COMPARISON OF STROMAL CD10 EXPRESSION IN BENIGN, BORDERLINE AND MALIGNANT PHYLLODES TUMORS

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

M.D. (PATHOLOGY)
BRANCH - III

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

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

APRIL 2015

CERTIFICATE

This is to certify that this Dissertation entitled **"COMPARISON OF STROMAL CD10 EXPRESSION IN BENIGN, BORDERLINE AND MALIGNANT PHYLLODES TUMORS"** is the bonafide original work of **Dr.HEMAVATHI M**, in partial fulfillment of the requirement for M.D., (Branch III) in Pathology examination of the Tamilnadu Dr.M.G.R Medical University to be held in April 2015.

Prof. Dr. R.Padmavathi,M.D.,D.G.O.,
PROFESSOR OF PATHOLOGY,
Institute of Pathology,
Madras Medical College,
Chennai – 600003.

Prof. Dr. M. Saraswathi, M.D.,
DIRECTOR,
Institute of Pathology,
Madras Medical College,
Chennai – 600003.

Prof. Dr. R.Vimala , M.D.,
DEAN,
Madras Medical College and
Rajiv Gandhi Government General Hospital,
Chennai – 600003.

DECLARATION

I **Dr.Hemavathi M**, solemnly declare that the dissertation titled "**COMPARISON OF STROMAL CD10 EXPRESSION IN BENIGN, BORDERLINE AND MALIGNANT PHYLLODES TUMORS** " is the bonafide work done by me at Institute of Pathology, Madras Medical College under the expert guidance and supervision of **Prof.Dr.R.Padmavathi, M.D., D.G.O.,** Professor of Pathology, Institute of Pathology, Madras Medical College. The dissertation is submitted to the Tamilnadu Dr. M.G.R Medical University towards partial fulfillment of requirement for the award of M.D., Degree (Branch III) in Pathology.

Place: Chennai
Date: **Dr. HEMAVATHI M**

ACKNOWLEDGEMENT

I express my sincere thanks to **Prof. Dr. R. VIMALA, M.D.**, Dean, Madras Medical College and Rajiv Gandhi Government General Hospital, for permitting me to utilize the facilities of the Institution.

I take this opportunity to express my heartfelt sincere gratitude to **Prof. Dr. M. SARASWATHI, M.D.,** Professor and Director of Institute of Pathology, Madras Medical College, Chennai, for her keen interest, constant encouragement and valuable suggestions throughout the study.

I express my sincere gratitude to my guide **Prof.Dr.R.PADMAVATHI, M.D., D.G.O.,** Professor of pathology, Madras Medical College, for being a constant source of inspiration and guidance, during the period of study, without which the dissertation would not have reached the final shape.

I am thankful to **Prof. Dr. P.KARKUZHALI, M.D.,** Professor and former Director of Institute of Pathology, Madras Medical College for her initial guidance and valuable suggestions during the study.

I am truly thankful to **Prof. Dr. SHANTHA RAVISANKAR M.D., D.C.P., Prof. Dr. V. RAMAMURTHY M.D., Prof. Dr. GEETHA DEVADAS M.D., D.C.P., Prof. Dr. M. P. KANCHANA M.D.,**

Prof. Dr. K. RAMA M.D., Prof. Dr. RAJAVELU INDIRA M.D., Prof. Dr. SUDHA VENKATESH M.D., and **Prof. Dr. S. PAPPATHI M.D., D.C.H** and all my **Assistant Professors**, my colleagues, technicians and staffs of Institute of Pathology, Madras Medical College for their co-operation and encouragements during my study period.

Big thanks to my parents for all the love and moral support they have given me throughout the years.

Finally, but most importantly I would like to thank my husband, my best friend Dr. Hariharan for his patience, endless support and encouragement. Thank you for being there with me in all my ups and downs.

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No : 044 25305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr. M.Hemavathi,
PG in Pathology,
Institute of Pathology,
Madras Medical College, Chennai-3.

Dear Dr. M. Hemavathi,

The Institutional Ethics Committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled **"Comparison of Stromal CD10 expression in benign, borderline and malignant Phyllodes Tumors "** No.27032014

The following members of Ethics Committee were present in the meeting held on 11.03.2014 conducted at Madras Medical College, Chennai-3.

1.	Dr. C. Rajendran, M.D.	-- Chairperson
2.	Prof. Kalaiselvi, MD Vice-Principal, MMC, Ch-3	-- Member Secretary
3.	Prof. Nandhini, M.D. Inst. of Pharmacology, MMC, Ch-3.	-- Member
4.	Prof. Bhavani Shankar, M.S. Prof & HOD of General Surgery, MMC, Ch-3.	-- Member
5.	Prof. V. Padmavathi, M.D. I/c Directory of Pathology, MMC, Ch-3.	-- Member
6.	Thiru. S. Govindasamy, BABL	-- Lawyer
7.	Tmt. Arnold Saulina, MA MSW	-- Social Scientist

We approve the proposal to be conducted in its presented form.

Sd/Chairman & Other Members

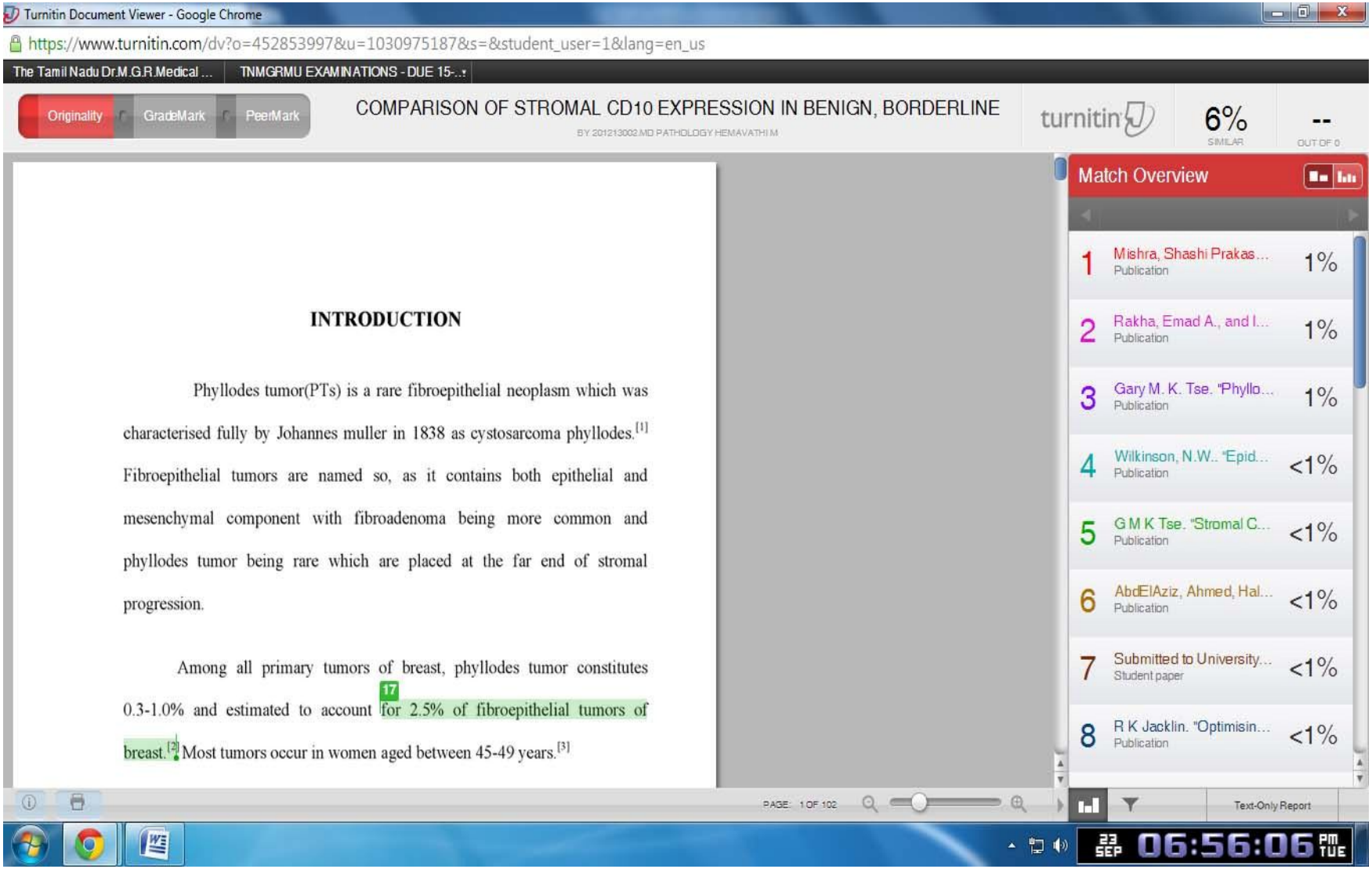
The Institutional Ethics Committee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and patients information / informed consent and asks to be provided a copy of the final report.

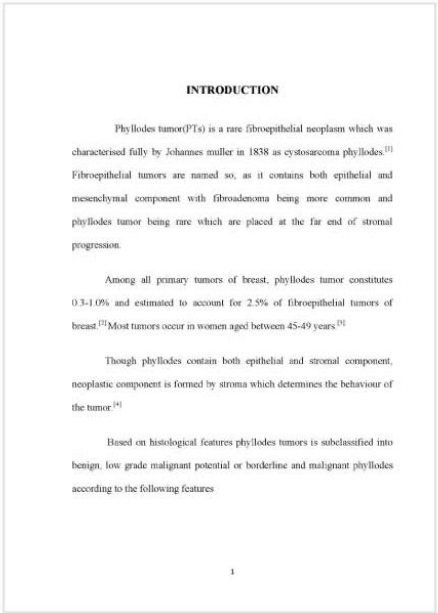
13/3/14

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE

Member Secretary, Ethics Committee

13/3/14


Turnitin Document Viewer - Google Chrome
https://www.turnitin.com/dv?o=452853997&u=1030975187&s=&student_user=1&lang=en_us
The Tamil Nadu Dr.M.G.R.Medical ...
TNMGRMU EXAMINATIONS - DUE 15-..
Originality
GradeMark
PeerMark
COMPARISON OF STROMAL CD10 EXPRESSION IN BENIGN, BORDERLINE
BY 201213002.MD PATHOLOGY HEMAVATHI M
turnitin
6%
SIMILAR
--
OUT OF 0
INTRODUCTION
Phyllodes tumor(PTs) is a rare fibroepithelial neoplasm which was characterised fully by Johannes muller in 1838 as cystosarcoma phyllodes.[1] Fibroepithelial tumors are named so, as it contains both epithelial and mesenchymal component with fibroadenoma being more common and phyllodes tumor being rare which are placed at the far end of stromal progression.
Among all primary tumors of breast, phyllodes tumor constitutes 0.3-1.0% and estimated to account for 2.5% of fibroepithelial tumors of breast.[2] Most tumors occur in women aged between 45-49 years.[3]
Match Overview
1 Mishra, Shashi Prakas... Publication 1%
2 Rakha, Emad A., and I... Publication 1%
3 Gary M. K. Tse. "Phyllo... Publication 1%
4 Wilkinson, N.W.. "Epid... Publication <1%
5 G M K Tse. "Stromal C... Publication <1%
6 AbdElAziz, Ahmed, Hal... Publication <1%
7 Submitted to University... Student paper <1%
8 R K Jacklin. "Optimisin... Publication <1%
PAGE: 1 OF 102
Text-Only Report
23 SEP 06:56:06 PM TUE



INTRODUCTION

Phyllodes tumor(PTs) is a rare fibroepithelial neoplasm which was characterised fully by Johannes muller in 1838 as cystosarcoma phyllodes.[1] Fibroepithelial tumors are named so, as it contains both epithelial and mesenchymal component with fibroadenoma being more common and phyllodes tumor being rare which are placed at the far end of stromal progression.

Among all primary tumors of breast, phyllodes tumor constitutes 0.3-1.0% and estimated to account for 2.5% of fibroepithelial tumors of breast.[2] Most tumors occur in women aged between 45-49 years.[3]

Though phyllodes contain both epithelial and stromal component, neoplastic component is formed by stroma which determines the behaviour of the tumor.[4]

Based on histological features phyllodes tumors is subclassified into benign, low grade malignant potential or borderline and malignant phyllodes according to the following features

1

ABBREVIATIONS

PTs	:	Phyllodes tumors
FA	:	Fibroadenoma
HPF	:	High power field
WHO	:	World health organization
WLE	:	Wide local excision
CALLA	:	Common acute lymphoblastic leukemia antigen
RT	:	Radiotherapy
CT	:	Chemotherapy
NCCN	:	National comprehensive cancer network
EGFR	:	Epidermal growth factor receptor
IHC	:	Immunohistochemistry
BIRADS	:	Breast imaging and reporting data system
H & E	:	Hematoxylin & Eosin

CONTENTS

COMPARISON OF STROMAL CD10 EXPRESSION IN BENIGN, BORDERLINE AND MALIGNANT PHYLLODES TUMORS

ABSTRACT

BACKGROUND :

Phyllodes tumor is a rare fibroepithelial tumor of the breast comprising less than 1% of all primary breast tumor. Phyllodes tumors are graded into benign, borderline and malignant based on histological criteria. Grading of phyllodes tumor is important as it determines the biological behaviour of the tumor.

AIMS:

The aim of the present study was to identify the incidence and clinicopathological features of benign, borderline and malignant phyllodes tumors and to compare the CD10 expression in benign, borderline and malignant phyllodes, in order to highlight its diagnostic significance.

MATERIALS AND METHOD:

The clinical and pathological findings of phyllodes tumors were retrieved from the surgical pathology records from January 2012 to June 2014.Totally 50 case were selected randomly (38 benign, 6 borderline and 6 malignant) and their representative formalin fixed paraffin embedded tissue samples were subjected to immunohistochemistry for CD10 expression.

RESULTS:

In the 38 cases of benign phyllodes tumors, only three cases (7.9%) were CD10 positive. Three out of six cases (50%) of borderline phyllodes tumors showed CD10 positivity, whereas five out of six cases (83.3%) of malignant phyllodes tumor showed CD10 positivity.

CONCLUSION:

CD10 expression correlated well with grade of phyllodes tumors, which is of statistical significance and therefore it can be used in the determination of tumor grade and this may pave way for development of targeted therapies.

INTRODUCTION

Phyllodes tumor(PTs) is a rare fibroepithelial neoplasm which was characterised fully by Johannes muller in 1838 as cystosarcoma phyllodes.[1] Fibroepithelial tumors are named so, as it contains both epithelial and mesenchymal component with fibroadenoma being more common and phyllodes tumor being rare which are placed at the far end of stromal progression.

Among all primary tumors of breast, phyllodes tumor constitutes 0.3-1.0% and estimated to account for 2.5% of fibroepithelial tumors of breast.[2] Most tumors occur in women aged between 45-49 years.[3]

Though phyllodes contain both epithelial and stromal component, neoplastic component is formed by stroma which determines the behaviour of the tumor.[4]

Based on histological features phyllodes tumors is subclassified into benign, low grade malignant or borderline and malignant phyllodes tumors according to the following features

- Stromal cellularity,
- Stromal overgrowth,
- Tumor margin,
- Cellular atypia and
- Number of mitosis per 10 high power field (HPF).

Benign phyllodes tumor being most common subtype, it accounts for 35% to 64% with remainder divided between intermediate and malignant subtypes.[5]

Grading of tumor is important as it determines the biological behaviour of the tumor with recurrence rate of 8 to 65% depending on grade.[6] Distant metastasis of tumor is encountered in up to 22% of malignant tumors[7] whereas metastasis is not reported in benign tumor.

There is interobserver variation in grading phyllodes especially in intermediate variant as tumors were more atypical than the benign but does not fulfil the criteria of malignancy. Hence, there is a need for a marker for proper grading and evaluating its clinical behaviour for proper treatment, as death is more common due to metastasis in borderline and malignant subtypes than local recurrence.

CD10 known as common acute lymphoblastic leukemia antigen is a zinc metalloproteinase which is normally expressed in myoepithelial cells of breast. It is commonly used in diagnosis of stromal malignancy especially in uterus to differentiate stromal tumors from smooth muscle tumors. Its expression in tumors facilitate metastatic potential of tumor with capacity to invade blood vessels thus indicating the presence of it commonly in higher grade tumors.

In this study CD10 expression in the stromal cells of benign, borderline and malignant phyllodes which are already histologically classified is studied and its role in grading of tumor is evaluated.

Aims and Objectives

AIMS AND OBJECTIVES

1. To evaluate the incidence of benign, borderline and malignant phyllodes.

2. To study the clinical and histopathological features of benign, borderline and malignant phyllodes.

3. To study and compare the expression of CD 10 in benign, borderline and malignant phyllodes.

Review of Literature

REVIEW OF LITERATURE

ANATOMY AND MICROANATOMY OF BREAST:

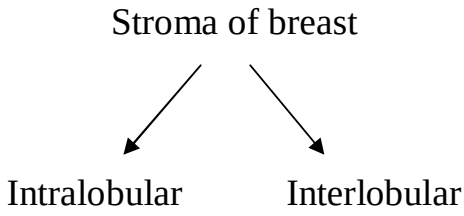
Mammary gland is a modified sweat gland which develops from milk line or mammary ridges which are epidermal thickening that appears on the ventral surface of foetus during 5^{th} week of gestation.[8] It rests over pectoralis muscle in the chest wall between second and sixth rib.

It is covered with skin and subcutaneous tissue and composed of epithelium, stromal cells and adipose tissue.

Mammary gland is composed of 15 to 25 lobes consisting of lactiferous duct which dilate to form lactiferous sinuses before terminating in nipple. Lactiferous ducts within a lobe divide repeatedly forming segmental, subsegmental and terminal duct.

Terminal duct leads to lobules which consist of multiple acini. These terminal ducts along with acini form terminal duct lobular unit.[9]

Entire ductal and lobular units are lined by two layers - luminal cuboidal or columnar epithelial cells and outer myoepithelial cells. Entire glandular structure is supported by fibrofatty tissue.



Interlobular stroma is composed mainly of dense fibrous connective tissue admixed with adipose tissue.[10,11]

In Intralobular stroma, it is less densely collagenised and contains more capillaries. Cells present in the stroma is found to have paracrine effect on the epithelium.[12]

Stroma also contains scattered inflammatory cells like lymphocytes, plasma cells, macrophages and mast cells. Ochrocytes lipofuscin containing periductal histiocytes are usually seen in association with proliferative breast disease, inflammatory condition and postmenopausal women.[13]

Both epithelium and stroma cells are hormonally responsive which is responsible for changes occurring during menstrual cycle, pregnancy and lactation.

Fibroepithelial tumor:

Fibroepithelial lesions are biphasic lesions composed of both epithelial and mesenchymal components in varying proportions.

Tumors included in this category are

- Fibroadenoma
- Phyllodes tumor
- Sclerosing lobular hyperplasia
- Hamartoma

Of these fibroadenoma is the commonest having both components and phyllodes being rare and are placed at the far end of stromal progression.

Phyllodes tumors:

Uncommon fibroepithelial tumor having double layered epithelial component forming clefts surrounded by hypercellular stromal component.[2] Though it contains both components, neoplastic component is formed by the stroma.

The term phyllodes were derived from word ‘phyllos’ in greek meaning ‘leaf’ as stroma shows leaf like growth pattern that projects into cleft like spaces which are lined by epithelium.[14]

Phyllodes tumor was first described as giant fibroadenoma in the year 1774.[1] It was first described by Chelius in the year 1827.[15] Later in 1838 it was fully characterised by Johannes Muller who first used the term cystosarcoma phyllodes based on its fleshy appearance and leaf like projection of stroma.[1]

It was considered to be benign until Cooper and Ackerman reported malignant potential of this tumor in the year 1943.[16] The term phyllodes tumor was adopted by WHO in the year 1981. Later it was subclassified based on histology into benign, low grade malignant potential (borderline) and malignant, with chances of recurrence and metastatic potential increasing with grade.

Epidemiology:

Phyllodes tumor constitute 0.3 to 1.0% of all primary breast tumors and accounts for upto 2.5% of fibroepithelial tumors.[2] In a population based study conducted in USA there was an annual incidence of 2.1 per million women.[3] Asian and latin American whites have increased incidence.[3,17]

It occurs commonly in women in the age group of 35-55 yrs with mean age of 45 years and when compared with fibroadenoma, it occurs 15-20 years later. Among Asians it occurs relatively at youger age group. Cases have also been reported in adolescents and in elderly women.

Though very rare, a few cases have been reported in men and it is invariably associated with estrogen induced gynecomastia.[18]

Majority of PTs are benign accounting for 35% to 65% with remainder of the cases divided between intermediate and malignant subgroups.[5] Although malignant PTs are rare it occurs more frequently in latino whites.

Etiology:

Phyllodes tumors are thought to arise from periductal or intralobular stroma. Exact etiology of PTs are unknown. Nearly 40% of phyllodes tumor has coexisting fibroadenoma[19], but its etiological role is unclear.

According to Noguchi et al.[20] Clonal analysis showed that, in fibroadenoma both epithelial and stromal component are polyclonal and should be considered hyperplastic rather than neoplastic. Whereas in phyllodes, epithelial cells are polyclonal and stromal cells are monoclonal suggesting it as a tumor of stromal origin. Since it has both components it has been suggested that phyllodes tumor begin as fibroadenoma with somatic mutation in stromal cell resulting in evolution of PTs as shown in fig.1.

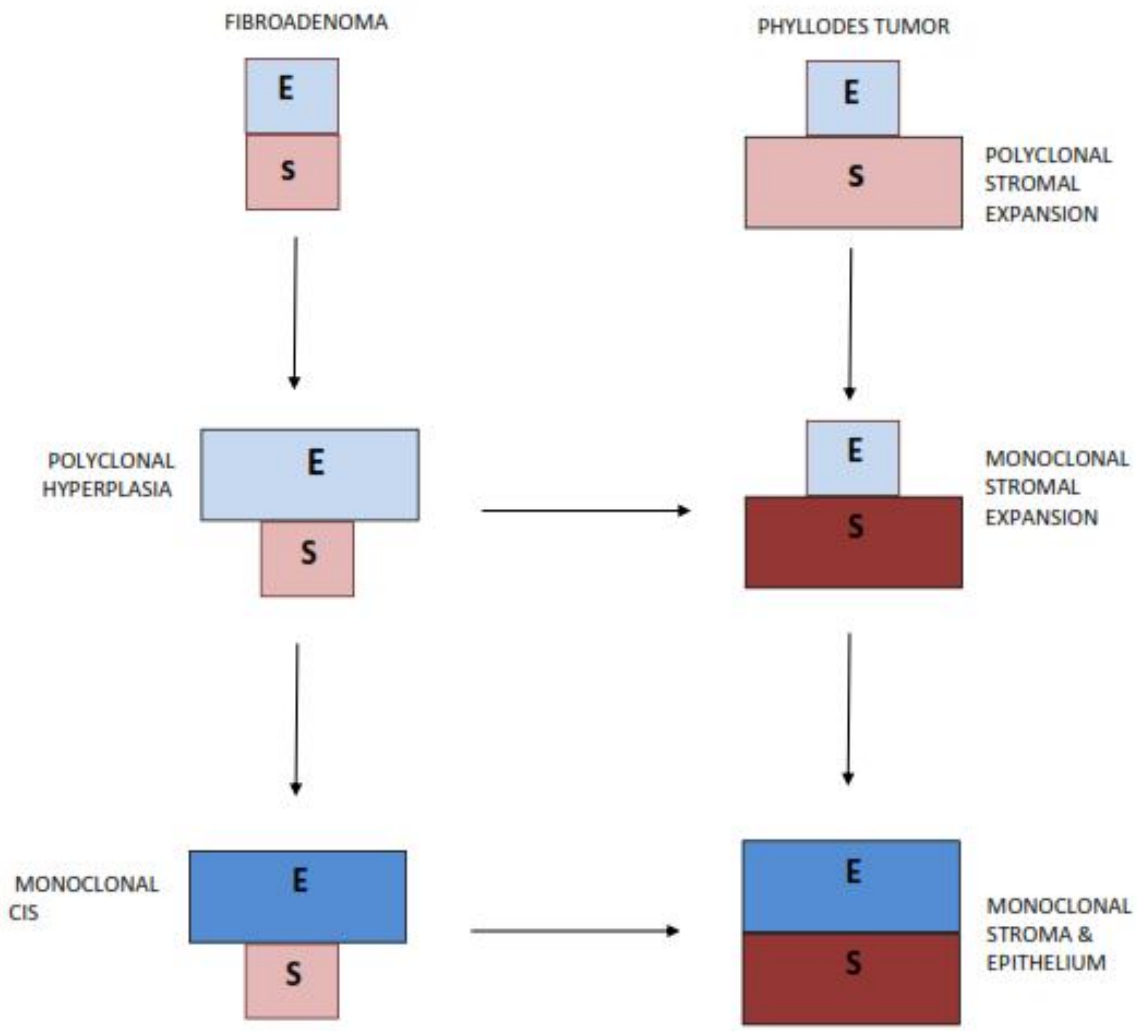


Fig.1. Pathogenesis of development of fibroepithelial lesion of breast
E- Epithelium, S- Stroma, CIS- Carcinoma In Situ

This hypothesis was supported by a study conducted by Kuijper et al.[21] using PCR assay targeted at human androgen receptor gene.

