixture to reverse the formaldehyde modification of nucleic acid.
5. Incubate the centrifuge tube at 55ºC for 15 minutes and 80ºC for 15 minutes.
6. 500μ of Buffer RBC was added to the mixture and vortex to adjust the binding condition.
7. All the centrifuge tube contents were transferred to gDNA eliminator spin column placed in 2 ml collection tube.
8. The tube is centrifuged at 13,000 rpm for 30 sec to filter the genomic DNA in the tissue sample.
9. The column with genomic DNA was discarded and the flow through was saved.
10. 1200ml of 100% ethanol was added to the flow through and mixed well to enable precipitation of RNA in sample.
11. RNA precipitates were then filtered in RNeasy Minelute spin column placed in 2 ml collection tube by centrifuging at 13,000 rpm for 15 seconds.
12. The column with RNA precipitate was transferred to another collection tube and 500 μl of Buffer RPE added and centrifuged at 13,000 rpm for 15 seconds and then the flow through was discarded.
13. Step 12 is repeated for 2 minutes.
14. Transfer RNeasy Minelute spin column to a new 2 ml collection tube and centrifuged at 13,000 rpm for 5 minutes with their lids open to remove the residual ethanol form RNA and the flow through was discarded.
15. Transfer RNeasy Minelute spin column to a new 1.5 ml collection tube and 30 μl of RNAse free water was added and centrifuged for 1 minute at 13,000 rpm to elute total RNA.
16. The total RNA was stored at -20ºC to -70ºC.

KEY TO MASTER CHART

SI	-	Skin infiltration
LI	-	Lymphatic invasion
VI	-	Vascular invasion
LCI	-	Lymphocytic infiltration
NEC	-	necrosis
LNS	-	lymph node status
P53	-	Protein 53
HoxD10	-	Relative Homeobox D10 gene expression in relation to actin B-1 gene
CDH1	-	Cadherin-1
MRM	-	Modified radical mastectomy
SM	-	Simple mastectomy
RM	-	Radical mastectomy
TM	-	Toilet mastectomy
PSM	-	Palliative simple mastectomy
A	-	Absent
N	-	Negative
P	-	Present / positive
M	-	Male
F	-	Female
R	-	Right
L	-	Left
NAC	-	neoadjuvant chemotherapy
IDC NOS	-	Invasive ductal carcinoma not otherwise specified
Sup	-	superior
Inf	-	inferior
Med	-	medial
Lat	-	lateral
Post	-	posterior
G	-	Grade

MASTER CHART

S.NO.	BIOPSY NO	AGE	SEX	SIDE	STAGE	SURGERY	SUBTYPE	SIZE	GRADE	SI	LI	VI	LCI	NEC	MARGINS	LNS	P53	HOX D10	CDH1 MUTATION
1	37/10	75	F	R	IIB	MRM	IDC -COMEDO	6	3	A	P	P	P	P	POST	0			
2	67/10	70	F	R	IIA	MRM	IDC -PAPILLARY	2.5		A	P	P	A	A	FREE	0			
3	71/10	65	F	L	IIA	MRM	MUCINOUS CARCINOMA	5		A	A	P	A	A	POST	0			
4	150/10	45	F	L	IIIB	PSM	LOBULAR CA	3		P	P	A	A	A	FREE	0			
5	238/10	74	F	R	IIIC	TM	IDC - COMEDO	7	3	A	P	P	A	P	POST,MED,LAT	6			
6	268/10	50	F	R	IIB	MRM	IDC - NOS	3.5	2	A	P	A	P	A	FREE	1			
7	281/10	39	F	R	IIA	MRM	IDC - NOS	5	3	A	A	A	A	P	FREE	0			
8	291/10	35	F	R	IIIC	MRM	LOBULAR CA	5		A	P	A	P	A	POST	7			
9	300/10	65	F	R	IIIC	MRM	IDC - NOS	7	3	A	P	A	A	A	POST	8			
10	349/10	63	F	R	IIIA	MRM	IDC - COMEDO	11	3	A	P	P	P	P	FREE	12			
11	354/10	60	F	R	IIIC	MRM	IDC- MICROPAPILLARY	8		A	P	P	P	A	POST	5			
12	367/10	55	F	R	IIIC	MRM	IDC - NOS	8	3	A	P	P	P	P	POST	7			
13	442/10	40	F	L	IIA	MRM	IDC - NOS	3.5	3	A	P	P	P	A	FREE	0			
14	523/10	42	F	L	IIIA	MRM	IDC -NOS	2	2	A	P	P	P	A	FREE	3			
15	544/10	35	F	L	IIIA	MRM	IDC - NOS	5.5	2	A	P	P	P	A	POST	14			
16	590/10	55	F	R	IIIA	MRM	IDC - NOS	2	1	A	P	P	A	A	FREE	5			
17	599/10	42	F	R	IIIB	SM	IDC- MUCINOUS	7		P	P	A	P	A	LAT	0			
18	673/10	35	F	R	IIA	NAC + MRM	IDC - NOS	3	1	A	A	A	A	A	POST	0			
19	698/10	60	F	R	IIA	MRM	IDC - COMEDO	4.5	3	A	A	A	P	P	POST	0			
20	705/10	50	F	L	IIB	MRM	IDC - COMEDO	3	3	A	P	A	P	P	FREE	1			
21	733/10	65	F	R	IIIA	NAC + MRM	IDC - NOS	3.5	2	A	P	A	A	A	FREE	3			
22	803/10	55	F	L	IIIA	MRM	IDC - NOS	6	1	A	P	A	A	P	POST,MED	1			
23	1031/10	65	F	L	IIIC	MRM	IDC - NOS	3.5	1	A	P	A	A	A	POST	8			
24	1123/10	43	F	R	IIB	MRM	IDC - NOS	4	1	A	P	A	A	A	FREE	1			
25	1192/10	50	F	R	IIIB	TM	IDC -NOS	5	2	P	P	A	A	A	FREE	3			
26	1234/10	69	M	L	IIIB	MRM	IDC - NOS	3	2	P	P	A	A	A	POST	3			
27	1285/10	55	F	R	IIIC	MRM	IDC - NOS	4	1	A	P	A	A	A	POST	4			
28	1315/10	40	F	L	IIIB	MRM	IDC - NOS	5	2	P	P	A	P	A	FREE	1			
29	1469/10	43	F	R	IIA	MRM	IDC - CRIBRIFORM	4.5		A	A	P	P	A	FREE	0			
30	1545/10	60	F	R	IIIA	MRM	IDC - NOS	1.5	2	A	P	P	A	A	FREE	3			

