"EXPRESSION OF VIMENTIN AS A MARKER OF EPITHELIAL-MESENCHYMAL TRANSITION IN INVASIVE BREAST CARCINOMA"

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LIST OF ABBREVIATIONS USED

AJCC	-	American Joint Committee on Cancers system
BCS	-	Breast conservative surgery
BRCA	-	Breast cancer gene
DCIS	-	Ductal carcinoma in situ
DPX	-	Dibutylphthalate Polystyrene Xylene
EMT	-	Epithelial-Mesenchymal Transition
ER	-	Estrogen receptor
E-cadherin	-	Epithelial cadherin
GLOBOCAN	-	Global cancer observatory
HER2	-	Human epidermal growth factor receptor
H&E	-	Haematoxylin and eosin
IARC	-	International Agency for Research on Cancer
IDC	-	Invasive ductal carcinoma
ILC	-	Invasive lobular carcinoma
IHC	-	Immunohistochemistry
LCIS	-	Lobular carcinoma in situ
LVI	-	Lympho-vascular invasion
MET	-	Mesenchymal to epithelial transition
MMP	-	Matrix metalloprotease
MRM	-	Modified radical mastectomy
N-cadherin	-	Neuronal cadherin
NGS	-	Nottingham grading system
PR	-	Progesterone receptor
SCC	-	Squamous cell carcinoma

TNM	-	Tumour, nodes and metastases
TDLU	-	Terminal duct lobular unit
TNBC	-	Triple Negative Breast cancer
WHO	-	World Health Organization

ABSTRACT

BACKGROUND:

Breast cancer stands as the leading and most fatal form of cancer among women worldwide, with invasion and metastasis being the primary culprits behind the majority of fatalities.

Many studies have been done to understand cancer progression and metastasis in breast cancers. EMT is one such process that is known to be reactivated in tumor progression. Numerous biomarkers have been used in studies to demonstrate EMT. One such marker is Vimentin, which is a cytoskeletal protein and has been conventionally used as a mesenchymal marker.

Its aberrant expression is seen in multiple carcinomas and recent studies have shown that it plays a significant role in the process of EMT in breast cancers.

OBJECTIVES

This study aimed to analyze Vimentin expression as a marker of EMT in invasive breast carcinomas and investigate any possible association between Vimentin expression and clinicopathological parameters including histological grading and immunohistochemical expression of ER, PR, Her2 and Ki67.

METHODS

A total of 60 breast carcinoma cases diagnosed during September 2022 to December 2023 were included in this study. Slides stained with H&E, and immunohistochemically for Vimentin were evaluated for histopathological examination. Results were subjected to appropriate statistical analysis.

RESULTS

The study showed Vimentin expression was significantly positive in high grade tumor. ($p<0.05$) Significant association was found between positive Vimentin expression and high Ki67%, negative ER and PR receptors while no significant association was found between Vimentin expression with tumor size and lymph node status. Additionally, triple negative cancers also showed increased Vimentin positivity.

CONCLUSION

In conclusion, the study shows increased expression of Vimentin is associated with more aggressive nature of tumor and TNBC molecular subtype. The findings of this study may give certain directions towards exploring Vimentin as a target for potential anticancer therapy.

KEYWORDS:

Breast carcinoma; Vimentin; Epithelial mesenchymal transition (EMT); Triple negative breast neoplasm; prognosis

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INTRODUCTION

Breast cancer is known to be the leading and most fatal cancer in women in the world, with invasion and metastasis being the primary culprits behind the majority of fatalities. In 2020, breast cancer diagnoses globally amounted to 2.3 million, with 685,000 deaths. By the year's end, the count of women surviving within five years of a breast cancer diagnosis totaled 7.8 million, solidifying its status as the most widespread cancer worldwide. Notably, breast cancer affects women in every country, with incidence rates rising notably in later stages of life[1].

As per GLOBOCAN predictions of incidence of cancer and death compiled by International Agency for Research on Cancer (IARC), the burden of newly diagnosed cases of invasive breast cancer globally is predicted to increase by over 40% by 2040, reaching nearly 3 million cases annually[2].

Despite considerable progress in research, breast cancer stands as a significant health issue and holds priority in medical research with multiple new prognostic markers being studied.

Multiple molecules and signaling pathways play roles in progression of malignancy. The epithelial to mesenchymal transition (EMT) is pivotal in embryonic development and wound healing, along with tumorigenesis and tumor progression, wherein it facilitates the loss of cellular adhesions, alterations in cell polarisation, detachment, migration, and metastasis. Morphologically, EMT is defined by the transition from epithelial to a mesenchymal phenotype, typically evidenced by increased expression of N-cadherin and Vimentin along with reduced E-cadherin expression [3].

Malignant epithelial neoplasms undergoing mesenchymal conversion are unfavorable and encourage more aggressive tumor behavior.

Raised Vimentin expression has been revealed in numerous cell lines in carcinoma breast according to recent researches. Vimentin is categorized as a type III intermediate filament, and is typically found in tissues originating from the mesenchyme[4]. Its aberrant expression has highlighted its significant role in the EMT process of breast carcinoma[5].

Comprehending the molecular mechanisms underlying the epithelial-mesenchymal transition EMT is crucial for developing inhibitors that may target this response. Such inhibitors could potentially be utilized to augment traditional chemotherapy treatments.

In a study by Shen et al., administration of a pan-inhibitor of Aurora kinases, danusertib, was shown to promote autophagy and suppressed EMT in tumor cells in breast, by modulating p38 MAPK/Erk1/1/Akt/Mtor pathways, indicating its potential as a promising anticancer treatment for breast cancer[6].

Therefore, directing therapies toward inhibiting EMT holds capacity as a therapeutic avenue, offering the potential for curing cancer through early recognition of tumor malignancy. Consequently, there is a critical need to investigate the presence of the EMT signature to forecast metastatic potential.

Vimentin, being a cost-efficient and readily accessible marker, its incorporation in the histopathology reports for routine use could significantly improve our understanding of tumor behavior and ultimately enhance patient care.

Thus, this study aimed to analyze Vimentin expression as a marker of EMT in breast carcinomas and investigate any possible association between Vimentin expression and clinicopathological parameters including histological grading and immunohistochemical expression of ER, PR, Her2 and Ki67. Herewith, the study sought to explore the potential role of the EMT in tumor invasiveness. The results of the study could facilitate in identification of

predictive and prognostic biomarkers, as well as development of novel treatment strategies in breast cancer patients.

OBJECTIVES

Primary objective

To study expression of Vimentin as a marker of epithelial to mesenchymal transition in invasive breast carcinoma.

Secondary Objective

1.To study its association with clinicopathological parameters including histological grading of Invasive breast carcinoma.

2.To study its association with immunohistochemical expression of hormonal receptors (ER and PR), Her2 and Ki67.

REVIEW OF LITERATURE

EMBRYOLOGY OF BREAST

Breast development begins around 5th week of intrauterine life with the appearance of mammary line which extends from axilla to groin on both sides of the body[7].

The mammary primordium develops from the cephalic 1/3rd of the mammary line while the caudal 2/3rd disappears. Solid cord of cells grows underneath the mesenchyme. At around 10-12 weeks, epithelial buds can be seen and gradually forms ducts in second and third trimester. By the time of birth, simple branched ducts develop with few lobular units[7].

ANATOMY OF THE BREAST

Female breasts extend from 2nd to 6th ribs, medially up to the sternal edge and laterally up to the anterior axillary line. An extension into the axilla is known as axillary tail of Spence[8,9].

There are three main components of breast – skin, adipose tissue and glandular tissue. The glandular tissue consists of parenchyma and stroma. The parenchyma contains 15-20 lobes which get drained by lactiferous ducts into the nipple. The main duct branches repeatedly within each lobe forming terminal ducts leading to lobules containing many acini. The terminal duct along with the lobule constitutes terminal duct lobular unit (TDLU). On the basis of morphology, breast lobules are classified into three types. Type 3 are the most developed and seen in parous and premenopausal women. Type 1 to type 3 progression involves additional branching and increased alveolar buds[8,9].

Blood Supply

Blood supply of the breast occurs in following ways –

- Second to fourth intercostal arteries perforating thoracic wall
- Superior thoracic, lateral thoracic, thoraco-acromial and subscapular arteries supplying lateral part, which are branches of axillary artery
- Branches of internal thoracic artery supplying medial part

Venous supply occurs through veins which are corresponding to arteries and drain into axillary, internal thoracic and intercostal veins[8,9].

Nerve Supply

Nerve supply occurs by anterior and the lateral cutaneous branches of fourth to sixtth intercostal nerves. The innervation of the nipple is via forth intercostal nerve[8,9].

Lymphatic Supply:

Skin overlying the breast is drained by superficial lymphatics. The nipple, areola and the parenchyma are drained by deep lymphatics.

The lymphatic supply hols significance since the metastatic spread occurs mainly by lymphatic channels in breast cancer[8,9].

PHYSIOLOGY:

The breast undergoes physiologic variations in morphology and function beginning from menarche until the menopause and also in each menstrual cycle. The changes are hormonally regulated by primarily estrogen, progesterone and prolactin. Breast development starts around puberty with branching and elongation of ducts. Also, during menstruation, pregnancy and lactation, it undergoes cyclical changes. During pregnancy, epithelial cells proliferate resulting in increased number and size of TDLUs[10].

After menstruation, major structural change occurs as epithelial atrophy. With loss of glandular epithelium, lobular basement membrane thickens and intralobular stroma collagenises[9,10].

HISTOLOGY

The ducts and acini are double layered- luminal cuboidal epithelium and abluminal myoepithelium[9].

The myoepithelium contains microfilaments and hence the contractile nature. This is responsible for milk ejection during lactation[11].

There is interlobular stroma and intralobular stroma. The interlobular stroma is dense collagenised and paucicellular as compared to the intralobular stroma[9].

The proportion of stroma and adipose tissue is variable with dense stroma being more in young women[11].

RISK FACTORS OF BREAST CARCINOMA

Factors known to increase risk of breast cancer include early menarche, late menopause, nulliparous women and absence of breastfeeding[12].

Other lifestyle factors are also known to contribute to increased risk such as lack of physical activity, obesity, smoking, alcohol intake, use of oral contraceptives, hormonal replacement therapy and radiation exposure[8,13].

Around 5-10 % of the breast cancer are known to arise because of gene mutations, of which inherited mutation in the BRCA1 and BRCA 2 genes is considered to be the most common as

per American Cancer society 2006. Besides, other genes shown to be associated are CHEK2, PTEN, CGH1, STK11 and PALB2[14].

HISTOLOGIC CLASSIFICATION

Traditionally, breast cancers have been classified on the basis of tumor cell type, architectural patterns, extracellular secretions and immunohistochemistry[15].

Breast tumors can be invasive or non-invasive on the basis of basement membrane invasion. Non-invasive tumors can be of two main forms- ductal carcinoma in situ (DCIS) and lobular carcinoma in situ (LCIS). Similarly invasive breast tumors can be divided into lobular and ductal types. The invasive ductal cancer makes up 50% to 70% of all invasive breast cancers[16].

For classifying invasive breast cancer, WHO classification is widely used. The latest 5th edition updates the classification with special focus on morphology. In the current WHO classification, mitotic count expression in defined area (in mm2) in denominator, addition of molecular classification and expression profile are among a few changes[17].

According to the update, invasive breast cancer is classified as follows:

- Infiltrating duct carcinoma -NST
- Invasive Lobular carcinoma
- Invasive Tubular carcinoma
- Invasive Cribriform carcinoma
- Invasive Micropapillary carcinoma
- Invasive Papillary carcinoma
- Carcinoma with apocrine differentiation
- Metaplastic carcinoma

- Mucinous cystadenocarcinoma
- Tall Cell carcinoma with reverse polarity[17]

National Health Service's calculation for 5,10,15-year survival with and without additional therapies has incorporated parameters like age, menopausal status, ER, HER2, Ki-67, tumour size, grade, and lymph node status[18].

Before treatment, breast cancer staging is done clinically, using physical examination and radiological investigations. After definitive surgical treatment, pathological staging is done using pathologic examination of primary tumour and regional lymph-node. Staging classifies individuals into risk groups defining prognosis and therapeutic options. The TNM classification system is done using the size of main tumour (T), regional lymph node status(N), and presence of distant metastases (M). The American Joint Committee on Cancers system (AJCC) is commonly used[16].

- Invasive Breast Carcinoma of No Special Type (NST)

The terminology for Invasive Carcinoma-NOS (not otherwise specified) has been changed to Invasive ductal carcinoma of no special type[18].

This is commonest type and comprises of tumors with no specific differentiating feature characteristic of other types of breast cancers. It is more common in post-menopausal women and arise from the TDLU[10].

Gross features: they are mostly firm to hard in consistency with irregular borders. On cutting they often produce grating sound due to central foci of microcalcification. Large tumours often show haemorrhage, necrosis and cystic changes[10].

Microscopic features: A variety of histologic appearances are seen. In well differentiated tumors, tubule formation is predominant, and small uniform nuclei are characteristic. In moderately differentiated carcinomas, tubules may be there along with solid clusters or singly scattered tumor cells[18]. Poorly differentiated carcinomas occur as nests or solid sheets of tumor cells with minimal tubule formation and irregular vesicular nuclei and areas of tumor necrosis[10].

- Invasive lobular carcinoma

Gross features: These are firm to hard tumors with irregular borders and often have scirrhous appearance. They show higher incidence of bilaterality. Usually cyst, haemorrhage, necrosis and calcification are not seen[10].

Microscopic features: Classically they present with loosely cohesive infiltrating cells, frequently in single file pattern and loose clusters[18]. Tubule formation is not seen and signet ring cells are common which contain intracytoplasmic mucin. This can be demonstrated with mucin and alcian blue stains. Desmoplasia is often absent or present minimally[10].

- Mucinous (Colloid) Carcinoma

These account for around 2 % invasive breast cancers and seen in elderly women (mean age of 71 years) and tend to progress gradually. They are also sometimes referred as gelatinous, colloid, mucus and mucoid carcinoma. They have a better prognosis and tend to be less aggressive[19].

Gross features: The tumor is mostly well defined with pushing or circumscribed borders. and soft-rubbery in consistency. On cut surface, it is glistening with pale grey-blue gelatinous appearance[10].

Microscopic features: there are tumor cells in clusters or small islands floating in lakes of mucin[19]. Calcification is less likely to be present[10].Tumour cells show low mitotic activity and mild-to-moderate degree of nuclear pleomorphism[18]. Insitu ductal component and marked mucin production can be seen[10].

- Tubular Carcinoma

The tubular pattern refers to the microscopic features in which there is neoplastic tubule formation similar in resemblance with breast ductules[18].

Gross features: Moderately well-defined lesion with stellate or grey-white cut surface[19].

Microscopic features: they show well-formed tubules with myoepithelial layer characteristically absent[19]. Apocrine snouts are often seen and intraluminal calcifications maybe present. They are almost always well differentiated with excellent prognosis[10].

- Papillary Carcinoma

Papillary carcinomas account for 1-2% of all breast carcinomas[19]. It has characteristic microscopic pattern of finger like projections or fronds having a fibrovascular core covered with epithelium. Myoepithelial cell layer is absent[18].

Gross features: often well circumscribed and soft to firm in consistency. The relative proportion of solid and cystic component is variable. Cut surface is tan or grey[10].

Microscopic features: Encapsulated variant has papillary configuration having single to multiple cell layers without myoepithelial layer[18]. Solid variant has appearance of multiple, circumscribed solid masses of cells, but at higher magnification, papillary lesion can be discerned[10]. Invasive papillary carcinomas have clusters of cells in micropapillary or tubule-

alveolar pattern in clear space or sometimes mucinous fluid[18]. The micropapillary variant lacks fibrovascular core having reverse polarity[10].

- Medullary Carcinoma

Medullary carcinomas constitute around 7% of all breast cancers[18]. Tumor cells are poorly differentiated and have scant stroma and marked lymphoid infiltration[19].

Gross features: It is a well-defined and circumscribed mass having soft fleshy grey tan cut surface. They range from 2.5-3cm in size[19]. The cut surface often tends to be lobular or nodular with bulging above the surrounding parenchyma. Haemorrhage and necrosis can be seen[10].

Microscopic features: It is characterised by solid, syncytium like clusters of tumour cells which are large having nuclear pleomorphism and prominent nucleoli constituting major component of the tumor mass. It has pushing non infiltrative borders. lymphocytic infiltration can be seen within and around the tumour mass[19].

- Metaplastic Carcinoma

They are a heterogenous set of tumors having an admixture of squamous cell carcinomas, matrix producing cancer cells and adenocarcinomas with spindle cell component[18].

It can be classified as:

1) Purely epithelial: Squamous cell carcinoma, adenocarcinoma with spindle cell differentiation, adeno-squamous carcinoma including muco-epidermoid carcinoma[10].
2) Mixed epithelial and mesenchymal: Cancers with chondroid or osseous metaplasia and carcinosarcoma[10].