In 2005 kujiper et al. found that there is an increase in cell cycle dysregulation of stromal component as the grade of phyllodes tumors increases.[22]

Risk factors:

- Ethnicity – Higher incidence of phyllodes tumor in asian and latina women[3] and it occurs at significantly younger age group in asian women.
- Fibroadenoma
- Li-fraumeni syndromre, a rare autosomal dominant syndrome characterised by multiple tumors are found to be associated with PTs also.

Clinical features:

Most common presentation is lump breast and they are indistinguishable from fibroadenoma**.** Usually presents as unilateral solitary mass and most commonly occurs in upper outer quadrant with propensity to occur equally in both the breasts. Rarely multifocal lesions can occur in same breast[19,23] or both breasts.[19,24,25] Rare cases of PTs in vulva[26] or the axilla[27] have been reported where it has been arising from ectopic breast tissue.

Clinically it presents as a well demarcated, fairly mobile, firm to hard palpable mass. Size of the tumor ranges from 1-20cm with an average size of 4-5cm. Larger size and rapid growth suggest PTs rather than fibroadenoma. Rapid growth in a pre-existing stable lesion suggests malignant progression. Studies show that there is direct relationship between increasing size and

malignancy. But there are exceptions with high grade malignant tumor smaller than 2cm and benign tumor being larger.

Other findings include pain, nipple discharge due to spontaneous tumor infarction, prominent dilated veins over the skin and skin ulceration mainly in larger size tumor due to pressure effect rather than invasion.

Radiological findings:

- **Ultrasonography**

PTs are ovoid or lobulated, well circumscribed with smooth margin with heterogenous internal echoes.[28]

Study conducted by Liberman et al. has shown that retrotumor acoustic enhancement and hypoechoic internal echoes is present in majority of benign as well as malignant phyllodes.[29]

Though some authors suggest intramural cysts within solid mass of breast as characteristic feature of phyllodes tumor[28,29] , such findings are also present in other well circumscribed neoplasms of breast, like medullary carcinoma which implies that this cannot be considered as pathognomic feature.

- **Color and pulse Doppler ultrasonography**

As microvessel density is increased in borderline and maligmant PTs, various flow indices aid in differentiating benign from malignant. Features favouring malignancy are

- ✓ Increased pulsatility index (PI),
- ✓ Increased Resistance index (RI),
- ✓ Increased systolic peak flow velocity (Vmax),
- ✓ Marked hypoechogenicity,
- ✓ Ill defined tumor margin ,
- ✓ Posterior acoustic shadowing.[30]

- **Mammography**

Shows well circumscribed lobulated or ovoid mass with radiolucent halo around the mass. Coarse calcification may be seen rarely.[31]

- **Magnetic resonance imaging**

- ✓ Lobulated mass with well defined margin and heterogeneous internal echoes.
- ✓ Hypointense on T1 and
- ✓ ISO/Hyperintense on T2 weighed images.

With contrast enhancement - benign and malignant can be differentiated.

- ✓ Benign tumors - initial slow enhancement with persistence of delayed phase.
- ✓ Malignant tumors - fast initial enhancement with plateau of delayed phase.[31,32,33,34]

Macroscopic appearance:

Phyllodes tumors are well circumscribed tumor, grow radially compressing the adjacent parenchyma forming pseudocapsule with pushing margin, whereas margin is infiltrative in malignant PTs. They present as single or multinodular mass with lobulated surface.

Cut surface

- grey to tan bulging mass giving cauliflower like appearance
- firm and rubbery in consistency sometimes soft with gelatinous areas
- shows characteristic whorling pattern with cleft like spaces
- large tumor shows cystic degeneration with area of hemorrhage and necrosis.

Microscopic appearance:

Grading:

Many grading system have been proposed that divide PTs into either two subgroups (benign and malignant) or three (benign, borderline and malignant) subgroups.

World Health Organization (WHO) classified phyllodes tumor into benign, borderline and malignant tumors based on the following histological features.

- Stromal cellularity
- Stromal overgrowth
- Cellular atypia
- Mitosis per 10 high power field (HPF)
- Tumor margin.

Cytology:

FNAC in the diagnosis of phyllodes tumors has limitations, with diagnostic accuracy of about 63%.[35]

As both epithelial and stromal components are present in both fibroadenoma and PTs, it is difficult to differentiate them cytologically rather than differentiating benign from malignant phyllodes tumors.

Features favouring diagnosis of PTs are

- ✓ Hypercellular cohesive stromal fragments
- ✓ Well delineation of fragment borders
- ✓ Bipolar naked nuclei
- ✓ Stromal nuclear atypia
- ✓ Blood vessels crossing stromal fragments
- ✓ Tumor giant cells and absence of apocrine metaplasia.

Deen et al.[36] and Jayaram and Sthaneshwar[37] classified cells on smear by comparing it with small lymphocytes as

Epithelial cells	-	small, round to oval with size two times smaller than size of lymphocyte
Stromal cells	-	long spindle cell, three times larger than size of Lymphocyte
Benign PTs	-	stromal fragment, singly dispersed stromal cells, naked stromal nuclei which are more numerous than epithelial cells.
Borderline PTs	-	stromal cell predominant with atleast two fragment in each field, large stromal cells and monomorphic naked nuclei.

Malignant PTs - cellular smear, stromal fragments with discohesive spindle cells, bizarre multinucleate giant cells and minimal or absent epithelial element.

Biopsy:

Core biopsy is preferred over FNAC as it provide architectural information and has a sensitivity of 99%, with 83% and 93% of positive and negative predictive value.[38]

Paddington clinicopathological suspicion score[39]-Criteria outlined in this help to identify patients for core biopsy in order to improve preoperative diagnosis.

Paddington clinicopathological suspicion score-

Clinical findings

i) Sudden increase in size in a longstanding breast lesion

ii) Apparent fibroadenoma > 3cm diameter or in patient >35 years

Imaging findings

i) Rounded borders/lobulated appearance at mammography

ii) Attenuation or cystic areas within a solid mass on Ultrasonography

FNAC findings

i) Presence of hypercellular stromal fragments

ii) Indeterminate features.

ANY 2 features mandate core biopsy.

Stromal features that has to be seen in core biopsy include nuclear atypia, mitosis, cellularity and amount of stroma compared to epithelium.[40] Of these, mitosis is the most significant feature in differentiating PTs from FA.

In benign PTs where it lacks nuclear atypia and mitosis prominent periductal proliferation of stromal cells, exaggerated intracanalicular growth pattern and heterogenous stromal cell help to differentiate it from FA with increased cellularity.

In case if it is not possible to differentiate it from FA in core biopsy, lesion should be designated as cellular fibroepithelial lesion to avoid underdiagnosis and should be recommend for exicision .

Grading should be done mainly in exicision biopsy to avoid undergrading due to sampling error. Histological criteria for grading into

borderline and malignant was described first by pietruszka and Barnes[41] and later Azzopardi[42] modified it and it is adopted by WHO.

Benign phyllodes tumor

Most common subgroup of phyllodes tumor accounting for 60% arise from periductal or inralobular stroma. Similar to fibroadenoma benign PT has both epithelial and stromal component but it shows stromal hypercellularity resembling exaggerated intracanalicular fibroadenoma with leaf like growth pattern of stroma into the cleft like space.

Epithelial component consists of luminal epithelial and basal myoepithelial cells. Epithelial hyperplasia is common and other changes taking place include squamous metaplasia and rarely apocrine metaplasia .

Stromal density is more and seen in the immediate vicinity of epithelial element, so called periductal stroma and these areas show increased mitotic activity. Stromal cells show heterogeneity within same lesion and differ from case to case. Myxoid stromal change and hyalinisation are more common finding and pseudoangiomatous stromal hyperplasia (PASH) are also seen in PTs. Rare changes occurring in PTs include lipomatous, cartilaginous and osseous metaplasia. Necrosis are usually seen in larger benign tumors.

Features to designate tumor as benign include[2]

- Low stromal cellularity
- Absent to mild stromal cell atypia
- Low mitotic count(0-4/10HPF)
- Minimal stromal overgrowth
- Well circumscribed pushing margin
- Rare heterologous stromal element.

It is usually difficult to differentiate it from FA. Features favouring PTs are

- Stromal hypercellularity
- Periductal stromal condensation
- Stromal heterogeneity (stroma is uniform in FA)
- Cellular atypia and Mitotic figure.

Benign features of this tumor does not rule out local recurrence as recurrence mainly depends on completeness of excision.

Borderline phyllodes tumor

Those tumors which histologically shows some features between benign and malignant but does not possess all the features for malignancy comes under this category.

This pose a great problem for both clinician and pathologist in assessing the likelihood of local recurrence and metastatic malignant potential.

Features favouring borderline PTs are[2]

- Moderate to marked stromal cellularity
- Moderate to marked stromal overgrowth
- Moderate atypia
- Number of mitosis 5-9/10HPF
- Pushing or Infiltrative margin.

Epithelial hyperplasia is more commonly found than in benign PTs and also shows increased microvessel density.

Malignant phyllodes tumor

This forms the other end of the spectrum of phyllodes tumor which accounts for about 20%.

Features favouring malignancy include[2]

- Marked cellularity
- Marked stromal overgrowth
- Marked stromal atypia
- Infiltrative margin

- Mitotic rate >10/10HPF
- Necrosis and hemorrhage
- Malignant heterologous element

Most common stromal sarcoma pattern is that of fibrosarcoma. Other heterologous differentiation include liposarcoma, chondrosarcoma, osteosarcoma, rhabdomyosarcoma and rarely angiosarcoma. Liposarcoma has been the most common among heterologous differentiation with good prognosis if completely excised.

Stromal overgrowth here will be extensive there by masking epithelial component and hence it warrants extensive sampling.

Some articles suggest a mitotic rate of >5/10HPF as an indicator of malignancy, in view of absence of worrying features mitotic rate of >10/10HPF would be more significant.

Local recurrence :

Both benign and malignant tumors have local recurrence which ranges from 10%-40%. Grading has some correlation with local recurrence which ranges from 10% to 25% in benign, 32% in borderline and upto 40% for malignant PTs.[17]

Features favouring local recurrences include

- Incomplete excision
- Infiltrative margin
- Secondary nodule at tumor periphery.

Usually local recurrences occur within first three years of surgery, but it occurs much earlier in malignant than benign tumors.

Rather than grading it is more correlated with the extent of primary surgery with more recurrence occurring in tumor with positive margin. Though size of tumor has no direct role in predicting local recurrence, it does determine the extent of surgery and marginal status.

Recurrent lesion may present as either biphasic having both epithelial and stromal elements or monophasic with only stromal component. Sometimes stromal cells in recurrent lesion may show increased cellularity with aggressive histological features when compared with the original tumor.

Metastasis :

Metastasis are usually seen in borderline and malignant PTs tumors with metastatic rate of about 22% have been reported in malignant lesion and is not seen in benign tumors .

According to Hawkins et al[43] , features that predict metastasis are;

- Nuclear pleomorphism
- High mitotic rate >10/10HPF
- Size >10cm
- Stromal overgrowth and
- Necrosis.

Usually these tumors metazise to distant site. As it spreads through hematogenous route, axillary nodes are very rarely involved (<1%).

Metastases are common in tumor showing chondro[44] or osteosarcoma[45] features and rare in tumor showing liposarcomatous feature.[46]

Most common sites of metastasis include lung, bone and abdominal viscera. Other rare sites like heart and central nervous system have also been described. For sites of metastasis, they usually contain the stromal component of the neoplasm. Metastasis indicate poor prognosis of tumor.

Prognosis and survival rate:

Poor prognostic factors include

- presence of malignant heterologous element

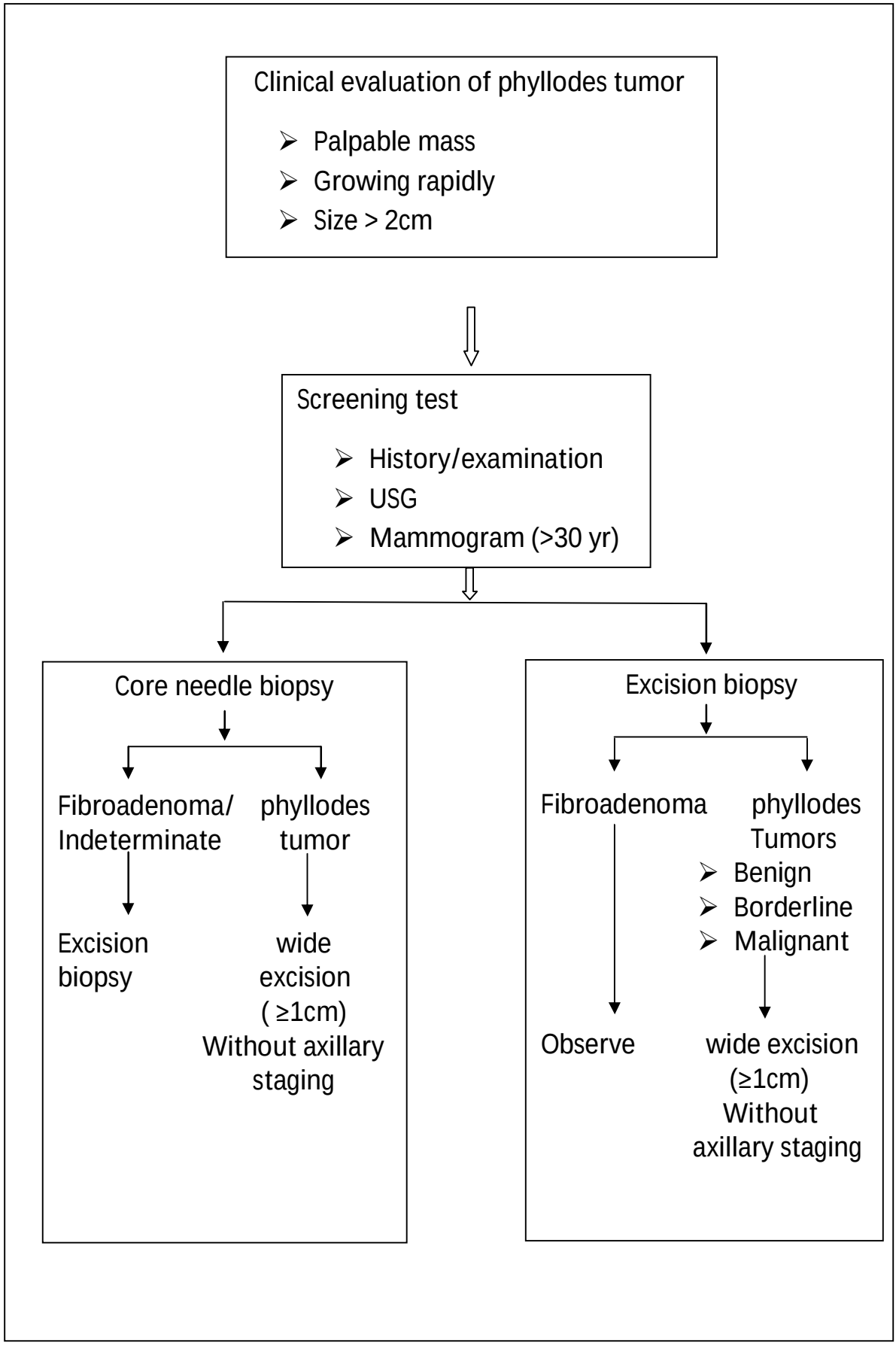
- metastasis (as it is a common cause of death).

According to Belkacemi et al[47], 5 and 10 year survival rate was found to be 97% and 96%, whereas it was reported to be 79% and 62% by Chaney et al.[48] Overall survival rate for malignant tumors varies from 42% to 95%.

Treatment:

Mainstay of treatment for PTs is surgery with adequate margin clearance. Depending on size it may be either wide local excision with 1 cm margin clearance or mastectomy.

National comprehensive cancer network (NCCN) has given guildeline for management of PTs which is shown below.


Clinical evaluation of phyllodes tumor
Palpable mass
Growing rapidly
Size > 2cm
Screening test
History/examination
USG
Mammogram (>30 yr)
Core needle biopsy
Fibroadenoma/ Indeterminate
phyllodes tumor
Excision biopsy
wide excision (≥1cm) Without axillary staging
Excision biopsy
Fibroadenoma
phyllodes Tumors
Benign
Borderline
Malignant
Observe
wide excision (≥1cm) Without axillary staging

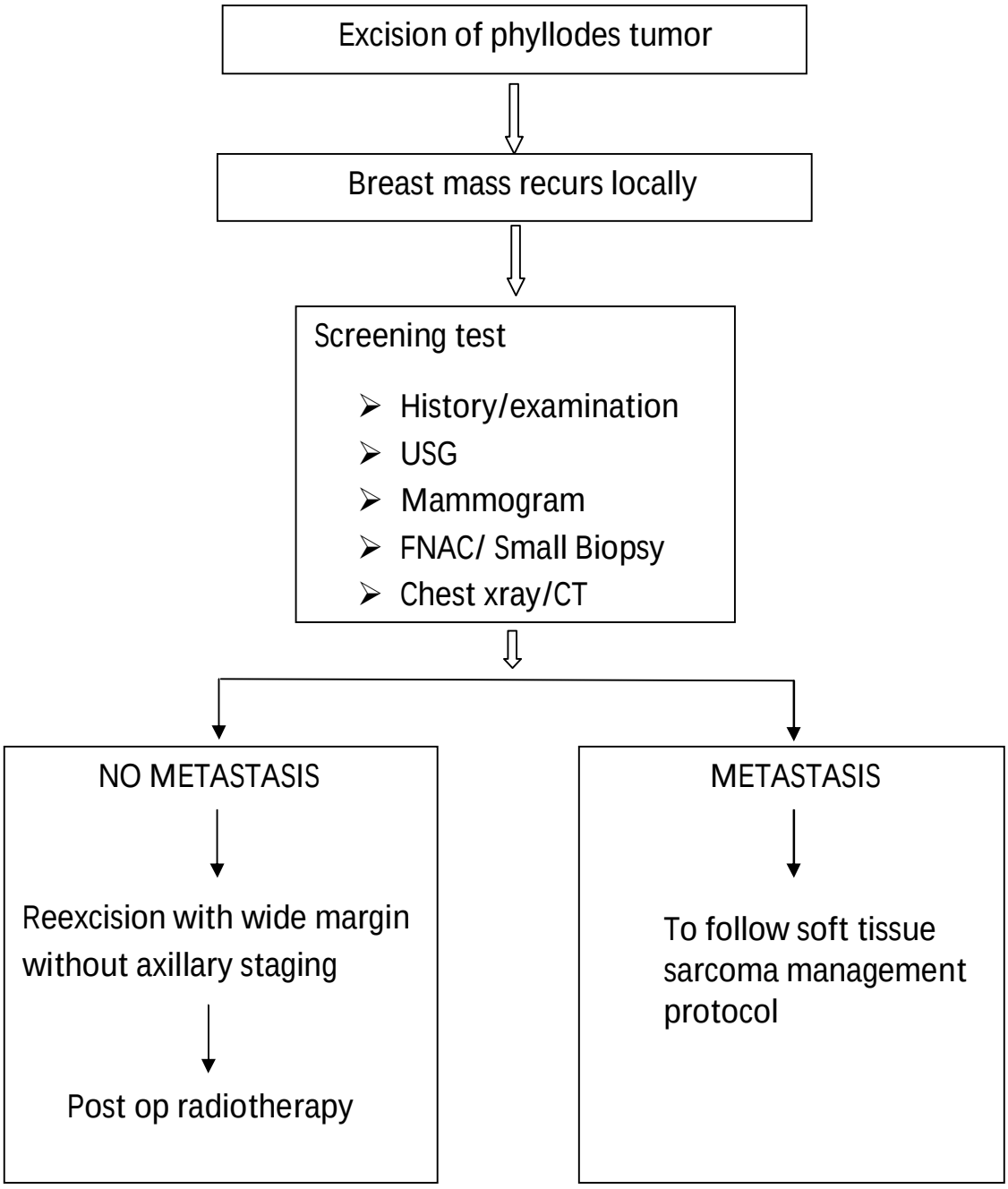
Smaller biopsy may help in deciding the type of surgery based on diagnosis.

Smaller lesions require only WLE with 1-2 cm margin clearance whereas larger lesion requires mastectomy. In younger patients who need reconstructive surgery partial mastectomy with lattismus dorsi flap have shown good results.

Nowadays breast conserving surgery and WLE are preferred over mastectomy. Guillot et al.[49] in a study of 165 patients, 97% underwent breast conserving surgery and only 5% had mastectomy. As lymph node metastasis is very rare there is no role for axillary sampling.

Whatever is the surgery marginal clearance plays an important role in preventing local recurrence. According to Barth's[50] even in malignant PTs there is no survival significance based on type of surgery.

Management of local recurrence based on NCCN guideline



Radiotherapy

There is a controversy over the use of radiation therapy and no firm data is available to support its use.

Similar to soft tissue sarcoma, adjuvant radiotherapy (RT) has been recommended to prevent local recurrence in malignant PTs. It has also been recommended for patients with adverse pathological features like tumor size >5cm and positive margin.[51] In a population based study conducted in 821 patients, it was found to have worse prognosis compared with patients treated with surgery alone.[52] Study conducted by Soumarova et al. showed local recurrence of 12% in patient treated with adjuvant radiotherapy compared to 25% of patients who have not received RT.[53]

Later in 2009 clinical trial was conducted, were patient with malignant PTs were treated with breast conservative surgery and adjuvant RT and followed up for 56 months for local recurrence. None showed local recurrence thereby concluded that margin negative resection combined with adjuvant RT forms effective management in preventing local recurrence in borderline and malignant phyllodes.[54]

Currently adjuvant RT is not routinely used and is recommended only where margin clearance cannot be achieved, inoperable local recurrence and metastatic tumors.

