"EXPRESSION OF PROSTATE SPECIFIC MEMBRANE ANTIGEN IN BREAST CANCER PATIENTS ATTENDING TERTIARY CARE HOSPITAL – A ONE YEAR CROSS SECTIONAL STUDY"

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LIST OF ABBREVIATIONS USED

PSMA	–	Prostate specific membrane antigen
H&E	–	Haematoxylin and Eosin
IHC	–	Immunohistochemistry
CD 31	–	Cluster of differentiation
WHO	–	World Health Organisation
TNM	–	Tumor stage, Nodal status, Metastasis
BCIR	–	Breast cancer incidence rate
BRCA	–	BReastCAncer gene
tp53	–	Tumorprotein 53
CHEK	–	Check point kinase
PTEN	–	Phosphatase and tensin homolog
CDH	–	Cadherin
STK	–	Serine/Threonine kinase
PALB	–	Partner and localiser of BRCA2
ER	–	Estrogen Receptor
PR	–	Progesteron Receptor
DCIS	–	Ductal carcinoma in situ
Her 2/neu	–	Human epidermal receptor 2 neuroblastoma
PIK3CA	–	Phosphatidylinositol- 4,5-biphosphate 3-kinase, catalytic subunit alpha
PET	–	Positron Emission tomography
CT	–	Computed tomography
SPECT	–	Single positron emission computarised tomography
MRI	–	Magnetic resonance imaging

3D CEU	–	3 dimensional contrast enhanced ultrasound in response
DC MRI	–	Diffusion contrast Magnetic resonance imaging
MVD	–	Micro vessel density
MMP	–	Matrix mettaloproteinase
VEGF	–	Vascular endothelial growth factor
CEA	–	Carcino-embryonic antigen
CA	–	Carbonic anhydrase
IBC	–	Infiltrating breast carcinoma
ILC	–	Infiltrating lobular carcinoma
EMA	–	Epithelial membrane antigen
PAP	–	Phosphatidic acid phosphatise
PSP	–	Phosphoserinephophatase
PECAM	–	Platelet endothelial cell adhesion molecule

ABSTRACT

Background and purpose: Breast carcinomas are challenging to treat and are also the leading cause of morbidity and mortality in women. Prostate specific membrane antigen (PSMA) is type II transmembrane glycoprotein having a 3 part structure. PSMA is expressed in a variety of normal tissues and its expression is dysregulated in different cancerous tissues. Only a few studies have demonstrated the expression of PSMA in tumor cells in breast carcinoma as well as in cytoplasm of endothelial cells lining tumormicrovessels and the results of the studies done have shown variable outcomes.

Till today, the PSMA expression assessment in primary breast cancer has not been explored well.

Thus, the aim in this study is to investigate the expression of PSMA in primary breast carcinomas, making it a potential target for specific anti-cancer therapy and will be a helping agent in the treatment of breast cancer and preventive agent in its metastasis.

Study design: Cross sectional study

Methodology: The present study was a prospective study which included 40 cases from January 2019 to December 2019. Clinical details pertaining to the patient with breast carcinoma admitted to the tertiary care hospital were obtained from the case papers, and from the medical records department of the hospital.

The details of the gross and histopathological findings were obtained from the requisition forms and report forms archived in the department of pathology.

Tissues were fixed in 10% formalin and processed to make paraffin blocks. Haematoxylin and Eosin (H&E) stained slides available from the department of pathology were studied for histopathological features.

For Immunohistochemistry (IHC), Sections 4 to 5µ thick were placed on coated slides. The sections were stained with the antibody directed against PSMA and CD31, as per the IHC staining protocol.

Results:PSMA expression in tumor cells appeared in 47.5% (19/40) of tested cases ranged between weak to strong. On the other hand, 100% (40/40) of tested cases showed PSMA expression in tumor associated neovasculature. But, no significant correlation was found with any clinicopathological parameters.

Conclusion: We came to a conclusion that PSMA staining is seen in tumor cells as well as tumor associated neovasculature. Tumor associated neovasculature showed prominent staining as compared to tumor cells and normal breast epithelial cells. Nevertheless, many more studies are still needed to establish a relationship between use of PSMA in breast carcinoma and its clinic-pathological correlation.

Keywords: PSMA, CD31,Breast carcinoma, IHC

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INTRODUCTION

Breast cancer is the most common malignancy in women in Asia and its incidence is rapidly intensifying. In developing countries, due to upsurging life expectancy, the adoption of western lifestyles and urbanization there is a rapid rise in the number of breast cancer cases. Therefore, to improve breast cancer outcomes, early detection remains an important element in breast cancer control and survival rate. Prompt referral and early diagnosis programs help in diagnosing and treating breast cancer. Population-based cancer screening is cost-effective if done with high-standard programs targeting large and specific cancer burden.[1]

Mammography screening is very expensive is the only proved an effective breast cancer screening method in countries with better infrastructure and affordable population-based organized screening programs. In limited resource settings, low-cost screening approaches, like clinical breast examination becomes a necessity.[2] WHO within the context of national cancer control programs and integrated to non-communicable disease prevention and control promotes breast cancer control.

Annual age standardized incidence rate of breast carcinoma as of 2018 was 18.1 per 10,00,000 women worldwide whereas in India it was 25.8 per 10,00,000 women.[3] In Karnataka state, according to Bengaluru registry area, the breast cancer incidence is as high as 34.4 per 10,00,000 women, which was accounting for 27.5% of all female cancers.[4,5]

The incidence and detection rate of breast cancer in India has increased in the past few years. Studies revealed that, the diagnosis of breast cancer is considerably

late due to which there is delay in the treatment, which has direct impact on morbidity and mortality.[1]

Thus, a better understanding of breast cancer is required for early diagnosis and to have knowledge about prognosis to modify the treatment.

In Pathology, histological grading of breast carcinomas is an independent prognostic factor and has a significant role to estimate the overall survival of the patients. In spite of a uniform grading given by Bloom and Richardson which was later modified by Nottingham and further modified by Scarff and Ellis-Elston, there still remains a subjective variability to decide the grade in many carcinomas.[6,7,8]

In the present day, use of IHC markers in detection and treatment of breast cancer is a routine practice. Various IHC markers are helpful in understanding the role of tumor and its nature of spread.

At times distinction between an invasive carcinoma and in situ carcinoma can be difficult, particularly in a core biopsy specimen. There is a considerable inter-observer disagreement in difficult cases when a diagnosis is made on histology alone.

PSMA a type 2 membrane protein is characterised by murine monoclonal antibody. It is expressed in all forms of prostate tissue including carcinoma prostate. The impact of enzymatic function of PSMA in prostatic tissue is clear while remains unclear in other human tissues.[9]

Formerly PSMA seems to be expressed not only in prostate cancer but also in other cancers. More specifically it is found to be related with neovasculature associated to cancer. Various studies assessed a wide range of carcinomas including

neuroendocrine, renal, bladder, colon and breast, which expressed PSMA in their neovasculature.[10]

Few researchers have used PSMA as an IHC marker in other tissues, which have variable results. But PSMA IHC expression in breast cancer is less explored.

In this study an attempt was made to find out the expression of PSMA in breast cancer tumor cells and tumor associated neovasculature which could be helpful in treating and prognosticating breast cancers at an early stage.

AIMS AND OBJECTIVES

1. To study the expression of PSMA in tumor epithelial cells and vascular endothelial cells in tumor area in Breast carcinoma

REVIEW OF LITERATURE

DEVELOPMENT OF BREAST:

Breast development happens in certain stages during a women's life: first before birth, at puberty and later in child bearing years, while some changes are also evident during menstrual cycle and menopause.[11]

Mammary glands are modified tubuloalveolar apocrine sweat glands with a cutaneous origin. It consists of parenchymal and stromal elements. Parenchyma forms a system of branching ducts, leading to secretary acini development. The stroma consists of connective and adipose tissue, while providing environment for development of parenchyma.[12, 13]

Parenchyma derives embryonically from surface ectoderm, stroma arises from surrounding mesenchyme. When inutero, ectodermal thickening in the chest area called mammary ridge or milk line or line of Schultz develops, extending from axilla to inguinal area which persists in the pectoral area and penetrates the mesenchyme. The origin of gland is ectodermal and of the stroma is mesodermal.[14]

After birth, nipples and milk duct system are formed. First to develop are lobes followed by glands. The entire system is solid and later gets canalised.[14]

Perinatal development: Breast development is divided in different stages.[15]

Table 1: Perinatal development of mammary gland[15]

Stage	Stages of Mammary gland development	Embryo-fetal stage
1	Ridge	< 5mm of embryo
2	Milk hill	>5.5 mm of embryo
3	Mammary disc	Around 10-11 mm
4	Lobule type	11-25 mm
5	Cone	25-30 mm
6	Budding	30-68 mm
7	Indentation	68 mm to 10 cm
8	Branching	10 cm fetus
9	Canalization	20-32 weeks of gestation
10	End vesicle	New born

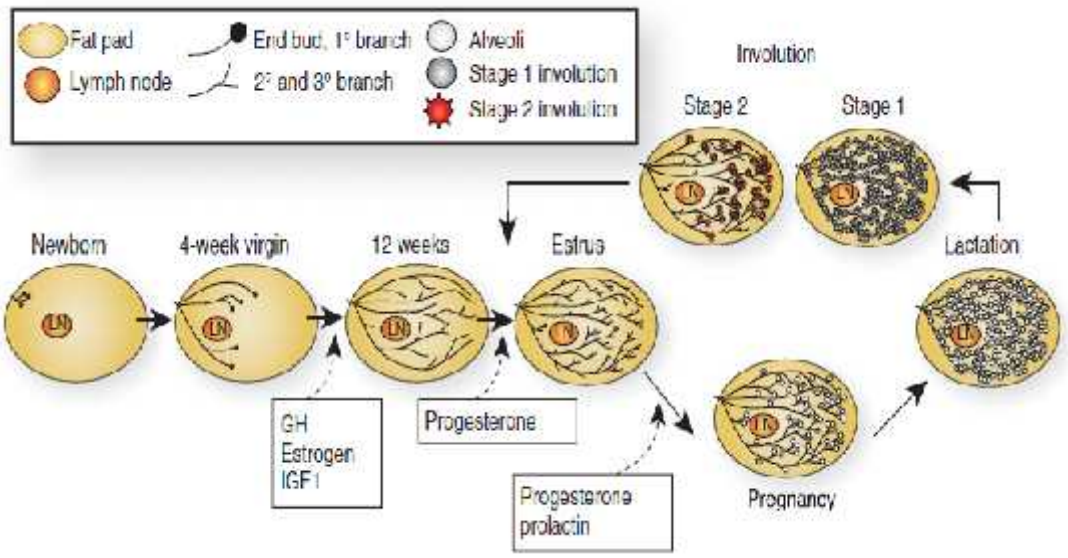


Fig 1: The stages of postnatal mammary gland development[16]

At puberty, the ovaries start to produce and release estrogen, fat in the connective tissue starts to buildup. This causes the breast to enlarge and duct system also starts to grow. When ovulation and menstruation starts, the breast begins to mature and glands form at the end of the milk duct. Estrogen controls the growth of ducts and progesterone controls the growth of glandular buds.[16]

During pregnancy, due to growth of milk duct system and formation of lobules there is enlargement of breast.[17]

At menopause, levels of estrogen and progesterone prepares for change. Estrogen levels drastically decrease leading to dehydration of breast's connective tissue and it remains no longer elastic.[17]

Table 2: Tanner's Stages of Breast Development[18]

Tanner's Breast Developmental Stages	
Stage I (Preadolescent)	Papilla elevation above the level of the chest wall.
Stage II (Breast budding)	Breast and papilla elevation, along with increased areola diameter.
Stage III	Ongoing enlargement of the breasts and areole.
Stage IV	Elevation of the areola and papilla above the breast mound.
Stage V (Mature breast)	Elevation of the papilla with regression of the areola

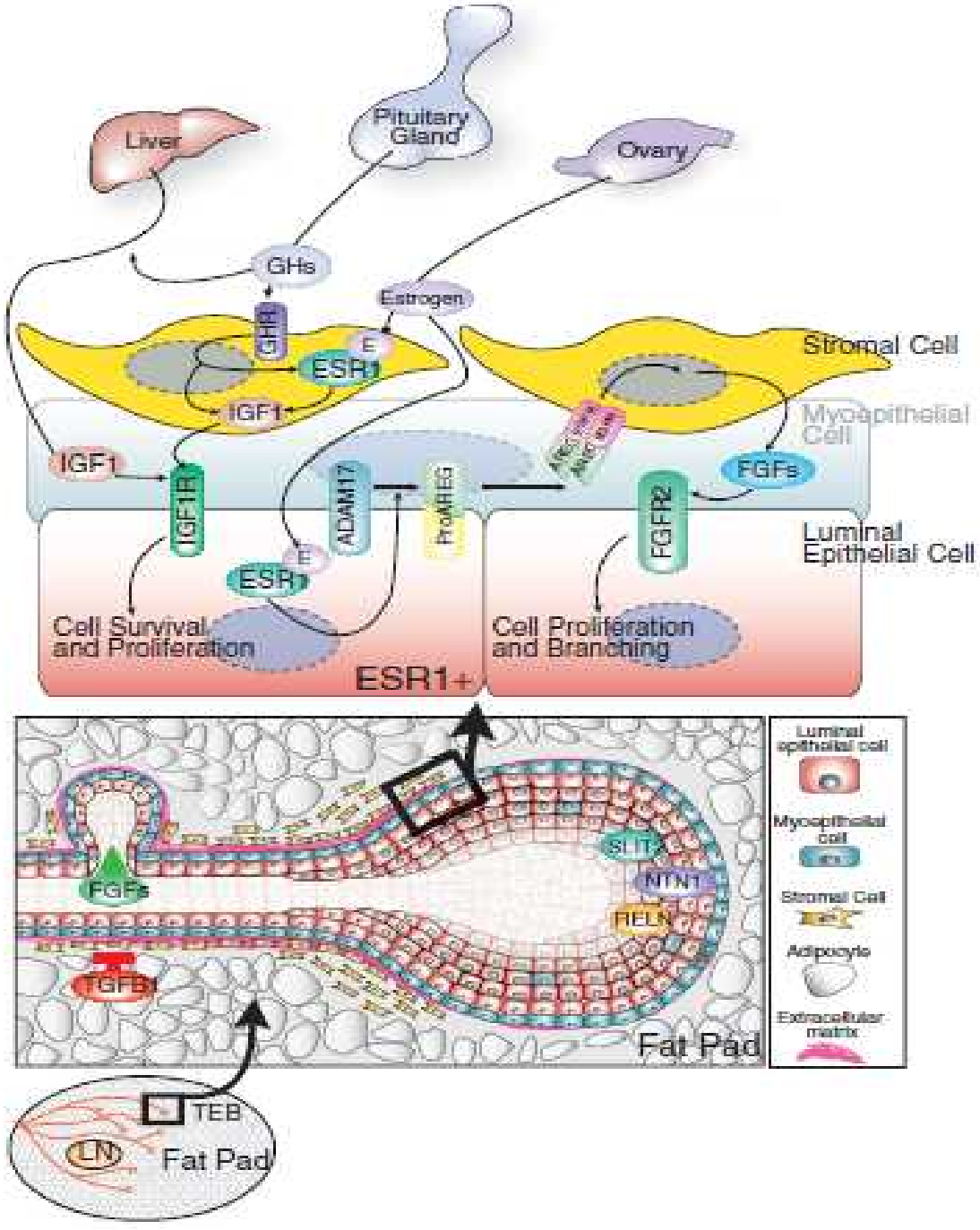


Fig 2: The physiological events occurring during pubertal development[18]

ANATOMY AND PHYSIOLOGY:

Breast extends from 2nd rib above to 6th rib below. Medially it borders the lateral edge of the body of sternum and laterally it reaches mid-axillary line. A tongue shaped superolateral extension of breast tissue projects into axilla called Axillary tail of Spence.[19]

Breast is divided into four quadrants, main bulk lies in the upper quadrant. This quadrant is more commonly involved in breast cancer.[19]

The Nipple is generally situated at fourth intercostal space. 15-20 lactiferous ducts open on to the nipple. It is surrounded by areola, consisting of large sebaceous glands called as Glands of Montgomery which are visible to naked eyes.[20]

Breast majorly lies on the pectoralis major, laterally on serratus anterior and inferiorly on upper part of rectus sheath. Considering fascial relationship, it mainly lies in a pocket of superficial fascia. This fascia lies under the dermis and allows superficial flaps to be separated from glandular mass in a avascular plane. This ensures dissection of breast tissue to the skin flaps.[21]

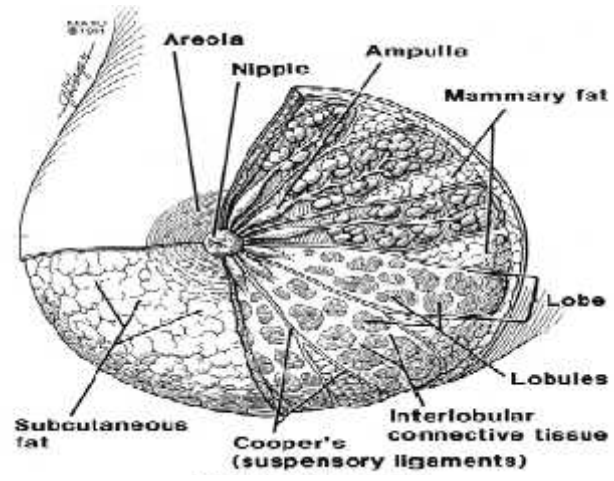


Fig 3: Anatomy of female breast[22]

Extension of fibrous process of this layer of fascia to the skin, nipple and upper part of breast forms suspensory ligament of cooper . Narrowing or tightening of this tissue due to malignant infiltration results in skin dimpling over carcinoma of breast. Deep layer of superficial fascia is thicker and covers breast plate.

Under the deep layer lies the layer of areolar tissue, which allows the breast to move freely on fascial covering of serratus anterior and pectoralis major. This layer forms retromammary space. Infiltration of tumor thorough this space into pectoralis displays the physical sign of deep tethering of malignant breast mass. Retromammary space enables bloodless dissection of deep aspect of breast during simple mastectomy.[22, 23]

Blood supply to the breast:

The Blood supply to the breast is composed of rich anastomosis derived from axillary, internal thoracic and intercostals arteries. [23]

Four branches of axillary artery:

1. Superior thoracic artery
2. Thoracoacromial artery
3. Lateral thoracic artery
4. Sub scapular artery

Internal Thoracic artery: Perforating branches to anteromedial breast

Intercostal arteries: Second to fourth anterior intercostals arteries. Second perforating artery also supplies nipple and areola.[24]

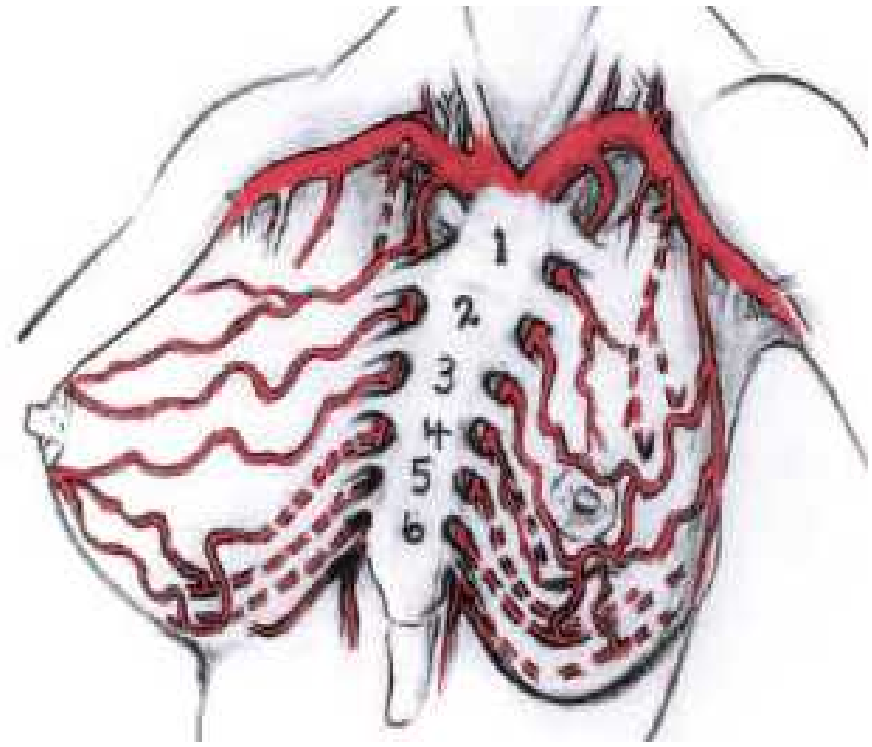


Fig 4: Blood supply of the breast by means of the internal thoracic artery[24]

Venous drainage:

Veins follow the arteries. First converges around the nipple to form an anastomosing venous circle and then form two sets of veins.

