RESPONSE TO NEOADJUVANT CHEMOTHERAPY IN TRIPLE NEGATIVE BREAST CANCER FOLLOWING 4 CYCLES OF AC WITH CISPLATIN FOLLOWED BY 4 CYCLES OF TAXANES VS 4 CYCLES OF AC FOLLOWED BY 4 CYCLES OF TAXANES

M.S DEGREE EXAMINATION

BRANCH I - GENERAL SURGERY

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MAY 2020

CERTIFICATE BY THE DEAN

I hereby certify that this dissertation entitled **"Response to Neoadjuvant chemotherapy in triple negative breast cancer following 4 cycles of AC with Cisplatin followed by 4 cycles of taxanes vs 4 cycles of AC followed by 4 cycles of taxanes"** is a record of work done by **Dr.A.Sakthinesh**, in the Department of General surgery, Rajaji hospital, Madurai Medical College, Madurai, during his postgraduate degree course period from 2017- 2020. This work has not formed the basis for previous award of any degree.

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I solemnly declare that this dissertation titled **"Response To Neoadjuvant Chemotherapy In Triple Negative Breast Cancer Following 4 Cycles Of AC With Cisplatin Followed By 4 Cycles of Taxanes Vs 4 Cycles of AC Followed By 4 Cycles of Taxanes"** submitted by me for the degree of M.S, is the record work carried out by me during the period of 2017-2020 under the guidance of **Prof. Dr.D.MARUTHUPANDIAN, M.S., FICS., FAIS.,** former Professor of General Surgery & **Prof. Dr.V.SELVARAJ, M.S,DCH.,** Professor of General Surgery, Department of General Surgery, Madurai Medical College, Madurai. The dissertation is submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai, towards the partial fulfilment of requirements for the award of M.S. Degree (Branch I) General Surgery examination to be held in May 2020.

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CONTENTS

INTRODUCTION

Breast cancer a devastating illness for tens of thousands of women around the world, Most common non-skin cancer in women worldwide with an estimated 1.68 million new cases, breast cancer make it a leading cause of death in women aged 35 to 55 years [1], Accounts for 23% of all cancers in women and responsible for 20% of cancer related death in women [2,3]. In India, it is the most common cancer reported from urban cancer registries, for about 30% of all cancers in females [4].Majority of the cases present at a younger age and with advanced disease [5]. Appears to be relatively on the increase due to late marriage, birth of the first child at a later age, short period of breast feeding [6].In India mostly 2/3rd of cancers detected are locally advanced disease [7]. Locally advanced breast cancer encompass a heterogenous group of patient that includes those with a neglected slow growing tumour as well as those with aggressive disease. LABC is relatively uncommon presentation in developed world accounting for only 5 to 20%. But in the developing world like India it constitutes about 50% of the cases. Aggressive local treatment such as surgery or radiotherapy did little to improve the survival rates, but resulted in increased complications [8,9,10,11]. Predominant pattern of failure in LABC is development of distant metastasis . With the development of systemic therapy to address micrometastasis, chemotherapy is routinely incorporated in the treatment of LABC [8,9,10,11]. It can be given

preoperatively/post operatively. Breast cancer is a complex and heterogeneous disease comprising of a various biological entities, that are classified based on specific morphological appearances, immune histochemical features and clinical behaviour. In recent years, it has become evident that this diversity is the result of genetic alterations.[12] The extensive analysis of gene expression profiles of breast cancers using DNA microarrays has led to the classification of breast cancer into molecular subtypes which have distinct clinical features, with markedly differing prognoses and clinical outcomes.[13] Based on the study of gene expression profiles, breast cancers are divided into five molecular subtypes: Luminal A(ER+), Luminal B (ER+),basal-like, normal breast-like and human epidermal growth factor 2 (HER2) overexpressing subtype.[14,15] Within these five subtypes, basal like 2 breast cancer is characterized by absence of expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor 2 (HER2). It is associated with aggressive histomorphology, poor prognosis and unresponsiveness to the hormonal chemotherapy, shorter survival and BRCA1 association. Although triple negative breast cancer (TNBC) is universally used as a surrogate marker, triple negative and basal like are not synonymous.[16] Triple-negative breast cancer (TNBC) is defined by the lack of expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor 2 (Her2*neu*) expression and associated with aggressive clinical course and poor prognosis. Two

subtypes of triple-negative breast cancers have been described: basal and nonbasal.5 Triple-negative breast cancer(TNBC) accounts for 10-17% of all breast cancers with increased incidence in premenopausal women.[17] Basal type TNBC characteristically exhibits high histological grade, p53 mutation, in most instances expresses basal cytokeratin (CK5/6, CK14 and CK17), epidermal growth factor receptor (EGFR) overexpression, is significantly associated with Ki-67 labelling index, p53 expression, and BRCA1 expression, and show a shorter survival than nonbasal type. Gene expression profiling is the gold standard for the identification of basal type TNBC. Since GEP is costly, cumbersome to perform and requires fresh or frozen samples, it is still not feasible to perform GEP in low resource settings. Hence Rakha et al. suggested the use of immunohistochemical surrogates panel for the identification of basal-like subtype of TNBC. The panel includes four markers – ER, her2, cytokeratin 5/6 and epidermal growth factor receptor EGFR which identifies basal-like TNBC with 100% specificity and 76% sensitivity.[18] Patients with TNBC derive no benefit from molecularly targeted treatments such as endocrine therapy or trastuzumab, because they lack the appropriate targets for these drugs. Although TNBC is characterized by aggressive behavior, it is particularly sensitive to cytotoxic chemotherapy (the so-called 'triple negative paradox')[19]. In the neoadjuvant setting, TNBC patients have higher response rates to standard chemotherapy when compared with women affected by hormone

receptor-positive breast cancer. Approximately 30%–40% of TNBC patients achieve a pathological complete response (pCR) after standard anthracycline plus cyclophosphamide- and taxanebased neoadjuvant chemotherapy [20]. The achievement of pCR in TNBC patients has a strong prognostic value, larger than in other breast cancer subtypes [21,22]. Therefore, neoadjuvant chemotherapy is currently considered the preferred approach for the majority of TNBC patients with early-stage disease [23]. Platinum agents (i.e. carboplatin and cisplatin) are cytotoxic DNA damaging compounds leading to DNA strand breaks and possible consequent cell apoptosis; this peculiar mechanism of action makes them specially active in cancer cells with DNA repair deficiency such as those harbouring deleterious mutations in the BRCA genes[24.] Based on the biological rationale for a heightened susceptibility of TNBC to DNA-damaging compounds [25] , several trials have investigated the possible role of platinum agents as a treatment option in TNBC patients. Although some of these studies have suggested a possible benefit for platinum-based neoadjuvant chemotherapy in TNBC patients .The primary goal of this study is to assess the efficacy of cisplatin as neoadjuvant chemotherapy in TNBC based on the pathologic complete response [pCR]), progression free survival (PFS), site-specific distribution of recurrence, post recurrence survival (PRS), and overall survival (OS).

AIM OF THE STUDY

The aim of this study is to assess the response to neoadjuvant chemotherapy in TNBC following 4 cycles of AC with Cisplatin followed by 4 cycles of Taxanes versus 4 cycles of AC followed by 4 cycles of taxanes to study the efficacy of cisplatin as neoadjuvant chemotherapy in TNBC based on the objective clinical and pathologic complete response [pCR]).

REVIEW OF LITERATURE

Anatomy of the breast

Embrology [26,27]

At sixth week of the fetal development, two ventral bands of thickened ectoderm are evident in the embryo. In mammals paired breasts develop along these ridges extending from base of the forelimb to the region of the hind limb. These ridges are not prominent and disappear after a short time, except for small portions that persist in the pectoral region.The breast develops when an ingrowth of ectoderm forms a primary tissue bud in the mesenchyme. The primary bud initiates development of 15 to 20 secondary buds. Epithelial cords develop from the secondary buds extending into the surrounding mesenchyme. Major ducts open into shallow mammary pits and the proliferation of mesenchyme transforms mammary pit into nipple.

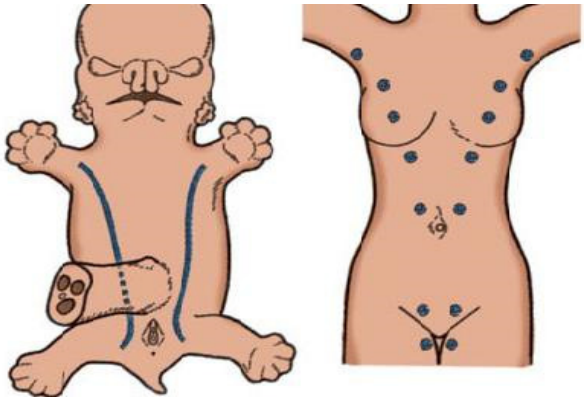


Fig: Milk line

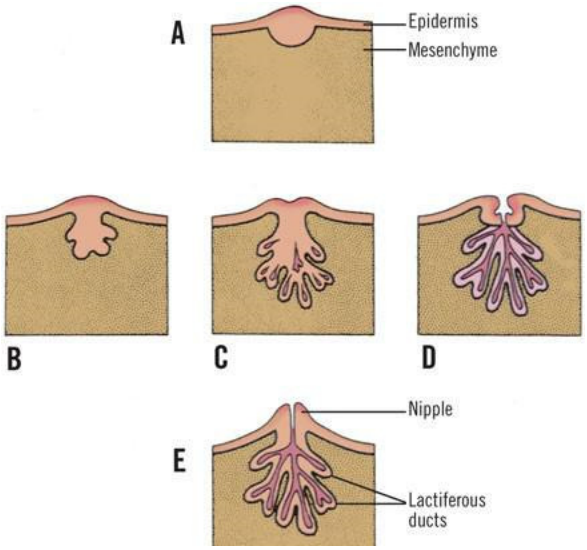


Fig: Development of Secretory unit

Gross anatomy[26,27]

Breast composed of 15 to 20 lobes and each composed of several lobules. Fibrous bands of connective tissue travels through the breast inserting perpendicularly into the dermis and it is called the suspensory ligaments of Cooper and provides structural support. In females mature breast extending from the level of infra mammary fold at the sixth or seventh rib and extends transversally from lateral border of the sternum till the anterior axillary line. The deep surface formed by the fascia of the pectoralis major, serratus anterior, external oblique abdominis, and the upper part of the rectus sheath. Axillary tail of Spence extending laterally across the anterior axillary fold. The upper outer quadrant of the breast has greater volume of the tissue than any other quadrant. Epidermis of the

nipple areola complex of breast is pigmented , variably corrugated, during puberty the pigment becomes darker, the nipple assumes an elevated configuration and during pregnancy the areola enlarges and pigment becomes further enhanced. Areola of the breast contains sebaceous glands, sweat glands and accessory glands. The dermal papillae at the tip of nipple contains sensory nerve endings and meissner's corpuscles. Smooth muscle bundle fibres lying circumferentially and longitudinally along the major ducts, and are responsible for the nipple erection.

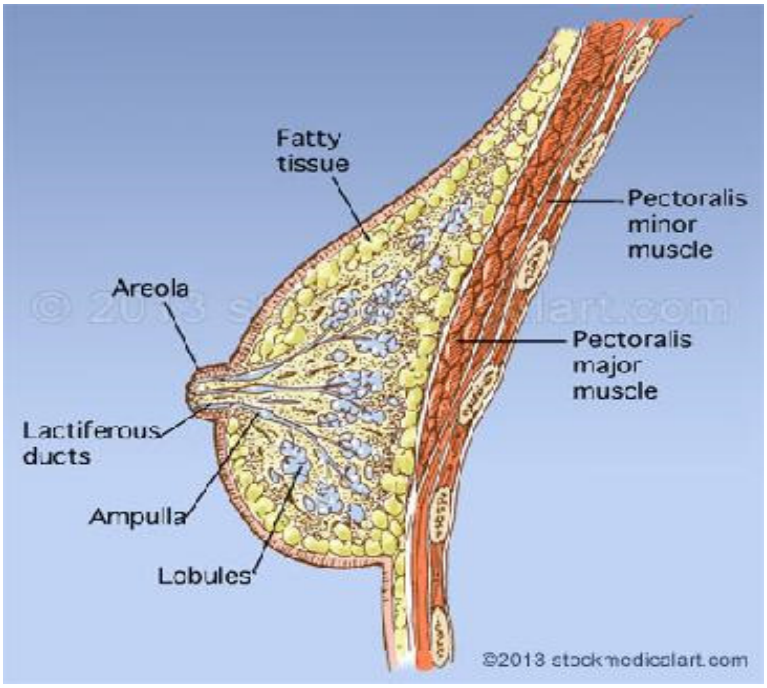


Fig: Anatomy of breast

Blood supply[28,29,30,31,32]

Blood supply of breast

a) Perforating branch of the internal mammary artery

b) Lateral branch of posterior intercostals arteries

c) Branches from the axillary artery including lateral thoracic and pectoral branches of the thoraco acromial artery.

The second, third, 4th anterior intercostals perforators and branches of the internal mammary artery. Lateral thoracic artery give rise to lateral mammary branches. Veins of the breast follows the course of the artery with the venous drainage towards axilla. Three principal groups of veins are perforating branches of

a) Internal thoracic vein

b) Posterior intercostals veins

c) Axillary veins

The vertebral plexus of veins called as Batson's plexus: It provides second route for the spread of breast carcinoma via veins. This venous plexus surrounds the vertebra extending from the base of skull to the sacrum. These veins do not have valves and making blood to flow through them in either direction. This venous communication is important in the breast as the posterior intercostals arteries are in direct continuity

with vertebral plexus. This potential pathway is the cause for metastasis of breast cancer to the vertebrae, skull, pelvic bone, lungs and the CNS.

Innervation of the breast.[33]

Lateral cutaneous branches of third through the 6th intercostals nerve supplies sensory inervation to the breast and the anterolateral chest wall and the anterior branch of the supraclavicular nerve supplies limited area of the skin over the upper part of breast.

Lymphatic drainage[34,35]

Six axillary lymph node groups

(a) Axillary vein group (lateral group)- consist of 4 to 6 lymph nodes, lying medial to axillary vein, receiving most of the lymph drainage from the upper extremity.

(b) External Mammary group: (Anterior or pectoral group) : consist of 5 to 6 lymph nodes , lying along the lower border of pectoralis minor muscle, receiving most of the lymph drainage from the lateral aspect of the breast.

(c) Scapular group: (Posterior or subscapular) : consists of 5 to 7 lymph nodes lying along posterior wall of the axilla, receiving lymph drainage from the lower posterior neck, posterior trunk and posterior shoulder.

(d) Central Group: consists of 3 to 4 lymph nodes lying immediately posterior to the pectoralis minor muscle, receiving lymph drainage from the axillary vein, external mammary and directly from the breast.

(e) Sub clavicular group(Apical): consists of 6 - 12 lymph nodes lying superior and posterior to upper part of the pectoralis minor, receiving lymph drainage from rest of the remaing group of axillary lymph nodes.

(f) The interpectoral group: (Rotter's) : consists of 1 to 4 lymph nodes, found between the pectoralis major and pectoralis minor muscle, receiving lymphatic drainage directly from the breasts. From interpectoral group of lymphnodes lymph fluid directly passes to the central and apical group of lymphnodes.

The axillary lymphnodes based on their relationship with the pectoralis minor muscle.Lymph nodes located lateral and below the lower border of the pectoralis minor muscle-level 1 consisting external mammary, axillary vein and scapular group of lymph nodes. Lymph nodes located posterior to the pectoralis minor muscle - level 2, including central and interpectoral group of lymph nodes. Lymph nodes located above and medial to the upper border of pectoralis minor muscle - level 3, including subclavicular group of lymph nodes.