Chemotherapy

There is no clear evidence suggesting beneficial role of chemotherapy(CT) in treating phyllodes tumors. Some case reports have shown a positive response to chemotherapy in metastatic tumors. Guideline for CT is same as that of soft tissue sarcoma. Drugs commonly used are ifosfamide, doxorubicin, cisplatin and etoposide.[55,56]

Adjuvant chemotherapy has no survival significance hence currently chemotherapy is not recommended.

Hormonal and biological therapy

Estrogen and progesterone receptor expression were assayed immunohistochemically and it was found that receptors were expressed in glandular epithelium and not expressed in stroma. Therefore it is unlikely that these tumors will respond to hormonal therpy.

Recent advancement and understanding of biological marker and its expression in phyllodes tumor (c-kit, EGFR,CD10...) might provide room for application of targeted therapy in the near future.

Role of Biological markers:

Many markers have been studied and most were found to have correlation with grading but no marker is able to predict recurrence or metastasis.

p53

Tumor suppressor gene mapped to chromosome 17p13 which regulate cell cycle.

Most commonly studied marker in PTs and its expression increases with grade of tumor. It is found to be associated with high mitotic rate, cellular atypia and stromal overgrowth.

Staining pattern - diffuse strong nuclear stain in malignant tumor especially in subepithelial and highly cellular area.[57]

Most studies show p53 expression is not associated with outcome, but Study conducted by yonemori et al. found that increased expression of p53 indicate poor prognosis.[58]

ki67

This non histone protein is a marker for cellular proliferation. Its exression varies from 5%-25% in benign to 15% to 100% in malignant tumor.[58,59] Association of increasing expression of this marker with increasing grade has been well documented and some studies suggest it to be a useful marker in predicting outcome.[58,59,60]

c-kit (CD117)

c-kit, a protooncogene which is important diagnostic marker for GIST encodes tyrosine kinase receptor. Its expression in PTs vary from 5% to 46% in benign to 46% to 100% in malignant.[61,62] Studies shows that, its expression is associated with recurrence.[61]

As its expression is more in higher grade, the role of tyrosine kinase inhibitor as treatment for higher grade lesion and recurrence has to be explored.

Hormone receptors

Estrogen α and progesterone receptors were expressed only in epithelial component and not seen in stromal component. Recent evidence shows the expression of estrogen β in stromal cells[63], but its role in PTs has not been established.

Angiogenesis

Increase in microvessel density from benign to malignant tumor was assessed by using CD31, its role in predicting outcome was not found. Tse et al. in his study shows an association between microvessel density, p53 expression and stromal cellularity.[64]

EGFR

Like other biological markers, EGFR expression also increases with increasing grade of tumor ranging from 12% to 16% in benign to 56% to 63% in malignant tumors, but its mechanism for overexpression remains unclear.

While egfr gene amplification was found only in 8-16% by FISH, the likely mechanism for its overexpression may include gene polysomy or activating mutation.

IMMUNOHISTOCHEMISTRY(IHC)

Albert coons et al. in 1941 first described this molecular technique. Principle of this technique is to identify antigen in cell by antigen antibody interaction. Original method consist of developing an antibody against an antigen in rabbit and then it is tagged with fluorescent dye isocyanate. when it binds to antigen in tissue it emit apple green fluorescence which is detected by fluorescent microscope, one of the limitation of this method which is overcome by the use of enzymes as labels.[65]

Since then numerous advancement has been made in the field of immunohistochemistry. Nakane and Pierce et al. in 1966, introduced the indirect labelling technique in which the unlabelled antibody is followed by second antibody or substrate. Most commonly used techniques include Peroxidise antiperoxidase technique described by Sternberger et al. (1970) and Biotin avidin technique described by Heggeness and Ash in 1977.[66,67]

ANTIGEN RETRIEVAL:

Antigen has to be retrieved as it is masked during formalin fixation and paraffin processing.

Antigen retrieval can be done by any one of the following techniques

1. Proteolytic enzyme digestion
2. Heat mediated antigen retrieval which include;

    Microwave antigen retrieval

    Pressure cooker

    Steamer

    Water bath and

    Autoclave

PROTEOLYTIC ENZYME DIGESTION:[68]

Huang et al in 1976 introduced this technique to breakdown formalin cross linkages and to unmask the antigen determinants. The most commonly used enzymes include trypsin and protease. Others that can also be used are proteinase K, chymotrypsin and pepsin. The disadvantages include over digestion, under digestion and antigen destruction. Therefore the optimal concentration of enzyme and incubation time needs to be validated. Advent of heat retrieval technique replaced this method.

HEAT INDUCED EPITOPE RETRIEVAL

Here the tissue sections are placed in the retrieval solution and heat is applied for varying period of time. This result in breakdown of protein cross-links formed by formalin fixation and recovers the tissue antigenicity. [69]

Commonly used retrieval solution is the Citrate buffer with pH 6.0. Other retrieval solutions include the TRIS-EDTA with pH 9.0 and EDTA with pH 8.0.

MICROWAVE ANTIGEN RETRIEVAL:

This is a new technique most commonly used in current practice. Microwave oven heating involves boiling formalin fixed paraffin sections in various buffers for rapid and uniform heating. Antibodies against Ki67 and MIB-1 work well after heat pretreatment in this method.

PRESSURE COOKER ANTIGEN RETRIVEL:

Miller et al. in 1995 compared and proved that pressure cooking method has fewer inconsistencies, less time consuming and can be used to retrieve large number of slides than in microwave method.[70]

PITFALLS OF HEAT PRETREATMENT:

i. Drying of sections at any stage after heat pretreatment destroys antigenicity.

ii. Nuclear details are damaged in poorly fixed tissues.

iii. Fibers and fatty tissues tend to detach from slides while heating.

iv. Not all antigens are retrieved by heat pretreatment and also some antigens like PGP 9.5 show altered staining pattern.

ANTIGEN DETECTION SYSTEMS:

After retrieval specific antibodies are added to the tissue section which binds to the antigens forming antigen antibody complex. The methods employed are the direct and indirect methods.

Direct method is a one step method in which primary antibody conjugated with the label directly react with antigen. Most commonly used labels are flouro-chrome, horse radish peroxidase and alkaline phosphatase.

Indirect method is a two-step method in which labelled secondary antibody reacts with primary antibody bound to specific antigen. This method is more sensitive than direct method as it has better signal amplification.[65]

USES OF IMMUNOHISTOCHEMISTRY IN BREAST PATHOLOGY [71]

- ✓ High molecular weight cytokeratins - Distinguish usual ductal hyperplasia from ductal carcinoma in situ.
- ✓ Myoepithelial markers to assess stromal invasion.
- ✓ E Cadherin - differentiate ductal from lobular carcinoma.
- ✓ To differentiate primary from secondary metastatic tumors
- ✓ To establish site of origin in metastatic tumors
- ✓ Cytokeratin stains to detect sentinel lymph nodes metastasis.
- ✓ Assessment of Estrogen, Progesterone receptor & HER2neu for prognostic significance
- ✓ For molecular classification of breast carcinoma
- ✓ To distinguish metaplastic carcinoma from mesenchymal lesion.

CD10 –common acute lymphoblastic leukemia antigen (CALLA)

It is a cell surface neutral endopeptidase expressed both in haematopoeitic and non haematopoeitic cells.

Haematopoeitic cells - it is taken up by precursor cell especially precursor B cell and in germinal centre. One of the first marker to identify ALL - hence its name.

Also expressed in other lymphomas like - angioimmunoblastic lymphoma, follicular lymphoma, Burkitt lymphoma, diffuse large B cell lymphoma, mantle and marginal zone lymphoma

Non haematopoeitic cells - It is expressed in variety of normal tissue including brush border of epithelial cells of small intestine and proximal tubule of kidney, myoepithelial cells of breast, endometrial stromal cells, liver..etc.

It is the specific marker for renal cell carcinoma especially of clear cell type and is the commonly used marker in metastatic tumor of renal origin.

Other neoplasm where it has been extensively studied is endometrial stromal tumor and its role in differentiating it from smooth musle tumor of the uterus.

Other lesion where CD10 used are transitional cell carcinoma, prostatic carcinoma, hepatocellular carcinoma, colonic carcinoma, mesonephric remanant, solid and pseudopapillary tumor of pancreas, leiomyosarcoma, and hemangiopericytoma.

CD10 is a metalloproteinase which degrades many bioactive peptides. Thus its expression may provide tumors the capacity to infiltrate adjacent tissue and invade blood vessels thereby increasing the metastatic potential of the tumor.

This hypothesis was supported by study conducted by Iwaya et al.[72] Where increased stromal expression of CD10 in invasive ductal carcinoma is associated with increased lymph node metastasis.

Similar observation was made by ogawa et al.[73] in colorectal carcinoma where its expression in severe dysplasia, intramucosal and invasive adenocarcinoma is significantly higher compared to adenoma with mild to moderate dysplasia. Furthermore invasive growth front shows the higher expression of CD10.

Role of CD10 in breast lesions

CD10 is expressed in myoepithelial cells of human breast and is considered specific as luminal epithelial cells and surrounding stromal cells are negative for CD10.

CD10 is found to be expressed in proliferating stromal cells of fibroadenoma, phyllodes tumor and epithelial cells exhibiting apocrine metaplasia.

In invasive breast carcinoma, study conducted by Iwaya et al. showed that stromal CD10 expression is significantly associated with lymph node metastasis rather than with histological grade or clinical staging.[72]

Only few studies are available regarding expression of CD10 in phyllodes tumors. Expression tends to occur in subepithelial location where stromal condensation occurs. CD10 expression was found to be varying, with low expression ranging 0 to 6% in benign PTs to 32 to 50% in borderline to malignant PTs [74,75] thus help in grading PTs.

Another study conducted by Ibrahim also shows the correlation between CD10 expression and tumor grade with CD10 positvity in 17% of benign, 60% of borderline and 80% of malignant PTs.[76]

Masri et al. found a significant association existing between CD10 expression and metastasis in phyllodes tumor. Metastasis is unlikely to occur in CD10 negative tumor indicating its prognostic significance.[77]

CD10 is a cytoplasmic stain. In phyllodes tumor CD10 expression is assessed by the percentage of stromal cells which takes the stain and how intensely it is stained (compared to myoepithelial cells). CD10 is considered positive if stromal cells show moderate to strong intensity of CD10 stain in greater than 20% of stromal cells.[74]

Materials and Methods

MATERIALS AND METHODS

This study is both prospective and retrospective study of Phyllodes tumors of breast in the Institute of Pathology, Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai during the period between January 2012 and June 2014.

A total of 28,178 specimens were received in the Institute of Pathology, Madras Medical College during the period of January 2012 – June 2014 for histopathological examination. Out of that, 1931 specimens were breast specimens.

Of these 1931 breast specimens 83 were phyllodes tumor. Among these 83 specimens, benign, borderline and malignant phyllodes tumors were 70, 6 and 7 respectively. Of these 83 phyllodes tumor specimens, 31 were simple mastectomy, 26 were wide local excision, 2 were lumpectomy, 15 were trucut biopsies and 9 were incisional biopsies.

SOURCE OF DATA:

The patients attending the surgical outpatient department with the complaint of lump or pain were subjected to incision/ trucut/wide local excision biopsies or simple mastectomy based on clinical presentation, radioimaging and FNAC report. Cases reported as benign, borderline and

malignant phyllodes tumor in the Institute of pathology, Madras medical college from January 2012 to june 2014 were taken.

INCLUSION CRITERIA:

- Phyllodes tumor reported in breast specimens irrespective of the age.
- Both small and large biopsy of phyllodes tumor irrespective of procedure done.

EXCLUSION CRITERIA:

- Fibroadenoma and other benign lesions.
- Other malignant tumors.

METHOD OF DATA COLLECTION:

Detailed history of the cases regarding age, clinical findings, site, radioimaging finding, FNAC and type of procedure done were obtained for all the phyllodes tumor reported during the period of study from surgical pathology records.

Hematoxylin and Eosin stained 4 µ thick sections of the paraffin tissue blocks of specimens were reviewed. The following clinical and pathological parameters were evaluated: Age, clinical findings, tumor size, tumour site, BIRADS score , FNAC, tumor grade and infiltration.

Phyllodes tumor cases were graded into benign, borderline and malignant based on cellular atypia, stromal cellularity, stromal overgrowth, mitosis and marginal status.

Out of 83 cases, 50 cases comprising 38 benign tumors, 6 borderline tumors and 6 malignant phyllodes tumor were randomly selected and their representative formalin fixed paraffin embedded tissue samples were subjected to immunohistochemistry for CD10.

IMMUNOHISTOCHEMICAL EVALUATION:

Immuohistochemical analysis of markers CD10 were done in paraffin embedded tissue samples using Super-sensitive polymer HRP system based on non-biotin polymeric technology. Four micron thick sections from formalin fixed paraffin embedded tissue samples were transferred onto gelatin coated slides. Heat induced antigen retrieval was done. The antigen was bound with mouse monoclonal antibody (Pathnsitu) against CD10 antigen and then detected by the addition of secondary antibody conjugated with horse radish peroxidase-polymer and diaminobenzidine substrate. The step by step procedure of Immunohistochemistry is given below.

Antigen	Vendor	Species(clone)	Dilution	Positive control
CD10	Pathnsitu	Mouse	Ready to use	Internal control (Myoepithelial cells of breast)

Immunohistochemistry procedure:

Slide Preparation:

1. Sections with a thickness of 4 µ were cut from formalin fixed paraffin embedded tissue samples and transferred to gelatin-chrome alum coated slides.
2. The slides were incubated for overnight at 58ºC.
3. The sections were deparaffinised in xylene for 15 minutes x 2 changes.
4. The sections were dehydrated with absolute alcohol for 5 minutes for 2 changes.
5. The sections were then washed in tap water for 10 minutes.
6. The slides were then immersed in distilled water for 5 minutes.

Antigen Retrieval:

1. Heat induced antigen retrieval was done with microwave oven in appropriate temperature with appropriate buffer for 20 minutes. This step unmasks the antigenic determinants of fixed tissue sections.

2. The slides were then cooled to room temperature for 20 minutes and washed in running tap water for 5 minutes.
3. The slides were then rinsed in distilled water for 5 minutes.
4. They were washed with appropriate wash buffer (phosphate buffer) for 5 minutes x 2 changes.
5. Peroxide block was applied over the sections for 5 minutes with polyexcel H2O2.
6. The slides were washed in phosphate buffer for 5 minutes x 2 changes.

Antibody application:

1. The sections were drained (without washing) and appropriate primary antibody was applied over the sections and incubated for 30 minutes.
2. The slides were washed in phosphate buffer for 5 minutes x 2 changes.
3. The slides were covered with target binder and incubated for 10 minutes at room temperature.
4. The slides were washed in phosphate buffer for 5 minutes x 2 changes.
5. The slides were covered with Polyexcel polyHRP for 10 minutes.

6. The slides were washed in phosphate buffer for 5 minutes x 2 changes.

Chromogen application:

1. DAB substrate was prepared by diluting 1 drop of polyexcel stunnDAB chromogen to 1 ml of StunnDAB buffer.
2. DAB substrate solution was applied on the sections for 5 minutes.
3. The slides were washed in distilled water for 2 minutes.
4. The sections were counterstained with Hematoxylin for 2 seconds.
5. The slides were washed in running tap water for 5 minutes.
6. The slides were air dried, cleared with xylene and mounted with DPX.

Alternate methods of antigen retrieval

- Pressure cooker antigen retrieval
- Microwave and trypsin antigen retrieval

INTERPRETATION AND SCORING SYSTEM:

The immunohistochemically stained slides were analyzed for the presence of reaction, cellular localization, percentage of cells stained and intensity of staining.

Evaluation of staining:[74]

Percentage of stomal cells stained was assessed- 0 to 100%

Intensity was graded (compared to myoepithelial staining intensity)

0 - no staining

1 - weak

2 - moderate

3 - strong

Interpretation

Positive- intensity 2 or 3 in >20% cells

Negative- intensity 0 or 1 even in >20 cells

- Intensity 2 or 3 in <20% cells.

Data entry:

All the data collected and the results obtained were entered into Excel 2007.

STATISTICAL ANALYSIS:

The statistical analysis was performed using statistical package for social science software version SPSS 17.0. The p value was considered significant if below 0.05.

Observation and Results

OBSERVATION AND RESULTS

In a study period of 30 months cases were taken both retrospectively and prospectively from January 2012 to june 2014. During this period, a total of 28,178 specimens were received in the Institute of pathology, Madras Medical College for histopathological examination. Of these 1931 were breast specimens constituting 6.9%.

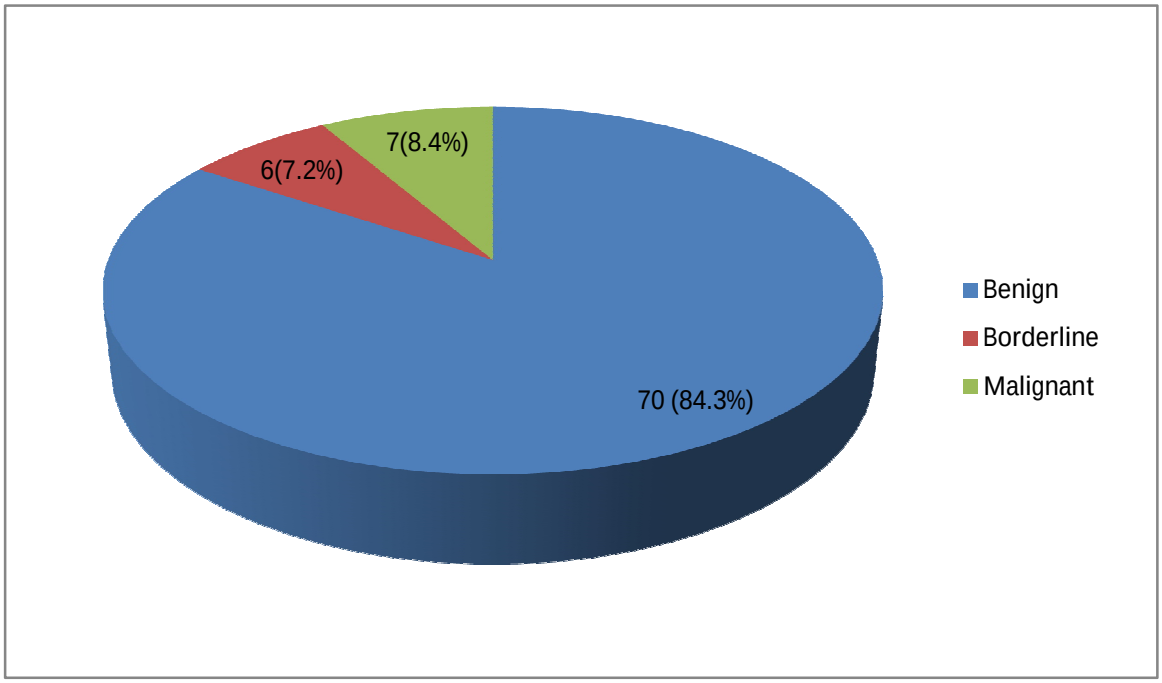
Out of 1931 breast specimen received 83 were reported as phyllodes tumor accounting for 4.2%.

Table 1: Distribution of cases based on histological subtype

S.No	Type of tumor	Total no of cases	Percentage
1	Benign	70	84.3%
2	Borderline	6	7.2%
3	Malignant	7	8.4%

The most common histological subtype of phyllodes tumor is benign constituting 84.3% whereas borderline and malignant constitute only 7.2% and 8.4% respectively (Table 1 & chart 1).

Chart 1: Distribution of cases based on histological subtypes

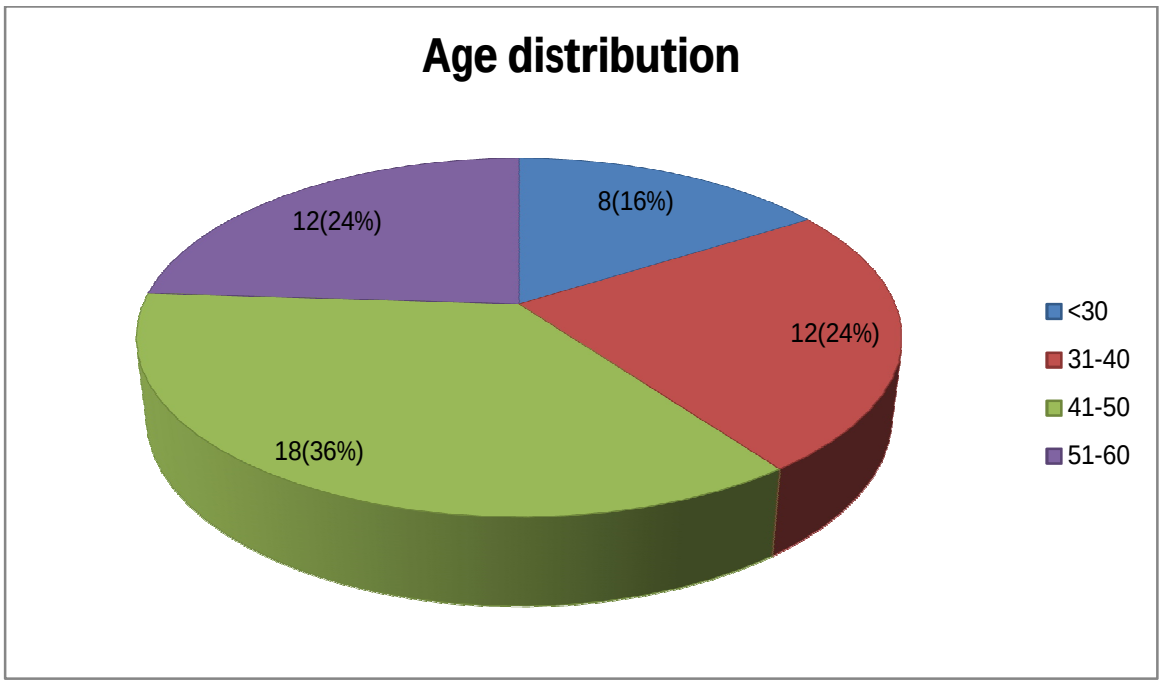


Out of the total 83 cases, 50 were taken randomly (38 benign, 6 borderline and 6 malignant tumor).

Table 2: Distribution of phyllodes tumor according to age

Age	No of cases	Percentage
≤30	8	16%
31-40	12	24%
41-50	18	36%
51-60	12	24%
Total no of cases	50	100%

Chart 2: Distribution of tumor according to age



In this study most of the phyllodes tumors occur in the age group of 41-50 comprising 36% followed by age group of 31-40 and 51-60 comprising 24% each age respectively. Only 16% occur in the age group less than 30 years (Table 2 and chart 2).

Benign tumor had a peak incidence in the age group of 41-50 years. The youngest and oldest age of presentation observed was 17 years and 57 years respectively with mean of 39.7.

In case of borderline peak incidence occur in the age group betwen 51and 60 years with youngest and oldest age of presentation of 35 and 60 years respectively with mean of 47.8 years.

