"COMPARISON OF P63 AND SMOOTH MUSCLE MYOSIN HEAVY CHAIN IMMUNOMARKERS IN DISTINGUISHING INVASIVE AND NON-INVASIVE BREAST LESIONS – A ONE YEAR OBSERVATIONAL STUDY"

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ABSTRACT

Background: Breast malignancies are one of the leading reasons for increase in morbidity and mortality among women.[1] Majority of the cases are easily diagnosed based on histopathology but at times presence invasion is difficult to comment upon.

Objectives: 1. To evaluate the efficacy of P63 and SMM-HC immunostaining in distinguishing invasive from non-invasive breast lesions.

2.To study the clinicopathological features of preinvasive and invasive breast lesions.

Methodology: The present study was a prospective as well as retrospective study which included 30 cases from January 2017 to December 2018. Clinical details pertaining to the patient with breast carcinoma admitted to the tertiary care hospital were obtained from the case papers, and from the medical records department of the hospital.

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

Tissues were kept for fixation in 10% formalin and processed, followed by paraffin embedding. Haematoxylin and Eosin (H&E) stained slides available from the department of pathology were studied for histopathological features.

For Immunohistochemistry (IHC), Sections 4 to 5µ thick were placed on coated slides. The sections were stained with the antibody directed against P63 and SMM-HC, as per the IHC staining protocol.

Result: Both P63 and SMM-HC were calculated to have similar sensitivity (94.74%) and specificity (93.33%). However, each had their limitations, P63 exhibited incomplete staining pattern whereas SMM-HC showed cross-reactivity with vascular smooth muscle cells (100%) and stromal myofibroblasts (40%).

Conclusion: We have come to the conclusion that both the markers have very high sensitivity (94.74%) and specificity (93.33%). Both these markers could be used in conjuncture with each other to diagnose cases in which clear infiltrative morphology of invasive carcinomas is not apparent or the tumour margin is obscured as a result of inflammation.

Key words: P63, SMM-HC, Myoepithelial cells, Invasive carcinoma

LIST OF ABBREVIATIONS USED

ADH	– Atypical Ductal Hyperplasia
AJCC	– American Joint Committee for Cancer
ALH	– Atypical Lobular Hyperplasia
ALK	– Anaplastic Lymphoma Kinase
AML	– Acute Myeloid Leukemia
ATM	– Ataxia Telangiectasia and Mantle cell lymphoma
BRCA1	– Breast Carcinoma 1
BRCA2	– Breast Carcinoma 2
CD10	– Cluster of Differentiation 10
CDH1	– E-Cadherin
CHEK2	– Checkpoint kinase 2
CK	– Cytokeratin
cm	– centimetre
DCIS	– Ductal Carcinima In-Situ
DNA	– Deoxyribonucleic Acid
EOD	– Extent of Disease
ER	– Estrogen Receptor
FA	– Fibroadenoma
FFPE	– Formalin Fixed, Paraffin Embedded
H & E	– Haematoxylin and Eosin
HER2	– Human Epidermal growth factor Receptor 2
hpf	– high power field
HPR	– Histopathology
HRT	– Hormone Replacement Therapy

IARC – International Agency for Research on Cancer

IDC – Invasive Ductal Carcinoma

IHC – Immunohistochemistry

inv – Inversion

IP – Intraductal Papilloma

k – kappa

Ki-67 – Kiel 67

LCIS – Lobular Carcinoma In-Situ

MALT – Mucosa Associated Lymphoid Tissue

Mf – Myofibroblasts

min – minutes

mm – millimetre

MRM – Modified Radical Mastectomy

NCI SEER – National Cancer Institute Surveillance Epidemiology and End Results

NOS – Not Otherwise Specified

NST – No Special Type

PALB2 – Partner And Localizer of BRCA2

PR – Progesterone Receptor

PTEN – Phosphatase and Tensin homolog

SA – Sclerosing Adenosis

SERM – Selective Estrogen Receptor Modulator

SMA – Smooth Muscle Actin

SMM-HC – Smooth Muscle Myosin - Heavy Chain

T4 – Fourth Thoracic nerve root

TDLU – Terminal Duct Lobular Unit

TNM – Tumour size, Nodal status, Metastasis

TP53 – Tumour Protein 53

Vsm – Vascular smooth muscle

WHO – World Health Organization

μ – microns

TABLE OF CONTENT

SR. NO.	TOPIC	PAGE NO.
1	INTRODUCTION	1-2
2	OBJECTIVES	3
3	REVIEW OF LITERATURE	4-34
4	METHODOLOGY	35-38
5	RESULTS	39-58
6	DISCUSSION	59-64
7	CONCLUSION	65
8	SUMMARY	66-67
9	BIBLIOGRAPHY	68-76
10	ANNEXURES	
	ANNEXURE I – CONSENT FORM	77-79
	ANNEXURE II – ETHICAL CLEARANCE	80
	ANNEXURE III – PROFORMA	81-82
	ANNEXURE IV – H & E STAINING PROTOCOL	83-84
	ANNEXURE V – IHC STAINING PROTOCOL	85-86
	ANNEXURE VI – KEY TO MASTER CHART	87
	ANNEXURE VII – MASTER CHART	88-89

LIST OF TABLES

TABLE NO.	TOPIC	PAGE NO.
1	Histologic Grading Using Nottingham Modification of Scarff-Bloom-Richardson System	26
2	The Scores of the Histological Grading	26
3	TNM Staging (AJCC)	28-29
4	Antibodies used in IHC	37
5	Distribution of clinic-pathological parameters	42
6	P63 stain positivity	46
7	SMM-HC stain positivity	46

LIST OF GRAPHS

GRAPH NO.	TOPIC	PAGE NO.
1	Age distribution	39
2	Age wise distribution of each lesion	40
3	Mean age of patients	40
4	Mean size of the lesion	41
5	Mean size for age	42
6	Comparison of P63 and SMM-HC staining	44
7	P63 staining score	44
8	SMM-HC staining score	45
9	Mean score for size	47
10	Mean score for age	47
11	Mean score for each diagnosis	48

LIST OF FIGURES

FIG. NO.	TOPIC	PAGE NO.
1	Structural anatomy of breast	5
2	Lymphatic drainage of breast	7
3	Terminal Duct Lobular Unit	8
4	WHO classification of Breast Tumours	14-16
5	SMM-HC outlining a normal duct	23
6	P63 nuclear staining in a normal duct	25

LIST OF PHOTOGRAPHS

PHOTOGRAPH NO.	TOPIC	PAGE NO.
1a	Atypical Ductal Hyperplasia	49
1b	P63 positivity in a case of ADH. IHC score – 2	49
1c	SMM-HC positivity in a case of ADH. IHC score – 3	50
2a	Ductal Carcinoma In-Situ	50
2b	P63 positivity in a case of DCIS. IHC score – 2	51
2c	SMM-HC positivity in a case of DCIS. IHC score – 3	51
3a	Invasive Ductal Carcinoma with a focus of DCIS(arrow)	52
3b	P63 positivity in a focus of DCIS in the above case	52
3c	SMM-HC positivity in a focus of DCIS in the above case	53
4a	Invasive Ductal Carcinoma	53
4b	Tumour foci negative for P63 in a case of IDC	54
4c	Tumour foci negative for SMM-HC in a case of IDC	54
5a	Intraductal Papilloma	55
5b	P63 positivity in a case of IP. IHC score – 3	55
5c	SMM-HC positivity in a case of IP. IHC score – 3	56
6a	Sclerosing Adenosis	56
6b	P63 positivity in a case of SA. IHC score – 3	57
6c	SMM-HC positivity in a case of SA. IHC score – 3	57
7	Vascular smooth muscle cells positive for SMM-HC	58
8	Myofibroblasts positive for SMM-HC	58

INTRODUCTION

Breast malignancies are one of the major causes of increased morbidity and mortality among women. It is the most frequently diagnosed carcinoma in women worldwide with approximately 1.38 million new cases every year.[1] Among Indian women as well, it remains the commonest cause of cancer.[2]

Annual age standardized incidence rate of breast carcinoma as of 2012 was 43.1 per 1,00,000 women worldwide whereas in India it was 25.8 per 1,00,000 women.[3] In Karnataka state the incidence is as high as 36.6 per 1,00,000 women.[4]

The incidence and detection rate of breast cancer in India has increased in the last decade. A major contributing factor for this is the introduction of breast cancer screening programs, but other factors such as lifestyle changes also played an important role.[5]

At times distinction between an invasive carcinoma and in situ carcinoma can be difficult, particularly in a core biopsy specimen. There is a considerable inter-observer disagreement in difficult cases when a diagnosis is made on histopathological examination alone.[6]

Ducts and lobules of breast are composed of a double layer of cells (an inner luminal cell layer and an outer myoepithelial cell layer).[7] Loss of peripheral myoepithelial cells layer is the hallmark of invasion.[8-10]

Numerous immunohistochemical markers have been used for the demonstration of these myoepithelial cells in order to identify invasion. Some of the markers used include S-100, CD10, SMA (Smooth Muscle Actin), Calponin and SMM-HC

(Smooth Muscle Myosin-Heavy Chain).[11] Among these markers, ones which are commonly used in practice include SMA, Calponin and SMM-HC.

Studies have shown that Calponin and SMM-HC are highly sensitive markers for myoepithelial cells. However, they show cross-reactivity with myofibroblasts in the stroma and smooth muscle cells in vascular wall which can lead to significant diagnostic difficulty in cases of desmoplasia or those tumors having a highly vascular stroma.[12-14]

On-going researches have shown that p63 is also expressed by myoepithelial cells and it does not show positivity with myofibroblasts or smooth muscle cells.[15] One study has suggested that P63 can be used as a complement to or can replace SMM-HC as a myoepithelial marker in diagnosing difficult cases of carcinoma breast.[16]

P63 is a member of the p53 family of genes which is expressed in the basal lamina of some organs such as the skin, prostate, cervix and also in few carcinomas.[17] Diagnostically it has been used to differentiate poorly differentiated carcinoma as squamous or transitional cell carcinoma[18] and also to identify invasion in the basal layer of prostatic epithelium in cases of adenocarcinoma.[19]

OBJECTIVES

1. To evaluate the efficacy of P63 and SMM-HC immunostaining in distinguishing invasive from non-invasive breast lesions.
2. To study the clinicopathological features of preinvasive and invasive breast lesions.

REVIEW OF LITERATURE

The breasts or the mammary glands are secondary sexual characters which are well developed in female and also serve as a source of nutrition for newborns. The breasts develop from the mammary ridge, also known as the milk line. They appear as a thickening of epidermis on ventral surface of body of the fetus in 5^{th} week of gestation. This thickening extends from the axillary region to the upper medial aspect of thighs bilaterally. In humans, the ridge disappears with development except in the anterior pectoral region. Nipple formation begins on day 56. Mammary sprouts, which form the primitive ducts start development on day 84 and they get canalaized at around day 150. Myoepithelial cells appear around these ducts between 23 and 28 weeks of gestation. [20]

Mature breast development begins at puberty and is termed as thelarche. It begins with cyclical estrogen and progesterone secretion. Branching of the mammary ducts occurs under the influence of estrogen. Accumulation of adipose tissue occurs in the parenchyma leading to breast enlargement.[20]

GROSS ANATOMY

Mature breast is a bilateral accessory organ located on the anterior chest wall. It's configuration is eccentric with the long axis running diagonally along the pectoralis major. It extends into axilla as the axillary tail of Spence. It lies within the superficial fascia which is continuous superiorly as the cervical fascia and inferiorly as the superficial abdominal fascia. The pectoralis major muscle fascia forms the suspensory ligament of axilla. Deeper to the breast tissue lies the retromammary space or the sub mammary space which contains loose areolar connective tissue.

The morphological and functional unit of the breasts are the lobes which are comprised of ducts, glands and terminal lobules in a fibrocollagenous stroma. It has been labelled as the terminal duct lubular unit (TDLU). TDLU is the milk secreting component of the mammary glands and is responsive to hormonal changes. It is also the most common site for a primary malignancy of the breast.

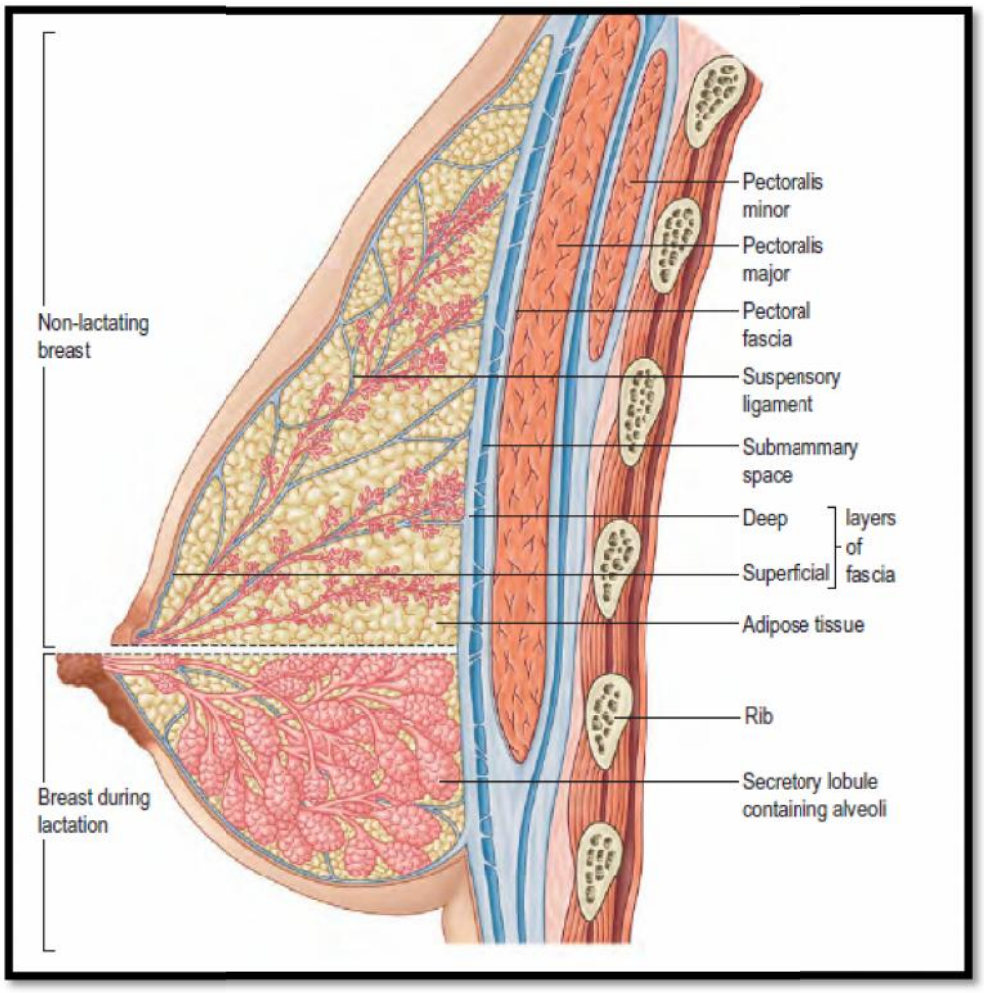


Fig. 1: Structural anatomy of breast[20]

Nipple Areola Complex: The terminal secretory ducts from each of the lobules converge just underneath the nipple and form the lactiferous sinus. The skin surrounding the nipple is known as the areola. Abundant numbers of sweat glands are present in this region which directly open into the skin surface. These glands can

become grossly visible near term pregnancy or in lactating mothers as circumferentially arranged elevations known as Montgomery's tubercles.

Arterial supply and venous drainage: The breast is supplied by branches from the internal thoracic, axillary and intercostal arteries. The internal mammary artery, which is a branch of the internal thoracic artery, is the major artery supplying the breast in most individuals.

Lymphatic drainage: Lymph fluid from the breasts drain into axillary group of lymph nodes via the lymphatics which run along the external thoracic vein and also into the internal mammary lymph nodes via the lymphatics which run along the internal thoracic vein. In the subareolar region lies a plexus of lymphatic vessels referred to as the subareolar plexus of Sappey.

Three important routes of drainage have been identified. Nearly 75% of the lymphatic fluid drains into the axillary group of lymph nodes. Around 25% is via the internal mammary lymph nodes. Posterior intercostal lymph nodes are the third draining sites for the breast.

The lymphatic route has been recognised as an important route for dissemination of breast cancer. Lymphatics from the right breast drain into the right subclavian vein and those from the left breast drain into the thoracic duct which terminates into the left subclavian vein.

The axillary group of lymph nodes are further sub-grouped as anterior, posterior, lateral and apical group of lymph nodes which range from 20-40 in number. Surgically these lymph nodes are described in 3 levels based on their relation to pectoralis minor muscle. Level 1 nodes are those which lie lateral to the pectoralis

minor, level 2 nodes lie posterior to the muscle whereas level 3 lymph nodes lie medial to the pectoralis minor. There may be one or two inter-pectoral group of lymph nodes, known as Rotter's nodes.

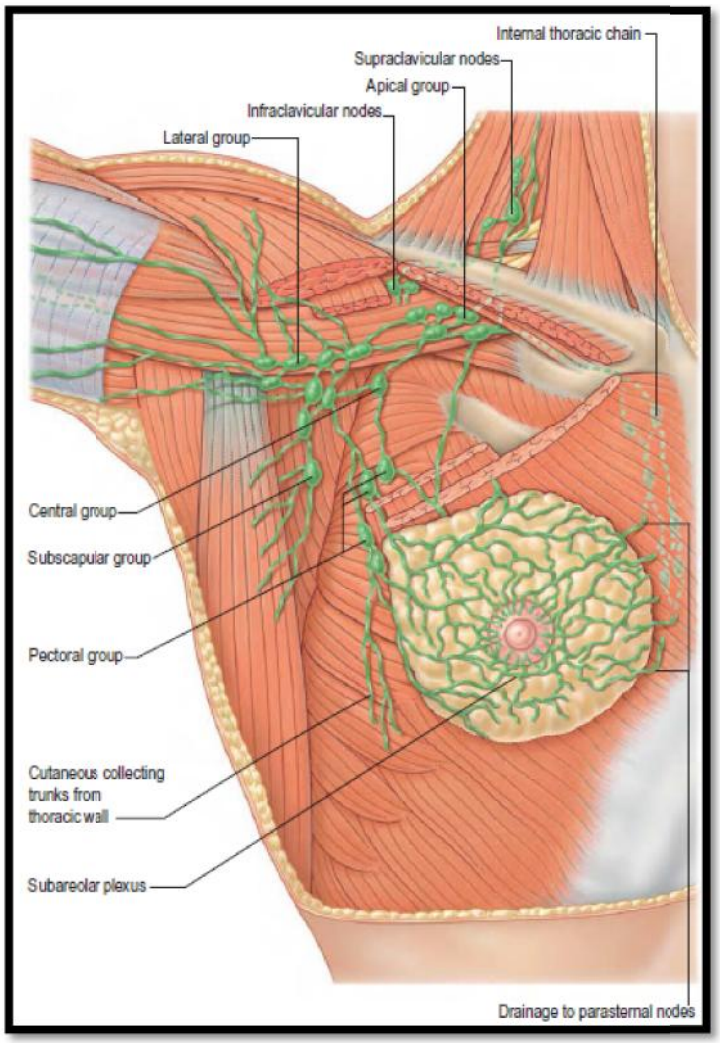


Fig. 2: Lymphatic drainage of breast[20]

First draining node of a malignant lesion is called the **Sentinal lymph node.**. Mapping of lymphatics and identifying this node is an essential step for staging of patients with early breast cancer. It is removed surgically and histopathological analysis is done to detect presence of matastasis. In cases where sentinel lymph node is negative, radical surgery can be avoided.[20]

Nerve supply: The mammary glands are innervated by branches of 4^{th} to 6^{th} intercostal nerves. The nipple areolar complex is supplied by lateral cutaneous branch of T4.

Microstructure: Each of the mammary glands constitute of 15 - 20 lobes of tubuloalveolar glands. These lobes are separated by dense collagenous tissue and adipose tissue. Each lobe in itself is a distinct gland with its own duct which opens individually into the nipple. Within the lobes, each duct branches to form multiple terminal ducts which ends in a lobule forming a Terminal duct-lobular unit (TDLU).

The lining of these ducts and acini is formed by double layer of cells; the inner layer formed by epithelial cells and the outer layer formed by myoepithelial cells. The luminal epithelial layer is formed by cuboidal cells in smaller ducts and acini whereas in the larger ducts it is formed by tall columnar cells. Surrounding these cells lies a layer of stellate shaped myoepithelial cells. During the reproductive ages, the ductal epithelium undergoes mild hormonal changes under the influence of ovary.