- Cribriform Carcinoma

It is a less common type in which >90% tumor should have cribriform pattern[19].

Gross features: well defined mass having grey white/stellate cut surface[19].

Microscopic features: characterised by mild to moderate pleomorphic tumor cells in a collagenous stroma[19]. Variable amounts of intraluminal mucin positive secretions are present, often containing microcalcifications[10]. Nodal metastases from classical tumors also show cribriform pattern.[10]

MOLECULAR CLASSIFICATION

Breast cancers have been classified molecularly by Perou, Sorlie and colleagues on the basis of similarities in gene profiles, using cDNA microarray technique[20,21,22]. Their studies showed that these subtypes exhibited specific phenotype, prognosis and directed systemic planning of treatment[15].

Accordingly, 4 clinically pertinent molecular subtypes have been given: Luminal-A, Luminal-B, HER 2enriched (HER2+), and Triple Negative cancers (TNBC)[23].

The genes used to subtype are associated to the expression of oestrogen receptor (ER), progesterone receptors (PR), HER2 (Human epidermal growth factor receptor-2), and cellular proliferation marker (Ki-67)[24]. Usage of Immunohistochemistry (IHC) panel having these biomarkers has been used to stratify these entities[15].

- Luminal A

This is the most common and constitute around 50% of the newly diagnosed breast cancers[25]. As per the revised update of St. Gallen in 2013, this subtype is includes immunohistochemically: ER+ (≥ 1 %), high expression of PR (≥ 20 %), HER2- (≤ 10 %), with low levels of Ki-67% (< 20%)[26]. They include low histological grade types like IDC-NST,

tubular, mucinous, cribriform, and classic ILC[27]. These tend to show indolent clinical course and have better prognosis[28]. Since the hormone receptor status is positive in these cases, patients have the benefit of endocrine therapies like Selective oestrogen receptor modulator (SERM) or aromatase inhibitor (anastrozole)[28].

- Luminal B

They account for 20-30% of all invasive breast cancers[29]. They are defined immunophenotypically as Luminal B (HER2 negative): ER+ (≥ 1 %), PR- or < 20 %, HER2 negative (≤ 10%) with high levels of Ki-67 (≥ 20%); or Luminal B (HER2 positive): ER+ (≥ 1 %), HER2+ (> 10 %) and any level of PR and Ki-67[26,30]. They include moderate histological grade cancers such as most IDC-NST and have intermediate prognosis[28,31].

- HER2+

It comprises of 15-20% of newly diagnoses cancer cases[32]. They are characterised by increased HER 2 expression (> 10 %), negative ER (< 1 %) and PR (< 20%), with high expression of Ki-67 % (> 20 %)[26]. These cases respond well to drugs blocking HER2 activity like humanized monoclonal antibodies (Trastuzumab) as well as tyrosine kinase inhibitors (Lapatinib)[15].

- Triple Negative

These tumors are highly proliferative and lack the expression of the hormone receptors (ER, PR) and HER 2 (≤ 10%).[26] This subtype comprise of 10-20% of all breast cancers[15].

PROGNOSTIC AND PREDICTIVE FACTORS

Prognosis for breast carcinoma patients varies greatly. While some women have only 10% chances of 5-year survival, most women have normal lifespan. This is with the exception of women who have distant metastasis (<10%) or inflammatory carcinoma (<5%), which have

worse prognosis irrespective of other factors. AJCC staging, states the classification for breast cancer patients with stages 0 to IV incorporating major prognostic factors[33].

1.Axillary Nodal Status

Involvement of axillary lymph node is the most significant factor for prognosis in breast cancer. Furthermore, number of lymph nodes is directly correlated to the risk of distant recurrence[34]. It is the most reliable factor used in decision making for adjuvant treatment with the patients with positive lymph nodes to be given adjuvant therapy[33].

2.Tumor Size

In addition to being an independent prognostic factor, tumour size relates with number of lymph nodes involved[33].

According to Rosen et al. there is a strong corelation between the size of tumor and 20-year recurrence free survival[35].

3.Tumor Type

The tumors pathologic features are important in determining prognosis. Compared to unspecified breast cancers, certain subtypes like tubular, mucinous and medullary have a better prognosis[33].

4. Tumor grade:

Elston and Ellis showed the importance of histologic grade and its correlation with prognosis in a series of around 1800 patients[36]. The Nottingham grading system (NGS) which is an Elston-Ellis modification of Bloom-Richardson grading classification has been widely used to guide management in breast cancer cases.[37,38,39]

It's based on assessment of 3 morphological characteristics: (i) degree of tubular or glandular formation, (ii) nuclear pleomorphism, and (iii) mitotic count[40].

4.Lymphatic and Vascular Invasion

It has been shown that peri-tumoral lymphatics and vascular invasion holds prognostic importance for risk of local as well as distant recurrence. According to a follow - up study of 20 years by Rosen et al. lymphovascular invasion exhibited an association with risk of recurrence and death[35]. Women with stage I LVI-positive had a recurrence rate of 38% whereas those with LVI-negative had recurrence rate of 22%. For patients with negative lymph node status and borderline tumor sizes, LVI serves as an important prognostic parameter for decision making[33].

5.Proliferation Markers

The proliferation rate of tumors has been measured using a variety of methods. Some of them are thymidine labelling index, S-phase fraction (SPF), mitotic index, besides IHC analysis of Ki-67 and proliferating cell nuclear antigen[33].

6.Age

Few studies have reported that patients below 35 years had worse prognosis[41,42].

7. ER/PR Status

Hormone receptors if present in breast cancers is a major predictive and prognostic indicator for assessment of treatment benefit from tamoxifen therapy[33].

8. HER2/*neu*

A transmembrane glycoprotein called $p185^{HER2}$ which is encoded by c-erbB-2 (HER2/*neu*) proto-oncogene on chromosome 17q21 has intrinsic tyrosine-kinase action similar to that of epidermal growth factor[43]. It's been found to be amplified or overexpressed in around 30% breast cancer cases in humans[44]. While the effect on patients who are node-negative is variable, in lymph node positive cases, the overexpression is linked to more tumor aggressiveness, increased recurrence rates and mortality[45,46,47,48].

9.Urokinase-Type Plasminogen Activator and Plasminogen Activator Inhibitor Type 1

There are two factors, urokinase type plasminogen activator (uPA) and its inhibitor, plasminogen activator inhibitor type 1(PAI-1) having prognostic and predictive application. Patients who have negative-node status with low uPA/PAI-1 show better prognosis with a 5-year DFS of 90%[33].

EPITHELIAL TO MESENCHYMAL TRANSITION (EMT):

The theory of EMT was initially introduced by Elizabeth Hay, who utilised chick primitive streak cells to demonstrate that epithelial cells undergo significant changes in their phenotype to transition into mesenchymal cells[49].

In normal epithelium, there is cell-to-cell contact which is crucial for the development during embryogenesis and for maintain structure and homeostasis of epithelial tissues in adulthood.

Epithelial cells are tightly packed together, resembling a group of defined, cobblestone-like cells. When cultured, these cells form layers closely connected through specialised structures in the membrane like tight junctions, adherens junctions, desmosome junctions and gap junctions[50]. Additionally, epithelial cells show apico-basal polarity, which is evident by the arrangement of molecules of cell surface like cadherins and integrins, organisation of cell-to-

cell junctions, polarised arrangement of actin cytoskeleton and formation of a basal lamina extra-cellular matrix at the basal surface[50,51] Various molecules that are present in epithelial cells, are known to be classic markers for identification of epithelial cell type, such as keratins and E-cadherin.

It is suggested that epithelial tumor cells lose these properties during tumorigenesis and acquire motility[52]. This process is known as Epithelial to mesenchymal transition.

Mesenchymal cells differ from epithelial cells in appearance and biological behaviour. These have amorphous shape, do not form organised cell layers, lack apico-basolateral organisation, show no polarization of cell surface molecules and exhibit a disturbed, undefined actin cytoskeleton[53].

The process of EMT involves a variety of cellular, phenotypic and functional changes. It is characterised by a series of changes that classically include alterations in gene expression (loss of epithelial markers like E-cadherin and acquisition of mesenchymal markers like Vimentin), decreased cell-cell adhesion, changes in cell matrix adhesion, changes in polarity of cells and cytoskeleton, changes in synthesis and assembly of ECM molecules, and an increase in production and activity of extracellular proteases such as matrix metalloproteases (MMPs)[52].

Understanding the mechanism contributing to this process is of immense interest given the importance of EMT in cancer progression[54]. In the biological context in which they occur, three distinct subtypes of EMT have been described: type 1 involved in embryogenesis and organ development; type 2 associated with regeneration of tissues and type 3 is linked to cancer progression and metastasis[55].

Type 1 EMT is responsible for producing mesenchymal cells to form new tissues with varied functions. For instance, in embryonic development neuroectoderm epithelial cells undergo EMT to generate neural crest cells with migratory capacity[56]. Type 2 EMT is depicted as a

repair mechanism following inflammatory injury to regenerate tissues and is related to wound healing and fibrosis. This is closely linked to fibrosis seen in chronic inflammatory conditions affecting organs like liver, lung, kidney and intestine[55]. Type 3 EMT, is the focus of this thesis work, and appears aberrantly in cancer cells due to genetic and epigenetic alterations, promoting clonal outgrowth and invasion from their primary site[55].

EMT In Normal Development

In early embryogenesis, there is a need for reprogramming epithelial cells to adopt a migratory character.

By the process of Gastrulation, simple spherical organisation of cells is rearranged into the three germ layers, which encompasses changes in cellular adhesion, shapes and motility. This is controlled by well-organised program of EMT[57].

According to a review by Thiery et al. EMT is crucial in neural crest formation which forms the craniofacial structures, peripheral nervous system, melanocytes, and endocrine cells[57]. In addition, organs such as liver, pancreas, and heart undergo morphogenesis of the epithelial structures by secondary or tertiary EMT to differentiate into specialised cells[57,58].

EMT In Cancer Metastasis

The ability of cancer cells to alter their shape and their detachment from surrounding cells and extracellular matrix, has been known as one of the hallmarks of cancer. This allows the tumor to progress to a more advanced state[59]. To gain the migration and invasion ability, is one of the early steps of metastasis which is initiated by the reactivation of type 3 EMT[60].

The mechanism by which epithelial cells in a specific region can dissociate and migrate to a different location is termed as EMT[61]. In this process the polarised epithelial cells undergo numerous changes and gain mesenchymal like phenotype wherein they have greater migratory

and invasive capabilities, resistance to apoptosis and enhance the production of extracellular matrix[55].

Even though EMT is thought to be the initial step in metastasis, its extent and timing is not well studied because of unavailability of advanced technologies to monitor EMT in vivo. With the recent imaging technologies, the movement of tumor cells from the primary site has been observed and also few studies have been able to prove the occurrence of EMT in tumor progression[62].

It has been stated that after the disseminated tumor cells reach a certain distant site, reversal of EMT, which is known as MET (mesenchymal to epithelial transition) occurs and is also known to be crucial during metastatic process[57,63].

EMT In Metastatic Breast Cancer:

Most deaths from breast cancer are due to metastases wherein secondary tumors are formed from disseminated cancer cells in distant organs like lungs, liver, brain and bones[64,65,66].

The process of metastasis has multiple steps requiring properties such as anoikis resistance and acquiring capacity to migrate and invade. Notably, these changes mostly occur during the process of EMT.

Below are the steps of the metastatic cascade elucidating role of EMT in this process:

- Detachment:

Breast carcinoma originates from epithelial cells which have a tendency to normally organise in a polarised sheet with attachment to the basement membrane. Hence, for metastasis to occur, cells need to detach from surrounding cells by downregulation of adhesion molecules like claudin, occludin and specifically E-cadherin[67]. E-cadherin, which is a transmembrane protein found in epithelial cells, is responsible for cell-to-cell adhesion by calcium dependent binding with the E-cadherin molecules of neighbouring cells. There is an intracellular interaction

between p120 and β-catenin that is crucial for cell adhesion and Wnt/ β-catenin signalling, stabilisation of E-cadherin in the cell membrane and establishing cell-to-cell contacts. It is known now that E-cadherin is important to maintain epithelial morphology, starting from the first epithelial lining during embryogenesis to the epithelial tissues in adults[68,58].

It has been reported that there is decreased E-cadherin expression in around half of invasive ductal carcinomas whereas there is total loss of expression in invasive lobular carcinomas[69]. In consistence with these findings, breast cancer cells with decreased CDH1 (gene encoding E-cadherin) expression have been shown to proliferate aggressively indicating cancer cell invasion to be related with loss of E-cahderin[68]. Loss of E-cadherin along with other junctional proteins relies on suppression of transcription of their genes by EMT- driving transcription factors such as Snail, Slug, Twist and Zeb1[70,71,72].

- Anoikis Resistance

In normal epithelial cells, there is a special form of cell death, known as Anoikis for the cells with inappropriate detachment. This is needed to preserve orderly tissue framework and avert growth of epithelial cells in ectopic locations[73,74]. Contrary to this, cancer cells tend to evade anoikis by numerous chief regulators of EMT. EMT transcription factors such as Slug and Twist reportedly inhibit anoikis via activation of anti-apoptotic pathways[67,75,76].

This sort of acquired anoikis resistance is of pivotal importance in cancer progression as most solid tumors arranged in multicellular masses and a few of these cells are bound to proliferate without basement membrane attachment[77]. Moreover, anoikis resistance is significant for metastasis since it permits individual cancer cells to survive in spite of being detached from primary tumor.

- Migration

Followed by this is the gain of capacity to migrate. Cells in carcinomas undergoing EMT exhibit changes in gene expression from epithelial to mesenchymal to attain migratory potential, in comparison to epithelial cells in development processes [78].

- Gain Of Mesenchymal Morphology

This shift in expression of genes comprises what is known as 'Cadherin switch' in which tumor, cells undergoing EMT do not just loose E-cadherin but also induce CDH2 expression (gene encoding N-cadherin) by the transcription factor of EMT, Twist[78,79,80]. It has been found that N-cadherin expression in cells increases their potential to migrate. Supporting this finding, N-cadherin upregulation has been documented in invasive breast cancer lines[81,82] and its ectopic expression in non- invasive MCF-7 and BT20 breast tumour cells has been reported to encourage motility[81].

Moreover, motile tumor cells display a shift in expression from epithelial keratin genes to mesenchymal vimentin, also regulated by EMT transcription factors[82,83]. Keratins are a type of intermediate filaments which provide mechanical stability to epithelial cells and hence maintain epithelial phenotype[84]. Notably, all epithelial tissue during transition to mesenchymal phenotype exhibit a downregulation of a specific set of keratins[85].

Vimentin, a structural protein of intermediate filaments present in mesenchymal cells supports migratory ability by formation of matrix adhesions and recycling of endocytosed Integrins that are vigorously turned over in migrating cells[86,87].

Apart from above changes, cancer cells in order to migrate need to make way through the dense extracellular matrix for invasion. Hence, they secrete proteolytic enzymes to digest these barriers and for remodelling of their surrounding tissue[88]. Increased activity of promoter of MMP is shown to be regulated by EMT transcription factors like Slug, Snail, and ZEB2 in oral cancers[89], squamous cell carcinomas[90] and hepatic cancers[91], respectively.

EMT markers and Transcriptional regulators-

The loss of E-cadherin is taken to be a key step in EMT process. Emergence of Vimentin, a cytoskeletal marker has been used as a marker to identify tumour cells undergoing EMT, and its expression has been positively associated with increased tumor invasion and metastasis[92]. EMT is regulated by various transcription factors that directly or indirectly supress CDH1. Factors such as Snail, Slug, Zeb1 and Zeb2 can inhibit CDH1 transcription by binding to its promoter[93,94,95].

VIMENTIN-

Vimentin is a 54kDa type III intermediate filament protein found in several cell types including endothelial cells, fibroblasts, neutrophils, lymphocytes and macrophages[96].

During embryogenesis, it is widely expressed in the embryo in all primitive cell types but its expression becomes limited with further differentiation[97,98,99].

Expression of Vimentin in migratory epithelial cells during processes such as embryogenesis, wound healing or tumor invasion, have been supported by various studies[100,101,102].

The association of Vimentin with metastatic progression and tumor invasion, alongside the aberrant expression of E-cadherin/ β-catenin complexes, highlights its role in these processes[103].

In invasive breast cancer, Vimentin expression has been a consistent finding[104,105,106]. In a study by Hemalatha et al., Vimentin positivity was reported to be associated with tumor aggressiveness[5].

Significance of EMT

Cells that go through the process of EMT may acquire the ability to metastasise but they make up a small percentage of tumor cell population. Tumour budding is when single cancer cell or

small group of cells are involved at the invasive front of the tumour mass. These have shown to exhibit downregulation of E-cadherin and upregulation of Vimentin[107,108].