31	1597/10	73	F	R	IIA	MRM	IDC- MUCINOUS	5		A	A	A	A	A	FREE	0			
32	1632/10	50	F	R	IIIB	NAC + MRM	IDC -NOS	4	2	P	P	P	P	A	FREE	2			
33	1675/10	56	F	R	IIIA	MRM	IDC - NOS	5	2	A	P	P	P	A	FREE	4			
34	1695/10	40	F	L	IIIA	MRM	IDC - NOS	3	1	A	A	A	P	A	POST	0			
35	1731/10	50	F	L	IIB	MRM	IDC - NOS	3	3	P	P	P	P	A	FREE	0			
36	1738/10	50	F	R	IIIC	MRM	IDC - NOS	10	3	A	P	A	P	A	POST	8	P	0.001	
37	1776/10	26	F	R	IIA	MRM	IDC - NOS	5	1	A	A	A	A	A	FREE	0			
38	1852/10	35	F	R	IIIA	MRM	IDC - NOS	10	2	A	P	A	P	A	FREE	1			
39	1859/10	60	F	R	IIIC	MRM	IDC - NOS	3.5	1	A	P	A	P	A	FREE	11			
40	2077/10	53	F	R	IIA	MRM	IDC - NOS	3.5	2	A	A	P	P	A	FREE	0			
41	2144/10	74	F	L	IIIA	MRM	IDC - NOS	2	2	A	P	A	A	P	FREE	3			
42	2401/10	25	F	L	IIIC	MRM	IDC - NOS	3	1	A	P	A	P	P	FREE	0			
43	2589/10	45	F	L	IIA	MRM	IDC - COMEDO	3	3	A	P	A	P	P	POST	0			
44	2640/10	42	F	L	IIIA	MRM	IDC - NOS	2	1	A	P	A	P	A	FREE	0			
45	2683/10	42	F	L	IIA	MRM	IDC - MICROPAPILLARY	4		A	P	A	P	A	FREE	0			
46	2703/10	47	F	R	IIB	MRM	IDC - NOS	5	2	A	P	A	P	A	FREE	1			
47	2757/10	65	F	L	IIA	MRM	IDC - NOS	1.75	2	A	P	A	A	A	FREE	1			
48	2780/10	46	F	L	IIIA	MRM	IDC - COMEDO	8	3	A	P	A	A	P	FREE	2			
49	2795/10	42	F	L	IIA	NAC + MRM	IDC - COMEDO	5	3	A	A	A	A	P	POST	0			
50	2870/10	38	F	L	IIB	MRM	IDC - MEDULLARY	6		A	A	A	P	P	FREE	0			
51	2909/10	55	F	R	I	MRM	IDC - NOS	2	2	A	A	A	P	A	POST	0			
52	2933/10	40	F	R	IIIA	MRM	IDC - NOS	8	2	A	P	A	P	A	POST	1			
53	3103/10	40	F	R	IIIB	MRM	IDC - NOS	2.5	2	P	A	A	P	A	FREE	1			
54	3162/10	58	F	L	IIIC	MRM	IDC - NOS	5	2	P	P	A	P	A	POST	6			
55	3357/10	50	F	R	IIA	MRM	IDC - NOS	2.5	2	A	P	A	A	A	FREE	0			
56	3582/10	50	F	L	IV	TM	IDC - NOS	18	2	P	P	A	A	P	POST	7			
57	3598/10	35	F	L	I	MRM	IDC - NOS	2	2	A	A	A	P	P	POST	0			
58	3694/10	65	F	R	IIA	TM	IDC - NOS	5	3	A	P	P	P	A	POST	0	P	0.093	
59	3822/10	43	F	L	IIIA	MRM	IDCNOS	7.5	2	A	P	P	P	P	POST	3			
60	4090/10	55	F	L	IIIC	MRM	IDCNOS	2	3	A	P	P	A	A	FREE	7			
61	4125/10	47	F	R	IIIA	MRM	IDCNOS	8	3	A	P	A	A	A	FREE	25			
62	4570/10	36	F	L	IIIC	MRM	IDCNOS	7	2	A	P	A	A	A	POST	7			
63	4608/10	50	F	L	IIB	NAC + MRM	IDCNOS	3	2	A	P	A	A	A	FREE	1			