Lymph flow: The plexus of lymph vessels in the breast arises from the inter lobular connective tissue and in walls of lactiferous ducts, communicating with subareolar plexus of lymph vessels. Efferent lymph vessels from breast pass around the lateral edge of pectoralis major, piercing the clavipectoral fascia continued as external mammary group of lymph nodes. Upper part of the breast few lymph vessels directly pass to the sub clavicular group of lymph nodes. Axillary lymph nodes receives more than 75% of the drainage from the breast and rest derived primarily from the medial aspect of the breast, flows through the lymph vessels that accompany the perforating branches of the internal mammary artery and enters the internal mammary group of lymph nodes.

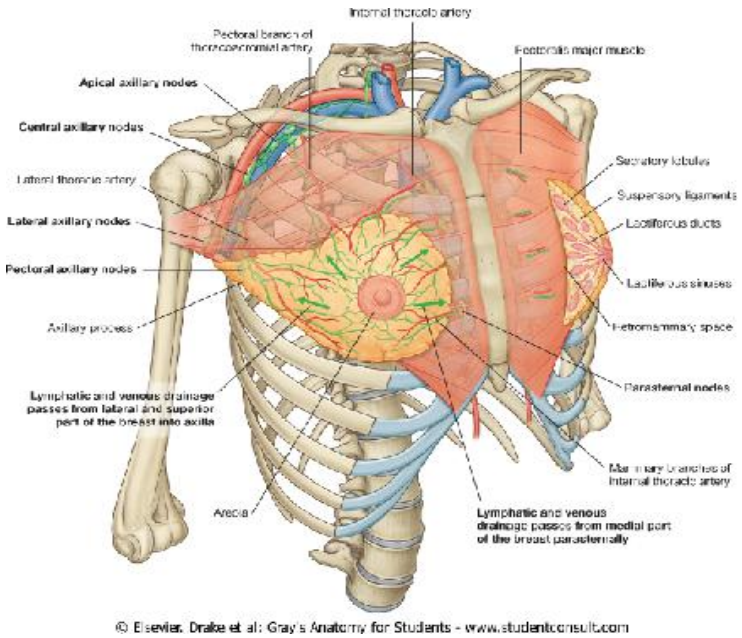


Fig: Relations of breast

CARCINOMA OF BREAST

Most common cancer among women in world[36].The increased utilization of mammography, resulted in an increase in the detection of breast cancers at earlier in the western countries. Eventhough there is a reasonable reduction in the breast cancer mortality in the developed countries due to the early diagnosis and advances in the treatment,but still there is rise in most of the Asian countries.[37]

EPIDEMIOLOGY

Breast cancer is the leading cause of cancer deaths across the world with more than one million cases annually.[37] It is now regarded as the most common cancer both in developed and developing regions with 690,000 new cases estimated in each region of the world. In India breast cancer in female is the most common cancer with an age-standardized incidence rate of 30.8 per 100000 population.[38]

CLINICAL PRESENTATION [39]

A large number of breast cancer cases present symptomatically. Recently, widely used screening methods including mammography, the incidence of asymptomatic cases has come to a rise. Symptomatic women present most commonly with breast lumps (60-70%), followed by pain (14- 18%), nipple abnormalities like retraction and discharge (7-9%).

Family history seen in about 3-14% cases. The breast tumours mostly involve upper outer quadrant of the breast (40-50%), followed by central, upper inner, lower outer and lower inner quadrant. All symptomatic breast diseases should be assessed using triple assessment including (i)clinical examination, (ii) imaging studies(mammography & ultrasound) and (iii)tissue sampling (fine needle aspiration cytology or core needle biopsy).

RISK FACTORS FOR BREAST CANCER

AGE [40]

Breast cancer rare in young age women below 25 years except some familial cases. Although breast cancer rare in young age, half of those affected are either ER negative or HER2/*neu* positive in the Western population. About 77% of breast cancers occur in women over 50 years of age, incidence rises throughout the lifetime of a woman.

AGE AT MENARCHE AND MENOPAUSE [41,42,43]

Early age at menarche has been associated with increased risk of breast cancer. Late menopause occurring after the age of 55 years doubles the risk of breast cancer than those attaining menopause before the age of 45 years.

AGE AT FIRST CHILDBIRTH AND PARITY [44]

Childbearing at younger age has associated with decreased risk of breast cancer. First childbirth at age of <30 years and multiple pregnancies has been associated with reduced risk of breast cancer. Relative risk of developing breast cancer estimated to increase by 3% for each year. The risk of breast cancer has been reduced by 7% with each full term delivery, overall multiparous women have a 30% lower risk of developing breast cancers than nulliparous women.

RACE AND ETHNICITY [45,46]

The incidence of mortality is more in African –American women presenting at a more advanced stage at time diagnosis when compared to white women. Large number of breast cancer cases diagnosed in black women were less than 40 years of age, the breast tumours occurring in black women exhibit higher nuclear grade, usually do not express hormone receptors, and have sporadic mutations in p53 gene.

BREAST FEEDING [43]

Breast feeding has been associated with reduction in the risk of breast cancer. The longer duration of breastfeeds, the greater is the protection against breast cancer, and the risk is reduces by 4% for every 12 months of breastfeeding. The risk of breast cancer in women who had

breastfed for more than two years was 33% lower than those females who had never breastfed.

PROLONGED EXPOSURE TO EXOGENOUS ESTROGEN [40]

Women undergoing hormone replacement therapy with combined estrogen and progestin shows an increased risk of developing breast cancer.

RADIATION THERAPY [47]

Ionized radiation forms an established risk factor for breast cancer. The risk of developing breast cancer was the highest in women who had increased number of exposures and at a younger age.

PREVIOUS BREAST DISEASE [48]

Women with history of benign breast diseases have increased risk of developing breast cancer. Women who had proliferative disease without atypia on biopsy specimens have a two-fold increase and proliferative disease with atypia have a five-fold increase in risk of breast cancer development.

GENETIC AND FAMILY HISTORY

Women having two or more first-degree relatives are affected at early age and having four-fold increase in the risk of development of

breast cancer. Several studies shown link between risk of breast cancer development and inherited mutations in many genes like BRCA1, BRCA2, p53, PTEN and ATM. The breast cancer genes, BRCA1, located on the long arm of chromosome 17 and BRCA2, located on the long arm of chromosome 13, identified and mutations in these genes have been the established risk factors for breast cancer. Inherited mutations in p53 and PTEN genes, associated with familial syndromes Li-Fraumeni and Cowden are at high risk of developing breast cancer[49].

OBESITY

Associated with increased incidence of breast cancers, risk is greater in postmenopausal women than in premenopausal women[50].

DIET

Several studies suggested that increased consumption of dietary fat is associated with an increase in breast cancer risk[51].

SMOKING AND ALCOHOL CONSUMPTION

A meta-analysis study found that there is higher risk in women started smoking at young age, before the age of 20 and before the birth of their first Child[52]. Alcohol intake associated with increased risk of breast cancer and the risk is dose-dependent[53].

PHYSICAL ACTIVITY

Several studies showed strong association between physical activity and breast cancer, about 15-20% reduction of risk of developing breast cancer in the most active women and strongest association shown for postmenopausal women[54].

INVASIVE CARCINOMA OF BREAST

Microscopically classified into various special types based on the particular pattern they exhibit. Approximately 75% of the carcinomas don't represent any special types and labelled as invasive ductal carcinoma, not-otherwise-specified (NOS) type.[37]

WHO CLASSIFICATION OF INVASIVE CARCINOMA OF BREAST

1. Invasive ductal carcinoma

a. Pleomorphic carcinoma

b. Carcinoma with melanotic features

c. Carcinoma with choriocarcinomatous features

d. Carcinoma with osteoclast like giant cells

2. Invasive lobular carcinoma

a. Classic lobular carcinoma

b. Solid lobular carcinoma

c. Alveolar lobular carcinoma

d. Pleomorphic lobular carcinoma

e. Tubulolobular lobular carcinoma

f. Mixed lobular carcinoma

3. Tubular carcinoma

4. Cribriform carcinoma

5. Carcinoma with medullary features

a. Medullary carcinoma

b. Atypical medullary carcinoma

c. Invasive carcinoma NST with medullary features

6. Metaplastic carcinoma of no special type

a. Low-grade adenosquamous carcinoma

b. Fibromatosis-like metaplastic carcinoma

c. Squamous cell carcinoma

d. Spindle cell carcinoma

e. Metaplastic carcinoma with mesenchymal differentiation

i. Chondroid differentiation

ii. Osseous differentiation

iii. Other types of mesenchymal differentiation

f. Mixed metaplastic carcinoma

g. Myoepithelial carcinoma

7. Mucinous carcinoma

8. Carcinoma with signet-ring-cell differentiation
9. Carcinoma with neuroendocrine features
10. Carcinoma with apocrine differentiation
11. Invasive papillary carcinoma
12. Invasive micropapillary carcinoma
13. Adenoid cystic carcinoma
14. Mucoepidermoid carcinoma
15. Salivary gland/skin adnexal type tumours
16. Polymorphous carcinoma
17. Inflammatory carcinoma
18. Bilateral breast carcinoma and non-synchronous breast carcinoma
19. Exceptionally rare types and variants

    Secretory carcinoma

    Oncocytic carcinoma

    Sebaceous carcinoma

    Lipid-rich carcinoma

    Glycogen-rich clear cell carcinoma

    Acinic cell carcinoma [55]

1. INVASIVE DUCTAL CARCINOMA

Invasive ductal carcinoma of NOS type, a heterogeneous group of tumours not having characteristics specific to any special type of

carcinoma to be classified as special types such as tubular, mucinous, medullary or lobular carcinoma. Most common type of infiltrating breast cancers constituting about 47-75% of the cases.[56] and considered to be the prototype of all breast cancers.[57]

GROSS FEATURES

Grossly, the tumour varies in size, shape and presents as ill circumscribed, firm to hard mass. The cut surface is greyishyellow with stellate outline formed by the trabeculae radiating into the surrounding fat. In larger tumours, there may be areas of necrosis, cystic degeneration and haemorrhage. The cut surface of these tumours, show few 'chalky streaks', due to the duct elastosis.

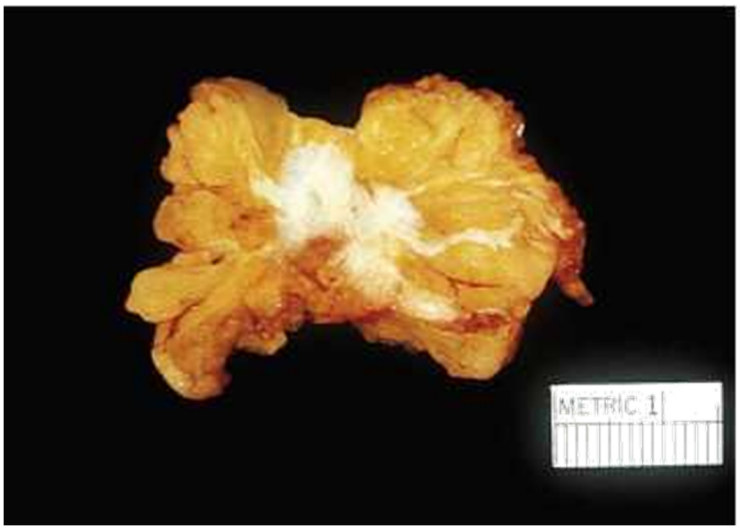


FIG. 1(A) - INVASIVE DUCTAL CARCINOMA- CUT SURFACE SHOWING WHITISH IRREGULAR MASS WITH CHALKY STREAKS.

MICROSCOPY

Microscopically, the tumour cells grow in different patterns like diffuse sheets, cords, nests, trabeculae and even as single cell. Glandular differentiation may be marked, focally present or totally absent. The tumour cells are large, pleomorphic, with multinucleated tumour giant cells, showing characteristic features of malignancy with prominent nuclei and nucleoli and numerous mitotic figures. About 60% cases show areas of necrosis. Focal areas of squamous, apocrine metaplasia or clear cell change may be present. The amount of stroma varies from empty to abundant. The stroma ranging from fibrotic to desmoplastic in appearance, with desmoplastic stroma being found in most of the cases. Stromal elastosis and foci of periductal and perivenous elastosis may also be present. Calcifications in the form of both coarse or fine granules and rarely psammoma bodies are seen in about 60% of the cases. The interphase between tumour and stroma, often shows lymphoplasmacytic inflammatory infiltrate. Fisher et al., found angioinvasion, perineural invasion and lymphatic invasion in 33%, 28% and 5% respectively.[37] In up to 80% of cases, foci of in situ component may be present, however, the relative proportion of invasive carcinoma and in situ component may largely vary in individual cases.[56]

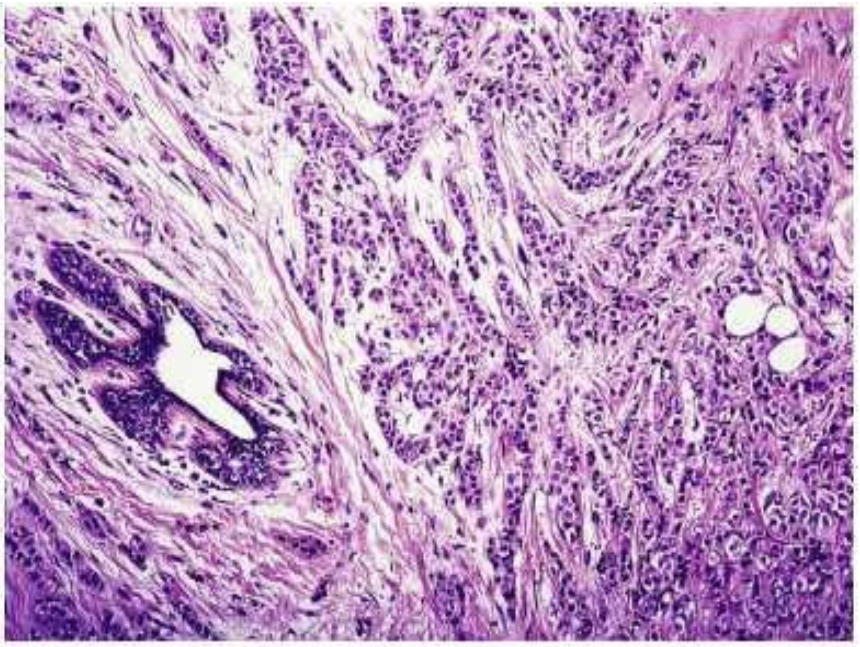


FIG. 1(B): PROTOTYPICAL INVASIVE DUCTAL CARCINOMA

2. INFILTRATING LOBULAR CARCINOMA

Infiltrating lobular carcinoma is the second most common type of breast cancer constituting to about 5-15% of the cases.[56]

HISTOLOGIC VARIANTS

CLASSIC FORM

The malignant cells are small, less pleomorphic and are arranged singly, in a linear single file pattern, narrow trabeculae within the stroma. It may also show a 'pagetoid'/ concentric growth pattern around the lobules exhibiting lobular neoplasia in situ. Often, the stroma appears dense and fibrous with periductal and perivenous elastosis.[37]

PLEOMORPHIC VARIANT

The growth pattern of this variant is similar to that of classic form with lack of cohesion. The tumour cells exhibit nuclei that are highly pleomorphic, anaplastic and show high mitotic rate. It sometimes, shows apocrine differentiation and signet ring morphology.[37]

SOLID VARIANT

It accounts for about 10% of lobular carcinoma cases. The tumour cells are similar to classic type, but are arranged in diffuse sheets rather than single files with very less amount of intervening stroma.[56]

ALVEOLAR VARIANT

It is an uncommon variant of lobular carcinoma in which the tumour cells with typical lobular features are arranged in globular clusters of 20 or more cells in each cluster.[56]

TUBULOLOBULAR VARIANT

In this variant, the overall infiltrative pattern of lobular carcinoma cells is admixed with small tubules showing minute or undetectable lumen. Often, there is presence of in situ component, which may be of ductal, lobular or mixed type.[37]

HISTIOCYTOID CARCINOMA

It is composed of tumour cells growing in a diffuse pattern with individual cells showing abundant, granular and foamy cytoplasm. It is currently considered a variant of invasive lobular carcinoma with apocrine differentiation.[37]

IHC

About 80-95% of invasive lobular carcinomas are positive for ER and 65-75% is positive for PR. 70-75% of the tumours are positive for both ER and PR. About 85-90% of invasive lobular carcinoma cases show loss of E-cadherin expression.[58]

3. TUBULAR CARCINOMA

Tubular carcinoma is an uncommon histologic type of breast carcinoma. It accounts for about 1-4% of all breast malignancies.[55]

GROSS

Macroscopically, tubular carcinoma is indistinguishable from the invasive ductal carcinoma. It has poorly circumscribed margins and hard in consistency. Characteristically, the size of the tumour is small ranging from 2mm to 1.5 cm. Most tumours are less than 1 cm in size.