1. Superficial vein which drains into internal thoracic veins and superficial vein of lower part of neck,

2. Deep vein which drains into internal thoracic, axillary and posterior intercostals vein.

Intercostal veins communicate with vertebral veins. This route is responsible for metastasis of Breast cancer to vertebral bodies, sacrum and pelvic bone.[24]

Nerve supply of breast:

Breast is supplied by fourth to sixth intercostal nerve by anterior and lateral cutaneous branches. Nipple is supplied by anterior branch of lateral cutaneous branch of T4. Its sensory fibers terminate close to the epithelium as free endings. Secretary activities of the glands are largely controlled by ovarian and hypophyseal hormones rather than by efferent motor fibers.[25]

Lymphatic drainage: The breast is an ectodermal tissue thus its lymphatic drainage is mostly parallel to the lymph flow of overlying skin. Lymphatic flow of the breast is significant because metastatic dissemination occurs mainly by lymphatic routes. [25]

Lymph node stations:

1. Axillary (85%): Anterior, posterior, central, lateral, apical, interpectoral
2. Internal mammary/ Para sternal (10%)
3. Others (5%): Supraclavicular, Cephalic, posterior intercostals, subdiaphragmatic, subpertoneal[25]

Table 3: Quadrant wise drainage:

Quadrant	Axilla (%)	Internal mammary chain (%)
Upper outer	95.8	10.4
Upper inner	93.1	32.4
Lower outer	97.7	29.5
Lower inner	88	52.7
Center	100	23.7

Lymphatic from left breast terminate in the thoracic duct and left subclavian vein. Right breast drains into right subclavian vein.[25]

Lymphatic vessels:

1. Superficial lymphatics – Skin over breast except nipple and areola
2. Deep lymphatics – Parenchyma, nipple and areola
3. Subareolar plexus of Sappey is a network of lymphatics in areola and nipple. It is a good sight for injecting dye during sentinel lymph node biopsy.

Sentinel lymph node biopsy was first used clinically for penile carcinomas. Its utility in Breast cancer was explored in 1970s. It is the first node in regional lymphatic to receive lymph from primary tumor. In Breast carcinoma, the usual site of sentinel lymph node is most lateral of anterior group of lymph node. Sentinel lymph node biopsy is thus indicated in patients with clinically node negative disease.[25]

Histology of Breast:

Breast has two types of epithelial cells i.e luminal and myoepithelial cells. The origin of these cells is thought to be from intermediate or basal cells, which acts as stem cells. Luminal cells are cuboidal to columnar in shape having small round to oval nuclei with inconspicuous nucleoli and have moderate amount of eosinophilic cytoplasm. It forms the inner most layer lining the ducts and acini. Myoepithelial cells forms the outermost layer between luminal cells and basement membrane. It is flattened with small round nuclei and clear to abundant cytoplasm. It helps to produce and maintain basement membrane.[26]

Breast has two types of stroma i.e interlobular and intralobular. It comprises of connective, fibrous and adipose tissue. The cellular components of interlobular stroma are fibroblasts, myofibroblasts, adipocytes, blood and lymphatic vessels. Intralobular stroma consists of lymphocytes and plasma cells.[27]

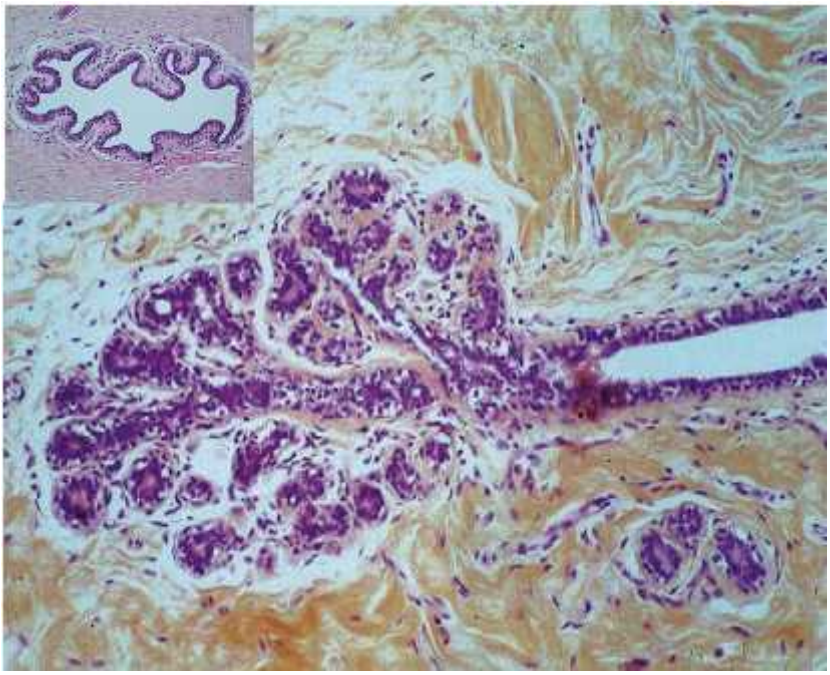


Fig 5: Histology of the Breast[27]

BREAST CANCER

Breast cancer is uncontrollable division of cells in the breast tissue, bringing about changes and resulting in lump or a mass. Most common site is the lobules or in the ducts which connects the lobules to nipples.[28]

Breast cancer is a heterogenous disease, which is comprehensive and inherent with various morphologies, molecular characteristics and clinical patterns.[29]

In 2018, the global cancer incidence was estimated as 18.1 million new cases and 9.6 million deaths. 5 out of 6 women and 1 of 8 men worldwide develop cancer during their lifetime, out of which 1 in 11 women die due to cancer. Most common cancers are lung, colorectal, breast, prostate and stomach. Breast cancer is second most common cancer after lung cancer, with 10.4% of all cancer incidences. It is 5th most common cause of cancer death. According to Globocan 2018 status, it is reported that overall burden of cancer has increased and trend might continue in coming years. It is estimated that approximately 1,797,900 women in India would have breast cancer by the end of 2020.[30, 31]

Considering Asian population, a recent insight by BCIR (Breast cancer incidence report, 2019) into breast cancer incidence trends among Asian counties, observed 1.1% increase in last decade. WHO says, the global load of breast cancer is gradually drifting from developed to developing countries, where western countries shows drop in incidence due to healthy lifestyle, tobacco resistance, awareness programs and better diagnostic facilities.[31] The cause for which is associated with social and economic development. In industrialised nation, due to rapid changes in lifestyle, it has become more prevalent.[32]

Union health ministry reports Indian females with breast cancer, ranks as the number one cancer with a rate of 25.8 per million women and mortality of 12.7 per million. The survival rate in India for breast cancer is low, with only 66.1%. While in countries like USA and Australia, survival rates from breast cancer are as high as 90%.[33]

RISK FACTORS OF BREAST CANCER:

Risk factor of breast cancer is complex and its progression is a multistep process. The consequences are from a series of epigenetic, genetic, endocrine, aging, familial history, lifestyle exposure to the environmental risk factor. It has been identified in the research 60-70% cases are with known risk factors while, remaining 30-40% of unknown risk factors. Common risk factors are age, geographical variation, previous benign breast cancer, menopause, oral contraceptives, breast feeding, breast density, familial history, personal history, radiation, tobacco, alcohol, diet, and hormonal changes.[34]

Aging is an important risk factor for breast cancer, because of its direct relation with incidence. According to American Cancer Society, prevalence of invasive breast cancer is higher in women of age 55 years and older, as compared to women under 45 years of age.[34]

The risk for developing breast cancer doubles if a woman has a positive history of breast cancer in her first degree relative i.e. mother, sister, daughter, father and brother. The risk is 5 folds when 2 or more first degree relatives develop breast cancer. Females with history of cancer in one breast, has greater risk of developing breast cancer in the other breast or another part of the same breast. Breast diseases

which are non cancerous in origin like, atypical hyperplasia or lobular carcinoma insitu are associated with higher risk of getting breast cancer.[35]

In the early menarche and late menopause, have greater risk of breast cancer. Menopause after 55 years of age, such females are more likely to develop breast cancer as compared to females getting menopause before 45 years of age. Only 40% of women, who undergo bilateral oophrectomy with normal age for menopause, thus are at higher risk for breast cancer.[36, 37]

Females using oral contraceptives are at risk of developing breast cancer than those using exogenous hormones. Irrespective of duration of use of oral contraceptives, ten years after stopping its use, is same as new users. This is of importance as these are generally used by young females, who are at low risk of developing breast cancer. Thus, using oral contraceptives might increase the risk in young females. Hormone replacement therapy if taken more than five years during menopause can increase the risk of breast cancer.[38]

In higher age group with nuliparity, increases risk of breast cancer. In pregnancy and breast feeding, due to persistent change in mammary gland, it becomes less susceptible to carcinogenic factors, which reduces the risk. Pregnancy in younger age is protective to breast cancer incidence. Breast feeding for longer durations has also been related with a greater reduction in risk of developing breast cancer.[39]

Variety of dietary factors are being examined which are having potential to cause breast cancer. Increased consumption of alcohol, red meat, processed meat, animal fat and decreased consumption of fruits and vegetables, calcium, vitamin D, vitamin C, vitamin E, soy and antioxidants such as beta carotene and other carotinoids increases risk of breast cancer. Biomarkers of inflammation, DNA damage, oxidative

stress has been proven to be influenced by mediterranean diet which in turn influences breast cancer.[40]

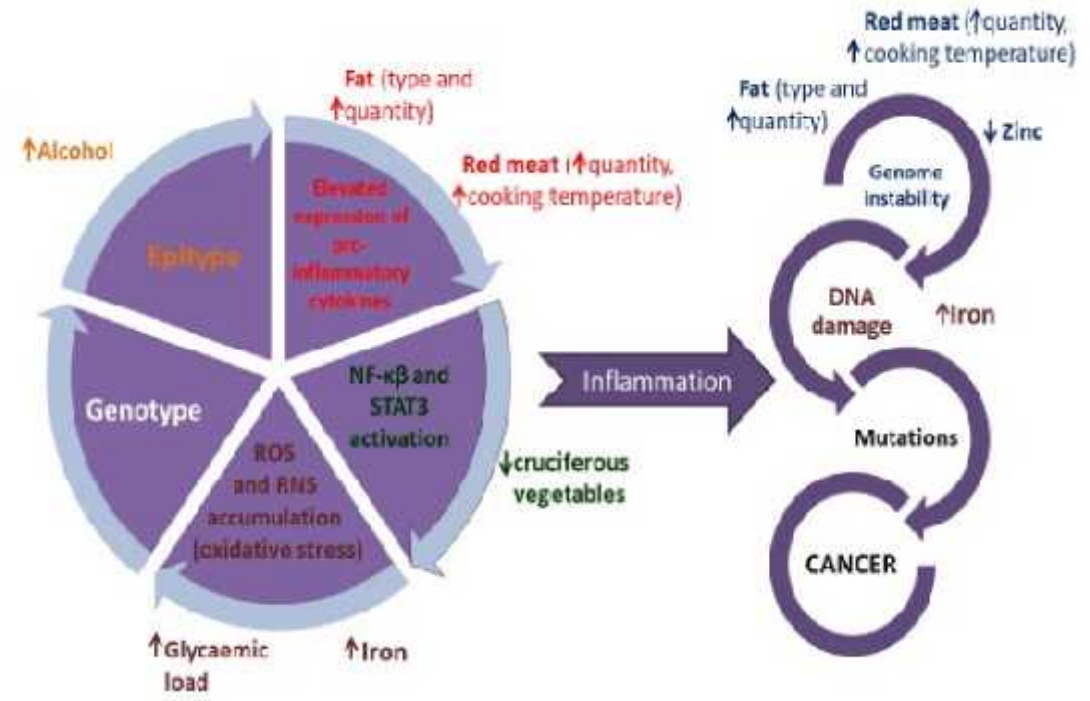


Fig 6: The influence of diet on the series of events that mediate inflammation-induced cancer initiation[40]

The ratio of omega 3 to omega 6 fatty acids, is also related to breast cancer. Some studies concluded, higher ratio is related to lower risk.[41]

Blood sugar response of body to standardised amount of carbohydrate in food is indicated by glycemic index. Amount of food consumed is glycemic load. Foods having high glycemic index and glycemic load can influence blood glucose level and insulin concentration, which increases the risk of breast cancer.[42]

Obesity has been associated with breast cancer according to many studies where the mechanism is related to insulin like growth factor 1 and its binding protein. Insulin like growth factor 1 helps in growth, progression and metastasis of breast cancer.[43, 44]

Five to ten percent cases of breast cancer are the result of genetic mutations inheredited from parents. BRCA 1 and BRCA 2 mutations are the most common cause of hereditary breast cancer. These genes helps in DNA damage repair while mutated version can lead to abnormal cell growth thus leading to cancer. 7 in 10 females with BRCA 1 or BRCA 2 gene mutation have a greater chance of getting breast cancer. These females are generally diagnosed at younger age and can develop cancer bilaterally. Other genetic mutations such as ATM, TP53, CHEK2, PTEN, CDH1, STK11 and PALB2 are also contributing to the risk factors of breast cancer.[45]

PATHOGENESIS:

Several pathways are followed in pathogenesis of breast cancer. Based on hormonal receptors and morphology, breast cancer initiation, transformation and progression have been described.

Considering ER+ and ER- Models, Flat epithelial atypia, atypical ductal hyperplasia and ER+ Ductal carcinoma insitu (DCIS) are non-obligate precursors of invasive and metastatic breast cancers, which has been noted in ER+ model. According to ER- model, ER - DCIS and microglandular adenomas are precursors for ER- breast carcinomas.[46]

Gains of 1q, loss of 16q, infrequent amplification of 17q12 genes are associated with ER+ pathway. These lesions expresses basal markers and lacks the over expression of Her2. This pathway consists of neoplastic lesions predominantly of low to intermediate grade phenotype, with small subset of morphologically defined high grade tumors.[46]

Loss of 13q, gain in chromosomal region 11q13, and amplification of 17q12 genes are associated with ER- pathway. This pathway consists mainly of morphologically defined intermediate and high grade tumors.[46]

In both the pathways, PIK3CA mutation occurs commonly. TP53 mutations are more commonly seen in ER- pathways. Both Her2+ and Her2- groups are seen in ER- breast cancers.[46]

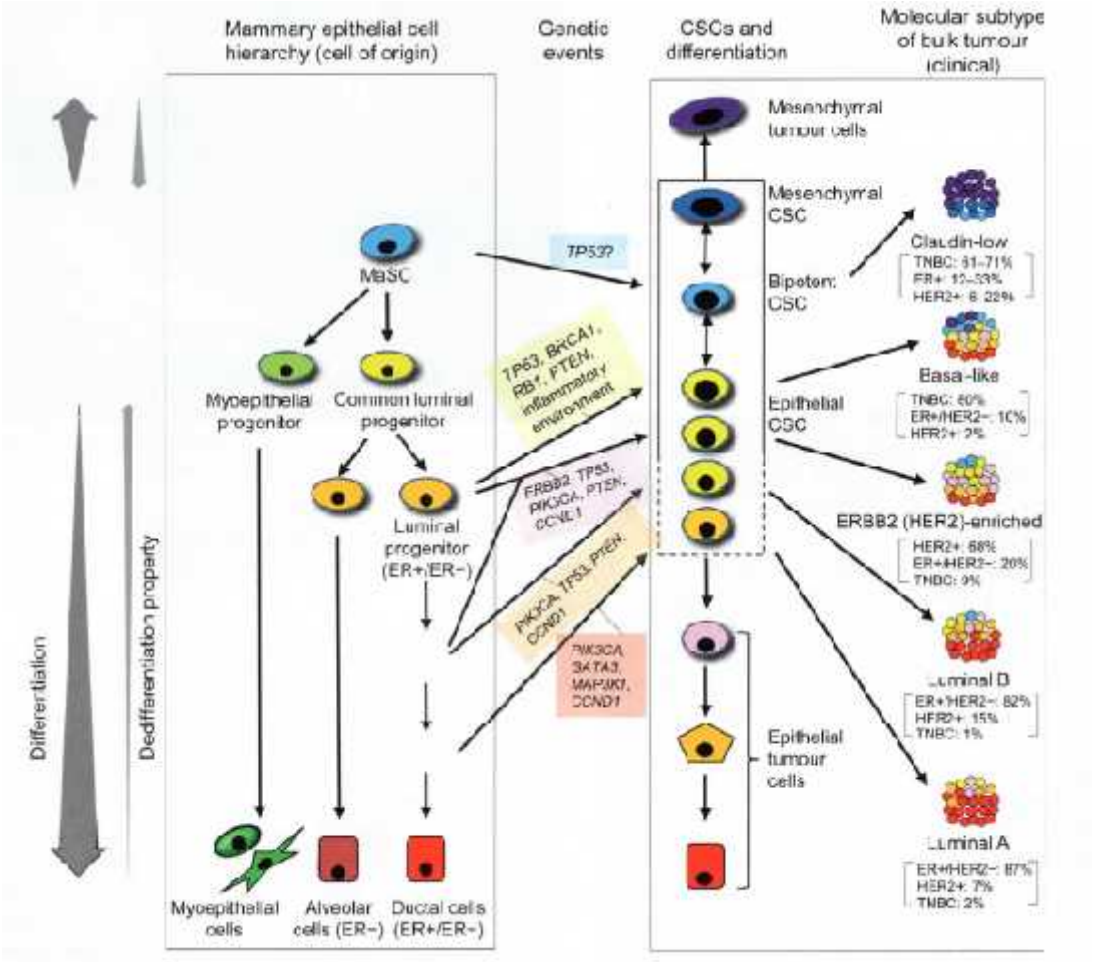


Fig 7: Flow chart depicting differentiation of normal cells and their genetic mutational transformation[46]

DIAGNOSTIC TECHNIQUES:

Various imaging techniques are utilised to detect and access the progression of breast cancer and response to treatment. It includes mammography, MRI (Magnetic resonance imaging), PET scan (Positron emission tomography), CT (Computed tomography) scan, SPECT (Single-photon emission computed tomography).

Mammography is considered to be gold standard technique in diagnosing breast cancer. It is considered to be having high sensitivity and specificity. It is inexpensive and well tolerated by the patients. According to recent studies, reduction in mortality due to breast cancer is observed by 19% with the help of mammography.[47]

Besides mammography, Ultrasound imaging technique is another common method to diagnose and to monitor response of treatment in breast cancer. As it does not use ionising radiations, it can be readily used in pregnant and breast feeding females. Techniques such as ultrasound contrast and elastography are utilised for breast interventions.[47]

Few newest techniques for assessing angiogenesis in breast tumours by 3D-CEUS (Contrast enhanced Ultrasound) and DC-MRI (Dynamic contrast-enhanced magnetic resonance imaging) are also utilised. These techniques show various tumour related vascular characteristics such as micro-vessel density (MVD), vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMP 2 and MMP 9) expression.[48]

Magnetic resonance imaging (MRI) is one of the important diagnostic tools for breast cancer. This is utilised to monitor the high risk patients, assessment of

breast cancer metastasis, response to therapy and recurrence. With the help of data by MRI various actions such as breast discharge, micro-calcification, premalignant lesion, staging of cancer and residual tumor can be assessed.[48]

PET and SPECT are other techniques used to diagnose breast cancer. Radioactive isotopes are used in PET techniques which emits positrons, while isotopes which emit gamma photons are used in SPECT technique. Studies suggest, these two can be used to detect breast cancer with bone metastasis.[48]

Diagnosis is possible with the help of mammogram and is confirmed on needle core or surgical biopsies. Selection of type of biopsy depends on size, location, preferences and resources.[48]

The American society of clinical oncology has updated its recommendation for use of tumor markers in prevention, screening, treatment and surveillance if breast cancer. Markers that showed evidence clinically includes CA 15-3, CEA, ER, PR, HER2, p53.[49]

Due to lack of sensitivity in early detection and lack of specificity, none of the available marker is of value in early diagnosis of breast cancer.[48]

The metastasis of breast cancer is observed in lung, liver, bones and brain, with brain being most prevalent organ. In 2019, 8.2 million global deaths were associated with cancer. The major cause of death from cancer worldwide is metastasis to distant organ.[48]

WHO HISTOLOGICAL CLASSIFICATION OF BREAST TUMORS 2019 [46]

1. Epithelial tumors of Breast

A. Benign epithelial proliferations and precursors

a. Usual ductal hyperplasia

b. Columnar cell lesions

c. Atypical ductal hyperplasia

B. Adenosis and Benign sclerosing lesions

a. Sclerosing adenosis

b. Apocine adenosis and adenoma

c. Microglandular adenosis

d. Radial scar/ Complex sclerosing lesion

C. Adenomas

a. Tubular adenoma

b. Lactating adenoma

c. Ductal adenoma

D. Epithelial-myoepithelial tumors

a. Pleomorphic adenoma

b. Adenomyoepithelioma

c. Malignant adenomyoepithelioma

E. Papillary Neoplasms

a. Intraductal papilloma

b. Papillary ductal carcinoma in situ

c. Encapsulated papillary carcinoma

d. Solid papillary carcinoma (Insitu/Invasive)

e. Invasive papillary carcinoma

F. Non invasive lobular Neoplasia

a. Atypical lobular hyperplasia

b. Lobular carcinoma insitu

G. Ductal carcinoma Insitu

H. Invasive Breast Carcinoma

a. Invasive Breast Carcinoma, No special type

b. Microinvasive carcinoma

c. Invasive Lobular carcinoma

d. Tubular carcinoma

e. Cribriform carcinoma

f. Mucinous carcinoma

g. Mucinous cystadenocarcinoma

h. Invasive micropapillary carcinoma

i. Carcinoma with apocrine differentiation

j. Metaplastic carcinoma

I. Rare and Salivary gland tumors

a. Acinic cell carcinoma

b. Adenoid cystic carcinoma

c. Secretory carcinoma

d. Mucoepidermoid carcinoma

e. Polymorphous adenocarcinoma

f. Tall cell carcinoma with reversed polarity

J. Neuroendocrine neoplasms

a. Neuroendocrine tumor

b. Neuroendocrine carcinoma

2. Fibroepithelial tumors and hamartomas of Breast

a. Hamartoma

b. Fibroadenoma

c. Phyllodes tumor

3. Tumors of Nipple

A. Epithelial tumors

a. Syringomatous tumor

b. Nipple adenoma

c. Paget's disease of breast

4. Mesenchymal tumors of Breast

A. Vascular tumors

a. Haemangioma

b. Angiomatosis

c. Atypical vascular lesions

d. Postradiation angiosarcoma of breast

e. Primary angiosarcoma of breast

B. Fibroblastic and Myofibroblastic tumors

a. Nodular fascitis

b. Myofibroblastoma

c. Desmoid fibromatosis

d. Inflammatory myofibroblastic tumor

C. Peripheral nerve sheath tumors

a. Schwannoma

b. Neurofibroma

c. Grannular cell tumors

D. Smooth muscle tumors

a. Leiomyoma

b. Leiomyosarcoma

E. Adipocytic tumors

a. Lipoma

b. Angiolipoma

c. Liposarcoma

F. Other mesenchymal tumors and tumor like conditions

a. Pseudoangiomatous stromal hyperplasia

5. Hematolymphoid tumors of Breast

A. Lymphoma

a. MALT Lymphoma

b. Follicular Lymphoma

c. DLBCL

d. Burkitt's Lymphoma

e. Breast implant associated – anaplastic large cell lymphoma

6. Tumors of Male Breast

A. Epithelial tumors

a. Gynaecomastia

b. Carcinoma in situ

c. Invasive carcinoma

7. Metastasis to Breast

8. Genetic tumor syndromes of Breast

a. BRCA1/2-associated hereditary breast and ovarian cancer syndrome

b. Cowden syndrome

c. Ataxia-telangiectasia

d. Li-Fraumeni syndrome, TP53-associated

e. Li-Fraumeni syndrome, CHEK2-associated

f. CDH1-associated breast cancer

g. PALB2-associated cancers

h. Peutz-Jeghers syndrome

i. Neurofibromatosis type 1

j. The polygenic component of breast cancer susceptibility

TNM STAGING OF MALIGNANT TUMORS, 2017, 8th Edition

TNM Clinical Classification[36]

T- Primary Tumour

TX- Primary tumour cannot be assessed

T0- No evidence of primary tumour

Tis- Carcinoma in situ

Tis- (DCIS) Ductal carcinoma in situ

Tis- (LCIS) Lobular carcinoma in situ

Tis- (Paget) Paget disease of the nipple not associated with invasive carcinoma and/or carcinoma in situ (DCIS and/or LCIS) in the underlying breast parenchyma.