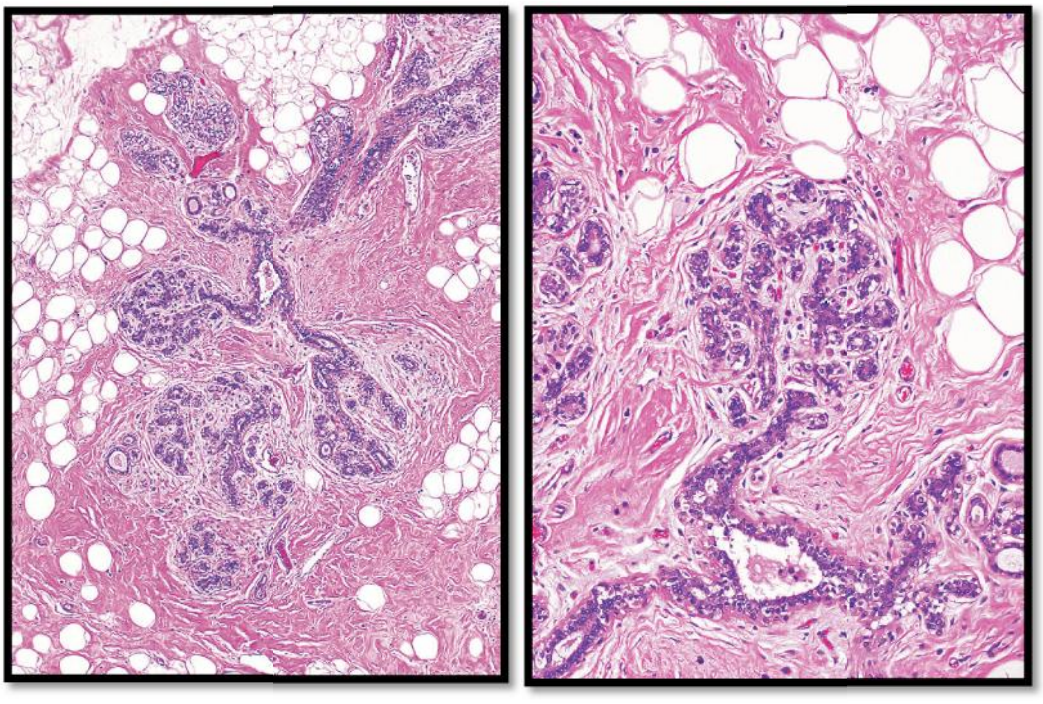


Fig. 3: Terminal Duct Lobular Unit[21]

Myoepithelial cells

Myoepithelial cells align parallel to the ducts and form a single continuous layer which lies in between the epithelial cells and adjoining stroma and it is also responsible for secretion of the basement membrane.[22-24] DCIS is a pre-invasive condition in which malignant epithelial cells are confined within the ducts which are surrounded by a layer of myoepithelial cells.[25] Disruption of this cell boundary can lead to invasive carcinoma.[26] It is believed that these cells form a physical barrier which must be traversed by tumour cells in order to lead to invasive carcinoma and there is substantial evidence to support that loss of normal regulatory role of myoepithelial cells results in transition from preinvasive DCIS to invasive carcinoma.[27]

BREAST CARCINOMA

Epidemiology: Approximately 1.7 million new cases of breast carcinoma are diagnosed yearly. This malignancy is the 5th most common cause of deaths related to cancer in the world.[28] In India, breast carcinoma is the commonest malignant cancer in women.[29] The annual age standardized incidence rate of breast cancer in India as of 2012 was 25.8 per 100000 women.[3] However, in Karnataka the rates were as high as 36.6 per 100000 women.[4]

On the basis of histology, breast carcinomas are divided into in-situ carcinomas and invasive carcinomas. Carcinoma in-situ can be further sub-classified as ductal and lobular, which are differentiated from each other based on cytological features and pattern of growth. Ductal carcinoma in-situ (DCIS) is a condition that is more common than Lobular carcinoma in-situ (LCIS) and it can be further sub-classified on

the basis of morphology as Solid, Papillary, Micropapillary, Cribriform and Comedo carcinoma.

In atypical ductal hyperplasia (ADH) and in-situ carcinoma, the proliferation of cells is limited to the lumen of the ductal system and there occurs no invasion of myoepithelial cell layer or the basement membrane.[28,30]

Among invasive malignancies, ductal carcinoma is the commonest variant, accounting for 47-80%. On gross examination, these tumours range from 0.5 to 10 cm in size. They are firm to hard in consistency with ill-defined margins. Microscopically, the neoplastic cells show arrangement in the form of sheets and cords or are diffusely infiltrative. These cells have hyper-chromatic, pleomorphic nucleus with prominent nucleolus and abundant eosinophilic cytoplasm. Atypical mitotic figures are also commonly seen. The surrounding stroma might be cellular or may show desmoplastic reaction. Foci of DCIS are also seen along with the invasive carcinoma.[31]

Risk Factors

- Age: The risk of breast carcinoma is increased with advancement of age.[32] Reproductive factor which may act as risk factors include early menarche (<12 years), delayed first birth (>30 years) and delayed menopause (>55 years).[33]
- Family history: The probability of carcinoma of breast is nearly double in those women whose sister or mother has had a history of breast carcinoma.[34]
- Genetic factors: The commonest cause of hereditary breast cancer is the BRCA1 gene on 17q chromosome and BRCA2 located on 13q. Mutation in the TP53 tumour suppressor protein gene (Li-Fraumeni syndrome) leads to an elevated risk of breast cancer development along with sarcomas, brain

tumours, leukemia, bone tumours and adrenal tumours. ATM (Ataxia Telangiectasia and Mantle cell lymphoma) gene codes for a DNA repair protein and an inherited abnormality leads to increased risk of breast cancer. Other genetic abnormalities that are observed along with breast cancer are PTEN tumour suppressor gene, CHEK2 (Checkpoint kinase 2), CDH1 (E-Cadherin) tumour suppressor gene and PALB2.[33]

- Weight: Obesity and weight gain results in elevated risk of development of breast cancer especially during the perimenopausal age.[33] In the postmenopausal age, marked increase in weight is seen to be associated with nearly double risk of development of breast cancer. Post-operative weight gain also increases the risk of relapse.
- Exercise: It has been observed that a physically active lifestyle may have a protective effect by lowering the levels of various hormones.[33] Some studies show that an active lifestyle after treatment of cancer reduces the chances of relapse and the overall risk of mortality.
- Diet: There is little consensus over the effect of individual dietary components as a risk factor for breast malignancy. There are studies which suggest that risk of ER negative tumours may be reduced by low fat intake and high vegetable intake.[35] Consumption of alcohol is associated with moderate increase in the risk of breast cancer. Tobacco play no significant role in the etiology of breast cancer.[36]

  Favourable lifestyle changes which include low calorie diet, increased exercise and reduced environmental exposure to disturbances of circadian rhythm can reduce breast cancer by one-third.[35]

- Breast feeding: Chances of breast cancer can be reduced by prolonged breast feeding.[37] There is 30% lower risk of breast cancer among women who first start breastfeeding at the age of 20-24 years as compared to those who have never breastfed, independent of the age of first child birth.[38]
- Parity: Low parity and late pregnancy are significant factors which contribute to increase in risk of breast cancer. Nulliparous women have a 30% higher risk of malignancy as compared to parous women. Women having their first child birth at age greater than 35 years have a 40% higher risk of development of breast cancer in comparison with those who give birth before the age of 20 years.[39]
- Hormone replacement therapy (HRT): The risk of breast cancer is higher in women taking combined estrogen and progestogen therapy. However, recent evidence suggests that HRT has no effect on breast cancer mortality.[36] Prolonged use (>5 years) of post menopauasal HRT is an important risk factor.[40]
- Estrogen exposure: Use of Selective Estrogen Receptor Modulator (SERM) have shown that the risk is reduced by 38% from the start of five year treatment plan up to 10 years.[35]
- Radiation: Radiation exposure in young women such as in cases of patients undergoing treatment for lymphoma constitutes a higher risk of development of a neoplasm and this increase in risk is directly proportional to the dose of radiation.[40]
- Precancerous conditions of the breast: The risk of development of malignancy is four-fold in patients with ADH or lobular hyperplasia. History of even a benign disease of the breast results in an increased risk. Patients having

increased breast density and benign proliferative breast diseases on biopsy have a greater relative risk of future development of breast malignancy.[40,41]

MANAGEMENT OF BREAST NEOPLASM

For in-situ cases, surgical resection followed by radiotherapy is the treatment approach of choice. There has been a shift from radical mastectomy towards minimally invasive procedures. Invasive breast carcinomas surgery is followed by irradiation of the chest wall or the breast. Systemic therapy is given to the patient either prior to the surgery, in cases where tumour to breast size ratio is more in order to achieve breast conserving therapy, or is given after the surgery.[42]

All patients with estrogen receptor positive are administered anti estrogens. There are two distinct classes of anti-estrogens: selective estrogen receptor modulators (Tamoxifen) and selective estrogen receptor downregulators (Fulvestrant).

Patients who are ER negative and positive for HER2 amplification are usually treated with Trustuzumab, either alone or it is combined with chemotherapy.[43]

WHO CLASSIFICATION OF BREAST TUMOURS 2012

The 2012 classification for breast tumours was put forth during a Consensus and Editorial meeting of a Working group at the International Agency for Research on Cancer (IARC), Lyon in September of 2011. This classification was the first attempt to separate breast tumours from those of the female genital tract. Primary focus of this classification is on morphology of the tumour however there is updated information regarding molecular pathology, expression profiling and molecular classification.[44,45]

- **Epithelial tumors**
  - Microinvasive carcinoma
- **Invasive breast carcinoma**
  - Invasive carcinoma of no special type (NST)
    - Pleomorphic carcinoma
    - Carcinoma with osteoclast like stromal giant cells
    - Carcinoma with choriocarcinomatous features
    - Carcinoma with melanotic features
  - Invasive lobular carcinoma
    - Classic lobular carcinoma
    - Solid lobular carcinoma
    - Alveolar lobular carcinoma
    - Pleomorphic lobular carcinoma
    - Tubulolobular carcinoma
    - Mixed lobular carcinoma
  - Tubular carcinoma
  - Cribiform carcinoma
  - Mucinous carcinoma
  - Carcinoma with medullary features
    - Medullary carcinoma
    - Atypical medullary carcinoma
    - Invasive carcinoma NST with medullary features
  - Carcinoma with apocrine differentiation
  - Carcinoma with signet ring differentiation
  - Invasive micropapillary carcinoma
  - Metaplastic carcinoma of no special type
    - Low-grade adenosquamous carcinoma
    - Fibromatosis like metaplastic carcinoma
    - Squamous cells carcinoma
    - Spindle cell carcinoma
    - Metaplastic carcinoma with mesenchymal differentiation
      - Chondroid differentiation
      - Osseous differentiation
      - Other types of mesenchymal differentiation
    - Mixed metaplastic carcinoma
    - Myoepithelial carcinoma
  - Rare types
    - Carcinoma with neuroendocrine features
      - Neuroendocrine tumor, well differentiated
      - Neuroendocrine carcinoma, poorly differentiated (small cell carcinoma)
      - Carcinoma with neuroendocrine differentiation
    - Secretory carcinoma
    - Invasive papillary carcinoma
    - Acinic cell carcinoma
    - Mucoepidermoid carcinoma
    - Polymorphous carcinoma
    - Oncocytic carcinoma
    - Lipid rich carcinoma
    - Glycogen rich clear cell carcinoma
    - Sebaceous carcinoma
    - Salivary gland / skin adnexal type tumors
      - Cylindroma
      - Clear cell hidradenoma
- **Epithelial-myoepithelial tumors**
  - Pleomorphic adenoma
  - Adenomyoepithelioma
    - Adenomyoepithelioma with carcinoma
  - Adenoid cystic carcinoma
- **Precursor lesions**
  - Ductal carcinoma in situ
  - Lobular neoplasia
    - Lobular carcinoma in situ

        - Classic lobular carcinoma in situ
        - Pleomorphic lobular carcinoma in situ
    - Atypical lobular hyperplasia

- **Intraductal proliferative lesions**
    - Usual ductal hyperplasia
    - Columnar cell lesions including flat epithelial atypia
    - Atypical ductal hyperplasia

- **Papillary lesions**
    - Intraductal papilloma
        - Intraductal papilloma with atypical hyperplasia
        - Intraductal papilloma with ductal carcinoma in sit
        - Intraductal papilloma with lobular carcinoma in situ
    - Intraductal papillary carcinoma
    - Encapsulated papillary carcinoma
        - Encapsulated papillary carcinoma with invasion
    - Solid papillary carcinoma
        - In situ
        - Invasive

- **Benign epithelial proliferations**
    - Sclerosing adenosis
    - Apocrine adenosis
    - Microglandular adenosis
    - Radial scar / complex sclerosing lesion
    - Adenomas
        - Tubular adenoma
        - Lactating adenoma
        - Apocrine adenoma
        - Ductal adenoma

- **Mesenchymal tumors**
    - Nodular fasciitis
    - Myofibroblastoma
    - Desmoids type fibromatosis
    - Inflammatory myofibroblastic tumor
    - Benign vascular lesions
        - Haemangioma
        - Angiomatosis
        - Atypical vascular lesions
    - Pseudoangiomatous stromal hyperplasia
    - Granular cell tumor
    - Benign peripheral nerve sheath tumors
        - Neurofibroma
        - Schwannoma
    - Lipoma
        - Angiolipoma
    - Liposarcoma
    - Angiosarcoma
    - Rhabdomyosarcoma
    - Osteosarcoma
    - Leiomyoma
    - Leiomyosarcoma

- **Fibroepithelial tumors**
    - Fibroadenoma
    - Phyllodes tumor
        - Benign
        - Borderline
        - Malignant
        - Periductal stromal tumor, low grade
    - Hamartoma

- **Tumors of the nipple**
    - Nipple adenoma
    - Syringomatous adenoma
    - Paget disease of the nipple

- **Malignant lymphoma**
    - Diffuse large B cell lymphoma
    - Burkitt lymphoma
    - T cell lymphoma
        - Anaplastic large cell lymphoma, ALK negative

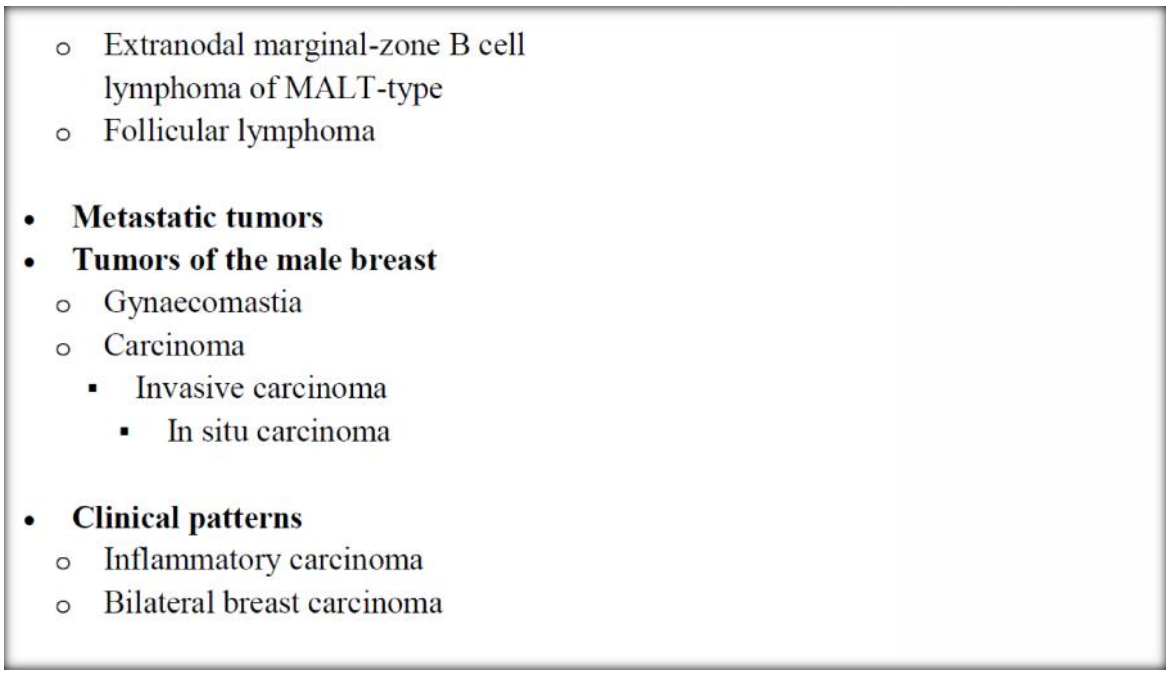


Fig. 4: WHO classification of Breast Tumours[45]

Molecular Classification

The WHO classification of breast tumours does not predict response to the newer targeted therapy. For this purpose, the molecular classification was introduced.

The term "Molecular Classification" in breast cancer was proposed by Perou and Sorlie in the year 2000[46]. There are five distinct subtypes of breast cancer based on the molecular classification, namely basal like, HER2/neu positive, Luminal A type, Luminal B type and normal breast like[R2,3,47,48]. These subtypes have distinct clinical presentation[49], histological features[50], response to chemotherapy[46,51] and outcome[52].

- **Luminal A type**: These tumours are positive for estrogen-receptor and/or progesterone-receptor, negative for HER2/*neu*, with low Ki-67 index. Luminal A cancers are low-grade and they have the best long term prognosis.

- **Luminal B type**: These tumours are positive for estrogen-receptor and/or progesterone-receptor, and either positive or negative for HER2/*neu*, and have high Ki-67 index. Luminal B breast cancers usually grow somewhat faster than luminal A cancers and have a prognosis which is somewhat worse than Luminal A.
- **Triple-negative/basal-like**:These breast cancers are negative for estrogen-receptor and progesterone-receptor and negative for HER2/*neu*. This type is commonly seen in cases with mutation of *BRCA-1* gene.
- **HER2-enriched** type: These tumours are negative for estrogen-receptor and progesterone-receptor and positive for HER2/*neu*. These cancers have worse prognosis as they tend to grow faster than luminal cancers. They can however be treated with targeted therapies such as Trastuzumab, Lapatinib or Pertuzumab.
- **Normal-like**: These tumours are similar to luminal A disease i.e. positive for estrogen-receptor and/or progesterone-receptor, negative for HER2/*neu*, and with low Ki-67 protein levels. Their prognosis is slightly worse than that of luminal A cancer.[53]

Histomorphology of Carcinoma Breast

Breast carcinoma can be classified into biological and clinical subgroups based on the histological grade[54] and histological type[55].

Histological grade is the degree of differentiation of the tumour and its proliferative activity and tells about its aggressiveness[54].

Histological type refers to the pattern of growth of the tumour. Certain specific morphological and cytological patterns were identified which were associated with distinctive clinical presentation and/or outcome.

Ductal Carcinoma In-Situ (DCIS)

"A neoplastic intraductal lesion characterized by increased epithelial proliferation, subtle to marked cellular atypia and an inherent but not obligate tendency for progression to invasive breast cancer."[45]

The involved acini and lobules are dilated and appear like small ducts, hence the name, Ductal carcinoma in-situ. The myoepithelial cell layer is intact in the involved ducts.

Architecturally DCIS has two subtypes – comedo and non comedo. DCIS with comedo pattern commonly presents as a vague mass. Histologically the neoplastic cells have high grade pleomorphic nuclei with areas of central necrosis. In non comedo DCIS there is absence of high grade nuclei as well as central necrosis. Focal calcifications, along with focal necrosis and intraluminal secretions maybe seen in non comedo DCIS.[37]

The neoplastic cells of low grade DCISare small, with minimal pleomorphism, having uniform sized nuclei with regular chromatin pattern and inconspicuous nucleoli. These cells can be arranged in micropapillary, cribriform or solid pattern. High grade DCIS are composed of pleomorphic atypical cells which may also form micropapillary, cribriform or solid pattern. However, the nuclei of these neoplastic cells are markedly pleomorphic with coarse clumped chromatin and prominent nucleoli.[45]

Lobular Carcinoma In-Situ (LCIS)

"LCIS is a clonal proliferation of cells within ducts and lobules that grow in a discohesive fashion, usually due to an acquired loss of tumour suppressive adhesion protein E-cadherin"[37]

Nearly 20-40% cases of LCIS are bilateral. It is almost always an incidental finding without any calcification or stromal reaction.

Morphologically, the lobules comprise of monomorphic population of tumour cells having round nuclei and small prominent nucleoli. Signet ring cells may also be seen. There is absence of formation of papillae and cribriform spaces.[37]

Invasive Lobular Carcinoma

Invasive lobular carcinomas constitute less than 5% of breast malignancies; however the proportion is greater in women with age greater than 75 years.[41] The presenting symptom in majority of the cases is a painless, ill-defined mass. On microscopically, these cells are seen to be arranged in linear cords and are seen infiltrating the adjacent stroma.

Invasive Ductal Carcinoma, not otherwise specified (NOS)

It is the most commonly diagnosed type of breast malignancy comprising approximately 75% of carcinomas.[41]

These tumours show marked variation in size, ranging from smaller than 0.10 cm to larger than 10 cm. These masses show a nodular consistency with irregular or stellate shaped outlines. There is absence of regularity of structure associated with tumour of any specific type.[33]

Invasive Papillary Carcinoma

It is the malignant counterpart of Papillaryintraductal carcinoma; however on invasion there is loss of papillary architecture and the appearance is nearly identical to that of invasive ductal carcinoma. A grossly well circumscribed invasive papillary carcinoma is seen in around 60-70% of the cases [(56)]. Microscopically, these tumours are circumscribed and exhibit delicate, blunt papillae along with areas of solid tumour mass. These cells may also show apocrine features.