MICROSCOPY

Microscopically, it is difficult to differentiate from benign conditions like radial scar and microglandular adenosis due to the well differentiated appearance of tubular glands, scarcity/absence of pleomorphism, necrosis and mitoses. The tubules are arranged haphazardly in the stroma and are often angulated. The stroma is often cellular and desmoplastic in nature. The tubules may show intraluminal basophilic secretions, apocrine snouts in the apical cytoplasm. The tumour may infiltrate into the adjacent adipose tissue, at the periphery.

The term 'tubular carcinoma' is best reserved for the tumours showing at least 90% of tubular pattern.[59] These tumours show a favourable prognosis. DCIS is frequently present in association with tubular carcinoma. The in situ component is usually of low grade showing cribriform or papillary pattern.[60] The tubular carcinoma can be seen associated with invasive ductal carcinoma, NOS type or sometimes with invasive lobular carcinoma, which leads to dilemma in making a diagnosis. In such instances, the term tubular NOS and tubular mixed can be employed. Mixed tubular carcinomas are associated with increased tumour size, increased nodal metastases and worse clinical outcome.[61]

Immunohistochemically, these tumours are 90% of these tumours are positive for ER, 70-80% are positive for PR and rarely they show HER2 overexpression.

4. CRIBRIFORM CARCINOMA

Invasive cribriform carcinoma is a relatively rare neoplasm of breast which is similar to tubular carcinoma and shares a favourable prognosis with it. This tumour shows a cribriform appearance of the invasive component, reminiscent of the in situ counterpart, which is frequently associated with it. Cribriform pattern is often associated with tubular formations, but the relative proportion of both determines the terminology of the tumour, as proposed by Page et al.,[37]

5. MUCINOUS CARCINOMA

Mammary mucinous carcinomas are also described as colloid, mucoid, or gelatinous carcinomas. It is characterized by the production of abundant mucin, both intracellular and extracellular.

Macroscopically, these tumours are characteristically sharply circumscribed, soft tumours with gelatinous and glistening cut surface.

Microscopically, the tumour cells are arranged in clusters and islands composed of 10-20 cells in lakes of extracellular mucin. The mucin stains positive with mucicarmine, MUC2 and MUC6. The term

mucinous carcinoma is employed only when at least 90% of the tumour shows mucinous appearance.[62]

Immunohistochemically, these tumours are always positive for hormone receptors whereas HER2neu is almost always negative.[63]

6. CARCINOMA WITH SIGNET-RING CELL DIFFERENTIATION

Signet-ring cell carcinoma is a type of malignancy in which the cells show abundant intracellular mucin which pushes the nucleus to one side of the cell, resembling the characteristic signet-ring appearance. It is important that primary invasive breast carcinomas with signet-ring cell morphology to be differentiated from metastases from other organs.[62]

7. CARCINOMA WITH MEDULLARY FEATURES

The tumours of the breast which show medullary features are typically associated with triple negative phenotype i.e., lack of expression of ER, PR and lack of her2 amplification. These tumours have been found to be associated with carriers of BRCA1 germ line mutation, which shows worse prognosis. The medullary-like carcinoma is associated with expression of basal cytokeratins, which leads to them being classified as basal-like breast cancers.[64]

Grossly, these tumours are well circumscribed and have soft and uniform consistency.

Microscopically, there are three major morphologic criteria, for a tumour to be classified as medullary-like carcinoma. They are, more than 75% of the tumour cells to be arranged in syncytial network, absence of fibrosis, prominent stromal lymphocytic infiltrates, lack of gland formation, and well circumscribed tumour with pushing margins rather than infiltrative margins. The cells have abundant cytoplasm, pleomorphic vesicular nuclei with one or more prominent nucleoli. Mitotic rate is high with numerous giant mitoses.[56]

8. METAPLASTIC CARCINOMA

Metaplastic carcinoma represents a group of rare primary breast malignancies, which exhibit either an admixture of epithelial and mesenchymal components including chondroid, osseous, spindle cell and rhabdomyoid cells, or purely the mesenchymal elements. Immunohistochemically, metaplastic carcinomas have been found to be negative for ER, PR, her2neu expression and they express basal cytokeratins like CK5/6 and EGFR.[62]

9. INVASIVE PAPILLARY CARCINOMA

Invasive papillary carcinoma of the breast is a very rare neoplasm, constituting about <1% of all breast malignancies.[59] The major differential diagnosis include papillary carcinoma in situ, encysted papillary carcinoma and benign papilloma with ductal carcinoma in situ.[56]

The diagnostic criteria include the presence of papillary pattern of tumour cells constituting more than 90% of the tumour area. Microscopically, the invasive component of the tumour shows papillary architecture with fibrovascular cores lined by malignant epithelial cells.[62]

10. INVASIVE MICROPAPILLARY CARCINOMA

Pure micropapillary carcinoma is an extremely rare tumour, constituting about <1% of all the tumours of breast. The histopathological appearance is distinctive, with formation of pseudo papillary structures without the fibrovascular cores and tubular structures floating in clear empty spaces. The tumour cell clusters exhibit a distinctive 'inside out' appearance due to the inversion of polarity which can be evidenced by the pattern of MUC staining. These tumours have increased propensity for lymphatic invasion with nodal metastases and high local recurrence and are associated with a poorer prognosis.[65]

Immunohistochemistry and genomic studies have identified that majority of invasive micropapillary carcinomas express ER and have led to the classification of these tumours as luminal A or B subtype.

11. CARCINOMA WITH APOCRINE DIFFERENTIATION

It is a very rare tumour that constitutes about 0.5% of invasive breast carcinomas. Apocrine differentiation has been found in association with invasive carcinoma NST and various special type carcinomas including lobular, tubular, papillary and medullary carcinomas. [55]

Microscopically, it is characterized by cells with abundant granular eosinophilic cytoplasm which is positive for PAS and diastase resistant or cells with foamy cytoplasm due to intracellular lipid or both. The nuclei are enlarged with prominent nucleoli.

Immunohistochemical staining shows characteristic ER and PR negativity and HER2 positivity. They are positive for androgen receptor and GCDFP-$_{15}$.66,67

12. SECRETORY CARCINOMA

Secretory carcinomas are exceptionally rare in the breast and they account for less than 0.15% of all breast cancers. It is primarily found in children and young adults.[55] Grossly, the tumour is usually small and well-circumscribed. Microscopically, it shows three patterns including

solid, tubular and microcystic. The presence of both intracellular and extracellular rounded vacuoles of varying sizes containing secretory material which stains positive with alcian blue and PAS with diastase digestion is diagnostic of secretory carcinoma.

Immunohistochemically, secretory carcinomas are usually triple negative that is ER, PR and HER2 negative along with p63 negativity.[68]

13. CARCINOMA WITH NEUROENDOCRINE FEATURES

Primary neuroendocrine tumours of the breast constitute for about 1-4% of all invasive breast malignancies. They are designated as neuroendocrine carcinomas when the tumours express neuroendocrine markers in more than 50% of its cell population.[55]

Histopathologically, these tumours are classified as well-differentiated neuroendocrine carcinomas, poorly differentiated/ small cell carcinoma, invasive breast carcinoma with neuroendocrine differentiation.

Immunohistochemical staining of these tumours shows expression of chromogranin, synatophysin and neuron specific enolase. Electron microscopy demonstrates the presence of dense core granules.[56]

14. SPREAD RELATED VARIANTS: INFLAMMATORY CARCINOMA

The term inflammatory carcinoma is entirely based on the characteristic clinical features in which the breast appears warm, red and shows edema of the overlying skin, reminiscent of mastitis. The appearance of inflammatory carcinoma is essentially due to the carcinomatous involvement of dermal lymphatic channels. The occurrence of clinical inflammatory appearance may or may not show microscopic dermal lymphatic invasion by tumour cells and its reverse is also possible, wherein the tumour showing histopathologic permeation by tumour cells, of the dermal lymphatic vessels may or may not have a clinical inflammatory carcinoma.

The presence of microscopic dermal lymphatic invasion is a sign of poor prognosis, irrespective of whether the patient has clinical features of inflammatory carcinoma or not.[37]

According to a study by Charafe-Jauffret et al., inflammatory carcinoma is found to be ER negative and positive for E-cadherin, MIB1, MUC1 and HER2neu.

PAGETS DISEASE

Paget disease of the nipple is a crusted lesion of the nipple that is almost always associated with high grade ductal carcinoma in situ, with or without stromal invasion. It occurs in about 2 % of all breast cancers, clinically presenting as an eczematous or erythematous ulcerating rash of the nipple. Clinically, it is indistinguishable from benign eczematous dermatitis. Hence, these patients should be thoroughly examined for breast malignancies.

Microscopically, the epidermis shows single or small clusters of large pleomorphic cells with abundant, often clear cytoplasm, usually in the basal layer. These cells stain positive for PAS positive diastase-resistant mucin.

Immunohistochemistry shows EMA, CEA, MUC1, CK7 and HER2 positivity, whereas the tumour cells are negative for high molecular weight cytokeratins and melanoma specific markers such as S-100, HMB45 and MELAN-A.[37]

PROGNOSTIC AND PREDICTIVE FACTORS IN BREAST CANCER

1. SIZE OF THE TUMOR

The gross tumour size is one of the most significant independent prognostic factors in breast cancers. With increasing size of the tumour,

the incidence of axillary nodal metastases increases and the survival decreases.

T1 Tumour≤20 mm in greatest dimension

T1mi Tumour≤1 mm in greatest dimension

T1a Tumour>1 mm but ≤5 mm in greatest dimension

T1b Tumour>5 mm but ≤10 mm in greatest dimension

T1c Tumour>10 mm but ≤20 mm in greatest dimension

T2 Tumour>20 mm but ≤50 mm in greatest dimension

T3 Tumour>50 mm in greatest dimension

T4a extension to chest wall, not includes pectoralis major

T4b Tumours that are of any size and show direct extension to the skin over the breast resulting in ulceration.

T4c Both T4a and T4b

T4d includes tumours of any size with permeation into dermal lymphatic channels, which is an ominous prognostic sign and inflammatory carcinoma.

According to the Nottingham/Tenovus Primary Breast Cancer Study (NTPBCS), the tumour size is considered an important independent variable and hence it forms an integral component of the

Nottingham Prognostic Index (NPI). NPI is an important prognostic factor for management purposes, which incorporates tumour size, lymph node status and histologic grade as a continuous variable.[59]

2. LYMPH NODE METASTASIS

Lymph node status is one of the most significant independent prognostic factors in breast carcinomas and it should be assessed on histopathological examination rather than clinical examination.

Many studies have confirmed that the patients who have microscopic involvement of regional lymph nodes have a poorer prognosis than those without regional node involvement.[69] Furthermore, the prognosis depends on the number of nodes involved, the presence or absence of extra nodal involvement and the size of the nodal metastasis. [56]

Lymph node staging is divided into three categories: stage 1 in which no node is involved; stage 2 which shows involvement in up to 3 axillary lymph nodes or a single internal mammary node involvement; stage 3 tumours which show 4 or more positive lymph nodes.

3. HISTOLOGIC TYPE

The tumours that belong to special types like tubular carcinoma,[60] mucinous carcinoma,[63] tubulolobular carcinoma,[70] invasive cribriform carcinoma,[59] medullary carcinoma, classic lobular carcinomas

are identified to have better prognosis than invasive carcinoma, NST type.[71] The variants like pleomorphic lobular carcinoma, basal-like carcinomas and signet ring cell type carcinomas, usually show a poorer prognosis.

4. HISTOLOGIC GRADE

The first description of histologic grading of ductal carcinoma was by Greenhough in the year 1925. The most commonly used grading system in the Scarff-Bloom-Richardson grading system. It is based on three morphologic features namely, tubule formation, nuclear grade and mitotic rate. It classifies breast carcinomas into two major groups, which includes low grade and high grade breast carcinomas.

Later, the Elston-Ellis modification of the Scarff-Bloom-Richardson system was made and till date, this has been the most preferred grading system all over the world, for the grading of breast cancers. This system uses scoring system for the morphologic features and hence it is a semi quantitative grading system. It uses score 1 to 3 for each of the features. It is also called as Nottingham grading system.[59] It is the recommended system for grading of breast cancer by various international bodies including world health organization (WHO) and American joint committee of cancer (AJCC).[55]

TABLE 1(A): SCORING OF TUBULE FORMATION

PERCENTAGE OF TUBULES WITHIN TUMOR	SCORE
>75%	1
10-75%	2
<10%	3

TABLE 1(B): SCORING OF NUCLEAR PLEOMORPHISM

NUCLEAR FEATURES	SCORE
Small, uniform nuclei	1
Moderate increase in size/ variation	2
Marked variation	3

TABLE 1(C): SCORING OF MITOTIC COUNT PER 10 HPF

FIELD DIAMETER 0.59MM/0.274mm^2	FIELD DIAMETER 0.44 mm/0.152mm^2	SCORE
0-9	0-5	1
10-19	6-10	2
>20	>11	3

TABLE 1(D): FINAL GRADING OF NOTTINGHAM MODIFICATION OF SCARFF BLOOM RICHARDSON SYSTEM

GRADE	TOTAL SCORE	DEGREE OF DIFFERENTIATION
I	3-5	Well differentiated
II	6-7	Moderately differentiated
III	8-9	Poorly differentiated

The Nottingham grading of breast cancer is used along with tumour size and lymph node stage in the Nottingham prognostic index, and is a effective tool for stratification of treatment strategies for various prognostic groups of patients.[56]

5. ANGIOLYMPHATIC INVASION

Lymphovascular invasion is one of the most significant prognostic factor in breast cancers, as it is one of the important step in the pathogenesis of metastatic breast cancer, leading to increased morbidity, mortality and decreased long-term survival of the patient.[72] Histopathologically, the lymphovascular invasion is assessed using Haematoxylin and Eosin stained sections of tumour and peritumoral region. The presence of tumour cells within the lumen of intratumoral and peritumoral lymphatics and blood vessels which are lined by endothelial cells is considered to be positive for lymphovascular invasion. But, whether the emboli are involving a blood vessel or lymphatic channel, cannot be differentiated by routine histopathological examination. The use of immunohistochemical stains specific for endothelial cells of lymphatics, like D2-40 and anti-podoplanin is applied for differentiating blood vessel and lymphatic emboli[73].