Of malignant lesions peak incidence was seen at 51- 60 years with youngest and oldest age of presentation observed was 51years and 60 years respectively with mean of 54 years. (Table 3 & 4 and chart 3 & 4)

Table 3: Age distribution in different subtypes

Age	Benign(%)	Borderline(%)	Malignant(%)
≤30	8 (21.05%)	--	--
31-40	11 (28.94%)	1 (16.67)	--
41-50	16 (42.11%)	2 (33.33)	--
51-60	3 (7.89%)	3 (50%)	6 (100%)
Total no of cases	38(100%)	6(100%)	6(100%)

Pearson chi square test : Test value - 27.290
P value - 0.001

Chart 3: Age distribution in different subtypes

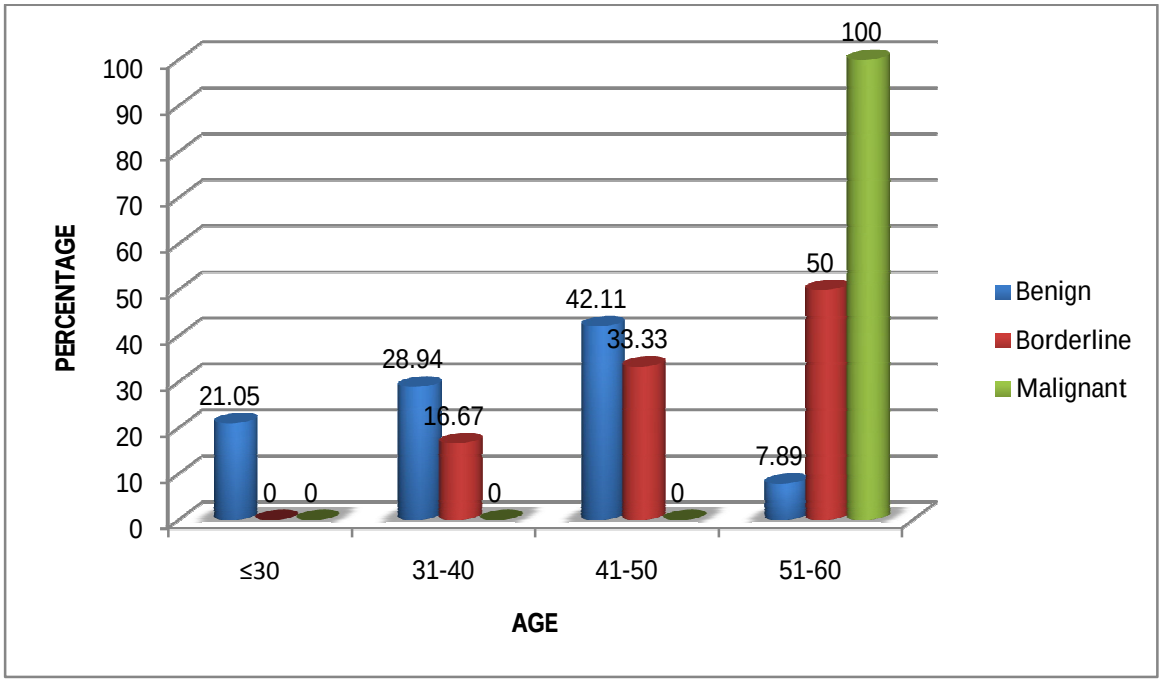
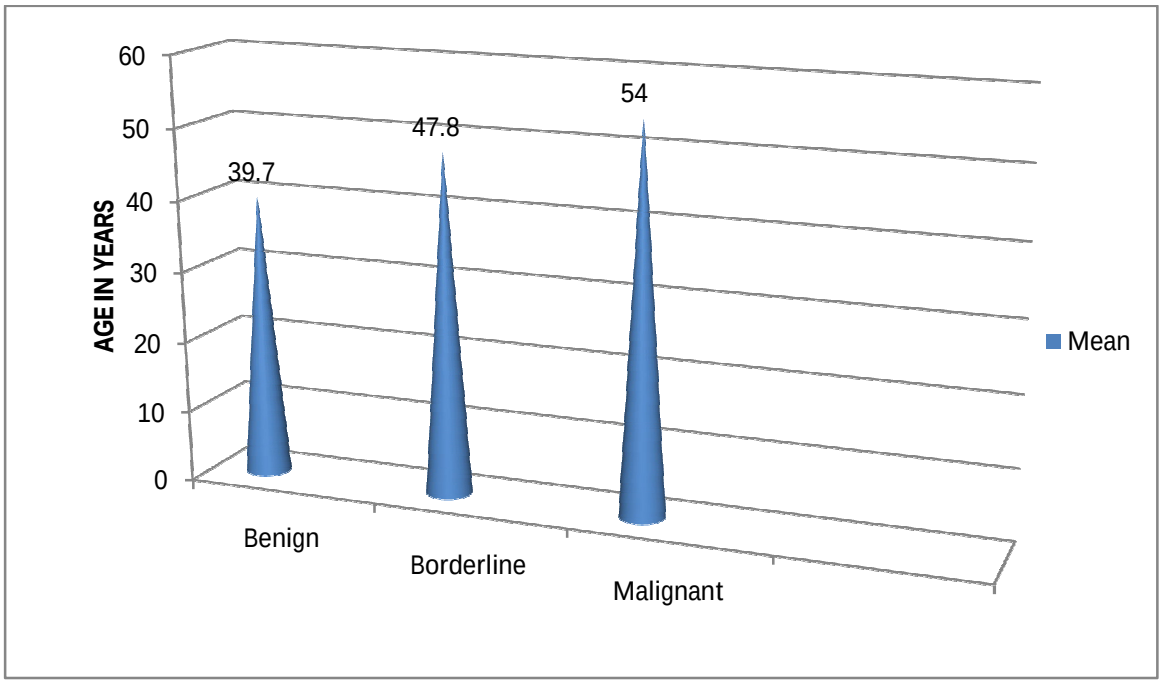


Table 4: Relationship between patients age and tumor grade

Tumor grade	Youngest age	Oldest age	Mean
Benign	17	57	39.7
Borderline	35	60	47.8
Malignant	51	60	54

Chart 4: Mean age distribution in different grade

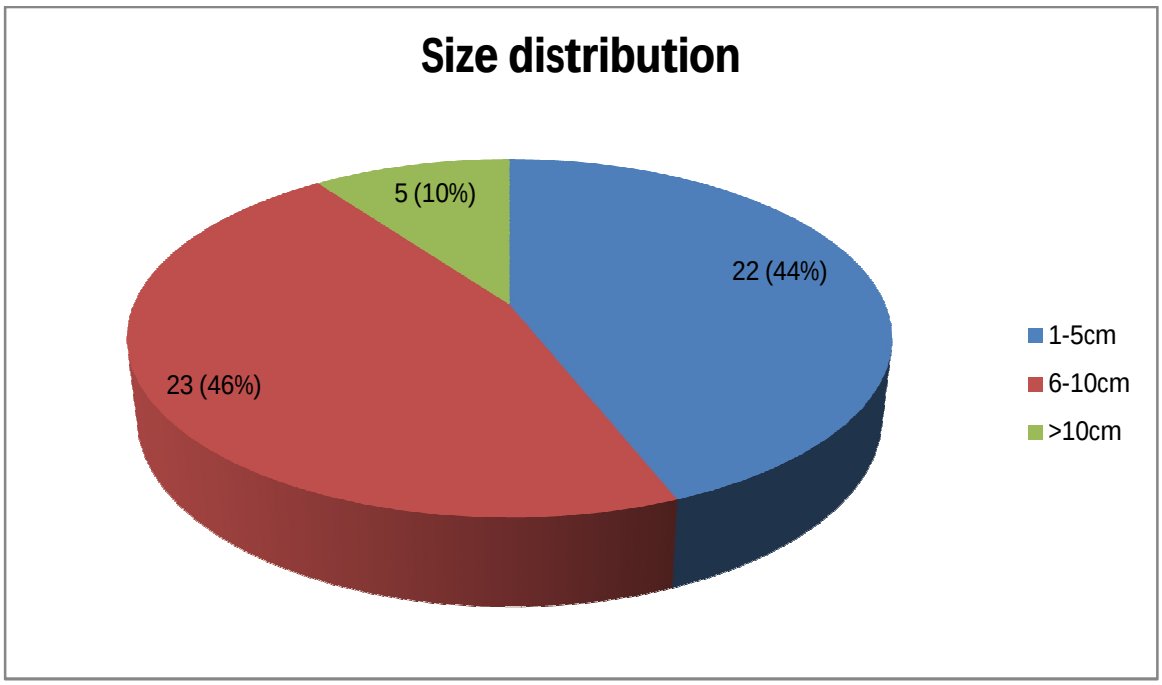


Depending on the size, distribution of cases were as shown in table 5 and chart 5. Most of the lesions (46%) had the size range of 6-10cm,44% had 1-5 cm size and only 10% of the cases were more than 10cm.

Table 5: Distribution of cases according to size

Size	No of cases	Percentage
1-5	22	44%
6-10	23	46%
>10	5	10%
Total no of cases	50	100%

Chart 5: Distribution of cases according to size



Most of the benign tumors were of the size 1-5cm (55.26%) followed by 39.47% were of size 6-10cm with only 5.265 had size greater than 10cm.

Of borderline tumors most (66.67%) were of size 6-10cm, with 16.67% is of size 1-5cm and greater than 10cm each respectively.

Among malignant tumors 66.67% of cases were of size 6-10cm, 33.33% were of size greater than 10cm and none of the cases were less than 5cm (Table 6 & Chart 6).

Table 6: Distribution of size in different subtypes

Size	Benign (%)	Borderline (%)	Malignant (%)
1-5	21 (55.3%)	1 (16.7%)	--
6-10	15 (39.5)	4 (66.7%)	4 (66.7%)
>10	2 (5.3%)	1 (16.7%)	2 (33.3%)
Total no of cases	38 (100%)	6 (100%)	6 (100%)

Pearson chi square test: Test value - 10.606
P value - 0.031

Chart 6: Distribution of size in different subtypes

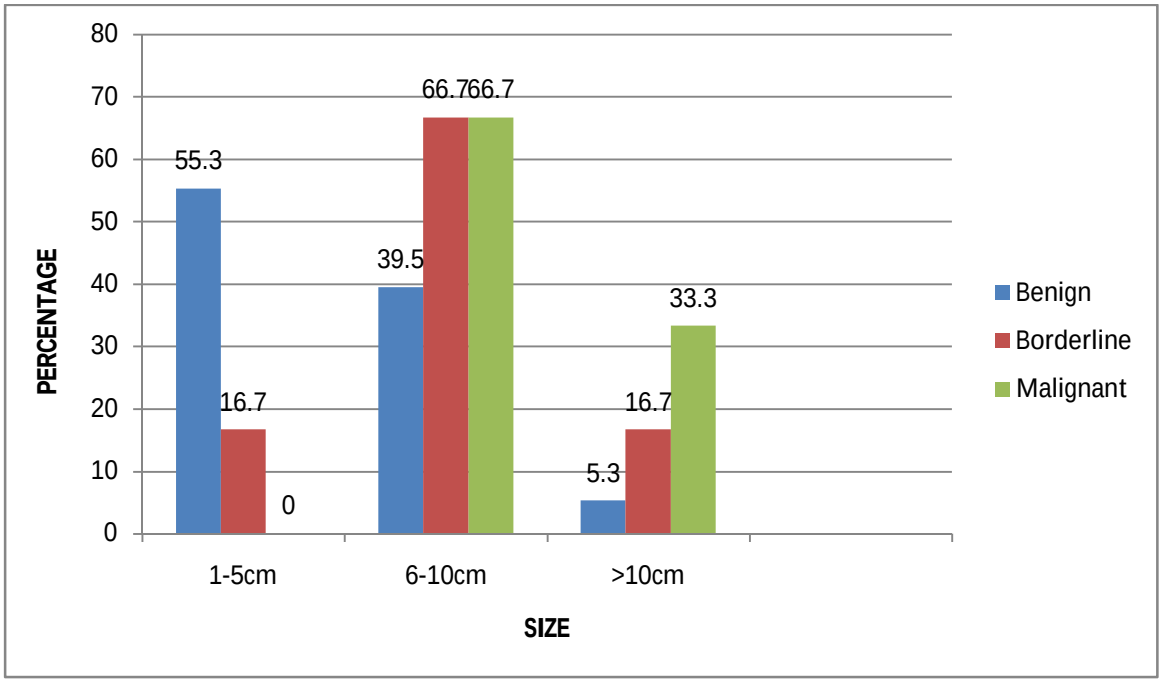


Table 7: Relationship between tumor size and tumor grade

Size	Smallest size (cm)	Largest size (cm)	Mean (cm)
Benign	3	15	5.6
Borderline	5	14	8.5
Malignant	7	19	10.8

In this study , benign tumor size ranges from 3cm to 15 cm in maximum diameter with mean size of 5.6cm. Size of borderline tumor ranges from 5cm to 14cm with mean of 8.5cm.

In 6 cases of malignant tumor the size ranged from 7cm upto 19cm with mean of 10.8cm (Table 7 and Chart 7).

Chart 7: Relation between tumor size and tumor grade

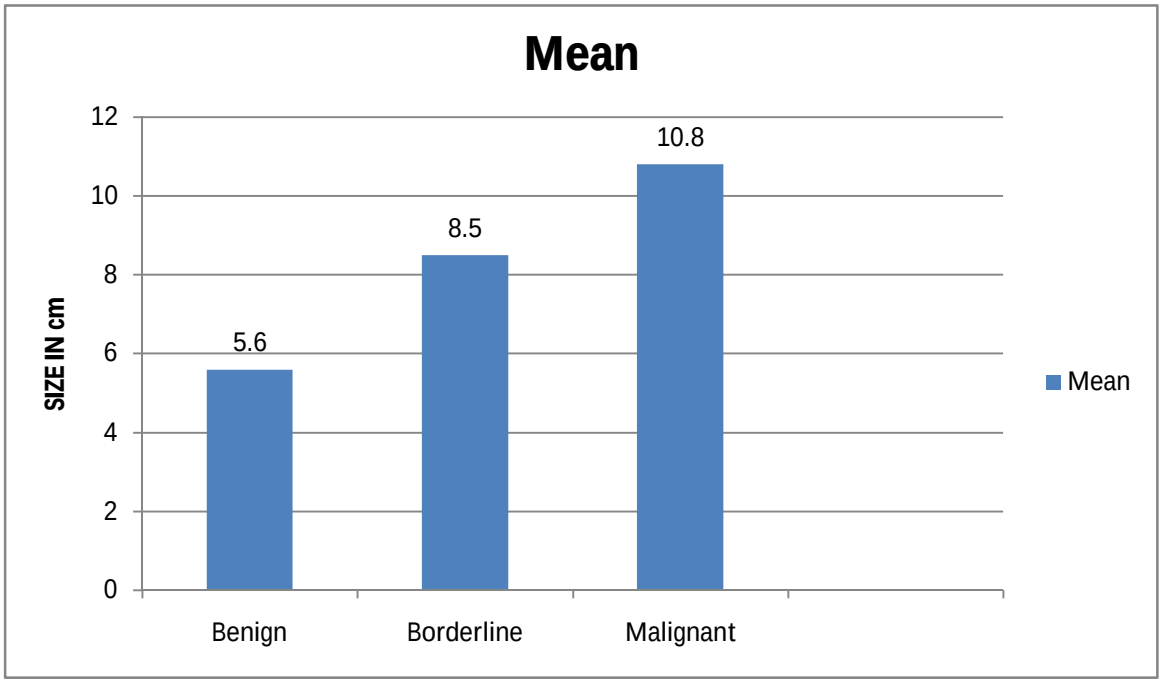
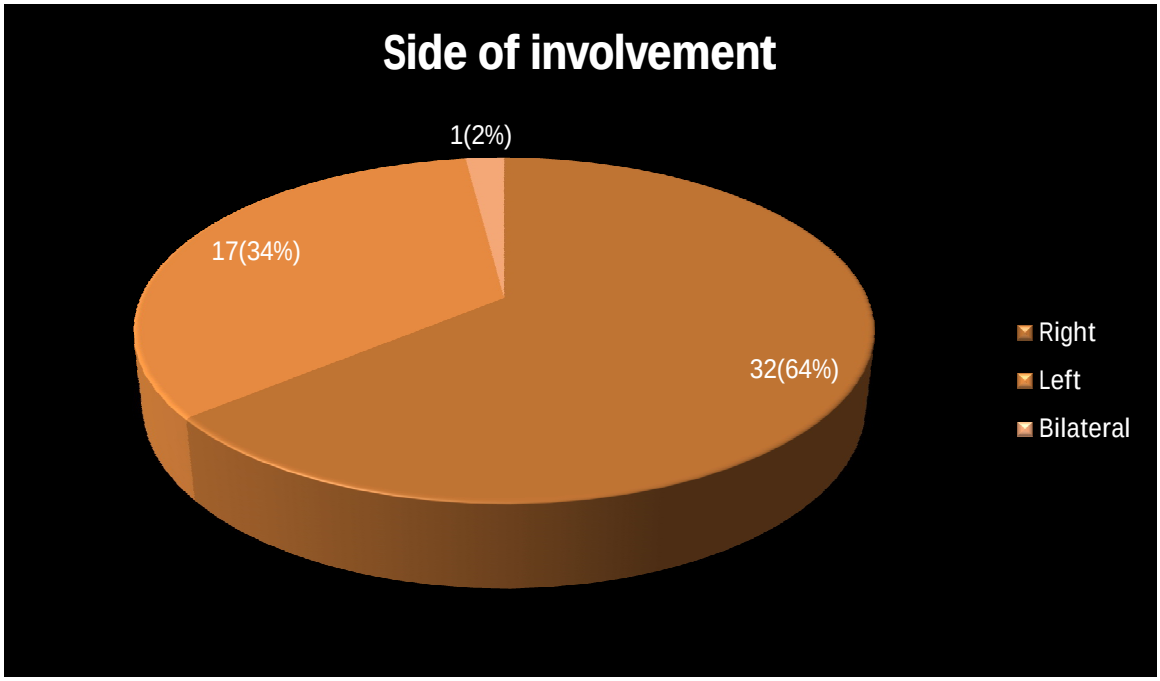


Table 8: Distribution of cases according to laterality

Side	No of cases	Percentage
Right	32	64%
Left	17	34%
Bilateral	1	2%
Total	50	100%

Chart 8: Distribution of cases according to laterality



One case of benign tumor was bilateral remaining cases most often occur in right side which accounts for 64% (32 cases) and left side with 17cases accounting for 34% (Table 8 & 9,chart 8 &9).

Table 9: Distribution of laterality in different grade

Side	Benign (%)	Borderline(%)	Malignant(%)
Right	26 (68.42%)	1(16.67%)	5(83.33%)
Left	11(28.94%)	5(83.33%)	1(16.67%)
Bilateral	1(2.63%)	--	--
Total no of cases	38 (100%)	6 (100%)	6 (100%)

Pearson chi square test: Test value - 7.993

P value - 0.092

Chart 9: Distribution of laterality in different grade

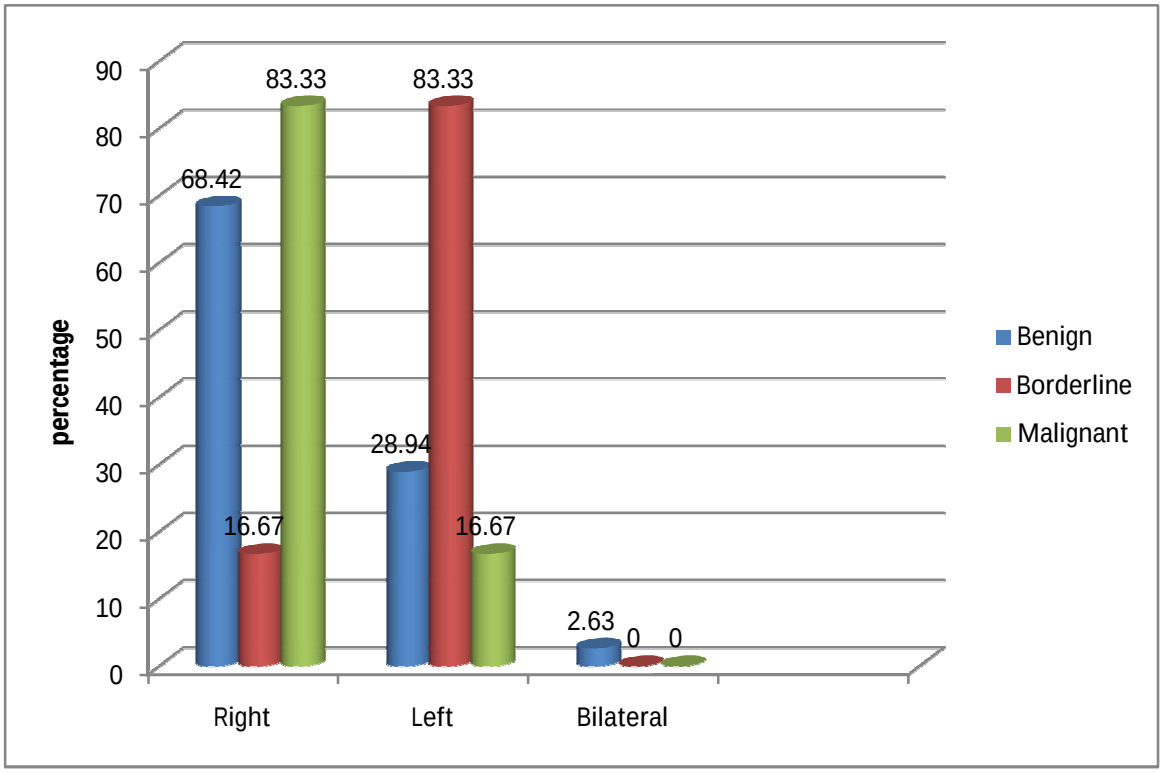
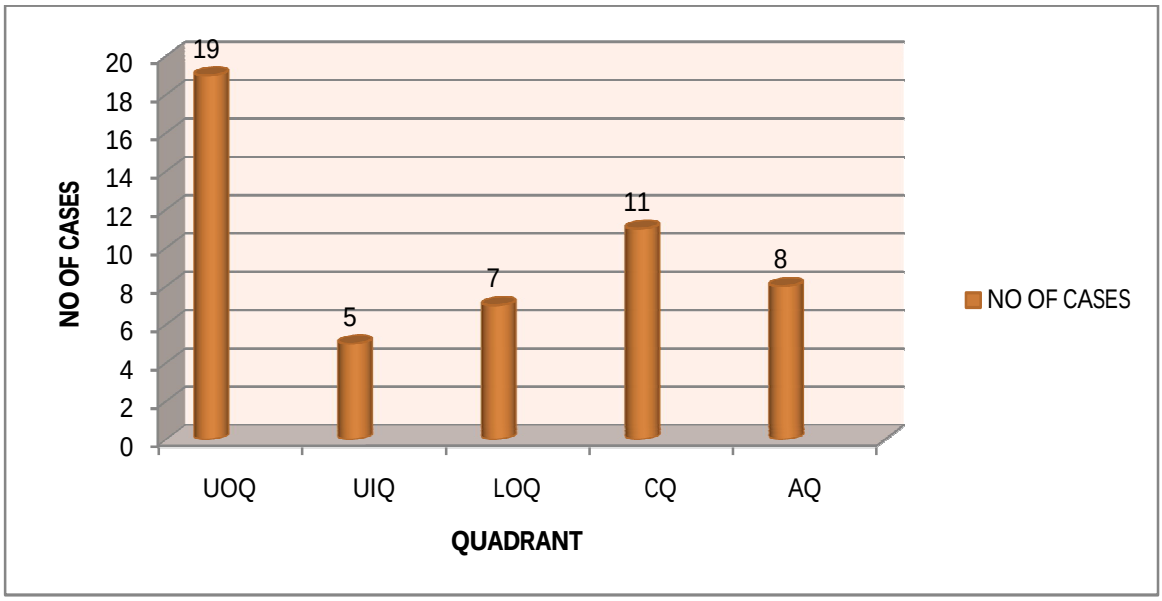


Table 10: Distribution of cases according to tumor location

Tumor location	No of cases	Percentage
Upper outer quadrant(UOQ)	19	38%
Upper inner quadrant(UIQ)	5	10%
Lower outer quadrant(LOQ)	7	14%
Central quadrant(CQ)	11	22%
All quadrant(AQ)	8	16%
Total	50	100%

Chart 10: Distribution of cases according to tumor location



In this study the most common site of tumor location was found to be the upper outer quadrant corresponding to 38%, followed by central quadrant with

22%,all quadrant with 16.0% , lower outer quadrant with 14.0%and the least common site was the upper inner quadrant with 10.0%. No cases were found in lower inner quadrant as shown in the Table 10 and Chart 10.