64	4637/10	53	F	R	IIIA	MRM	COMBINED	5		A	P	P	P	P	FREE	6			
65	4651/10	38	F	R	IIIB	MRM	IDCNOS	5	2	A	P	A	P	A	POST	1			
66	4660/10	37	F	L	I	MRM	IDCNOS	1	2	A	A	A	P	A	FREE	0			
67	4756/10	75	F	L	I	MRM	IDCNOS	2	1	A	A	A	P	A	POST	0			
68	4899/10	50	F	L	IIIC	MRM	IDCNOS	8	1	A	P	A	P	A	FREE	5			
69	4905/10	50	F	R	IIIC	NAC + MRM	IDCNOS	3.5	1	A	P	A	A	P	FREE	4			
70	4957/10	60	F	L	IIIB	MRM	IDCNOS	2.5	2	P	P	A	A	A	FREE	2			
71	5005/10	39	F	L	IIA	MRM	IDCNOS	2.5	1	A	P	P	A	P	FREE	0			
72	5162/10	47	F	R	IIIC	MRM	IDCNOS	3.5	2	A	P	A	A	A	FREE	5			
73	5222/10	45	F	R	IIIC	MRM	IDCNOS	1.5	2	A	P	P	P	P	FREE	5			
74	5297/10	43	F	L	IIB	MRM	IDCNOS	9	2	A	A	A	A	A	FREE	0			
75	5299/10	55	F	R	IIIA	NAC +MRM	IDCNOS	2.5	2	A	P	A	A	A	FREE	10			
76	5432/10	60	F	R	IIIC	MRM	IDCNOS	4.5	3	A	P	A	P	P	POST	6	N	0.021	
77	5509/10	38	F	L	IIIA	MRM	IDCNOS	3	2	A	P	A	P	A	FREE	4			
78	5525/10	56	F	R	IIB	MRM	METAPLASTIC CA	6		A	A	P	A	P	FREE	0			
79	5737/10	60	F	R	IIIA	MRM	IDCNOS	5	1	A	P	A	P	A	FREE	21			
80	5816/10	35	F	L	IIB	MRM	MAL PHYLLODES	10		A	A	A	A	P	FREE	0			
81	5875/10	60	F	R	IIA	SM	PAPILLARY CA	4		A	A	A	A	A	FREE	0			
82	6011/10	48	F	R	IIA	MRM	IDC - NOS	3	2	A	A	A	A	A	FREE	0			
83	6315/10	70	F	L	IIIB	MRM	IDC - NOS	3	1	P	P	A	P	A	POST	1			
84	6354/10	47	F	R	IIA	MRM	IDC - NOS	3	1	A	A	A	A	A	FREE	0			
85	6412/10	48	F	R	IIIB	NAC + MRM	IDCNOS	5	2	P	P	P	P	P	FREE	2			
86	6493/10	45	F	R	IIB	MRM	IDCNOS	4	1	A	P	A	P	P	FREE	1			
87	6520/10	38	F	L	IIIA	MRM	IDCNOS	8	3	A	P	A	P	P	POST	10			
88	6626/10	45	F	R	IIB	MRM	IDCNOS	17	2	A	A	A	A	A	POST	0			
89	6688/10	54	F	R	IIB	MRM	IDCNOS	4	1	A	P	A	A	A	FREE	1			
90	6791/10	70	F	R	IIB	MRM	APOCRINE CA	7		A	A	A	P	P	FREE	0			
91	6952/10	50	F	L	IIIB	TM	IDCNOS	24	2	P	A	A	A	P	FREE	0			
92	6964/10	56	F	R	IIB	MRM	APOCRINE CA	5		A	P	A	A	A	FREE	2			
93	7033/10	55	F	R	IIB	MRM	IDCNOS	4	3	A	P	A	A	P	FREE	2	N	0.085	
94	7073/10	50	F	L	IIA	MRM	IDCNOS	3	1	A	A	A	P	A	FREE	0			
95	7110/10	75	F	L	IIIC	MRM	IDCNOS	4.5	1	A	P	A	A	A	FREE	7	P	0.016	
96	7141/10	48	F	L	IIA	NAC + MRM	IDCNOS	4	1	A	A	A	P	P	FREE	0			