Lymphatic invasion has been observed in 44% of LN negative and 86% of LN positive accounting to 66% of overall breast cancer patients, as demonstrated by Kahn et al.,[74,75]

The presence of blood vessel invasion is associated with an increased risk of early recurrence in operated lymph node negative breast cancer patients. Thus, lymphovascular invasion forms an important

predictive factor for local recurrence and nodal metastasis after conservative surgeries in early breast cancers and flap recurrence in mastectomised patients.[76]

6. TUMOR NECROSIS

Spontaneous tumour necrosis is associated with high histologic grade and earlier lymph node metastasis. It is also associated with decreased long term survival. Tumour necrosis is commonly seen in tumour of ductal type with high histologic grade and basal phenotype.[37]

7. STROMAL FEATURES

Tumours which show absence or decreased inflammatory infiltrates at the invasive front are associated with better prognosis, lesser incidence of lymphnode metastasis and survival.[37]Stromal fibrosis has been associated with variable prognosis, according to various semi-quantitative studies, from favourable outcome to poor disease-free survival. Stromal elastosis is yet another variable stromal elastic fibres in cases of breast cancer. Elastosis can be periductal or diffuse in nature. Stromal elastosis, by itself is not an independent prognostic factor, though some studies have shown good prognosis in cases with elastosis.

8. INSITU COMPONENT

Some studies have shown that the presence of prominent in situ component associated with invasive carcinomas bodes better prognosis and decreased lymph node metastases.

9. OTHER HISTOLOGIC PROGNOSTIC FACTORS

INFLAMMATION

Prominent lymphocytic or lymphoplasmacytic infiltration has been associated with good prognosis. Grade 3 ductal carcinoma NST with prominent inflammation has been found to have better prognosis than grade 3 ductal carcinoma NST without prominent inflammation. [77]

MICROVESSEL DENSITY

Increased microvascular density is found to be associated with earlier lymph node metastasis and decreased long-term survival in node-negative carcinomas.[78]

APOPTOTIC INDEX

Apoptosis is characterized by shrinkage of cells and pyknotic and karyorrhectic nuclei with cytoplasmic blebbing. Apoptotic index is counted on H&E stained sections similar to mitotic index. Immunohistochemical stains can also be used to count mitotic index, like

caspase 1 and 3 annexin V cleaved CK18 and CD95, and terminal deoxynucleotidyl transferase–mediateddigoxigenin-11 – deoxyuridine triphosphate nick end labeling (TUNEL) method. Although High apoptotic index is associated with higher grade of tumour, higher proliferative activity and lack of expression of hormone receptors,[79] it does not qualify to be an independent prognostic factor for breast cancers. [80]

PERINEURAL INVASION

Perineural invasion is found in about 10-25% of breast carcinomas and is associated with high grade tumours and lymphovascular invasion. But it is not proved to be independently associated with prognosis.

IMMUNOHISTOCHEMICAL TUMOR MARKERS

HORMONE RECEPTORS

Estrogen and progesterone are steroid hormones that play an important role in the normal glandular development and in breast cancer progression. The presence of hormone receptors namely estrogen and progesterone receptors, in the tumour tissue of breast correlates with the response to hormonal and chemotherapy. Estrogen and progesterone mediate their effects on breast tissue by binding to specific nuclear receptors, leading to transcriptional regulation (activation or repression)

of target genes[81]. Estrogen receptor occurs in two different forms namely α and β. While ERα plays an important role in ductal elongation during puberty, PR and ERβ are important for lactational development of lobules[82].

Studies have found that ER-negative breast malignancies are associated with histologic grade 3, pushing margins, comedo-necrosis, central fibrosis and lymphocyte-rich stroma[83]. Mucinous carcinoma, tubular and lobular carcinomas are associated with increased ER-positivity whereas most of the cases of medullary, apocrine and metaplastic carcinomas show ER-negativity.[55]

ER is positive in 70% - 95% of invasive lobular carcinomas and 70% - 80% of invasive ductal carcinomas, whereas PR is positive in 60%- 70% of invasive breast carcinomas[84]. Although the presence of ER and PR in an invasive breast carcinoma is considered as an independent prognostic and predictive factor[85]; Liu et al., 2010), both lose their prognostic value after long-term follow-up.[86-88]

Hormone receptors are evaluated in formalin fixed paraffin-embedded breast tissues using various methods including immunohistochemistry, fluorescent in situ hybridization and PCR,[37] of which immunohistochemistry is the most widely used technique.

ASSESSMENT OF HORMONE RECEPTORS

Immunohistochemical assessment of hormone receptors is performed by using two parameters, namely the number of positively stained tumour cell nuclei and the intensity of staining. Many scoring systems have been proposed for assessing both the parameters, of which Allred scoring system is the simpler and most widely accepted and recommended method. The number of stained tumour cell nuclei is expressed as percentage of total tumour cell population.

TABLE 2(A): SCORING OF PROPORTION OF STAINED NUCLEI

SCORE FOR PROPORTION OF POSITIVE NUCLEI	PERCENTAGE OF STAINED TUMOR CELL NUCLEI
0	No staining
1	<1%
2	1-10%
3	11-33%
4	34-66%
5	67-100%

TABLE 2(B): SCORING OF INTENSITY OF STAINING

SCORE FOR INTENSITY	INTENSITY OF STAINING
0	No staining
1	Weak staining
2	Moderate staining
3	Strong staining

Both the score for proportion of stained cell nuclei and score for intensity of staining are summed up to give a maximum score of 8.[89]

The presence of these steroid receptors has been found to be very powerful predictor of response to breast cancer hormonal therapy and has significantly improved the long term clinical outcomes of patients with ER/PR-positive tumours. The response rate to cancer hormonal therapy for ER/PR positive tumours is approximately 80%[90]. ER-positive tumours show better prognosis and show good response to adjuvant chemotherapy with tamoxifen[37].

HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR TYPE 2 (Her2)

The human epidermal growth factor receptor 2 (HER2) is a transmembrane tyrosine kinase receptor, homologous to the epidermal growth factor receptor and is encoded by the gene ERBB2 on chromosome 17q12.[91] Her-2 amplification and/or overexpression is observed in about 15-30% of human breast cancers leading to increased activity of tyrosine kinase and downstream pathways stimulating tumour growth.[92] It is associated with clinically aggressive nature of tumour with morphologically high histologic grade, negative ER status, increased rates of recurrence and mortality. The prognosis of patients with her-2 positive tumours has been found to be significantly worse than those with her-2 negative tumours[93,94]. The development of targeted chemotherapy using anti-Her2 monoclonal antibody Trastuzumab (Herceptin) is a major breakthrough in the management of breast cancers. Trastuzumab is useful in the treatment of early stage breast cancers and has been found to reduce the risk of recurrence after surgery[95]. Various studies have shown that the use of Trastuzumab with chemotherapy in advanced breast cancers and metastatic disease has shown increase in overall response rates, decrease in risk of death, longer disease free survival and longer duration of disease progression[94-97].

MOLECULAR CLASSIFICATION OF BREAST CANCER

Conventionally, breast cancers have been classified based on morphological characteristics until the year 2000, when Perou and colleagues first described the genomic and molecular nature of breast cancers based on cDNA microarray and gene expression profiling. They demonstrated that the expression pattern of a set of genes within the tumour determine the molecular signature of breast cancer and that the intrinsic molecular signatures predict the clinical outcome of the disease[14,15]. Breast cancers can be broadly classified into ER – positive and ER- negative which are further divided into five molecular subtypes:

1. Luminal A
2. Luminal B
3. Her-2 overexpressing
4. Basal-like
5. Normal breast-like.[14,15]

Luminal A tumours have the best prognosis and basal and Her-2 positive tumours have the worst prognosis[15]. The luminal A subtype is the most frequent type constituting about 24%-39% of breast cancers, followed by basal-like (17%-37%), luminal B (10%- 18%), HER2 (4-10%) and normal-like (0-5%)[98,99].

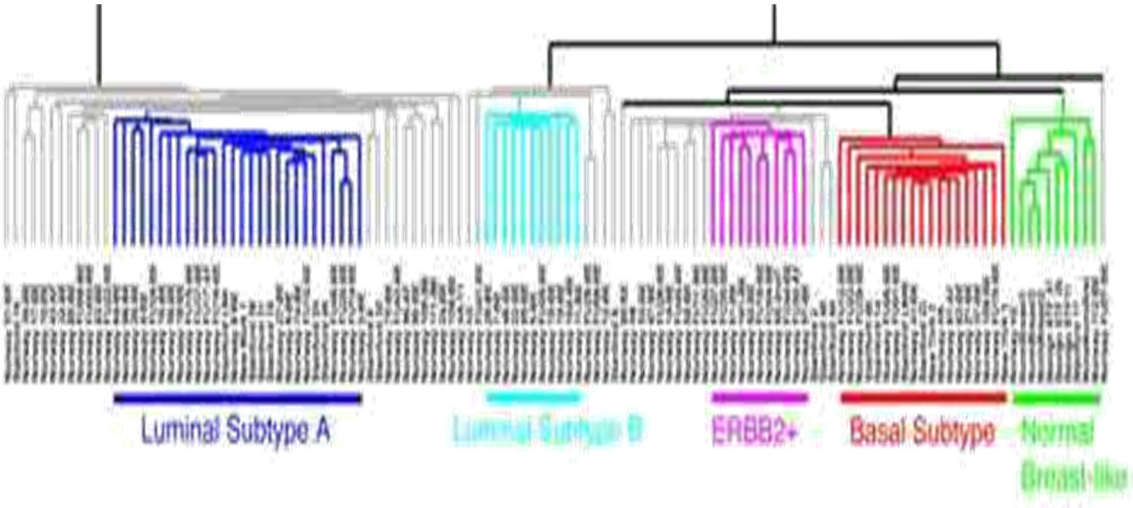


FIGURE 2: HIERARCHICAL CLUSTERING OF 115 TUMOUR TISSUES AND 7 NON-MALIGNANT TISSUES USING THE "INTRINSIC" GENE SET. EXPERIMENTAL DENDROGRAM SHOWS THE CLUSTERING OF THE TUMOURS INTO FIVE SUBGROUPS. BRANCHES CORRESPONDING TO TUMOURS WITH LOW CORRELATION TO ANY OF THE SUBTYPE ARE SHOWN IN GREY.[98]

TABLE 3: RECEPTOR STATUS OF MOLECULAR SUBTYPES OF BREAST CANCERS

MOLECULAR SUBTYPE	RECEPTOR STATUS
Luminal A	ER+/PR+/HER2-
Luminal B	ER+/PR+/HER2+
HER2 overexpressing	ER-/PR-/HER2+
Basal-like	ER-/PR-/HER2-

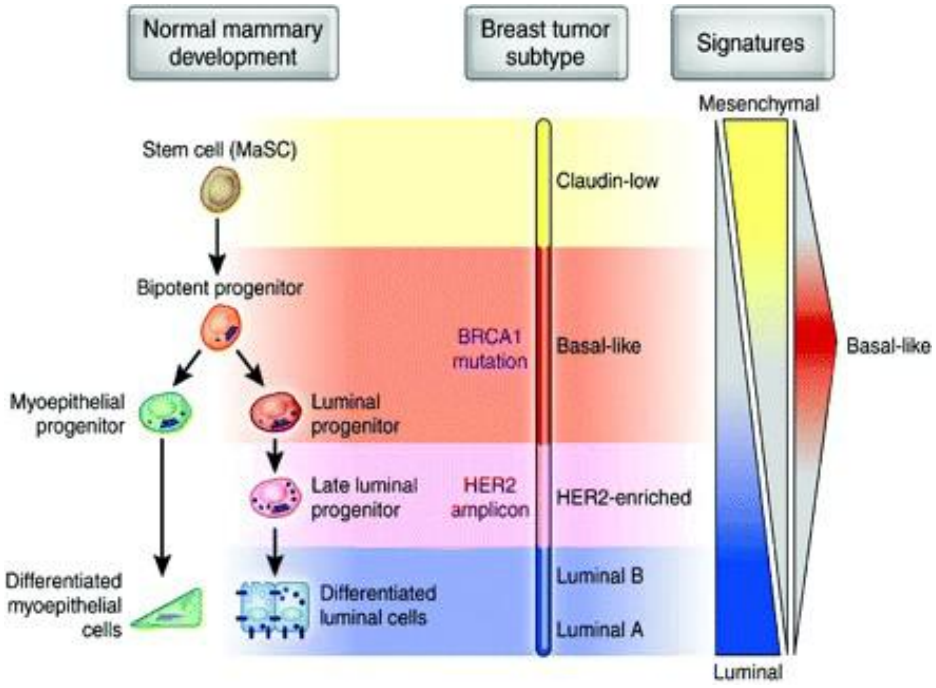


Fig Mammary Cell Development and Stages Where Malignancy Develops

Luminal A tumours are characterized by higher levels of ER and PR expression, negative expression of HER2, low Ki67 staining, and expression of luminal epithelial cytokeratins CK8 and 18 by IHC[100]. Luminal A tumours frequently exhibit low histological grade, and good prognosis[101].

Luminal B tumours are also ER-positive and express CK8 and 18. They are frequently associated with a more aggressive phenotype and worse prognosis, higher histological grade, lower PR expression, and exhibit higher Ki67 staining [101,102]

HER2+ overexpressing tumours are found to be associated with high tumour proliferation, high histological grade, and frequent p53 mutations[103]. Triple negative breast cancer (TNBC) subtype is one of the breast cancer subtype which constitutes about 10 to 20% of tumors[101]. These tumours show lack of expression of ER, PR, or HER2. Basal-like breast tumours show expression of basal/ myoepithelial markers, such as CK 5/6, 14, and 17[104]. TNBCs account for about 75% of all BRCA1-deficient tumours, frequently associated with p53 mutations, exhibit genomic instability, show high histological grade, and high Ki67 positivity[101].

Thus, molecular classification using gene expression profiling has produced a paradigm shift in the understanding of the pathology, clinical outcome and prognosis of breast cancers and has been applied in the development of targeted therapy for use as part of personalised medicine.

MULTIGENE PROGNOSTIC AND PREDICTIVE TESTS

Many studies have led to the development of commercialized multigene assays for the prognostication and selection of treatment for individual patients[105]. These include Oncotype DX and MammaPrint genomic tests.

The Oncotype DX is a reverse transcription polymerase chain reaction (RTPCR)-based assay that analyses a panel of 21genes expression in formalin fixed paraffin-embedded tumour tissues. It was formulated to predict the likelihood of disease recurrence in patients with node-negative, ER+ breast cancer who undergo treatment with Tamoxifen. The 21 genes are associated with ER pathway, proliferation, HER2 and invasion, and determines a risk of recurrence (ROR) score ranging from 0 to 100, which is an independent prognostic factor.[106] The Oncotype DX is the most widely used molecular test in the clinical setting for making treatment decisions and is recommended by the St. Gallen Consensus[107].

Mamma-Print® is one another commercially available 70-gene test that provides prognostic information for patients with stage 1 or 2, node-negative invasive breast cancer of tumour size < 5 cm. It quantifies the risk of metastasis in early breast cancers and divides patients into two groups- low and high risk of recurrence irrespective of ER status and prior chemotherapy[108]. Other commercially available prognostic signatures are 76-gene signature named as Veridex, breast cancer index and PAM50 signature.