In a study of oral squamous cell carcinomas by Jing ping et al, E-cadherin and Vimentin positivity was proven to be associated with tumor metastasis, supporting the phenomenon of EMT during the development of SCC[109].

Hence in this study the main focus is on Vimentin as an EMT marker and determine its association with clinicopathological parameters including tumor grading and immunohistochemical expression of hormone receptors, Her2, and Ki67, if any.

MATERIALS AND METHODS

A total of 60 specimens of breast carcinoma cases from patients diagnosed clinically and histopathologically at KLEs Dr. Prabhakar Kore Hospital and Research Center, Belagavi during September 2022 to December 2023 were included in this study. Detailed clinicopathological information was taken from patient records. The specimens included modified radical mastectomy and breast conservative surgery specimens.

Study design: Hospital based observational study

Study period: September 2022 to December 2023

Study population: Patients clinically and histologically diagnosed with breast carcinoma at Dr. Prabhakar Kore Hospital during the study period.

Inclusion criteria: Histologically proven cases of breast carcinoma, modified radical mastectomy (MRM) specimens and breast conservative surgery (BCS) specimens-irrespective of age were included in this study.

Exclusion criteria:

1. Inadequate biopsies/core biopsies

2. Improperly fixed specimens

Sample size: Sixty breast carcinoma cases in total which fulfilled the inclusion criteria were taken in the study (Universal sampling).

Ethical clearance: The present study was approved by Jawaharlal Nehru medical college's Institutional Ethics committee on Human Subjects research. (Ref.: MDC/JNMCIEC/135).

Data collection:

The breast carcinoma cases that were operated in Dr. Prabhakar Kore Hospital were taken for this study. These specimens were grossed according to the standard procedure, and bits were given. Slides were stained with H&E, and slides were screened. Suitable blocks were selected for Vimentin IHC staining with clone V9 (Dako) antibody.

Information regarding IHC expression of ER, PR, HER2 and Ki67 were taken from the laboratory records. Information regarding clinical data were obtained from Medical Records Department (MRD) records.

Histopathology Evaluation:

All slides were evaluated and histological grading was done as per The Modified Bloom-Richardson system[40].

IHC Staining Procedure:

Thin tissue sections of 3-4 microns were cut with microtome and then placed on coated slides.

Steps for IHC staining for Vimentin is as follows:

1.The slides were baked at 60 degree centigrade for 1 hour prior to start deparaffinisation.

2. Then deparaffinised in fresh xylene for two changes 10minutes each.

3. Rinsed in absolute alcohol for two changes 10minutes each.

4. Then were rinsed in water for-5minutes, followed by distilled water for-1minute.

5. They were subjected to antigen retrieval using pressure cooker method using TRIS EDTA buffer solution.

6. After that they were allowed to cool at room temperature for 15minutes.

7. Rinsed with wash buffer 2times with gap of 30seconds each.

8. Placed in 0.3% Hydrogen peroxide-8 to 10 minutes to inhibit endogenous peroxidase activity.

9. Then washed with wash buffer 3times with gap of 30seconds each.

10. Primary antibody incubated for 45-60 minutes at room temperature in a closed room

11. Then they were washed with wash buffer 3times with gap of 30seconds each

12. HRP polymer was applied and incubated for 25-30 minutes at room temperature in a closed chamber

13. Washed with wash buffer 3times with gap of 30seconds each

14. DAB substrate was applied to sections for 10minutes

15. Washed in water-2minute, followed by wash with distilled water-1minute

16. Counter stained with Haematoxylin-3minutes

17.Washed in running water for 2 minutes.

18. Blotted and cleared in xylene & mounted with DPX.

Antibody in the study:

Antibody: Vimentin
Localisation: Cytoplasmic
Clonality: Monoclonal Mouse Anti Vimentin Clone V9
Dilution: Ready to use
Manufacturer: Dako

Negative control (without adding primary antibody) was done in all batches. Fibroblasts, endothelial lining were taken as positive internal control for expression of Vimentin.

Evaluation of immunoreactivity:

Sections were examined to look for cytoplasmic immunoreactivity in tumor cells. Vimentin expression was considered positive if ≥10% of the tumour cells showed distinct granular cytoplasmic immunoreactivity[5]. Evaluation of Vimentin expression was done in area of maximum Vimentin positivity following examination of the entire section. Other positive non-tumour cells (Lymphocytes, stromal fibroblast) were excluded by morphologic assessment.

Statistical analysis:

It was done using SPSS software version 23.0. Chi-square was used to study association between analysed Vimentin expression and clinicopathological parameters including histological grading and immunohistochemical expression of ER, PR, Her2 and Ki67.

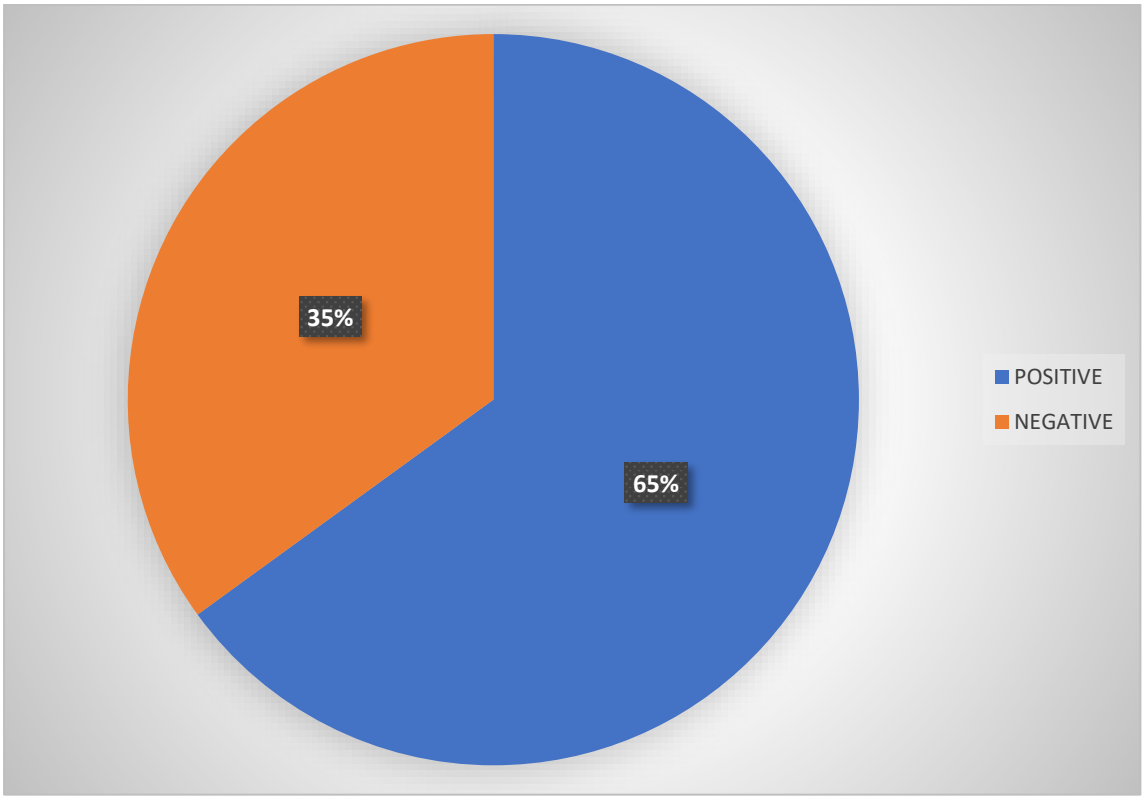
RESULTS

In the present study, a total of 60 cases of histologically proven breast cancer were taken. The cases included 51 modified radical mastectomy specimens and 9 breast conserving surgery specimens which were received in section of histopathology in the Department of Pathology, Dr. Prabhakar Kore Hospital, Belgaum during the study period.

All the 60 cases were subjected to immunohistochemical study for Vimentin.

Positive Vimentin expression was seen in 39 of the 60 (65%) cases.

Graph 1: Vimentin expression in breast cancer cases



The age of the patients in the study ranged from 32 to 95 with a mean value of 54±11.6 years.

There was no significant association found between age of the patient and Vimentin expression ($p>0.05$)

Table 1: Vimentin Expression and Age of The Patient

VIMENTIN STATUS		age
NEGATIVE	Mean	56.190
	N	21
	Std. Deviation	13.4001
POSITIVE	Mean	53.359
	N	39
	Std. Deviation	10.6388
Total	Mean	54.350
	N	60
	Std. Deviation	11.6457
	P value	0.374

50 out of the 60 cases (83%) were post-menopausal women

Expression of Vimentin and menopausal status did not show any significant association ($p>0.05$).

Table 2: Vimentin expression and menopausal status

		VIMENTIN STATUS		
		NEGATIVE	POSITIVE	Total
MENOPAUSAL STATUS	POST MENOPAUSAL	18	32	50
		36.0%	64.0%	100.0%
	PRE-MENOPAUSAL	3	7	10
		30.0%	70.0%	100.0%
Total		21	39	60
		35.0%	65.0%	100.0%

Chi sq = 0.132 p value = 0.717

Majority of cases i.e. 42 of the 60 cases (70%) were of T2 size, followed by 9 cases (15%) of T3 size and 8 cases (13%) of T1 size. The study results showed no significant association of tumor size with Vimentin expression ($p>0.05$).

Table 3: Vimentin expression and tumor size

		VIMENTIN STATUS		
		NEGATIVE	POSITIVE	Total
T STAGE	T1	1	7	8
		12.5%	87.5%	100.0%
	T2	18	24	42
		42.9%	57.1%	100.0%
	T3	2	7	9
		22.2%	77.8%	100.0%
	T4	0	1	1
		0.0%	100.0%	100.0%
Total		21	39	60
		35.0%	65.0%	100.0%

Chi sq = 4.104 p value = 0.250

Histologically, majority of the cases i.e. 56 out of 60 cases (93%) were invasive ductal carcinoma-NST, the remaining 7% included 2 cases (3.3%) of papillary carcinoma, 1 case (1.6%) of invasive lobular carcinoma (ILC), and 1 case (1.6%) of mucinous carcinoma.

In this study, 36 of the 56 cases (64.3%) of IDC-NST showed Vimentin positivity. There was one case of invasive lobular carcinoma which did not show positivity for Vimentin. There was one case of mucinous carcinoma which showed Vimentin positivity and two cases of papillary carcinoma, which were also positive for Vimentin.

However, there was no significant association found between Histological Type and Vimentin Status ($p>0.05$).

Table 4: Vimentin Expression and Histologic Type

		VIMENTIN STATUS		
		NEGATIVE	POSITIVE	Total
HISTOLOGICAL TYPE	INVASIVE DUCTAL CARCINOMA(NST)	20 35.7%	36 64.3%	56 100.0%
	OTHER-INVASIVE LOBULAR CARCINOMA	1 100.0%	0 0.0%	1 100.0%
	OTHER-MUCINOUS CARCINOMA	0 0.0%	1 100.0%	1 100.0%
	OTHER-PAPILLARY CARCINOMA	0 0.0%	2 100.0%	2 100.0%
Total		21 35.0%	39 65.0%	60 100.0%

Chi sq. = 3.485 p value = 0.323

There were 8 cases of grade I, 39 cases of grade II, and 13 cases of grade III tumors. All 13 cases (100%) of Grade III showed Vimentin positivity, followed by 22 cases (56.4%) of the 39 Grade II tumors and 4 cases (50%) out of 8 Grade I tumors.

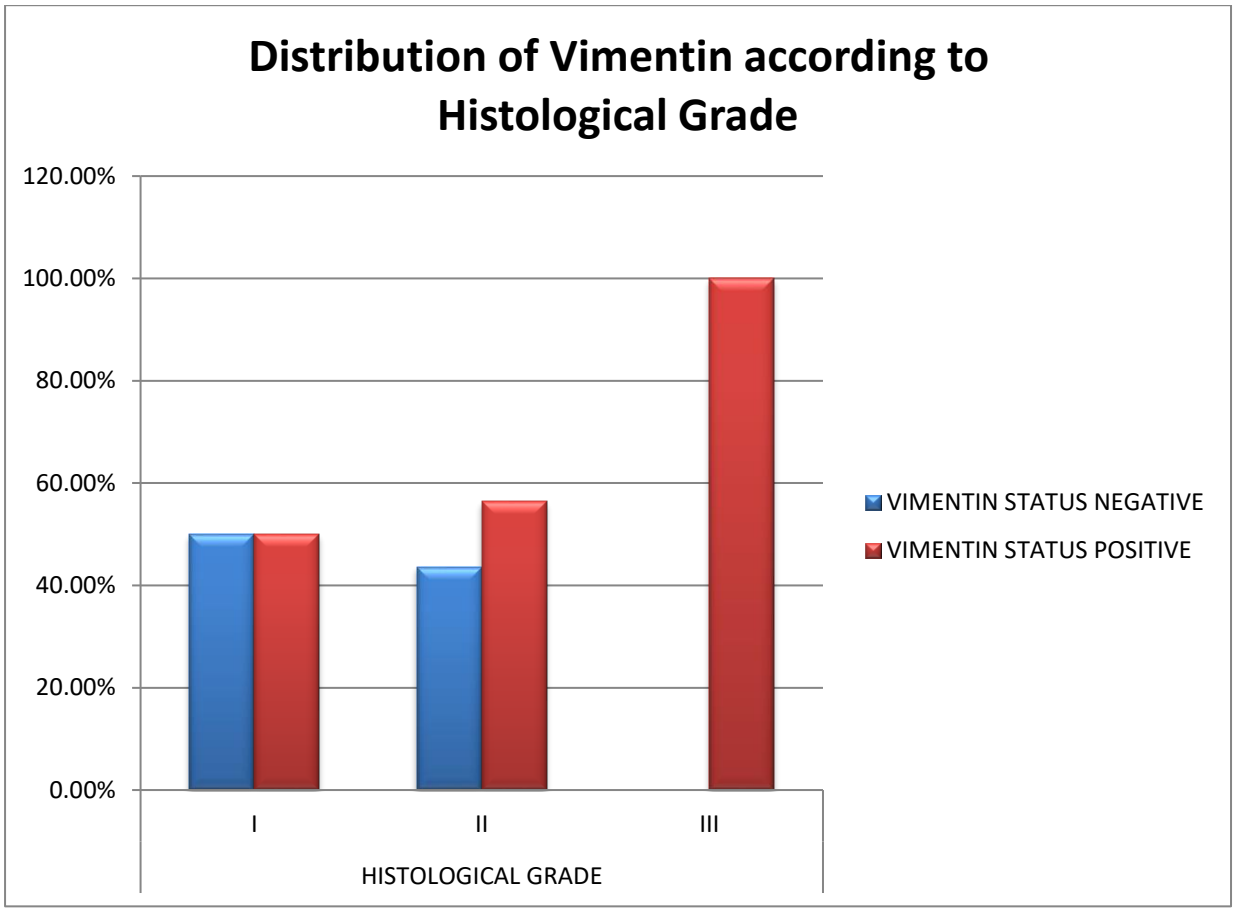
The p value was significant indicating increased Vimentin positivity in high grade tumours ($p<0.05$).

Table 5: Vimentin Expression and Histological Grade (Nottingham grading)

		VIMENTIN STATUS		
		NEGATIVE	POSITIVE	Total
HISTOLOGICAL GRADE	I	4	4	8
		50.0%	50.0%	100.0%
	II	17	22	39
		43.6%	56.4%	100.0%
	III	0	13	13
		0.0%	100.0%	100.0%
Total		21	39	60
		35.0%	65.0%	100.0%

Chi sq = 9.056 p value = 0.011*

Graph 2: Distribution of Vimentin expression according to Histological grade



Out of the 60 cases, lymph nodes were retrieved in 56 cases.

32 out of 56 cases (57%) had positive lymph node status.

Among the 32 cases that had lymph node metastasis, 22 (68.8%) were positive for Vimentin whereas 17 out of the 24 (70.8%) lymph node negative cases were Vimentin positive.

There was no significant association found between Lymph Node Status and Vimentin Status ($p>0.05$).

Table 6: Vimentin expression and Lymph node status

		VIMENTIN STATUS		
		NEGATIVE	POSITIVE	Total
LYMPH NODE STATUS (n=56)	NEGATIVE	7	17	24
		29.2%	70.8%	100.0%
	POSITIVE	10	22	32
		31.3%	68.8%	100.0%
Total		17	39	56
		30.4%	69.6%	100.0%

Chi Sq. = 0.028 p value = 0.867

Vimentin positivity was seen in 82% of the ER negative cases as compared to 50% of ER positive cases.