97	7142/10	50	F	R	IIB	NAC+ MRM	IDCNOS	8	1	A	A	A	A	A	FREE	0			
98	7270/10	32	F	R	IIA	MRM	IDCNOS	4	1	A	A	A	P	A	FREE	0			
99	7331/10	37	F	L	IIIC	MRM	IDC - NOS	4	1	A	P	A	A	P	POST	5	N	0.321	
100	7459/10	68	F	L	IIA	MRM	IDC - NOS	3	1	A	A	A	P	P	FREE	0			
101	7538/10	48	F	R	IIIC	NAC + MRM	IDC - NOS	10	2	P	P	A	A	A	FREE	6			
102	7612/10	47	F	R	IIA	MRM	IDC - NOS	4	2	A	A	A	A	P	FREE	0			
103	7912/10	40	F	L	IIIB	NAC + MRM	IDC - NOS	4	2	P	A	A	A	P	FREE	0			
104	7934/10	57	F	R	IIA	MRM	IDC - NOS	2.5	1	A	A	A	A	P	FREE	0	P	12.32	
105	7961/10	70	F	L	IIB	MRM	IDC - NOS	8	1	A	P	A	A	P	FREE	0			
106	8176/10	65	F	L	IIA	MRM	IDC - NOS	3	1	A	A	A	A	A	FREE	0			
107	8197/10	56	F	L	IIA	MRM	IDC - NOS	3	2	A	A	A	A	P	FREE	0			
108	8391/10	40	F	L	IIB	MRM	IDC - NOS	4	1	A	P	A	A	P	FREE	2			
109	8440/10	43	F	L	IIIA	MRM	IDC - NOS	2	1	A	P	P	A	A	POST	8			
110	8440/10	43	F	R	I	MRM	IDC - NOS	2	1	A	A	A	A	A	FREE	0			
111	8449/10	64	F	L	I	MRM	IDC - NOS	2	2	A	A	A	A	P	FREE	0			
112	8519/10	63	F	L	IIIC	NAC + MRM	IDC - NOS	1	1	P	P	A	A	A	FREE	6			
113	8666/10	50	F	L	IIA	MRM	IDC - NOS	3	1	A	A	A	A	A	FREE	0			
114	8781/10	52	F	L	IIIB	NAC + MRM	IDC - NOS	3	1	A	P	A	A	A	POST	2	P	2.06	
115	8812/10	65	F	R	IIA	MRM	IDC - NOS	3	2	A	P	A	A	A	FREE	0			
116	9043/10	58	F	L	IIA	SM	IDC - NOS	3	2	A	A	A	P	A	FREE	0			
117	9053/10	52	F	R	IIIB	TM	IDC - NOS	8	3	P	P	P	A	P	POST	0	P	0.001	
118	9060/10	55	F	R	IIIC	NAC+TM	IDC - NOS	4	1	A	P	A	A	A	FREE	4	N	1.563	
119	9065/10	60	F	L	IIIA	MRM	IDC - NOS	2	2	A	P	A	A	P	FREE	3			
120	9078/10	60	F	L	IIIA	MRM	IDC - NOS	7	2	A	P	A	P	A	FREE	3			
121	9110/10	45	F	L	IIA	MRM	IDC - NOS	4	2	A	A	A	P	A	FREE	0			
122	9361/10	52	F	L	IIB	MRM	IDC - NOS	6	2	A	A	A	A	P	FREE	0			
123	9404/10	65	F	L	IIA	NAC + MRM	PAPILLARY CA	4		A	A	A	A	A	FREE	0	P	0.21	
124	9413/10	55	F	L	IIIB	TM	IDC - NOS	9.5	3	P	A	A	P	P	POST,MED	0	P	0.124	
125	9438/10	50	F	L	I	MRM	IDC - NOS	2	1	A	A	A	P	A	FREE	0	P	0.014	
126	9459/10	50	F	R	IIB	MRM	IDC - NOS	9	2	A	A	A	P	A	FREE	0			
127	229/11	44	F	L	IIA	MRM	IDC - NOS	3.5	1	A	A	A	P	A	FREE	0			
128	281/11	38	F	L	IIIC	MRM	IDC - NOS	5	2	P	P	P	P	A	FREE	4			
129	303/11	40	F	L	IIA	MRM	IDC - NOS	4.5	2	A	A	A	P	A	FREE	0	P	0.52	N