TRIPLE NEGATIVE BREAST CANCER

Triple negative breast cancers are defined by the negative expression of estrogen receptor, progesterone receptor and lack of overexpression and amplification of HER2 gene. They account for about 10-20% of all breast cancers and also account for most of the deaths due to breast cancer[16]. These tumours have been found to show poor prognosis despite good response to neoadjuvant chemotherapeutic agents and lack of targeted therapy[109].

EPIDEMIOLOGY

TNBC more frequently affects younger patients(<50 years),[110-112] more prevalent in African-American women, increased incidence of distant visceral metastases and a unique mechanism of haematogenous spread, more frequently presents as interval cancers between consecutive mammograms and are more aggressive than tumours of other molecular subgroups[110,112]. TNBC accounts for 39% of all breast cancers that occur in pre-menopausal African-American women under the age of 50 years, whereas only 16% of breast cancers that are diagnosed in Caucasian women of the same age group. It has been observed that about 14% of breast cancers occurring in post-menopausal African-American women are found to be TNBC[113]. More than 75% of breast cancers arising from

mutation in BRCA1 gene belong to triple-negative phenotype, basal-like subtype or both[114]

PATHOLOGIC FEATURES

The prototypical features of triple negative breast cancers are summarised in the table 5.

TABLE 5: PROTOTYPICAL FEATURES OF TRIPLE NEGATIVE BREAST CANCER[115]

Morphological	High histologic grade
features	Lack of tubule formation
	Prominent nuclear abnormalities
	High mitotic count
	Broad pushing borders
	Necrotic and fibrotic areas
	Prominent lymphoplasmacytic infiltrate

Biological features	Lack of expression of ER and PR
	HER2 negative
	High Ki-67 index
	P53 mutation
	Positive immunostaining for basal cytokeratins,
	vimentin,
	p-cadherin, EGFR, PDGFR, IGF-IR and c-kit
Molecular	Most commonly basal-like
classification	

Histologically, TNBC are often high grade tumours with lack of tubule formation, high mitotic count and high nuclear atypia[110]. They show broad pushing margins and more commonly have large areas of necrosis, fibrosis and prominent lymphocytic infiltrate. Most of the TNBC are high grade invasive ductal carcinoma, no special type, metaplastic carcinoma and medullary carcinomas.[116,117].Methylated BRCA1 promoter region has been found in medullary and metaplastic types of breast cancer[118]. There is no convincing association between the prevalence of lymph node involvement at the time of diagnosis and triple negative breast cancers, as various studies show varying results[16]. Only a weak correlation has been observed between tumour size and lymph node

metastasis in triple negative breast tumours in a study conducted by Dent et al.[110]

MOLECULAR SUBTYPES OF TNBC

Lehmann et al have identified 6 different molecular subtypes of triple-negative breast cancer based on gene expression profiling of TNBC including

Two basal-like (BL1 and BL2),

Immunomodulatory (IM),

Mesenchymal (M),

Mesenchymal stem-like (MSL), and

Luminal androgen receptor (LAR) subtypes.

These subtypes are thought to be driven by various distinct pathways which can be effectively targeted by specific drugs[119].

CLINICAL COURSE AND OUTCOME

Triple-negative breast cancers are generally aggressive with shorter disease-free interval with adjuvant chemotherapy and aggressive clinical course with earlier nodal and distant metastases with significant shortening of overall survival of the patients, despite their sensitivity and

response to initial adjuvantand neoadjuvant chemotherapy with taxanes and anthracyclines[109].

BASAL-LIKE BREAST CANCER

Basal-like breast cancer have been identified by the hierarchical clustering of the variations in the gene expression profile of 496 genes, which is called the "intrinsic subset"[14]. Approximately 80% of triple negative breast cancers are basal-like breast cancers[120] and they account for about 15% of all breast cancers[14]. It is a unique and aggressive breast cancer subtype[15]. Basal-like breast cancers arise from epithelial/ myoepithelial progenitor stem cell of breast[121]. Although most of the triple negative breast cancers and basal-like carcinomas are of high histologic grade, a small subset of them belong to low histologic grade like secretory carcinoma, acinic cell, adenoid cystic and apocrine carcinomas.

EPIDEMIOLOGY AND CLINICAL FEATURES

Basal-like breast cancer is found to be seen more frequently in women who attain early menarche, women who are overweight and obese, high parity and women who had breastfed for a shorter duration during their lifetime[114].

CISPLATIN

Cisplatin is a widely used anti-cancer drug that is exceptionally effective against testicular cancer. trans-DDP, the geometric isomer of cisplatin, is ineffective as a chemotherapeutic agent. The anti-tumour activity of cisplatin is generally attributed to its formation of DNA adducts, both intrastrand and interstrand crosslinks, which induce structural distortions in DNA. The DNA adducts of cisplatin are thought to mediate its cytotoxic effects by inhibiting DNA replication and transcription and, ultimately, by inducing programmed cell death, or apoptosis. The adducts of both cis- and trans-DDP are removed from DNA by the nucleotide-excision-repair pathway. Cellular proteins possessing certain DNA-binding motifs, including the HMG domain, bind selectively to DNA modified by cisplatin, but not to DNA adducts of trans-DDP; evidence suggests a possible role for these proteins in modulating cisplatin cytotoxicity. Both intrinsic and drug-induced resistance often limit the success of cisplatin; several specific mechanisms of cisplatin resistance have been identified.

Platinum agents (i.e. carboplatin and cisplatin) are cytotoxic DNA damaging compounds leading to DNA strand breaks and possible consequent cell apoptosis; this peculiar mechanism of action makes them specially active in cancer cells with DNA repair deficiency such as those

harbouring deleterious mutations in the BRCA genes[24]. Based on the biological rationale for a heightened susceptibility of TNBC to DNA-damaging compounds[25], several trials have investigated the possible role of platinum agents as a treatment option in TNBC patients.

MATERIALS AND METHODS

This study was carried out in the Department of Surgery, Government Rajaji Hospital Madurai, during the period August 2017 to November 2019 in collaboration with Department of Medical Oncology, Government Rajaji Hospital, Madurai.

The patients presenting with inoperable triple negative breast cancer with M0(no metastasis) in GRH Madurai will be recruited for this study. Following consent, a questionnaire will be filled to record the patient's demographic data, duration of disease, symptoms, & treatment history.

Patients coming to GRH, Madurai with lump breast cancer are evaluated based on receptor status, USG Abdomen ,USG breast ,FNAC breast, trucut biopsy from the lump, skeletal survey ,routine blood investigations are done. Patient are categorised under TRIPLE NEGATIVE BREAST carcinoma based on their investigation reports.

Patients are divided into group A and group B regimen. Regimen A (4 Cycles Of AC with Cisplatin Followed By 4 Cycles Of Taxanes) and Regimen B (4 Cycles Of AC Followed By 4 Cycles Of Taxanes) will be given to the group of patients accordingly. Response to the

treatment is assessed based on the tumour size, nodal status, staging and MRI.

CRITERIA FOR PATIENT SELECTION

INCLUSION CRITERIA

1. Female patients who are >18 years of age
2. Patients consented for inclusion in the study according to designated proforma.
3. The tumor must be invasive carcinoma of the breast on histologic examination.
4. No clinical evidence of metastasis.
5. The tumor must have been determined to be HER2-negative.
6. The tumor must have been determined to be ER- and PR-negative,
7. Bone marrow function Hb: ≥ 10.0 g/dL ANC: ≥ 1,500/µL Platelet count: ≥ 10×10^4/µL
8. Renal function Creatinine: ≤ 1.5 × UNL or Creatine clearance (Ccr) >50 ml/min
9. Hepatic function Total Bilirubin: ≤ 1.5 × UNL AST/ALT: ≤ 2.5 × UNL 10) Ability and willingness to comply with the study visits, treatment, testing, and with the protocol, as per investigator's judgment

EXCLUSION CRITERIA

1. Any prior systemic treatment for primary invasive breast cancer
2. Evidence of metastatic breast cancer.
3. Patients considered a poor medical risk due to a serious, uncontrolled medical disorder or uncontrolled infection.
4. Pregnant or breastfeeding women.
5. Patients unwilling for the study.

By above mentioned inclusion and exclusion criteria **60** patients had been enrolled for the study. All the women enrolled in the study were subjected to the following protocol. They had been explained about the chemotherapy regimen and insisted to attend chemotherapy regimen regularly. They had been told about surgical and radiation therapy following the neoadjuvant chemotherapy.

Patients are divided into group A(cases) and group B (control) regimen.

- **<u>Regimen A</u> (4 CYCLES OF AC WITH CISPLATIN FOLLOWED BY 4 CYCLES OF TAXANES**)
- **<u>Regimen B</u> (4 CYCLES OF AC FOLLOWED BY 4 CYCLES OF TAXANES**) will be given to the group of patients accordingly.
- Response to the treatment is assessed based on the tumour size, nodal status, staging by MRI.

<u>REGIMEN A (CASE)</u>

4 CYCLES OF AC (Anthracyclines Cyclophosphamide) with Cisplatin

⇩

MRM (Modified Radical Mastectomy)

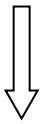
⇩

4 CYCLES OF TAXANES

REGIMEN B (CONTROL)

4 CYCLES OF AC (Anthracyclines Cyclophosphamide)

MRM (Modified Radical Mastectomy)



4 CYCLES OF TAXANES

Response to the treatment is assessed based on the tumour size, nodal status, staging by MRI.

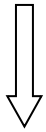
BEFORE CHEMOTHERAPY

Clinical examination	Tumour size(cms)	Nodal status (N)
USG Breast		
MRI		
TNM staging		

4 cycles of neoadjuvant chemotherapy

AFTER 4 CYCLES OF CHEMOTHERAPY

Clinical examination	Tumour size(cms)	Nodal status (N)
USG Breast		
MRI		
TNM staging		



HPE REPORT FOLLOWING MRM

Tumour size(cms)	Nodal status (N)	Pathological TNM staging

NEO ADJUVANT CHEMOTHERAPY REGIMEN:

INJ CYCLOPHOSPHAMIDE**:** an alkylating agent given as 600mg/m2 of body surface area.

INJ DOXORUBICIN**:** an anthracyclin antibiotic administered in the dose of 50mg/ m2 of the body surface area.

INJ CISPLATIN: an alkylating agent under platinum group given as 30mg/m2 of body surface area.

INJ PACLITAXEL: dose administered 80mg/m2 of body surface area.

8mg of inj Ondansetron (5HT3 Antagonist) and 8mg of inj dexamethasone was given intravenously half an hour before chemotherapy to prevent vomiting. The patient was given another dose of Ondansetron 4 hours later to prevent breakthrough vomiting. Patients were subjected to the above mentioned chemotherapy regimen once in 21 days till maximum response was achieved or till response became plateau or if patients were detected to have intolerable toxicity to the drugs given during chemotherapy. Every time before the next cycle of chemotherapy was given, the patient was assessed for response to chemotherapy and toxicity to chemotherapy. To rule out the toxic side effects, complete hemogram and liver function tests were done, the patients with intractable toxicity like uncontrolled vomiting, myelosuppression, cerebellar ataxia, cardiomyopathy, were withdrawn from the chemotherapy regimen and were subjected to surgical intervention. Modified radical mastectomy (MRM) was done for these patients.

Based on the response the patients were categorized into 4 groups.

GROUP I - *COMPLETE CLINICAL RESPONSE*:

Here there was no evidence of measurable tumour or new disease for a specified interval usually 4 weeks. .

GROUP II – ***PARTIAL CLINICAL RESPONSE***:

Tumour size decreased 50% or more than 50% determined by two observations not less than 4 weeks apart.

GROUP III – *NO RESPONSE* or ***STABLE DISEASE***

Tumour size decreased less than 50%

GROUP IV – ***PROGRESSIVE DISEASE***:

If 25% or greater increase was seen in the product of one or more measurable lesion or appearance of new lesion, was termed progressive disease.

MODIFIED RADICAL MASTECTOMY (MRM)

AUCHINCLASS :

Procedure:

1. Patient was positioned on the operative table in the supine position with rolled sheet under the ipsilateral hemithorax so as to allow the motion of arm without limitation.
2. Incision was ideally made transversely from lateral border of sternum to just below anterior axillary fold. This incision included the nipple areola complex 5 cms skin around the lesion and the scar of the previous biopsy if any.
3. Skin flap was elevated in the plane between the subcutaneous fat and the mammary fat. Initially cephalic flap was raised upto subclavius muscle. Pectoralis fascia was dissected from pectoralis muscle in the plane parallel to the course of the muscle bundle. Perforators of the lateral thoracic and anterior intercostal arteries were ligated.
4. Lateral flap was elevated upto anterior border of lattismus dorsi.
5. Inferior flap was raised to upto 3 cms below inframammary fold. After elevating the breast from the chest wall, the breast was attached only to the axilla.

6. Axillary vein was identified at lateral axillary space while anterior border of lattismus dorsi was dissected from inferior to superior direction.
7. Shoulder was abducted and arm was extended to facilitate the dissection of inferior and lateral margin of pectoralis major. Pectoralis major was retracted to identify the pectoralis minor.Inter pectoral nodes were removed preserving the medial pectoral nerve.
8. Loose areolar tissue at the junction of the axillary vein with the anterior margin of lattismus dorsi was swept inferiorly to include the lateral group of axillary nodes, thoraco dorsal vessels and nerve were preserved. Subscapular group of nodes between the thoraco dorsal nerve and chest wall, were dissected enbloc.
9. Central group of nodes were dissected enbloc along with pectoral group, and the Long thoracic nerve of Bell was preserved.
10. After removal of specimen hemostasis was obtained. Two vacuum drains, one for the flap and the another for the axilla were inserted.
11. Skin closure by vertical mattress technique with 2-0 ethilon.

ASSESSMENT OF PATHOLOGIC RESPONSE:

Mastectomy specimens from the patients who underwent MRM were sent for histopathological assessment. In histo pathological examination the following factors were studied.

1. The presence of tumour cells.

2. Whether resected margins were free of tumour

3. Status of lymph node metastasis.

Depending on the pathologic response the patient was categorized into two groups

PATHOLOGICAL COMPLETE RESPONSE (PCR): No tumour cells were detected in the resected specimen.

PATHOLOGICAL NON RESPONDERS (PINV) : Presence of tumour cells in the resected specimen.

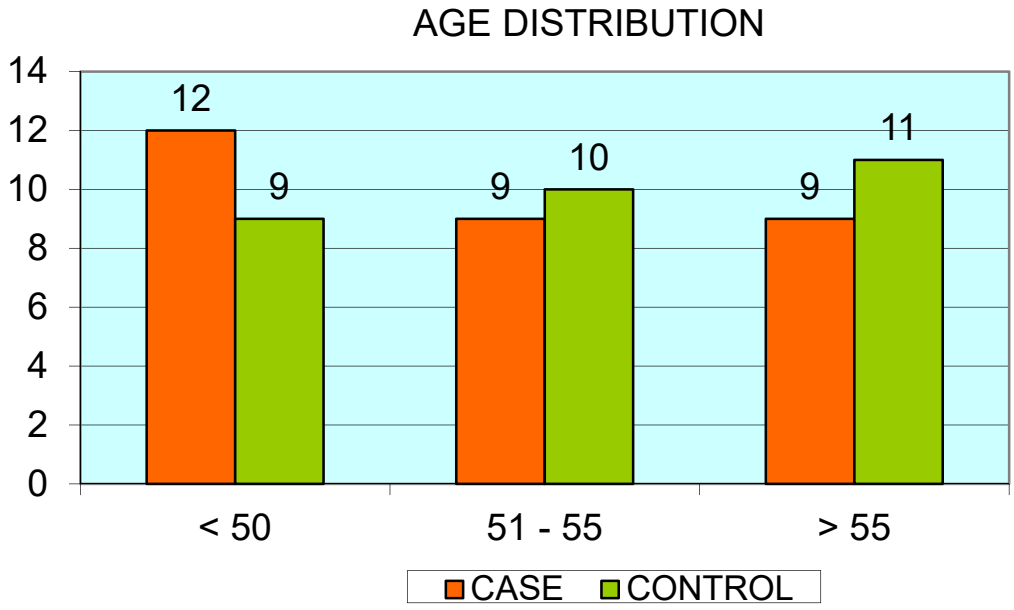
FOLLOW UP:

In the first two years after surgery, the patients were seen atleast once in every 6 months. In the following 3 years they were followed up for every 6 to 12 months.