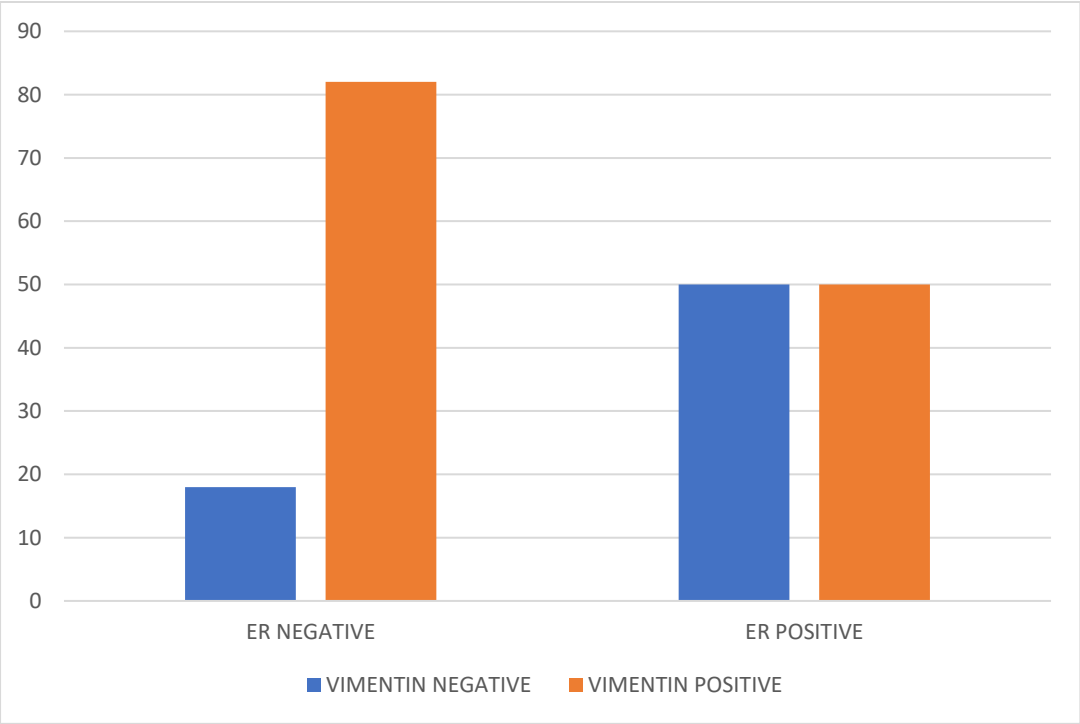
The results showed a statistically significant association between ER Expression and Vimentin Status (p-value<0.05). Hence, positive expression of Vimentin was significantly associated with ER negativity.

Table 7: Vimentin expression and ER receptor status

		VIMENTIN STATUS		
		NEGATIVE	POSITIVE	Total
ER EXPRESSION	NEGATIVE	5	23	28
		17.9%	82.1%	100.0%
	POSITIVE	16	16	32
		50.0%	50.0%	100.0%
Total		21	39	60
		35.0%	65.0%	100.0%

Chi sq. = 6.782 p value = 0.009*

Graph 3: Distribution of Vimentin expression according to ER expression



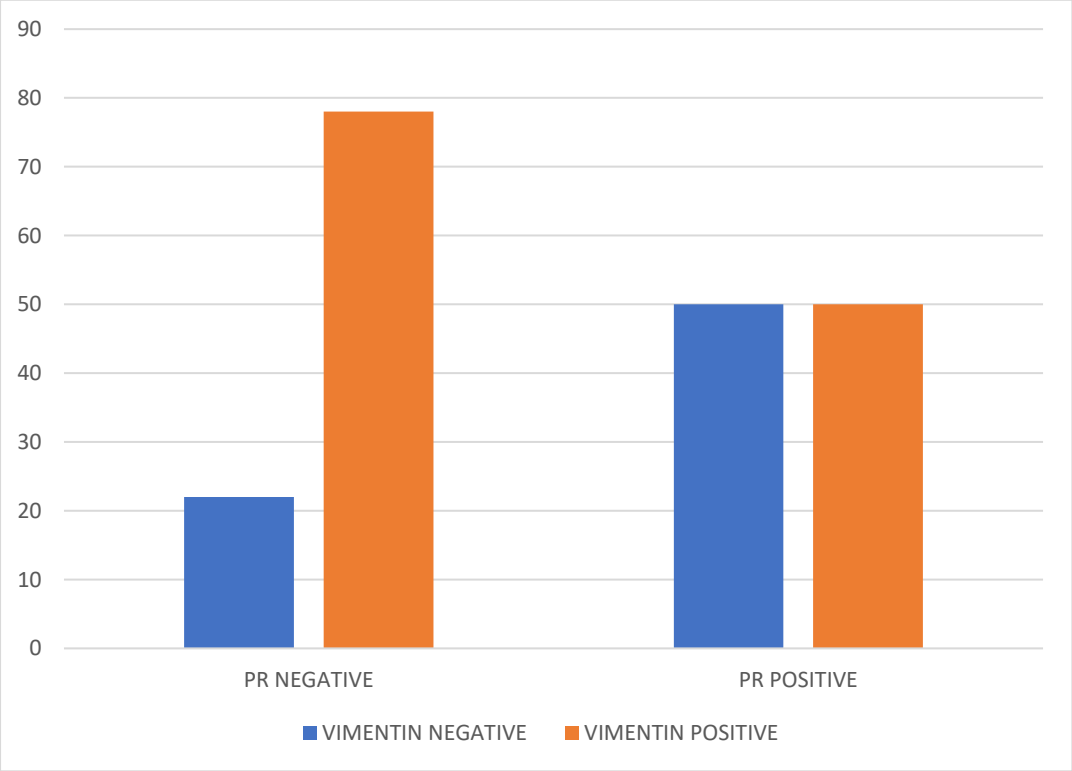
Vimentin positivity was seen in 78% of the PR negative cases as compared to 50% of PR positive cases. The results showed a statistically significant association between PR Expression and Vimentin Status ($p<0.05$). Hence, positive Vimentin expression was significantly associated with PR negativity.

Table 8: Vimentin expression and PR receptor status

		VIMENTIN STATUS		
		NEGATIVE	POSITIVE	Total
PR EXPRESSION	NEGATIVE	7	25	32
		21.9%	78.1%	100.0%
	POSITIVE	14	14	28
		50.0%	50.0%	100.0%
Total		21	39	60
		35.0%	65.0%	100.0%

Chi sq = 5.192 p value = 0.023*

Graph 4: Distribution Of Vimentin Expression According to PR Expression



There was no significant association found between HER2 Expression and Vimentin expression ($p>0.05$).

Table 9: Vimentin Expression and Her2 Expression

		VIMENTIN STATUS		
		NEGATIVE	POSITIVE	Total
HER 2 Expression	NEGATIVE	18	34	52
		34.6%	65.4%	100.0%
	POSITIVE	3	5	8
		37.5%	62.5%	100.0%
Total		21	39	60
		35.0%	65.0%	100.0%

Chi sq = 0.025 p value = 0.873

Out of 19 tumors with high Ki67 index, 16(84.2%) were Vimentin positive as compared to Vimentin positivity of 56% in low Ki67% tumors.

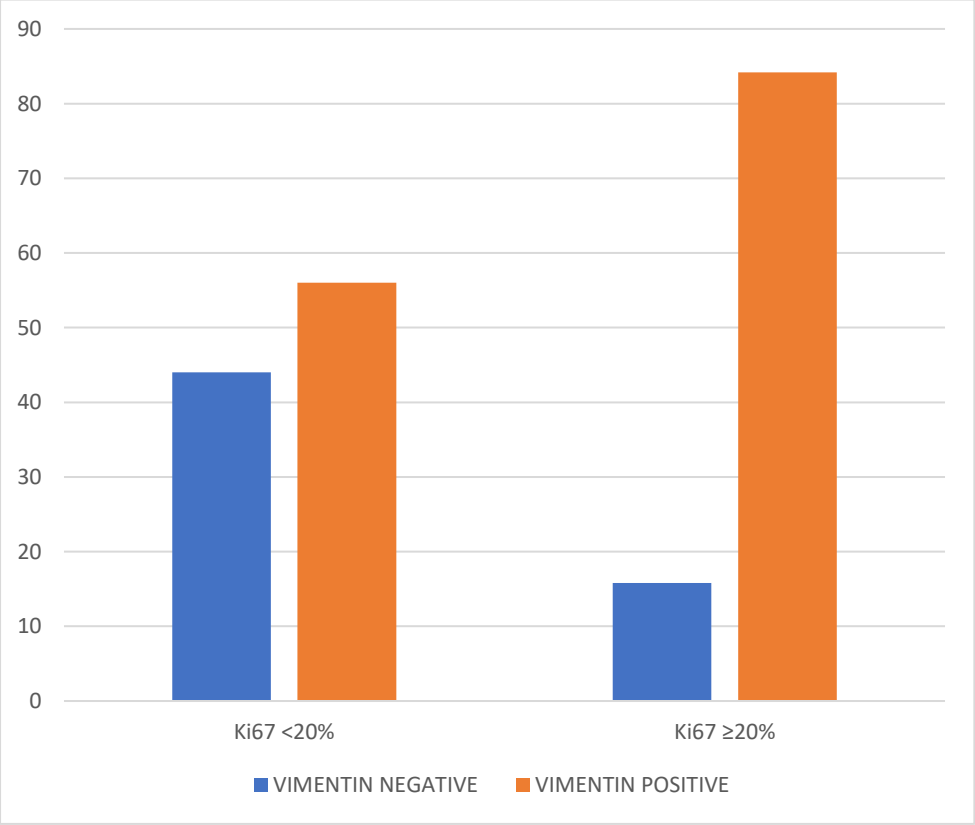
The association between Ki67 % and Vimentin expression was statistically significant($p<0.05$).

Table 10: Vimentin expression and Ki 67% (Proliferation index)

		VIMENTIN STATUS		
		NEGATIVE	POSITIVE	Total
Ki67%	<20%	18	23	41
		43.9%	56.1%	100.0%
	≥ 20%	3	16	19
		15.8%	84.2%	100.0%
Total		21	39	60
		35.0%	65.0%	100.0%

Chi sq = 4.510 p value = 0.034*

Graph 5: Distribution Of Vimentin Expression According to Ki67%



Out of 24 Triple Negative cancers, 20 showed Vimentin positivity (83.3%), 3 out of 4 (75%) cases of Her2 Enriched showed Vimentin positivity and 9 out of 13 (69.2%) cases of Luminal B cancers showed Vimentin positivity. There was statistically significant association between Molecular Subtype and Vimentin Status ($p<0.05$).

Table 11: Vimentin expression and Molecular subtype

		VIMENTIN STATUS		
		NEGATIVE	POSITIVE	Total
MOLECULAR SUBTYPE	HER2 ENRICHED	1	3	4
		25.0%	75.0%	100.0%
	LUMINAL A	12	7	19
		63.2%	36.8%	100.0%
	LUMINAL B	4	9	13
		30.8%	69.2%	100.0%
	TRIPLE NEGATIVE	4	20	24
		16.7%	83.3%	100.0%
Total		21	39	60
		35.0%	65.0%	100.0%

Chi sq = 10.446 p value = 0.015*

Graph 6: Distribution Of Vimentin Expression According to Molecular subtype

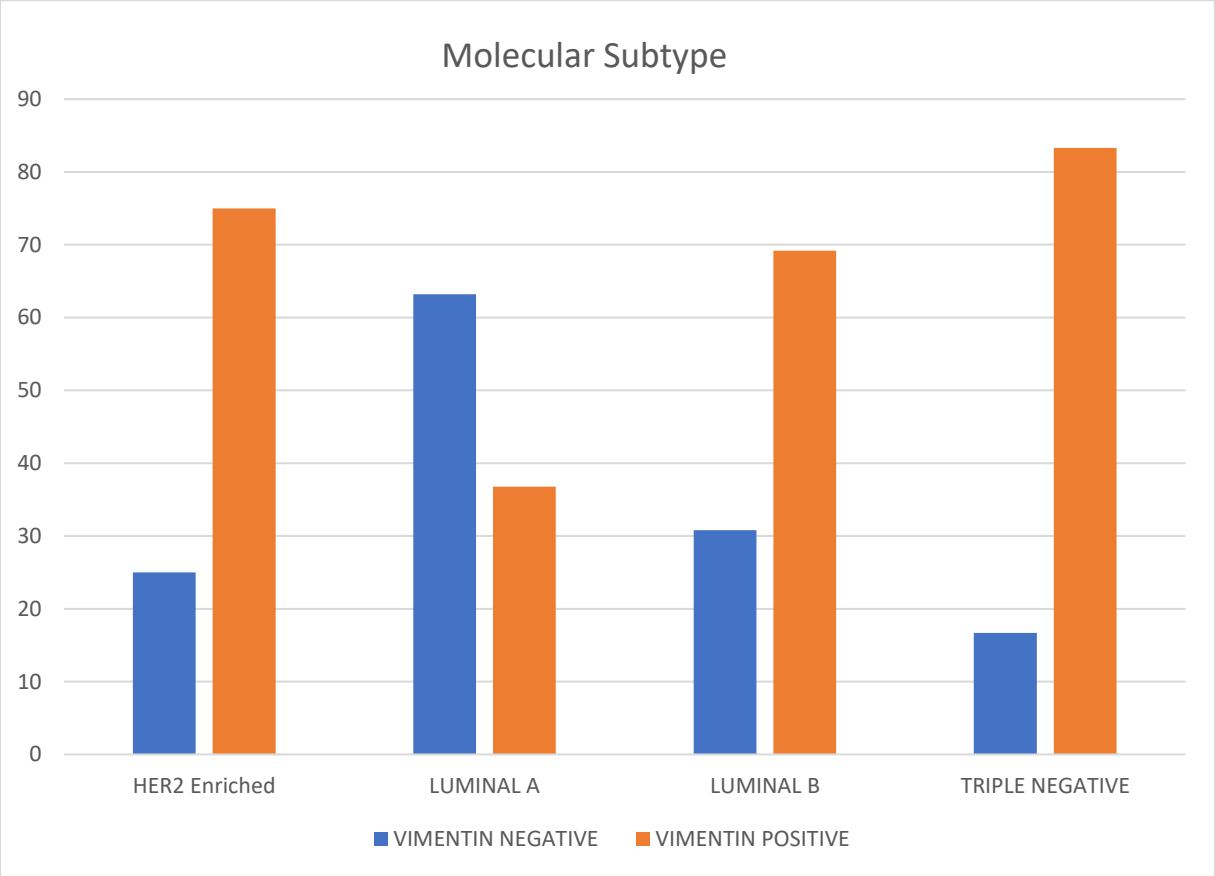
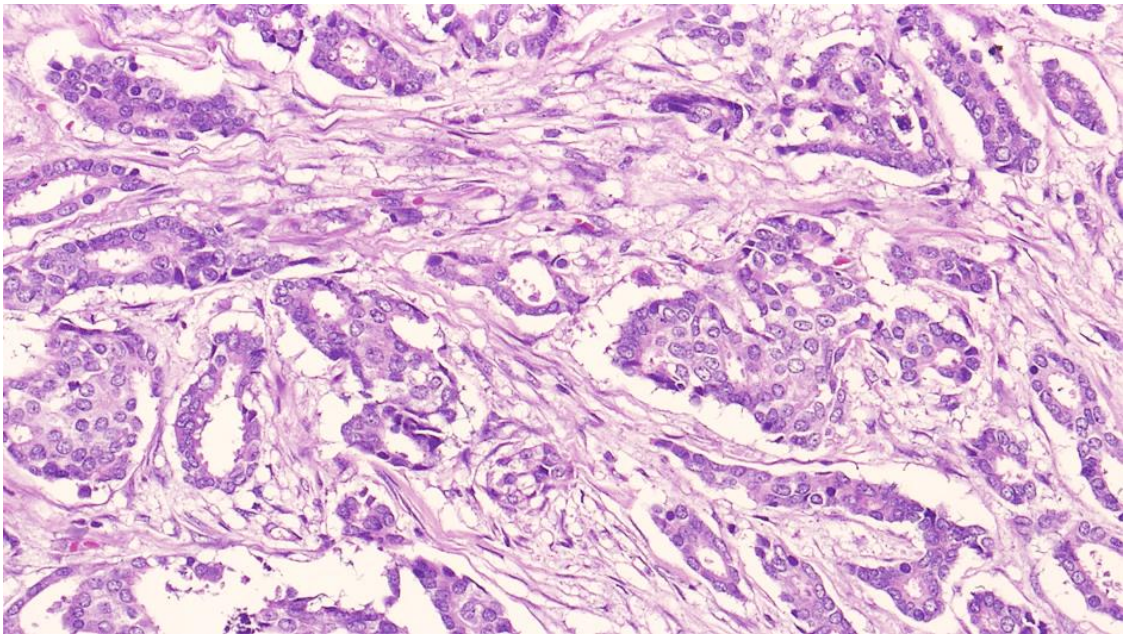
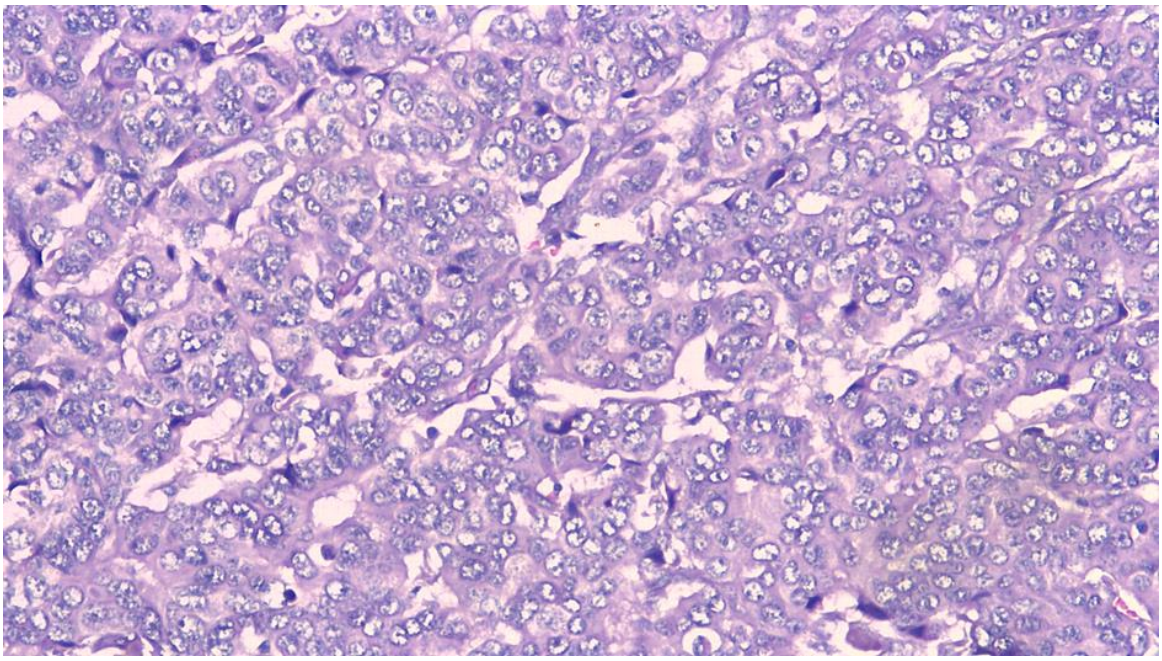