T1- Tumour 2 cm or less in greatest dimension

T 1mi- Microinvasion 0.1 cm or less in greatest dimension

T1a- More than 0.1 cm but not more than 0.5cm in greatest dimension

T1b- More than 0.5cm but not more than 1 cm in greatest dimension

T1c- More than 1 cm but not more than 2cm in greatest dimension

T 2- Tumour more than 2 cm but not more than 5 cm in greatest dimension

T 3- Tumour more than 5 cm in greatest dimension

T 4- Tumour of any size with direct extension to chest wall and/or to skin (ulceration or skin nodules)

T4a- Extension to chest wall (does not.Include pectoralis muscle invasion only)

T4b- Ulceration, ipsilateral satellite skin nodules, or skin oedema (including peau d'orange)

T4c- Both 4a and 4b

T4d- Inflammatory carcinoma

pN - Pegional Lymph Nodes

pNX - Pegional lymph nodes cannot be assessed (e.g., previously removed, or not removed for pathological study)

pN0- No regional lymph node metastasis

pN1- Micrometastases; or metastases in 1 to 3 axillary ipsilateral lymph nodes; and/or in internal mammary nodes with metastases detected by sentinel lymph node biopsy but not clinically detected

pN1mi - Micrometastases (larger than 0.2 mm and/or more than 200 cells, but none larger than 2.0 mm)

pNla - Metastasis in 1-3 axillary lymph node(s), including at least one larger than 2 mm in greatest dimension

pNlb - Internal mammary lymph nodes not clinically detected

pNlc - Metastasis in 1-3 axillary lymph nodes and internal mammary lymph nodes not clinically detected

pN2 - Metastasis in 4-9 ipsilateral axillary lymph nodes, or in clinically detected ipsilateral internal mammary lymph node(s) in the absence of axillary lymph node metastasis

pN2a - Metastasis in 4-9 axillary lymph nodes, including at least one that is larger than 2 mm

pN2b - Metastasis in clinically detected internal mammary lymph node(s), in the absence of axillary lymph node metastasis

pN3

pN3a - Metastasis in 10 or more ipsilateral axillary lymph nodes (at least one larger than 2 mm) or metastasis in infraclavicular lymph nodes/ level III lymph nodes

pN3b - Metastasis in clinically detected internal ipsilateral mammary lymph node(s) in the presence of positive axillary lymph node(s); or metastasis in more than 3 axillary lymph nodes and in Internal mammary lymph nodes with microscopic or macroscopic metastasis detected by sentinel lymph node biopsy but not clinically detected

pN3c - Metastasis in ipsilateral supraclavicular lymph node(s)

pM - Distant Metastasis

M0 - No distant Metastasis

pM1- Distant metastasis microscopically confirmed

Table 4: TNM Classification of Breast tumors[36]

Stage 0	Tis	N0	M0
Stage 1a	T1	N0	M0
Stage 1b	T0	N1mi	M0
	T1	N1mi	M0
Stage 2a	T0	N1	M0
	T1	N1	M0
	T2	N0	M0
Stage 2b	T2	N1	M0
	T3	N0	M0
Stage 3a	T0	N2	M0
	T1	N2	M0
	T2	N2	M0
	T3	N1	M0
	T3	N2	M0
Stage 3b	T4	N0	M0
	T4	N1	M0
	T4	N2	M0
Stage 3c	Any T	N3	M0
Stage 4	Any T	Any N	M1

INVASIVE BREAST CARCINOMA

The term "invasive breast carcinoma (lBC)" refers to a large and heterogeneous group of malignant epithelial neoplasms of the glandular elements of the breast.

Clinical features:

Most common clinical sign of invasive breast cancer are nipple inversion, nipple discharge and skin retraction. Less common signs are change in size, shape, color, texture of the overlying skin. Even skin ulceration can occur in extreme cases.[46]

Gross features:

Invasive breast cancers can be visualised or palpated as hard and gritty masses with irregular stellate outline or nodular configuration with poorly defined edges. When the tumor size and extent are not macroscopically obvious, radiological reference is highly recommended. It is important to note skin ulceration, nipple changes and separate skin nodules for staging purposes.[46]

Tissue handling parameters such as time of removal of specimen, time it was placed in the fixative, time between the fixation and tissue sampling process is important to assess cold ischemia time. These parameters are important as it affects ER/PR and Her2 testing, grading and lymphovascular invasion assessment.[46]

Microscopy/ Histopathology:

In invasive breast cancer, the important four histological features to be assessed are:

1. Histological subtype based on tumour architecture, cytonuclear features, and stromal features
2. Nottingham grade
3. The presence or absence of spread in the vessels and lymphatics

4. An associated in-situ component

Other features include tumour size, distance to margins, stromal changes and tumour-infiltrating lymphocytes.[46]

Table 5: Semi-quantitative method for assessing histological grade in breast. From elston and ellis {modified scarff–bloom - richardson scoring system}[36]

FEATURES	**SCORE**
1. TUBULE AND GLAND FORMATION	
Majority of tumor >75%	1
Moderate degree 10-75%	2
Little or none <10%	3
2. NUCLEAR PLEOMORPHISM	
Small, regular uniform cells	1
Moderate increase in size and variability	2
Marked variation	3
3. MITOTIC COUNTS	
Dependent on microscopic field area	1-3

MITOTIC COUNT / 10 high power fields	
1 point	0-9
2 points	10-19
3 points	>20

TOTAL SCORE	**FINAL GRADING**
3 -5	Grade 1
6 or 7	Grade 2
8 or 9	Grade 3

The well differentiated IBC are composed of bland cells forming tubular or ductal structure. The poorly differentiated IBC are composed of cells with substantial nuclear pleomorphism and are arranged in sheets.

In majority of IBC are high grade with tumor cells displaying high N:C ratio, solid growth pattern, frequently pushing borders and geographical necrosis.

The immune infiltrates in tumor also known as TILs, is gaining importance in IBC as a prognostic marker. High number of TILs is associated with better response and better outcomes with neoadjuvant therapies.

Tumor cells are arranged in clusters, cords and trabecular. Wide range of apoptotic, mitotic activity and necrosis is seen.

IBCs are accompanied by marked fibrosis in a scirrhous pattern with diffuse infiltration. It is irregular shaped speculated mass. Foci of elastosis may also be present with occasionally cyst like cavities. 20-30% of IBCNST shows lymphovascular invasion or perinural invasion, while in 80% cases ductal carcinoma insitu are present.[46]

Special morphological patterns included in IBC NST are:

Carcinoma with medullary pattern, Neuroendocrine differentiation, Pleomorphic, Choriocarcinomatous and Melanocytic pattern.

IHC: Invasive breast cancer cells are generally positive for low molecular weight cytokeratins, EMA, ECadherin, BCL2 and GATA3.

Some invasive breast cancers also show positivity for GCDFP-15, mammaglobin, milk fat globule, lactalbumin, CEA and B72.3.

In invasive breast cancers, it is important to evaluate ER expression to predict clinical benefit from endocrine therapy and various other clinical therapies. As PR expression varies more than ER expression, which helps furthermore in stratification of ER expression.

In invasive breast cancer, approximately 10-20% of the cases show Her2 gene amplification. This results in over expression of Her2 at cell surface which makes the canver more aggressive. It is because of increase in cancer proliferation, cell mobility and angiogenesis.

Her2 amplification can also be assessed by Insitu hybridization.[46]

Table 6: Biomarkers IHC staining criteria (ASCO/CAP)[36]

ER	Positive		>1% of invasive cancer has nuclear staining of intensity
	Negative		<1% or 0% of invasive cancer has nuclear staining of intensity
PR	Positive		>1% of invasive cancer has nuclear staining of intensity
	Negative		<1% or 0% of invasive cancer has nuclear staining of intensity
ERBB2 (Her2 neu)	Positive	3+	Circumferential membrane staining that is complete, intense, and in >10% of tumor cells
	Equivocal	2+	Weak to moderate complete membrane staining observed in >10% of tumor cells
	Negative	1+	Incomplete membrane staining that is faint / barely perceptible and in >10% of tumor cells
		0	No staining is observed, or incomplete membrane staining that is faint / barely perceptible and in <10% of tumor cells

INVASIVE LOBULAR CARCINOMA

It is composed of dyscohesive cells, that most often are individually dispersed or arranged in single file linear pattern.

Localisation:

Centrally located as compared to IDC NST

Clinical features:

Poorly defined palpable mass with architectural distortion, which is difficult for palpation.

Gross:

Irregular with poorly defined tumor mass as it has diffuse growth pattern.

Microscopy/Histopathology:

It is characterized by proliferation of small cells that lack cohesion. Throughout the fibrous connective tissue it appears dispersed individually or arranged in single file of linear cords that invades the stroma. Generally presents as concentric pattern around a normal duct. Host reaction or disturbance of background architecture is sometimes seen. Neoplastic cells have round or notched ovoid nuclei. The rim of surrounding cytoplasm is thin with intra-cytoplasmic lumen seen sometimes. Mitosis are less common. Lympho-vascular invasion is less common.[46]

IHC:

ILCs show 80-95% of ER positivity. Her2 amplification and over-expression is rare in ILC. Most common molecular alternation in ILC is loss of expression of cell to cell adhesion molecule E-cadherin. The expression of TP53, basal markers and myoepithelial markers are rare in ILC. [46]

Various patterns are seen in ILC that share either cytological pattern or growth pattern, but all lack cell to cell cohesion. Various patterns are classical, solid, alveolar, pleomorphic.[46]

PAPILLARY NEOPLASM OF BREAST

These neoplasms are predominantly composed of papillary architecture. Depending upon the type of papillary neoplasms, papillae consisting of fibrovascular cores are covered by epithelium with or without myoepithelial layer. Epithelial atypia is assessed by considering the space between the adjacent fibrovascular cores as equivalent to duct space.

Most papillary neoplasms are confined within the ducts, which tends to be distended and are cystic with a thick wall.[46]

Papillomas, papilloma with atypical ductal hyperplasia, papillary ductal carcinoma insitu, solid and encapsulated papillary carcinoma insitu are included in Intraductal papillary neoplasms of Breast. These benign Intraductal papillary neoplasms are surrounded by a continuous layer of myoepithelium.

Invasive and solid papillary carcinomas are devoid of continuous myoepithelial layer.

Invasive papillary carcinoma shows a frankly invasive growth pattern with mildly dilated ducts and microcysts containing papillary formation. [46]

IHC markers used for myoepithelial cells are Calponin, SMA, p63 and SMMHC.[46]

MOLECULAR CLASSIFICATION OF BREAST

Global gene expression profiles are used to express different molecular or hormonal subtypes of breast cancer. It comprises of Luminal A, Luminal B, Basal cell like, HER 2 Enriched.[46, 50, 51]

Table 7: Molecular classification of Breast cancers[50]

Subtypes	Hormonal	HER2
Luminal A	ER+ and/or PR+	-
Luminal B	ER+ and/or PR+	+
Basal cell like	ER- / PR-	-
HER2 enriched	ER- / PR-	+

Luminal A

It is found in approximately 40% of breast cancer women. These are slower growing and less aggressive then other types. These patients show most positive short term prognosis. As they express hormonal receptors, it is predicted to be responsive to hormonal therapy.

Luminal B

It constitutes 10-20% of breast cancer women. These tumors show elevated rate of proliferation.

Basal cell like

It constitutes 10-20% of breast cancer women. It is also termed as Triple negative breast cancer. These are more commonly found in afroamerican women, premenopausal females and females with BRCA 1 gene alteration. These patients have poorer short term prognosis, since there are no targeted therapies.

HER2 Enriched

It comprises of 10% of breast cancer cases. It produces excess growth promoting protein called Her2. This type tends to grow and spread much more aggressively than other types. Therefore has poorer short term prognosis. Targeted therapies are under research.

Intrinsic Subtype	Gene Profile	Molecular Findings	IHC Phenotype	Histologic Subtypes	Integrative Cluster	DNA Architecture	Survival
Luminal A	High expression of luminal epithelial genes and ER-related genes	Mutations in PIK3CA, MAP3K1, and GATA3; CCND1 amplification w/o corresponding activation of PI3K pathways	ER+, PR≥20%, HER2−, Ki-67low	Tubular Carcinoma, low-grade IDC-NST, classic ILC	IntClust 2	11q13/14 amplification; fractional pattern of high-level copy number gains	Poor
					IntClust 3	Low genomic instability	Good
					IntClust 4	CNA devoid	Good
					IntClust 6	High genomic instability; 8p12 amplification	Intermediate
					IntClust 7	16p gain, 16q loss, 8q amplification	Good
					IntClust 8	1q gain, 16q loss	Good
Luminal B	Lower expression of luminal epithelial genes and ER-related genes, but higher level of proliferation and HER2 related genes than luminal A	Similar to luminal A but with a higher prevalence of TP53 and RB pathway inactivation as well as Myc related and FOXM1 related transcription	ER+, PR <20%, or HER2 (+) or Ki67high	IDC-NST, micropapillary carcinoma, pleomorphic ILC	IntClust 1	High genomic instability; 17q23 amplification; GATA2 mutation	Intermediate
					IntClust 2	See above	
					IntClust 5	HER2 amplification	Poor
					IntClust 6	See above	
					IntClust 9	8q gain, 20q amplification	Intermediate
HER2 OE	High expression of HER2-related genes; low expression of ER-related genes	HER2 amplicon and EGFR/HER2 signature	ER−, PR−, HER2+	High grade IDC NST, pleomorphic ILC	IntClust 5	See above	
Basal like	High expression of basal epithelial and proliferation genes; low expression of HER2-related and ER-related genes	Mutations in TP53, losses in RB1 and BRCA1; amplification of MYC; high PI3K/AKT pathway activation	ER−, PR−, HER2−	High-grade IDC-NST, metaplastic carcinoma, medullary carcinoma, adenoid cystic carcinoma	IntClust 10	5q loss, 8c gain, 10p gain, 12p gain; high genomic alterations with survivorship pattern	Poor
					IntClust 4	See above	

ER indicates estrogen receptor; HER2, human epidermal growth factor receptor 2; IDC-NST, infiltrating duct carcinoma, no special type; IHC, immunohistochemical; ILC, invasive lobular carcinoma; PR, progesterone receptor.

Fig 8: Details of molecular classification of breast cancers[51]

Estrogen Receptors

Estrogen receptors belongs to intracellular receptors and is referred as ER alpha and beta. ESR 1 encodes ER alpha on chromosome 6 while, ESR 2 encodes ER beta on chromosome 14. DNA-binding transcription factor regulates gene expression which is the main role of estrogen receptor. ER alpha is normally seen in stroma of

prostate and uterus, theca cells of ovary, leydig cells of testis, epididymis, bone, breast, various regions of brain, liver and white adipose tissue. ER beta is seen in epithelium of prostate, testis, granulose cells of ovary, salivary gland, bone marrow, colon, vascular endothelium and certain regions of brain. Research has shown that part of ER alpha resides in the nucleus of ER-negative breast cancer epithelial cells. Recent research proposes that ER beta is associated with proliferation and poor prognosis. These 2 Estrogen receptors are over expressed in 60-70% of breast cancer cases and are ER positive and estrogen dependent. Binding of estrogen to ER stimulates the proliferation of mammary gland resulting in increased cell division.[52, 53]

Progesterone receptors

It is also known as NR3C3. It is located in the Chromosome 11 long arm. It consists of two main nuclear isoforms A and B. Mutation in expression of co-regulators effects normal function. Thus, affecting the normal growth of mammary gland and causing breast cancer.[53, 54]

ERBB-2/HER2 neu

It is also known as ERBB2. It is located in the long arm of Chromosome 17. It belongs to family of tyrosine kinases, which is involved in signal transduction pathway that regulates cell growth and proliferation. HER2 is clinically important because monoclonal antibody, Trastuzumab targets it.[55]

PSMA

Prostate specific membrane antigen (PSMA) is a type 2 membrane protein featured by murine monoclonal antibody 7E11; C5.3. It is located on short arm of chromosome 11. It is expressed in all forms of prostate tissue, including prostatic carcinoma. PSMA is also known as carboxypeptidase II (GCPII, EC3.4.17.21), N-acetyl-α- linked acidic dipeptidase I (NAALADase or folatehydrolase), is a typeII transmembrare protein. It is hooked in cell membrane of prostate epithelial cells. [56]

The cDNA of PSMA codes for glycoprotein of 750 aminoacids with 100kDa of molecular mass. It has 3 part structure: a 19-amino-acid internal portion, a 24-amino-acid transmembrane portion, and a 707-amino-acid external portion. The extracellular part folds into 3 structural and functional domains: a protease domain (56-116), an apical domain (117-351), and C-terminal domain (592-750). It is also expressed as compact homo-dimer. PSMA contains bionuclear zinc site. It is known to catalyze the hydrolytic cleavage of glutamate from glutamate folate.[56]

Thus plays an important role in folate metabolism. Unlike other antigens like Prostate specific antigen (PSA), prostatic acidic phosphatase (PAP) or prostate secretory protein (PSP), PSMA is not secreted into circulation. PSMA undergoes constitutive internalisation after antibody binding and is therefore has transport function.[57]

PSMA expression is highly organ specific. Its expression was detected in secretary cells of salivary glands, in cryptic cells of duodenal brush border, subset of proximal renal tubules. In addition, its expression was found in brain and colon, but these results were controversial.[57]

PSMA has a unique anti-angiogenetic target as it is expressed in neovascularisation of solid tumors as in bladder, kidney, breast, pancreas, lungs, melanoma, but not in normal blood vessels. In endothelial cells, PSMA regulates tumor angiogenesis by modulating integrin signal transduction and cell invasion.[58]

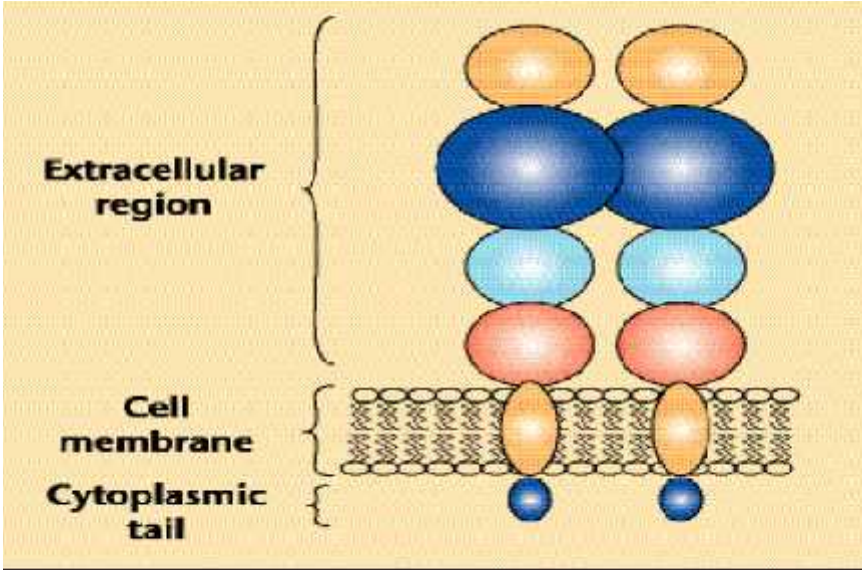


Fig 9: Membranous portion of Psostate specific membrane antigen[58]