The minimum requirement for follow up were, physical examination, locoregional evaluation, performance scale assessment, mammography, MRI, usg breast.

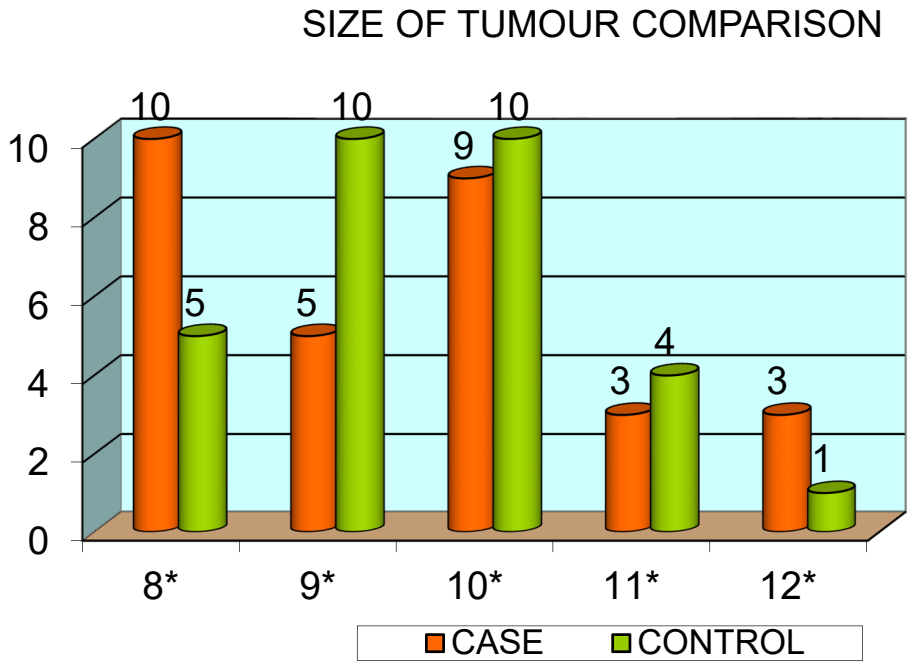
RESULTS

AGE	CASE	CONTROL
< 50	12	9
51 - 55	9	10
> 55	9	11
TOTAL	30	30
Mean	52.633	53.7
SD	4.832	4.669



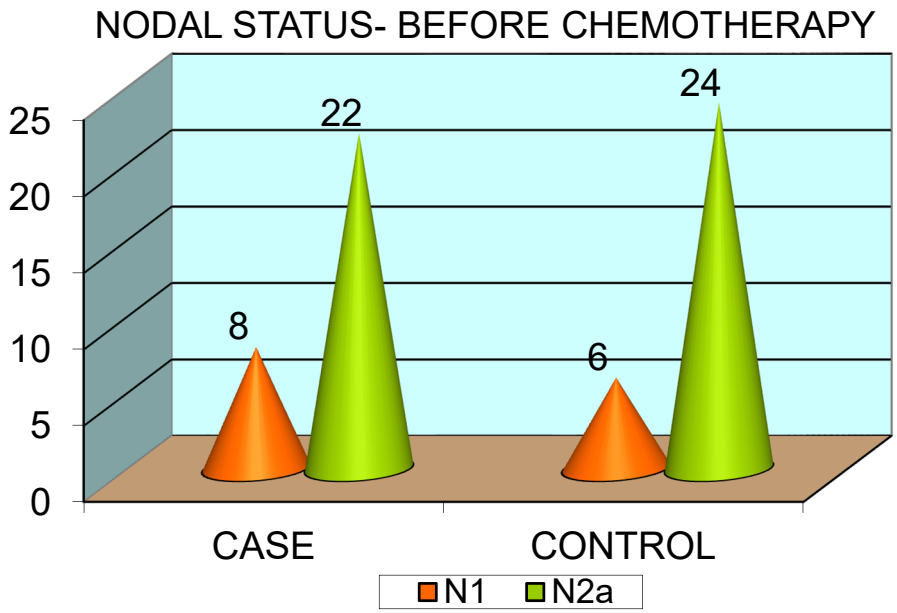
Out of 30 patients in case group, 12 patients in case group belong to less than 50 age group, 9 patients in case group belong to age group 51 to 55 years and rest of the patients (9 patients) belong to age group more than 55 years.

Before neoadjuvant chemotherapy		
size of tumor(cm) app~	**CASE**	**CONTROL**
8*	10	5
9*	5	10
10*	9	10
11*	3	4
12*	3	1
TOTAL	30	30



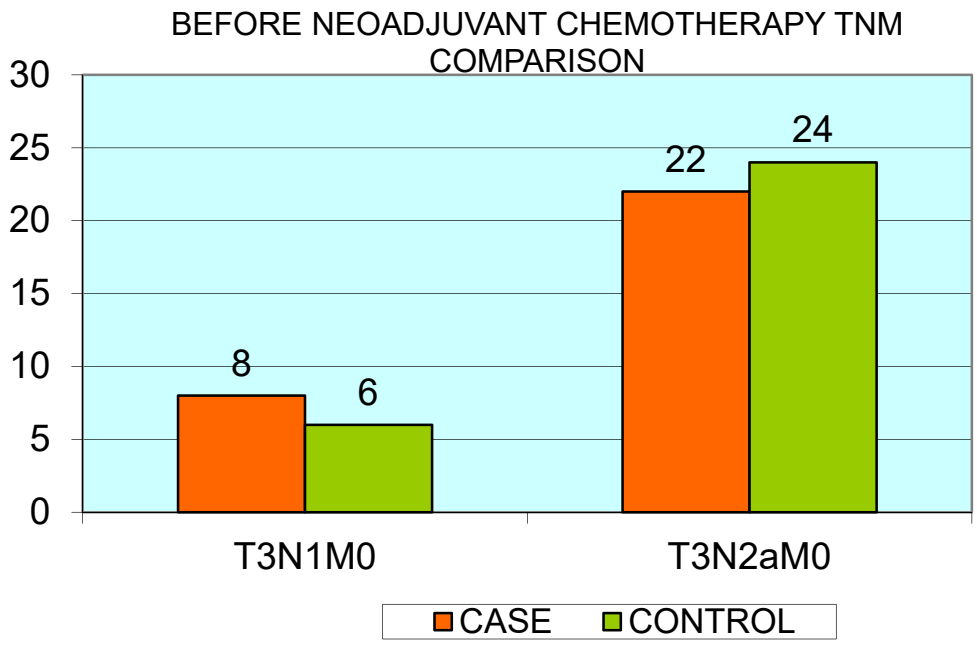
Among 30 patients in study group, 10 of them belong to tumour size 8 cms approximately, and 9 of them belong to tumour size 10 cms app, remaining 9 belong to 9-12 cms. Pre chemotherapy size was assessed and tabulated, to assess the post chemotherapy response.

Before neoadjuvant chemotherapy		
Nodal status	**CASE**	**CONTROL**
N1	8	6
N2a	22	24
TOTAL	30	30



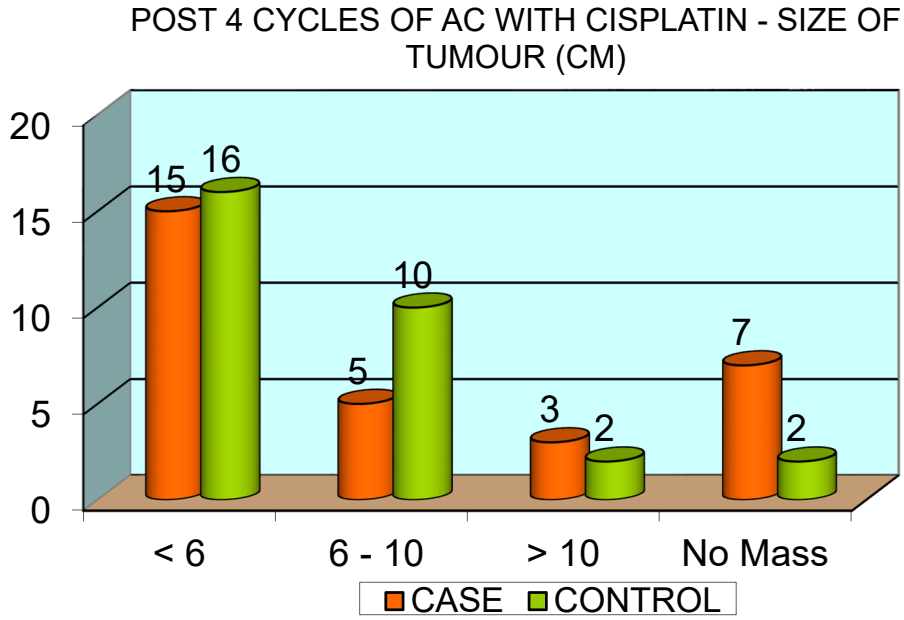
As this study is conducted among LABC patients, case and control group are selected as they belong to approximately equal nodal status as depicted in the bar chart.

Before neoadjuvant chemotherapy		
TNM	**CASE**	**CONTROL**
T3N1M0	8	6
T3N2aM0	22	24
TOTAL	30	30



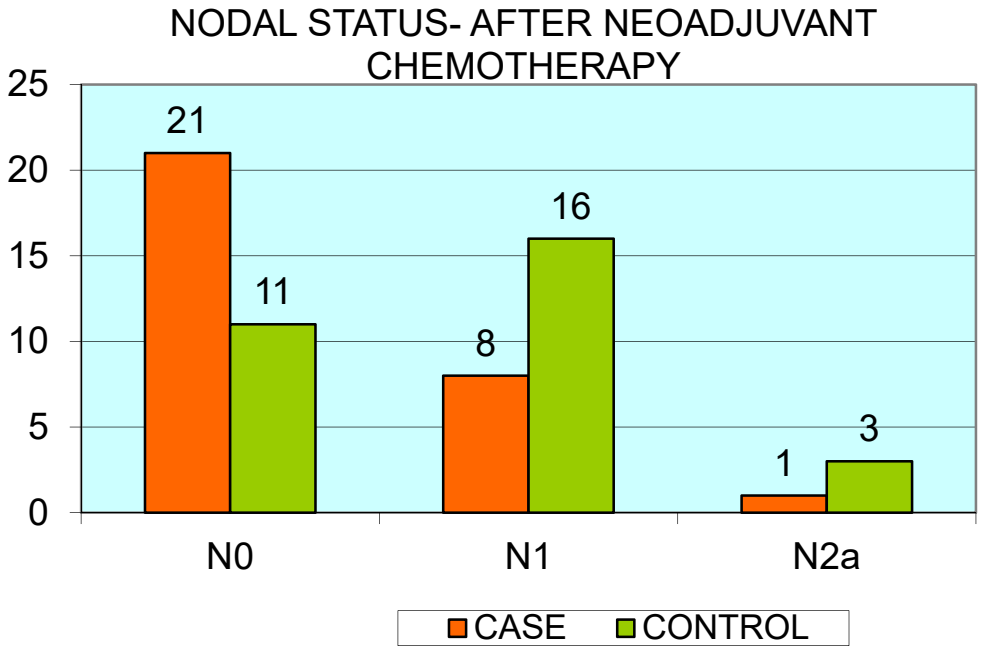
Patients were chosen in a way nearly 22 patients in case group and 24 patients in control group belongs to T3N2aM0 stage and 8 patients in case group and 6 patients in control group belongs to T3N1M0 stage, so that both study group will have patients with equal disease staging.

Post 4 cycles of AC with Cisplatin		
Size of tumor(cm)	**CASE**	**CONTROL**
< 6	17	13
6 - 10	6	10
> 10	0	5
No Mass	7	2
TOTAL	30	30
P'value	0.025 Significant	



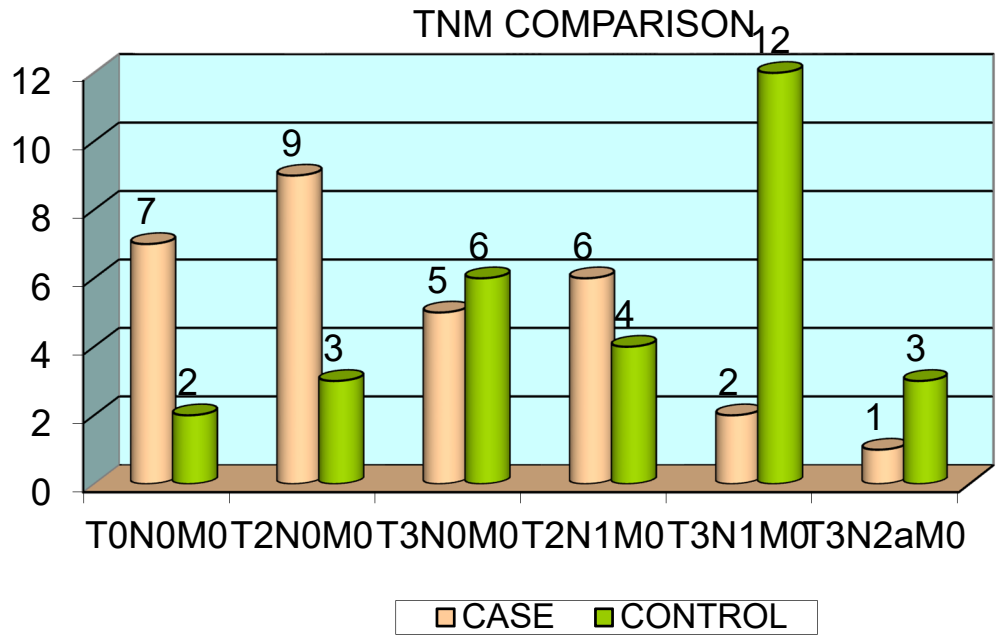
Out of 30 patients in case group who received cisplatin, 7 patients showed complete response with no detectable mass after 4 cycles of AC with Cisplatin which accounts for 23.3%. p value for this response is 0.025 which is significant and implying that cisplatin has good response in tumour regression.