Invasive Micropapillary Carcinoma

Micropapillary carcinomas have a lobulated appearance because of expansive growth. The neoplastic cells appear to form tubules with narrowed or obliterated lumen. It is an extremely aggressive tumour with poor long term prognosis. 95% patients at the time of presentation have lymph node metastasis.[(57)]

Tubular Carcinoma

These carcinomas constitute <2% of all breast malignancies. These tumours are smaller in size as compared to ductal carcinoma-NOS, apart from this there is no characteristic differentiating feature. Microscopically, the most characteristic feature is the presence of tubules lined by monolayered epithelial cells with a clear central lumen.[(45)]

Invasive Cribriform Carcinoma

Invasive cribriform carcinomas constitute 0.80-3.50% of breast carcinomas with an average presenting age of 53-58 years. The tumour is commonly clinically occult but may present as a breast lump. On microscopy, more than 90% of the tumour comprises of an invasive cribriform pattern or a sieve like pattern.[(33)]

Paget's disease of the Nipple

This manifestation of carcinoma of breast is seen in 1-4% of the cases. It presents as an erythematous eruption over the nipple along with scale of crust which is unilateral. Pruritis is a common feature and the lesion mimics eczema. There is involvement of the nipple by the neoplastic cells due to disruption of the epithelial barrier which allows fluid to collect onto the outer surface of the nipple.

PREINVASIVE BREAST LESIONS

Certain benign proliferative breast diseases have increased relative risk of development of invasive carcinomas. These include conditions like Complex fibroadenoma, Solitary intraductal papilloma, Sclerosingadenosis, Atypical ductal and Atypical lobular hyperplasia.[41]

Complex Fibroadenoma

When a fibroadenoma is associated with foci of sclerosingadenosis (SA), papillary apocrine hyperplasia, cysts, or epithelial calcifications, they are labelled as "complex". At least one of these histologic features must be present for the lesion to be classified as complex FA. Among these SA is the most commonly associated lesion.[41]

Sclerosing Adenosis

It is a type of glandular proliferation which is characterized by variable degree of epithelial cell atrophy along with intralobular fibrosis and an intact myoepithelial cell layer. In some cases the proliferating glands exhibit an infiltrative pattern of growth within the stroma and the surrounding fat making it difficult to differentiate from infiltrating carcinoma particularly in a specimen obtained by needle core biopsy.[33]

Intraductal Papilloma

Papillomas are benign neoplasms of the epithelium lining the mammary ducts, most commonly found in the subareolar region and are associated with nipple discharge. These can be solitary or multiple. Multiple papilloma are seen in younger age group whereas solitary papilloma are commonly seen in 5^{th} decade of life and the later also carries increased risk of malignant transformation.[41,58]

Atypical Ductal Hyperplasia (ADH)

"Atypical ductal hyperplasia is defined as a proliferation of monomorphic, evenly placed epithelial cells involving terminal-duct lobular units (TDLUs)". Morphologically ADH shows resemblance to low grade DCIS. Proliferation of cells in this condition is usually in the form of solid pattern of growth; however, microacini, micropapillary or cribriform pattern can also be seen. A woman with ADH has 3-5 times higher relative risk of cancer development as compared to those without ADH.[33]

Atypical Lobular Hyperplasia (ALH)

This is a pre-invasive condition on the spectrum of lobular neoplasm with its distinction from LCIS being the extent of individual lobe involvement. It is important to make this distinction from LCIS as the relative risk of development of carcinoma for ALH is half that of LCIS.[33]

MYOEPITHELIAL MARKERS

An intact myoepithelial cell layer is used by pathologists to distinguish between pre-invasive DCIS and an invasive malignancy. Numerous immune-histochemical antibodies have been used to distinguish myoepithelial cells from luminal epithelial

cells in the breast, including S100 protein, α-smooth muscle actin (SMA), smooth muscle myosin - heavy chain (SMM-HC), P63, P-cadherin, CK-5, CK-14, CK-17, h-caldesmon and maspin,.[59] In some cases of DCIS, myoepithelial cells show reduced sensitivity for some of these markers.[11,33,60] Therefore, a combination of SMMHC and p63 are routinely used in common practice.[61]

SMOOTH MUSCLE MYOSIN – HEAVY CHAIN (SMM-HC)

SMM-HC is a cytoplasmic muscle protein encoded by MYH11 gene on 16 chromosome. It is a subunit of a hexameric contractile protein. This protein is present in the cytoplasm and cell membrane of myoepithelial cells, smooth muscle cells and myofibroblasts. It is useful in differentiating benign and malignant breast and lung (bronchoalveolar carcinoma) lesions. In cases of AML M4 with inv(16) nuclear positivity for SMMHC can be seen.[62]

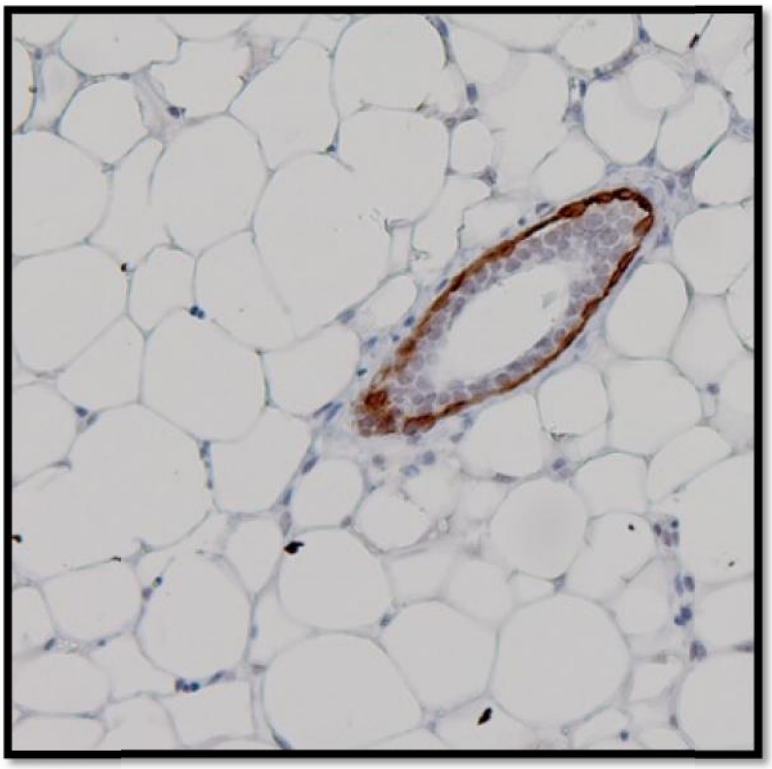


Fig. 5: SMM-HC outlining a normal duct[61]

P63

P63 is a member of p53 family of genes, but it doesn't appear to function as a tumor suppressor gene. It seems to have a role in development and growth of epithelial organs. It exists mainly in two isoforms; TAp63 and deltaNp63. The former is highly expressed in majority of benign tumors and is negative or weak in majority of carcinomas. However, the later isoform is negative or weak in majority of benign tumors and has high expression in adenoid cystic, mucoepidermoid and myoepithelial carcinomas. P63 typically shows a nuclear staining pattern.

Cells which normally show positive staining with p63 include Breast myoepithelium, mature cervical, vaginal and vulval basal squamous epithelium, transformation zone reserve cells, bronchial reserve cells, basal cells of skin, basal cells of prostate, thymic epithelial cells and urothelial cells. It is used by pathologists to exclude invasion in breast tumors and salivary tumours by identifying myoepithelial cells. It is also used to distinguish high grade prostate cancers which are often p63− from high grade infiltrating urothelial cancers which are often p63+.[(63)]

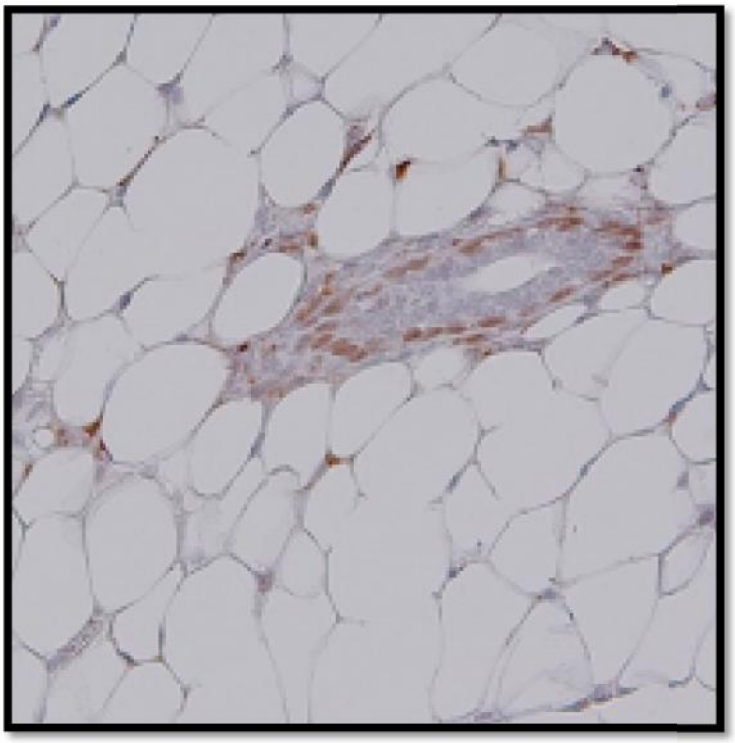


Fig. 6: P63 nuclear staining in a normal duct[61]

HISTOLOGIC GRADING

Histologic grading is done on the basis of Nottingham score which is also referred to as Scarf-Bloom-Richardson scoring system.

The grading system for carcinoma of breast was introduced by Greenhough based on the assessment of eight morphological parameters. Scarff and his colleagues emphasized on the amount of tubule formation, inequality in the size of nuclei and hyperchromatism. Bloom followed Scarff's grading and added an additional criterion of mitotic figures for the grading and along with Richardson gave the numerical scoring system. The Scarff method, modified by Bloom Richardson displayed grade and prognosis and was later adopted by WHO as the preferred grading system.[64]

The Nottingham modification of Bloom-Richardson method was published by Elston and Ellis which included the percent of tubule formation, degree of nuclear pleomorphism and mitotic count.(65)

Table 1: Histologic Grading Using Nottingham Modification of Scarff-Bloom-Richardson System (65)

Criteria	Score 1	Score 2	Score 3
Tubule formation	>75% of tumour	10-75% of tumour	<10% of tumour
Nuclear pleomorphism	Minimal variation in shape and size of nuclei	Moderate variation in shape and size of nuclei	Marked variation in shape and size of nuclei
Mitotic count per 10hpf(0.44mm)	0-5	6-10	>11

Table 2: The Scores of the Histological Grading(65)

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

TNM STAGING

The TNM (primary tumour [T], regional lymph nodes [N], distant metastasis [M]) staging was introduced by the American Joint Committee for Cancer (AJCC) in the year 1959.[66]

It is the most widely accepted staging system which is used to provide information to the patients. It is periodically updated according to the advancements in the field of oncology.[67]

Table 3: TNM Staging (AJCC)[67] Primary Tumour (T)

Tx	Primary tumour cannot be assessed
T0	No evidence of primary tumour
Tis	Carcinoma in situ
Tis(DCIS)	Ductal carcinoma in situ
Tis(LCIS)	Lobular carcinoma in situ
Tis(Pagets)	Pagets disease of the nipple NOT associated with invasive carcinoma and/or carcinoma in situ (DCIS and/or LCIS) in the underlying breast parenchyma. Carcinomas in the breast parenchyma associated with Pagets disease are categorized based on the size and characteristic of the parenchymal disease, although presence of Pagets disease should still be noted.
T1	Tumour $\leq$ 20 mm in greatest dimension
T1mi	Tumour $\leq$ 1 mm in greatest dimension
T1a	Tumour > 1 mm but $\leq$ 5 mm in greatest dimension
T1b	Tumour > 5 mm but $\leq$ 10 mm in greatest dimension
T1c	Tumour > 10 mm but $\leq$ 20 mm in greatest dimension
T2	Tumour > 20 mm but $\leq$ 50 mm in greatest dimension
T3	Tumour > 50 mm in greatest dimension
T4	Tumour of any size with direct extension to the chest wall and/or to the

	skin (ulceration or skin nodules) Note: Invasion of dermis alone does not qualify as T4
T4a	Extension to the chest wall, not including only pectoralis muscle adherence/invasion
T4b	Ulceration and/or ipsilateral satellite nodules and/or edema (including peau d'orange) of the skin, which do not meet the criteria for inflammatory carcinoma
T4c	Both T4a and T4b
T4d	Inflammatory carcinoma

Regional Lymph Nodes (N) CLINICAL

Nx	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastases
N1	Metastases to movable ipsilateral level I, II axillary lymph node(s)
N2	Metastases in ipsilateral level I, II axillary lymph nodes that are clinically fixed or matted; or in clinically detected* ipsilateral internal mammary nodes in the absence of clinically evident axillary lymph node metastases
N2a	Metastases in ipsilateral level I,II axillary lymph nodes fixed to one another (matted) or to other structures
N2b	Metastases only in clinically detected* ipsilateral internal mammary nodes and in the absence of clinically evident level I, II axillary lymph node metastases
N3	Metastases in ipsilateral infraclavicular (level III) axillary lymph node(s) with or without level I, II axillary lymph node involvement; or in clinically detected* ipsilateral internal mammary node(s) with clinically evident level I, II axillary lymph node metastases; or metastases in ipsilateral supraclavicular lymph node(s) with or without axillary or internal mammary lymph node involvement
N3a	Metastases in ipsilateral infraclavicular lymph node(s)
N3b	Metastases in ipsilateral internal mammary lymph node(s) and axillary lymph node(s)
N3c	Metastases in ipsilateral supraclavicular lymph node(s)

Regional Lymph Nodes (N) PATHOLOGICAL

pNx	Regional lymph nodes cannot be assessed
pN0	No regional lymph node metastases
pN1mi	Micrometastases (larger than 0.2 mm, but not larger than 2 mm in greatest dimension)
pN1	Metastasis in 1-3 ipsilateral axillary lymph node(s), and/or in internal mammary nodes with microscopic metastasis detected by sentinel lymph node dissection but not clically apparent
pN1a	Metastasis in 1-3 ipsilateral axillary lymph node(s), including at least one larger than 2 mm in greatest dimension
pN1b	Internal mammary lymph nodes with microscopic metastasis detected by sentinel lymph node dissection but not clinically apparent
pN1c	Metastasis in 1 - 3 axillary lymph nodes and internal mammary lymph nodes with microscopic metastasis detected by sentinel lymph node dissection but not clinically apparent
pN2	Metastasis in 4 - 9 ipsilateral axillary lymph nodes, or in clinically apparent ipsilateral internal mammary lymph node(s) in the absence of axillary lymph node metastasis
pN2a	Metastasis in 4-9 axillary lymph nodes, including at least one that is larger than 2 mm
pN2b	Metastasis in clinically apparent internal mammary lymph node(s), in the absence of axillary lymph node metastasis
pN3	Metastasis in 10 or more ipsilateral axillary lymph nodes; or in

	infraclavicular lymph nodes; or in clinically apparent ipsilateral internal mammary lymph nodes in the presence of one or more positive axillary lymph nodes; or in more than 3 axillary lymph nodes with clinically negative, microscopic metastasis in internal mammary lymph nodes; or in ipsilateral supraclavicular lymph nodes
pN3a	Metastasis in 10 or more axillary lymph nodes (at least one larger than 2 mm) or metastasis in infraclavicular lymph nodes
pN3b	Metastasis in clinically apparent internal mammary lymph node(s) in the presence of one or more positive axillary lymph node(s); or metastasis in more than 3 axillary lymph nodes and in internal mammary lymph nodes with microscopic metastasis detected by sentinel lymph node dissection but not clinically apparent
pN3c	Metastasis in supraclavicular lymph node(s)

Distant Metastases (M)

M0	No clinical or radiographic evidence of distant metastases
cM0(i+)	No clinical or radiographic evidence of distant metastases, but deposits of molecularly or microscopically detected tumor cells in circulating blood, bone marrow, or other nonregional nodal tissue that are no larger than 0.2 mm in a patient without symptoms or signs of metastases
M1	Distant detectable metastases as determined by classic clinical and radiographic means and/or Histologically proven larger than 0.2mm

Other Staging Systems

There are two other staging systems

Summary Stage System – it is used by state cancer registries. It classifies the cancer as “localised”, “regional” or “distant”. Information about the incidence and the cancer extension at the time of diagnosis is provided by this staging system.

The other system is the Extent of Disease System (EOD system). The National Cancer Institute Surveillance Epidemiology and End Results Program (NCI SEER) developed this system in the 1970s.[67]

MYOEPITHELIAL MARKERS FOR IDENTIFICATION OF INVASION

SMA was a commonly used myoepithelial marker for diagnosis in breast pathology. It has a very high sensitivity but lacks specificity for myoepithelial cells as it also stains stromal myofibroblasts and vascular smooth muscle. Hence, it was recommended to use a panel of markers which included P63, Calponin, SMM-HC and CD10.[68]

Kalof et al compared the staining characteristics of SMM-HC and CD10 and arrived to the conclusion that SMM-HC exhibits higher sensitivity and specificity for myoepithelial cells and is also more cost effective when compared to CD10.[69]

Abdallah DM et al used p63, SMA, CD10 and Calponin for comparison. They concluded that Calponin and P63 were sensitive markers, in comparison with CD10 and SMA, with Calponin exhibiting somewhat higher sensitivity than P63. Specificity for myoepithelial cells was maximum with P63 and least with SMA. Calponin also exhibited less specificity as it also stained myofibroblasts and smooth muscle of blood vessels.[16]

In a similar study conducted by Werling RW et al, Calponin was proved to have higher sensitivity when compared to p63 and SMM-HC. However, p63 did not show any cross-reactivity with either myofibroblasts or vascular smooth muscle cells, as shown by both Calponin and SMM-HC.[11]

Korker MM et al in 2004 also conducted a study on expression of P63 in various malignancies of breast. They came to a conclusion that only 7.4% of invasive breast tumours were positive for P63 of which majority were metaplastic carcinomas.[70]

METHODOLOGY

The present study has been conducted at the Dept. of Pathology of KAHER's JN Medical College, and Dr. Prabhakar Kore Hospital, and Research Centre, Belagavi.

Study design: This is a Cross Sectional study

Inclusion criteria: Well fixed surgically resected and biopsy specimens of breast pathology.

Exclusion criteria:

1. Specimens which had not been fixed optimally.
2. Male breast carcinoma
3. Benign neoplastic conditions like fibroadenoma
4. Inflammatory breast diseases
5. Invasive carcinomas other than invasive ductal carcinoma

Sample Size Calculation: The sample size was calculated using the formula 4pq/d2

p - prevalence

q - 100-p

d- Sample error (10)

Substituting the values in the above formula we obtained a sample size of 30.

Ethical clearance: The present study was approved by Jawaharlal Nehru Medical College's Institutional Ethics Committee on Human subjects Research. (Ref.:MDC/DOME/03)(Annexure II)

Sampling Procedure: All surgically resected and biopsy specimens of breast pathology during the period of January-2017 to December-2018 were included in the study.

Case Selection: Thirty surgically resected specimens of breast carcinoma were collected from KAHER's Dr. Prabhakar Kore hospital and MRC during the period of 2017 to 2018 and were studied in the Dept. of pathology, KAHER's JN medical college.

The clinicopathological parameters, including age, tumor size, clinical diagnosis were obtained from the patient's outpatient and inpatient recordsand requisition forms as per proforma given. The specimens were adequately fixed using 10% neutral buffered formalin. Sections of 4-5 micron thickness were cut from formalin fixed, paraffin embedded (FFPE) blocks. A section from each of these blocks was taken and stained with Haematoxylin and eosin (H&E). (Annexure IV)

The H&E stained slides were evaluated for

- Diagnosis of lesion
- Pattern of growth
- Myoepithelial cell layer
- Invasion
- Blood vessels
- Stromal myofibroblasts

Immunohistochemistry: All cases were studied for the expression of P63 and SMM-HC. For immunohistochemical analysis, tissue sections of 3-4 micron thickness were prepared from FFPE blocks, on saline coated slides. Slides were air dried for 2 hours

at 58^{o}C. Slides were deparafinised, dehydrated and rehydrated. The rehydrated slides were subjected to antigen retrieval using heat in a decloaking chamber. Slides were incubated for 15 min on high heat after adding distilled water. After 15 min, the chamber was opened and slides were immediately transferred to room temperature. Slides were then washed with IHC wash buffer. The sections were then stained according to the IHC procedure. Incubation of these slides was carried out with respective optimized primary antibody for 60 min. A brown precipitate is produced on addition of substrate chromogen. Slides were removed. Appropriate controls were stained with each batch of the study slides. (Annexure V)

Table 4: Antibodies used in IHC

Sr. No.	Antibody	Company	Positive Control
1	P63	Dako	Breast tissue
2	SMM-HC	Dako	Breast tissue

Assessment of expression of P63

Nuclear staining in myoepithelial cells was considered positive, regardless of the intensity. All non specific cytoplasmic staining was ignored.[(11)]

Assessment of expression of SMM-HC

Cytoplasmic staining in myoepithelial cells was considered positive, regardless of the intensity. Staining was also observed in cytoplasm of myofibroblasts in the stroma and vascular smooth muscle cells. All non specific background staining was ignored.[(11)]

Scoring of Immunoreactivity

The reactivity of myoepithelial cells with both these antibodies was scored separately in each case and a score was assigned.[11]

- Score-0: negative (−),
- Score-1: (<25% of target cells positive),
- Score-2: (26–90% of target cells were labeled), or
- Score-3: (91–100% of target cells).