Among the subtypes , benign occur more commonly in upper outer quadrant (44.7%) whereas most (50%) of borderline and malignant involves any of the quadrants of breast (Table 11).

Table 11 :Association of tumor location with different subtypes

Tumor location	Benign (%)	Borderline(%)	Malignant (%)
UOQ	17(44.7%)	1(16.7%)	1(16.7%)
UIQ	4(10.5%)	1(16.7%)	--
LOQ	6(15.8%)	--	1(16.7%)
CQ	9(23.7%)	1(16.7%)	1(16.7%)
AQ	2(5.3%)	3(50.0%)	3(50.0%)
Total no of cases	38 (100%)	6 (100%)	6 (100%)

Pearson chi square test: Test value - 15.338
P value - 0.053

Out of 50 cases, most of the cases belonged to BIRADS IV comprising 46%,followed by BIRADS III 38% and BIRADS II & IV each constituting 8% respectively (table 12 and chart 11).

Most of the benign tumors belong to BIRADS IV and III (44.7% and 42.1%) with only few cases have BIRADS II and V.

Borderline also shows similar findings with most of the cases belonging to BIRADS IV and III with 66.7% and 33.3% respectively whereas most of malignant tumors belong to BIRADS V with 50.0% followed by BIRADS IV and III, found to be 33.3% and 16.7% each (Table13 and chart 12).

Table 12: Distribution of cases according to BIRADS

BIRADS	No of cases	Percentage
1	--	--
2	4	8%
3	19	38%
4	23	46%
5	4	8%
Total	50	100%

Chart 11:Distribution of cases according to BIRADS

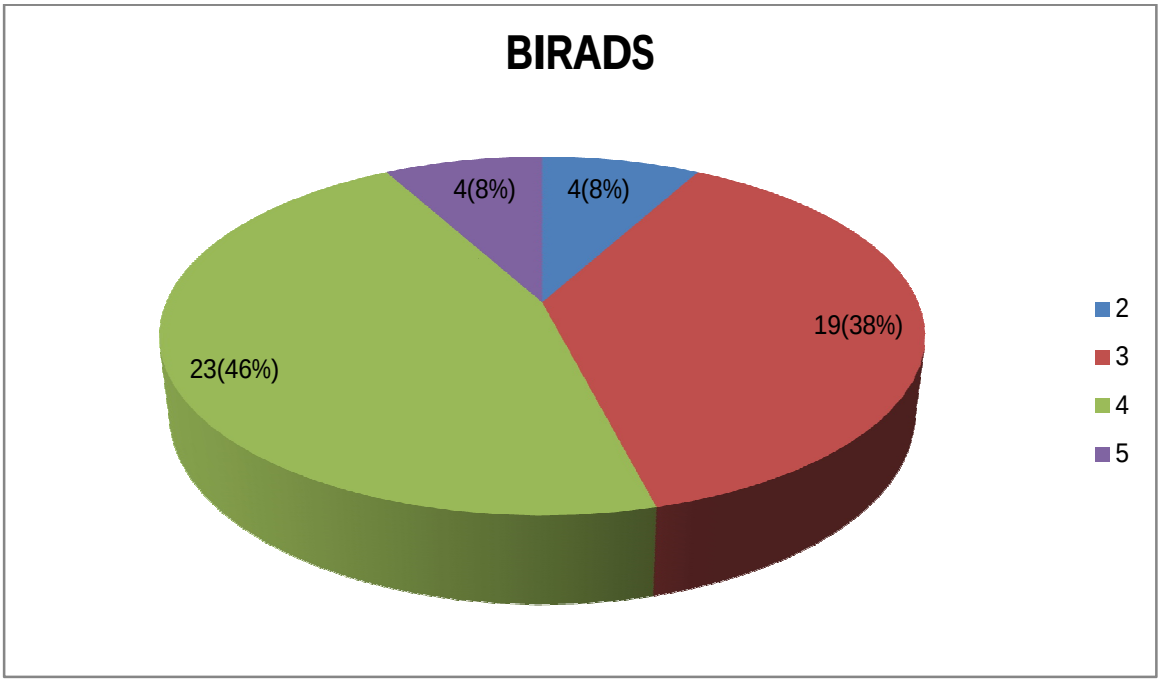


Table 13: BIRADS findings in individual subtypes

BIRADS	Benign(%)	Borderline (%)	Malignant (%)
1	--	--	--
2	4(10.5%)	--	--
3	16(42.1%)	2(33.3%)	1(16.7%)
4	17(44.7%)	4(66.7%)	2(33.3%)
5	1(2.6%)	--	3(50.0%)
Total no of cases	38 (100%)	6 (100%)	6 (100%)

Pearson chi square test: Test value - 18.043

P value - 0.006

Chart 12: BIRADS finding in individual cases

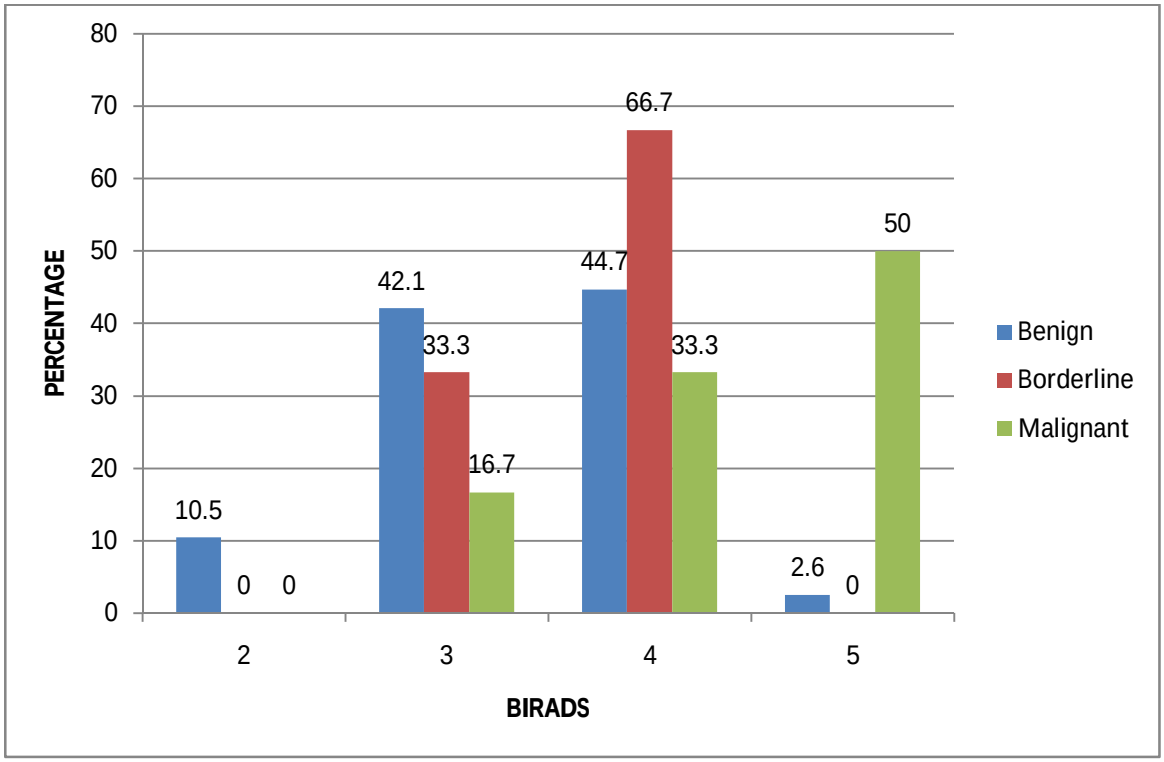


Chart 13: Distribution of cases based on clinical features

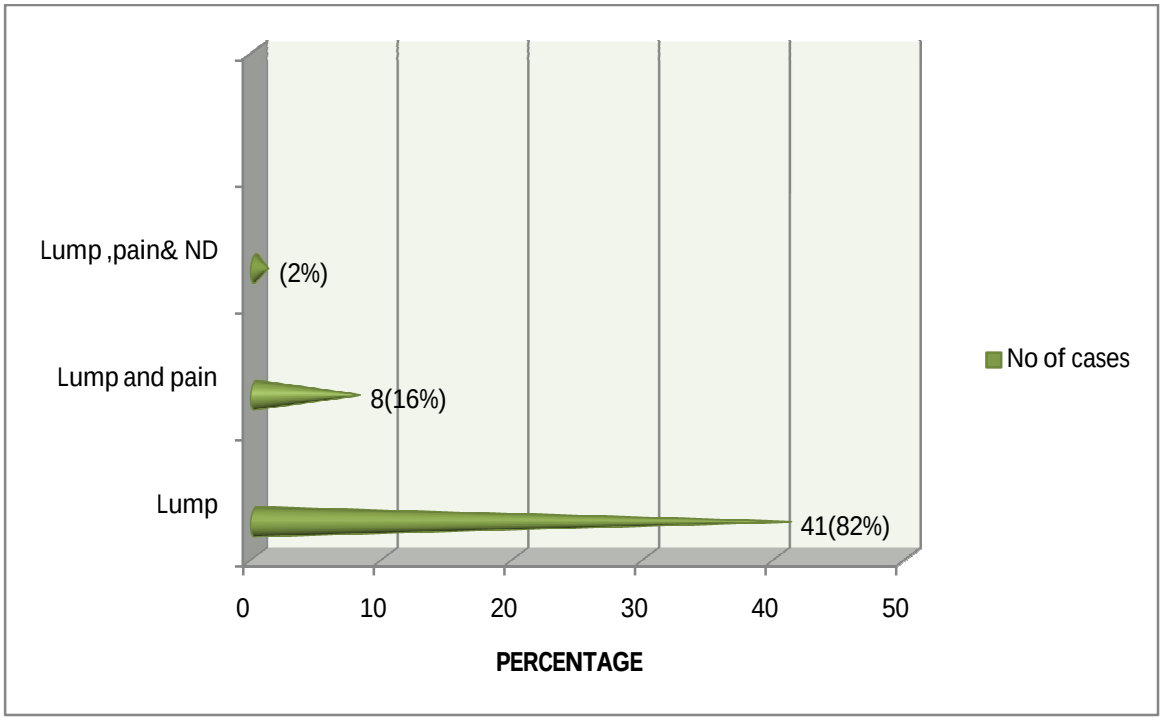


Table 14: Distribution of cases based on clinical features

Clinical features	No of cases	Percentage
Lump	41	82%
Lump & pain	8	16%
Lump, pain & nipple discharge	1	2%
Total	50	100%

On analysing 50 cases, 41 cases (82%) presented only with lump and 8cases (16%) presented with both lump and pain. Only one case (2%) presented with nipple discharge (ND) along with lump and pain and was found to belong to malignant category(Table 14 &15 and chart 13).

Table 15: Distribution of clinical presentation in different subtypes

Clinical features	Benign (%)	Borderline (%)	Malignant (%)
Lump	34(89.5%)	4(66.7%)	3(50.0%))
Lump & pain	4(10.5%)	2(33.3%)	2(33.3%)
Lump, pain & nipple discharge	--	--	1(16.7%)
Total no of cases	38 (100%)	6 (100%)	6 (100%)

Pearson chi square test : Test value - 11.478

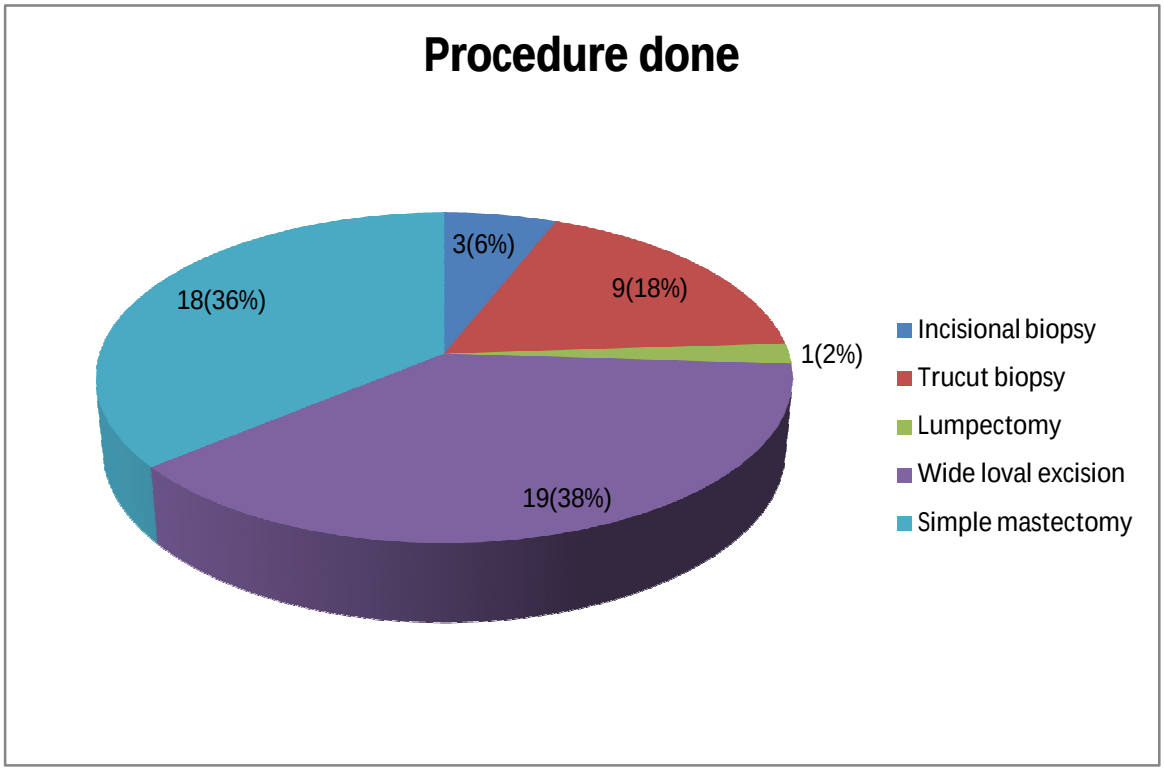
P value - 0.022

Table 16: Distribution of cases in relation to surgical biopsy specimens

Procedure done	No of cases	Percentage
Incision biopsy	3	6%
Trucut biobsy	9	18%
Lumpectomy	1	2%
Wide local excision	19	38%
Simple mastectomy	18	36%
Total	50	100%

Of the 50 cases, resected specimens include 38 cases whereas small biopsy were 12 case. Of the resected specimens commonest type of surgical biopsy was wide local excision constituting 38% followed by simple mastectomy which constitutes 36%(Table 16 and chart 14).

Chart 14: Distribution of cases in relation to surgical biopsy specimens



Gross involvement of margins are assessed only in resected specimens and small biopsies are not included. Out of 50 cases, 38 were resected specimen and 1 was lumpectomy for which marginal status could not be assessed.

Out of 37 cases 9 cases show infiltration into adjacent breast tissue whereas 28 cases shows pushing margin (Table 17 And chart 15).

Table 17: Gross margin in different subtypes

Gross margin	Benign (%)	Borderline (%)	Malignant (%)
Infiltrative	--	4(66.7%)	5(83.3%)
Pushing	28 (73.7%)	--	--
Margin not known	10(26.3%)	2 (33.3%)	1(16.7%)
Total no of cases	38 (100%)	6 (100%)	6 (100%)

Pearson chi square test: Test value - 38.132

P value - 0.001

Chart 15: Gross margin in different subtypes

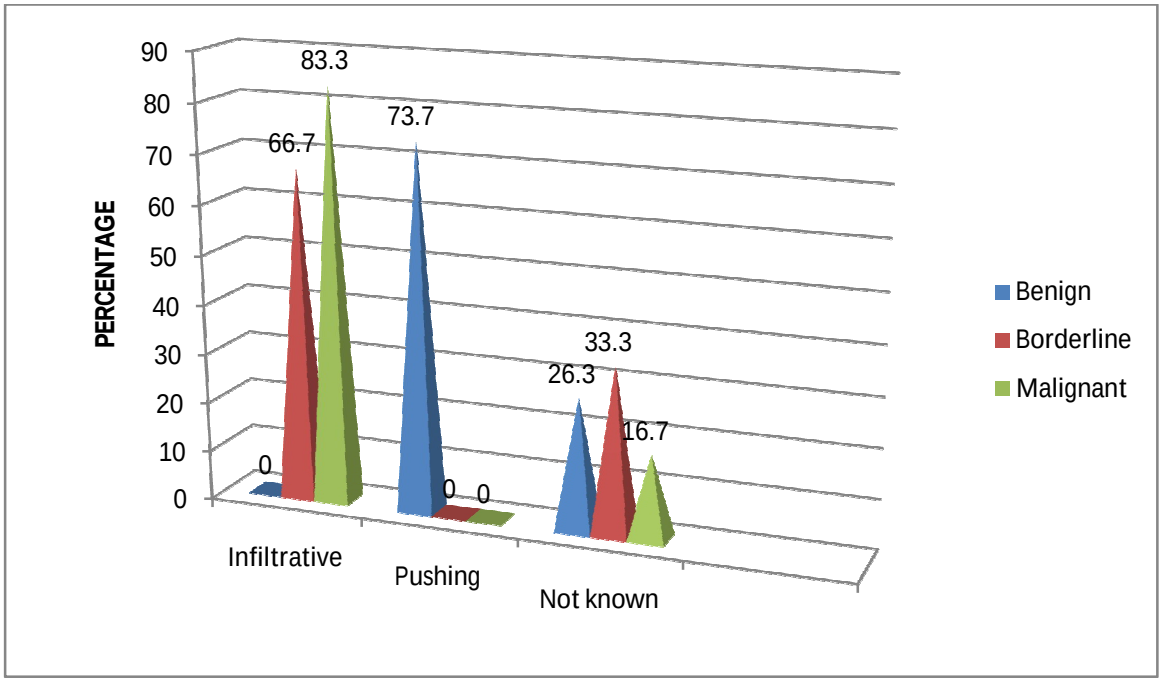


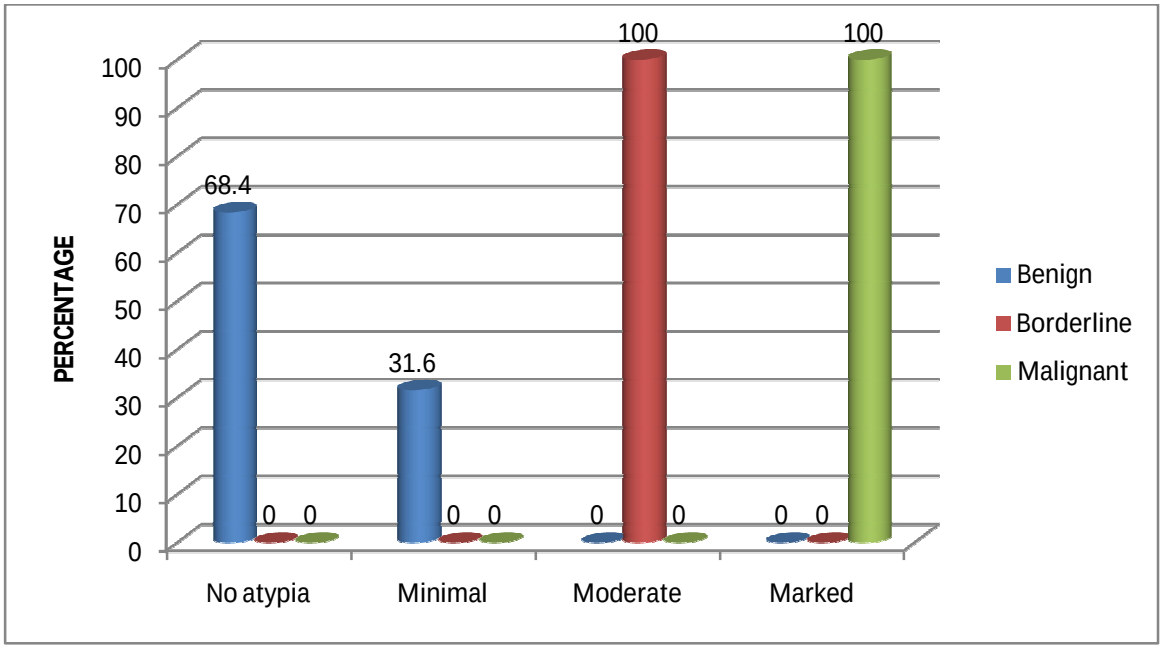
Table 18: Association of atypia in different subtypes

Atypia	Benign (%)	Borderline (%)	Malignant(%)
No atypia	26(68.4%)	--	--
Minimal	12(31.6%)	--	--
Moderate	--	6(100%)	--
Marked	--	--	6(100%)
Total no of cases	38 (100%)	6 (100%)	6 (100%)

Pearson chi square test: Test value - 100

P value - 0.001

Chart 16: Association of atypia in different subtypes



Among different subtypes atypia is more maked in all cases (100%) of malignant phyllodes tumor , with moderate atypia in all cases of borderline tumor. In benign tumors 68.4% shows no atypia of stromal cells but 31.6% shows minimal atypia(Table 18 and chart 16).