130	340/11	32	F	L	IIIA	MRM	IDC - NOS	1.5	2	A	P	P	P	A	POST	10	P	1.113	N
131	430/11	51	F	L	IIB	MRM	IDC - NOS	6	2	A	A	A	A	A	POST	0			
132	511/11	47	F	R	IIA	MRM	IDC - NOS	2.5	1	A	A	P	P	A	FREE	0			
133	512/11	51	F	L	IIIA	MRM	IDC - NOS	3	2	A	P	P	P	A	FREE	17			
134	549/11	37	F	L	IIB	MRM	MAL PHYLLODES	8.5		A	A	A	A	P	FREE	0			
135	701/11	42	F	L	IIIA	MRM	IDC - NOS	7	2	A	P	P	A	A	POST	6			
136	738/11	55	F	L	IIIA	SM	IDC - NOS	3	2	A	P	P	P	A	FREE	8			
137	784/11	38	F	R	IIA	MRM	IDC - NOS	2.5	2	A	A	A	P	A	POST	0			
138	1117/11	62	F	L	IIA	MRM	IDC - NOS	2.5	1	A	A	A	P	P	FREE	0			
139	1170/11	52	F	L	IIA	MRM	IDC - NOS	5	1	A	A	A	P	P	POST	0	P	1.22	P
140	1257/11	70	F	R	IIA	MRM	IDC - NOS	2	2	A	P	P	P	A	FREE	1			
141	1274/11	38	F	L	IIIC	TM	IDC - NOS	6	2	A	P	P	P	A	POST	6			
142	1327/11	59	F	R	IIA	MRM	IDC - NOS	2	1	A	P	P	P	A	FREE	2			
143	1358/11	55	F	L	IIIC	MRM	IDC - NOS	8	2	A	P	P	P	A	FREE	4	P	8.131	P
144	1395/11	40	F	R	IIB	MRM	IDC - NOS	3	2	A	P	P	P	A	FREE	1	P	5.549	P
145	1444/11	48	F	L	IIIA	MRM	IDC - NOS	2.5	2	A	P	P	P	A	FREE	13			
146	1460/11	75	F	L	IIIA	MRM	IDC - NOS	4.5	3	A	P	P	P	A	FREE	13	P	0.031	P
147	1525/11	61	F	R	IIIB	TM	IDC - NOS	6	2	P	P	P	P	P	POST	0			
148	1542/11	52	F	R	IIIC	MRM	IDC - NOS	2.5	3	A	P	P	P	A	FREE	4	P	0.014	P
149	1627/11	27	F	L	IIIB	MRM	IDC - NOS	1	1	P	A	A	P	P	FREE	0			
150	1947/11	35	F	L	IIIB	NAC + TM	IDC - NOS	10	2	P	P	P	P	A	ALL	0			
151	1997/11	45	F	R	I	SM	IDC - NOS	2	2	A	P	P	P	A	LAT	0			
152	2117/11	30	F	L	IIIA	MRM	IDC - NOS	2	2	A	P	P	P	A	FREE	12			
153	2174/11	50	F	R	IIB	MRM	METAPLASTIC CA	4		A	P	A	A	P	FREE	1			
154	2237/11	60	F	R	IIA	MRM	IDC - NOS	4	2	A	A	A	P	A	FREE	0			
155	2376/11	45	F	R	IIA	MRM	IDC - NOS	2.5	1	A	A	A	P	A	FREE	0			
156	2516/11	40	F	L	IIA	NAC + MRM	IDC - NOS	2	2	A	P	A	P	A	FREE	2			
157	2638/11	57	F	R	IIA	MRM	PAPILLARY CA	5		A	A	A	P	A	FREE	0			
158	2676/11	50	F	L	IIB	MRM	IDC - NOS	2.5	2	A	P	A	P	A	LAT	1	N	2.907	
159	2787/11	44	F	R	I	MRM	IDC - NOS	2	1	A	A	A	P	A	POST	0	P	2.563	
160	2869/11	35	F	R	IIIA	MRM	IDC - NOS	5	1	A	P	P	P	P	FREE	3	P	8.032	N
161	3022/11	85	F	R	IIB	MRM	MUCINOUS CA	8		A	A	A	A	A	FREE	0			
162	3193/11	40	F	R	I	MRM	IDC - NOS	2	1	A	A	A	A	A	FREE	0	P	2.34	