Statistical Analysis

Mean and standard deviations were calculated for the continuous quantitative variables. Categorical data were expressed in terms of rates, ratios and percentages.

Sensitivity and specificity were calculated to assess the diagnostic accuracy of P63 and SMM-HC.

Microsoft excel was also used for the formulation of graphs.

RESULTS

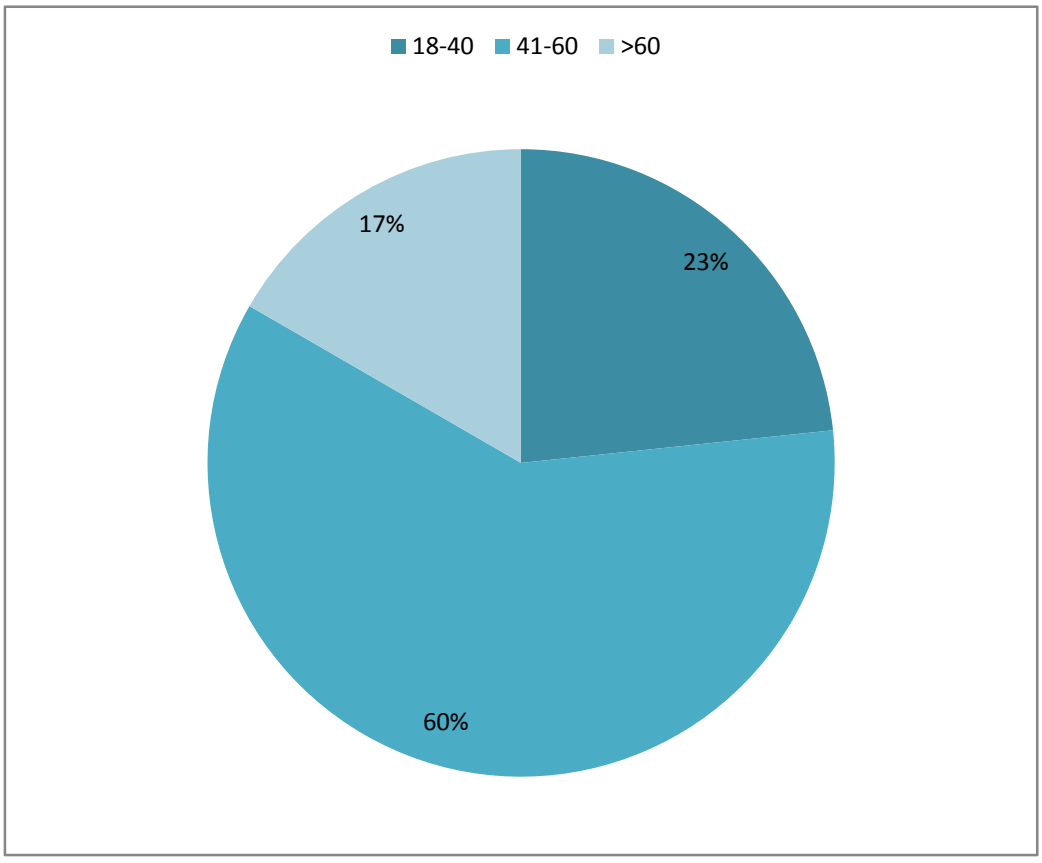
A cross sectional study was conducted in the Dept. of Pathology of KAHER's JN Medical College, and Dr. Prabhakar Kore Hospital, and Research Centre, Belagavi to study the expression P63 and SMM-HC in breast lesions.

A total of 30 cases were evaluated.

Data that was obtained from this study was compiled; tabulated and statistical analysis was performed. The results are presented here under the headings of the various parameters considered for the study.

The patient's age ranged from 18 years to 80 years, with the mean age of 48.8 years. Majority of the women belonged to the age group of 40-65 years.

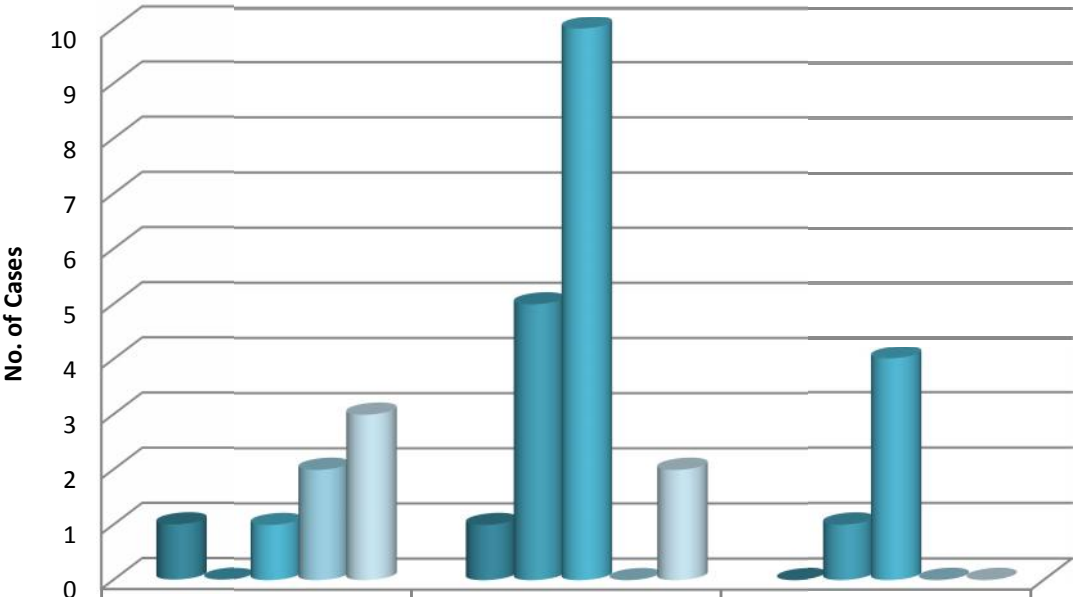
Graph 1: Age distribution



Different lesions which were included in the study were

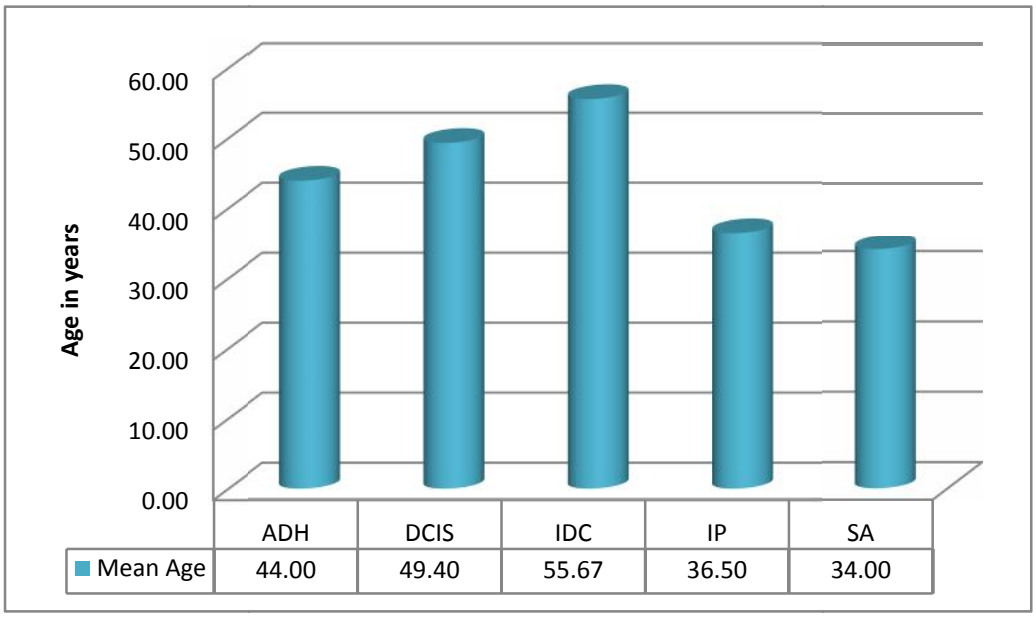
- SclerosingAdenosis - (SA)
- Intraductal Papilloma - (IP)
- Atypical Ductal Hyperplasia - (ADH)
- Ductal Carcinoma In-Situ - (DCIS)
- Invasive Ductal Carcinoma - (IDC)

Graph 2: Age wise distribution of each lesion



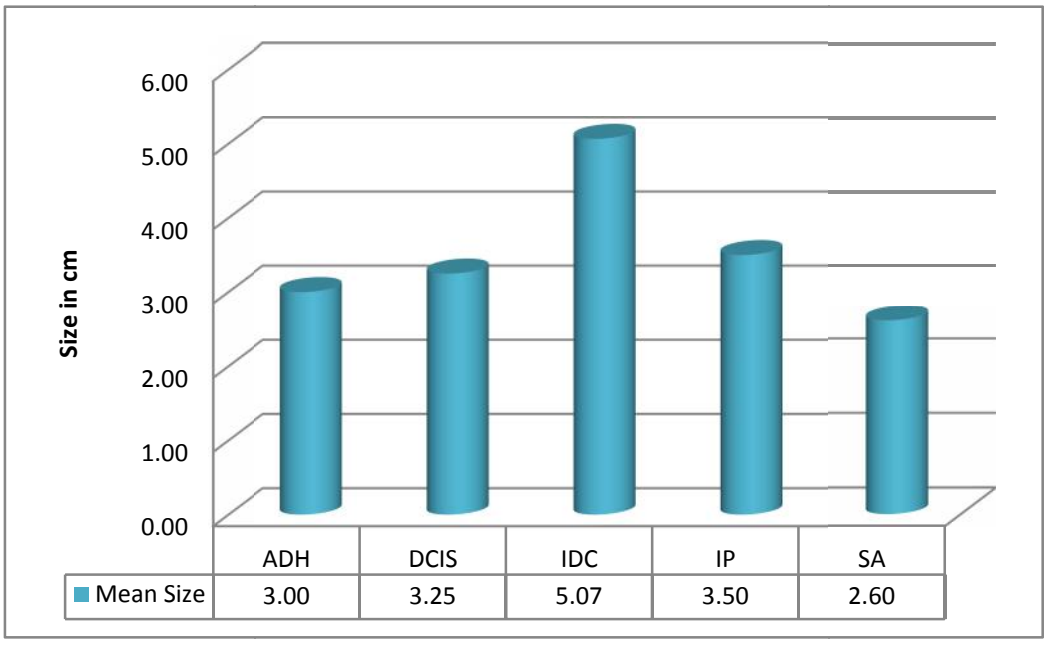
	18-40 years	41-60 years	>60 years
ADH	1	1	0
DCIS	0	5	1
IDC	1	10	4
IP	2	0	0
SA	3	2	0

Graph 3: Mean age of patients



The specimen received varied in size ranging from 1-9 cm in maximum dimension.

Graph 4: Mean size of the lesion



Graph 5: Mean size for age

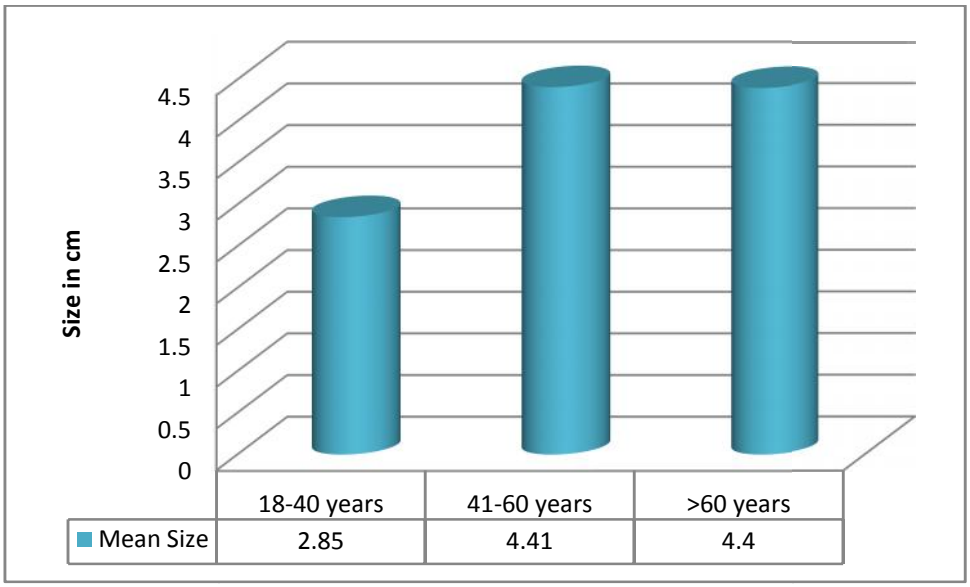


Table 5: Distribution of clinic-pathological parameters

Parameter	Frequency	Percentage
1. Side		
Left	12	40%
Right	18	60%
2. Surgery		
Lumpectomy	14	46.7%
MRM	16	53.3%
3. Clinical diagnosis		
Benign breast lump	14	46.7%
Carcinoma	16	53.3%
4. HPR diagnosis		
IDC	15	50%
Non IDC (SA, IP, DCIS)	15	50%

18 cases (60%) had tumour on the right side and 12 cases (40%) with tumour on left side.

Out of the 30 cases, 14 were clinically diagnosed to have a benign breast lump and 16 were diagnosed to have carcinoma. These 16 cases (53.3%) underwent MRM while the remaining 14 cases (46.7%) underwent lumpectomy.

All the lumpectomy specimen were confirmed to be benign (IP, SA) or pre-invasive (DCIS) lesions on histopathological examination. Of the 16 MRM specimen received, 15 were confirmed to have IDC and one case was diagnosed as DCIS. The overall clinic-histopathological correlation was 96.6%

Out of the 15 cases of IDC, 4 cases were such that they also exhibited a component of DCIS. These cases had a mean value for age as 50.75 years and they showed a mean tumour size of 3.5 cm.

(For analysis of IHC staining, both these entities were considered separately and hence the total sample size was considered to be 34)

Expression of Myoepithelial markers

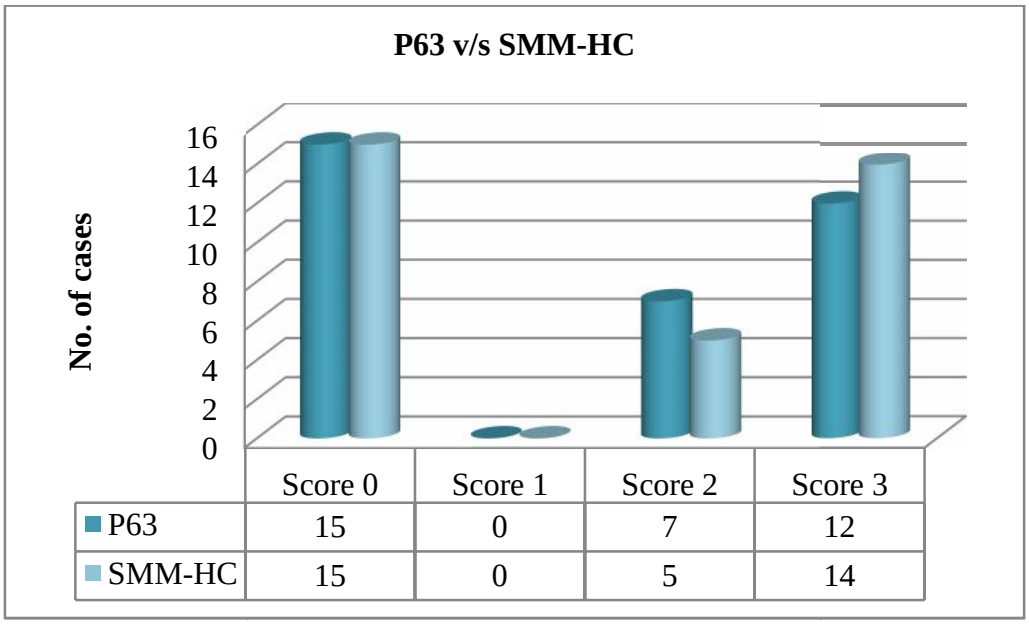
All the cases were stained with P63 and SMM-HC immunohistochemical stains to demonstrate myoepithelial cells.

For a semi-quantitatively analysis of these markers, positivity was scored according to the percentage of cells showing nuclear staining in P63 and cytoplasmic in case of SMM-HC.

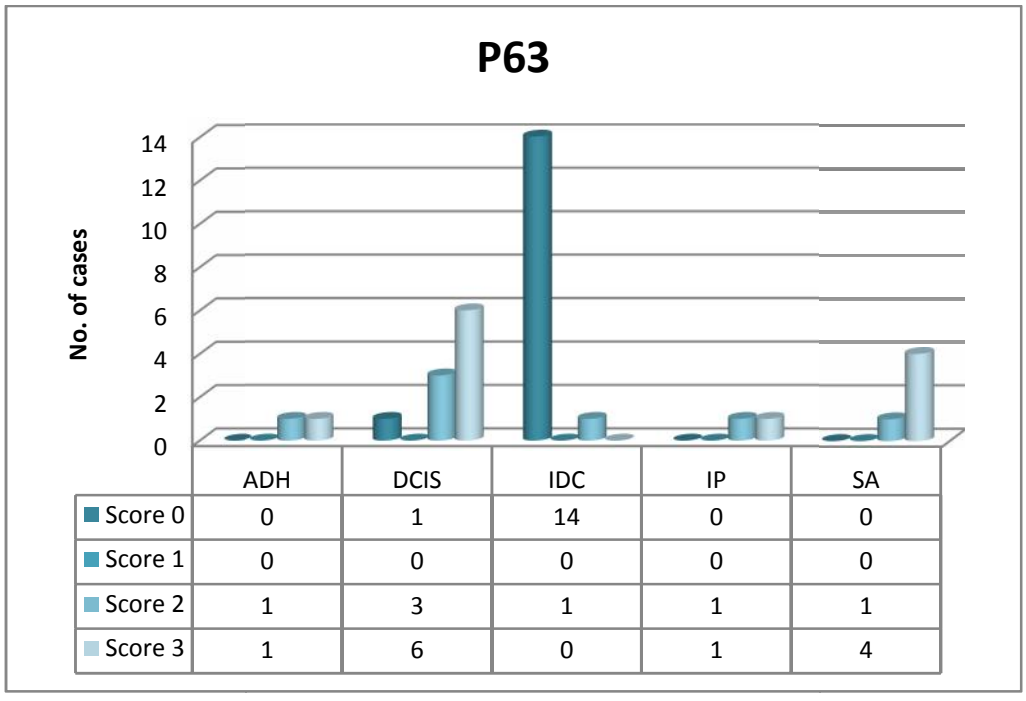
- Score-0 – No staining
- Score-1 – 1-25% of the target cells stain positive
- Score-2 – 26-75% of the target cells stain positive
- Score-3 – >75% of the target cells stain positive

Scores ≥1 were considered positive, and a score of 0 was considered negative.