Figure 1



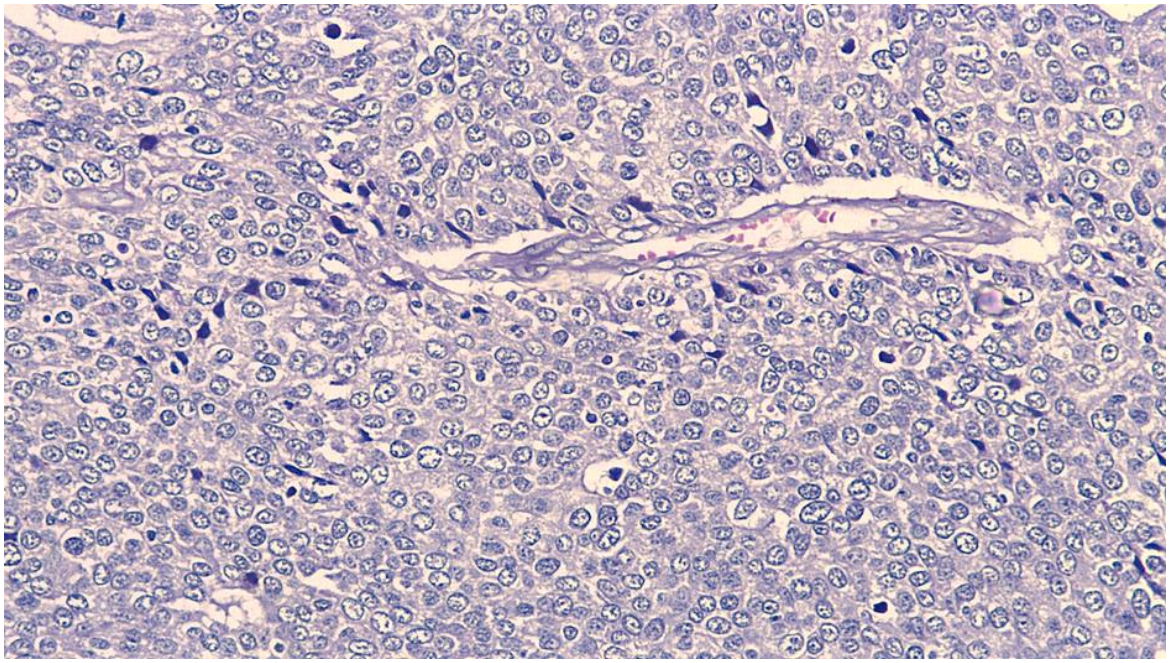
Invasive breast carcinoma- Grade I (H&E,200x)

Figure 2



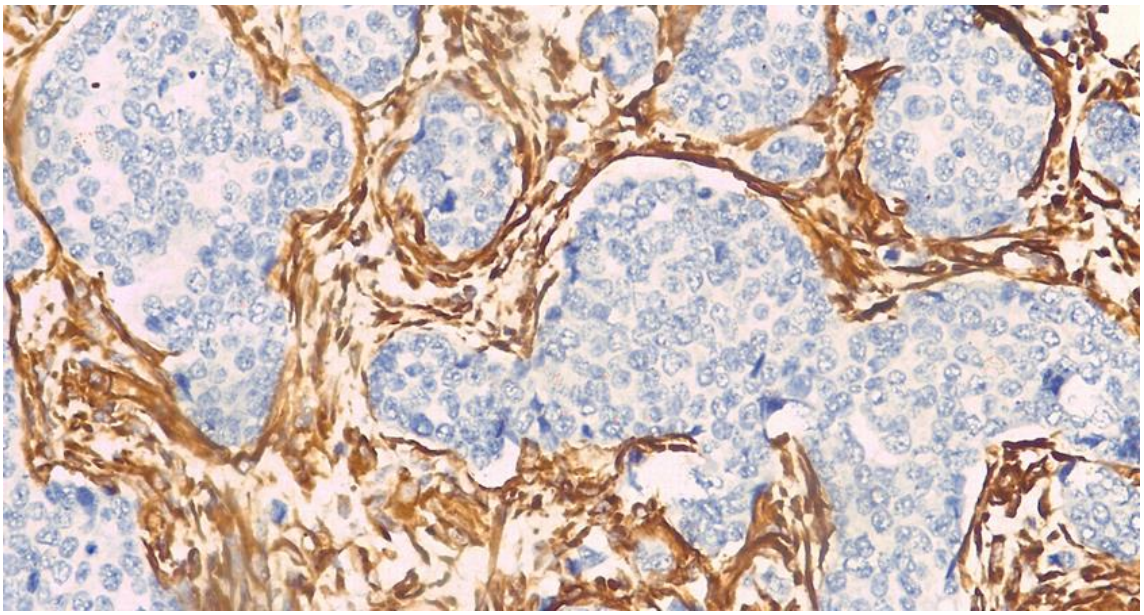
Invasive breast carcinoma- Grade II (H&E,200x)

Figure 3



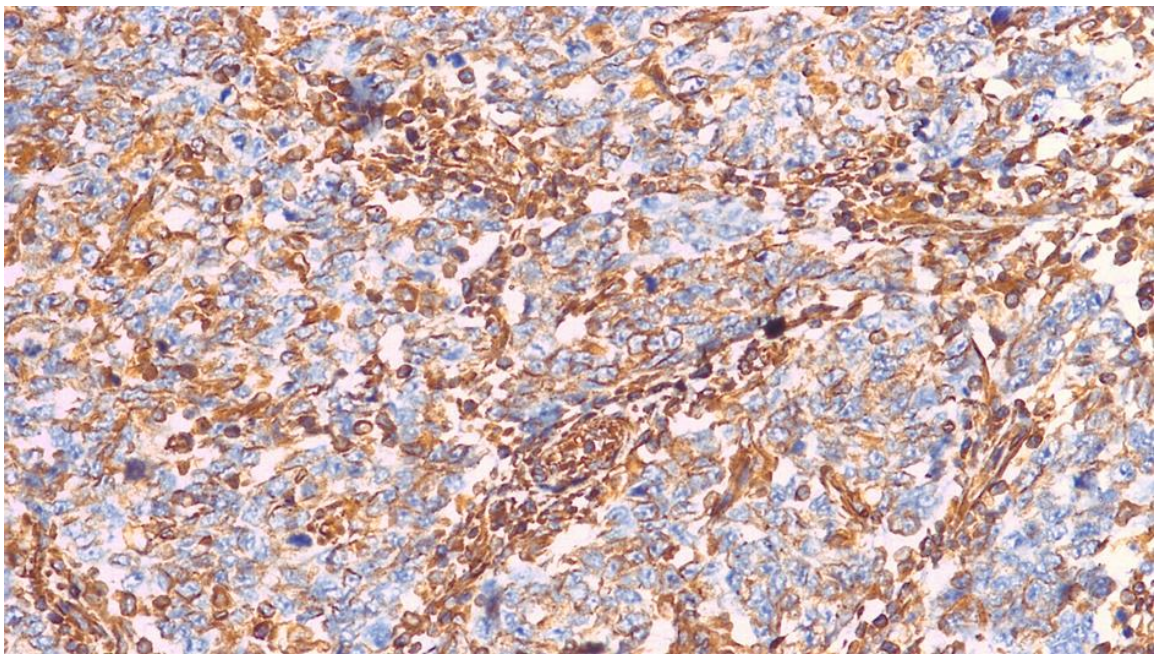
Invasive ductal carcinoma- Grade III (H&E,200x)

Figure 4



Vimentin Negativity in Tumor Cells, IHC 200x

Figure 5



Cytoplasmic Vimentin Positivity in Tumor Cells, IHC 200x

DISCUSSION

Breast cancer shows a spectrum of clinical behaviour. In spite of the advent of various biomarkers, its progression and chances of metastasis are difficult to be predicted with certainty. In around 30% patients diagnosed with primary breast carcinoma, recurrence is seen within 10 years[110,111,112]. The main reason causing shorter survival in these cases is metastatic disease and thus holds concern[113]. Many studies had been done for understanding cancer progression and metastasis of breast cancers. EMT is one such process that is known to be reactivated in tumor progression of certain carcinomas[114]. This finding is supported by multiple reports linking EMT with tumor progression and metastasis[115,116,117,118].

The level of expression; distribution and function of proteins changes during EMT, allowing them to be used as biomarkers of EMT. Some of the already known markers are transcription factors (SNAIL and TWIST), growth factors (TFG-β and Wnts), adhesion molecules (cadherins) and cytoskeletal molecules(vimentin)[119].

Mesenchymal proteins in the cytoskeleton like Vimentin have been found aberrantly in certain epithelial tumors and has been researched since long[120].

Routinely, Vimentin is used as a mesenchymal marker. Its aberrant expression not just 'marks' the EMT stamp on a tumor cell but also possibly directs changes in shape, motility and reduced adhesive property; all of which have pro-metastatic effect[121].

Its expression in breast carcinoma was first described by Raymond et al. in 1989[120]. Recent researches showed Vimentin is pivotal in the process of EMT in breast cancers, besides its downregulation causes reduction in genes linked to breast cancer metastasis, like receptor tyrosine kinase, Axl[122].

This study had been done to study Vimentin expression as a marker of epithelial to mesenchymal transition in invasive breast cancer cases. A significant Vimentin positivity was found in 39 cases (65%) of the total 60 cases in this study. In the literature, the expression of Vimentin in breast cancer cases varies from 7 to 91 %[123].

Most of the studies on expression of Vimentin in breast cancers have taken a cut off of 10% positivity in cytoplasm of tumor cells as significant. On the same lines, 10% positivity was taken as a cut off in this study[5]. Keeping a 10% cut off allows the non-tumorous cells in the tumor micro-environment like fibroblasts and lymphocytes which are Vimentin positive to be segregated[124].

In this study no significant correlation was seen between expression of Vimentin and tumour size. This is similar to the results found in other studies[5,125].

In this study, 36 of the 56 cases (64.3%) of IDC-NST showed Vimentin positivity. There was one case of mucinous carcinoma which showed Vimentin positivity and two cases of papillary carcinoma, which were also positive for Vimentin. There was one case of invasive lobular carcinoma which did not show positivity for Vimentin. However, the association of Vimentin expression with histologic subtype was not statistically significant. In a study by Domagala et al[126]., it was concluded that Vimentin expression was associated with ductal and not lobular differentiation. In their study, its expression was seen in medullary carcinoma, infiltrative ductal NOS but not in lobular carcinomas. Since our study included mainly IDC-NST and very few cases of other types of invasive breast carcinoma, further studies are required focusing on other histological types to draw conclusions.

Grading of cancer indicates its aggressive character and is assessed morphologically[40]. The present study included 8 cases of grade I, 39 cases of Grade II, and 13 cases of Grade III tumors. Vimentin expression was found to be associated significantly to high grade tumours ($p<0.05$)

indicative of epithelial to mesenchymal transition in tumour cells. All 13 grade III tumors showed significant Vimentin positivity (100%). This was in line with studies by Hemalatha et al., Korsching et al[5,106].

The results were in accordance with the EMT theory wherein cancer cells of low grade tend to retain properties of adhesion attributing to the presence of E-cadherin & similar proteins. When the grade of tumor cells increases, it undergoes transition into mesenchymal phenotype along with loss of adhesion and produces increased Vimentin[127].

Table 12: Comparison of Vimentin expression and tumor grade with other studies

S. No.	Study	Grade I (%)	Grade II (%)	Grade III (%)
1.	Raymond and Leong[120]	1/16(6.2)	2/23(8.7)	5/11(45.5)
2.	Sheshadri et al.[128]	4/38(10.5)	10/105(9.5)	26/86(30.2)
3.	Korsching et al.[106]	-	-	19/21(90.5)
4.	Hemalatha et al.[5]	0/22(0)	2/20(10)	7/8(87.5)
5.	Khillare et al.[123]	9/15(60)	19/19(100)	25/25(100)
6.	Present study	4/8(50)	22/39(56.4)	13/13(100)

Of the 39 cases showing Vimentin positivity in primary tumour of this study, 22(56.4%) cases had metastasis in lymph nodes. However, this was not statistically significant. This was in concordance with many other studies[5,128,129], except from a study by Vora et al.[130] which reported increased Vimentin expression in patients with lymph node metastases.

In spite of the fact that involvement of the axillary lymph nodes is one of the major prognostic factors, it is often seen in advanced disease state. This also implies that even in absence of positive lymph nodes, possibility of aggressive tumor behaviour cannot be dismissed.[131]

In the present study, significant association was found between positive Vimentin status and ER negativity ($p<0.05$). This finding was in concordance with the observation that tumors with increased Vimentin expression were frequently ER negative as reported in study by Khillare et al.[123]

There was significant association found between positive Vimentin expression and negative PR Expression ($p<0.05$).

This was in line with the study conducted by Seshadri et al. where they found that high Vimentin expression was associated with negative PR receptor expression.[128] However, the study by Yamashita et al and Khillare et al. reported no significant association of Vimentin expression and PR receptor expression.[132,123]

Interestingly, a hypothesis has been studied by multiple authors which states that loss of estrogen receptor contributes to epithelial to mesenchymal transition resulting in aggressive tumor behaviour and chances of metastasis[133].

Moreover, studies have shown that Luminal cancers tend to retain their epithelial character whereas non luminal cancers exhibit mesenchymal properties[134,135].

There was no significant association found between expression of HER 2 and Vimentin expression ($p>0.05$) in this study. This was in line with studies done by Yamashita *et al*[132,123].

There was statistically significant association found between high Ki67 index and positive Vimentin expression with 16 of the 19 (84.2%) tumors with high Ki67 index showing vimentin positivity ($p <0.05$). These observations correlated with other studies done by Raymond et al., Domagala et al., Thomas et al., Hemalatha et al[120,125,131,5].

In this study, positivity rate for Vimentin was higher in Triple negative breast cancers (83.3%) followed by Her2 Enriched (75%) and Luminal B (69.2%). Similarly, in another study the positive expression rate of Vimentin in triple-negative breast cancer was 81.2 %, higher than 66.7 % in the HER2 enriched type, and 33.3 % in the luminal B type[136].

TNBC tend to be biologically aggressive and patients do not avail advantage with hormonal medication due to absent hormone receptors. The prognosis is poor partly due to reduced

disease-free interval in adjuvant and neoadjuvant setting and an aggressive progression in metastatic scenario[132,137,138].