163	3241/11	48	F	R	IIIA	MRM	IDC - NOS	3	2	A	P	P	P	A	FREE	3	P	1.019	N
164	3408/11	60	F	R	IIIB	TM	IDC - NOS	7	3	P	P	P	P	A	POST	0	P	0.057	N
165	3422/11	42	F	R	IIA	NAC+TM	IDC - NOS	2	2	A	P	P	P	A	POST	0	P	12.19	N
166	3488/11	60	F	L	IIIC	MRM	IDC - NOS	2.5	1	A	P	P	A	A	POST	5	P	1.11	P
167	3693/11	45	F	L	IIIA	MRM	IDC - NOS	1	2	A	P	A	A	A	FREE	3	P	6.7	P
168	3800/11	39	F	R	I	MRM	IDC - NOS	1.5	1	A	A	A	P	A	FREE	0			
169	3929/11	55	F	R	IIB	MRM	IDC - NOS	3	1	A	P	P	P	A	FREE	2			
170	4157/11	70	F	R	IIA	MRM	IDC - NOS	4	1	A	A	A	P	A	POST	0	N	3.412	
171	4216/11	57	F	R	IIIC	MRM	IDC - NOS	3	1	A	P	P	P	A	FREE	5	P	24.01	
172	4349/11	70	F	R	IIIB	TM	IDC - NOS	3.5	2	P	P	P	P	A	LAT,POST	0	P	1.002	N
173	4362/11	48	F	R	IIA	MRM	IDC - NOS	3	2	A	A	A	P	A	FREE	0	P	0.613	N
174	4371/11	33	F	R	IIIB	TM	IDC - NOS	5	2	P	P	P	P	A	POST	0	P	0.342	P
175	4371/11	33	F	L	IIIB	MRM	IDC - NOS	11	2	P	P	P	P	A	FREE	14	P	4.972	P
176	4378/11	37	F	L	IIIA	NAC+MRM	IDC - NOS	10	3	A	P	P	P	P	FREE	2	P	0.069	P
177	4430/11	38	F	L	IIIC	NAC+MRM	IDC - NOS	4	3	A	P	P	P	A	FREE	5	N	0.078	P
178	4511/11	65	F	R	IIIC	MRM	IDC - NOS	3	2	A	P	P	P	A	FREE	4	N	2.683	N
179	4552/11	60	F	L	IIA	NAC+MRM	IDC - NOS	2	2	A	P	A	A	A	FREE	1			
180	4555/11	40	F	R	IIIB	TM	IDC - NOS	6.5	2	A	P	P	A	P	SUP,MED,LAT,POST	0	P	6.744	P
181	4750/11	33	F	L	IIA	NAC+TM	IDC - NOS	2	2	A	P	A	P	A	FREE	2	N	9.008	N
182	4765/11	45	F	R	IIA	MRM	IDC - NOS	4	2	A	A	A	P	A	FREE	0	P	2.852	P
183	4807/11	68	F	L	IIB	MRM	IDC - NOS	4	2	A	P	A	P	A	FREE	2			
184	4930/11	47	F	L	IIIC	MRM	IDC - NOS	4	2	A	P	A	P	A	FREE	4	P	11.58	P
185	4988/11	60	F	R	IIIB	TM	IDC - NOS	7	3	P	A	A	P	A	FREE	0	P	0.011	P
186	4998/11	45	F	R	IIA	MRM	IDC - NOS	4	2	A	A	A	P	A	FREE	0	P	3.97	
187	5107/11	70	M	L	IIIA	MRM	IDC - NOS	7	3	A	P	P	P	A	POST,SUP,INF	6			
188	5147/11	75	F	R	IIIC	MRM	IDC - NOS	2	1	A	P	P	P	A	FREE	5			
189	5170/11	60	F	L	IIA	MRM	IDC - NOS	2	2	A	P	P	P	A	FREE	1			
190	5176/11	38	F	R	IV	NAC+MRM	IDC - NOS	17	2	P	P	P	P	A	POST	6			
191	5324/11	60	F	L	IIA	MRM	IDC - NOS	2.5	2	A	A	A	P	A	FREE	0			
192	5450/11	60	F	L	IIA	MRM	IDC - NOS	3	2	A	A	A	P	A	FREE	0	P	10.49	
193	5451/11	54	F	R	IIIC	MRM	METAPLASTIC CA	7		A	P	P	P	A	POST	6			
194	5536/11	49	F	R	IIB	MRM	IDC - NOS	2.5	2	A	P	P	P	A	FREE	2			
195	5598/11	58	F	L	IIIA	MRM	IDC - NOS	5	3	A	P	P	P	A	FREE	8	P	0.002	