Post 4 cycles of AC with Cisplatin		
nodal status	**CASE**	**CONTROL**
N0	21	11
N1	8	16
N2a	1	3
TOTAL	30	30
P'value	0.034 Significant	

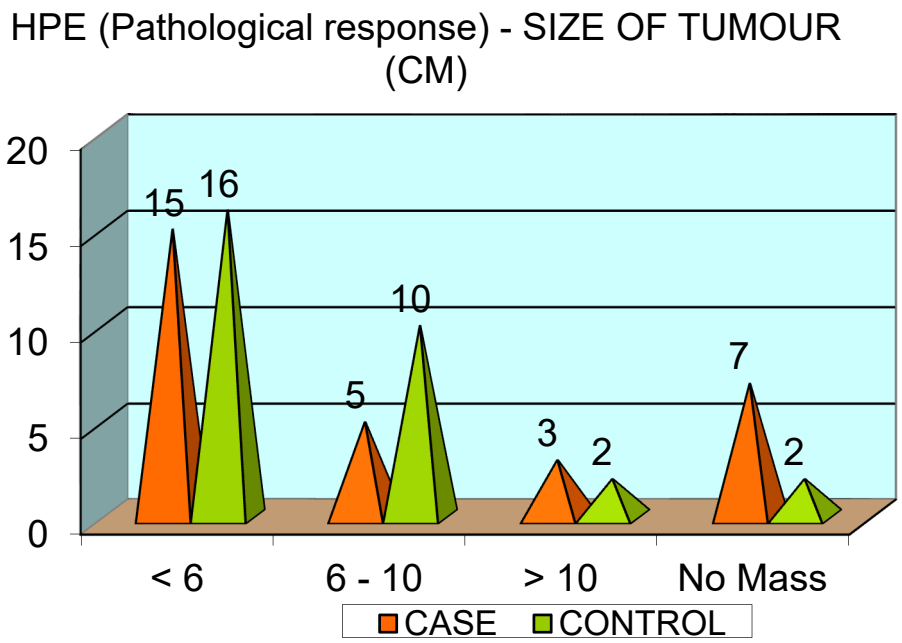


According to nodal status after receiving chemotherapy nearly 21 patients in case group falls under N0 status, which accounts for 70%. p value for nodal response is 0.034 which is significant. Nearly 22 patients in case group belong to N2a status prior to neoadjuvant chemotherapy out of which only one patient stay back in N2a status, remaining 21 patients were downstaged with cisplatin therapy.

Post 4 cycles of AC with cisplatin		
TNM	**CASE**	**CONTROL**
T0N0M0	7	2
T2N0M0	9	3
T3N0M0	5	6
T2N1M0	6	4
T3N1M0	2	12
T3N2aM0	1	3
TOTAL	30	30
P'value	0.013 Significant	

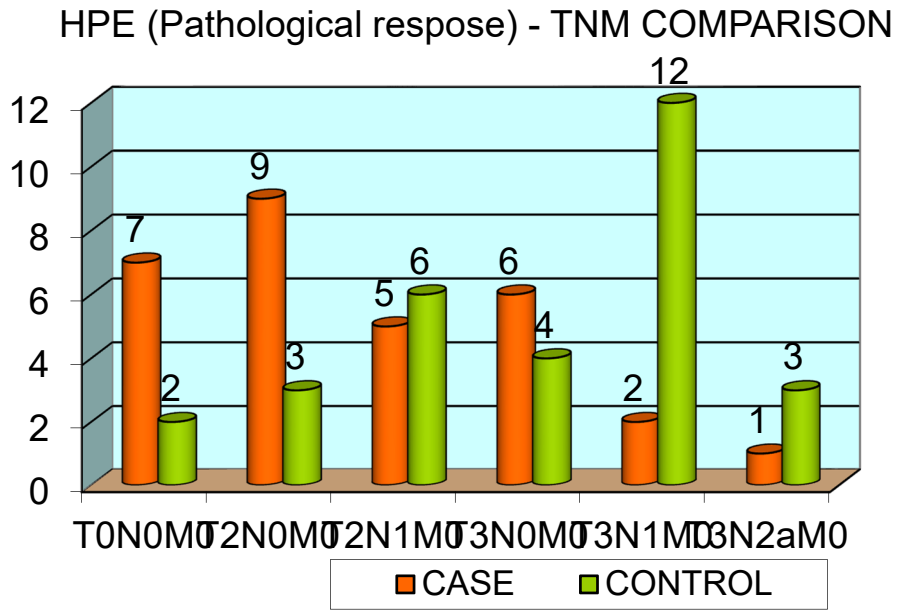


HPE (Pathological response)		
Size of tumor(cm)approximately	**CASE**	**CONTROL**
< 6	17	13
6 - 10	6	10
> 10	0	5
No Mass	7	2
TOTAL	30	30
P'value	0.025 Significant	



Post operative histopathology report correlates with post chemotherapy clinical assessment of tumour size which is proven through no detectable tumour in HPE for 7 patients in case group. p value is 0.025 which is significant.

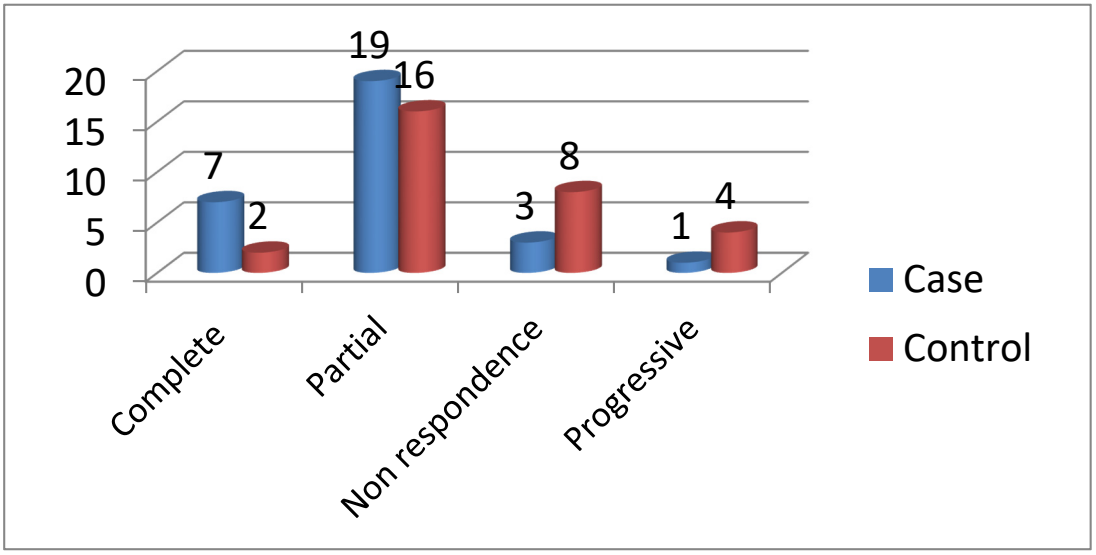
HPE (Pathological response)		
TNM	**CASE**	**CONTROL**
T0N0M0	7	2
T2N0M0	9	3
T2N1M0	5	6
T3N0M0	6	4
T3N1M0	2	12
T3N2aM0	1	3
TOTAL	30	30
P'value	0.013 Significant	



Out of 30 patients in control group, 19 patients stayed back in T3 status even after neoadjuvant chemotherapy, but only 9 patients in case group retained in T3 status, which shows addition cisplatin to neoadjuvant chemotherapy has significant role in downstaging of the tumour.

PATHOLOGICAL RESPONSE

Response	Complete	Partial	Non respondence	Progressive	Total	pvalue
Case	7	19	3	1	30	0.035 Sig
Control	2	16	8	4	30	



Out of the 30 patients in case group the overall clinical and pathological response of 86.66% was observed. Complete clinical response of 23.33% (7 Patients) was noted, Partial clinical response was noted in 19 among 30 patients (63.33%). No response(<50% response) was observed in 3 patients (10%). However 1 patient(3.33%) showed progressive disease and developed vertebral metastasis post- MRM.

In our study, percentage of complete clinical response(23.33%) was higher for patients in case group than the percentage of complete clinical response(6.67%) for the patients in control group. p value is 0.035 which is significant.

DISCUSSION

REGIMEN A (CASES)

Patient characteristics	Number of patients	Percentage
Age		
< 50 years	12	40%
> 50 years	18	60%
Stage III		
Total	30	100%
Tumour size		
< 10cm	15	50%
>10cm	15	50%
Axillary node status		
N1	8	26.67%
N2	22	73.33%

In the current study, after following the inclusion and exclusion criteria, 30 patients were enrolled under case group (regimen A), and the remaining 30 patients were enrolled under control group (regimen B), all these patients were having locally advanced breast cancer.

The above table summarizes the patient characteristics and the clinical features. Out of the 30 subjects enrolled, 12 (40%) patients were less than 50 year age group and 18 patients (60%) were aged above 50

years. Among 30 patients grouped under case group,15 patients (50%) had tumour size less than 10 cms and 15 patients (50%) had tumour size more than 10 cms.

In the study group of 30 patients under case group(regimen A), all of them had palpable axillary node.Single node was palpable in 8 patients (26.67%). Remaining 22 patients(73.33%) had axillary node status of more than one node palpable.

REGIMEN A (CASE GROUP)

Clinical response	**Number of patients**	**Percentage**
Clinical complete Response	7	23.33
Clinical partial Response	19	63.33
No response or stable Disease	3	10
Progressive Disease	1	3.33

REGIMEN B (CONTROL GROUP)

Clinical response	Number of patients	Percentage
Clinical complete Response	2	6.67
Clinical partial Response	15	50
No response or stable Disease	9	30
Progressive Disease	4	13.33

Evaluation of the clinical response of primary tumour and lymph node to neoadjuvant cisplatin regimen A (cases) compared with regimen B(control) was one of the primary objective of study. The product of two greatest perpendicular diameter was measured both manually (clinically) and using ultrasonogram & MRI before and after every cycle of Neoadjuvant chemotherapy as defined by criteria.

The clinical response of 30 patients under case study was observed and recorded. Out of the 30 patients in case group the overall objective clinical response of 86.66% was observed. Complete clinical response of 23.33% (7 Patients) was noted, Partial clinical response was noted in 19

among 30 patients (63.33%). No response (<50% response) was observed in 3 patients (10%). However 1 patient (3.33%) showed progressive disease and developed vertebral metastasis post- MRM.

In similar studies conducted by Aguilar Martinez et al[122] showed an over all objective response of 86.3% was observed. In their study there were no progressive disease observed after Neoadjuvant chemotherapy.

B. Sirohi et al conducted a study in Breast unit, Royal Marsden NHS foundation Trust, London which showed an overall clinical response of 88% was observed[123].

In our study, percentage of complete clinical response (23.33%) was higher for patients in case group than the percentage of complete clinical response(6.67%) for the patients in control group. In similar studies conducted by Aguilar Martinez et al[122] and B. Sirohi et al, it was reported that complete clinical response after platinum based compond added to the standard Neoadjuvant chemotherapy in TNBC was better. This correlated with the present study.

The second objective in our study was to evaluate the pathological response of the primary tumour and lymph node to preoperative chemotherapy. The pathological response was classified into 2 categories, namely pathological complete response and PINV (invasive cells seen).

PCR constituted a group of patients who showed no invasive cells detected.Second group consisted of patients who were termed pathological non responders. (PINV), since their mastectomy specimen showed invasive cells on Histopathological examination.

Case group (regimen A)

Pathological response	***No of patients***	***% of pathological Response***
Pathological complete response (PCR)	7	23.33
PINV (pathological non responders)	23	76.67
PINV (pathological non responders)		

In the present study, 7 patients (23.33%), showed complete pathological response after cisplatin neoadjuvant chemotherapy. Invasive cells were detected in the mastectomy specimen of 23 patients on HPE (76.67%).

Control group (regimen B)

Pathological response	***No of patients***	***% of pathological Response***
Pathological complete response (PCR)	2	6.67%
PINV (pathological non responders)	28	93.33%
PINV (pathological non responders)		

In control group (regimen B), 2 patients (6.67%), showed complete pathological response after standard neoadjuvant chemotherapy. Invasive cells were detected in the mastectomy specimen of 28 patients on HPE (93.33%). From the study it was confirmed that the pathological complete response in case group (23.33%) was better than control group (6.67%).

In similar study conducted by Daniel P. Silver et al [124] , it was reported that complete pathological response (28%) after platinum based compond added to the standard Neoadjuvant chemotherapy in TNBC was better. This correlated with the present study.

CONCLUSION

From this study it is evident that cisplatin based neoadjuvant chemotherapy in locally advanced triple negative breast cancer

1) Showed a significant increased pCR rates in patients at the cost of worse haematological toxicities.
2) Downstage the disease so as to make the inoperable tumour to operable one.
3) It also provides an opportunity to analyze biological markers as predictors of response to CT.

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RESPONSE TO NEOADJUVANT CHEMOTHERAPY IN TRIPLE NEGATIVE BREAST CANCER FOLLOWING 4 CYCLES OF AC FOLLOWED BY 4 CYCLES OF TAXANES VS 4 CYCLES OF AC WITH CISPLATIN FOLLOWED BY 4 CYCLES OF TAXANES AT MADURAI GRH.

PROFORMA

GROUP :

PRE CHEMOTHERAPY

NAME: AGE/SEX: IP NO:

FNAC: D.O.A:

TRUCUT:

CO MORBIDITIES:

DURATION OF BREAST DISEASE:

U/S BREAST:

U/S ABDOMEN:

SKELETAL SURVEY:

BLOOD INVESTIGATION:

HB PCV

RBS

LFT:

RFT:

SIZE OF LUMP
NODAL STATUS
STAGING
MRI

RESPONSE TO NEOADJUVANT CHEMOTHERAPY IN TRIPLE NEGATIVE BREAST CANCER FOLLOWING 4 CYCLES OF AC FOLLOWED BY 4 CYCLES OF TAXANES VS 4 CYCLES OF AC WITH CISPLATIN FOLLOWED BY 4 CYCLES OF TAXANES AT MADURAI GRH.

PROFORMA

GROUP :

NAME: AGE/SEX: IP NO:

FNAC: D.O.A:

TRUCUT:

CO MORBIDITIES:

DURATION OF BREAST DISEASE:

U/S BREAST:

U/S ABDOMEN:

SKELETAL SURVEY:

BLOOD INVESTIGATION:

HB PCV

RBS

LFT:

RFT:

CHEMOTHERAPY REGIMEN/CYCLES COMPLETED:

SIZE OF LUMP
NODAL STATUS
STAGING

DIAGNOSIS:

REMARKS:

RESPONSE TO NEOADJUVANT CHEMOTHERAPY IN TRIPLE NEGATIVE BREAST CANCER FOLLOWING 4 CYCLES OF AC FOLLOWED BY 4 CYCLES OF TAXANES VS 4 CYCLES OF AC WITH CISPLATIN FOLLOWED BY 4 CYCLES OF TAXANES AT MADURAI GRH.

PROFORMA

GROUP :

NAME: AGE/SEX: IP NO:

FNAC: D.O.A:

TRUCUT:

CO MORBIDITIES:

DURATION OF BREAST DISEASE:

U/S BREAST:

U/S ABDOMEN:

SKELETAL SURVEY:

BLOOD INVESTIGATION:

HB PCV

RBS

LFT:

RFT:

CHEMOTHERAPY REGIMEN/CYCLES COMPLETED:

SIZE OF LUMP
NODAL STATUS
STAGING
MRI

DIAGNOSIS:

REMARKS:

RESPONSE TO NEOADJUVANT CHEMOTHERAPY IN TRIPLE NEGATIVE BREAST CANCER FOLLOWING 4 CYCLES OF AC FOLLOWED BY 4 CYCLES OF TAXANES VS 4 CYCLES OF AC WITH CISPLATIN FOLLOWED BY 4 CYCLES OF TAXANES AT MADURAI GRH.

PROFORMA

GROUP :

NAME: AGE/SEX: IP NO:

FNAC: D.O.A:

TRUCUT:

CO MORBIDITIES:

DURATION OF BREAST DISEASE:

U/S BREAST:

U/S ABDOMEN:

SKELETAL SURVEY:

BLOOD INVESTIGATION:

HB PCV

RBS

LFT:

RFT:

POST CHEMOTHERAPY

SIZE OF LUMP
NODAL STATUS
STAGING
MRI

DIAGNOSIS:

PROCEDURE DONE:

HPE REPORT:

REMARKS:

ஆராய்ச்சி தகவல் அறிக்கை

மதுரை அரசு இராசாசி மருத்துவமனையில் வரும் நோயாளிக்கு ஒரு ஆராய்ச்சி இங்கு நடைபெற்று வருகிறது. நீங்களும் இந்த ஆராய்ச்சியில் பங்கேற்க விரும்பிகிறோம் .