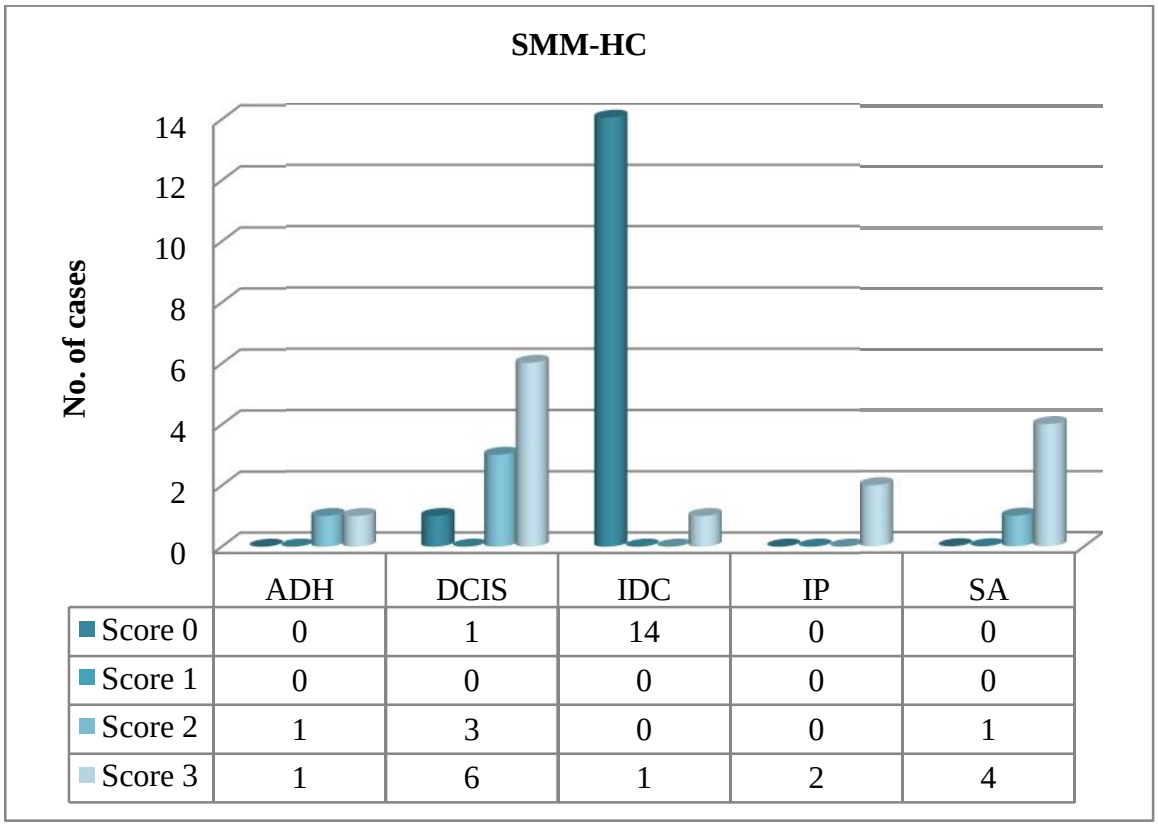
Graph 6: Comparison of P63 and SMM-HC staining



Graph 7: P63 staining score



Graph 8: SMM-HC staining score



	ADH	DCIS	IDC	IP	SA
Score 0	0	1	14	0	0
Score 1	0	0	0	0	0
Score 2	1	3	0	0	1
Score 3	1	6	1	2	4

A histopathological diagnosis of IDC means that there is loss of the myoepithelial cell layer and the lesion is invading into the stromal tissue. Hence, for calculation of Sensitivity and Specificity of the myoepithelial markers, a diagnosis of IDC is considered as negative and non-IDC diagnosis is considered positive.

Table 6: P63 stain positivity

	HPR Diagnosis		
P63	**Non-IDC**	**IDC**	**Total**
Positive	18	1	19
Negative	1	14	15
Total	19	15	34

Sensitivity of P63 – 94.74% with a confidence interval of 84.70%.

Specificity of P63 – 93.33% with a confidence interval of 80.71%.

Positive predictive value of P63 – 94.74% with a confidence interval of 84.70%.

Negative predictive value of P63 – 93.33% with a confidence interval of 80.71%.

Table 7: SMM-HC stain positivity

	HPR Diagnosis		
SMM-HC	**Non-IDC**	**IDC**	**Total**
Positive	18	1	19
Negative	1	14	15
Total	19	15	34

Sensitivity of SMM-HC – 94.74% with a confidence interval of 84.70%.

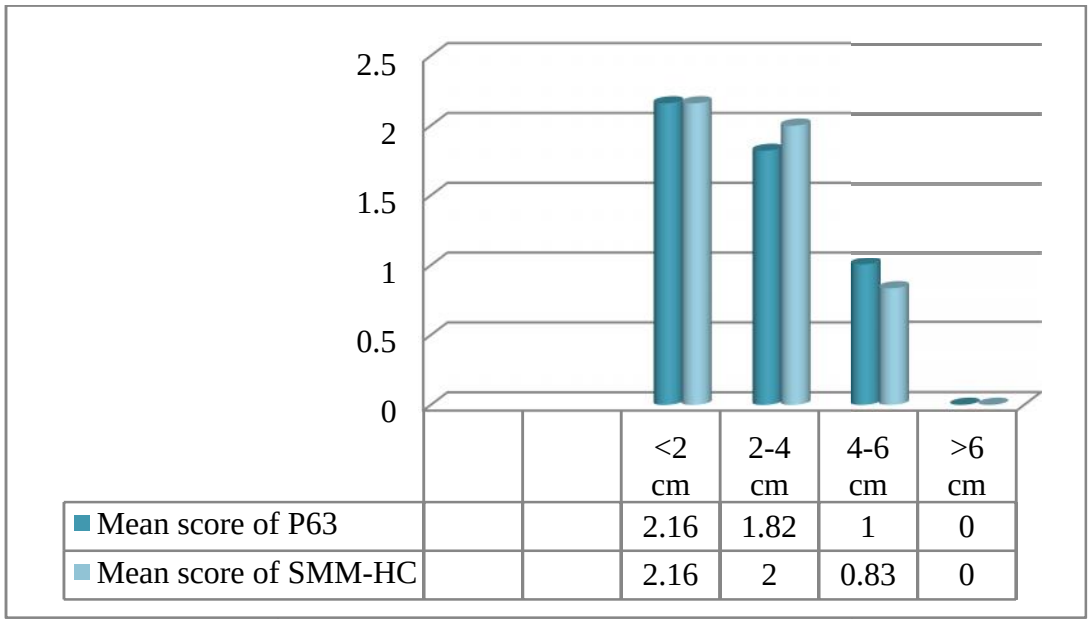
Specificity of SMM-HC – 93.33% with a confidence interval of 80.71%.

Positive predictive value of SMM-HC – 94.74% with a confidence interval of 84.70%.

Negative predictive value of SMM-HC – 93.33% with a confidence interval of 80.71%.

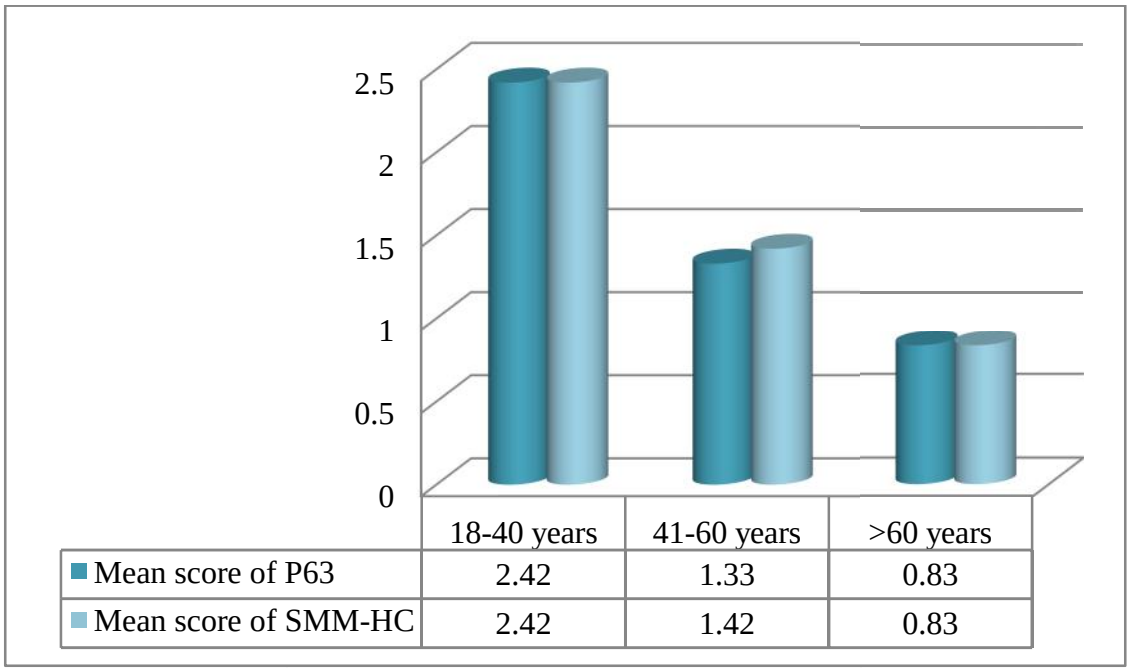
k value – 0.88 (confidence interval of 0.73)

Graph 9: Mean score for size



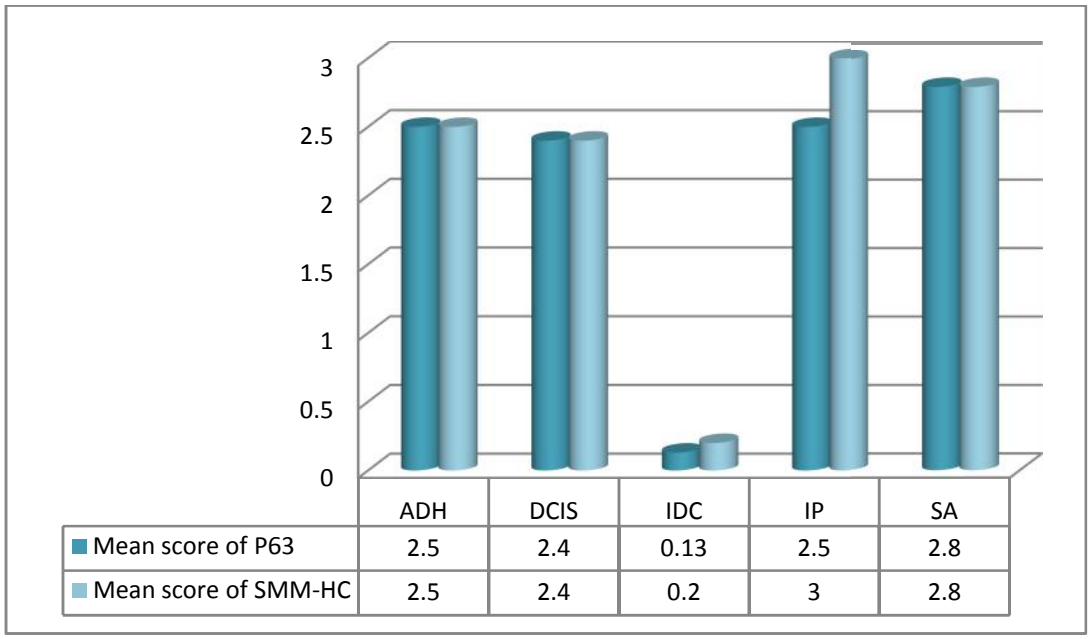
			<2 cm	2-4 cm	4-6 cm	>6 cm
Mean score of P63			2.16	1.82	1	0
Mean score of SMM-HC			2.16	2	0.83	0

Graph 10: Mean score for age



	18-40 years	41-60 years	>60 years
Mean score of P63	2.42	1.33	0.83
Mean score of SMM-HC	2.42	1.42	0.83

Graph 11: Mean score for each diagnosis

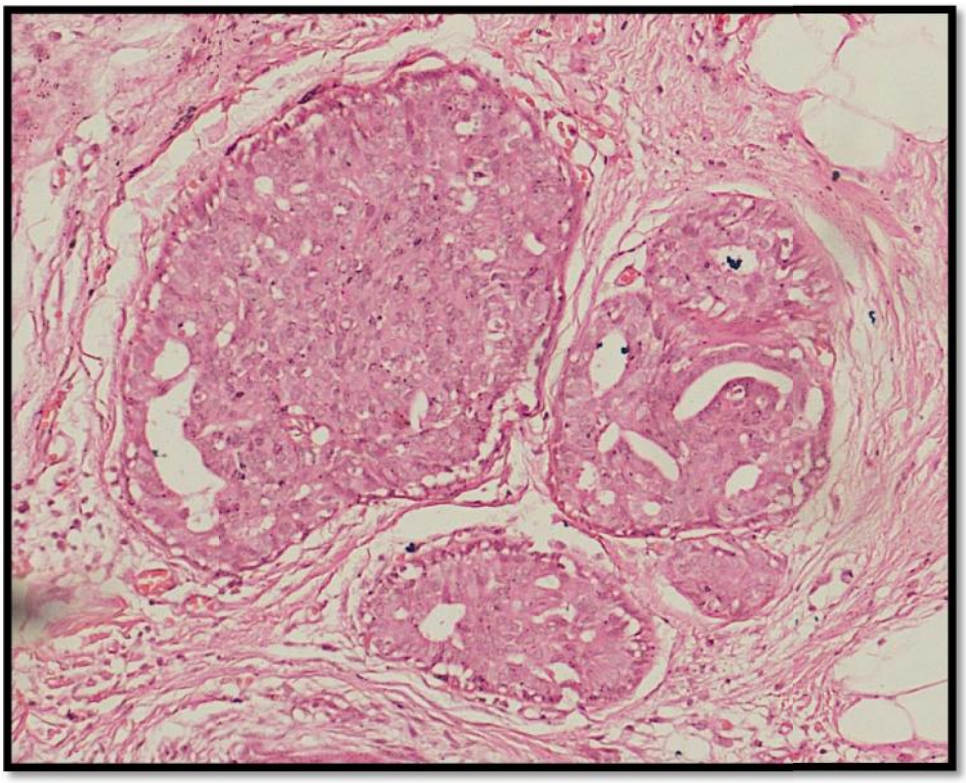


	ADH	DCIS	IDC	IP	SA
Mean score of P63	2.5	2.4	0.13	2.5	2.8
Mean score of SMM-HC	2.5	2.4	0.2	3	2.8

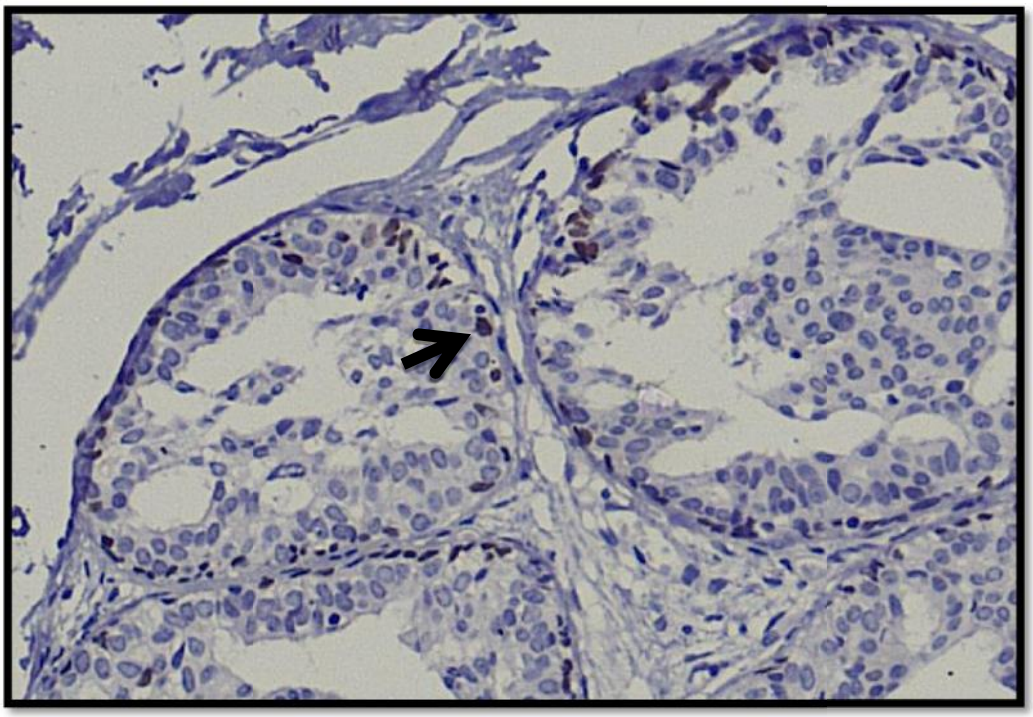
Cross-reactivity of SMM-HC

Vascular smooth muscle cell positivity – 100% (30 out of 30 cases)

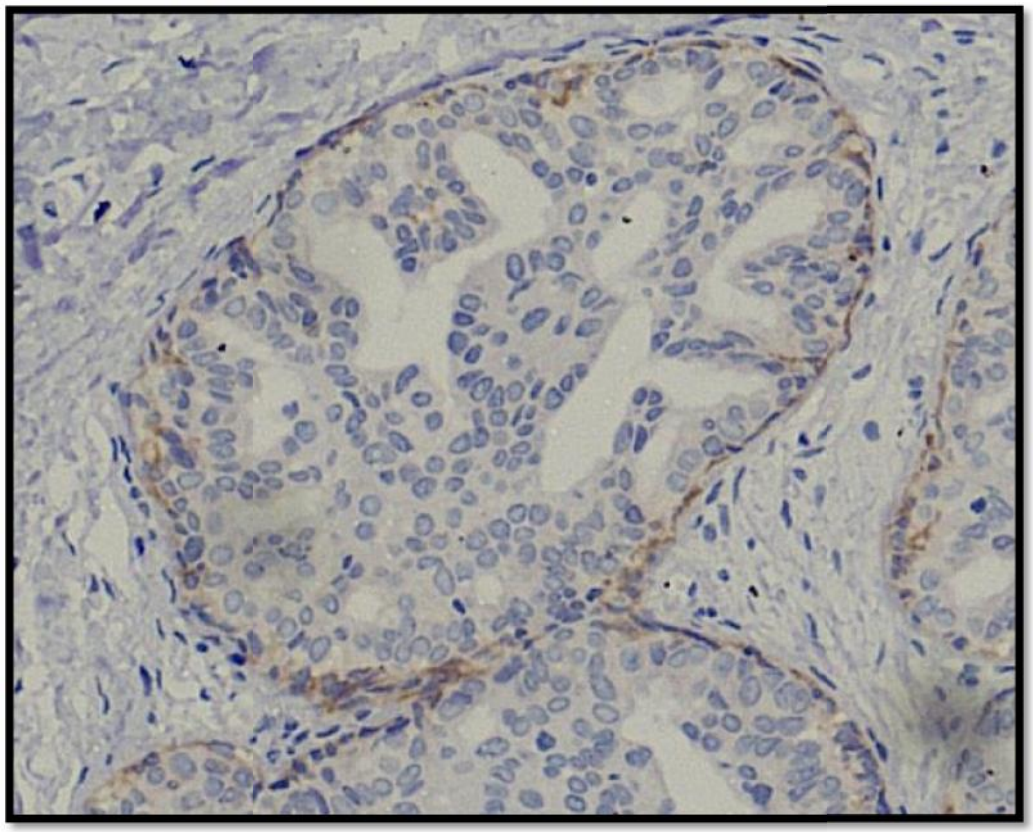
Stromal myofibroblasts positivity – 40% (12 out of 30 cases)



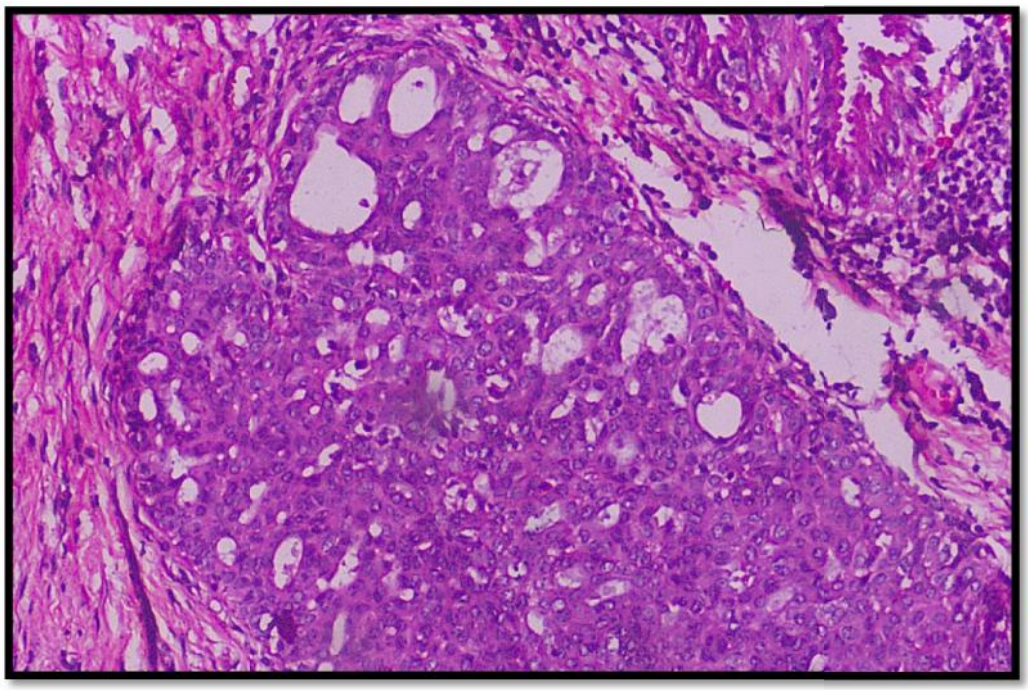
Photograph 1a: Atypical Ductal Hyperplasia (H&E stain 20x)