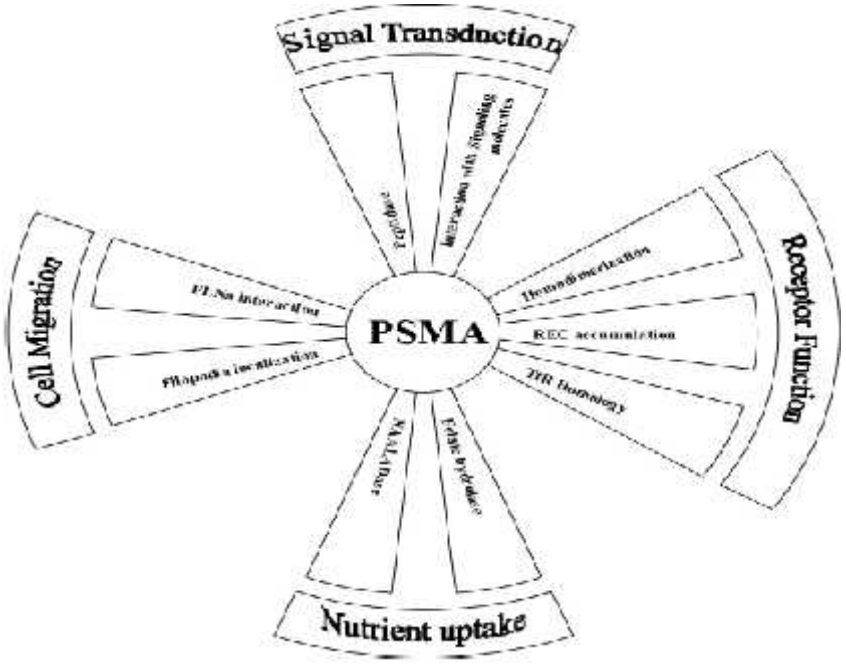


Fig 10: Functions of Prostate specific membrane antigen[59]

CD31/PECAM-1

The endothelial cells that line the basal membrane along the vessel wall can be labelled by Immunohistochemical markers, which is evident of angiogenesis in tumor. In malignant tumors, emboli are diagnosed with the help of labelling vessels. The capacity of tumor to produce blood borne metastasis is reflected by neoangiogenesis. Therefore, quantification of tumor vessels can be utilised as a prognostic marker to evaluate neoangiogenesis.[60]

CD31 or PECAM1 (Platelet endothelial cell adhesion molecule 1) is a 130 kDa cell surface molecule. It belongs to immunoglobulin super family and expressed by endothelial cells, platelets, monocytes, polymorphonuclear cells as well as circulating lymphocytes. Function of CD31 is to adhere the receptor molecule, thus playing an important role in leucocyte trafficking, mechanotransduction and vascular permeability across the endothelial layer. PECAM-1 maintains vascular integrity and resists mechanical force under conditions of fluid shear stress because of extensive homophilic contacts between aminoacid located in aminoterminal immunoglobulin.[61]

PECAM-1 was first found to be involved in cell migration and angiogenesis, when antiPECAM-1 antibodies inhibited the ability of endothelial cells to form tube like structures. This was later supported by observation that antibodies specific for PECAM-1 inhibited tumor induced angiogenesis. The mechanism by which PECAM-1 promoted cell migration is because of its ability to alter cytoskeleton both by dephosphorylating focal adhesion kinase and by altering the activity of small G Protein. Thus PECAM-1 is used in endotheliopathies, such as tumor angiogenesis. While inhibiting the activation of circulating platelets and leukocytes, it can help in tumor angiogenesis. PECAM-1 also supports integrity of endothelial cells via cell junctions and providing protection of vascular bed to apoptotic stimuli.[61]

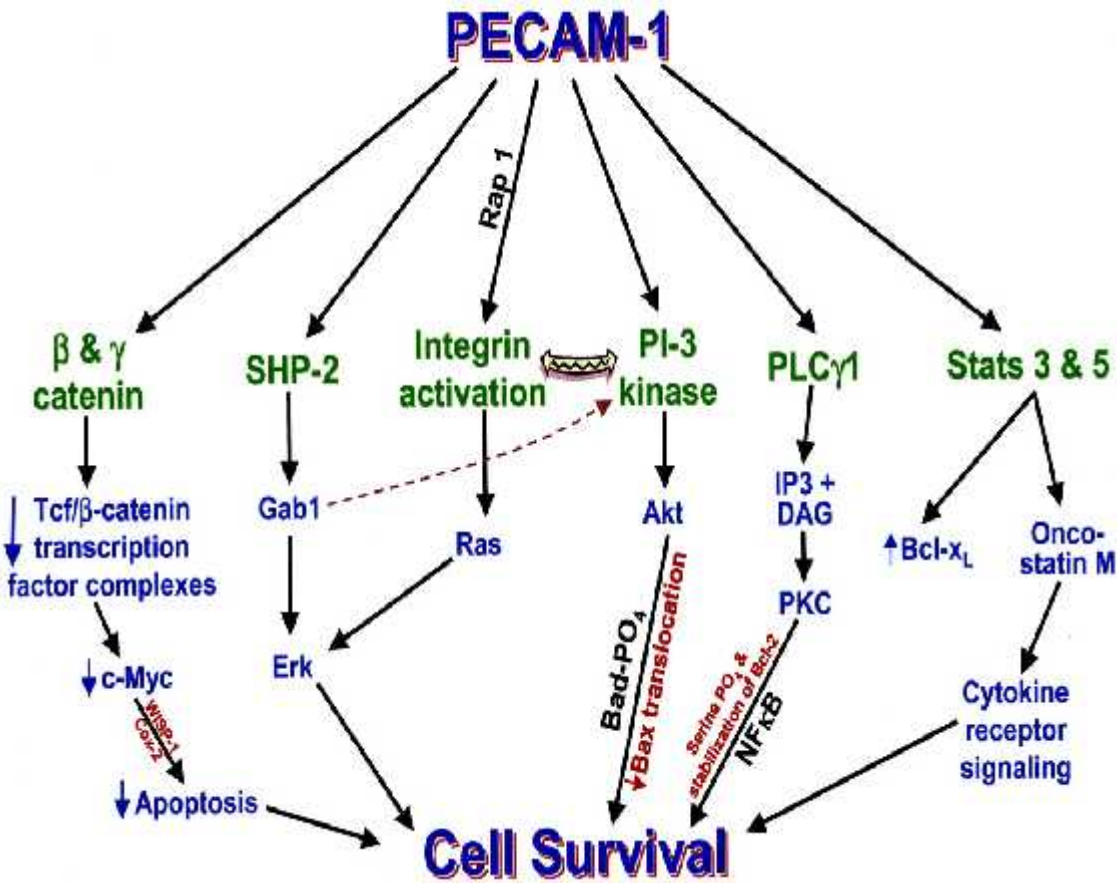


Fig 11: Signalling pathways emerging from PECAM-1 which mediates resistance to apoptosis[62]

METHODOLOGY

A total of 40 cases of modified radical mastectomy specimens received for histopathological evaluation at the Department of Surgical Pathology, KAHER's JNMC and KLES Dr Prabhakar Kore Charitable Hospital and MRC, from January 2019 to December 2019 were included in this one year cross-sectional study.

Sample Size Calculation: The sample size was calculated using the formula $4pq/d^2$

p– expected prevalance

q- 100-p

d- Sample error (10)

Substituting the values in the above formula and through universal sampling method, we obtained a sample size of 40.

Ethical clearance: The present study was approved by Jawaharlal Nehru Medical College's Institutional Ethics Committee on Human Subjects Research.

INCLUSION CRITERIA:

Modified radical mastectomy specimens from female patients of all ages, with breast carcinoma, were included in the study.

EXCLUSION CRITERIA:

1. Cases where only a trucut biopsy or limited surgery had been done, as in such cases all the parameters were not made available for assessment.

2. Cases where there was extensive tumor necrosis without sufficient viable tumor cells for accurate evaluation of the immunohistochemical results.

The detailed clinical history and results of relevant investigations were collected from the patient's case record files with consent. For cases, the mastectomy and lymph node dissection specimen were received in the Histopathology section in 10% formalin. In every case, the standard protocol for surgical grossing of modified radical mastectomy specimens was followed. Specimen was kept for fixation for 24 to 48 hrs. After a detailed specimen description, multiple sections were taken from the tumor proper, all surgical margins, nipple and areola, non- neoplastic peritumoral breast, and all the lymph nodes. After conventional processing in the Leica 1020 model histokinette and embedding in paraffin wax, sections of 4µm thickness were cut using Leica JUNG RM 2025 model rotator microtome and stained using haematoxylin and eosin (H & E) for histopathological study.

In addition, 4µm sections were cut from the paraffin blocks containing tumor tissue and taken on 2 glass slides coated with adhesive (silane) for immunohistochemistry (IHC) to detect PSMA and CD31 staining. For retrospective cases, the histopathology reports, slides and paraffin blocks were retrieved from the archives. Sections were cut from the paraffin blocks in a similar manner.

PROCESSING FOR IMMUNOHISTOCHEMISTRY:

Sections were 4µm thick to cut from the paraffin blocks with tumor tissue and taken on 2 glass slides coated with adhesive (3- aminopropyltriethoxysilane) for immunohistochemistry (IHC) of PSMA and CD31.

Technique for IHC included antigen retrieval in TRIS buffer in a microwave oven, [heat induced antigen retrieval breaks the formalin induced cross links and

exposes the epitopes to the antibody]. Retrieval solution used was TRIS-EDTA at a pH of 9, for 10 minutes for 3 cycles

This was followed by blocking endogenous peroxidase with 3% hydrogen peroxide, incubating with primary mouse monoclonal antibody (PSMA mAb-clone 3E6 and CD31 mAb- clone JC70A from DAKO), linking with rabbit anti mouse secondary antibody (DAKO), enzyme labelling with streptavidin- horseradish peroxidase (DAKO), developing chromogen with deaminobenzidine (DAB) and counterstaining with haematoxylin. Positive and negative controls were run for PSMA and CD31 respectively.

The hematoxylin and eosin stained slides were studied for the histopathological features of tumour, histological type, Modified Scarff-Bloom-Richardson (MSBR grade), lymph node metastasis etc. The immunostained slides were examined for membranous and cytoplasmic staining of tumor epithelial cells and endothelial cells in tumor associated neovasculature with PSMA and CD31 staining was studied for identification of tumor associated neovasculature of breast carcinoma cases. In each case, the proportion of positive staining tumor cells (expressed in percentage) and the average intensity of staining (expressed as 0, 1+, 2+ or 3+) was evaluated. The relationship between various clinicopathological parameters obtained after analysis such as age, duration of disease presentation, tumor size, tumor extent, histologic type, histologic grade, lymphnode status, the expression of ER, PR, and HER2/neu was studied.

ASSESSMENT OF PSMA EXPRESSION:

Immunohistochemical staining was evaluated by a pathologist unaware of the clinical and pathological data. All tissue sections were analyzed under Pentahead light microscope and pictures were taken using Progres gryphax software.

PSMA expression in tumor cells:

We identified the percentage of tumor cells positive for PSMA on each tested slide. PSMA expression was quantified according to Remmele and Stegner method.[64] Staining intensity was scored semi quantitatively as negative (0), weak (1), moderate (2) or strong (3). Extent of staining was scored as percentage of cells stained (0, 0% of cells; 1, < 10% of cells; 2, 10–50% of cells; 3, 51–80% of cells; 4, > 80% of cells). The final immune reactive score (IRS) was determined by multiplying the scores of intensity and extent of staining, ranging from 0 to 12. IRS < 2 was considered as negative staining, 3–4 as weak staining, 6–8 as moderate staining and 9–12 as strong staining.

PSMA expression in tumor associated neovasculature:

We identified and marked 5 areas (hotspots) that had the highest concentration of microvessels. The number of CD31 and PSMA positively stained microvessels in each of these hot-spots were calculated using CD31 stained slides and PSMA stained slides respectively. Counted vessels were chosen according to Weidner's method.[65] The percentage of PSMA expression in tumor vessels was calculated using the following equation: Percentage of PSMA expression in tumor vessels = (Average of vessels numbers stained for PSMA / Average of vessels numbers stained for CD31) × 100. PSMA staining intensity in the neovasculature was graded on a 0–3 scale (0, no

staining; 1, weak; 2, moderate; and 3, strong). Then, the final score of PSMA expression in the neovasculature was obtained by multiplying the intensity grade with the percentage of PSMA expression in tumor vessels obtaining a range of 0 to 12 (< 2 was considered as negative staining, 3–4 as weak staining, 6–8 as moderate staining and 9–12 as strong staining).

STATISTICAL ANALYSIS:

Statistical analysis was done using Spearman rank correlation, Karl pearson's correlation coefficients, student’s t test, wilcoxon t test and fisher’s exact test to find out correlation between PSMA staining in tumor cells and tumor associated neovasculature with clinicopathological factors.

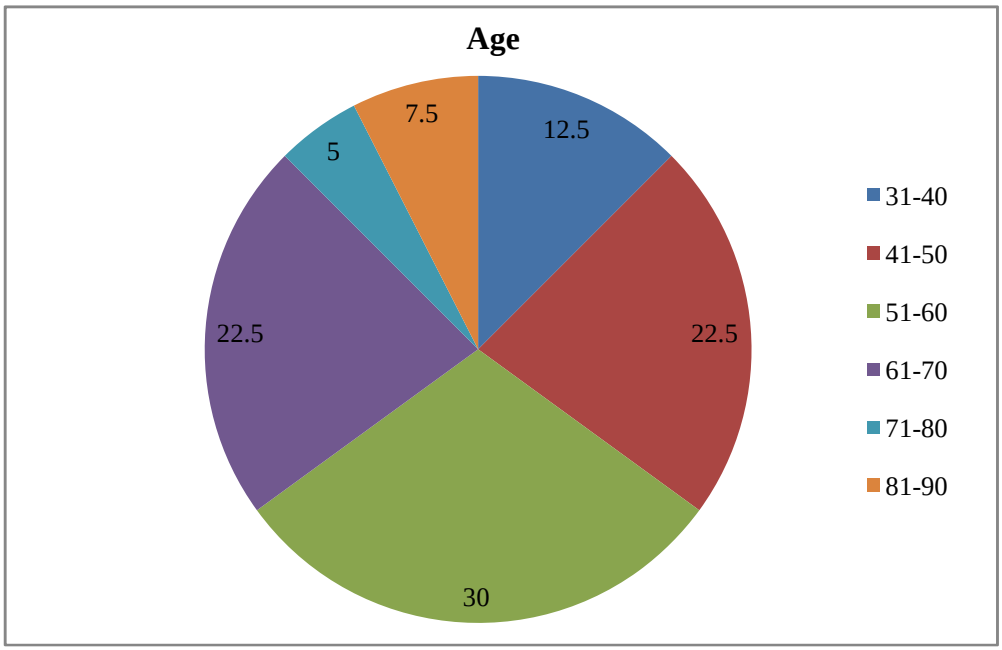
RESULTS

A cross sectional study of 40 patients with breast cancer is undertaken to study the expression of PSMA in tumor cells and tumor associated neovasculature and its correlation with various clinicopathological parameters in breast cancer.

Table 8: Age Distribution of Breast carcinoma

AGE IN YEARS	NUMBER OF PATIENTS	PERCENTAGE
31-40	5	12.5
41-50	9	22.5
51-60	12	30
61-70	9	22.5
71-80	2	5
81-90	3	7.5

Graph 1: Age distribution of Breast carcinoma



Mean age = 56.45 ± 13.25

In this study, age ranged from 31-90 years and mean age ± SD was 56.45±13.25 years. Majority (12 cases, 30%) of cases occurred in 51-60 years of age group.

Gender distribution

In this study, all 40 cases were females, i.e 100%.

Table 9: Distribution of Clinical Presentation

Clinical Presentation	Number of patients	Percentage
Breast lump	27	67.5
Breast lump with nipple retraction	5	12.5
Breast lump with nipple discharge	3	7.5
Breast lump with pain	5	12.5
Total	40	100

In this study, breast lump alone was seen in 27 cases (67.5%) followed by breast lump with nipple retraction or pain each consisting of 5 cases (12.5%) and breast lump with nipple discharge in 3 cases (7.5%).

Graph 2: Distribution of Clinical Presentation

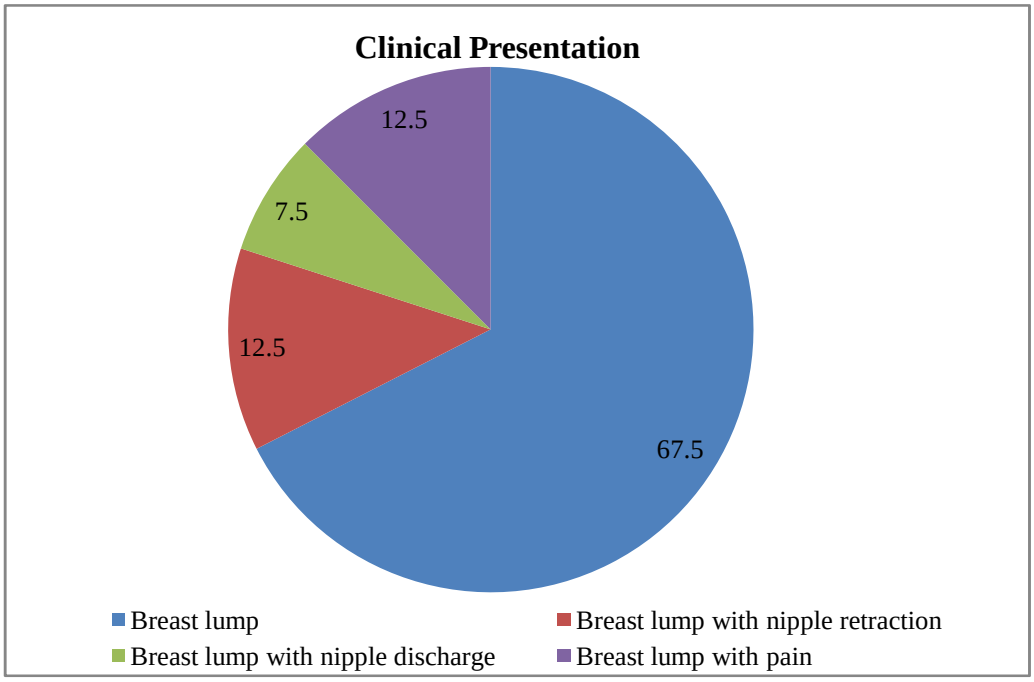


Table 10: Distribution of Laterality

Laterality	Number of patients	Percentage
Right side	19	47.5
Left side	21	52.5
Total	40	100

In this study, 21 cases (52.5%) occurred on the left side and 19 cases (47.5%) on right side.