Tumor cells with significant Vimentin expression showed poorly differentiated morphology and indicated association with TNBC, highlighting the likelihood that Vimentin is a potential predictor of worse prognosis in breast cancer[139]. Additionally, increased Vimentin expression rates in TNBC cancers as compared to non-triple-negative cases, suggests that the extent of EMT is more in triple-negative breast cancer cells[140].

Strengths and Challenges:

A major strength of this study is its considerable sample size allowing for significant results.

Apart from the tumor grade, the study compared Vimentin expression with numerous clinicopathological variables such as tumour size and lymphnode status.

Additionally, comparison of Vimentin expression with hormone receptors and molecular subtypes is another strength of this study which permitted to evaluate its significance in triple negative breast cancer cases.

One of the main observations of the study, was the inherent Vimentin positivity in stromal fibroblasts and lymphocytes which made detecting Vimentin positivity in tumor cells amidst fibroblasts challenging. Even though utmost focus was given to differentiate them based on morphology, error in estimation of positive tumor cells is still possible[141].

Future prospects

Altogether, EMT is an important indicator of morphological and molecular changes seen in tumour invasion and metastases. In this study, high grade tumors were seen to express Vimentin significantly. The study showed significant correlation between Vimentin expression

with ER, PR negativity and high Ki67 % while no significant association was seen between Vimentin expression with tumour size and lymph node status.

Regarding Vimentin as a biomarker for EMT and its significance clinically, further research is mandated to study role of Vimentin in tumorigenesis and tumor progression. Hence, novel therapies might add on to the existing treatment modalities improving patient management. This has special importance in cases of poor prognostic groups such as Triple negative breast cancers.

CONCLUSION

In conclusion the study shows increased expression of Vimentin is associated with more aggressive nature of tumor including high tumor grade, lack of hormone receptors (ER, PR), high Ki67% and TNBC molecular subtype.

Consequently, on the basis of these findings, it can be suggested that Vimentin can be used as a biomarker for assessing tumor progression and prognosis. Its expression can be useful to stratify subgroup of patients with poor prognosis. Moreover, its expression in breast cancer can be a potential and novel target for breast anticancer therapy.

Further studies with larger sample size especially with follow up periods would be helpful in exploring the role of Vimentin in tumor progression and defining a precise cut off of Vimentin positivity.

SUMMARY

The present study was done to study the expression of Vimentin as a marker of epithelial to mesenchymal transition in invasive breast carcinoma cases. The results of the study showed significant Vimentin positivity in 39 cases (65%) of the total 60 breast carcinoma cases.

Apart from the tumor grade, the study compared Vimentin expression with clinicopathological parameters including tumor grading and immunohistochemical expression of hormone receptors (ER, PR), Her2, and Ki67.

The study showed Vimentin expression was significantly positive in high grade tumors indicating the occurrence of EMT with tumor progression. Significant association was found between positive Vimentin expression and poor prognostic factors such as high Ki67%, absence of hormone receptors (ER and PR) while no significant association was found between Vimentin expression with tumor size and lymph node status. Additionally, triple negative cancers also showed increased Vimentin positivity.

The findings of the study suggest that Vimentin can be used as a biomarker for assessing tumor progression and prognosis.

BIBLIOGRAPHY

1. World Health Organization. Breast cancer Available from: https://www.who.int/news-room/fact-sheets/detail/breast-cancer Accessed on 13.12.2023.
2. Arnold M, Morgan E, Rumgay H, Mafra A, Singh D, Laversanne M, et al. Current and future burden of breast cancer: Global statistics for 2020 and 2040. Breast. 2022 Dec; 66:15–23.
3. Scimeca M, Antonacci C, Colombo D, Bonfiglio R, Buonomo OC, Bonanno E. Emerging prognostic markers related to mesenchymal characteristics of poorly differentiated breast cancers. Tumor Biology. 2016 Apr; 37:5427-35.
4. Satelli A, Li S. Vimentin in cancer and its potential as a molecular target for cancer therapy. Cell Mol Life Sci. 2011 Sep;68(18):3033–46.
5. Hemalatha A, Suresh TN, Harendra Kumar ML. Expression of vimentin in breast carcinoma, its correlation with Ki67 and other histopathological parameters. Indian J Cancer. 2013;50(3):189–94.
6. Li JP, Yang YX, Liu QL, Zhou ZW, Pan ST, He ZX, et al. The pan-inhibitor of Aurora kinases danusertib induces apoptosis and autophagy and suppresses epithelial-to-mesenchymal transition in human breast cancer cells. Drug Des Devel Ther. 2015; 9:1027–62.
7. Singh I, Pal GP. Breast. In: Singh, Pal (eds), Human embryology.11th ed. India. Macmillan India Ltd; 2020.
8. Johnson D, Shah P, Wigley C, Collins P.Thorax. In:Standring S, Ellis H, Healy JC, Johnson D, Williams A, Collins P, editors.Gray's anatomy: The anatomical basis of clinical practice. 39th ed. London: Elsevier Churchill Livingstone; 2005.
9. Chaurasia BD. Thorax. Chaurasia's Human Anatomy. Vol. 1.6th ed. India. CBS Publishers & Distributers Pvt Ltd;2020.

10. Rosen PP Anatomic and physiologic morphology. In: Rosen PP (ed) Rosen's breast pathology, 4nd edn. Lippincott Williams & Wilkins Inc, Wolters Kluwer;2014.
11. Young, Barbara, Geraldine O'Dowd, Phillip Woodford. Wheater's Functional Histology. 6th ed., Churchill Livingstone, 2013.p.263-66.
12. Perry CS, Otero JC, Palmer JL, Gross AS. Risk factors for breast cancer in East Asian women relative to women in the West. Asia-pacific Journal of Clinical Oncology. 2009 Dec;5(4):219-31
13. Parkin DM, Boyd L, Walker LC. 16. The fraction of cancer attributable to lifestyle and environmental factors in the UK in 2010. British journal of cancer. 2011 Dec;105(2): S77-81.
14. Youn HJ, Han W. A review of the epidemiology of breast cancer in Asia: focus on risk factors. Asian Pacific journal of cancer prevention: APJCP. 2020 Apr;21(4):867.
15. Tsang JY, Gary MT. Molecular classification of breast cancer. Advances in anatomic pathology. 2020 Jan 1;27(1):27-35.
16. Alkabban FM, Ferguson T. Breast Cancer. [Updated 2022 Sep 26]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK482286/
17. Agarwal I, Blanco L. WHO classification. PathologyOutlines.com website. https://www.pathologyoutlines.com/topic/breastmalignantwhoclassification.html. Accessed May 13th, 2024
18. Longacre TA. Mills and Sternberg's Diagnostic Surgical Pathology. London: Wolters Kluwer Health; 2021.p.965-1007.
19. Ackerman LV, Goldblum JR, Rosai J. Rosai and Ackerman's surgical pathology. Elsevier; 2004.

20. Perou CM, Sørlie T, Eisen MB, Van De Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge Ø. Molecular portraits of human breast tumours. nature. 2000 Aug 17;406(6797):747-52.
21. Sørlie T, Perou CM, Tibshirani R, Aas T, Geisler S, Johnsen H, Hastie T, Eisen MB, Van De Rijn M, Jeffrey SS, Thorsen T. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proceedings of the National Academy of Sciences. 2001 Sep 11;98(19):10869-74.
22. Sørlie T, Tibshirani R, Parker J, Hastie T, Marron JS, Nobel A, Deng S, Johnsen H, Pesich R, Geisler S, Demeter J. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proceedings of the national academy of sciences. 2003 Jul 8;100(14):8418-23.
23. Harbeck, N., Penault-Llorca, F., Cortes, J. et al. Breast cancer. Nat Rev Dis Primers 5, 66 (2019).
24. Lukong KE. Understanding breast cancer–The long and winding road. BBA clinical. 2017 Jun 1; 7:64-77.
25. Makki J. Diversity of breast carcinoma: histological subtypes and clinical relevance. Clinical medicine insights: Pathology. 2015 Jan;8: CPath-S31563.
26. Goldhirsch A, Winer EP, Coates AS, Gelber RD, Piccart-Gebhart M, Thürlimann B, Senn HJ, Albain KS, André F, Bergh J, Bonnefoi H. Personalizing the treatment of women with early breast cancer: highlights of the St Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2013. Annals of oncology. 2013 Sep 1;24(9):2206-23.
27. Gao JJ, Swain SM. Luminal a breast cancer and molecular assays: a review. The oncologist. 2018 May 1;23(5):556-65.

28. Hashmi AA, Aijaz S, Khan SM, Mahboob R, Irfan M, Zafar NI, Nisar M, Siddiqui M, Edhi MM, Faridi N, Khan A. Prognostic parameters of luminal A and luminal B intrinsic breast cancer subtypes of Pakistani patients. World journal of surgical oncology. 2018 Dec; 16:1-6.
29. Feng Y, Spezia M, Huang S, Yuan C, Zeng Z, Zhang L, Ji X, Liu W, Huang B, Luo W, Liu B. Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. Genes & diseases. 2018 Jun 1;5(2):77-106.
30. Cheang MC, Chia SK, Voduc D, Gao D, Leung S, Snider J, Watson M, Davies S, Bernard PS, Parker JS, Perou CM. Ki67 index, HER2 status, and prognosis of patients with luminal B breast cancer. JNCI: Journal of the National Cancer Institute. 2009 May 20;101(10):736-50.
31. Tsoutsou PG, Vozenin MC, Durham AD, Bourhis J. How could breast cancer molecular features contribute to locoregional treatment decision making? Critical reviews in oncology/hematology. 2017 Feb 1; 110:43-8.
32. Cho N. Molecular subtypes and imaging phenotypes of breast cancer. Ultrasonography. 2016 Oct;35(4):281-8.
33. Cianfrocca M, Goldstein LJ. Prognostic and predictive factors in early-stage breast cancer. The oncologist. 2004 Nov 1;9(6):606-16.
34. Saez RA, McGuire WL, Clark GM. Prognostic factors in breast cancer. Semin Surg Oncol. 1989;5(2):102–10.
35. Rosen PP, Groshen S, Kinne D, Norton L. Factors influencing prognosis in node-negative breast carcinoma: analysis of 767 T1N0M0/T2N0M0 patients with long-term follow-up. Journal of clinical oncology. 1993 Nov;11(11):2090-100.

36. Elston CW, Ellis IO. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology. 1991 Nov;19(5):403-10.
37. Curigliano G, Burstein HJ, Winer EP, Gnant M, Dubsky P, Loibl S, Colleoni M, Regan MM, Piccart-Gebhart M, Senn HJ, Thürlimann B. De-escalating and escalating treatments for early-stage breast cancer: the St. Gallen International Expert Consensus Conference on the Primary Therapy of Early Breast Cancer 2017. Annals of Oncology. 2017 Aug 1;28(8):1700-12.
38. Rakha EA, El-Sayed ME, Lee AH, Elston CW, Grainge MJ, Hodi Z, Blamey RW, Ellis IO. Prognostic significance of Nottingham histologic grade in invasive breast carcinoma. Journal of clinical oncology. 2008 Jul 1;26(19):3153-8.
39. Rakha EA, Reis-Filho JS, Baehner F, Dabbs DJ, Decker T, Eusebi V, Fox SB, Ichihara S, Jacquemier J, Lakhani SR, Palacios J. Breast cancer prognostic classification in the molecular era: the role of histological grade. Breast cancer research. 2010 Aug; 12:1-2.
40. Rakha EA, Ahmed MA, Aleskandarany MA, Hodi Z, Lee AH, Pinder SE, Ellis IO. Diagnostic concordance of breast pathologists: lessons from the National health service breast screening programme pathology external quality assurance scheme. Histopathology. 2017 Mar;70(4):632-42.
41. Albain KS, Allred DC, Clark GM. Breast cancer outcome and predictors of outcome: are there age differentials? Journal of the National Cancer Institute. Monographs. 1994 Jan 1(16):35-42.
42. Nixon AJ, Neuberg D, Hayes DF, Gelman R, Connolly JL, Schnitt S, Abner A, Recht A, Vicini F, Harris JR. Relationship of patient age to pathologic features of the tumor and prognosis for patients with stage I or II breast cancer. Journal of clinical oncology. 1994 May;12(5):888-94.

43. Schechter AL, Stern DF, Vaidyanathan L, Decker SJ, Drebin JA, Greene MI, Weinberg RA. The neu oncogene: an erb-B-related gene encoding a 185,000-M r tumour antigen. Nature. 1984 Dec 6;312(5994):513-6.
44. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. science. 1987 Jan 9;235(4785):177-82.
45. Borg Å, Tandon AK, Sigurdsson H, Clark GM, Fernö M, Fuqua SA, Killander D, McGuire WL. HER-2/neu amplification predicts poor survival in node-positive breast cancer. Cancer research. 1990 Jul 15;50(14):4332-7.
46. Winstanley J, Cooke T, Murray GD, Platt-Higgins A, George WD, Holt S, Myskov M, Spedding A, Barraclough BR, Rudland P. The long-term prognostic significance of c-erbB-2 in primary breast cancer. British journal of cancer. 1991 Mar;63(3):447-50.
47. Paterson MC, Dietrich KD, Danyluk J, Paterson AH, Lees AW, Jamil N, Hanson J, Jenkins H, Krause BE, McBlain WA, Slamon DJ. Correlation between c-erb B-2 amplification and risk of recurrent disease in node-negative breast cancer. Cancer research. 1991 Jan 15;51(2):556-67.
48. Clark GM, McGuire WL. Follow-up study of HER-2/neu amplification in primary breast cancer. Cancer Res 1991; 51:944-948.
49. Hay ED. An overview of epithelio-mesenchymal transformation. Acta Anat (Basel). 1995;154(1):8–20.
50. Thiery JP, Sleeman JP. Complex networks orchestrate epithelial-mesenchymal transitions. Nat Rev Mol Cell Biol. 2006 Feb;7(2):131–42.
51. Shook D, Keller R. Mechanisms, mechanics and function of epithelial-mesenchymal transitions in early development. Mech Dev. 2003 Nov;120(11):1351–83.

52. Iwatsuki M, Mimori K, Yokobori T, Ishi H, Beppu T, Nakamori S, et al. Epithelial-mesenchymal transition in cancer development and its clinical significance. Cancer Sci. 2010 Feb;101(2):293–9.
53. Mackenzie NC, Raz E. Found in translation: A new player in EMT. Developmental cell. 2006 Oct 1;11(4):434-6.
54. Bates RC, Mercurio AM. Tumor necrosis factor-alpha stimulates the epithelial-to-mesenchymal transition of human colonic organoids. Mol Biol Cell. 2003 May;14(5):1790–800.
55. Kalluri R., Weinberg R.A. The basics of epithelial-mesenchymal transition. J Clin Invest. 2009;119(6):1420–1428.
56. Bhatt S, Diaz R, Trainor PA. Signals and switches in Mammalian neural crest cell differentiation. Cold Spring Harb Perspect Biol. 2013 Feb 1;5(2):a008326.
57. Thiery JP, Acloque H, Huang RYJ, Nieto MA. Epithelial-mesenchymal transitions in development and disease. Cell. 2009 Nov 25;139(5):871–90.
58. Yang J, Weinberg RA. Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. Dev Cell. 2008 Jun;14(6):818–29.
59. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011 Mar 4;144(5):646–74.
60. Micalizzi DS, Farabaugh SM, Ford HL. Epithelial-mesenchymal transition in cancer: parallels between normal development and tumor progression. J Mammary Gland Biol Neoplasia. 2010 Jun;15(2):117–34.
61. Nieto MA. The snail superfamily of zinc-finger transcription factors. Nat Rev Mol Cell Biol. 2002 Mar;3(3):155–66.

62. Trimboli AJ, Fukino K, de Bruin A, Wei G, Shen L, Tanner SM, et al. Direct evidence for epithelial-mesenchymal transitions in breast cancer. Cancer Res. 2008 Feb 1;68(3):937–45.

63. Tsai JH, Donaher JL, Murphy DA, Chau S, Yang J. Spatiotemporal regulation of epithelial-mesenchymal transition is essential for squamous cell carcinoma metastasis. Cancer Cell. 2012 Dec 11;22(6):725–36.

64. Er O, Frye DK, Kau SWC, Broglio K, Valero V, Hortobagyi GN, et al. Clinical course of breast cancer patients with metastases limited to the liver treated with chemotherapy. Cancer J. 2008;14(1):62–8.

65. Greenberg PA, Hortobagyi GN, Smith TL, Ziegler LD, Frye DK, Buzdar AU. Long-term follow-up of patients with complete remission following combination chemotherapy for metastatic breast cancer. J Clin Oncol. 1996 Aug;14(8):2197–205.

66. Ries LG, Young JL, Keel GE, Eisner MP, Lin YD, Horner MJ. SEER survival monograph: cancer survival among adults: US SEER program, 1988-2001, patient and tumor characteristics. National Cancer Institute, SEER Program, NIH Pub. 2007;7(6215):193-202.