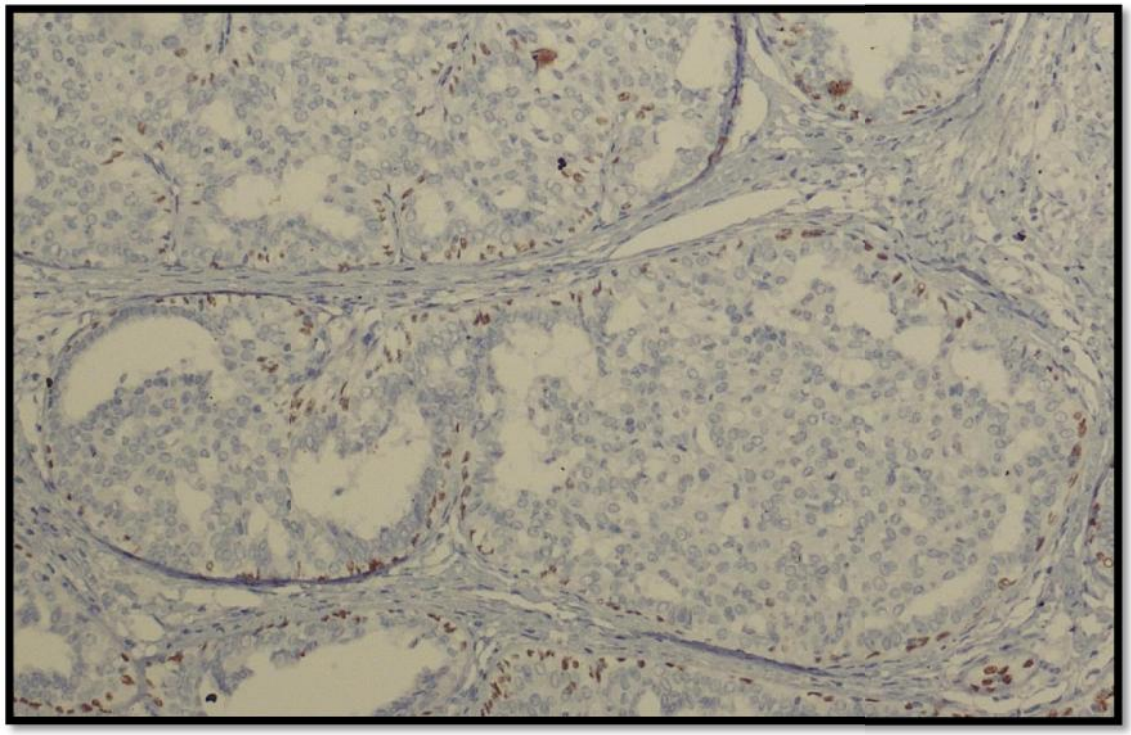
Photograph 1b: P63 positivity in a case of Atypical Ductal Hyperplasia. IHC score – 2 (IHC stain 40x)



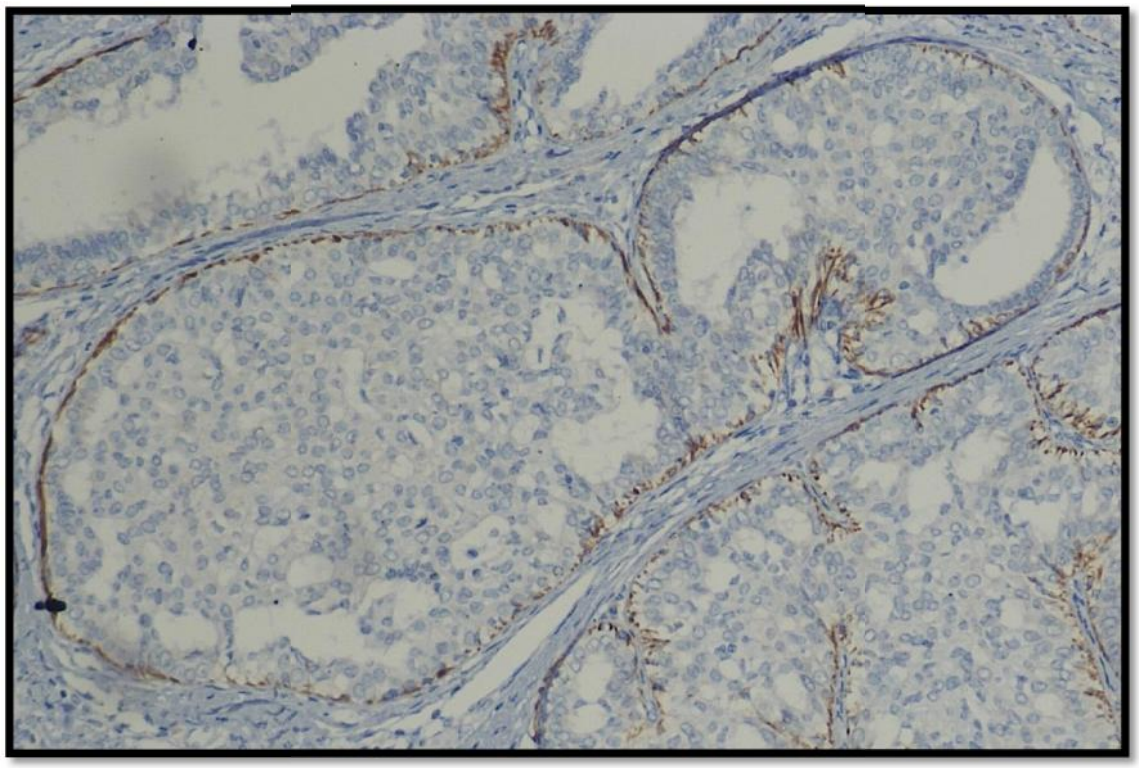
Photograph 1c: SMM-HC positivity in a case of Atypical Ductal Hyperplasia. IHC score – 3 (IHC stain 40x)



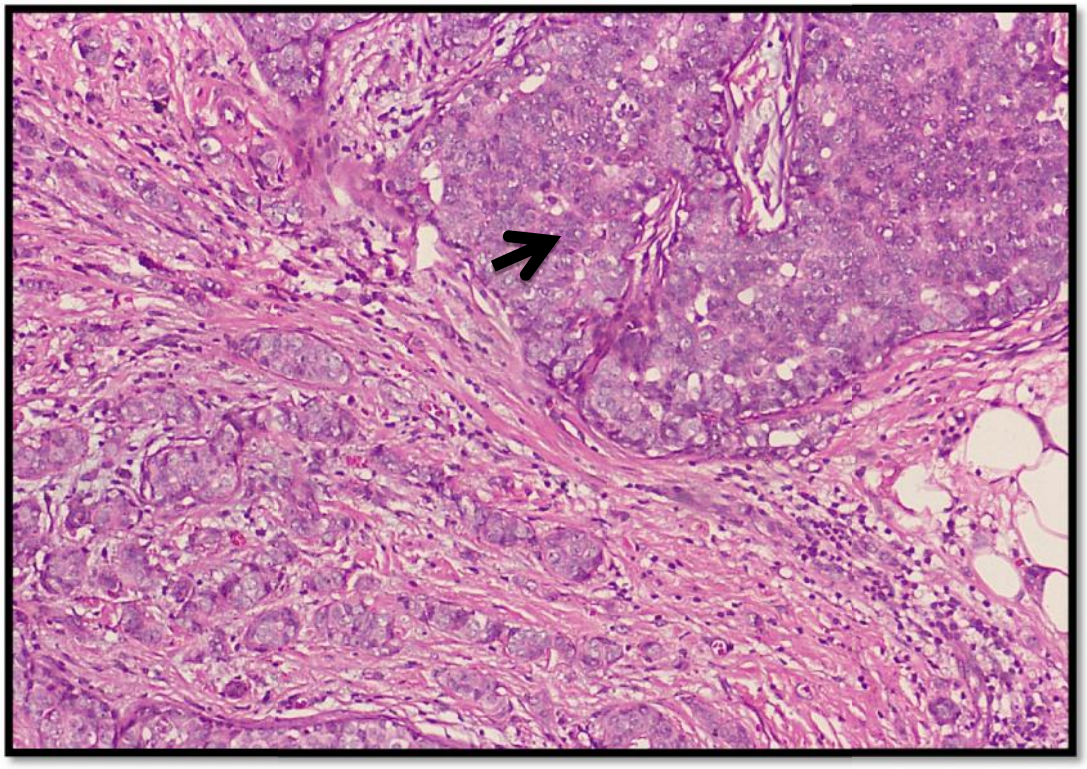
Photograph 2a: Ductal Carcinoma In-Situ (H&E 20x)



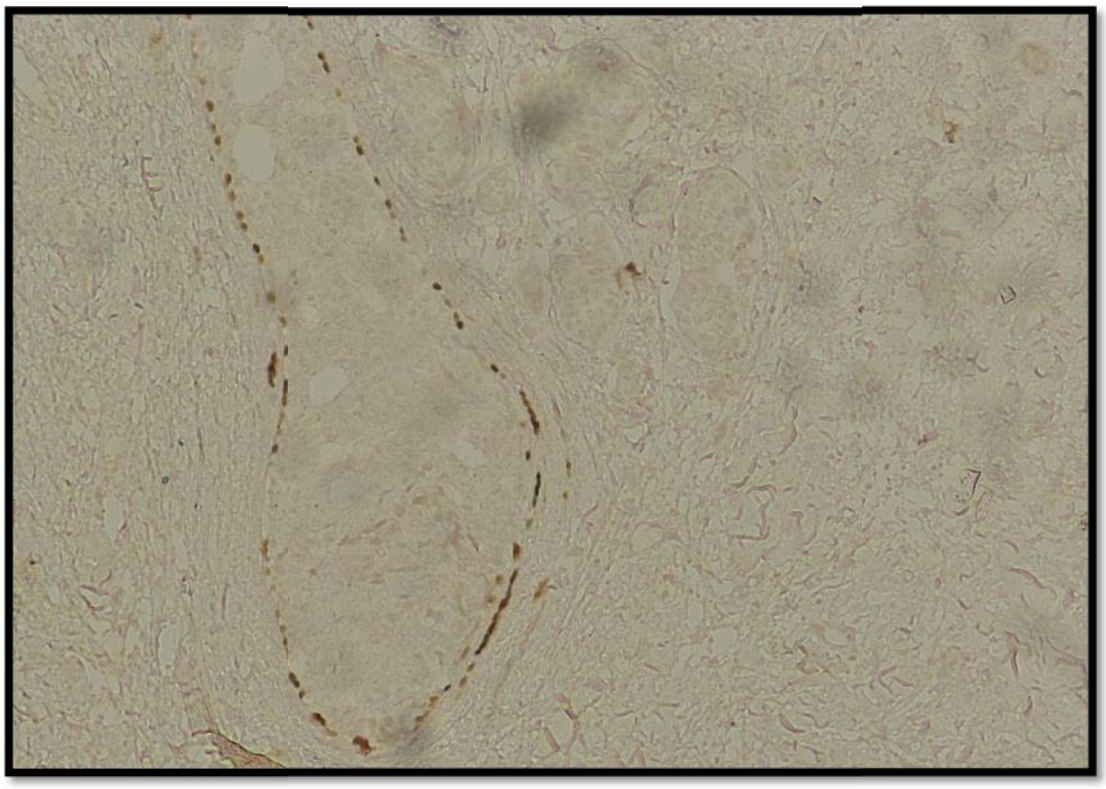
Photograph 2b: P63 positivity in a case of DCIS. IHC score – 2 (IHC stain 10x)



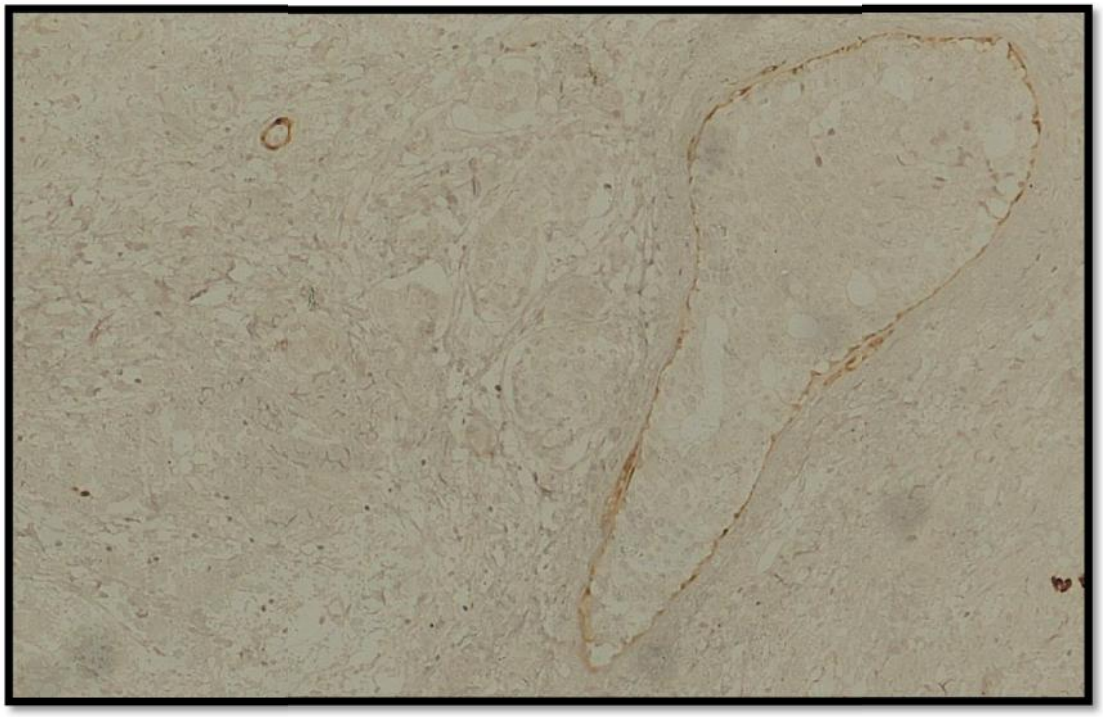
Photograph 2c: SMM-HC positivity in a case of DCIS. IHC score – 3 (IHC stain 10x)



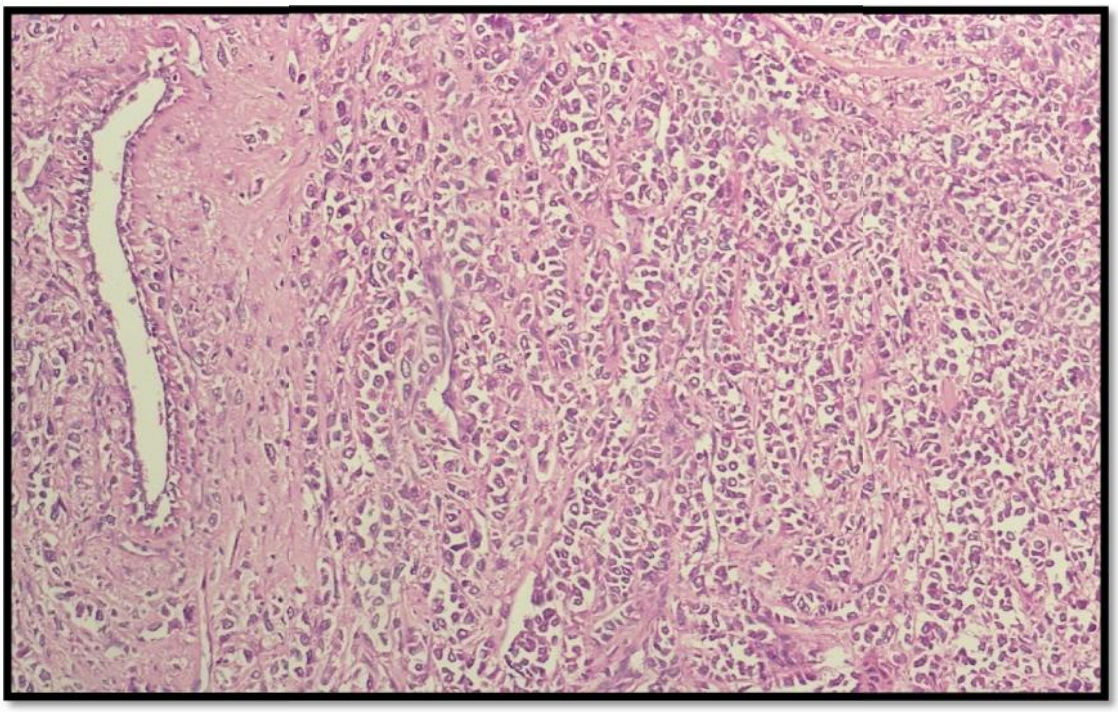
Photograph 3a: Invasive Ductal Carcinoma with a focus of DCIS(arrow) (H&E 10x)



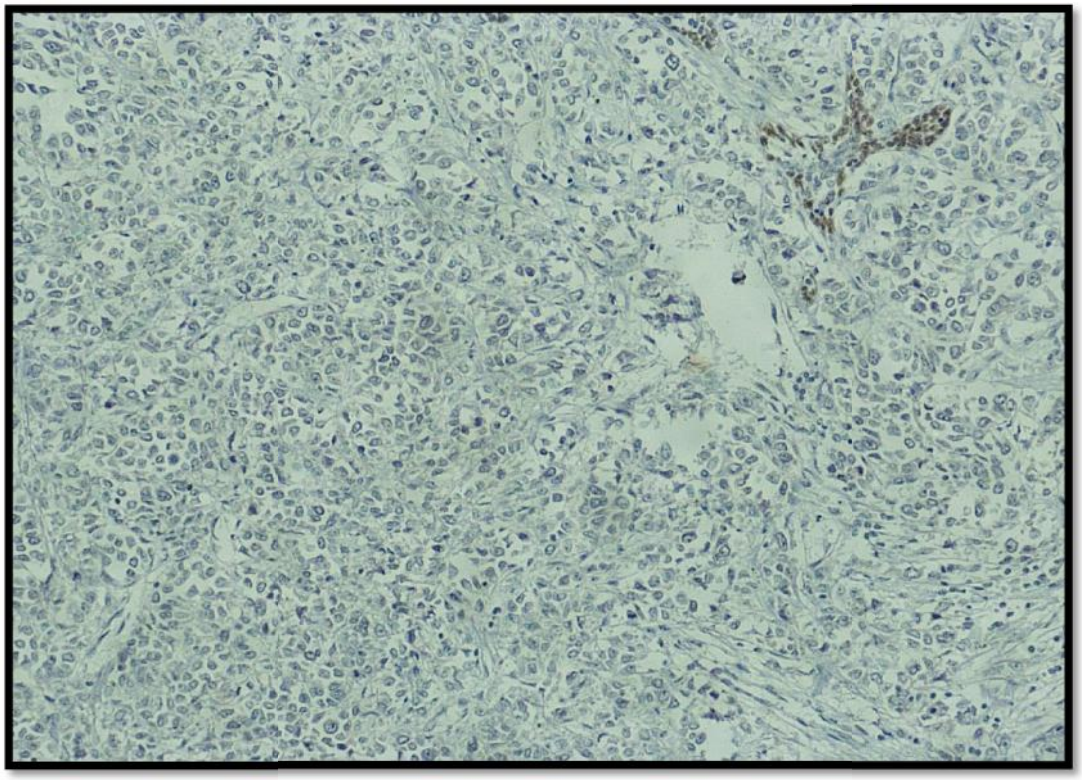
Photograph 3b: P63 positivity in a focus of DCIS in the above case (IHC 10x)



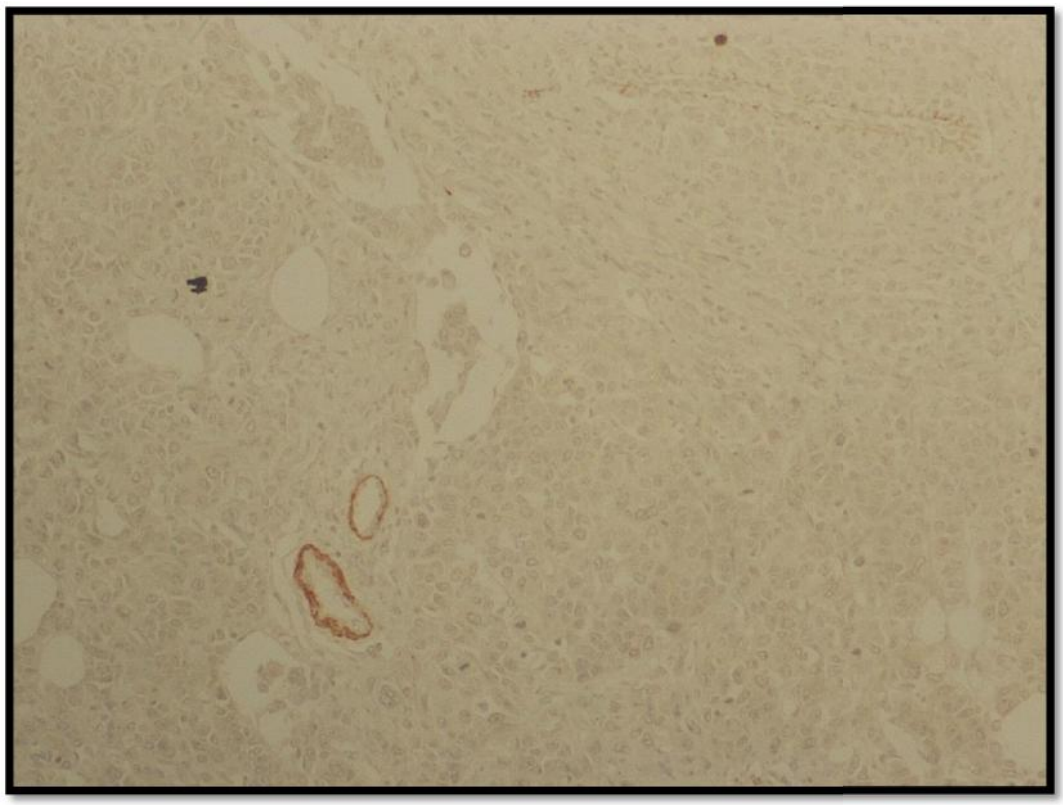
Photograph 3c: SMM-HC positivity in a focus of DCIS in the above case (IHC 10x)