Graph 3: Distribution of Laterality

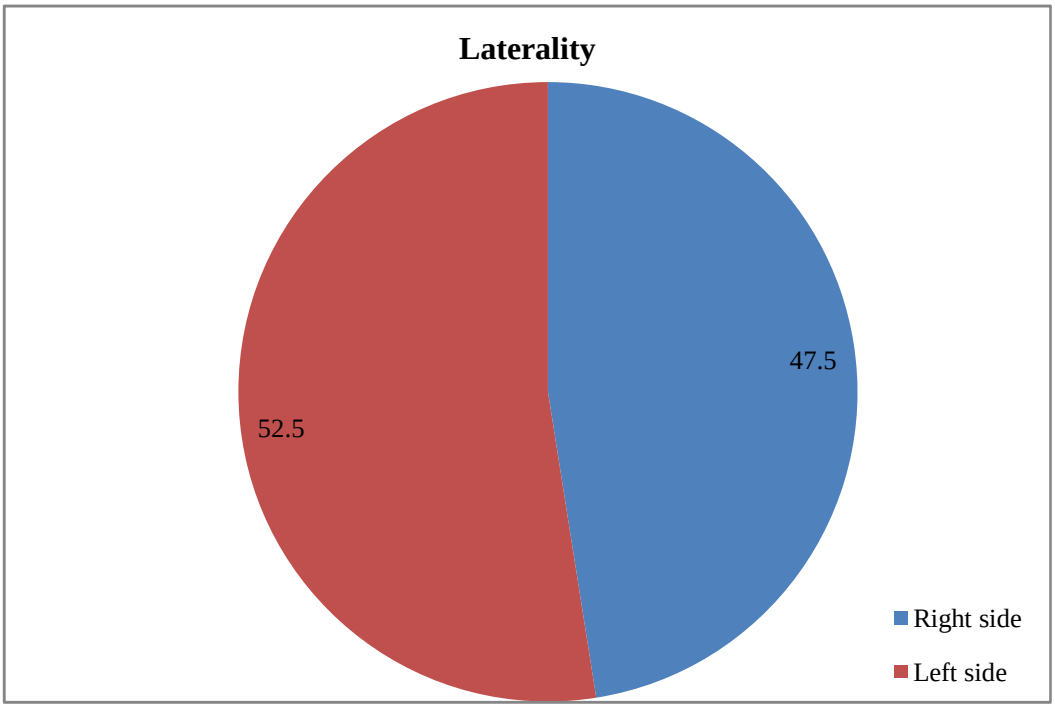
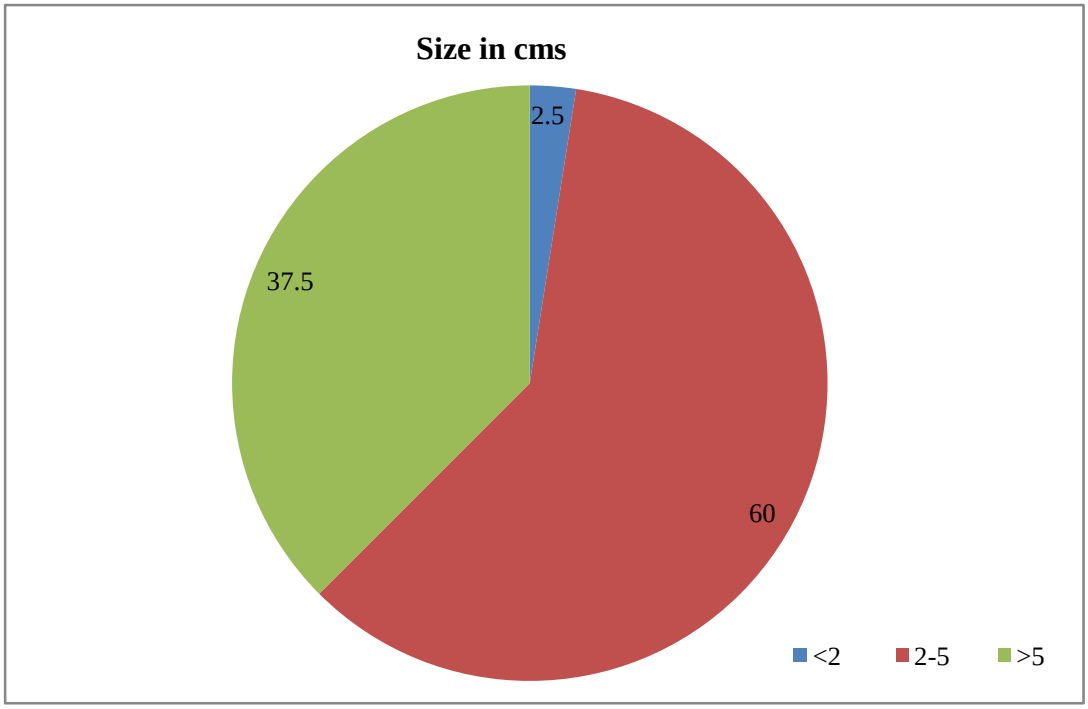


Table 11: Distribution of Tumor size

Size in cms	Number of patients	Percentage
<2	1	2.5
2-5	24	60
>5	15	37.5
Total	40	100

In this study, tumor size was taken in centimetres with majority (24 cases, 60%) having size between 2-5 cms, followed by tumor size more than 5 cms (15 cases, 37.5%) with only 1 case less than 2 cms (2.5%).

Graph 4: Distribution of Tumor size



Tumor Type

All the cases (n=40) included in the study were infiltrative ductal carcinoma nos (IDC-NOS).

Table 12: Distribution of Histological grade in breast carcinoma (Elston and Ellis modification of Scraff-Bloom-Richardson grading system)

Histological grade	Number of patients	Percentage
Grade 1	00	00
Grade 2	33	82.5
Grade 3	7	17.5
Total	40	100

In this study, majority of cases were having grade 2 tumor (33 cases, 82.5%) and 7 cases (17.5%) were having grade 3 tumor.

Graph 5: Distribution of Histological grade

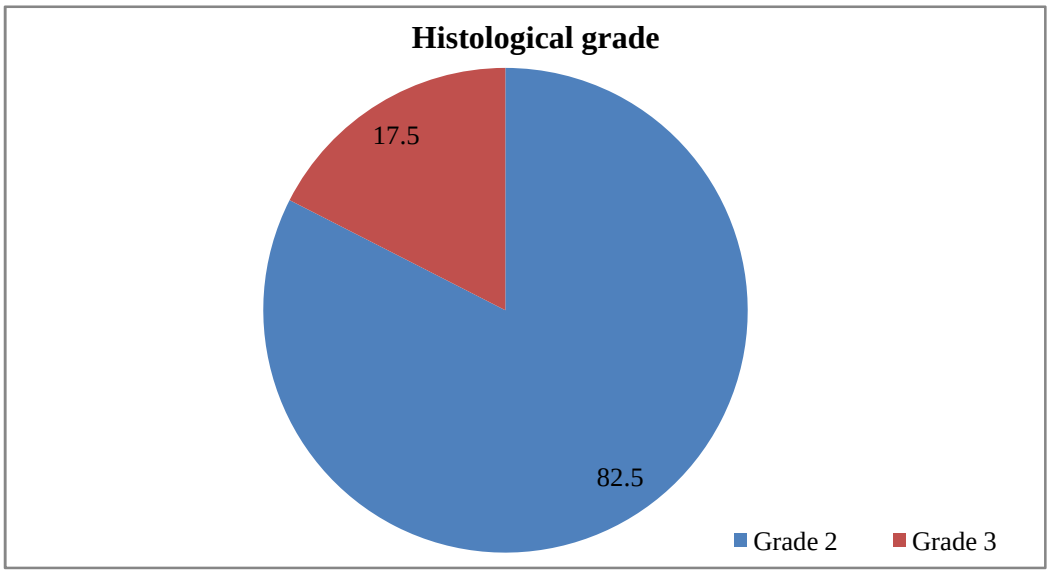


Table 13: Distribution of cases according to TNM staging

T stage	Number of cases	Percentage
T2	22	55.0
T3	13	32.5
T4	5	12.5
N stage		
N0	14	35.0
N1	17	42.5
N2	8	20.0
N3	1	2.5
M Stage		
Mx	40	100

Graph 6: Distribution of cases according to TNM staging

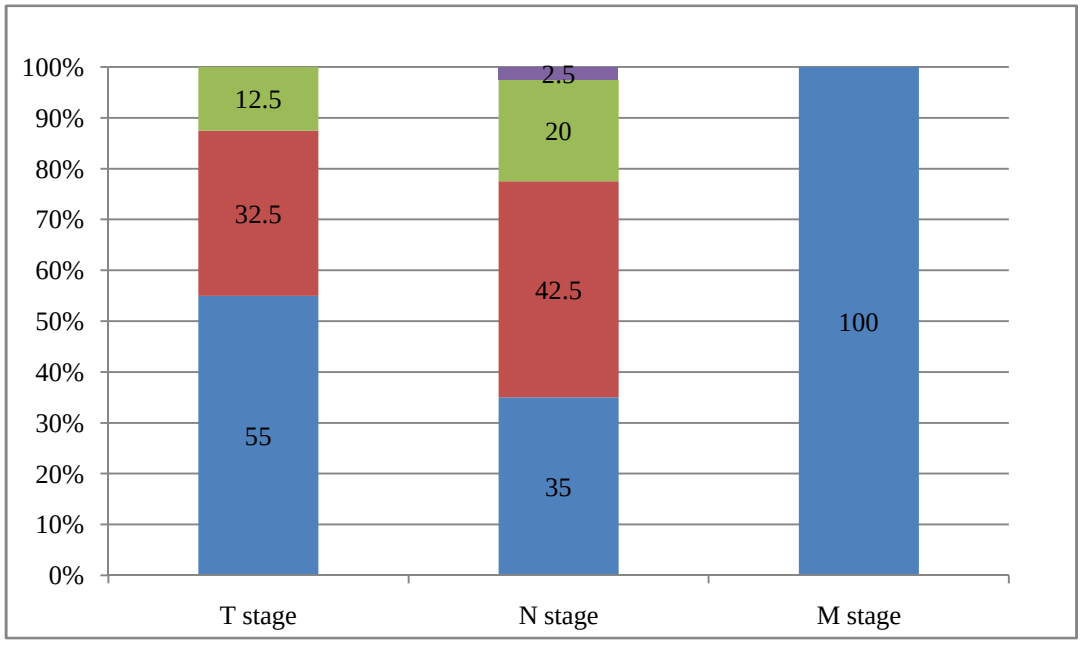


Table 14: Distribution of Microscopic feature: Necrosis

NECROSIS	NUMBER	PERCENTAGE
Negative	13	32.5
Positive	27	67.5
Total	40	100.0

In this study, 27 cases (67.5%) had necrosis and 13 cases (32.5%) were negative for necrosis.

Graph 7: Distribution of Microscopic feature: Necrosis

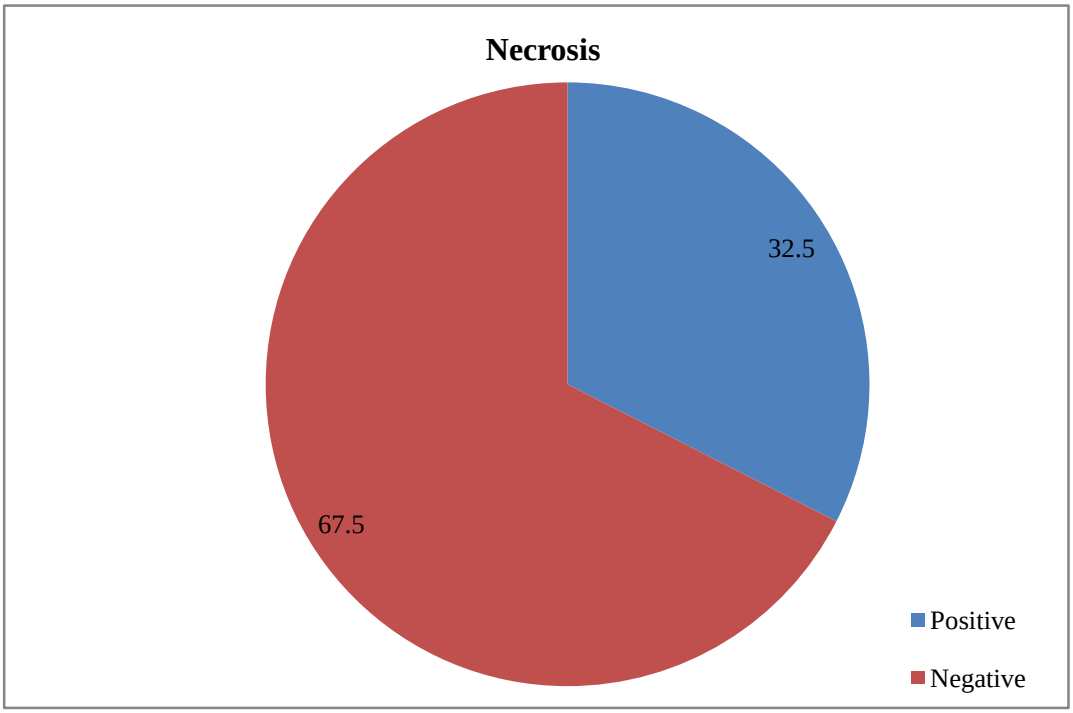


Table 15: Distribution of Microscopic feature: Lympho-vascular invasion and Peri-neural invasion

In this study, all cases showed LVI positivity and 39 cases (97.5%) showed PNI.

	Number of cases n=40	Percentage
LVI	40	100
PNI	39	97.5

Table 16: Distribution of Lymphnode Status

Lymphnode status	Number of cases	Percentage
Negative for metastasis	11	27.5
Positive for metastasis	29	72.5
Total	40	100.0

In this study, 29 cases (72.5%) showed lymph-node positivity and 11 cases (27.5%) lacked lymph-node positivity.

Graph 8: Distribution of Lymphnode Status

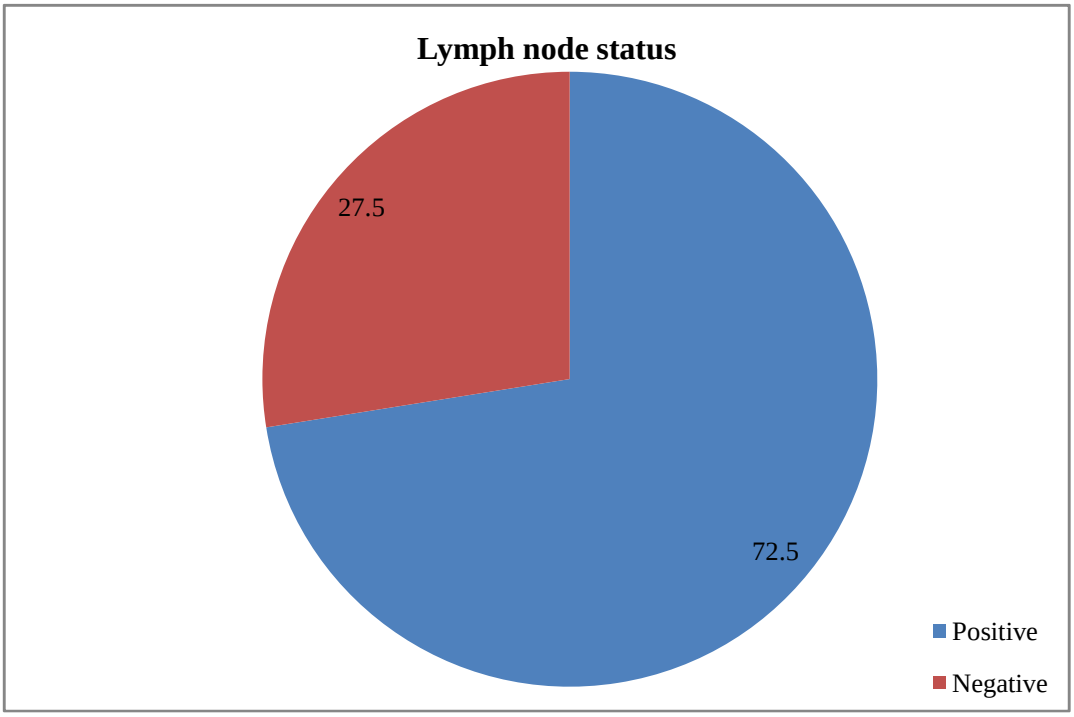


Table 17: Distribution of Hormone status

RECEPTOR STATUS (UNKOWN 32 CASES)		
ER+	4	50.0
ER-	4	50.0
PR+	4	50.0
PR-	4	50.0
HER2 NEU		
NEG	2	25.0
POS	3	37.5
EQUIVOCAL	3	37.5
TRIPLE NEGATIVE	2	25.0

Out of total cases (n= 40), 8 cases underwent hormone status testing (ER, PR, Her2 neu).

Among the cases in which tests were done, 4 cases were positive for ER and PR (50%), while 4 cases were negative for ER and PR (50%).

Her 2 neu test for the 8 cases showed positivity in 3 cases (37.5%), 2 were negative (25%) and 3 were equivocal (37.5%).

2 out of 8 cases (25%) were triple negative.

Table 18: Expression Pattern OfPsma In Breast Cancer

	PSMA Staining pattern in primary breast carcinoma				
	Negative	Weak	Moderate	Strong	TOTAL
	IRS: 0–2	IRS: 3–4	IRS: 6–8	IRS: 9–12	
PSMA in tumor cells	21 (52.5%)	10 (25%)	8 (20%)	1 (2.5%)	40 (100%)
PSMA in tumor associated neovasculature	0 (0%)	18 (45%)	16 (40%)	6 (15%)	40 (100%)

PSMA staining pattern in breast tumors was defined as follow:

Negative (IRS: 0–2)

Weak (IRS: 3-4)

Moderate (IRS: 6-8)

Strong (IRS: 9-12)

PSMA expression was observed in tumor cells in 19 cases (47.5%), the intensity of staining ranging from weak to strong. On the other hand, all 40 cases (100%) showed PSMA expression in tumor associated neovasculature.

PSMA in tumor cells was classified into the above described 4 groups based on IRS. PSMA staining in tumor cells was negative in 21 cases (52.5%), intensity being weak in 10 cases (25.0%), 8 cases (20%) were moderate and 1case (2.5%) was strong.

PSMA in tumor associated neovasculature was also described under 4 groups. All cases were positive for PSMA (100%). Among which 18 cases (45%) showed weak staining, 16 cases (40%) showed moderate staining and 6 cases (15%) showed strong staining.

Out of 40, 21 cases (52.5%) which were negative for PSMA expression in tumor cells expressed PSMA in tumor associated neovasculature.

No statistically significant correlation was observed between PSMA expression in tumor cells and in tumor associated neovasculature ($p=0.5068$ and $R^2=0.1081$).

All the epithelial cells in the peritumoral normal breast tissue, stained negative for PSMA.

Table 19 ;Corelation Between Psma Immune-Reactivity And Clinicopathological Factors

PSMA expression in primary breast tumors							
Tumor cells/tumor-associated neovasculature							
Factor	Cases	-/- Number Of Cases	+/- Number Of Cases	-/+Number Of Cases	+/+Number Of Cases	P value	Inference
AGE (YEARS)							
< 55	17	2	1	7	7		
≥ 55	23	6	1	7	9	0.71	NS
T STATUS							
T1	0	0	0	0	0		
T2, T3, T4	40	8	2	14	16	-	-
N STATUS							
N0	14	3	0	6	5		
N1, N2, N3	26	5	2	8	11	0.6645	NS
G STATUS							
1	0	0	0	0	0		
2, 3	40	8	2	14	16	-	-
TUMOR SIZE							
< 5	25	5	1	10	9		
≥ 5	15	3	1	4	7	0.8312	NS
LVI							
POSITIVE	40	8	2	14	16		
NEGATIVE	0	0	0	0	0	-	-
PNI							
POSITIVE	39	8	2	13	16		
NEGATIVE	1	0	0	1	0	0.5924	NS

METS/ NO OF LYMPHNODES							
POSITIVE	29	5	2	10	12		
NEGATIVE	11	3	0	4	4	0.7487	NS
NECROSIS							
POSITIVE	27	5	2	10	10		
NEGATIVE	13	3	0	4	6	0.7208	NS
ER RECEPTOR							
ER+	4	0	0	0	4		
ER-	4	0	0	1	3	-	-
PR RECEPTOR							
PR+	4	0	0	0	4		
PR-	4	0	0	1	3	-	-
HER2 NEU							
NEG	2	0	0	0	2		
POS	3	0	0	0	3		
EQUIVOCAL	3	0	0	1	2	-	-

The corelation of PSMA immune-reactivity with clinicopathological parameters is reflected in table 3. All the cases were classified into 4 groups depending on presence or absence of PSMA expression in tumor cells and in tumor associated neovasculature as mentioned earlier.

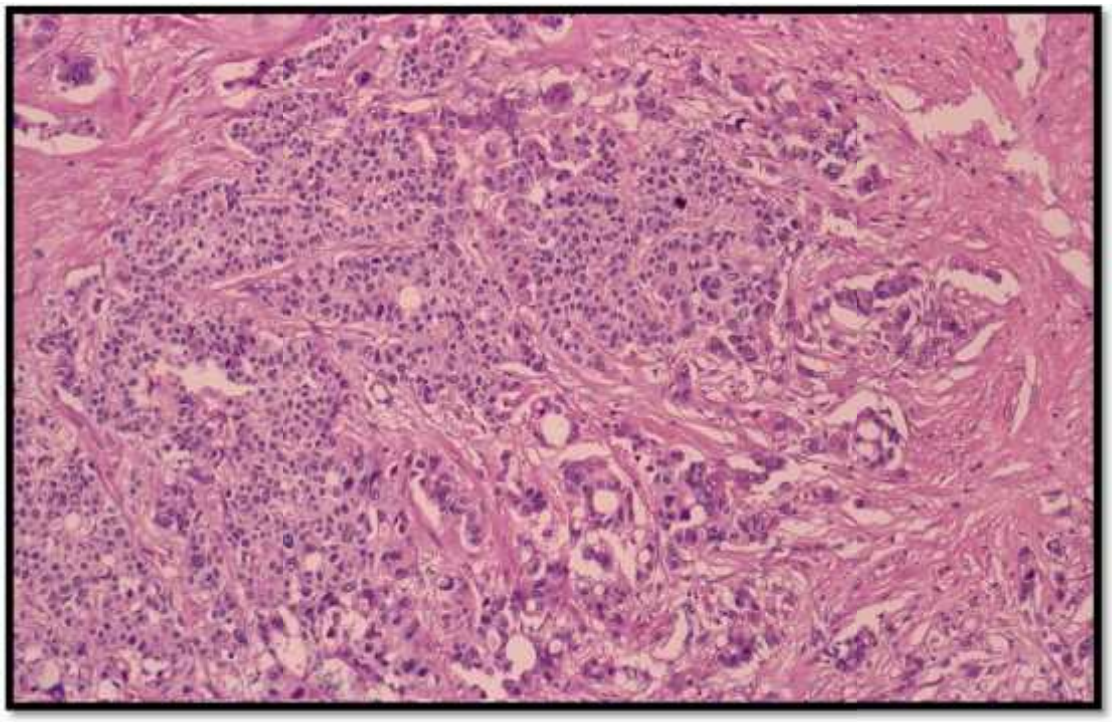
Statistically no significant correlation was demonstrated between PSMA expression and any of the clinicopathologcal parameters i.e age, staging, grading, tumor size, LVI, PNI, number of lymphnodes, ER, PR and Her2 neu.