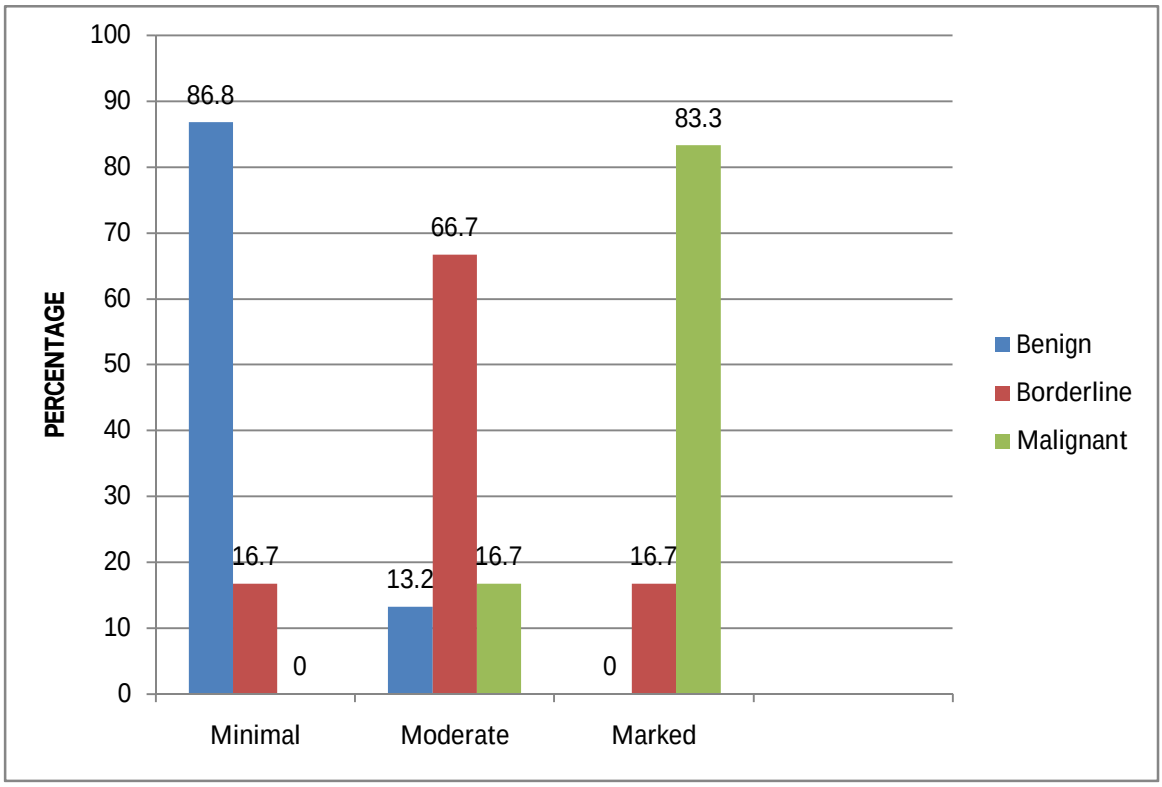
Table 19: Association of stromal cellularity in different subtypes

Stromal cellularity	Benign (%)	Borderline(%)	Malignant(%)
Minimal	33(86.8%)	1(16.7%)	--
Moderate	5(13.2%)	4(66.7%)	1(16.7%)
Marked	--	1(16.7%)	5(83.3%)
Total no of cases	38 (100%)	6 (100%)	6 (100%)

Pearson chi square test: Test value - 100

P value - 0.001

Chart 17: Association of stromal cellularity in different subtypes



Regarding stromal cellularity, benign tumors shows minimal cellularity in 86.8% and moderate cellularity in 13.2%. Nine of benign show marked cellularity. Most of borderline tumors have moderate cellularity in 66.7%, with mild and marked cellularity seen in 16.7% of cases each. Most of malignant tumors have marked cellularity accounting for 83.3% followed by 16.7% showing moderate cellularity(Table 19 and chart 17).

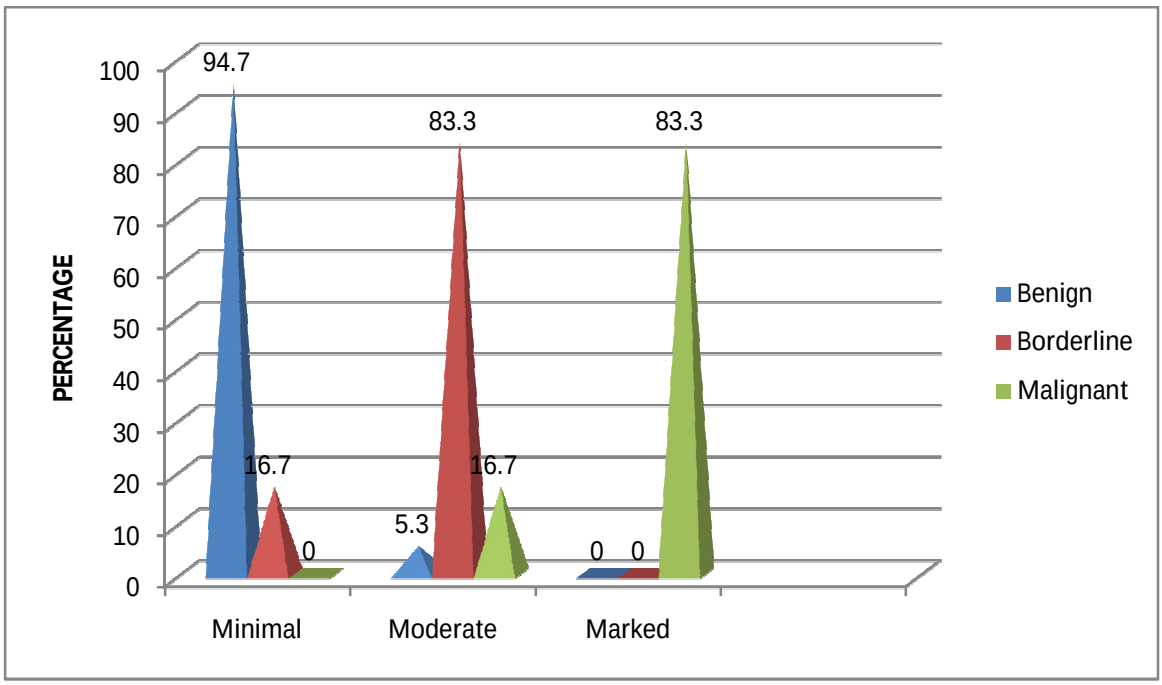
Table 20: Association of stromal overgrowth in different subtypes

Stromal overgrowth	Benign (%)	Borderline (%)	Malignant (%)
Minimal	36(94.7%)	1(16.7%)	--
Moderate	2(5.3%)	5(83.3%)	1(16.7%)
Marked	--	--	5(83.3%)
Total no of cases	38 (100%)	6 (100%)	6 (100%)

Pearson chi square test: Test value - 65.721

P value - 0.001

Chart 18: Association of stromal overgrowth in different subtypes



Stromal overgrowth is marked in most (83.3%) of the malignant tumors with only 16.7% showing moderate overgrowth. Whereas most of benign tumors show minimal cellularity (94.7% of cases), with only 5.3% have moderate cellularity. Among borderline 83.3% show moderate cellularity and 16.7% show minimal cellularity (Table 20 and chart 18).

As that of gross margin, microscopic involvement of tumor margin are assessed only in 37 cases of resected margin. Out of 5 cases of malignant tumor four showed margin involvemen . Out of 4 cases of borderline two showed margin involvement whereas in 28 cases of benign only three showed margin involvement (Table 21 and chart 19).

Table 21: Association of microscopic margins in different subtypes

Microscopic margin	Benign(%)	Borderline(%)	Malignant(%)
Uninvolved	25(65.8%)	2(33.3%)	1(16.7%)
Involved	3(7.9%)	2(33.3%)	4(66.7%)
Margin not known	10(26.3%)	2(33.3%)	1(16.7%)
Total no of cases	38 (100%)	6 (100%)	6 (100%)

Chart 19: Association of microscopic margins in different subtypes

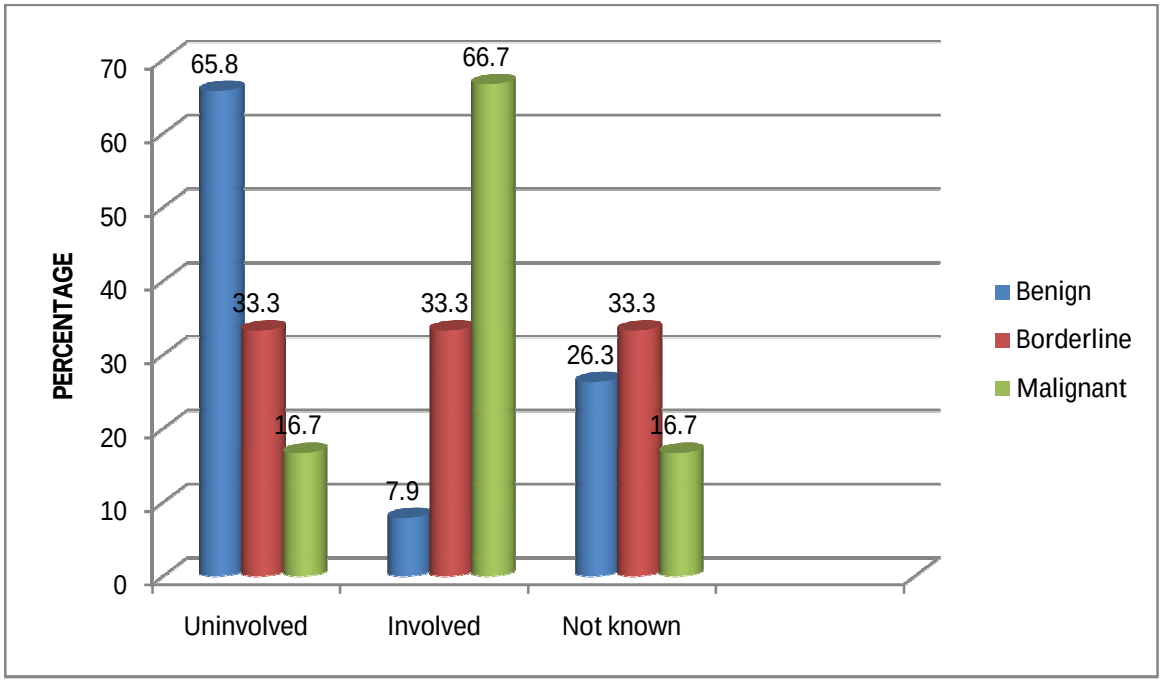


Table 22: Association of mitosis in different subtypes

	Benign(%)	Borderline (%)	Malignant(%)
0-4/10Hpf	38(100.0%)	--	--
5-9/10Hpf	--	6(100.0%)	--
>10/10Hpf	--	--	6(100.0%)
Total no of cases	38 (100%)	6 (100%)	6 (100%)

Pearson chi square test: Test value - 100

P value - 0.000

IHC interpretation

Table 23: CD10 Intensity of stain in different subtypes

	Benign (%)	Borderline (%)	Malignant(%)
No staining	18(47.4%)	1(16.7%)	--
Weak	14(36.8%)	1(16.7%)	--
Moderate	5(13.2%)	1(16.7%)	2(33.3%)
Strong	1(2.6%)	3(50.0%)	4(66.7%)
Total no of cases	38 (100%)	6 (100%)	6 (100%)

Pearson chi square test: Test value - 26.151

P value - 0.000

CD10 stain intensity was compared with myoepithelial cells and intensity grade was given as weak, moderate and severe.

Among benign tumor most cases(47.4%) show no staining in stromal cells whereas 36.8%,13.2% and 2.6% of cases show weak, moderate and intense staining .

In borderline tumor 50.0% cases show strong intensity of stain in stromal cells.

Of malignant tumors most cases (83.3%) show strong intensity and only 33.3% shows moderate intensity (Table 23 and chart 20).

Chart 20: CD10 Intensity of stain in different subtypes

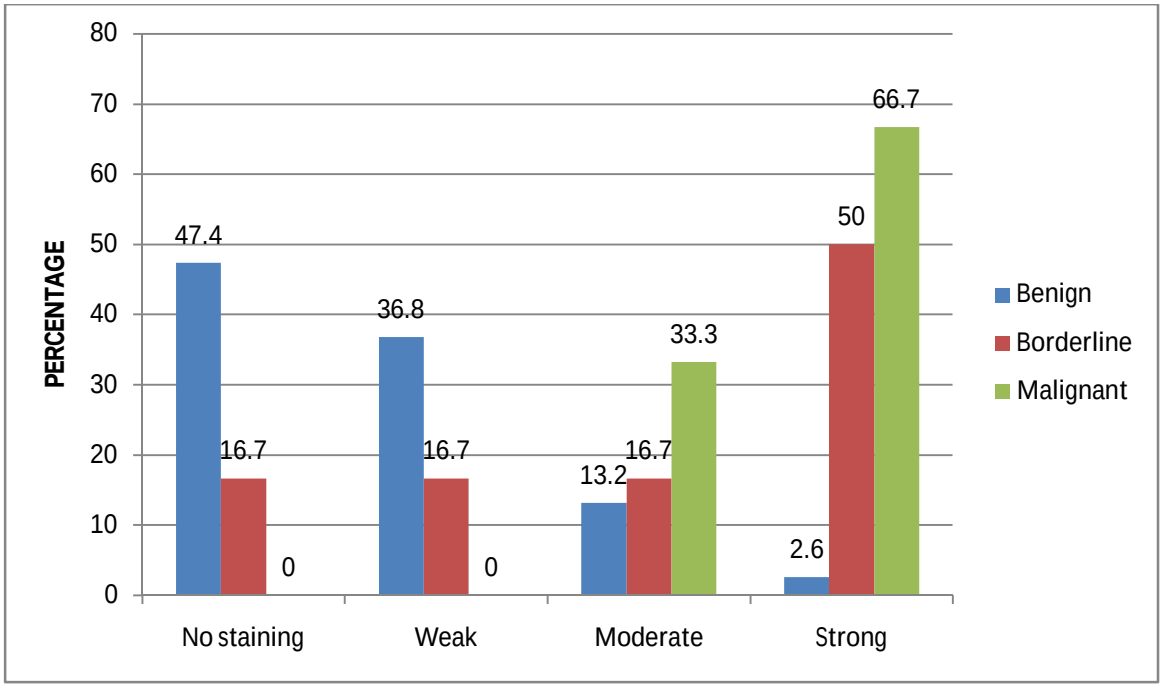


Table 24: percentage of cells stained in different subtypes

	Benign(%)	Borderline(%)	Malignant(%)
Nil	18(47.4%)	1(16.7%)	--
<20% cells	13(34.2%)	1(16.7%)	1(16.7%)
>20% cells	7(18.4%)	4(66.7%)	5(83.3%)
Total no of cases	38 (100%)	6 (100%)	6 (100%)

Pearson chi square test: Test value - 14.083
P value - 0.007

Out of 6 cases of malignant tumor, in five cases greater than 20% of cells have taken stain and only one case show staining in less than 20% of cells. In borderline tumor also 50% (3 out of 6 cases) cases shows staining in greater than 20% of cells.

Among benign out of 38 cases only 7 cases(13.4%) show staining in greater than 20% of stromal cells (table 24 and chart 21).

Chart 21: percentage of cells stained in different subtypes

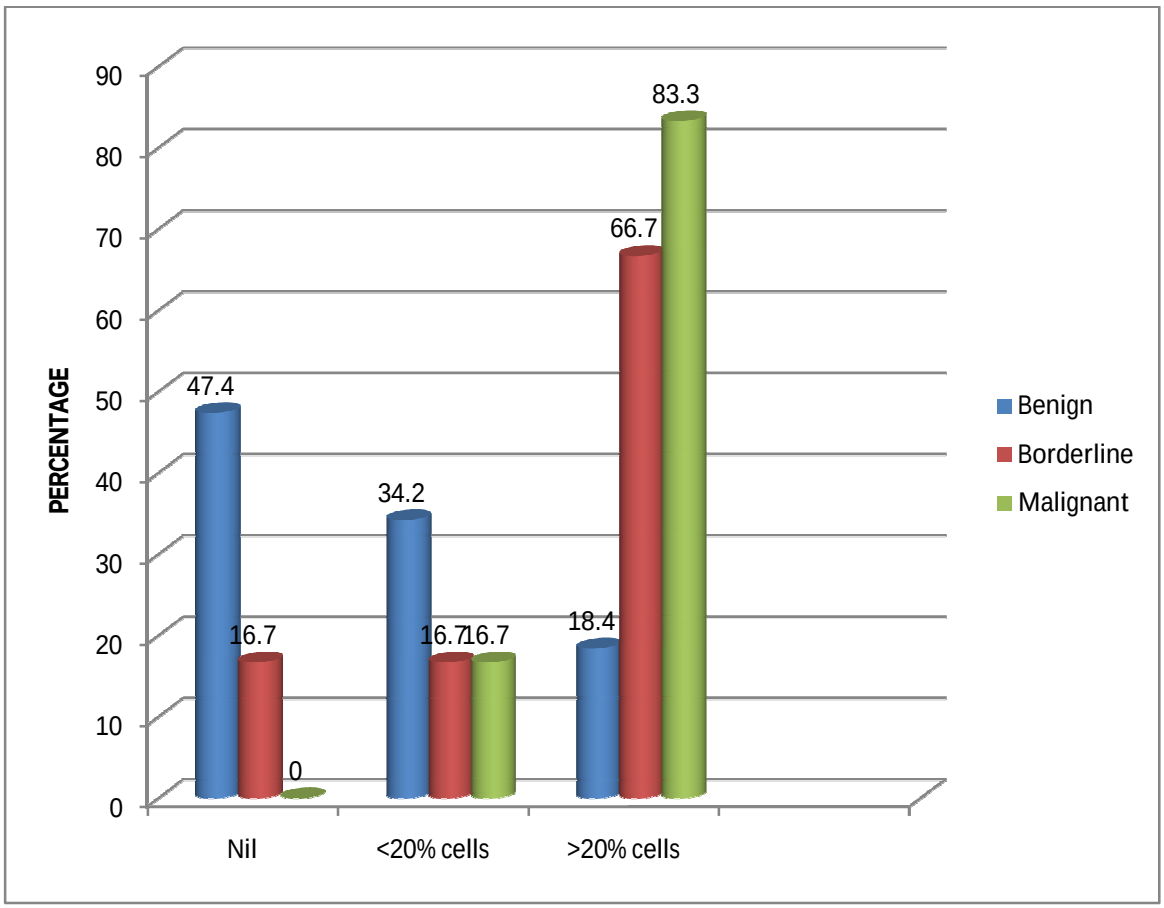


Table 25: CD10 interpretation

	No of cases	Percentage
CD10 positive	11	22%
CD10 negative	39	78%
Total	50	100%

Out of 50 cases of phyllodes tumor 11 cases show CD10 positive accounting for 22% and 39 cases are CD10 negative accounting for 78% (Table 25 and Chart 22)

Chart 22: CD10 interpretation

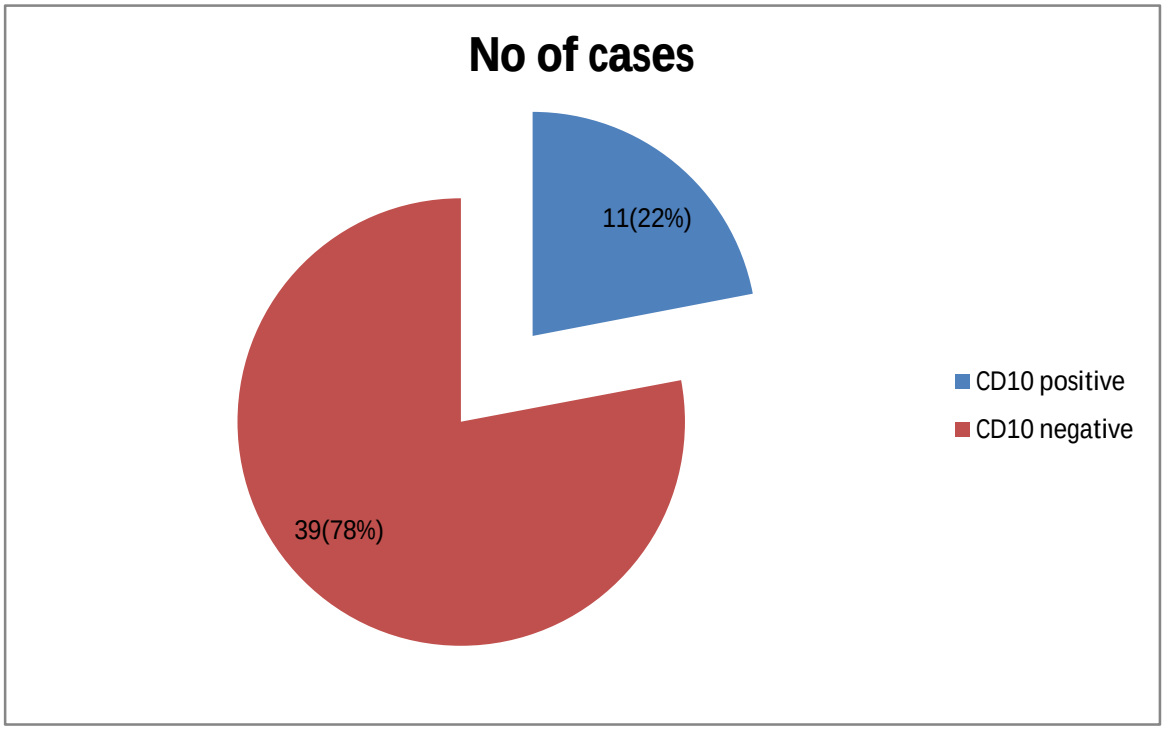


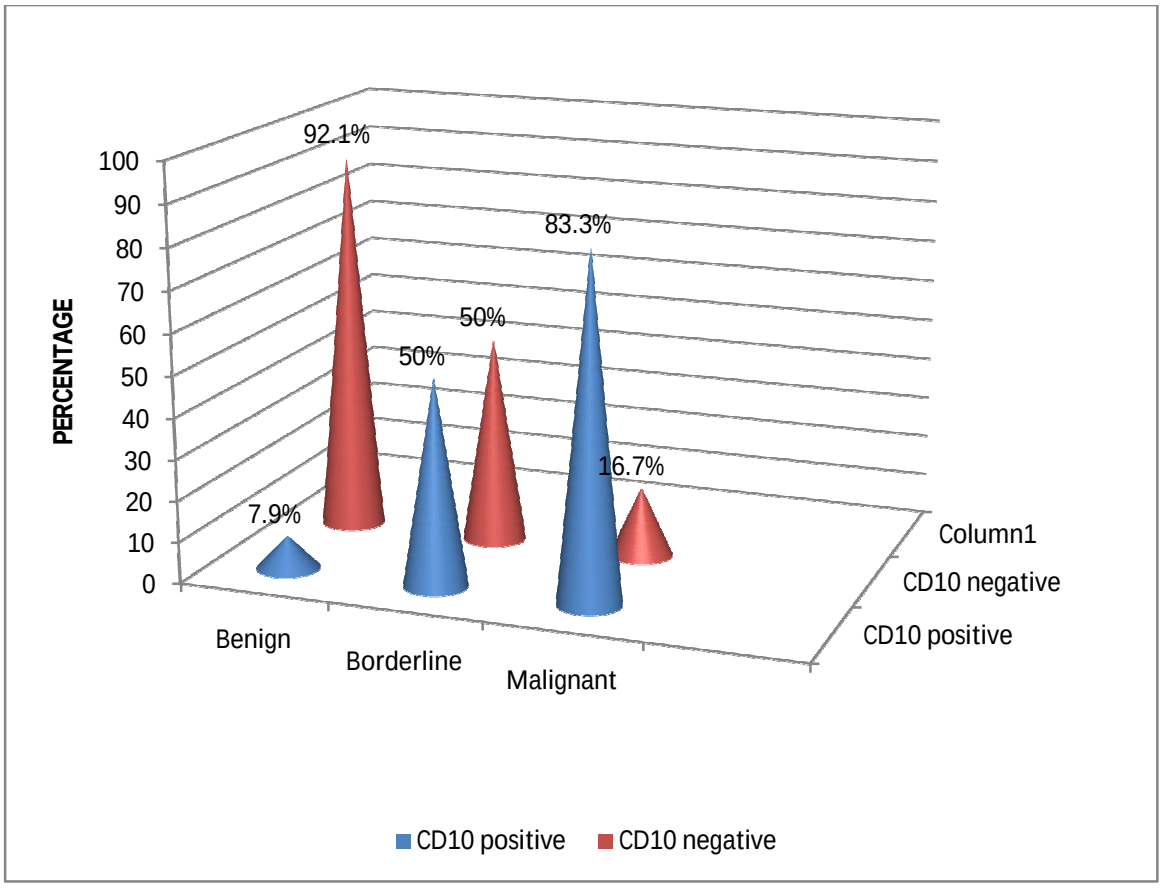
Table 26: CD10 positivity/negativity in different subtypes

	Benign (%)	Borderline (%)	Malignant (%)
CD10 positive	3(7.9%)	3(50.0%)	5(83.3%)
CD10 negative	35(92.1%)	3(50.0%)	1(16.7%)
Total no of cases	38 (100%)	6 (100%)	6 (100%)

Pearson chi square test: Test value - 20.300

P value - 0.000

Chart 23: CD10 positivity/negativity in different subtypes



Out of 50 cases of phyllodes tumor, CD10 positivity was more in case of malignant tumor (6 out of 5) accounting for 83.3% with only one case (16.7%) being CD10 negative.