67. Onder TT, Gupta PB, Mani SA, Yang J, Lander ES, Weinberg RA. Loss of E-cadherin promotes metastasis via multiple downstream transcriptional pathways. Cancer Res. 2008 May 15;68(10):3645–54.

68. Oka H, Shiozaki H, Kobayashi K, Inoue M, Tahara H, Kobayashi T, et al. Expression of E-cadherin cell adhesion molecules in human breast cancer tissues and its relationship to metastasis. Cancer Res. 1993 Apr 1;53(7):1696–701.

69. De Leeuw WJ, Berx G, Vos CB, Peterse JL, Van de Vijver MJ, Litvinov S, et al. Simultaneous loss of E-cadherin and catenins in invasive lobular breast cancer and lobular carcinoma in situ. J Pathol. 1997 Dec;183(4):404–11.

70. Eger A, Aigner K, Sonderegger S, Dampier B, Oehler S, Schreiber M, et al. DeltaEF1 is a transcriptional repressor of E-cadherin and regulates epithelial plasticity in breast cancer cells. Oncogene. 2005 Mar 31;24(14):2375–85.
71. Peinado H, Ballestar E, Esteller M, Cano A. Snail mediates E-cadherin repression by the recruitment of the Sin3A/histone deacetylase 1 (HDAC1)/HDAC2 complex. Mol Cell Biol. 2004 Jan;24(1):306–19.
72. Vesuna F, van Diest P, Chen JH, Raman V. Twist is a transcriptional repressor of E-cadherin gene expression in breast cancer. Biochem Biophys Res Commun. 2008 Mar 7;367(2):235–41.
73. Frisch SM, Francis H. Disruption of epithelial cell-matrix interactions induces apoptosis. J Cell Biol. 1994 Feb;124(4):619–26.
74. Frisch SM, Ruoslahti E. Integrins and anoikis. Curr Opin Cell Biol. 1997 Oct;9(5):701–6.
75. Leong KG, Niessen K, Kulic I, Raouf A, Eaves C, Pollet I, et al. Jagged1-mediated Notch activation induces epithelial-to-mesenchymal transition through Slug-induced repression of E-cadherin. J Exp Med. 2007 Nov 26;204(12):2935–48.
76. Eckert LB, Repasky GA, Ulkü AS, McFall A, Zhou H, Sartor CI, et al. Involvement of Ras activation in human breast cancer cell signaling, invasion, and anoikis. Cancer Res. 2004 Jul 1;64(13):4585–92.
77. Rosen K, Rak J, Leung T, Dean NM, Kerbel RS, Filmus J. Activated Ras prevents downregulation of Bcl-X(L) triggered by detachment from the extracellular matrix. A mechanism of Ras-induced resistance to anoikis in intestinal epithelial cells. J Cell Biol. 2000 Apr 17;149(2):447–56.

78. Mironchik Y, Winnard PT, Vesuna F, Kato Y, Wildes F, Pathak AP, et al. Twist overexpression induces in vivo angiogenesis and correlates with chromosomal instability in breast cancer. Cancer Res. 2005 Dec 1;65(23):10801–9.

79. Yang J, Mani SA, Donaher JL, Ramaswamy S, Itzykson RA, Come C, et al. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell. 2004 Jun 25;117(7):927–39.

80. Ackland ML, Newgreen DF, Fridman M, Waltham MC, Arvanitis A, Minichiello J, et al. Epidermal growth factor-induced epithelio-mesenchymal transition in human breast carcinoma cells. Lab Invest. 2003 Mar;83(3):435–48.

81. Nieman MT, Prudoff RS, Johnson KR, Wheelock MJ. N-cadherin promotes motility in human breast cancer cells regardless of their E-cadherin expression. J Cell Biol. 1999 Nov 1;147(3):631–44.

82. Tripathi MK, Misra S, Chaudhuri G. Negative regulation of the expressions of cytokeratins 8 and 19 by SLUG repressor protein in human breast cells. Biochem Biophys Res Commun. 2005 Apr;329(2):508–15.

83. Bindels S, Mestdagt M, Vandewalle C, Jacobs N, Volders L, Noël A, et al. Regulation of vimentin by SIP1 in human epithelial breast tumor cells. Oncogene. 2006 Aug 17;25(36):4975–85.

84. Moll R, Divo M, Langbein L. The human keratins: biology and pathology. Histochem Cell Biol. 2008 Jun;129(6):705–33.

85. Schwarz SM, Gallicano GI, McGaughey RW, Capco DG. A role for intermediate filaments in the establishment of the primitive epithelia during mammalian embryogenesis. Mech Dev. 1995 Nov;53(3):305–21.

86. Ivaska J, Vuoriluoto K, Huovinen T, Izawa I, Inagaki M, Parker PJ. PKCepsilon-mediated phosphorylation of vimentin controls integrin recycling and motility. EMBO J. 2005 Nov 16;24(22):3834–45.

87. Eckes B, Dogic D, Colucci-Guyon E, Wang N, Maniotis A, Ingber D, et al. Impaired mechanical stability, migration and contractile capacity in vimentin-deficient fibroblasts. J Cell Sci. 1998 Jul 1;111(13):1897-907.

88. Chabottaux V, Ricaud S, Host L, Blacher S, Paye A, Thiry M, et al. Membrane-type 4 matrix metalloproteinase (MT4-MMP) induces lung metastasis by alteration of primary breast tumour vascular architecture. J Cell Mol Med. 2009 Sep 29;13(9b):4002–13.

89. Joseph MJ, Dangi-Garimella S, Shields MA, Diamond ME, Sun L, Koblinski JE, et al. Slug is a downstream mediator of transforming growth factor-β1-induced matrix metalloproteinase-9 expression and invasion of oral cancer cells. J Cell Biochem. 2009 Oct 15;108(3):726–36.

90. Yokoyama K, Kamata N, Fujimoto R, Tsutsumi S, Tomonari M, Taki M, et al. Increased invasion and matrix metalloproteinase-2 expression by Snail-induced mesenchymal transition in squamous cell carcinomas. Int J Oncol. 2003 Apr;22(4):891–8.

91. Miyoshi A, Kitajima Y, Sumi K, Sato K, Hagiwara A, Koga Y, et al. Snail and SIP1 increase cancer invasion by upregulating MMP family in hepatocellular carcinoma cells. Br J Cancer. 2004 Mar 22;90(6):1265–73.

92. Satelli A, Li S. Vimentin in cancer and its potential as a molecular target for cancer therapy. Cell Mol Life Sci. 2011 Sep;68(18):3033–46.

93. Batlle E, Sancho E, Francí C, Domínguez D, Monfar M, Baulida J, et al. The transcription factor snail is a repressor of E-cadherin gene expression in epithelial tumour cells. Nat Cell Biol. 2000 Feb;2(2):84–9.

94. Cano A, Pérez-Moreno MA, Rodrigo I, Locascio A, Blanco MJ, del Barrio MG, et al. The transcription factor snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. Nat Cell Biol. 2000 Feb;2(2):76–83.

95. Comijn J, Berx G, Vermassen P, Verschueren K, van Grunsven L, Bruyneel E, et al. The two-handed E box binding zinc finger protein SIP1 downregulates E-cadherin and induces invasion. Mol Cell. 2001 Jun;7(6):1267–78.

96. Luangdilok S, Box C, Patterson L, Court W, Harrington K, Pitkin L, et al. Syk tyrosine kinase is linked to cell motility and progression in squamous cell carcinomas of the head and neck. Cancer Res. 2007 Aug 15;67(16):7907–16.

97. Duprey P, Paulin D. What can be learned from intermediate filament gene regulation in the mouse embryo. Int J Dev Biol. 1995 Jun;39(3):443–57.

98. Hendrix MJ, Seftor EA, Seftor RE, Trevor KT. Experimental co-expression of vimentin and keratin intermediate filaments in human breast cancer cells results in phenotypic interconversion and increased invasive behavior. Am J Pathol. 1997 Feb;150(2):483–95.

99. Dandachi N, Hauser-Kronberger C, Moré E, Wiesener B, Hacker GW, Dietze O, et al. Co-expression of tenascin-C and vimentin in human breast cancer cells indicates phenotypic transdifferentiation during tumour progression: correlation with histopathological parameters, hormone receptors, and oncoproteins. J Pathol. 2001 Feb;193(2):181–9.

100. Steinert PM, Roop DR. Molecular and cellular biology of intermediate filaments. Annu Rev Biochem. 1988; 57:593–625.

101.Ramaekers FC, Haag D, Kant A, Moesker O, Jap PH, Vooijs GP. Coexpression of keratin- and vimentin-type intermediate filaments in human metastatic carcinoma cells. Proc Natl Acad Sci U S A. 1983 May;80(9):2618–22.

102.Gilles C, Polette M, Piette J, Delvigne AC, Thompson EW, Foidart JM, et al. Vimentin expression in cervical carcinomas: association with invasive and migratory potential. J Pathol. 1996 Oct;180(2):175–80.

103.Behrens J, von Kries JP, Kühl M, Bruhn L, Wedlich D, Grosschedl R, et al. Functional interaction of beta-catenin with the transcription factor LEF-1. Nature. 1996 Aug 15;382(6592):638–42.

104.Ackland ML, Newgreen DF, Fridman M, Waltham MC, Arvanitis A, Minichiello J, et al. Epidermal Growth Factor-Induced Epithelio-Mesenchymal Transition in Human Breast Carcinoma Cells. Laboratory Investigation. 2003 Mar;83(3):435–48.

105.Gilles C, Polette M, Mestdagt M, Nawrocki-Raby B, Ruggeri P, Birembaut P, et al. Transactivation of vimentin by beta-catenin in human breast cancer cells. Cancer Res. 2003 May 15;63(10):2658–64.

106.Korsching E, Packeisen J, Liedtke C, Hungermann D, Wülfing P, van Diest PJ, et al. The origin of vimentin expression in invasive breast cancer: epithelial-mesenchymal transition, myoepithelial histogenesis or histogenesis from progenitor cells with bilinear differentiation potential? J Pathol. 2005 Aug;206(4):451–7.

107.Christiansen JJ, Rajasekaran AK. Reassessing epithelial to mesenchymal transition as a prerequisite for carcinoma invasion and metastasis. Cancer Res 2006; 66: 8319–26.

108.Garber K. Epithelial-to-mesenchymal transition is important to metastasis, but questions remain. J Natl Cancer Inst 2008; 100: 232–39.

109.Zhou J, Tao D, Xu Q, Gao Z, Tang D. Expression of E-cadherin and vimentin in oral squamous cell carcinoma. Int J Clin Exp Pathol. 2015;8(3):3150-3154.

110.Mouridsen HT, Bjerre KD, Christiansen P, Jensen MB, Møller S. Improvement of prognosis in breast cancer in Denmark 1977–2006, based on the nationwide reporting to the DBCG Registry. Acta Oncol. 2008 Jan 1;47(4):525-36.

111.Pan H, Gray R, Braybrooke J, Davies C, Taylor C, McGale P, Peto R, Pritchard KI, Bergh J, Dowsett M et al (2017) 20-year risks of breast-cancer recurrence after stopping endocrine therapy at 5 years. N Engl J Med 377(19):1836–1846

112.Early Breast Cancer Trialists' Collaborative Group. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. The Lancet. 2005 May 14;365(9472):1687-717.

113.Lambert AW, Pattabiraman DR, Weinberg RA (2017) Emerging biological principles of metastasis. Cell 168(4):670–691

114.Jørgensen, C.L.T., Forsare, C., Bendahl, PO. et al. Expression of epithelial-mesenchymal transition-related markers and phenotypes during breast cancer progression. Breast Cancer Res Treat 181, 369–381 (2020).

115.Micalizzi DS, Ford HL. Epithelial–mesenchymal transition in development and cancer. Future oncol. 2009 Oct;5(8):1129-43.

116.Wang Y, Zhou BP. Epithelial-mesenchymal transition—a hallmark of breast cancer metastasis. Cancer hallm. 2013 Mar 1;1(1):38-49.

117.Berrondo C, Flax J, Kucherov V, Siebert A, Osinski T, Rosenberg A, Fucile C, Richheimer S, Beckham CJ. Expression of the long non-coding RNA HOTAIR correlates with disease progression in bladder cancer and is contained in bladder cancer patient urinary exosomes. PloS one. 2016 Jan 22;11(1): e0147236.

118.De Craene B1, Berx G Regulatory networks defining EMT during cancer initiation and progression. Nat Rev Cancer 2013;13: 97-110.

119.Thompson EW, Newgreen DF, Tarin D Carcinoma invasion and metastasis: a role for epithelial-mesenchymal transition? Cancer Res 2005;65: 5991-5995.

120.Raymond WA, Leong AS Vimentin–a new prognostic parameter in breast carcinoma? J Pathol 1989; 158:107–114

121.Roussos ET, Keckesova Z, Haley JD, Epstein DM, Weinberg RA, Condeelis JS AACR special conference on epithelial-mesenchymal transition and cancer progression and treatment. Cancer Res 2010; 70(19):7360–7364.

122.Gjerdrum C, Tiron C, Hoiby T, Stefansson I, Haugen H, Sandal T, Collett K, Li S, McCormack E, Gjertsen BT, Micklem DR, Akslen LA, Glackin C, Lorens JB Axl is an essential epithelial-to-mesenchymal transition-induced regulator of breast cancer metastasis and patient survival. Proc Natl Acad Sci USA 2010; 107(3):1124–1129.

123.Khillare CD, Sinai Khandeparkar SG, Joshi AR, Kulkarni MM, Gogate BP, Battin S. Immunohistochemical expression of vimentin in invasive breast carcinoma and its correlation with clinicopathological parameters. Niger Med J 2019; 60:17-21.

124.Niveditha SR, Bajaj P. Vimentin expression in breast carcinomas. Indian J Pathol Microbiol 2003; 46:579-84.

125.Domagala W, Lasota J, Bartkowiak J, Weber K, Osborn M. Vimentin is preferentially expressed in human breast carcinomas with low estrogen receptor and high Ki-67 growth fraction. Am J Pathol. 1990 Jan;136(1):219.

126.Domagala W, Wozniak L, Lasota J, Weber K, Osbornt M. Vimentin Is Preferentially Expressed in High-grade Ductal and Medullary, But Not in Lobular Breast Carcinomas. Am J Pathol 1990;137: 107-14.

127.Tomaskovic-Crook E, Thompson EW, Thiery JP. Epithelial to mesenchymal transition and breast cancer. Breast cancer res. 2009 Dec; 11:1-0.

128.Sheshadri R, Raymond WA, Leong AS, Horsfall DJ, McCaul K. Vimentin expression is not associated with poor prognosis in breast cancer. Int J Cancer 1996; 67:353-6.

129.Bhuvaneshwari A K, Vani D. Expression of vimentin in breast carcinoma and its correlation with histopathological parameters. Indian J Pathol Oncol 2021;8(2):207-212.

130.Vora HH, Patel NA, Rajvik KN, Mehta SV, Brahmbhatt BV, Shah MJ, Shukla SN, Shah PM. Cytokeratin and vimentin expression in breast cancer. Int J Biol Mark. 2009 Jan;24(1):38-46.

131.Thomas PA, Kirschmann DA, Cerhan JR, Folberg R, Seftor EA, Sellers TA, et al. Association between Keratin and Vimentin Expression, Malignant Phenotype, and Survival in Postmenopausal Breast Cancer Patients. Clin Cancer Res 1999; 5:2698-703.

132.Yamashita N, Tokunaga E, Iimori M, Inoue Y, Tanaka K, Kitao H, Saeki H, Oki E, Maehara Y. Epithelial paradox: clinical significance of coexpression of E-cadherin and vimentin with regard to invasion and metastasis of breast cancer. Clin breast cancer. 2018 Oct 1;18(5): e1003-9.

133.Al Saleh S, Al Mulla F, Luqmani YA. Estrogen receptor silencing induces epithelial to mesenchymal transition in human breast cancer cells. PloS one. 2011 Jun 21;6(6): e20610.

134.Prat, A.; Perou, C.M. Desconstructing the molecular portraits of breast cancer. Mol. Oncol. 2011, 5, 5–23.

135.Pomp, V.; Leo, C.; Mauracher, A.; Korol, D.; Guo, W.; Varga, Z. Differential expression of epithelial-mesenchymal transition and stem cell markers in intrinsic subtypes of breast cancer. Breast Cancer Res. Treat. 2015, 154, 45–55.

136.Chen Z, Fang Z, Ma J. Regulatory mechanisms and clinical significance of vimentin in breast cancer. Biomedicine & Pharmacotherapy. 2021 Jan 1; 133:111068

137.Bae YK, Choi JE, Kang SH, Lee SJ (2015) Epithelial-mesen chymal transition phenotype is associated with clinicopatho logical factors that indicate aggressive biological behaviour and poor clinical outcomes in invasive breast cancer. J Breast Cancer 18(3):256–263

138.Wahba HA, El-Hadaad HA. Current approaches in treatment of triple-negative breast cancer. Cancer Biol Med. 2015; 12:106–16.