However, PSMA expression in intra-tumoralneovasculature is positively correlated with CD31 ($p = < 0.0001$) while, no significant correlation was seen in extra-tumoralneovasculature between PSMA and CD31 ($p = 0.7993$).

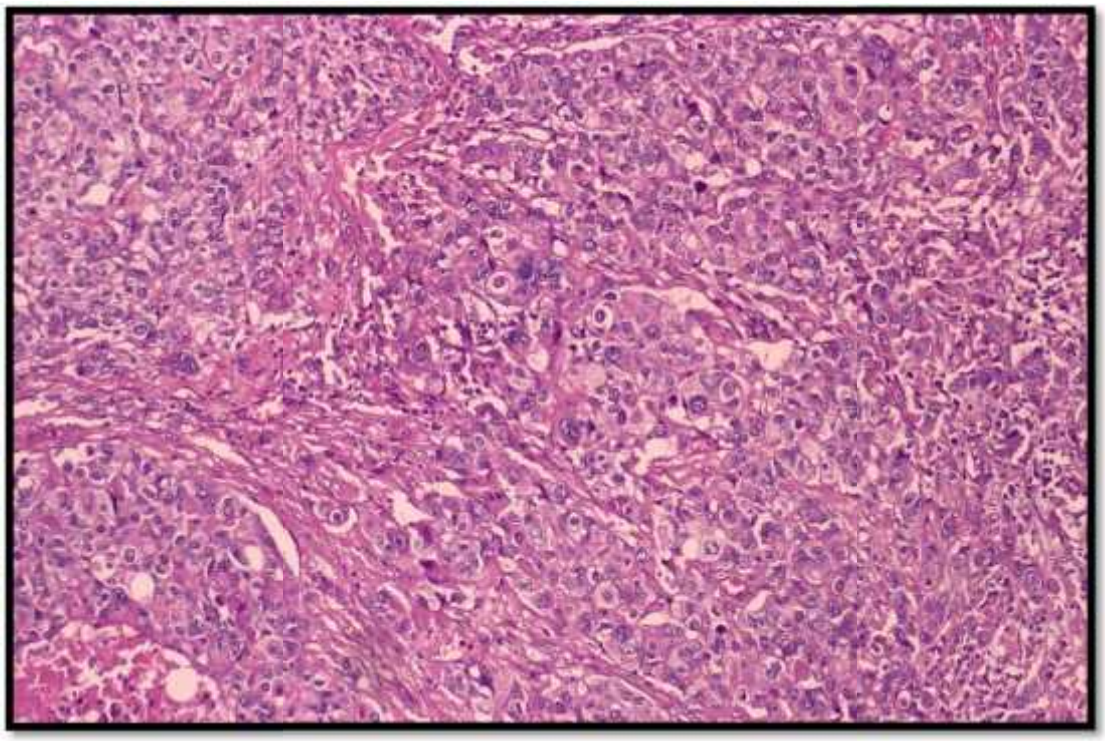
Table 20 : Karl Pearson's Correlation Coefficients

BETWEEN	p value	Inference
Intra-Tumoral AVG of PSMA expression AND Extra-Tumoral AVG of PSMA expression in tumor vessels	0.3526	NS
Intra-Tumoral Percentage of PSMA expression AND Extra-Tumoral Percentage of PSMA expression in tumor vessels	0.4084	NS

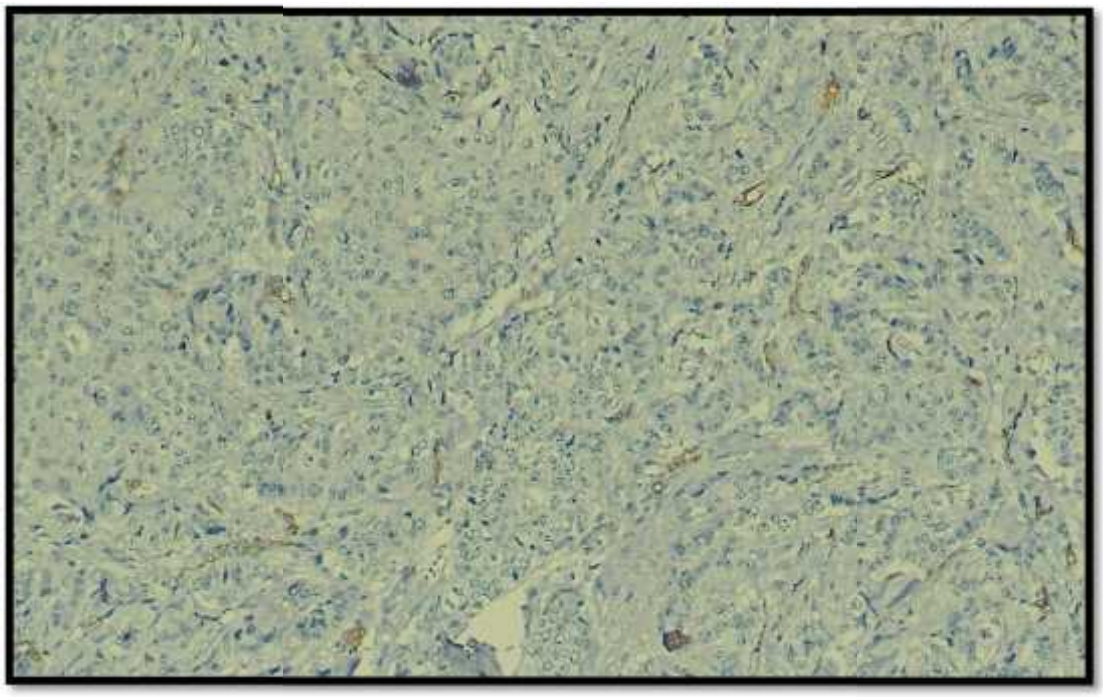
Using Karl pearson's correlation coefficients, no statistically significant corelation was found between PSMA expression in intra-tumoralneovasculature and extra-tumoralneovasculature in averages as well as in percentage.



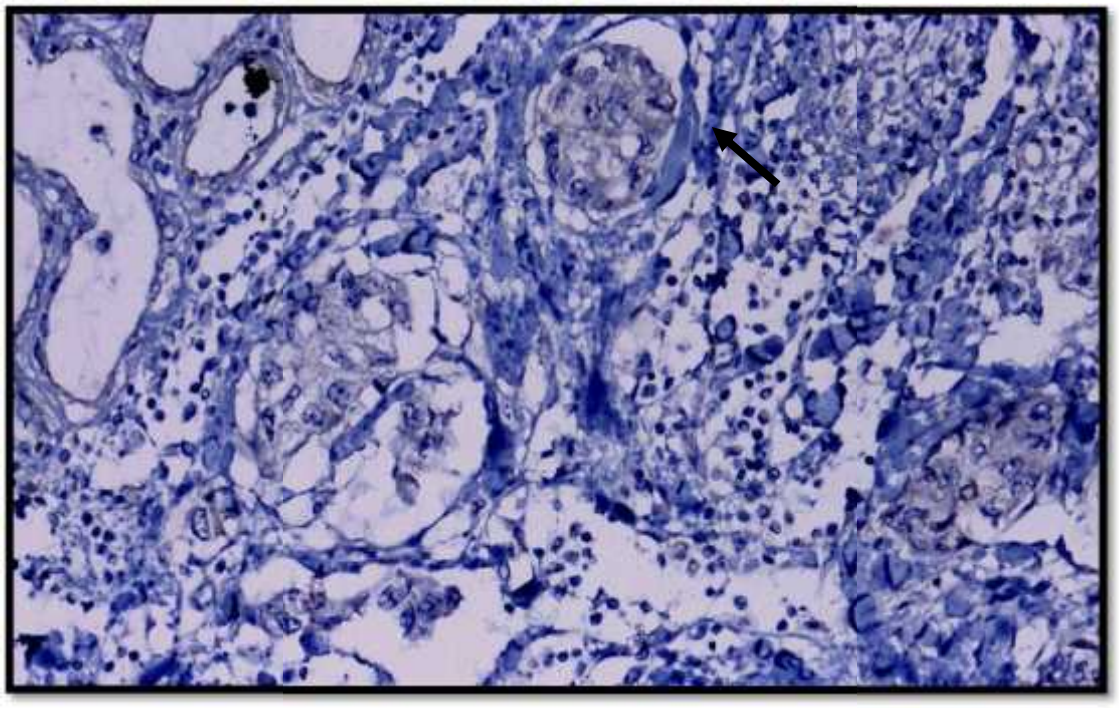
Pictomicrograph 1: Invasive Ductal Carcinoma NOS grade 2 (H&E, X200)



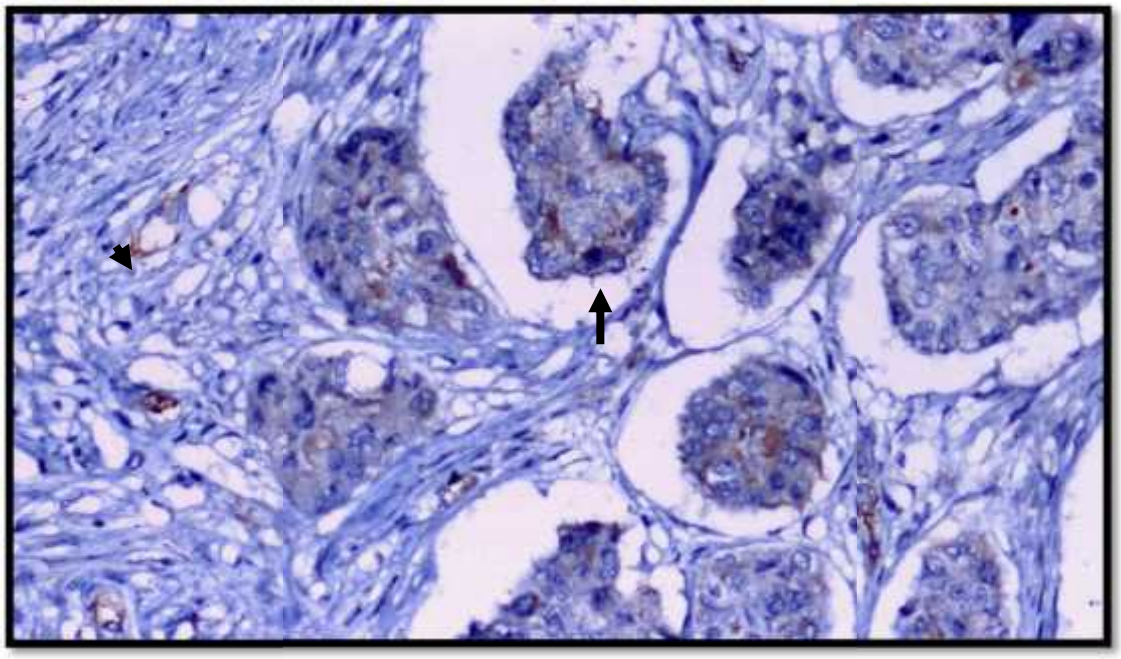
Pictomicrograph 2: Invasive Ductal Carcinoma NOS grade 3 (H&E, X400)



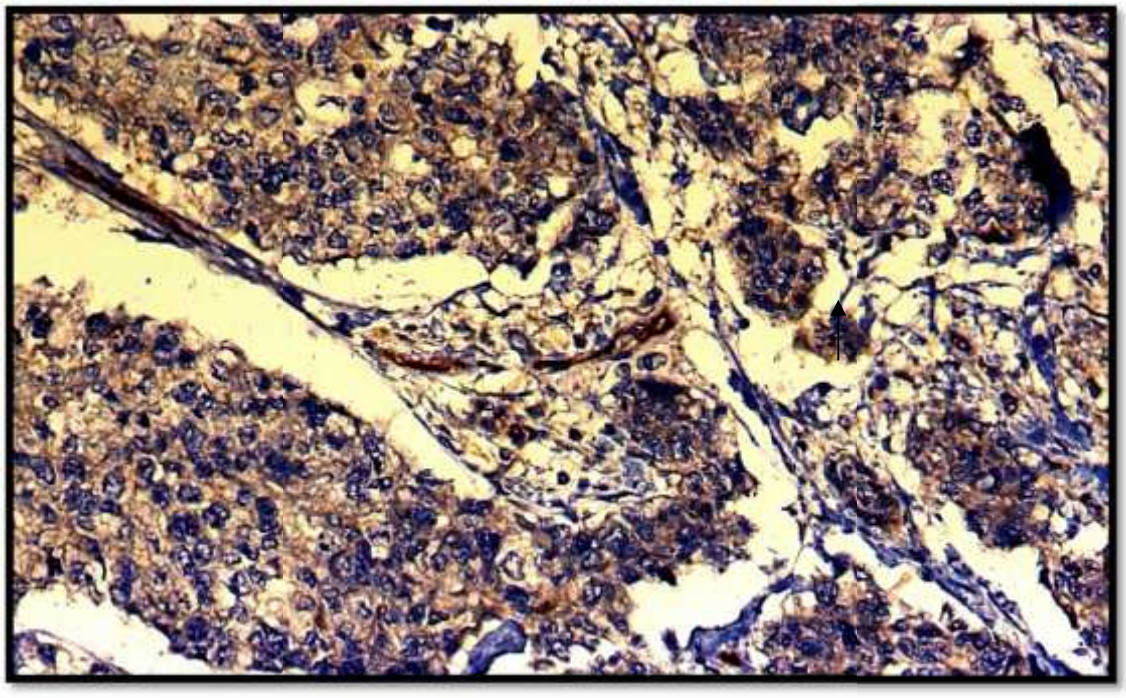
Pictomicrograph 3: PSMA expression: Negative in tumor epithelial cells and positive in tumor associated endothelial cells of neovasculature (IHC-PSMA, X200)



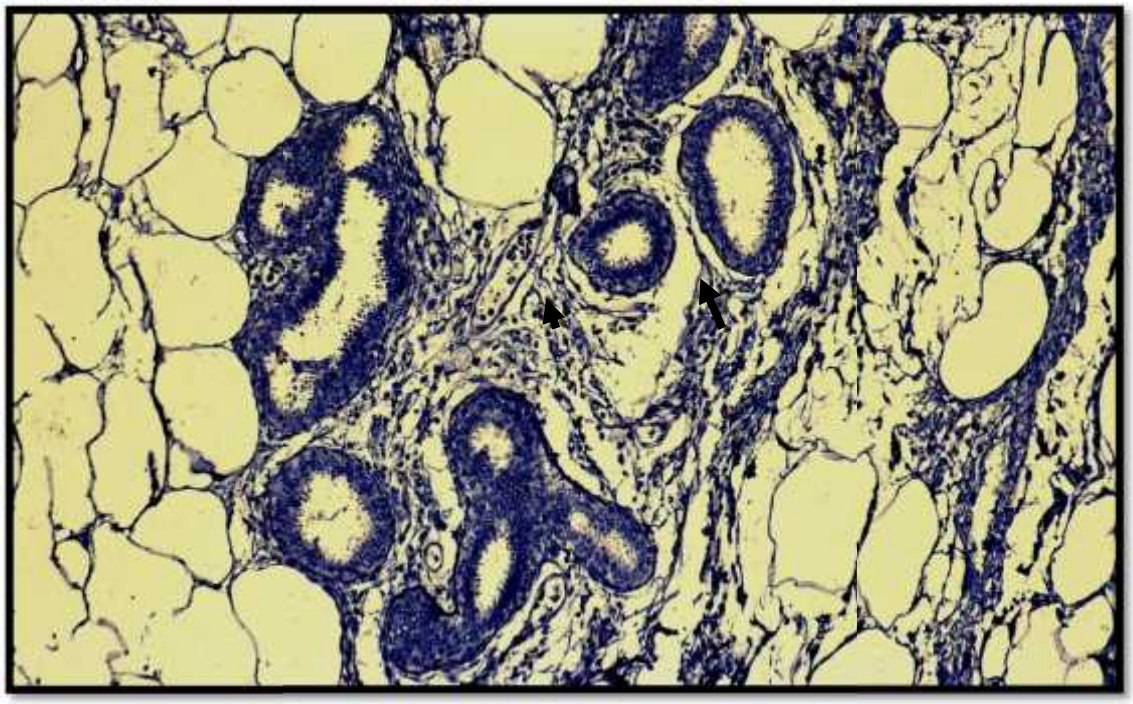
Pictomicrograph 4: Weak PSMA expression in tumor epithelial cells (IHC-PSMA, X400)



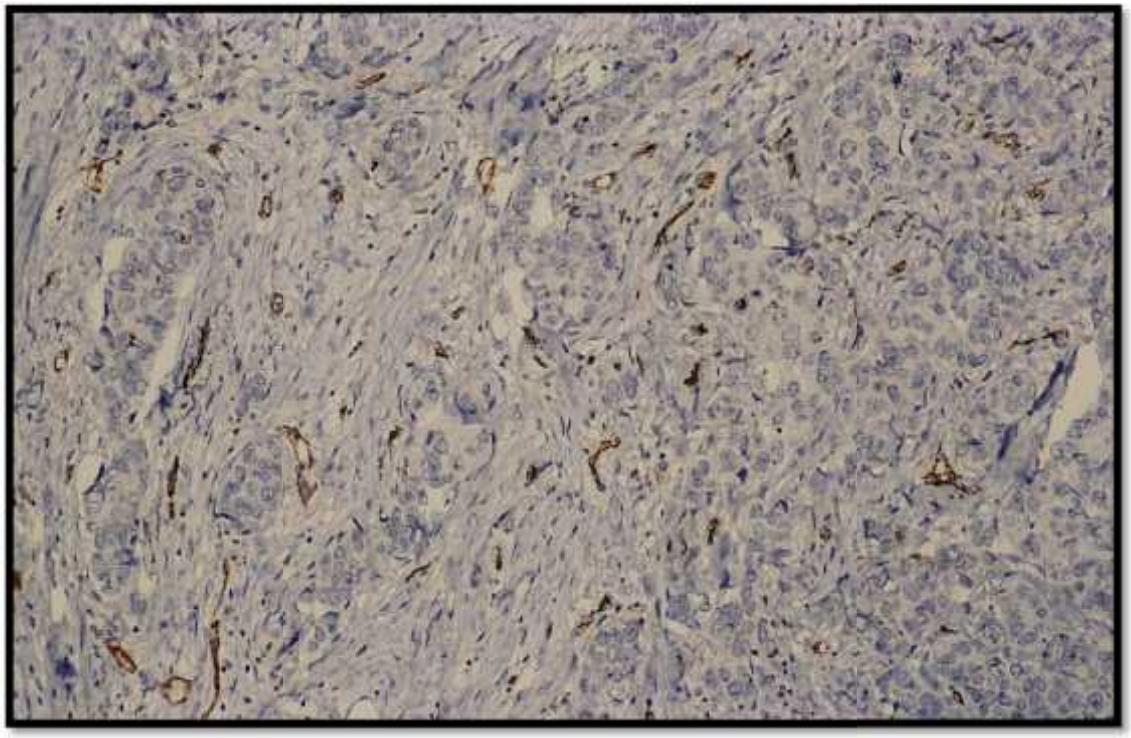
Pictomicrograph 5: Moderate PSMA expression in tumor epithelial cells and in endothelial cells of tumor associated neovasculature (IHC-PSMA, X400)



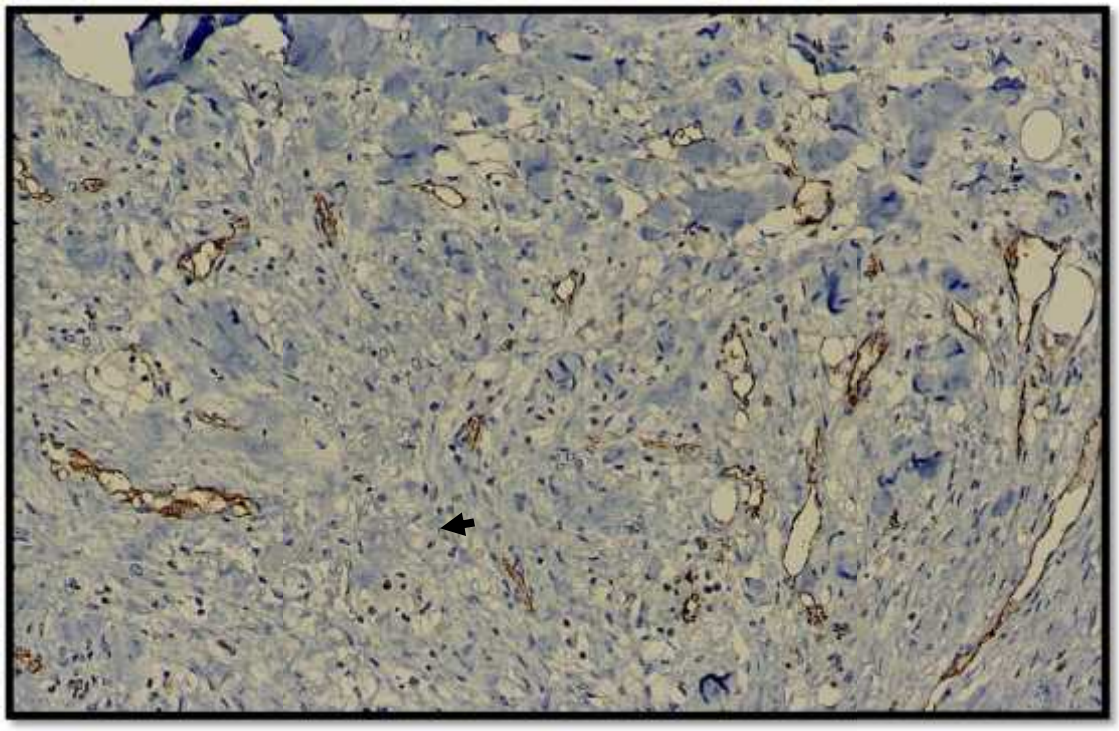
Pictomicrograph 6: Strong PSMA expression in tumor epithelial cells and in endothelial cells of tumor associated neovasculature (IHC-PSMA, X400)