196	5791/11	65	F	L	IIIA	MRM	IDC - NOS	3	1	A	P	A	A	A	FREE	10			
197	5814/11	50	F	L	I	MRM	IDC - NOS	1.5	1	A	A	A	P	A	FREE	0			
198	5858/11	50	F	L	IIIC	MRM	IDC - NOS	2	2	A	P	A	P	A	FREE	5			
199	5871/11	48	F	R	IIA	NAC+SM	IDC - NOS	3	2	A	A	A	A	P	FREE	0			
200	5949/11	55	F	R	IIIB	TM	IDC - NOS	12	3	P	P	A	P	A	FREE	0			
201	5988/11	48	F	L	IIA	NAC+MRM	IDC - NOS	2.5	2	A	P	P	P	A	FREE	0			
202	5992/11	55	F	L	I	MRM	PAPILLARY CA	1.5		A	A	A	A	A	FREE	0			
203	6051/11	51	F	R	I	MRM	IDC - NOS	1.5	1	A	A	A	P	A	FREE	0			
204	6157/11	50	F	R	IIB	MRM	IDC - NOS	4	2	A	P	A	P	A	FREE	1			
205	6162/11	45	F	L	IIIB	SM	IDC - NOS	7.5	2	P	A	A	P	A	POST	0			
206	6308/11	50	F	R	IIA	MRM	IDC - NOS	3	2	A	P	P	P	A	POST	0			
207	6524/11	42	F	R	IIIA	NAC+MRM	IDC - NOS	3	3	A	P	P	P	P	FREE	3	P	0.013	
208	6595/11	76	F	L	IIIA	MRM	IDC - NOS	5	1	A	P	P	P	P	FREE	3			
209	6609/11	47	F	R	IIA	MRM	IDC - NOS	4.5	2	A	A	A	P	A	FREE	0			
210	6646/11	54	F	R	IIIA	MRM	IDC - NOS	3	3	A	P	P	A	A	FREE	3			
211	6685/11	40	F	R	IIB	MRM	IDC - NOS	6	1	A	A	A	A	A	FREE	0			
212	6707/11	45	F	R	IIB	NAC+MRM	IDC - NOS	3	2	A	P	P	P	P	FREE	2			
213	6722/11	25	F	R	IIA	NAC+MRM	IDC - NOS	2	2	A	A	A	P	A	FREE	0			
214	6854/11	50	F	L	IIIB	NAC+MRM	IDC - NOS	15	3	P	P	P	A	A	POST	10	N	0.062	
215	6885/11	35	F	R	IIA	MRM	IDC - NOS	3	2	A	P	A	P	A	FREE	0			
216	6908/11	60	F	R	IIIA	MRM	IDC - NOS	3	1	A	P	P	P	P	FREE	3			
217	6973/11	43	F	R	I	MRM	APOCRINE CA	1.5		A	A	A	A	P	FREE	0			
218	7040/11	55	F	L	IIB	MRM	IDC - NOS	10	1	A	P	P	P	A	FREE	0			
219	7075/11	30	F	L	IIA	RT+NAC+MRM	IDC - NOS	1.5	3	A	P	P	P	A	FREE	2	P	0.025	
220	7231/11	45	F	L	IIIC	NAC+MRM	IDC - NOS	5	2	A	P	P	P	A	POST	7			
221	7274/11	50	F	R	IIA	NAC+MRM	IDC - NOS	1.5	2	A	P	A	P	P	FREE	2			
222	7278/11	45	F	R	IIIB	MRM	IDC - NOS	3.5	2	P	P	P	P	A	FREE	13			
223	7301/11	35	F	L	IIA	NAC+MRM	IDC - NOS	4	2	A	A	A	P	A	FREE	0			
224	7466/11	40	F	R	IIIC	MRM	IDC - NOS	3	2	A	P	P	P	A	FREE	5			
225	7495/11	65	F	L	IIIB	MRM	IDC - NOS	9	1	P	A	A	A	P	FREE	0	N	0.043	
226	7692/11	58	F	L	IIIB	MRM	METAPLASTIC CA	10		P	P	P	P	P	FREE	0			
227	7709/11	45	F	L	IIB	MRM	MEDULLARY CA	2.5		A	P	A	A	A	FREE	1			
228	7764/11	70	F	R	IIB	MRM	MUCINOUS CA	3.5		A	P	A	A	A	FREE	1			

229	7935/11	57	F	L	IIIB	MRM	IDC - NOS	4	2	P	A	A	A	P	FREE	0			
230	7947/11	60	F	L	IIA	MRM	IDC - NOS	3	1	A	P	P	P	P	FREE	0	N	3.2	
231	7973/11	40	F	L	I	MRM	IDC - NOS	2	2	P	A	P	A	A	FREE	0	N	14.62	
232	7977/11	63	F	L	IIIB	MRM	IDC - NOS	10	1	P	P	A	P	P	SUP,POST	3	P	1.003	
233	7989/11	55	F	R	IIIB	PSM	IDC - NOS	4	2	P	A	A	P	P	POST	0	N	16.36	
234	8044/11	55	F	R	IIIB	NAC+MRM	IDC - NOS	2.5	2	P	P	A	P	A	FREE	3			
235	8104/11	72	F	R	I	MRM	IDC - NOS	1.5	1	A	P	P	A	A	FREE	0	N	2.316	
236	8112/11	58	F	L	IIA	NAC+MRM	IDC - NOS	3	2	A	A	P	A	A	FREE	0			
237	8313/11	45	F	R	IIIB	NAC+MRM	IDC - NOS	3	2	P	P	P	A	A	POST	0			
238	8433/11	47	F	R	IIA	MRM	IDC - NOS	4	1	A	P	P	A	A	FREE	0			
239	8489/11	46	F	R	IIIB	NAC+MRM	IDC - NOS	1	2	P	P	A	A	A	FREE	1			
240	8544/11	56	F	L	IIB	NAC+MRM	IDC - NOS	5	1	A	P	P	A	A	FREE	2			
241	8672/11	42	F	L	IIA	MRM	IDC - NOS	3.5	1	A	A	A	P	P	FREE	0			
242	8740/11	37	F	L	IIB	MRM	IDC - NOS	4	1	A	P	A	P	A	FREE	1			
243	8758/11	50	F	R	IIIB	MRM	IDC - NOS	9	3	P	P	A	P	A	POST	1	P	0.031	
244	8794/11	58	F	R	IIIA	MRM	IDC - NOS	3	2	A	P	P	P	A	FREE	11			
245	8835/11	48	F	L	IIIB	MRM	IDC - NOS	2	1	P	A	A	A	A	FREE	0			
246	8848/11	45	F	R	IIA	MRM	IDC - NOS	3	2	A	A	A	P	A	FREE	0			
247	8849/11	50	F	R	IIIB	NAC+MRM	IDC - NOS	1	2	P	P	A	A	A	FREE	0			
248	8923/11	53	F	L	IIB	MRM	IDC - NOS	3	2	A	P	A	P	A	FREE	1			
249	9128/11	38	F	L	IIIC	NAC+MRM	IDC - NOS	5	3	A	P	P	P	P	FREE	5	P	0.24	
250	9218/11	58	F	L	IIIA	MRM	IDC - NOS	8	1	A	P	A	A	A	POST	2	N	1.1	
251	9311/11	50	F	R	IIIC	MRM	IDC - NOS	3	1	A	P	P	P	A	FREE	5			
252	9344/11	55	F	L	IIIB	TM	IDC - NOS	9	2	P	P	A	A	A	FREE	1			
253	9406/11	60	F	L	IIIB	NAC+MRM	IDC - NOS	2.5	1	P	P	P	P	A	FREE	2			
254	9492/11	35	F	R	IIA	MRM	IDC - NOS	4	1	A	P	A	P	A	FREE	0			
255	9552/11	46	F	L	IIIC	MRM	IDC - NOS	6	2	P	P	P	P	A	FREE	8			
256	9598/11	46	F	R	IIIB	NAC+MRM	IDC - NOS	6	2	P	P	P	P	A	POST	0			
257	9734/11	68	F	L	IIA	MRM	IDC - NOS	3	1	A	A	A	A	A	FREE	0			
258	9744/11	48	F	L	IIIA	MRM	IDC - NOS	2	2	A	P	P	A	P	FREE	3			
259	9852/11	60	F	R	IV	TM	IDC - NOS	12	2	P	P	P	P	A	POST	9			
260	172/12	48	F	R	IIIC	MRM	IDC - NOS	2.5	2	A	P	P	A	A	FREE	5			
261	306/12	40	F	R	IIIA	MRM	IDC - NOS	2.5	2	A	P	P	A	A	POST	3			