Among borderline 50% show CD10 positive while remaining 50% were negative.

Of benign tumors, out of 38 cases 35 (92.1%) were CD10 negative with only 3 cases accounting for 7.9% were CD10 positive (Table 26 and chart 23).

Colour Plates

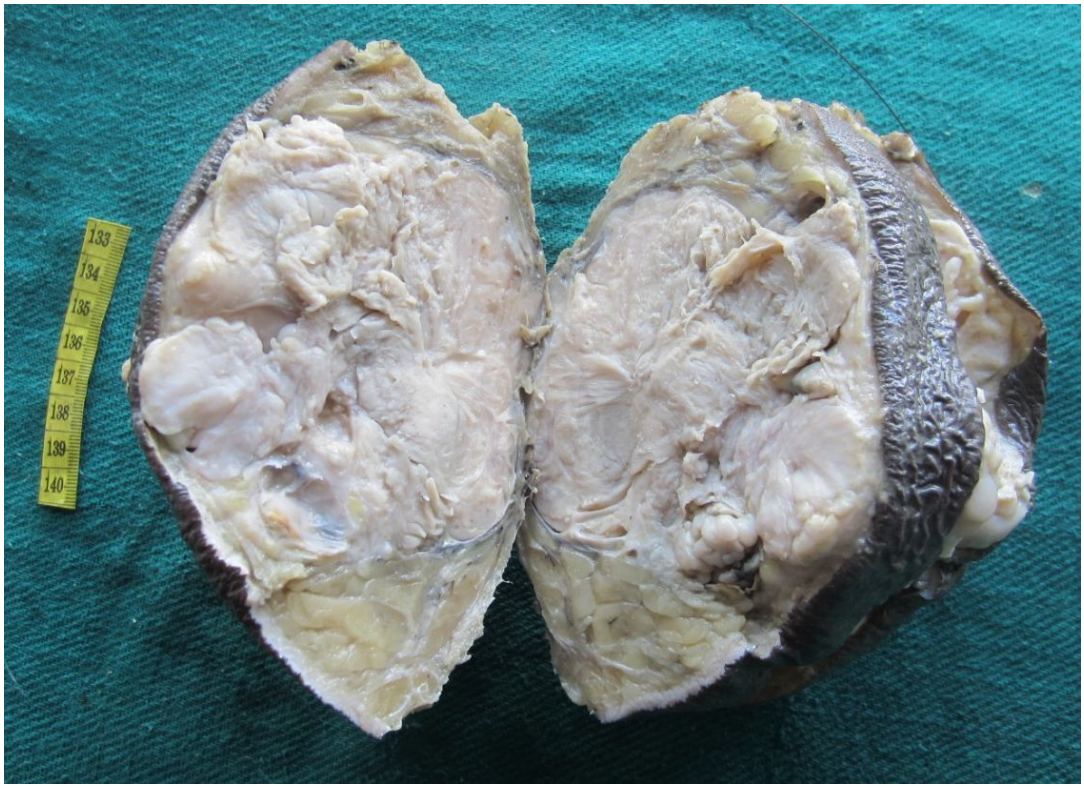


Fig.2: 3728/14- Benign phyllodes tumor- solid homogenous circumscribed mass with with pushing margin

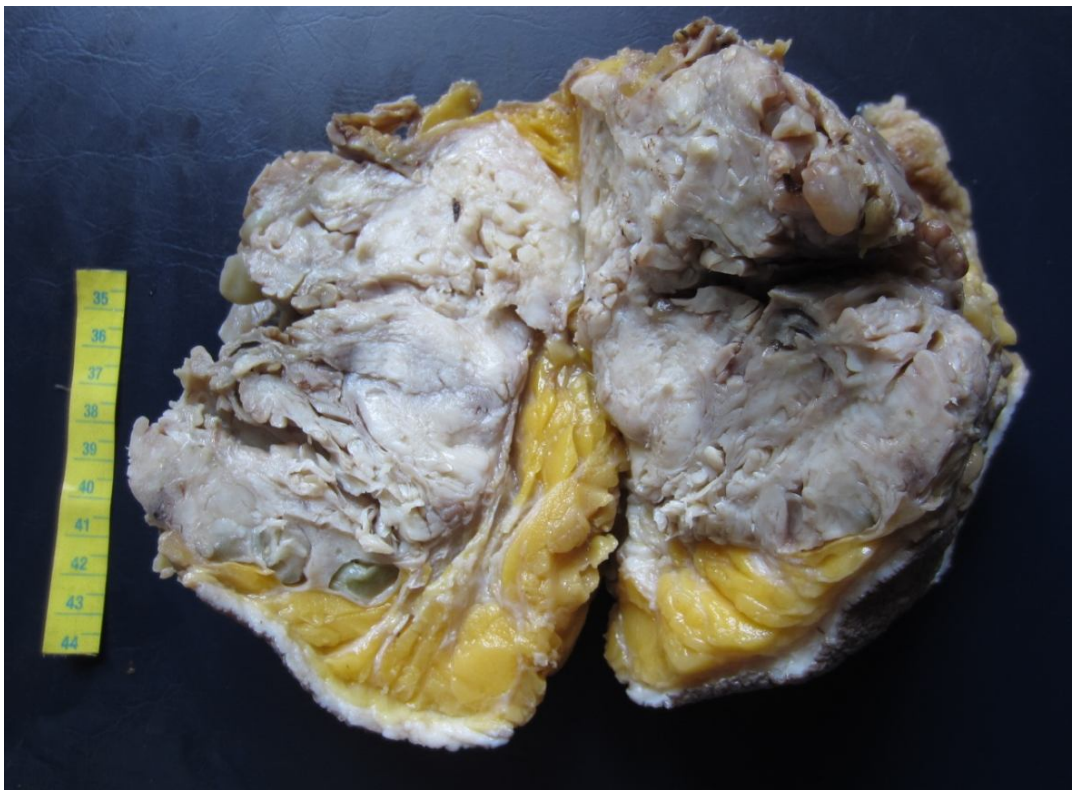


Fig 3:5695/12- Borderline phyllodes tumor- solid growth with pushing margin

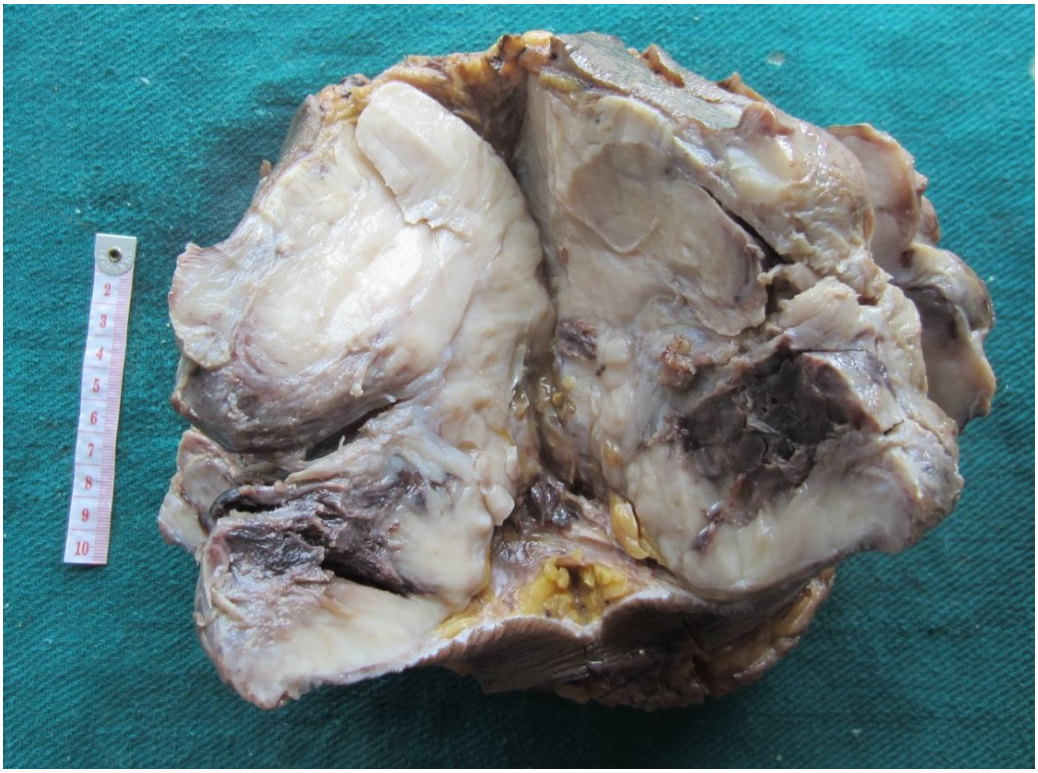


Fig 4: 6119/14- Malignant phyllodes tumor-solid growth with focal cystic spaces with infiltrative margin.

BENIGN PHYLLODES TUMOR

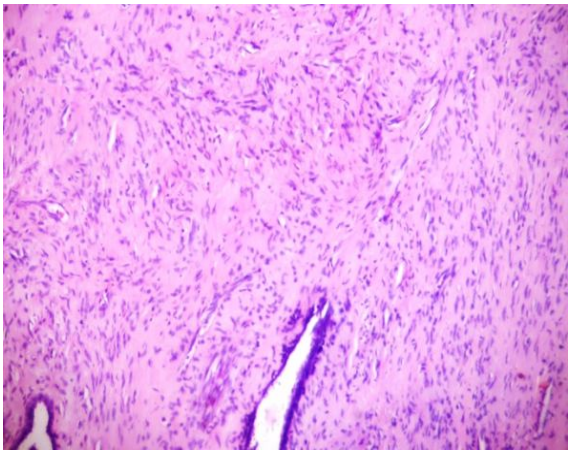


Fig 5: Benign tumor-Increased tromal cellularity with monotonous spindle cell. 100x, HPE- 7917/13

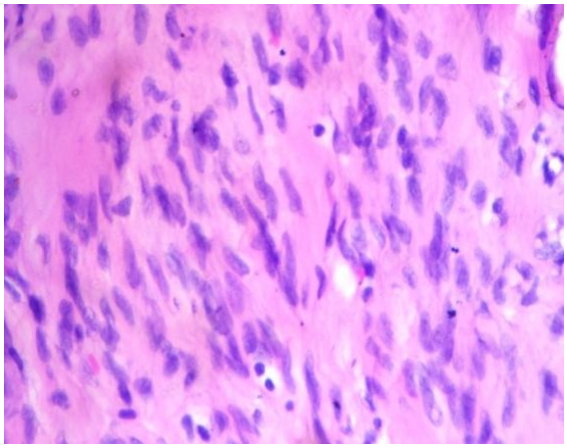


Fig 6: spindle cell with bland nuclei. No atypia and mitosis seen. 400x, HPE- 7917/13

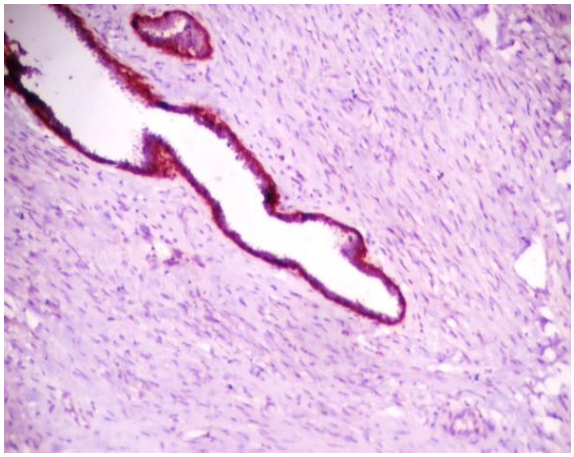


Fig 7: IHC –CD10 positive only in myoepithelial cells. 100x, HPE- 7917/13

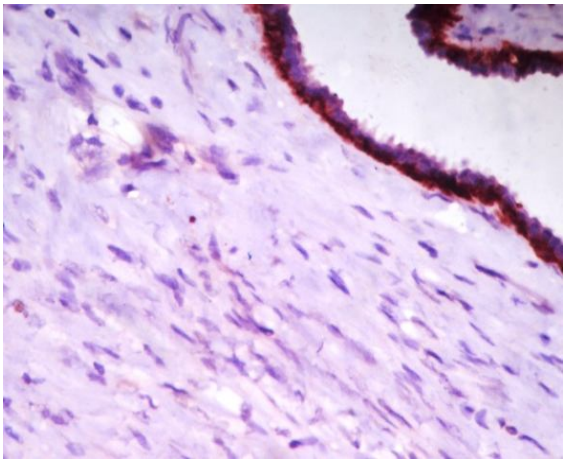


Fig 8: IHC- Stromal cells negative for CD10 . 400x, HPE- 7917

BENIGN PHYLLODES TUMOR

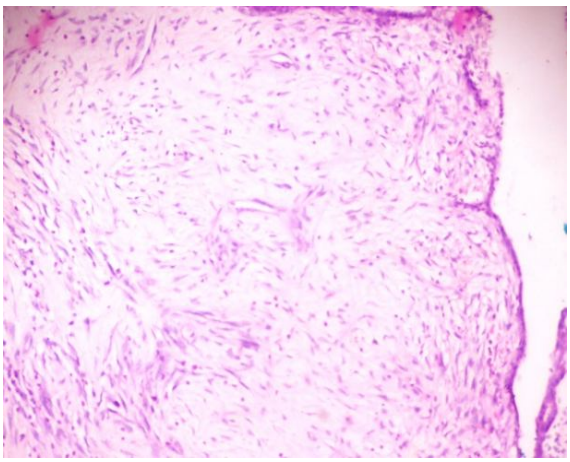


Fig 9: Benign tumor –Increased stromal cellularity . 100x, HPE- 2914/14

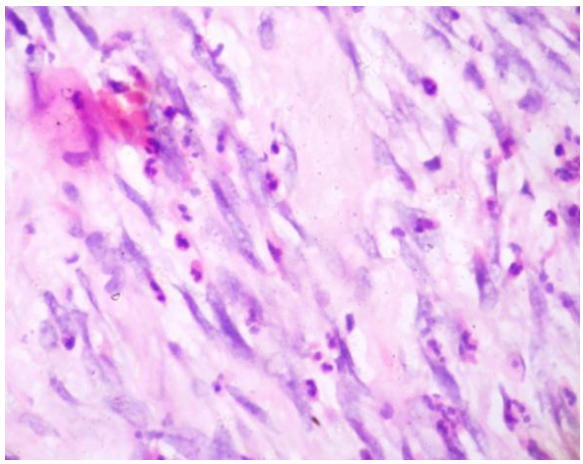


Fig 10: Benign spindle cells, no atypia . 400x, HPE- 2914/14

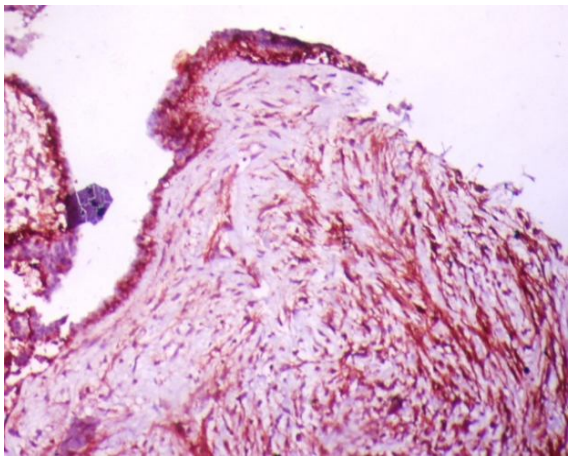


Fig 11: IHC:CD10 positive in Myoepithelial cells and >20% stromal cell taken stain . 100x, HPE- 2914/14

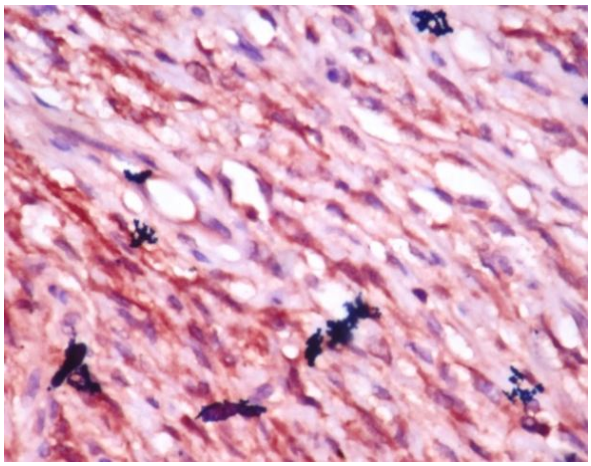


Fig 12: IHC CD10 - 2+ staining in >20% stromal cells. 400x, HPE- 2914/14

BORDERLINE PHYLLODES TUMOR

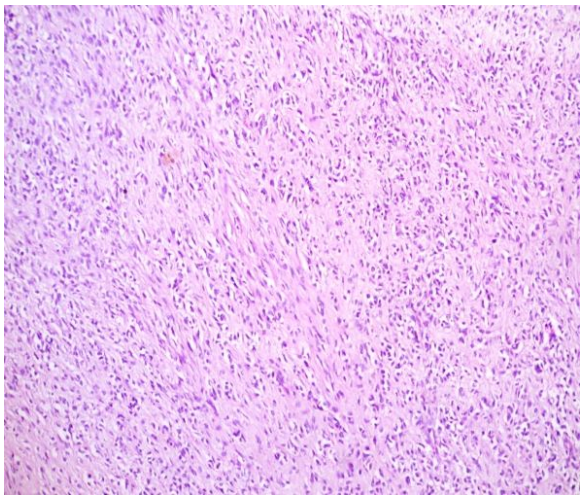


Fig 13: Borderline tumor –Increased stromal cellularitywith atypia . 100x, HPE- 8626/13

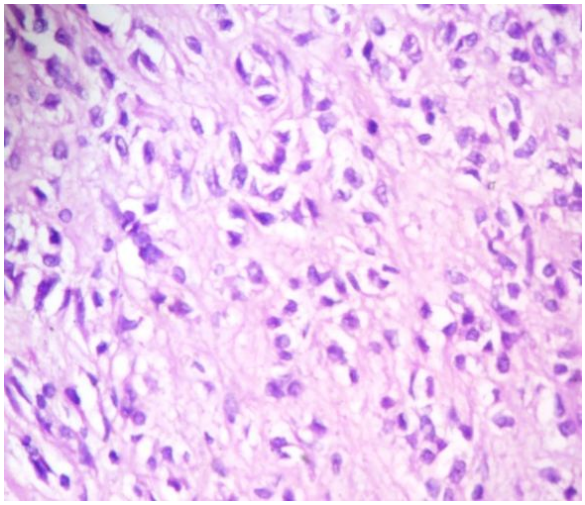


Fig 14: spindle cell with mild to moderate atypia with occasional mitosis . 400x, HPE- 8626/13

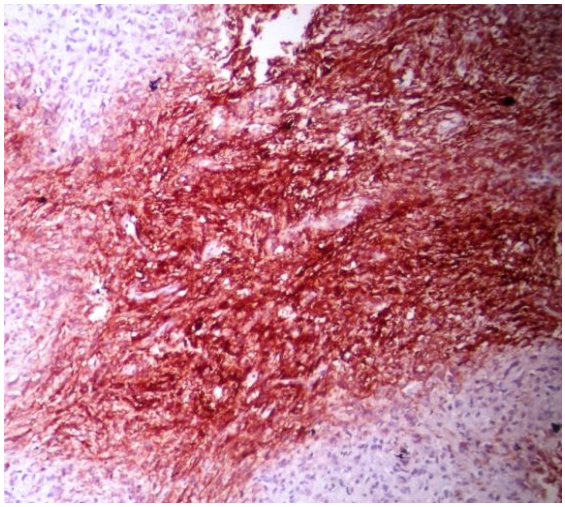


Fig 15: IHC->20% stromal cells positive for CD10. 100x, HPE- 8626/13

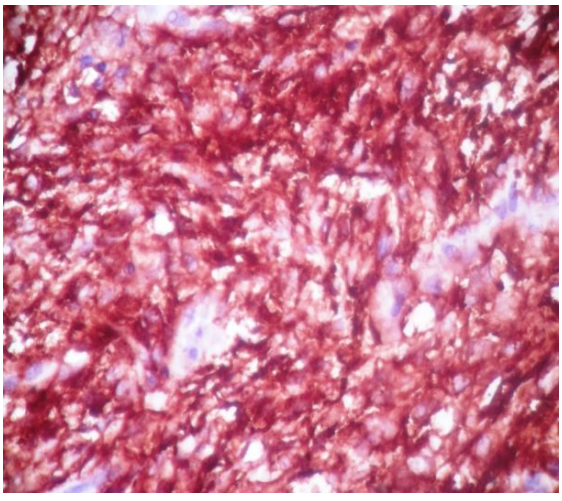


Fig 16: CD10 – 3+ staining in >20% stromal cells. 400x, HPE- 8626/13

BORDERLINE PHYLLODES TUMOR

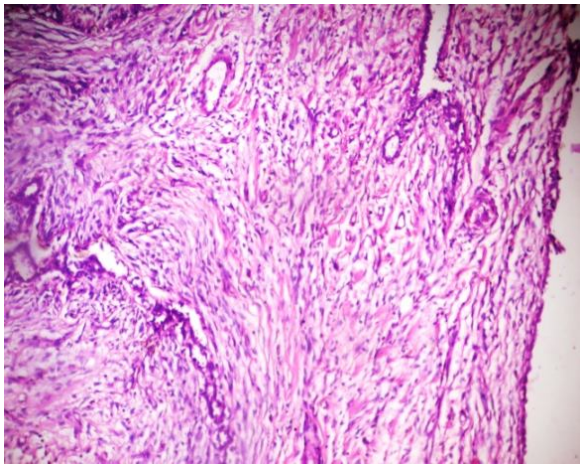


Fig 17: Borderline tumor –Increased stromal cellularity and stromal overgrowth. 100x, HPE- 9524/12

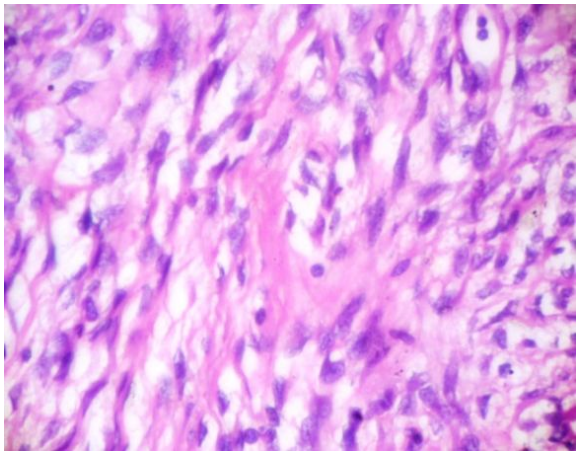


Fig 18: spindle cell with mild atypia with occasional mitosis . 400x, HPE- 9524/12