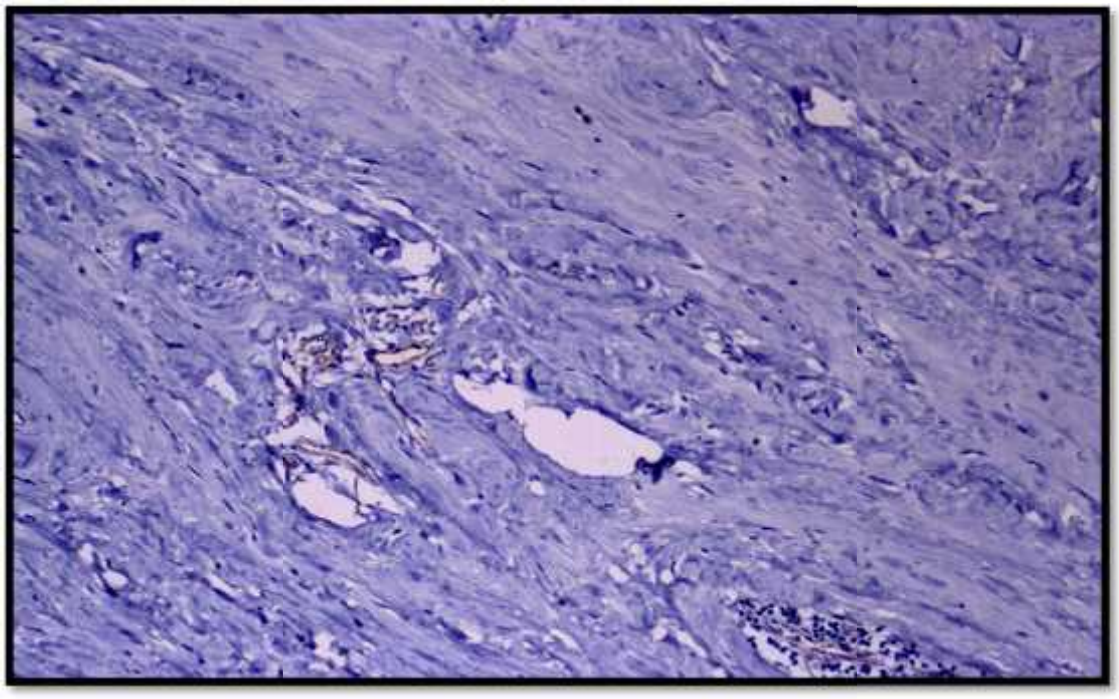
Pictomicrograph 7: Negative PSMA expression in normal breast epithelial cells and endothelial cells (IHC-PSMA, X200)



Pictomicrograph 8: Hot-spot area; PSMA positivity in intra tumoral vessels with negative PSMA staining in tumor cells (IHC-PSMA, X200)



Pictomicrograph 9: CD 31 expression in endothelial cells of tumor associated neovasculature (intra-tumoral)(IHC- CD31, X200)



Pictomicrograph 10: CD 31 expression in endothelial cells in peri-tumoral tissue (extra-tumoral)(IHC- CD31, X200)

DISCUSSION

Various studies have suggested PSMA as a tool for diagnosis and therapy of Prostate cancer. It is a safe therapy and has low toxicity profile.[65, 66]

PSMA is not secreted in circulation. Most common invasive cancer in females is breast cancer, while in males its prostate cancer. Both Breast and prostate are different anatomically as well as physiologically, but tumors in them have biological similarities. Thus, this study is targeted to evaluate PSMA expression and its significance in breast cancer.[67]

PSMA is highly organ specific and was detected in secretary cells of salivary glands, cryptic cells of duodenal brush border, subset of proximal renal tubule, brain and colon with contradictory outcomes. It has an antiangiogenetic target as its expression is seen in neovascularisation of solid tumors but not in normal blood vessels.[68]

PSMA expression in tumor cells and tumor associated neovasculature in patients with breast cancer was studied in detail. Using IHC markers, PSMA and CD 31 were applied on corresponding formalin fixed paraffin embedded blocks of breast cancer patients. To avoid inconsistent and contradictory results, common mAbs of PSMA were used for IHC detection. In this study, most commonly used mAb and breast cancer specific PSMA IHC were utilized. As PSMA is present in endothelium of tumor cells, baseline marker for neovascularisation was done with the help of CD 31. Then PSMA staining in neovasculature was compared to the baseline of CD 31 staining. To study the neo vascularisation in detail intra and extra tumoral vessels were observed. [69]

Only three studies have been carried out till date that assesses PSMA expression in patients with breast cancer. One being done by Wernicke et al which included 92 cases[70], second done by Kasoha et al with 72 cases[71] and third done by Tolkach et al with 315 cases.[72]

Table 21: Comparison of number of cases between various studies

	Wernicke et al	Kasoha et al	Tolkach et al	Kinoshita et al	Present study
Number of cases	92	72	315	5	40

In the present study PSMA expression was assessed according to final IRS value, which was calculated by multiplying the scores of intensity. The extent of staining, ranging was from 0 to 12. PSMA immunoreactivity was positive both in tumor cells and tumor associated neo vasculature (47.5% and 100% of tested cases). It was noted that frequency of PSMA staining in tumor associated neovasculature was more than in tumor cells. Our findings are in concordance with the study done by Wernicke et al where in they observed PSMA positivity in tumor associated neovasculature in 90/92 cases (98%), with remaining 2 cases showing weak staining in tumor cells as well as normal tissue and other study done by Tolkach Y et al where 189/315 cases (60%) showed PSMA expression in tumor associated neovasculature, out of which 87 cases (27.6%) showed weak PSMA intensity in endothelial cells.[70, 72] However, we found PSMA staining in tumor cells as negative in 21 cases (52.5%), remaining 18 positive cases showed weak to moderate staining intensity with 1 strong intensity, but all the cases showed positive expression in tumor associated neovasculature.

In contrast to our study, a similar study by Kasoha et al showed PSMA staining tumor cells had more extent with less intensity compared to staining in tumor associated neovasculature.[71]

Table 22: Comparison of PSMA staining in tumor cells and tumor associated neovasculature between various studies

	Wernicke et al (n=92)	Tolkach et al (n=315)	Kasoha et al (n=72)	Kinoshita et al (n=5)	Present study (n=40)
Positivity PSMA in tumor cells (epithelial)	0 (0%)	10 (3%)	50(72%)	0 (0%)	18(47.5%)
Positivity PSMA in tumor associated neovasculature (endothelial cells)	90(98%)	189(60%)	31(46%)	1(20%)	40(100%)

Kasoha et al had 39/72 cases having normal breast tissue. Out of those 39 cases, 26 cases (67%) showed PSMA expression in normal glandular breast epithelial cells and no PSMA staining in associated vessels of benign tissue, while in present study it was seen that normal breast epithelial cells were not stained by PSMA with weak staining intensity of PSMA in associated neovasculature.[71]

Study done by Tolkach Y et al showed weak expression of PSMA in tumor epithelial cells in 10/315 cases (3%) with weak PSMA expression in tumor associated neovasculature in endothelial cells in 87 cases (27.6%).[72]

As discussed above, PSMA staining in this study for tumor associated neovasculature showed 100% positivity. Similar finding was seen in study done by Chang et al who used 5 different PSMA mAbs and found that PSMA was positive in neovasculature but not in tumor cells of 5/6 breast carcinoma cases.[73]

Table 23: Comparison of PSMA staining in epithelial cells of normal breast tissue

	Wernicke et al (n=92)	Tolkach et al (n=315)	Kasoha et al (n=72)	Kinoshita et al (n=72)	Present study (n=40)
Positivity of PSMA in normal glandular breast tissue (epithelial cells)	nil	100 (32%)	26(67%)	6(100%)	nil

A study done by Kinoshita et al showed moderate staining of PSMA expression in normal breast epithelial cells in 6/6 cases while weak staining in 1/5 cases with ductal carcinoma.[74] This finding was also seen in the study done by Tolkach Y et al.[72] PSMA staining in epithelial cells was completely negative in 8/8 breast phylloids tumors in a study done by Mhawech-Fauceglia et al.[75]

Ross et al found association between PSMA expression in endothelial cells of neovasculature in 7/10 cases (70%) of infiltrating ductal breast tumors.[76]

Present study showed less expression of PSMA in breast cancer tumor cells as compared to tumor associated neovasculature. The reason for which is unclear but using different anti PSMA mAbs recognising different epitopes of protein could be a reason for variable staining. Even the differences in histological components of tested mammary tissue might add up to the reason. PSMA expression in tumor associated neovasculature is not consistent with some of the previous studies which show less staining in neovasculature and more in tumor cells. This might be because of restriction of PSMA expression in neovasculature to endothelial cells within pathologically defined tumor area.

Other studies have interpreted that PSMA expression in breast cancer showed no significant correlation with clinicopathological parameters like age, tumor type, staging, grading, tumor size, LVI, PNI, number of lymphnodes, ER, PR and Her2 neu. Study by Kasoha et al[71] showed PSMA expression in primary breast tumor had significant correlation with histological subtype and tumor grading. Such similar findings were also found in study done by Tolkach et al[72], additionally with negative hormone status and triple negavtive tumors. While, study by Wernicke et al[70] showed similar results like in this study with increase PSMA score in tumor associated neovasculature. These contradictory readings might be due to methodology differences.

Table 24: Comparison of PSMA expression and correlation with clinic-pathological parameters

	Wernicke et al (n=92)	Kasoha et al (n=72)	Tolkach et al (n=315)	Present study (n=40)
Significant correlation with clinicopathological parameters	No statistically significant correlation	Histological subtype and tumor grading (p-value= 0.026)	Histological subtype(NST), higher tumor grading, hormone receptor-negative (ER, PR, Her2-positive), and triple-negative tumors (p-value=0.002)	No statistically significant correlation

The function and utility of PSMA is yet unknown, recent study by Bradbury et al with invitro model concluded that depletion of PSMA in two metastatic human breast cancer resulted in reduction of proliferative and adhesive ability of cells. It also demonstrated reduced invasiveness and migratory capacity of tumor cells.[77]

Liu et al in an invitro model demonstrated that PSMA expression on cultured human umbilical vein endothelial cells (HUVECs was specifically limited to growth condition with tumor conditioned medium (TCM derived from human breast cancer cells in matrigel.[78]

PSMA has a transport function as it helps in internalisation after antibody binding.[79]

Nguyen et al tested invitro PSMA expression in conditioned media from several human cancer cells lines in HUVEC and coimplanted in mouse model through induction by regulatory factors which are secreted by tumor cells. This study also

showed a mAb in HUVECs having the capacity to internalize, the extracellular domain of PSMA named J591 which is seen by PSMA expression in tumor cells.[80]

PSMA expression in tumor associated neovasculature has been proved in various tumors including bladder cancer[81], colorectal cancer[82] and renal cancer[83, 84] which suggested role of PSMA in endothelial cell biology. Thus it suggests that endothelial cells lacking PSMA shows less efficient matrix invasion. Godron et al suggested that PSMA mediated folate uptake might be required to regenerate essential co factor of endothelial nitric oxide synthase that is required for angiogenesis.[85]

In past years, there have been rise in development of both diagnostic and therapeutic advantages of expression and activity of PSMA in various tumours but still remains unexplored in many areas. Though its utilisation in prostate cancer is good and studied well but it use in breast cancer is still less and is to be learnt more with better number of samples.

Handful of studies[70, 71, 74] show PSMA expression in tumor cells but PSMA expression in tumor associated neovasculature is limited and has a great scope.

A limited sample size restricts or limits our study; this may effect to hold down the establishment of statistically significant results. The need at this hour is for studies having larger sample size and an observational standard for interpretation of PSMA in tumor epithelial cells and endothelial cells in tumor associated neovasculature. Tackling this will further provide an accurate and validated score which would result to reduce the application cost and use of PSMA in much better ways.

CONCLUSION

Our study was an attempt to investigate PSMA expression in breast tumor cells and associated neovasculature and its correlation with clinicopathological parameters.

In this study, with primary breast cancer tumor epithelial cells showed positive staining with PSMA and all cases showed positivity in tumor associated neovasculature in endothelial cells.

PSMA expression in intra-tumoral neovasculature is positively correlated with CD31.

There was no correlation between PSMA and other clinicopathological parameters.

Thus, we conclude that use of PSMA in breast carcinomas should be done meticulously and many more studies with larger sample size would be needed to establish an association with any of the clinicopathological parameters.

Also, a standard for interpretation of PSMA staining should be devised for accurate, better, consistent and constant reporting.

SUMMARY

This was a one year prospective cross sectional study from January 2019 to January 2019 done in department of pathology, J N Medical College, Belagavi.

- A total of 40 histopathologically diagnosed breast carcinoma cases were included in our study. In all cases, the study was performed on modified radical mastectomy specimens (100%).
- Maximum number of cases belonged to 51-60 years of age (12 cases; 30%) with a mean age of 56.45 years.
- All cases were females (40 cases; 100%).
- Majority of them presented with only breast lump (27 cases; 67.5%).
- Greater number of cases had left sided breast involvement (21 cases; 52.5%).
- All cases were of modified radical mastectomy (100%).
- Majority of tumours were between 2-5 cms in greatest dimension (24 cases; 60%).
- All cases were invasive ductal carcinoma no special type (100%).
- Most of the cases were of invasive ductal carcinoma grade 2 (32 cases; 82.5%).
- Almost all cases showed LVI and PNI positivity
- Lymph-node positivity was seen in 29 cases (72.5%).
- Hormone studies were done only for 8 cases. Out of which 2 were triple negative breast carcinoma (25%).
- PSMA expression in tumor cells appeared in 47.5% (19 cases) of tested cases ranged between weak to strong. On the other hand, 100% (40 cases) of tested cases showed PSMA expression in tumor associated neovasculature.

- Cases which were negative for PSMA expression in tumor cells (21 cases; 52.5%) had positive expression of PSMA in tumor associated neovasculature.
- No significant correlation between PSMA expressions in tumor cells and in tumor associated neovasculature ($p=0.5068$ and $R^2=0.1081$) was found.
- All the epithelial cells in the peritumoral normal breast tissue, stained negative for PSMA.
- Statistically no significant correlation was demonstrated between PSMA expression and clinicopathologcal parameters i.e age, staging, grading, tumor size, LVI, PNI, number of lymphnodes, ER, PR and Her2 neu.
- PSMA expression in intra-tumoral neovasculature is positively correlated with CD31 ($p = < 0.0001$) while, no significant correlation was seen in extra-tumoral neovasculature between PSMA and CD31 ($p = 0.7993$).
- No statistical significance was found between PSMA expression between intra-tumoral neovasculature and extra-tumoral neovasculature in averages as well as in percentage.

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ANNEXURE - I

ETHICAL CLEARANCE CERTIFICATE

K.L.E. ACADEMY OF HIGHER EDUCATION AND RESEARCH
(Deemed – to- be- University)
Accredited 'A' Grade by NAAC (2nd Cycle) Placed in Category 'A' by MHRD (GoI)
JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)
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Ref: MDC/DOME/ 22 **Date: 24/11/2018**

To,

REG. NO: BN0118005

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"EXPRESSION OF PROSTATE SPECIFIC MEMBRANE ANTIGEN IN BREAST CANCER PATIENTS ATTENDING TERTIARY CARE HOSPITAL – A ONE YEAR CROSS SECTIONAL STUDY",** is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

(Dr. Arathi Darshan)
Member Secretary
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

(Dr. Roopa M Bellad)
Chairman,
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

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ANNEXURE II – CONSENT FORM

INFORMED CONSENT

EXPRESSION OF PROSTATE SPECIFIC MEMBRANE ANTIGEN IN BREAST CANCER PATIENTS ATTENDING TERTIARY CARE HOSPITAL – A ONE YEAR CROSS SECTIONAL STUDY

Purpose of the study: The purpose of this study is to determine the role of PSMA in tumor cells in invasive breast cancer. This study will help in determining a better diagnostic tool for invasiveness of cancers. Either those who have underwent mastectomy for breast cancer, or those who are about to undergo mastectomy, can volunteer to take part in this study.

Procedure: During this study, you will only be asked questions regarding your age, I.P number and any treatments undergone if any. If you agree to enroll yourself in this study, the breast tissue sample received in the hospital will be examined for the presence of PSMA expression in tumor epithelial cells and tumor associated neovasculature in endothelial cells.

Risks and benefits: There are no risks involved in taking part in this study and benefit is we will be able to know a better way to diagnose invasive cancers which is essential for providing appropriate treatment.

Alternatives: Taking part in this study is voluntary. You may choose not to take part in this study or if you decide to take part now, you still possess the right to withdraw from the study later on, in case you change your mind. The investigator may terminate your participation in this study anytime for any valid reason.

Privacy and confidentiality: All information collected about you during the course of this study will be kept confidential to the extent permitted by law. The code numbers will identify you in this research record. Information from this study will be published but your identity will be confidential in any publication. No information about you or information provided by you during research will be disclosed to other without your written permission except:

1. In emergency to protect your rights and welfare.
2. If required by law.

Financial incentives for participation: You will not be paid / offered any gift /incentives for participating in this study.

Authorization to publish results: The results of this study would be forwarded to the KAHER, Belagavi as a part of requirement towards the completion of MD degree, review and publishing.

Questions: In case you have any questions related to the study in future you can contact:

1. If you have any queries about your rights as a study subject, you may call Dr.RoopaBellad, Professor, Department of Paediatrics, Chairman of J.N. Medical College Institutional Ethical Committee of Human Subjects Research, Ph No- 9448113403, at J.N. Medical College, Belagavi

CONSENT STATEMENT

I voluntarily agree to take part in this study by signing below. I may withdraw at any time. I am not giving up any legal rights by signing this form. My signature below indicates that I have read, or it has been read to me, this entire consent form and have had all my questions answered.

In case of the queries during the study or in future you may contact following person.

Principal Investigator: _____

Guide :_____

If you have any queries about your rights as a study subject, you may call Dr.RoopaBellad, Professor, Department of Pediatrics, Chairman of J.N. Medical College Institutional Ethical Committee of Human Subjects Research, Ph No-9448113403, at J.N. Medical College, Belagavi

Name of the participant:

(signature/thumbprint)

Name of the witness : (signature)

Name of the investigator: (signature)

Date:

Address:

Phone no:

ANNEXURE III

PROFORMA

PATIENT HISTORY

Name : Age : IP no. :

History :

Gross :

Risk factor (if any) :

Median age :

Median size of tumor (in cm) :

Tumor type : Primary / Metastatic

Histological subtype : Ductal / Invasive / Other

T status : T1 / T2 / T3 / T4

N status : N0 / N1 / N2 / N3

M status : M0 / M1

G status : G1 / G2 / G3

Lympho-vascular Invasion : Present / Absent

Number of lymphnodes positive for metastasis :

ER receptor : Positive / Negative

PR receptor : Positive / Negative

Hormone status : Triple negative / Not triple negative

PSMA expression in tumor cells : Negative / Weak / Moderate / Strong

PSMA expression in tumor associated neovasculature : Negative / Weak / Moderate / Strong

ANNEXURE - IV

H&E STAINING

1. De-paraffinised sections on the slides dipped in 100% ethanol for 2mins.
2. Slides dipped in 70%ethanol for 2mins.
3. Slides taken down to water for 2mins.
4. Thereafter, stained with Harris" Hematoxylin for 10-15mins.
5. Down to water for 2min.
6. Dip in 1%acidalcohol
7. Down to water2min
8. One dip in lithiumcarbonate
9. Down towater
10. Stain with eosin two to threemin
11. Down to water 2min
12. Dehydrate in 100% alcohol onedip
13. Blotting, flaming and clearing inxylene.
14. Thereafter, mount using DPX andcoverslip.

ANNEXURE - V IHC STAINING

1. Incubate sections on polylysine coated slides marked PSMA and CD 31 overnight at 56^{o}c.

2. De paraffinise sections in xylene, for 10 min, twocycles.

3. Rehydrate in alcohol, 100% for two min, 70% for twomin.

4. Hydrate in water for 10 min.

5. Place slides in Antigen retrieval solution$^{\wedge}$ in the racks and place in the microwave$^{\$}$, taking care that the rack is correctly aligned to the temperature sensitive probe.

6. Balance rack with a similar rack filled with water to the same level as that of rack with slides.

7. Set temperature for 99^{o}c and timer for 8 minutes and start the firstcycle.

8. Repeat two more such cycles; refill the level of solution asrequired.

9. Bring slides to room temperature and wash in tap water for 5min.

10. Blot out excess moisture from sides of the sections and put Peroxide block* and place in humidified cabinet for 30min.

11. Wash in TRIS buffer for 5 min, two changes.

1. Blot out excess moisture again and put Power block* for 20 min in the humidified chamber.

2. Blot out excess moisture and put ER, PR, Her2Neu monoclonal antibodies[&] on respective marked sections, place in humidified chamber for 45min-1hr.