262	384/12	51	F	L	IIA	NAC+MRM	IDC - NOS	1	3	A	P	A	A	A	FREE	1			
263	474/12	48	F	L	IIIB	MRM	IDC - NOS	5	2	P	P	P	A	P	FREE	2			
264	689/12	45	F	R	I	MRM	IDC - NOS	1.5	1	A	A	A	P	A	FREE	0			
265	733/12	71	F	R	IIB	MRM	IDC - NOS	3.5	2	A	P	A	A	A	FREE	1			
266	792/12	56	F	L	IIA	NAC+MRM	IDC - NOS	2.5	3	A	P	P	A	P	FREE	0	N	0.017	
267	853/12	65	F	R	IIA	NAC+MRM	IDC - NOS	2.5	1	A	A	A	A	P	FREE	0			
268	948/12	45	F	R	IIA	MRM	IDC - NOS	4	3	A	A	A	P	P	FREE	0	P	0.114	
269	1156/12	48	F	R	IIA	MRM	IDC - NOS	2.5	2	A	P	P	A	P	FREE	0			
270	1164/12	39	F	R	IIA	MRM	NEUROENDOCRINE CA	4		A	A	A	A	P	FREE	0			
271	1249/12	48	F	R	IIIA	MRM	IDC - NOS	2	2	A	P	P	A	A	FREE	3			
272	1353/12	44	F	R	IIIB	MRM	APOCRINE CA	8		P	A	A	A	A	FREE	0			
273	1562/12	54	F	L	IIB	MRM	IDC - NOS	3	2	A	P	A	A	A	FREE	1			
274	1655/12	40	F	L	IIIA	MRM	IDC - NOS	3.5	2	A	P	P	A	A	FREE	3			

ABSTRACT

AIM:

The aim of the present study was to identify the incidence and distribution of breast carcinoma in patients admitted in the Government General Hospital, Chennai fromJanuary 2010 to February 2012 and to evaluate the expression of p53 by IHC and to study the E Cadherin and HoxD10 gene expression by PCR in invasive ductal carcinoma NOS and correlate the findings withhistological grade and other clinicopathologicalparameters.

MATERIALS AND METHODS:

20 cases of each grade from Invasive ductal carcinoma NOS subtype who had undergone mastectomy were randomly selected from thetotal cases and their representative formalin fixed paraffin embedded tissue samples were subjected to p53 immunohistochemical analysis and to HoxD10 & E Cadherin gene analysis by PCR.The results were correlated with histological grade and other clinicopathological features.

RESULTS:

P53 over expression and HoxD10 gene down regulation showed statistically significant association with high grade tumours. Though not statistically significant, CDH1 gene mutation was more frequent among tumours with

increasinggrade. The mean relative concentration of HoxD10 mRNA was lower in the p53 and CDH1 mutation positive group.CDH1 gene mutation was found to be more common among thep53 positive cases than the p53 negative cases.

CONLUSION:

Molecular analysis of breast carcinoma may serve as an important prognostic tool to predict patient outcome and for the development of targeted therapy in this new era of early cancer detection.

KEYWORDS: Breast carcinoma, p53, HoxD10, E Cadherin.