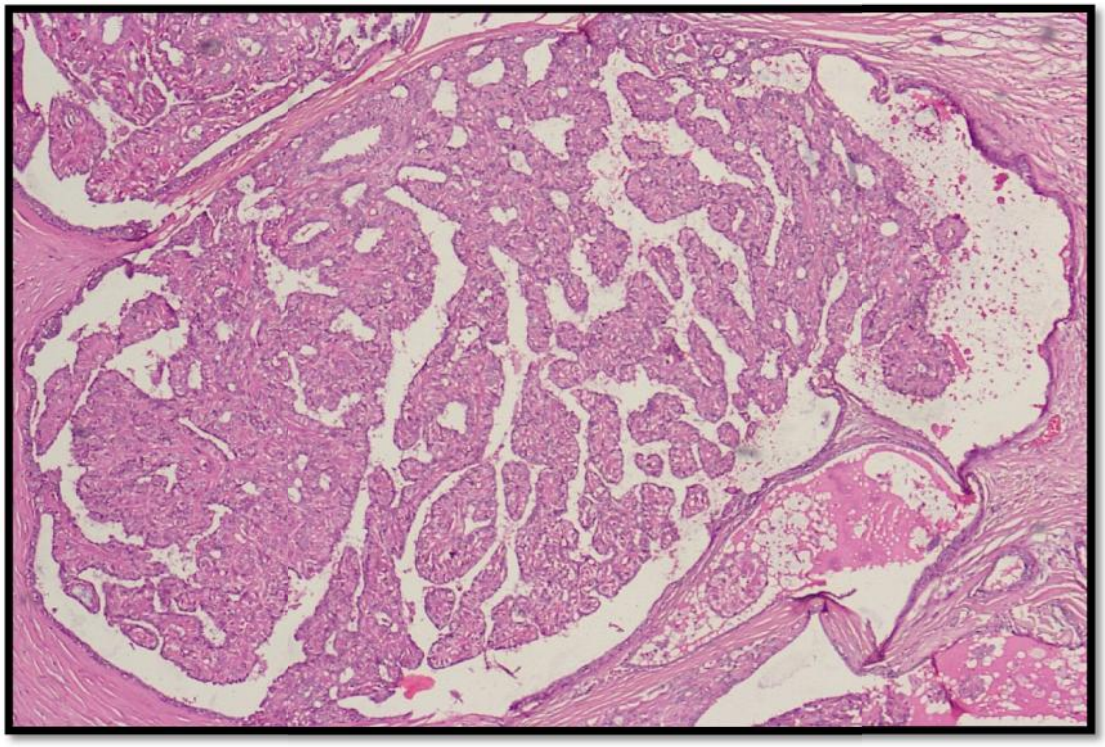
Photograph 4a: Invasive Ductal Carcinoma (H&E 10x)



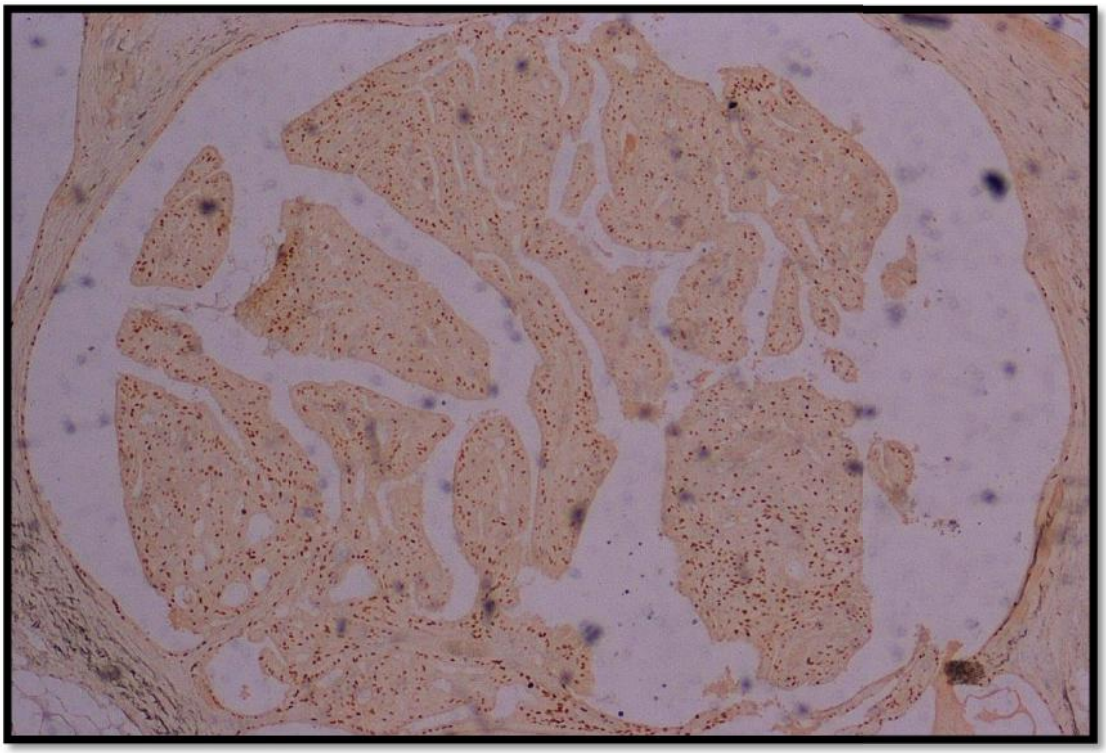
Photograph 4b: Tumour foci negative for P63 in a case of IDC (IHC 10x)



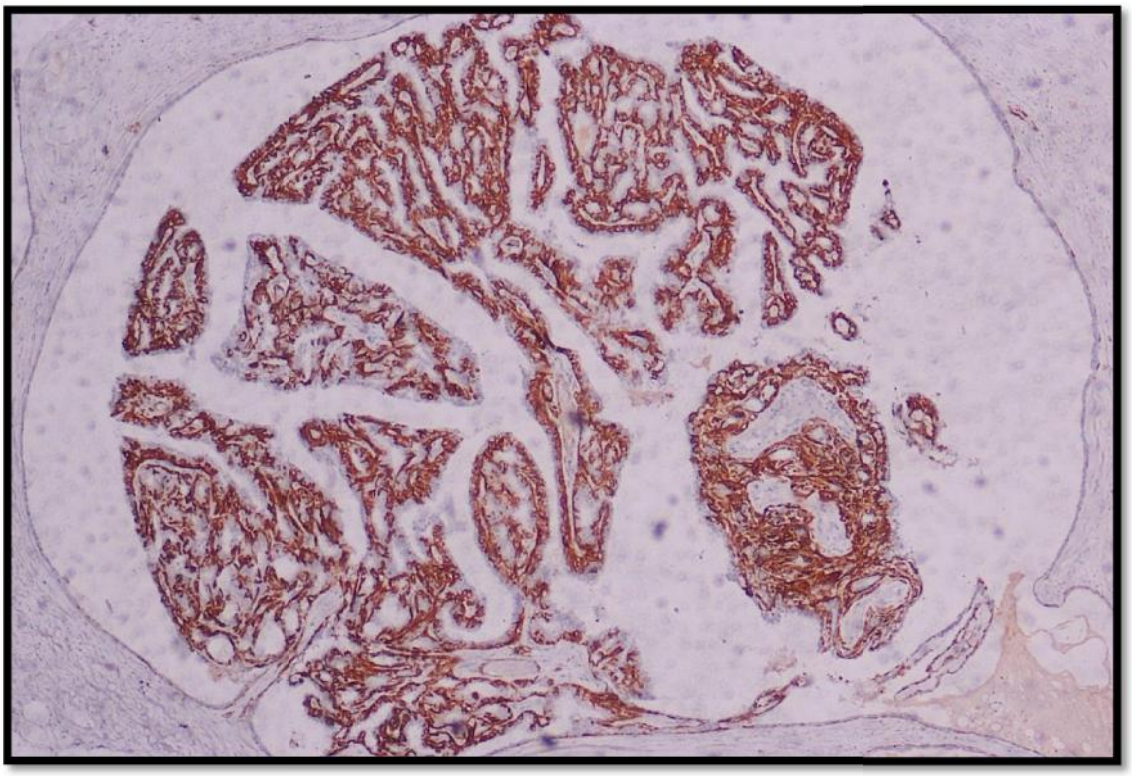
Photograph 4c: Tumour foci negative for SMM-HC in a case of IDC (IHC 10x)



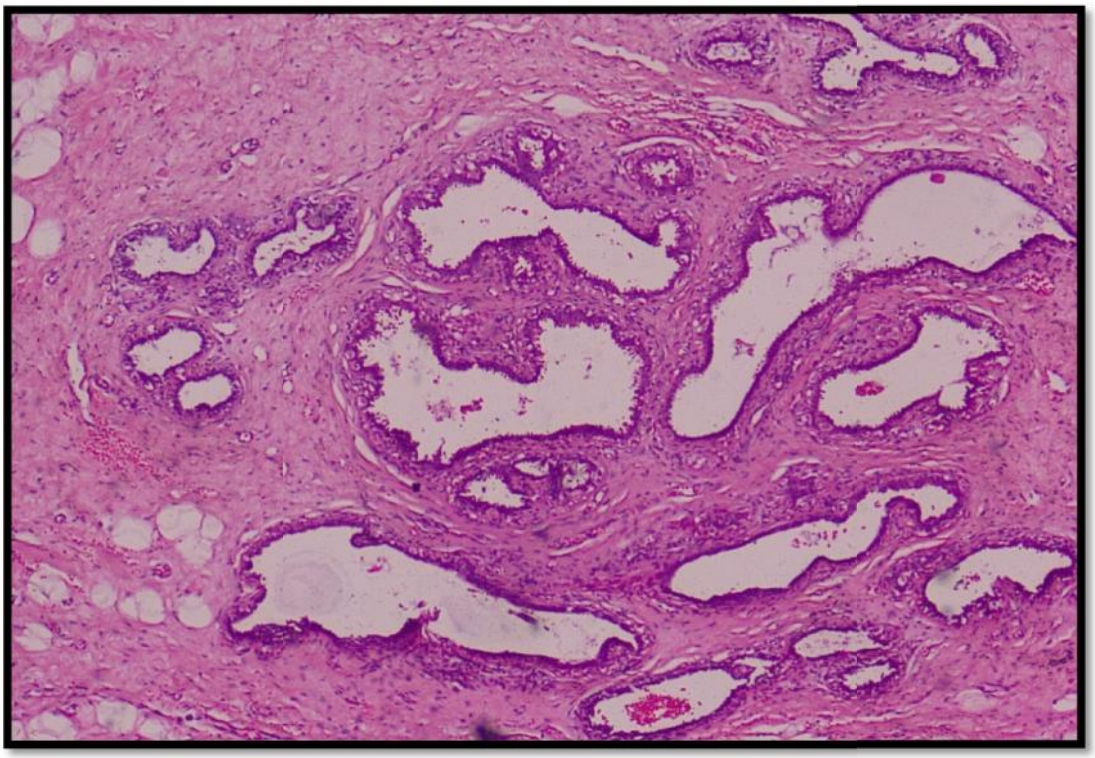
Photograph 5a: Intraductal Papilloma (H&E 10x)



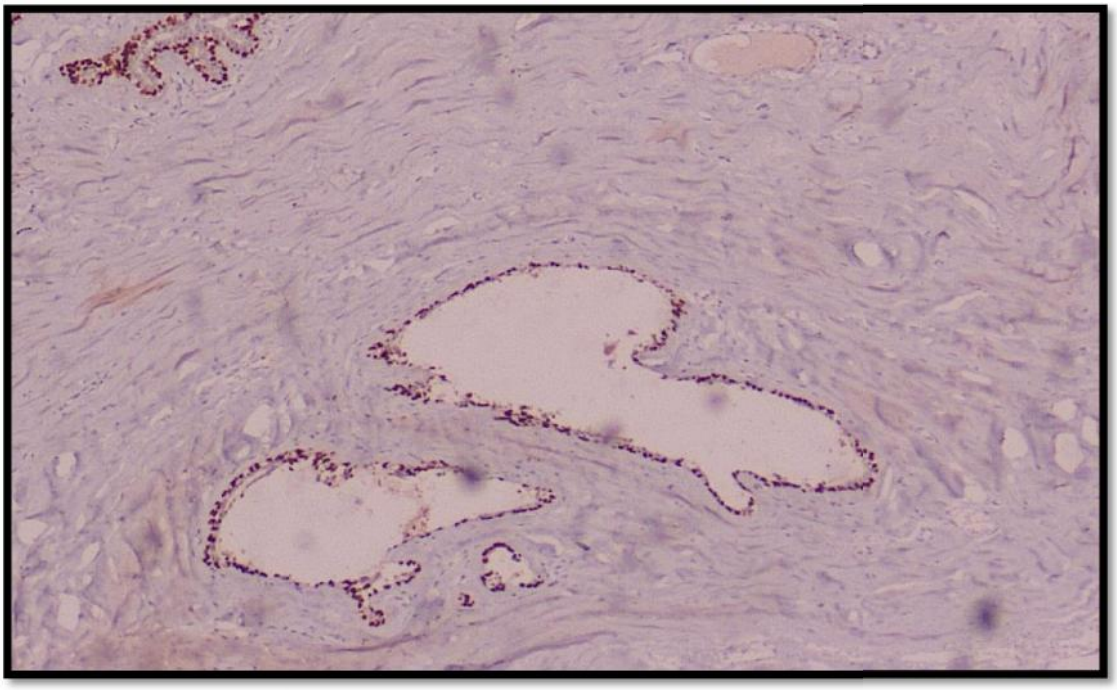
Photograph 5b: P63 positivity in a case of Intraductal Papilloma. IHC score – 3 (IHC stain 10x)



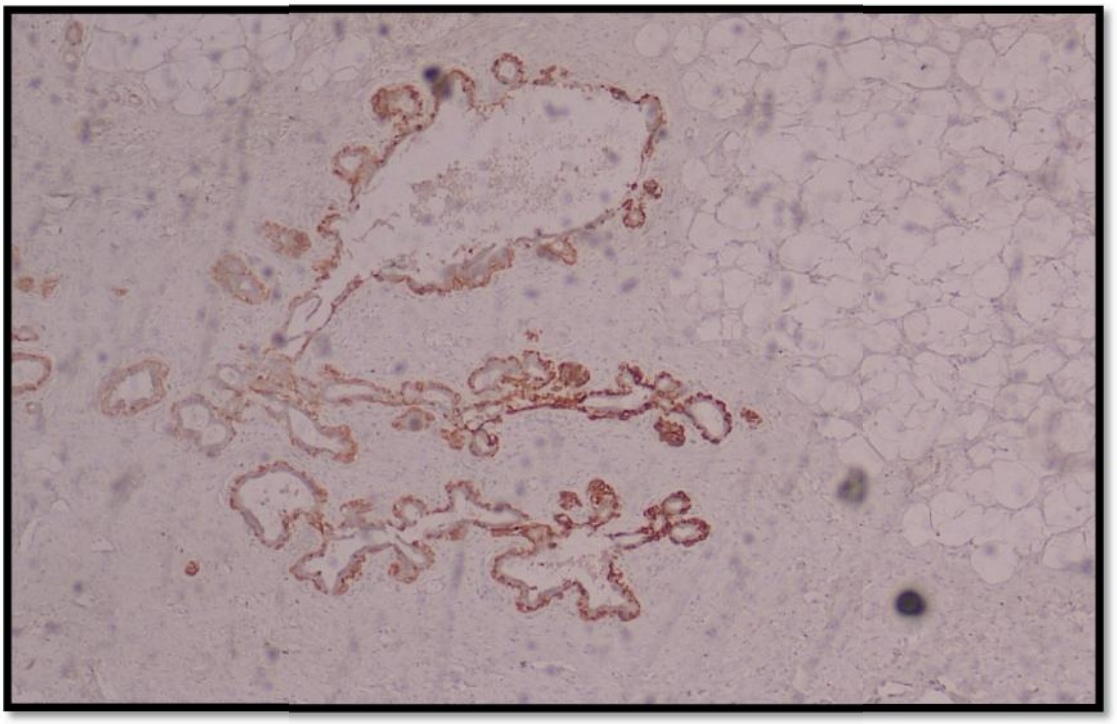
Photograph 5c: SMM-HC positivity in a case of Intraductal Papilloma. IHC score – 3 (IHC stain 10x)



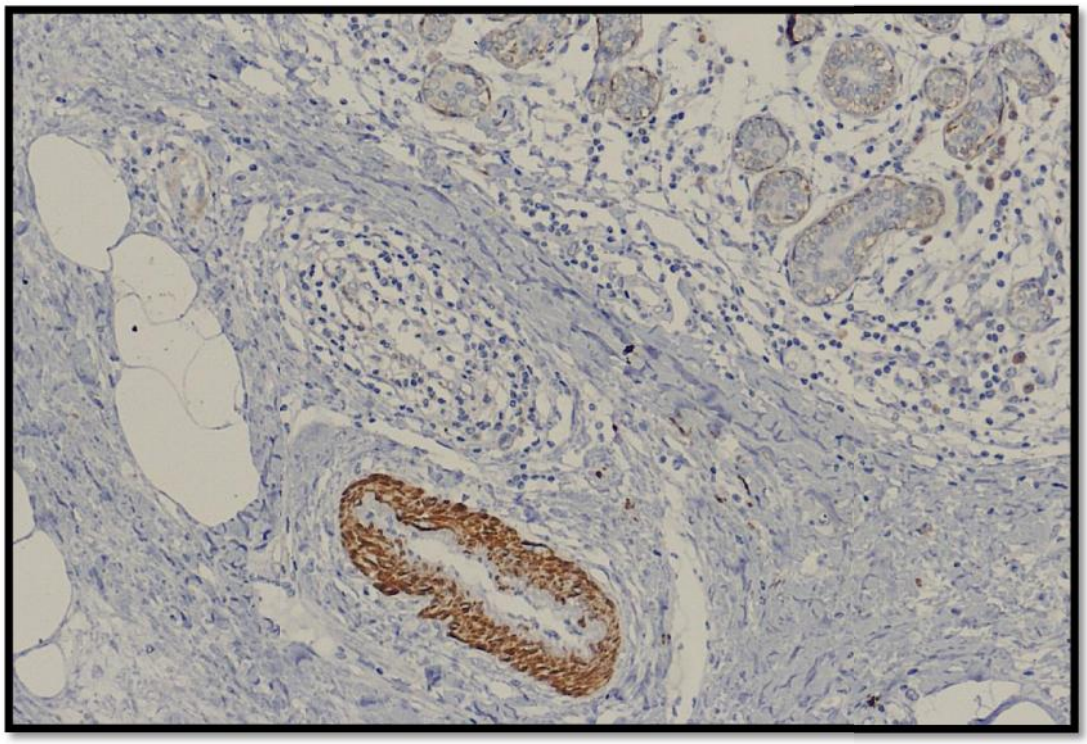
Photograph 6a: Sclerosing Adenosis (H&E 10x)



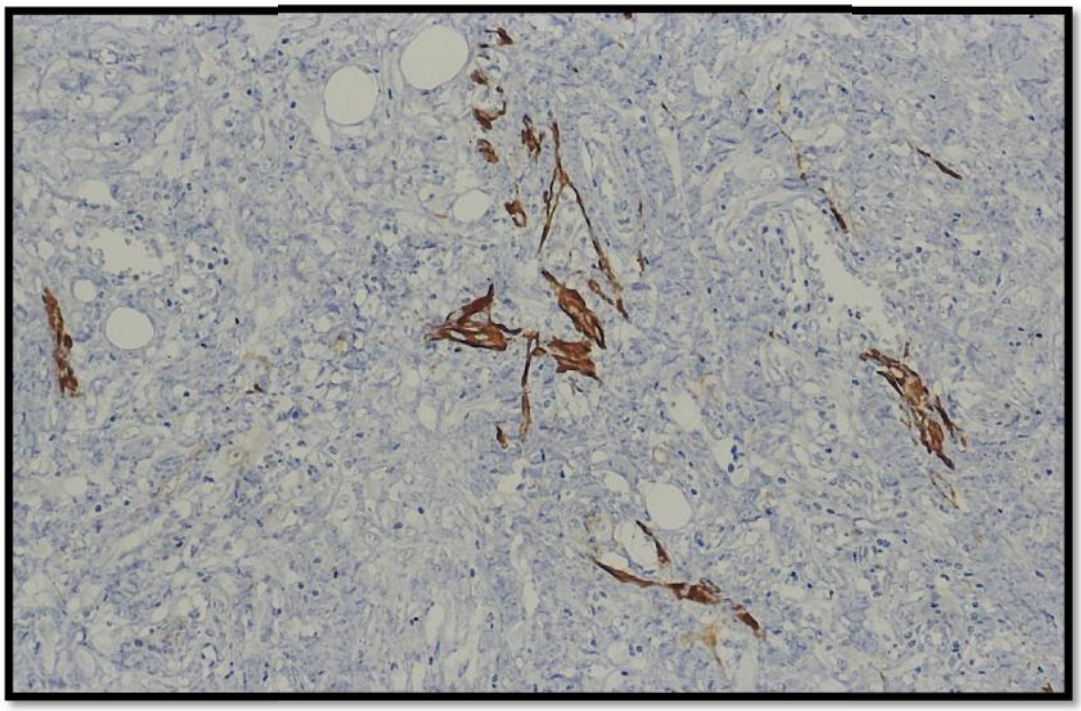
Photograph 6b: P63 positivity in a case of Sclerosing Adenosis. IHC score – 3 (IHC stain 10x)