உங்களை சி சிறப்பு பரிசோதனைக்கு உட்படுத்தி அதன் தகவல்களை ஆராய்வோம். அதனால் தங்களது நோயின் ஆய்வரிகையோ அல் து சிகிச்சையோ பாதிப்பு ஏற்படாது என்பதை தெரிவித்து கொள்கிறேன்.

முடிவுகளை வெளியிடும் போது அல் து ஆராய்ச்சியின் போதோ தங்களது பெயரோ அல் து அடையாளங்களோ வெளியிடமாட்டோம் என்பதை தெரிவித்து கொள்கிறோம்.

இந்த ஆராய்ச்சியில் பங்கேற்பது தங்களுடைய விருப்பத்தின் பேரில் தான் நடக்கும். மேலும் நீங்கள் எந்நேரமும் இந்த ஆராய்ச்சியில் இருந்து பின் வாங்க ாம் என்பதையும் தெரிவித்து கொள்கிறோம்.

இந்த சிறப்பு பரிசோதனை முடிவுகளை ஆராய்ச்சியின் போது அல் து ஆராய்ச்சியின் முடிவின் போது தங்களுக்கு அறிவிப்போம் என்பதையும் தெரிவித்து கொள்கிறோம்.

பங்கேற்பாளர் கையொப்பம்

ABBREVIATIONS

LABC	-	Locally advanced breast cancer
NACT	-	Neoadjuvant chemotherapy
MRM	-	Modified radical mastectomy
TNBC	-	Triple Negative Breast Cancer
CCR	-	Clinical complete response
CPR	-	Clinical Partial response
CNR	-	Clinical No response
PD	-	Progressive disease
PCR	-	Pathological complete response
PINV	-	Pathologically invasive cells
CT	-	Chemotherapy
AJCC	-	American Joint committee on cancer
UICC	-	International union against cancer

MASTER CHART

CASE GROUP (REGIMEN A)

s.no.	age	before neoadjuvant chemotherapy			post 4 cycles of AC with cisplatin			MRM	HPE			4 cycles of taxanes
		size of tumor(cm)	nodal status	TNM	size of tumor(cm)	nodal status	TNM		size of tumor(cm	nodal status	TNM	
1	55	8*8	N1	T3N1M0	4*3	N0	T3N0M0	done	4*2	N0	T3N0M0	given
2	56	9*8	N2a	T3N2aM0	5*4	N1	T3N1M0	done	5*4	N1	T3N1M0	given
3	54	11*9	N2a	T3N2aM0	6*5	N1	T3N1M0	done	6*4	N1	T3N1M0	given
4	52	12*8	N2a	T3N2aM0	6*4	N1	T3N1M0	done	6*3	N1	T3N1M0	given
5	48	8*6	N1	T3N1M0	no mass	N0	T0N0M0	done	no mass	N0	T0N0M0	given
6	60	8*4	N1	T3N1M0	4*2	N0	T2N0M0	done	4*2	N0	T0N0M0	given
7	60	10*6	N1	T3N1M0	5*3	N0	T3N0M0	done	5*2	N0	T0N0M0	given
8	58	10*8	N2a	T3N2aM0	no mass	N0	T0N0M0	done	no mass	N0	T0N0M0	given
9	60	9*7	N2a	T3N2aM0	7*6	N1	T3N1M0	done	7*6	N1	T3N1M0	given
10	45	8*6	N1	T3N1M0	4*3	N0	T2N0M0	done	4*2	N0	T2N0M0	given
11	49	8*6	N2a	T3N2aM0	9*8	N2a	T3N2aM0	done	9*7	N2a	T3N2aM0	given
12	50	10*7	N2a	T3N2aM0	8*6	N1	T3N1M0	done	8*5	N1	T3N1M0	given
13	52	9*7	N2a	T3N2aM0	5*3	N1	T3N1M0	done	5*3	N1	T3N1M0	given

14	49	10*8	N2a	T3N2aM0	5*3	N1	T3N1M0	done	5*3	N1	T3N1M0	given
15	45	8*9	N1	T3N1M0	no mass	N0	T0N0M0	done	no mass	N0	T0N0M0	given
16	50	8*7	N1	T3N1M0	4*3	N0	T2N0M0	done	4*3	N0	T2N0M0	given
17	55	8*8	N2a	T3N2aM0	4*4	N0	T2N0M0	done	4*3	N0	T2N0M0	given
18	57	10*9	N2a	T3N2aM0	5*4	N0	T3N0M0	done	5*4	N0	T3N0M0	given
19	59	8*6	N1	T3N1M0	no mass	N0	T0N0M0	done	no mass	N0	T0N0M0	given
20	58	12*7	N2a	T3N2aM0	6*3	N1	T3N1M0	done	6*2	N1	T3N1M0	given
21	57	8*6	N2a	T3N2aM0	6*5	N1	T3N1M0	done	8*6	N1	T3N1M0	given
22	55	10*6	N2a	T3N2aM0	no mass	N0	T0N0M0	done	no mass	N0	T0N0M0	given
23	45	11*7	N2a	T3N2aM0	5*3	N1	T3N1M0	done	5*2	N1	T3N1M0	given
24	46	11*8	N2a	T3N2aM0	5*4	N1	T3N1M0	done	5*4	N1	T3N1M0	given
25	52	12*8	N1	T3N1M0	6*4	N0	T3N1M0	done	6*3	N0	T3N0M0	given
26	52	9*6	N2a	T3N2aM0	no mass	N0	T0N0M0	done	no mass	N0	T0N0M0	given
27	47	10*7	N1	T3N1M0	5*3	N0	T3N0M0	done	5*2	N0	T3N0M0	given
28	55	9*8	N2a	T3N2aM0	5*3	N0	T3N0M0	done	5*3	N0	T3N0M0	given
29	48	10*6	N2a	T3N2aM0	4*3	N0	T2N0M0	done	4*3	N0	T2N0M0	given
30	50	10*6	N1	T3N1M0	5*3	N0	T3N0M0	done	5*3	N0	T3N0M0	given

MASTER CHART

CONTROL GROUP (REGIMEN B)

s.no.	age	before neoadjuvant chemotherapy			post 4 cycles of AC			MRM	HPE			4 cycles of taxanes
		size of tumor(cm	nodal status	TNM	size of tumor(cm)	nodal status	TNMc		size of tumor(cm	nodal status	TNMp	
1	56	8*7	N2a	T3N2aM0	4*3	N0	T2N0M0	done	4*3	N0	T2N0M0	given
2	57	10*8	N2a	T3N2aM0	8*6	N1	T3N1M0	done	8*5	N1	T3N1M0	given
3	60	10*8	N2a	T3N2aM0	5*3	N1	T3N1M0	done	5*3	N1	T3N1M0	given
4	45	11*9	N1	T3N1M0	9*8	N0	T3N0M0	done	9*7	N0	T3N0M0	given
5	49	11*6	N1	T3N1M0	6*3	N0	T3N0M0	done	6*3	N0	T3N0M0	given
6	52	10*7	N2a	T3N2aM0	8*6	N1	T3N1M0	done	8*6	N1	T3N1M0	given
7	55	9*7	N2a	T3N2aM0	5*3	N1	T3N1M0	done	5*2	N1	T3N1M0	given
8	59	10*6	N2a	T3N2aM0	12*7	N2a	T3N2aM0	done	12*7	N2a	T3N2aM0	given
9	49	8*6	N2a	T3N2aM0	4*3	N1	T2N1M0	done	4*3	N1	T2N1M0	given
10	50	9*8	N2a	T3N2aM0	8*7	N1	T3N1M0	done	8*6	N1	T3N1M0	given
11	50	9*8	N1	T3N1M0	5*4	N0	T3N0M0	done	5*3	N0	T3N0M0	given
12	60	8*5	N1	T3N1M0	no mass	N0	T0N0M0	done	no mass	N0	T0N0M0	given
13	56	9*7	N2a	T3N2aM0	5*3	NO	T3N0M0	done	5*3	N0	T3N0M0	given
14	47	9*6	N2a	T3N2aM0	11*7	N1	T3N1M0	done	11*6	N1	T3N1M0	given
15	51	10*7	N1	T3N1M0	5*3	N0	T3N0M0	done	5*3	N0	T3N0M0	given
16	55	9*7	N2a	T3N2aM0	7*6	N1	T3N1M0	done	7*5	N1	T3N1M0	given

17	59	11*8	N2a	T3N2aM0	5*4	N1	T3N1M0	done	5*4	N1	T3N1M0	given
18	49	10*6	N1	T3N1M0	8*6	N1	T3N1M0	done	8*5	N1	T3N1M0	given
19	60	10*8	N2a	T3N2aM0	5*4	N1	T3N1M0	done	5*3	N1	T3N1M0	given
20	59	8*8	N2a	T3N2aM0	10*9	N2a	T3N2aM0	done	10*9	N2a	T3N2aM0	given
21	55	9*7	N2a	T3N2aM0	5*3	N1	T3N1M0	done	5*3	N1	T3N1M0	given
22	54	9*8	N2a	T3N2aM0	5*4	N1	T3N1M0	done	5*4	N1	T3N1M0	given
23	53	10*6	N2a	T3N2aM0	no mass	N0	T0N0M0	done	no mass	N0	T0N0M0	given
24	60	10*7	N2a	T3N2aM0	5*3	N0	T3N0M0	done	5*3	N0	T3N0M0	given
25	51	11*6	N2a	T3N2aM0	9*5	N1	T3N1M0	done	9*4	N1	T3N1M0	given
26	45	10*6	N2a	T3N2aM0	5*3	N1	T3N0M0	done	5*3	N1	T3N1M0	given
27	49	12*8	N2a	T3N2aM0	10*7	N1	T3N1M0	done	10*6	N1	T3N1M0	given
28	52	9*7	N2a	T3N2aM0	4*3	N0	T2N0M0	done	4*3	N0	T2N0M0	given
29	55	9*6	N2a	T3N2aM0	11*8	N2a	T3N2aM0	done	11*8	N2a	T3N2aM0	given
30	59	8*7	N2a	T3N2aM0	4*3	N0	T2N0M0	done	4*3	N0	T2N0M0	given

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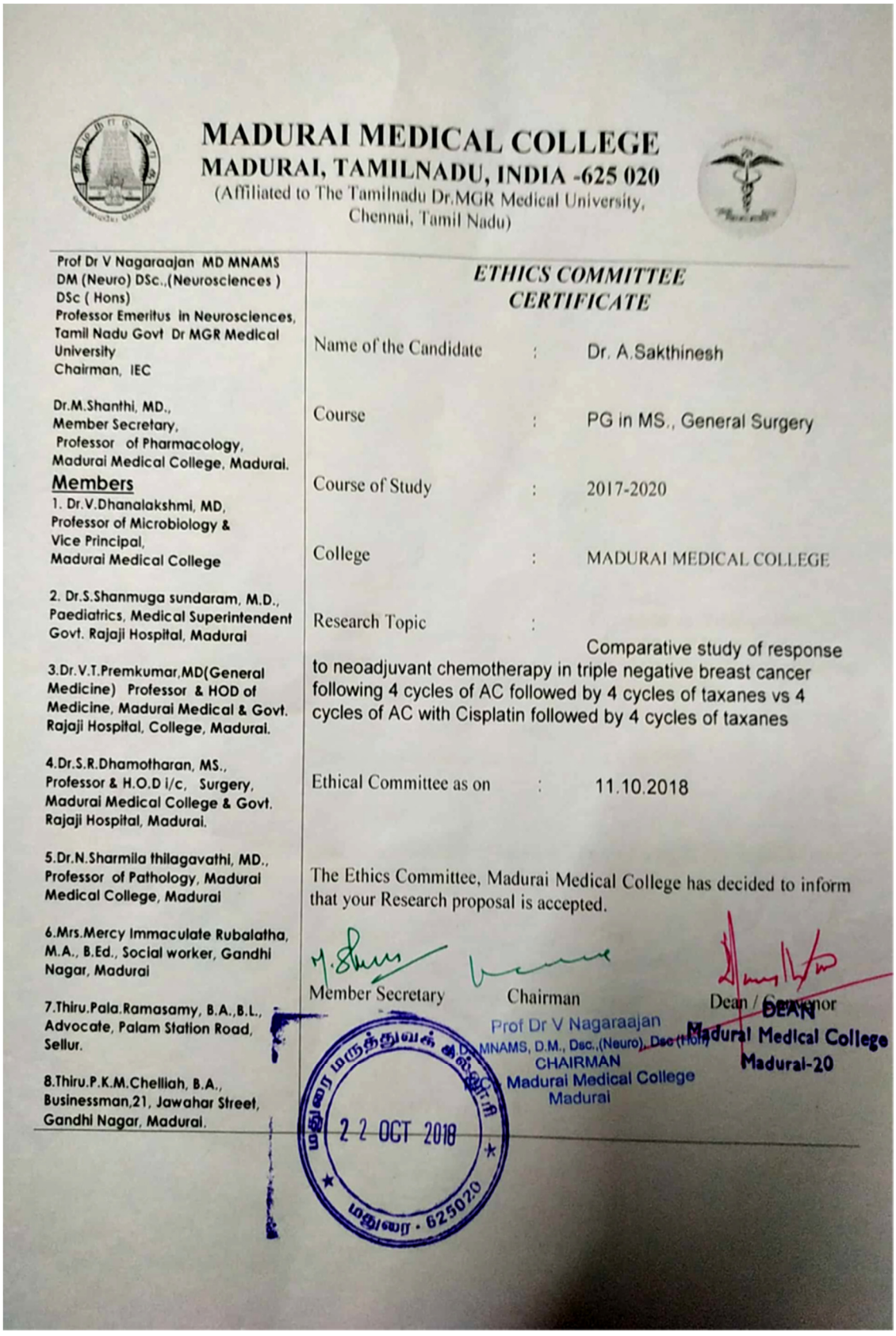

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ETHICS COMMITTEE CERTIFICATE

Name of the Candidate : Dr. A.Sakthinesh

Course : PG in MS., General Surgery

Course of Study : 2017-2020

College : MADURAI MEDICAL COLLEGE

Research Topic :

Comparative study of response to neoadjuvant chemotherapy in triple negative breast cancer following 4 cycles of AC followed by 4 cycles of taxanes vs 4 cycles of AC with Cisplatin followed by 4 cycles of taxanes

Ethical Committee as on : 11.10.2018

The Ethics Committee, Madurai Medical College has decided to inform that your Research proposal is accepted.

Member Secretary

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ANTI-PLAGIARISM CERTIFICATE

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CERTIFICATE II

This is to certify that this dissertation work titled, entitled ***"R*ESPONSE TO NEOADJUVANT CHEMOTHERAPY IN TRIPLE NEGATIVE BREAST CANCER FOLLOWING 4 CYCLES OF AC WITH CISPLATIN FOLLOWED BY 4 CYCLES OF TAXANES VS 4 CYCLES OF AC FOLLOWED BY 4 CYCLES OF TAXANES"** submitted by **Dr.A.SAKTHINESH**, with registration number 221711123 for the award of MASTER DEGREE in the branch of GENERAL SURGERY has been personally verified by me in urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **8** percentage of plagiarism in the dissertation.

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