139.Javir G, Joshi K, Khedkar V, Rojatkar S. 6 α-Hydroxy-4 [14], 10 [15]-guainadien-8β, 12-olide induced cell cycle arrest via modulation of EMT and Wnt/β-catenin pathway in HER-2 positive breast cancer cells. J. Steroid Biochem. Mol. Biol. 2020 Mar 1; 197:105514.

140.Su Y, Hopfinger NR, Nguyen TD, Pogash TJ, Santucci-Pereira J, Russo J. Epigenetic reprogramming of epithelial mesenchymal transition in triple negative breast cancer cells with DNA methyltransferase and histone deacetylase inhibitors. J. Exp. Clin. Cancer Res.2018 Dec; 37:1-8.

141.Kokkinos MI, Wafai R, Wong MK, Newgreen DF, Thompson EW, Waltham M (2007) Vimentin and epithelial-mesenchymal transition in human breast cancer-observations in vitro and in vivo. Cells Tissues Organs 185:191–203

142.Shah AN, Summy JM, Zhang J, Park SI, Parikh NU, Gallick GE. Development and characterization of gemcitabine-resistant pancreatic tumor cells. Ann Surg Oncol. 2007 Dec; 14:3629-37.

143.Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N. Bevacizumab plus irinotecan,

fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med. 2004 Jun 3;350(23):2335-42.

144.Shimono Y, Zabala M, Cho RW, Lobo N, Dalerba P, Qian D et al (2009) Downregulation of miRNA-200c links breast cancer stem cells with normal stem cells. Cell 138:592–603

145.Sun M, Estrov Z, Ji Y, Coombes KR, Harris DH, Kurzrock R (2008) Curcumin (diferuloylmethane) alters the expression profiles of microRNAs in human pancreatic cancer cells. Mol Cancer Ther 7:464–473

146.Zeisberg M, Neilson EG. Biomarkers for epithelial-mesenchymal transitions. The Journal of clinical investigation. 2009 Jun 1;119(6):1429-37.

147.Wu S, Liu S, Liu Z, Huang J, Pu X, Li J, Yang D, Deng H, Yang N, Xu J. Classification of circulating tumor cells by epithelial-mesenchymal transition markers. PloS one. 2015 Apr 24;10(4):e0123976.

148.Tachtsidis A, McInnes LM, Jacobsen N, Thompson EW, Saunders CM. Minimal residual disease in breast cancer: an overview of circulating and disseminated tumour cells. Clin Exp Metastasis. 2016 Aug; 33:521-50.

149.Desai K, Aiyappa R, Prabhu JS, Nair MG, Lawrence PV, Korlimarla A, Ce A, Alexander A, Kaluve RS, Manjunath S, Correa M. HR+ HER2− breast cancers with growth factor receptor–mediated EMT have a poor prognosis and lapatinib downregulates EMT in MCF-7 cells. Tumor Biol. 2017 Mar;39(3):1010428317695028.

ANNEXURE I- INFORMED CONSENT FORM

"EXPRESSION OF VIMENTIN AS A MARKER OF EPITHELIAL-MESENCHYMAL TRANSITION IN INVASIVE BREAST CARCINOMA"

Objective: The purpose of this study is to evaluate the expression of vimentin in breast carcinomas and assess its role in pathogenesis and invasiveness of breast carcinoma.

Introduction: Breast carcinoma is the most common and deadly malignancy of women globally and the majority of deaths are caused by invasion and metastasis. This study may help to evaluate the potential role of the epithelial–mesenchymal transition in tumor invasiveness, hence leading to development of potential predictive and prognostic biomarkers as well as novel treatment interventions.

Explanation of procedure: During this study, you will be asked questions regarding history and background and you are supposed to answer to the best of your knowledge. If you agree to enroll yourself in this study, you will be interviewed regarding your present, past and family history and your clinical manifestations.

Withdrawal from participation in the study: Participation in this study is voluntary. You will be free to decide whether to participate in this study or continue participation once enrolled. In case you decide to withdraw your participation, you are free to do so. However, please convey the decision to the principal investigator.

Possible benefits from participating in the study: You will/will not have nor get any benefits by participating in this study. The data gathered will help the population at large.

Possible risks from participating in the study: There are no risks involved in participating in this study.

Privacy and confidentiality: The information collected from you will be coded, to prevent any person from identifying you. Your identity will never be revealed. The data collected

from you will be kept confidential and only processed or aggregated data will be used for publication.

Financial incentives: You will not receive any payment for participating in this study.

Authorization for publication of aggregated data: Results obtained after processing of the aggregated data will be published for scientific purposes and or presented to scientific groups. However, your identity will never be revealed.

Questions: If you have any question or complaints with regard to your right as study participant you may contact Dr Harsha Hegde, Chairperson, Ethical committee of JNMC, 0831-2473777 Extension 4052.

Legal rights: By signing this consent form, we are not waving any of your legal rights.

ANNEXURE II- PROFORMA

NAME:

AGE:

BRIEF CLINICAL HISTORY:

SURGICAL PROCEDURE DONE:

DATE OF COLLECTION:

PAST HISTORY:

MENOPAUSAL STATUS:

H/O MEDICAL ILLNESS:

EXAMINATION:

- *SIDE*
- *SIZE*
- *SKIN CHANGES*
- *AXILLARY LYMPH NODE INVOLVEMENT*
- *NIPPLE, AREOLA*
- *OTHERS*

- **GROSS FINDINGS**

    - Size-
    - Quadrant involved-
    - Margins-
    - Nipple and areola-
    - Skin involvement-

- **HAEMATOXYLIN AND EOSIN FINDINGS**

    - Histological type-
    - Grade-
    - Necrosis-
    - Desmoplastic response-
    - In-situ component-
    - Lympho-vascular involvement status-
    - Lymph node metastasis-
    - Perineural invasion-
    - Any other-

IMMUNOHISTOCHEMISTRY:

- Vimentin
- ER
- PR
- HER2
- Ki67

CONSENT STATEMENT

I am making a voluntary decision to participate in the study "EXPRESSION OF VIMENTIN AS A MARKER OF EPITHELIAL-MESENCHYMAL TRANSITION IN INVASIVE BREAST CARCINOMA". My signature below indicates that I have decided to participate and I have read the information provided above or the information provided above has been read to me in the language that I understand best. I was given the opportunity to ask questions and that they have been answered to my satisfaction.

Name of the participant:

Signature or left thumb impression of the participant:

Name of the witness:

Signature or left thumb impression of the witness:

Name of the investigator:

Signature of the investigator:

ANNEXURE -III MASTERCHART

SERIAL NUMBER	AGE	MENOPAUSAL STATUS	TUMOR SIZE(p)	HISTOLOGICAL TYPE	HISTOLOGICAL GRADE	LYMPH NODE STATUS	VIMENTIN STATUS	ER EXPRESSION	PR EXPRESSION	HER2 EXPRESSION	KI67%	Ki67 STATUS	MOLECULAR SUBTYPE
1	60	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	III	NEGATIVE	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	25%	POSITIVE	TRIPLE NEGATIVE
2	45	PRE-MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	II	POSITIVE	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	5%	NEGATIVE	TRIPLE NEGATIVE
3	54	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	I	POSITIVE	POSITIVE	POSITIVE	NEGATIVE	NEGATIVE	10	NEGATIVE	LUMINAL A
4	59	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	II	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	5	NEGATIVE	TRIPLE NEGATIVE
5	69	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	II	NEGATIVE	POSITIVE	POSITIVE	NEGATIVE	NEGATIVE	30	POSITIVE	LUMINAL B
6	32	PRE-MENOPAUSAL	T3	INVASIVE DUCTAL CARCINOMA(NST)	III	POSITIVE	POSITIVE	POSITIVE	POSITIVE	NEGATIVE	10	NEGATIVE	LUMINAL A
7	50	POST MENOPAUSAL	T3	INVASIVE DUCTAL CARCINOMA(NST)	I	NEGATIVE	POSITIVE	POSITIVE	POSITIVE	POSITIVE	10	NEGATIVE	LUMINAL B
8	56	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	III	POSITIVE	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	40	POSITIVE	TRIPLE NEGATIVE
9	73	POST MENOPAUSAL	T3	INVASIVE DUCTAL CARCINOMA(NST)	II	NEGATIVE	NEGATIVE	POSITIVE	POSITIVE	NEGATIVE	25	POSITIVE	LUMINAL B
10	46	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	III	POSITIVE	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	30	POSITIVE	TRIPLE NEGATIVE
11	47	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	III	NEGATIVE	POSITIVE	NEGATIVE	NEGATIVE	POSITIVE	30	POSITIVE	HER2 ENRICHED
12	49	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	I	POSITIVE	NEGATIVE	POSITIVE	POSITIVE	NEGATIVE	10	NEGATIVE	LUMINAL A
13	38	PRE-MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	II	NEGATIVE	POSITIVE	NEGATIVE	NEGATIVE	POSITIVE	10	NEGATIVE	HER2 ENRICHED
14	52	POST MENOPAUSAL	T1	INVASIVE DUCTAL CARCINOMA(NST)	II	POSITIVE	POSITIVE	POSITIVE	POSITIVE	POSITIVE	10	NEGATIVE	LUMINAL B
15	44	PRE-MENOPAUSAL	T1	INVASIVE DUCTAL CARCINOMA(NST)	II	POSITIVE	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	10	NEGATIVE	TRIPLE NEGATIVE
16	43	PRE-MENOPAUSAL	T3	INVASIVE DUCTAL CARCINOMA(NST)	I	NEGATIVE	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	10	NEGATIVE	TRIPLE NEGATIVE
17	46	POST MENOPAUSAL	T1	INVASIVE DUCTAL CARCINOMA(NST)	II	NOT RETRIEVED	NEGATIVE	POSITIVE	POSITIVE	NEGATIVE	10	NEGATIVE	LUMINAL A
18	45	POST MENOPAUSAL	T2	OTHER-PAPILLARY CARCINOMA	I	NEGATIVE	POSITIVE	POSITIVE	POSITIVE	NEGATIVE	10	NEGATIVE	LUMINAL A
19	64	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	III	POSITIVE	POSITIVE	NEGATIVE	NEGATIVE	POSITIVE	50	POSITIVE	HER2 ENRICHED
20	63	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	II	NEGATIVE	NEGATIVE	POSITIVE	POSITIVE	NEGATIVE	10	NEGATIVE	LUMINAL A
21	43	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	II	NEGATIVE	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	30	POSITIVE	TRIPLE NEGATIVE
22	45	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	II	NEGATIVE	NEGATIVE	POSITIVE	POSITIVE	NEGATIVE	10	NEGATIVE	LUMINAL A
23	53	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	II	POSITIVE	NEGATIVE	POSITIVE	POSITIVE	NEGATIVE	10	NEGATIVE	LUMINAL A
24	50	POST MENOPAUSAL	T1	INVASIVE DUCTAL CARCINOMA(NST)	II	NEGATIVE	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	10	NEGATIVE	TRIPLE NEGATIVE
25	46	POST MENOPAUSAL	T3	INVASIVE DUCTAL CARCINOMA(NST)	III	NEGATIVE	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	80	POSITIVE	TRIPLE NEGATIVE
26	51	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	II	POSITIVE	POSITIVE	POSITIVE	POSITIVE	NEGATIVE	25	POSITIVE	LUMINAL B
27	50	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	II	POSITIVE	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	10	NEGATIVE	TRIPLE NEGATIVE
28	38	PRE-MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	II	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	POSITIVE	25	POSITIVE	HER2 ENRICHED
29	58	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	I	POSITIVE	NEGATIVE	POSITIVE	POSITIVE	NEGATIVE	10	NEGATIVE	LUMINAL A
30	67	POST MENOPAUSAL	T3	INVASIVE DUCTAL CARCINOMA(NST)	II	NEGATIVE	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	10	NEGATIVE	TRIPLE NEGATIVE
31	50	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	III	NEGATIVE	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	15	NEGATIVE	TRIPLE NEGATIVE
32	62	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	II	POSITIVE	NEGATIVE	POSITIVE	POSITIVE	POSITIVE	10	NEGATIVE	LUMINAL B
33	59	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	II	POSITIVE	NEGATIVE	POSITIVE	NEGATIVE	NEGATIVE	10	NEGATIVE	LUMINAL A
34	68	POST MENOPAUSAL	T2	OTHER-INVASIVE LOBULAR CARCINOMA	I	NOT RETRIEVED	NEGATIVE	POSITIVE	POSITIVE	POSITIVE	5	NEGATIVE	LUMINAL B
35	60	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	II	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	10	NEGATIVE	TRIPLE NEGATIVE
36	32	PRE-MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	II	POSITIVE	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	25	POSITIVE	TRIPLE NEGATIVE
37	81	POST MENOPAUSAL	T1	INVASIVE DUCTAL CARCINOMA(NST)	II	NEGATIVE	POSITIVE	POSITIVE	POSITIVE	NEGATIVE	25	POSITIVE	LUMINAL B
38	46	POST MENOPAUSAL	T4	INVASIVE DUCTAL CARCINOMA(NST)	III	POSITIVE	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	10	NEGATIVE	TRIPLE NEGATIVE
39	60	POST MENOPAUSAL	T1	INVASIVE DUCTAL CARCINOMA(NST)	II	POSITIVE	POSITIVE	POSITIVE	POSITIVE	NEGATIVE	10	NEGATIVE	LUMINAL A

40	38	PRE-MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	III	POSITIVE	POSITIVE	POSITIVE	POSITIVE	NEGATIVE	20	POSITIVE	LUMINAL B
41	66	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	II	POSITIVE	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	10	NEGATIVE	TRIPLE NEGATIVE
42	65	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	II	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	10	NEGATIVE	TRIPLE NEGATIVE
43	95	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	II	NEGATIVE	NEGATIVE	POSITIVE	POSITIVE	NEGATIVE	5	NEGATIVE	LUMINAL A
44	42	PRE-MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	II	NOT RETRIEVED	NEGATIVE	POSITIVE	POSITIVE	NEGATIVE	10	NEGATIVE	LUMINAL A
45	37	PRE-MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	I	POSITIVE	NEGATIVE	POSITIVE	POSITIVE	NEGATIVE	20	POSITIVE	LUMINAL B
46	63	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	II	NEGATIVE	NEGATIVE	POSITIVE	POSITIVE	NEGATIVE	10	NEGATIVE	LUMINAL A
47	46	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	II	POSITIVE	NEGATIVE	POSITIVE	POSITIVE	NEGATIVE	10	NEGATIVE	LUMINAL A
48	67	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	II	POSITIVE	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	10	NEGATIVE	TRIPLE NEGATIVE
49	62	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	II	POSITIVE	POSITIVE	POSITIVE	POSITIVE	NEGATIVE	10	NEGATIVE	LUMINAL A
50	45	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	II	NOT RETRIEVED	NEGATIVE	POSITIVE	NEGATIVE	NEGATIVE	10	NEGATIVE	LUMINAL A
51	62	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	II	POSITIVE	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	10	NEGATIVE	TRIPLE NEGATIVE
52	62	POST MENOPAUSAL	T3	INVASIVE DUCTAL CARCINOMA(NST)	II	NEGATIVE	POSITIVE	POSITIVE	POSITIVE	NEGATIVE	15	NEGATIVE	LUMINAL B
53	60	POST MENOPAUSAL	T1	INVASIVE DUCTAL CARCINOMA(NST)	II	NEGATIVE	POSITIVE	POSITIVE	POSITIVE	NEGATIVE	10	NEGATIVE	LUMINAL A
54	58	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	III	NEGATIVE	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	20	POSITIVE	TRIPLE NEGATIVE
55	60	POST MENOPAUSAL	T2	OTHER-MUCINOUS CARCINOMA	II	POSITIVE	POSITIVE	POSITIVE	POSITIVE	NEGATIVE	15	NEGATIVE	LUMINAL B
56	53	POST MENOPAUSAL	T1	INVASIVE DUCTAL CARCINOMA(NST)	II	NEGATIVE	POSITIVE	POSITIVE	POSITIVE	NEGATIVE	10	NEGATIVE	LUMINAL A
57	65	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	III	POSITIVE	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	20	POSITIVE	TRIPLE NEGATIVE
58	53	POST MENOPAUSAL	T2	INVASIVE DUCTAL CARCINOMA(NST)	III	POSITIVE	POSITIVE	POSITIVE	POSITIVE	NEGATIVE	25	POSITIVE	LUMINAL B
59	54	POST MENOPAUSAL	T3	OTHER-PAPILLARY CARCINOMA	II	POSITIVE	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	10	NEGATIVE	TRIPLE NEGATIVE
60	54	POST MENOPAUSAL	T3	INVASIVE DUCTAL CARCINOMA(NST)	II	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	15	NEGATIVE	TRIPLE NEGATIVE