Photograph 6c: SMM-HC positivity in a case of Sclerosing Adenosis. IHC score – 3 (IHC stain 10x)



Photograph 7: Vascular smooth muscle cells positive for SMM-HC (IHC 10x)



Photograph 8: Myofibroblasts positive for SMM-HC (IHC 4x)

DISCUSSION

Breast cancer is one of the most frequently diagnosed malignancies in women all over the world, with an estimated 1.7 million cases and 521,900 deaths in 2012. Developed countries show a relatively high prevalence, accounting for nearly half the cases of breast cancer.[71,72]

Differentiating a pre-invasive breast lesion from invasive carcinoma holds paramount prognostic importance. Myoepithelial cell layer plays a crucial role in aiding this distinction as loss of myoepithelial cells is the hallmark of invasion.[8-10]

Over the past decade, numerous immunohistochemical markers for myoepithelial cells have been identified. An ideal marker is one that would manifest nearly absolute sensitivity and specificity for these cells. It would not cross-react with any other type of cell in the breast tissue, such as stromal myofibroblasts, smooth muscle cells of the blood vessels, carcinoma cells or benign epithelial cells.[11] The earlier myoepithelial cell markers still in wide use include S-100 protein, high molecular weight CK, and smooth muscle actins. These markers have low sensitivities and specificities that make them unsuitable candidate as myoepithelial markers.[11] Another marker which is widely used is SMM-HC. It demonstrates higher sensitivity and specificity when compared to previously mentioned markers, however, it also shows cross-reactivity with smooth muscle cells of blood vessels and myofibroblasts in the stroma. All the markers currently in use are not ideal, mainly because of their cross-reactivities or because they have low sensitivity and specificity.[73]

P63 is the first nucleus staining antigen for myoepithelial cells. Barbareschi et al noted the difference between patterns of staining of the nucleus with anti-p63

antibodies from that seen with antibodies to cytoplasmic proteins. Nuclear pattern has an advantage of "cleaner" staining but there can be difficulty in interpretation as there is no direct continuity between nuclei of two adjacent myoepithelial cells, therefore, a discontinuous pattern of P63 can be observed even if continuous myoepithelial cell layer is present.[73]

The purpose of our present study was to compare the efficacy of SMM-HC, which as of today is considered to have the maximum sensitivity and specificity against P63 and also to study the clinicopathological parameters of certain pre-invasive and invasive breast lesions.

Our analysis of 30 women with breast lesions comprised of 15 cases of IDC and 15 cases with diagnosis other than IDC (ADH, DCIS,IP and SA). There was 96.6% clinico-histopathological correlation as all but one case were shown to have confirmed the clinically suspected lesion. Concurrent presence of foci of DCIS was seen in 4 out of the 15 cases of IDC. For evaluation of P63 and SMM-HC both the lesions in these cases were considered separately.

Immunohistochemical staining with both P63 and SMM-HC was negative in a total of 15 lesions out of 34 lesions which were considered for evaluation of IHC staining, of which 14 were IDC and 1 was a focus of DCIS present in a case of IDC. One case of IDC showed false positive result for P63 as well as SMM-HC.

In our study sensitivity and specificity of both P63 and SMM-HC were identical as only one false negative and one false positive result were seen in both the stains. (Sensitivity – 94.74%; Specificity – 93.33%) A k-value of 0.88 was calculated which suggests almost perfect agreement between the two raters.

Staining of myoepithelial cells with both these immunohistochemical markers was scored on a scale of 0-3 as mentioned earlier. Mean score for SMM-HC was observed to be 1.52 while that for P63 was 1.47, stating that staining pattern of myoepithelial cells with SMM-HC is slightly more complete when compared with that of P63. Discontinuous staining was observed in 37% of the cases with P63 and in 31% of the cases with SMM-HC. The nuclei of neighbouring myoepithelial cells are not in perfect alignment with each other. This can be a reason for reduced score for P63 when compared to SMM-HC.[11]

P63 did not demonstrate any cross-reactivity with any other cell in the tissue. SMM-HC, being a component of muscle protein also stained smooth muscle cells of the blood vessels and myofibroblasts present in the stroma. Cross-reactivity was 100% with vascular smooth muscle cells and it was 40% with stromal myofibroblasts (12 out of 30 cases).

A study conducted by Werling et al reported that SMM-HC has a slightly higher sensitivity (93%) when compared with P63 (90%), however, P63 demonstrated higher specificity for myoepithelial cells. This study also compared both these markers with Calponin and concluded that Calponin has the highest sensitivity and the lowest specificity among the three markers. Discontinuous staining was observed in 10% of the cases with P63 and 7% of the cases with SMM-HC. They also observed 10% of the neoplasms demonstrated ductal cells which stained positive for P63. The ductal cells positivity for P63 was not observed in the current study.[11]

A comparison study between CD10 and SMM-HC was conducted by Kalof et al in 2004. The researchers in this study emphasised on staining patterns of both these

markers and concluded that SMM-HC when compared to CD10 has increased specificity and sensitivity for myoepithelial cells and is also more cost effective.[69]

Abdallah et al in the year 2017 published a study comparing P63 with SMA, CD10 and Calponin. They stated that among these four markers, highest sensitivity was seen with Calponin (65%), followed by P63 (54%). In this study as well P63 demonstrated highest specificity for myoepithelial cells and it did not exhibit any cross-reactivity. CD10 although showed high specificity for myoepithelial cells, its sensitivity was very poor (19.5%). SMA on the other hand demonstrated poor specificity as well as sensitivity.[16]

Another study conducted by Koker et al exclusively studied expression of P63 in various breast malignancies. They have stated that 7.4% (14 out of 189 cases) of the carcinomas express P63 positivity, of which majority (13 cases) were metaplastic carcinomas. In these cases, the tumour cells that did stain positive were predominantly spindle shaped cells. Rest of the cases did not show any P63 staining in the tumour cells.[69]

In the present study, we also studied the staining in all the different morphological entities. Mean score with both the antibodies was high in benign conditions like SA and IP. In conditions like ADH and DCIS, it was slightly lower and in frankly invasive carcinoma it was nearly zero.

When the score was compared with age of the patient and size of the lesion, it was observed that the mean value decreases with increase in age and an increase in size. This can be attributed to the fact that incidence of IDC increases in elderly patients,[32] as was observed in the study.

The mean age of patients diagnosed with IDC was 55.67 years. In the present study, we observed that in the age group of >60 years all the patients were diagnosed with IDC except one case, which was diagnosed as DCIS. In the age group of 40-60 years, the proportion of patients with IDC was 55.55% with other common diagnosis being DCIS. Majority of the benign lesions were seen in patients younger than 40 years with just one case in this age group being diagnosed as invasive carcinoma.

When each of the diagnoses in the study were plotted against the mean size of the lesion, it was observed that IDC presented as the largest masses with a mean size of 5.07 cm in maximum dimension. Marked variations can be seen in the size of IDC which can range from a lesion <1 cm to as large as 10 cm.[45] However, conditions like intraductal papillomas are rarely larger than 5 cm and sclerosing adenosis is usually only a few centimetres in size. The mean sizes of both these lesions in our study were, 3.5 cm and 2.6 cm respectively.

The mean size of the lesion was higher in the age group of more than 60 years (4.4 cm) as compared to those in the age group of 18-40 years (2.85 cm). This observation is also attributed to higher prevalence of IDC in older patients.

16 out of the 30 patients underwent Modified Radical Mastectomy (MRM) as a treatment for suspected invasive carcinoma. Of these 15 patients were confirmed to have IDC on histopathological examination. One of the patients who underwent MRM was diagnosed to have DCIS and this procedure could have been avoided if a pre-operative diagnosis would have been established. The other 14 cases in which clinical diagnosis of benign breast lesions was made correlated with the histopathological findings as well.

Our study also included 4 cases of IDC which showed presence of foci of DCIS. P63 and SMM-HC scoring for both the morphological entities in these cases was done separately. One of these cases showed complete absence of myoepithelial cell staining with both the above mentioned antibodies in the foci of IDC as well as DCIS. Complete absence of myoepithelial cells suggests that the focus which morphologically resembles DCIS is an area of well demarcated invasive carcinoma making it difficult to differentiate.

There is need for more studies with larger sample size and with more diverse morphological entities in order to provide more detailed information regarding the role of myoepithelial markers in distinguishing invasive and non-invasive lesions.

CONCLUSION

Myoepithelial cells are present around the ductal epithelial cells in the terminal duct lobular unit of the breast and these cells form a boundary between the duct and the stroma. Breach of this boundary is the hallmark sign of invasion.

In the present study we conclude that both the immunostains for myoepithelial cells, P63 and SMM-HC have very high sensitivity (94.74%) and specificity (93.33%) in differentiating invasive ductal carcinoma from non-invasive lesions.

However, the limitations of both these stains are that the former shows incomplete staining pattern whereas the later shows cross-reactivity with vascular smooth muscle cells and myofibroblasts.

Both these markers could be used in conjuncture with each other to diagnose cases in which clear infiltrative morphology of invasive carcinomas is not apparent or the tumour margin is obscured as a result of inflammation.

Our study showed 96.6% clinical and histopathological correlation.

SUMMARY

A total of 30 cases of breast lesions were studied for expression of myoepithelial markers of P63 and SMM-HC. These cases included frank IDC along with benign and pre-invasive lesions like DCIS, ADH, IP and SA.

It was observed that positive staining was seen in majority of benign lesions and it was absent in frank carcinomas. The data was tabulated and sensitivity and specificity were calculated. Both the markers demonstrated equal sensitivity (94.74%) and specificity (93.33%). SMM-HC showed cross-reactivity with vascular smooth muscle cells (100%) and stromal myofibroblasts (40%). This was not seen with P63.

Majority of the patients were in the age group of 41-60 years. Malignant diagnosis like IDC and pre-invasive diagnosis like DCIS were more common in the older age group as compared to those patients in the age group of 18-40 years who presented with diseases like SA and IP.

Mean size of the lesion was also higher in the older age group as these patients commonly manifested invasive carcinomas. Size of lesion in SA and IP were usually less than 5 cm.

There were four cases of IDC which showed concurrent presence of foci of DCIS. In one of these cases, the DCIS focus showed absence of peripheral myoepithelial cell layer as demonstrated by negative staining with both P63 and SMM-HC.

We have concluded that both these myoepithelial markers can be used for differentiating invasive carcinoma from non-invasive lesions. However, it is advised to use them concurrently as both of them complement each other for making a conclusive diagnosis.

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ANNEXURE I - INFORMED CONSENT

COMPARISON OF EFFICACY OF P63 AND SMOOTH MUSCLE MYOSIN HEAVY CHAIN IMMUNOMARKERS IN DISTINGUISHING INVASIVE AND NON INVASIVE BREAST LESIONS – A ONE YEAR OBSERVATIONAL STUDY.

Purpose of the study: You are being asked to enroll in this study as you are eligible for participation in this study. If you undergo mastectomy or lumpectomy for a breast lesion you will be included in this study.

The purpose of this study is to determine the efficacy of p63 and SMMHC in distinguishing invasive and non-invasive breast lesions. This study will help in determining a better diagnostic tool for invasiveness of cancers.

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. The principal investigator of the study is Dr._____ under the guidance of Dr._____guide).

If you agree to enroll yourself in this study, you will be interviewed regarding your present, past and family history and your clinical manifestations.

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

Alternatives: Taking part in this study is voluntary. You may choose not to take part in this study or if you decide to take part now, you can later change your mind and

withdraw from the study. The study doctor or sponsor may terminate your participation in this study anytime.

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

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

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

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

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

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

CONSENT STATEMENT

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

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

Principal Investigator:

Guide :

Name of the participant:

(signature/thumbprint):

Name of the witness:

(signature)

Name of the investigator:

(signature)

Date:

Address:

Phone no.

ANNEXURE II – ETHICAL CLEARANCE

K.L.E.UNIVERSITY'S
JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)
(Accredited 'A' Grade by NAAC)

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Principal: 2471701
Fax No. +91 (0)831 – 2470759

Ref: MDC/DOME/03 **Date: 22/11/2017**

To,

PG student in Pathology,
J.N.Medical College,
BELAGAVI.

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"COMPARISON OF EFFICACY OF P63 AND SMOOTH MUSCLE MYOSIN HEAVY CHAIN IMMUNOMARKERS IN DISTINGUISHING INVASIVE AND NON INVASIVE BREAST LESIONS – A ONE YEAR OBSERVATIONAL STUDY"**, is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

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

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

ANNEXURE III - PROFORMA

QUESTIONNAIRE

IP number:

Contact number:

IDENTIFICATION DATA

Name:

Age:

Proforma

EXAMINATION

- Side: Right / Left
- Size:

INVESTIGATION

HPR

- HPR diagnosis:
- Cells seen
    - Myoepithelial cells: Y / N
    - Vascular smooth muscle cells: Y / N
    - Myofibroblasts: Y / N

IHC (P63 and SMM-HC)

- Myoepithelial cells: Score: 0 / 1 / 2 / 3
- Other cells:
    - Vascular smooth muscle cells
        - P63: Y / N
        - SMM-HC: Y / N
    - Myofibroblasts
        - P63: Y / N
        - SMM-HC: Y / N

ANNEXURE - IV - HEMATOXYLIN AND EOSIN STAINING PROTOCOL

Bancroft D, Laylon C. The haematoxylin and eosin, In: Kim SS Ed, Bancroft's Theory and practice of histopathological techniques. &th Ed., China, Churchill Livingstone; 2013: p173-87.

1. Deparaffinize in Xylene I and II and III changes. (III change use warmed xylene) (5 minutes in each)
2. Rehydrate using
    a. Absolute ethanol 100% (5 minutes)
    b. Absolute Ethanol 100% (5 minutes)
3. Rinse in distilled water (5 minutes)
4. Rinse in running tap water (5 minutes)
5. Stain in Harris's haematoxylin by progressive method (2 minutes) Fresh andfiltered
6. Rinse in running tap water (20 minutes)
7. Decolorize in 1% acid alcohol (1 second)
8. Rinse well in tap water (5 minutes)
9. Immerse in hot water bath, 55˚C for blueing (3 seconds)
10. Rinse in tap water (5 minutes)
11. Counterstain in Eosin (15 seconds)
12. Dehydrate with absolute alcohol 100% (2-4 dips)
13. Clear in xylene I and II (5 minutes)
14. Mount with DPX.

Stock solution – Eosin:

Stock – 1% aqueous Eosin – Y

Stock – 1% aqueous Phloxin B

Working Solution – Eosin:

100ml stock Eosin

10 ml stock Phloxin B

780 ml 95% Ethanol

4 ml glacial acetic acid

Working Solution – Hematoxylin

Harris Hematoxylin, 1 litre

Working solution – 0.25% Acid alcohol

95% Ethanol, 2578 ml

dH2O, 950 ml

HCl, 9ml

Result: Nuclei – blue, cytoplasm – pink, RBCs – red.

ANNEXURE V – IHC STAINING PROTOCOL

1. Cut the sections at approximately 2- microns
2. Float on to the positive charged slides
3. Slides were air dried for 2 hours at 58 ºC.
4. Two changes of xylene of 10 minutes each for deparaffinization
5. Two changes of absolute alcohol of 5 minute each for rehydration
6. Wash in distilled water for 5 minutes
7. Antigen retrieval by heat, using microwave using TRIS EDTA Buffer
8. Cooling of sections to room temperature
9. Rinse in distilled water for 3 minutes
10. Wash in TBS buffer two times for 3 minutes each
11. Treatment with peroxide block for 10 minutes to block endogenous peroxidase enzyme
12. Wash in TBS buffer two times for 3 minutes each
13. Treatment with primary antibody (Dako P63 and Dako SMMHC monoclonal anti human antibody) for 60 minutes to identify the myoepithelial cells by antigen-antibody reaction
14. Wash in TBS buffer two times for 3 minutes each
15. Treatment with Target binder for 10 minutes
16. Wash in TBS buffer two times for 3 minutes each
17. Treatment with HRP Polymer for 10 minutes
18. Wash in TBS buffer two times for 3 minutes each
19. Treatment with DAB (secondary antibody) for 3-5 minutes to give brown color to antigens

20. Wash in distilled water for 3 minutes
21. Counter stain with Harris haematoxylin for 30 seconds to 1 minute
22. Wash in tap water for 3 minutes to remove excess stain
23. Two changes of absolute alcohol for 2 minute each for dehydration
24. Clearing with xylene for two minutes. Dry the slides and mount with DPX

Preparation of reagents

1. Antigen retrieval Buffer

TRIS EDTA Buffer- pH: 8.5 to 9.0

Preparation:

TRIS Base- 1.21 gram

EDTA (atomic number:372)- 0.37 gram

Dissolve in 1000ml of water

2. Wash buffer

TRIS BUFFERED SALINE (TBS)-pH: 7.2 to 7.6

Preparation:

TRIS Base- 8.6 gram

NaCl- 9.6 gram

Dissolve in 1000ml of water.

Adjust pH by using concentrated HCl

ANNEXURE VI – KEY TO MASTER CHART

ADH	– Atypical Ductal Hyperplasia
Ca	– Carcinoma
cm	– centimetre
DCIS	– Ductal Carcinoma In-Situ
HPR	– Histopathology
IDC	– Invasive Ductal Carcinoma
IHC	– Immunohistochemistry
IP	– Intraductal Papilloma
L	– Left
Mf	– Myofibroblasts
N	– Negative staining
R	– Right
SA	– SclerosingAdenosis
SMMHC	– Smooth Muscle Myosin Heavy Chain
Vsm	– Vascular Smooth Muscle
Y	– Positive staining

ANNEXURE VII – MASTER CHART

Sr. no.	Age (in years)	Diagnosis			Size (in cm)	Site	Myoepithelial cell IHC score		Vsm (SMMHC)	Mf (SMMHC)
		Clinical	HPR				P63	SMMHC		
1	54	Ca breast	IDC		4	R	2	3	Y	Y
2	48	Breast lump	DCIS		5.5	L	3	3	Y	Y
3	35	Breast lump	IP		3	R	3	3	Y	N
4	55	Ca breast	IDC		7	L	0	0	Y	Y
5	50	Breast lump	SA		2	R	2	2	Y	Y
6	80	Ca breast	IDC		4	R	0	0	Y	N
7	39	Breast lump	SA		2.5	L	3	3	Y	N
8	52	Ca breast	IDC		9	R	0	0	Y	N
9	42	Ca breast	IDC with DCIS	IDC	1.5	L	0	0	Y	Y
				DCIS			2	2		
10	47	Breast lump	DCIS		1	R	3	3	Y	Y
11	36	Ca breast	IDC		9	L	0	0	Y	N
12	18	Breast lump	SA		1.5	R	3	3	Y	Y
13	38	Breast lump	ADH		3.5	L	3	2	Y	N
14	50	Breast lump	ADH		2.5	L	2	3	Y	N
15	38	Breast lump	IP		4	R	2	3	Y	N
16	45	Breast lump	DCIS		3	R	2	3	Y	Y
17	60	Ca breast	IDC		3.5	R	0	0	Y	N
18	55	Ca breast	IDC		4.5	R	0	0	Y	N
19	63	Ca breast	IDC with DCIS	IDC	4	L	0	0	Y	N
				DCIS			2	2		
20	65	Breast lump	DCIS		1	L	3	3	Y	Y
21	53	Ca breast	IDC		4	L	0	0	Y	N
22	18	Breast lump	SA		3	R	3	3	Y	N
23	64	Ca breast	IDC		6	R	0	0	Y	N

24	48	Ca breast	DCIS		4	L	3	3	Y	Y
25	55	Ca breast	IDC with DCIS	IDC	5	R	0	0	Y	N
				DCIS			0	0		
26	58	Ca breast	IDC		5	R	0	0	Y	N
27	45	Breast lump	DCIS		5	R	3	2	Y	Y
28	43	Ca breast	IDC with DCIS	IDC	2.5	L	0	0	Y	N
				DCIS			3	3		
29	65	Ca breast	IDC		7	L	0	0	Y	Y
30	45	Breast lump	SA		4	R	3	3	Y	N