3. Wash with TRIS buffer for 5 min, twocycles.

4. Blot out excess moisture and put Super enhancer* for 20 min in humidifiedchamber.

5. Wash with TRIS buffer for 5 min, twocycles.

6. Blot out excess moisture and put Secondary antibody* for 20 min in humidified chamber.

7. Wash with TRIS buffer for 5 min, twocycles.

8. Blot out excess moisture and put DAB chromogen[#] for 15min.

9. Wash in water for 10 min, 3-4 dips in Harris[‟] Hematoxylin, then down to water for 10 min.

10. Serially dehydrate in 70%alcohol followed by 100% alcohol for 2 mineach.

11. Blot, flame and place in Xylene forclearing.

12. Mount using DPX andcover slip.

* Super Sensitive Polymer HRP IHC detection system- Biogenex

$ EZ Retriever System V.2 Biogenex, microwave

^ EZ AR concentrated AR 4 Solution, 100 ml in 900ml of distilled water.

& Mouse Monoclonal Antibodies in PBS with carrier protein and preservative, Anti PSMA, Anti CD 31

One drop of DAB chromogen in 1 ml of DAB wash buffer in mixing vial, all supplied in the Super Sensitive Polymer HRP IHC detection system- Biogenex

ANNEXURE-VI

KEY TO MASTER CHART

Sr no	Ip Number	Biopsy no	Age	Tumor size	Histologic type	Histologic grade	Stage	Necrosis	LVI	PNI	METS/ NO OF LYMPHNODES	ER	PR	HER2 NEU
1	3009791	224/19	50	2x2x1.8	IDC	2	pT2N0Mx	2	POS	POS	NEG			
2	924002	289/19	41	4.5X3X3	IDC	2	pT2N0Mx	2	POS	POS	NEG			
3	3009496	394/19	49	4X4X2.5	IDC	2	pT2N1Mx	2	POS	POS	POS-2/17			
4	927605	510/19	57	2x1.5x1.3	IDC	2	pT2N0Mx	2	POS	NEG	NEG			
5	927476	515/19	63	3x2x2	IDC	2	pT2N0Mx	neg	POS	POS	NEG			
6	925152	544/19	53	2.5x2x1.5	IDC	3	pT2N1Mx	1	POS	POS	POS-1/8			
7	932111	835/19	50	2x2x1.5	IDC	2	pT2N1Mx	1	POS	POS	POS - 2/24			
8	932714	870/19	36	2.5X2X1.8	IDC	2	pT2N1Mx	1	POS	POS	POS-3/23			
9	3010478	911/19	65	3x2x2	IDC	3	pT2N3Mx	1	POS	POS	POS-24/25			
10	933057	951/19	31	3x3x2.5	IDC	2	pT2N0Mx	neg	POS	POS	NEG			
11	935205	1062/19	68	8x4x2	IDC	2	pT4N1Mx	2	POS	POS	POS-4/13			
12	3010815	1347/19	60	6x5x4	IDC	3	pT3N0Mx	2	POS	POS	NEG			
13	941817	1573/19	86	3x3x1	IDC	2	pT2N1Mx	neg	POS	POS	POS-3/16			
14	5248437	1788/19	61	4.5x3.5x3.5	IDC	2	pT2N0Mx	neg	POS	POS	NEG			
15	3011387	1984/19	55	3x1x1	IDC	2	pT2N2Mx	neg	POS	POS	POS- 10/29			
16	3012153	2739/19	72	1x1x1	IDC	2	pT2N0Mx	2	POS	POS	NEG			
17	958816	2792/19	68	6x6x3	IDC	2	pT3N2Mx	neg	POS	POS	POS-5/12			
18	5350892	2856/19	65	5.5x4.5x3	IDC	2	pT4N1Mx	neg	POS	POS	POS-3/5			
19	961415	2937/19	59	6x3.5x3	IDC	3	pT4N2Mx	2	POS	POS	POS-11/13			
20	961202	2951/19	54	3x3x2.5	IDC	2	pT2N1Mx	1	POS	POS	POS-13/22			
21	3012465	3024/19	62	3x3x1.8	IDC	2	pT2N1Mx	1	POS	POS	POS-1/10			
22	963752	3061/19	58	4.5x3x1.5	IDC	2	pT4bN1Mx	neg	POS	POS	POS-2/17			
23	965969	3194/19	60	2.5x2.x1.5	IDC	2	pT2N0Mx	1	POS	POS	NEG	NEG	NEG	NEG
24	3012647	3210/19	45	3x2.5x2	IDC	2	pT2N0Mx	neg	POS	POS	NEG			
25	969132	3338/19	45	7.5x7x3.5	IDC	3	pT3N2Mx	2	POS	POS	POS-19/26			
26	969337	3351/19	58	3x3x3	IDC	3	pT2N1Mx	1	POS	POS	POS-1/18	NEG	NEG	NEG

27	3012848	3380/19	46	2.5x2.5x1	IDC	3	pT2N1Mx	2	POS	POS	POS- 3/11			
28	5417756	3435/19	40	3x2.5x2.5	IDC	2	pT2N1Mx	1	POS	POS	POS-6/24	POS	POS	POS
29	983405	4262/19	50	6x5.5x4	IDC	2	pT3N2Mx	1	POS	POS	POS - 3 /17			
30	986874	4485/19	74	4x3x2	IDC	2	pT3N2Mx	2	POS	POS	POS - 19/23	POS	POS	EQUIVOCAL
31	5496017	4490/19	63	6.5x6x3	IDC	2	T4N2Mx	neg	POS	POS	POS -2/3	POS	POS	POS
32	3014056	4578/19	87	6.5X5.5X4	IDC	2	pT3N0Mx	1	POS	POS	NEG			
33	3014132	4628/19	43	4x3.5x2	IDC	2	pT3N1Mx	1	POS	POS	POS-5/12	POS	POS	EQUIVOCAL
34	988827	4633/19	64	3.5x3x2.5	IDC	2	pT2N1Mx	2	POS	POS	POS-14/19	NEG	NEG	POS
35	3014130	4646/19	40	7x4x4	IDC	2	pT3N1Mx	1	POS	POS	POS - 1/20			
36	3014245	4707/19	35	5.8x4.3x3	IDC	2	pT3N2Mx	1	POS	POS	POS - 2/26			
37	3014364	4834/19	51	6x4.5x3	IDC	2	pT3N0Mx	neg	POS	POS	POS-17/20			
38	993227	35/19	55	7x4x3.5	IDC	2	pT3N1Mx	1	POS	POS	POS-7/9	NEG	NEG	EQUIVOCAL
39	999107	415/19	57	12x10x3	IDC	2	pT3N0Mx	neg	POS	POS	POS-4/16			
40	999112	458/19	83	5X3.5X3	IDC	2	pT3N0Mx	neg	POS	POS	POS-4/8			

SL NO	Biopsy no.	PSMA																								
		IntraTumoral																		Extratumoral/Normal						
		VASCULATURE POSITIVE								TUMOR CELLS POSITIVE								IRS	VASCULATURE POSITIVE							
		HOT SPOT AREAS							Total no of vessels positive on whole section	Intensity	% in Positivity					Intensity				HOT SPOT AREAS							Total no of vessels positive on whole section
		1	2	3	4	5	Total	Average			0	<10%	10-50%	50-80%	>80%	Weak	Moderate	Strong		1	2	3	4	5	Total	Average	
1	224/19	25	20	15	15	10	85	17	298	2		1				1			1	5	8	5	3	2	23	4.6	38
2	289/19	18	8	10	10	7	53	10.6	225	2			2				2		4	2	2	5	3	2	14	2.8	32
3	394/19	30	30	25	17	9	111	22.2	442	3		1				1			1	5	2	3	5	2	17	3.4	27
4	510/19	20	15	12	8	5	60	12	198	2		1				1			1	3	3	2	2	1	11	2.2	21
5	515/19	25	20	15	10	8	78	15.6	240	2		1				1			1	5	3	3	2	2	15	3	28
6	544/19	45	32	21	9	8	115	23	328	2		1				1			1	1	5	3	2	1	12	2.4	20
7	835/19	46	30	24	18	12	130	26	403	3	0					1			0	8	5	3	3	1	20	4	32
8	870/19	20	12	10	8	7	57	11.4	185	1	0					1			0	8	3	3	1	1	16	3.2	28
9	911/19	40	32	25	12	12	121	24.2	372	3	0					1			0	10	7	7	3	2	29	5.8	35
10	951/19	34	28	21	13	7	103	20.6	297	2			2				2		4	8	5	3	2	2	20	4	31
11	1062/19	15	13	8	5	5	46	9.2	119	1				3			2		6	5	3	3	3	2	16	3.2	30
12	1347/19	19	7	7	5	3	41	8.2	122	1		1				1			1	5	3	2	1	1	12	2.4	28
13	1573/19	16	13	7	4	4	44	8.8	116	1	0					1			0	8	5	3	2	2	20	4	36
14	1788/19	26	21	15	10	8	80	16	225	2	0					1			0	5	2	3	2	2	14	2.8	31
15	1984/19	18	15	10	8	8	59	11.8	189	1	0					1			0	10	2	5	3	3	23	4.6	36
16	2739/19	15	13	10	8	5	51	10.2	149	1	0					1			0	8	3	7	2	2	22	4.4	30
17	2792/19	25	20	18	12	8	83	16.6	212	2					4		2		8	2	2	2	3	2	11	2.2	28
18	2856/19	17	13	10	5	3	48	9.6	147	1	0					1			0	7	3	3	1	1	15	3	32
19	2937/19	38	21	20	15	14	108	21.6	317	2	2					1			2	7	5	1	1	1	15	3	31
20	2951/19	20	15	13	10	3	61	12.2	182	1				3			2		6	8	3	3	1	1	16	3.2	25
21	3024/19	28	19	14	8	5	74	14.8	214	2		1				1			1	8	5	3	2	1	19	3.8	32
22	3061/19	36	32	28	17	15	128	25.6	385	2					4			3	12	5	3	3	3	2	16	3.2	28
23	3194/19	42	30	20	10	6	108	21.6	310	3			2				2		4	5	2	2	1	1	11	2.2	30
24	3210/19	45	32	26	19	10	132	26.4	411	3					4		2		8	7	3	3	1	1	15	3	39
25	3338/19	49	37	28	12	8	134	26.8	469	3				3			2		6	5	5	3	1	2	16	3.2	38

26	3351/19	39	28	21	10	9	107	21.4	373	2				3		1			3	5	5	3	2	2	17	3.4	35
27	3380/19	21	15	10	8	8	62	12.4	161	1		1				1			1	5	3	3	3	1	15	3	26
28	3435/19	28	18	10	10	8	74	14.8	274	2				3			2		6	7	5	3	2	1	18	3.6	38
29	4262/19	48	30	26	12	9	125	25	388	3					4		2		8	7	3	3	1	1	15	3	24
30	4485/19	32	28	26	10	5	101	20.2	337	3				3		1			3	6	6	5	2	1	20	4	37
31	4490/19	36	20	10	8	8	82	16.4	336	3				3			2		6	8	6	5	4	2	25	5	26
32	4578/19	22	20	10	5	3	60	12	143	1	0					1			0	6	6	5	3	2	22	4.4	38
33	4628/19	20	15	14	10	8	67	13.4	153	2	0					1			0	6	4	3	2	2	17	3.4	33
34	4633/19	42	32	28	10	5	117	23.4	318	2			2				2		4	7	6	4	4	3	24	4.8	34
35	4646/19	40	32	14	10	10	106	21.2	299	2		1				1			1	9	7	5	3	1	25	5	40
36	4707/19	35	30	26	12	9	112	22.4	286	2			2				2		4	8	6	5	2	2	23	4.6	37
37	4834/19	30	20	18	15	10	93	18.6	237	2		1				1			1	6	4	2	2	1	15	3	35
38	35/19	25	20	18	15	10	88	17.6	240	2			2				2		4	5	3	2	1	1	12	2.4	30
39	415/19	34	26	22	15	8	105	21	305	2			2				2		4	9	5	5	3	1	23	4.6	37
40	458/19	25	20	13	10	5	73	14.6	222	2		1				1			1	7	6	3	3	1	20	4	31

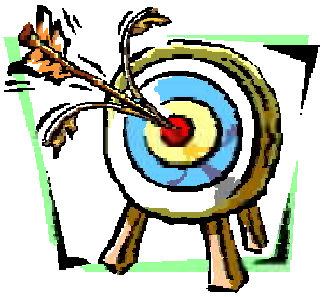
SL NO	Biopsy no.	CD31																	
		IntraTumoral										Extratumoral/Normal							
		VASCULATURE POSITIVE							IRS			VASCULATURE POSITIVE							
		HOT SPOT AREAS						Total no of vessels positive on whole	Intensity			HOT SPOT AREAS						Total no of vessels positive on whole
		1	2	3	4	5	Total	Average	section			1	2	3	4	5	Total	Average	section
1	224/19	49	40	45	35	28	197	39.4	500	3		36	27	19	15	12	109	21.8	214
2	289/19	52	45	25	20	18	160	32	417	3		30	24	18	14	10	96	19.2	196
3	394/19	60	48	40	28	19	195	39	705	3		41	33	26	14	10	124	24.8	303
4	510/19	42	28	38	25	17	150	30	398	3		24	21	19	10	7	81	16.2	178
5	515/19	65	43	56	29	18	211	42.2	486	3		29	20	17	14	12	92	18.4	225
6	544/19	86	48	35	23	12	204	40.8	578	3		32	30	24	18	14	118	23.6	251
7	835/19	69	56	45	37	35	242	48.4	786	3		46	35	27	21	16	145	29	365
8	870/19	62	45	35	22	20	184	36.8	342	3		28	21	15	12	8	84	16.8	159
9	911/19	60	48	45	39	28	220	44	621	2		36	24	21	15	11	107	21.4	287
10	951/19	58	47	41	35	22	203	40.6	505	3		30	23	17	16	11	97	19.4	236
11	1062/19	45	44	35	25	20	169	33.8	205	2		21	14	12	5	2	54	10.8	88
12	1347/19	39	32	28	23	19	141	28.2	282	2		23	16	11	6	4	60	12	107
13	1573/19	35	26	21	19	18	119	23.8	250	2		21	15	7	5	3	51	10.2	96
14	1788/19	48	42	31	24	19	164	32.8	426	3		36	28	15	12	8	99	19.8	189
15	1984/19	45	39	28	20	17	149	29.8	415	3		30	24	13	10	5	82	16.4	176
16	2739/19	32	27	24	18	17	118	23.6	254	2		24	22	14	9	4	73	14.6	148
17	2792/19	56	39	21	20	17	153	30.6	456	3		36	24	20	14	8	102	20.4	263
18	2856/19	47	32	31	25	20	155	31	348	3		30	24	17	11	7	89	17.8	179
19	2937/19	58	47	33	27	24	189	37.8	564	3		42	33	25	14	10	124	24.8	264
20	2951/19	42	39	26	21	20	148	29.6	361	2		37	28	21	14	9	109	21.8	188
21	3024/19	58	43	38	27	22	188	37.6	452	3		48	32	27	19	11	137	27.4	242
22	3061/19	69	55	49	38	27	238	47.6	684	3		58	46	39	25	18	186	37.2	327
23	3194/19	58	49	42	37	29	215	43	541	3		47	36	23	18	10	134	26.8	279
24	3210/19	72	63	50	41	23	249	49.8	566	3		49	37	24	20	16	146	29.2	286
25	3338/19	78	59	52	43	36	268	53.6	689	3		55	41	33	24	12	165	33	297
26	3351/19	70	62	55	41	31	259	51.8	645	3		51	44	34	25	18	172	34.4	304
27	3380/19	42	33	27	24	20	146	29.2	324	2		31	26	18	12	7	94	18.8	169
28	3435/19	63	47	36	27	23	196	39.2	546	3		39	31	29	20	14	133	26.6	227

29	4262/19	70	62	51	40	33	256	51.2	650	3		49	40	35	23	16	163	32.6	387
30	4485/19	69	52	39	30	21	211	42.2	554	3		36	29	22	17	11	115	23	284
31	4490/19	73	65	42	35	23	238	47.6	623	3		48	41	23	17	11	140	28	337
32	4578/19	38	33	24	23	19	137	27.4	350	2		25	21	19	14	11	90	18	166
33	4628/19	33	26	22	19	16	116	23.2	289	3		20	14	9	8	5	56	11.2	105
34	4633/19	56	43	38	33	24	194	38.8	542	3		36	24	20	18	10	108	21.6	249
35	4646/19	55	48	41	36	27	207	41.4	588	3		29	22	17	12	7	87	17.4	252
36	4707/19	52	45	36	27	22	182	36.4	564	3		35	28	21	14	10	108	21.6	258
37	4834/19	50	39	36	29	26	180	36	523	2		24	20	15	11	10	80	16	217
38	35/19	44	35	28	21	20	148	29.6	412	3		29	16	14	10	7	76	15.2	200
39	415/19	58	46	37	26	15	182	36.4	528	3		32	25	17	11	8	93	18.6	232
40	458/19	40	34	28	20	16	138	27.6	347	3		25	18	12	9	8	72	14.4	164

Intra Tumoral						Extra Tumoral					
AVG of PSMA exp	AVG of CD31 exp	Percentage of PSMA expression tumor vessels	Grading	Intensity	IRS	AVG of PSMA exp	AVG of CD31 exp	Percentage of PSMA expression tumor vessels	Grading	Intensity	IRS
17	39.4	43.14720812	2	2	4	4.6	21.8	21.10091743	2	2	4
10.6	32	33.125	2	2	4	2.8	19.2	14.58333333	2	2	4
22.2	39	56.92307692	3	3	9	3.4	24.8	13.70967742	2	3	6
12	30	40	2	2	4	2.2	16.2	13.58024691	2	2	4
15.6	42.2	36.96682464	2	2	4	3	18.4	16.30434783	2	2	4
23	40.8	56.37254902	3	2	6	2.4	23.6	10.16949153	2	2	4
26	48.4	53.71900826	3	3	9	4	29	13.79310345	2	3	6
11.4	36.8	30.97826087	2	1	2	3.2	16.8	19.04761905	2	1	2
24.2	44	55	3	3	9	5.8	21.4	27.10280374	2	3	6
20.6	40.6	50.73891626	3	2	6	4	19.4	20.6185567	2	2	4
9.2	33.8	27.21893491	2	1	2	3.2	10.8	29.62962963	2	1	2
8.2	28.2	29.07801418	2	1	2	2.4	12	20	2	1	2
8.8	23.8	36.97478992	2	1	2	4	10.2	39.21568627	2	1	2
16	32.8	48.7804878	2	2	4	2.8	19.8	14.14141414	2	2	4
11.8	29.8	39.59731544	2	1	2	4.6	16.4	28.04878049	2	1	2
10.2	23.6	43.22033898	2	1	2	4.4	14.6	30.1369863	2	1	2
16.6	30.6	54.24836601	3	2	6	2.2	20.4	10.78431373	2	2	4
9.6	31	30.96774194	2	1	2	3	17.8	16.85393258	2	1	2
21.6	37.8	57.14285714	3	2	6	3	24.8	12.09677419	2	2	4
12.2	29.6	41.21621622	2	1	2	3.2	21.8	14.67889908	2	1	2
14.8	37.6	39.36170213	2	2	4	3.8	27.4	13.86861314	2	2	4
25.6	47.6	53.78151261	3	2	6	3.2	37.2	8.602150538	1	2	2
21.6	43	50.23255814	3	3	9	2.2	26.8	8.208955224	1	3	3
26.4	49.8	53.01204819	3	3	9	3	29.2	10.2739726	2	3	6
26.8	53.6	50	3	3	9	3.2	33	9.696969697	1	3	3
21.4	51.8	41.31274131	2	2	4	3.4	34.4	9.88372093	1	2	2
12.4	29.2	42.46575342	2	1	2	3	18.8	15.95744681	2	1	2

14.8	39.2	37.75510204	2	2	4	3.6	26.6	13.53383459	2	2	4
25	51.2	48.828125	2	3	6	3	32.6	9.202453988	1	3	3
20.2	42.2	47.86729858	2	3	6	4	23	17.39130435	2	3	6
16.4	47.6	34.45378151	2	3	6	5	28	17.85714286	2	3	6
12	27.4	43.79562044	2	1	2	4.4	18	24.44444444	2	1	2
13.4	23.2	57.75862069	3	2	6	3.4	11.2	30.35714286	2	2	4
23.4	38.8	60.30927835	3	2	6	4.8	21.6	22.22222222	2	2	4
21.2	41.4	51.20772947	3	2	6	5	17.4	28.73563218	2	2	4
22.4	36.4	61.53846154	3	2	6	4.6	21.6	21.2962963	2	2	4
18.6	36	51.66666667	3	2	6	3	16	18.75	2	2	4
17.6	29.6	59.45945946	3	2	6	2.4	15.2	15.78947368	2	2	4
21	36.4	57.69230769	3	2	6	4.6	18.6	24.7311828	2	2	4
14.6	27.6	52.89855072	3	2	6	4	14.4	27.77777778	2	2	4

Introduction



Objectives

Review of Literature

Methodology

Results

Discussion

Conclusion

Summary

Bibliography

Annexure-I

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Annexure-II

Annexure-III

Annexure-IV

Annexure-V

Annexure-VI