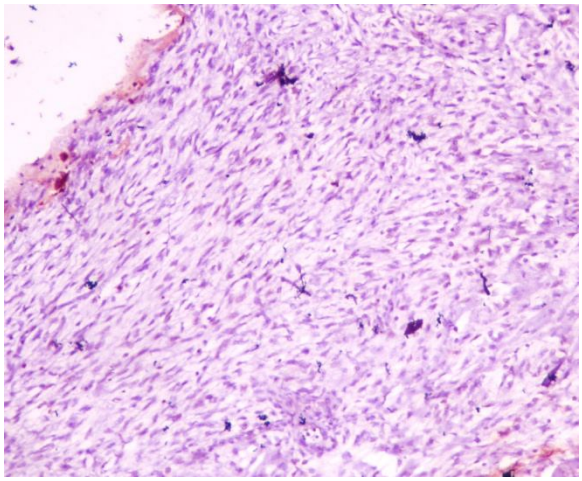


Fig 19:IHC- Stromal cells negative for CD10 . 100x, HPE- 9524/12

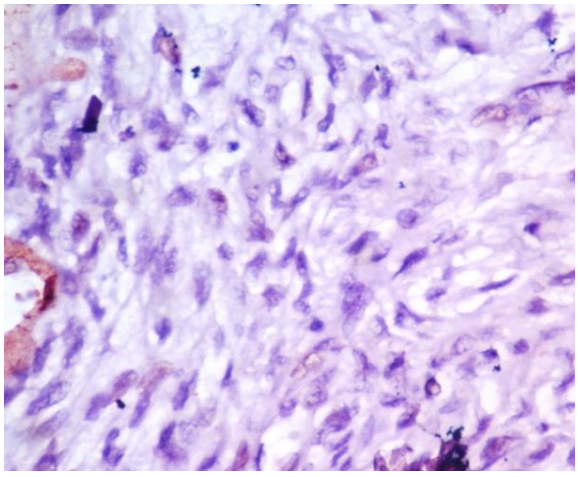


Fig 20:IHC-Stromal cells negative for CD10 . 400x, HPE- 9524/12

MALIGNANT PHYLLODES TUMOR

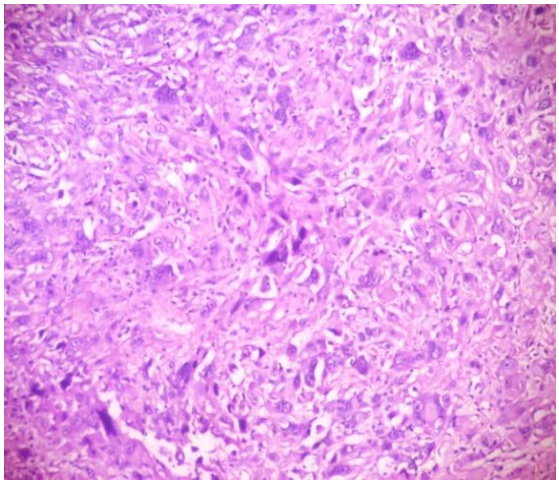


Fig 21: Malignant tumor –Marked stromal overgrowth with increased mitosis . 100x, HPE- 5025/14

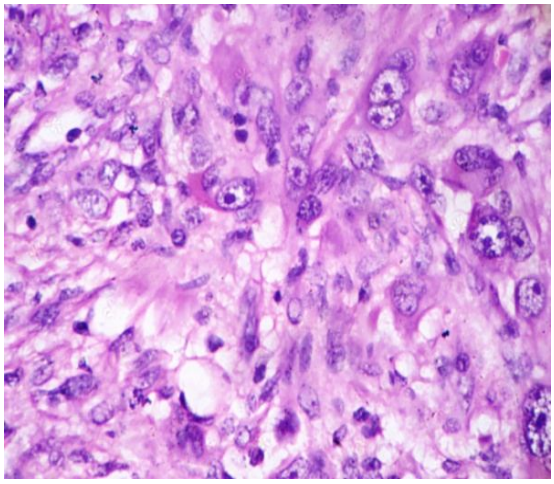


Fig 22: Marked atypia of cells with prominent nucleoli and tumor giant cells seen. 400x, HPE- 5025/14

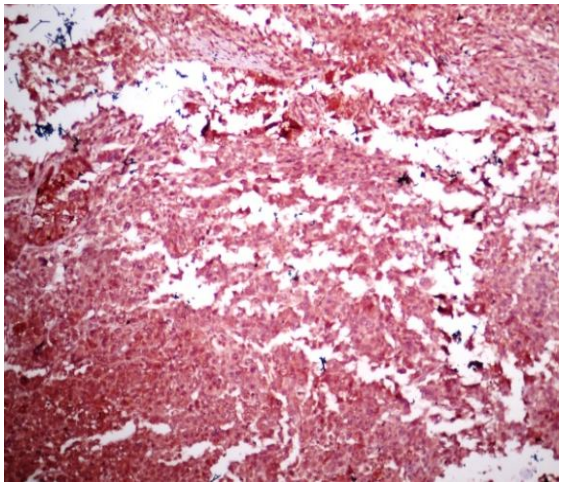


Fig 23: IHC – >20% stromal cells positive fot CD10. 100x, HPE- 5025/14

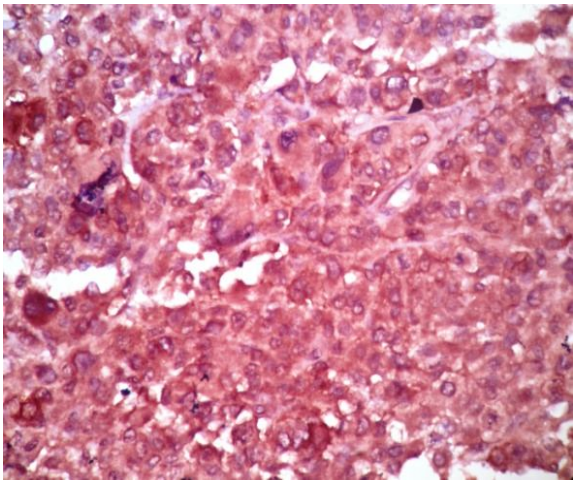


Fig 24: IHC CD10– 3+ staining in >20% stromal cells . 400x, HPE- 5025/14

Discussion

DISCUSSION

Phyllodes tumor is a rare fibroepithelial tumor accounting for <1% of all primary breast tumors and constitute 2.5% of fibroepithelial tumors. Annual incidence is estimated to be 2.1 per million women in a population based study conducted in USA.

Age group most commonly affected are between 35 and 55 years with peak incidence of 45 years.

Behaviour of Phyllodes tumor varies from completely benign to highly malignant tumor. Thus phyllodes tumor has been subclassified into benign and malignant with some category not fitting into both will come under borderline category.

This classification is mainly based on histological features including cellularity, cellular and nuclear atypia, overgrowth of stroma, marginal status whether infiltrative or pushing margin and number of mitosis per 10 high power field.

Though benign tumors are indolent, it sometimes has aggressive growth with local recurrence but do not metastasize whereas it is common in malignant phylodes tumor. Thus grading of tumor has important prognostic significance.

In the current study an attempt has been made to study the clinical, radiological and histomorphological features of 50 cases of Phyllodes tumors and to determine the role of immunohistochemical markers in determining the grading of these tumors.

Madras medical college being a tertiary referral care centre, the relative proportion of breast specimens received for histopathological examination was 6.9% (1931 out of 28,178) over a period of 30 months.

Of these 1931 bresat specimens, 83 were reported as phyllodes tumor accounting for 4.2% of breast lesions.

In the current study out of 83 cases, 50 cases were selected in which 38 were benign, 6 were bordrerline and 6 were malignant. Clinical, histomorphological features of these were evaluated and immunohistochemical analysis were done using CD10 marker.

Most of the phyllodes tumors are benign and its proportion varies from study to study accounting for 35% -65% and the remainder being borderline and malignant. Variation in proportion is mainly due to the subjective variation in interpretation criteria.

In current study out of 83 cases reported 70 (84.3%) were benign, 6(7.2%) were borderline and 7(8.4%) were malignant and all were female patients.

COMPARISON OF AGE WITH SUBTYPES OF PHYLLODES TUMORS:

Table 27: Relation between Mean age of patients in different subtypes

	Benign(yrs)	**Borderline(yrs)**	**Malignant(yrs)**
Ibrahim WS et al[76]	32.5	49.2	50.2
Tse GMK et al[74]	40	45	46
Kucuk et al[78]	31.88	--	48
Current study	39.7	47.8	54

According to Ibrahim et al[76], the mean age of benign, borderline and malignant tumors were 32.5, 49.2 and 50.2 years respectively (Table 27).

According to Tse GMK et al[74] the mean age of benign tumors was 40 years, whereas mean age of borderline and malignant phyllodes tumors were 45 and 46 years respectively (Table 27). Overall mean age is 42 years.

In the study by Kucuk et al[78], only benign and malignant tumors were compared mean age was found to be 31.88 and 48years respectively (Table 27).

In the current study the age range of benign tumor was 17 to 57years with mean age of 39.7years. In case of borderline tumors, age ranges from 35 to 60 years with mean of 47.8 years whereas in malignant phyllodes tumor age ranges from 51 to 60years with mean of 54 years.

In the current study most of benign and borderline tumors occur in the age group of 41 to 50 years whereas most malignant tumors occur in age group 0f 51 to 60 years. Overall, phyllodes tumor is common in age group of 41 to 50 years with mean of 42.4 years according to our study.

When we compare age with subtypes we have significant P value of 0.001 which is less than 0.05. Thus in the current study, age increases with increasing grade of tumor which is similar to other studies conducted.

COMPARISON OF SIZE WITH SUBTYPES:

Table 28: comparison of mean size with subtypes

	Benign (cm)	**Borderline(cm)**	**Malignant(cm)**
Ibrahim WS et al[76]	2.8	8.1	16
Tse GMK et al[74]	4	5.4	6.5
Current study	5.6	8.5	10.8

According to Ibrahim WS et al[76], the mean size was 2.8cm in case of benign tumors. In borderline tumors mean size is about 8.1 cm and malignant tumors shows a mean size of 16 cm (Table 28).

According to Tse GMK et al[74], the mean size of benign, borderline and malignant tumors were 4cm, 5.4cm and 6.5cm respectively. Overall mean size of phyllodes tumor in this study is 4.8cm (Table 28).

According to Masri et al[77], mean size of phyllodes tumor was 7.1 cm whereas according to Onkendi EO et al[79] mean size is about 8.3cm.

In the current study the minimum and maximum size of benign, borderline and malignant tumor were 3-15cm, 5-14cm and 7-19cm

respectively. Mean size of benign tumors was 5.6 cm, borderline was 8.5 cm and malignant tumor were 10.8cm with overall mean of 6.6cm.

In the current study most of benign tumors were of size 1-5cm, with most of borderline and malignant tumors were of size 6-10cm.

Most of the studies show no correlation between size and subtypes of phyllodes tumor.

When we compare size of tumor with subtypes in present study we have a P value of 0.031, thus showing association between tumor size and tumor grade.

COMPARISON OF SIDE OF PHYLLODES TUMOR:

Table 29: comparison of side of phyllodes tumor

	Right(%)	**Left(%)**	**Bilateral(%)**	**Total**
Chao TC et al[30] (%)	22(61.1%)	14(38.9%)	--	36(100%)
Onkendi EO[79] (%)	37(55.2%)	30(44.7%)	--	67(100%)
Current study (%)	32(64%)	17(34%)	1(2%)	50(100%)

According to Chao et al[30], out of 36 cases twenty two tumors occur in right side accounting for 61.1% whereas 14 cases accounting for 38.9% occur in the left side (Table 29).

According to Onkendi et al[79], out of 67 cases 37 had tumor in the right side accounting for 55.2% whereas thirty had tumor in left side of breast accounting for 44.7% (Table 29).

In a study conducted by Zissis et al[80], out of 84 cases 2 had bilateral involvement accounting for 2.4%.

In the current study out of 50 cases 32 cases had occurance in right side accounting for 64% and 17 had left side accounting for 34%. 0f 50 cases one show bilateral occurence accounting for 2%.

One case with bilateral lesion is found to be benign.

In the current study when side is compared with phyllodes tumor we had a P value of 0.092, hence there is no significant association between the two.

COMPARISON OF TUMOR LOCATION WITH SUBTYPES:

According to Mishra SP et al[5], phyllodes tumor most commonly found in upper outer quadrant.

In the current study most common location of phyllodes tumor is upper outer quadrant estimated to be 38% followed by central quadrant with 22%, involving all quadrant in 16%, lower outer quadrant in 14% and upper inner quadrant with 10%.

In the current study most common location is upper outer quadrant which correlated with the above study.

Most of benign tumor occur in upper outer quadrant whereas borderline and malignant involve all quadrants of breast which may be due to larger size of the tumor in these lesion.

P value is not found to be significant when location is compared with different subtypes.

COMPARISON OF CLINICAL PRESENTATION WITH SUBTYPES:

Lump is the most common clinical presentation accounting for 82% with lump and pain being next common presentation of about 16% in the current study.

In one case, in addition to pain and lump, it is associated with nipple discharge which may be due to some associated lesion.

COMPARISON OF RADIOIMAGING WITH SUBTYPES:

In the current study, based on BIRADS score most of the phyllodes tumor come under score IV.

Among benign most belong to BIRADS IV and III accounting for 44.7% and 42.1% respectively. In borderline most belong to BIRADS IV whereas most of malignant tumors belong to BIRADS V.

P value of 0.006 is obtained when compared with subtypes which is significant.

COMPARISON OF TYPE OF SURGERY WITH SUBTYPES:

In a study conducted by Onkendi EO et al[79] , out of 67 cases wide local excision was done for 32 cases accounting for 47.8% whereas 35 were treated by mastectomy amounting 52.2%

In this study out of 50 cases, 38 were resected specimen and most common surgery done in our institution for phyllodes tumor are wide local

excision and mastectomy which constitute 50% and 47.4% of resected specimen respectively.

There is no significance in the type of surgery done in both studies, as it depend mainly on the size of tumor.

COMPARISON OF MICROSCOPIC APPEARANCE WITH SUBTYPES:

Cellularity:

According to Ho SK et al[80] , most of benign tumors show mild cellularity with 72.5% and borderline tumor have moderate cellularity with 68.75% and malignant tumor have both moderate and marked cellularity with 52.9% and 47.1%.

Similar findings are obtained in the current study in benign and borderline, with 86.8% of benign have minimal cellularity and 66.7% show moderate cellularity whereas malignant shows marked cellularity in 83.3% with only 16.7% have moderate cellularity.

With P value of 0.001 there is strong association between stromal cellularity and tumor grade.

According to Ho SK et al[80], increase in stromal cellularity is absent in most of benign and borderline tumors and only 17.6% show marked stromal overgrowth.

Whereas in current study stromal overgrowth is minimal or absent in most of benign tumors , with minimal to moderate in borderline with marked cellularity in malignant with 83.3% with P value of 0.001.

Atypia:

Ho SK et al[80] in his study showed most of benign tumors displaying mild atypia accounting for 95.8%. In borderline 70.8% have mild atypia and 29.1% have moderate atypia. In malignant PTs 70.5% show moderate atypia with only 17.64% show severe atypia.

In the current study, most of benign tumors have no or mild atypia with all cases of borderline and malignant have moderate and marked atypia respectively.

Mitosis:

Mitosis are also more in malignant lesion with almost all cases have high mitotic rate of greater than 10 per 10 high power field whereas both benign and borderline tumors have less than ten per ten high power field. Both

atypia and mitotic figure have significant P value of 0.001 and 0.000 in the current study.

Micoscopic margin:

In the current study microscopic margin was considered to be positive when any one of the resected margin is found to have tumor cells. Here only resected specimens (37 cases) were considered excluding one lumpectomy specimen.

Out of 5 malignant lesion 4 (80%) shows positive microscopic margin whereas marginal involvement of margins is less in case of benign tumors (3 out of 28 cases accounting for 10.7%). In borderline 50% (2 out of 4) cases show involvement of margin.

Microscopic margin involvement plays significant role in local recurrence. Out of 50 cases, except two cases which were recurrent phyllodes all were presenting for the first time. Both recurrent cases were diagnosed to be benign phyllodes with one case having positive margin in previous biopsy.

Because of poor compliance, lack of adequate data and shoter duration of follow up local recurrence data could not be obtained.

IMMUNOHISTOCHEMISTRY:

CD10 staining intensity:

Table 30: Comparison of intensity of CD10 staining in different subtypes

	Masri MA et al[77]			Current study		
	Benign (%)	**Borderline (%)**	**Malignant (%)**	**Benign (%)**	**Borderline (%)**	**Malignant (%)**
No staining	0(0%)	4(40%)	1(5.9%)	18(47.4%)	1(16.7%)	0(0%)
Weak	8(50%)	0(0%)	2(11.8%)	14(36.8%)	1(16.7%)	0(0%)
Moderate	3(18.8%)	1(10%)	4(23.5%)	5(13.2%)	1(16.7%)	2(33.3%)
Strong	5(31.3%)	5(50%)	10(58.8%)	1(2.6%)	3(50.0%)	4(66.7%)
Total	16(100%)	10(100%)	17(100%)	38(100%)	6(100%)	6(100%)

According to Masri MA et al[77], most of the benign tumor shows weak CD10 staining in 50% cases followed by strong (31.3%) and moderate.

In borderline most cases (50%) show strong CD10 stain and 40% cases show no staining. In case of malignant tumors most of the cases (58.8%) show strong staining with P value of 0.15% (Table 30).

In the current study CD10 staining intensity was graded by comparing it with staining intensity of myoepithelial cells. Benign tumor shows no staining in 18 cases (47.4%) followed by weak, moderate and strong intensity of stain in 36.8%, 13.2% and 2.6% respectively. In borderline tumors 50% of cases show strong intensity of stain. Malignant tumor cells show either strong or moderate staining intensity accounting for 66.7% and 33.3%.

In the current study when we compare intensity of stain with different subtypes we get p value of 0.000. So it was found that there is correlation between intensity of stain and subtype of tumor.

For CD10 to be considered positive, greater than 20% of cells should have moderate or strong intensity of stain .

In the current study out of 6 cases of malignant lesions 5 cases (83.3%) show staining in greater than 20% of stromal cells whereas (4 out of 6)66.7% of borderline and (7out of 28)18.4% of benign tumors also shows staining in greater than 20% of cells. Out of these 16 cases those tumors with cells showing moderate to strong intensitry of stain are considered positive.

CD10 EXPRESSION IN SUBTYPES OF PHYLLODES TUMORS:

Table 31: comparison of CD10 expression and tumor grade

	Benign(%)		Borderline(%)		Malignant(%)	
	CD10 +ve	**CD10 -ve**	**CD10 +ve**	**CD10 -ve**	**CD10 +ve**	**CD10 -ve**
Ibrahim WS et al[76]	4 (16.7%)	20 (83.3%)	3 (60%)	2 (40%)	4 (80%)	1 (20%)
Tse GMK et al[74]	6 (5.9%)	96 (94.1%)	16 (31.4%)	35 (68.6%)	14 (50%)	14 (50%)
Masri MA et al[77]	7 (43.8%)	9 (56.3%)	6 (60%)	4 (40%)	14 (82.4%)	3 (17.6%)
Current study	3 (7.9%)	35 (92.1%)	3 (50%)	3 (50%)	5 (83.3%)	1 (16.7%)

According to Ibrahim WS et al[76], most of benign tumor are CD10 negative with only 16.7% show positivity whereas in borderline 60% show CD10 positive remaining 40% show CD10 negative. In case of malignancy 80% of tumors show CD10 positivity and remaining 20% were negativity (Table 31).

CD10 immunoreactivity in benign, borderline and malignant tumors were 16.7%, 60% and 80% with P value of 0.0001 showing significant correlation between CD10 expression and tumor grade.

According to Tse GMK et al[74], most of benign tumors show CD10 negativity accounting for 96.7% with borderline also showing CD10 negativity in 68.6% of tumor . In malignant tumors 50% show CD10 postivity and remaining 50% tumor show CD10 negativity (Table 31).

Here CD10 positivity in benign, borderline and malignat were 5.9%, 31.4% and 50% with p value of <0.001 showing increasing trend of CD10 expression with increasing grade.

According to Masri MA et al[77], CD10 positivity in benign , borderline and malignant phyllodes tumor were 43.8%,60% and 82.4% respectively with p value of 0.02 showing significant correlation.

In the current study percentage of cases showing CD10 positivity in benign, borderline and malignant tumors were 7.9%, 50% and 83.3%. Remaining 92.1% of benign tumors were CD10 negative with 50% and 16.7% of borderline and malignant tumor were also negative.

When we compare CD10 expression with tumor grade in the current study, we get a highly significant P value of 0.000.

Thus our study also confirms the increase in CD10 expression with increasing grade of phyllodes tumor.

Summary

SUMMARY

- The percentage of breast specimens among the 28,178 surgical samples received at Madras Medical College from January 2012 to june 2014 is 6.9%.

- Of this 83 cases were phyllodes tumor accounting for 4.2%.

- Most common among the cases reported as phyllodes tumor were benign tumors constituting 84.3%, followed by malignant and borderline constituting 8.4% and 7.2% respectively.

- All were female patients with no male cases being reported.

- Phyllodes tumor has peak incidence in the age group of 41 to 50 years with mean age of 42.5 years and most cases range in size from 6 to 10 cm with mean size of 6.6cm. Incidence of malignancy increases with increasing age and larger size of the tumor.

- In benign tumors age group ranges from 17 to 57 years with mean age of 39.7 years and size varies from 3cm to 15cm witn mean size of 5.6cm.

- In Borderline tumor age group ranges from 35 to 60 years with mean age of 47.8 years and size varies from 5 to 14cm with mean size of 8.5cm.

- Among Malignant tumors age group ranges from 51 to 60 years with mean age of 54 years and size varies from 7 to 19cm with mean size of 10.8cm.

- Statistically significant association was seen between age, sex and tumor grade.

- Right sided tumors are more common than left sided tumors accounting for 64%, with one case of benign showing bilateral involvement.

- Upper outer quadrant was found to be commonly involved.

- In radioimaging most of phyllodes tumors come under BIRADS score IV while most of malignant lesion have BIRADS V.

- Most common clinical presentation is lump in breast followed by pain.

- Stromal cellularity, stromal overgrowth, cytological atypia, nature of margin and mitotic rate increases with increasing grade.

- CD10 intensity of staining is more in malignant lesions with almost all cases showing moderate to strong staining, while only 6 cases (15.8%) of benign tumors show moderate to strong intensity of stain.

- Statistically significant association was seen between intensity of staining and tumor grade.

- CD10 expression was seen in 11 cases of phyllodes tumor, out of which 5 case (83.3%) were malignant, 3 cases (50%) were borderline and 3 cases(7.9%) were benign tumors.

- The association between CD10 expression and tumor grade is found to be statistically significant.

Conclusion

CONCLUSION

This is a hospital based study and may not represent the true incidence in the community. Phyllodes tumor account for 4.2% of all breast specimens received in the institute of pathology from January 2012 to june 2014. Peak incidence occurs in the age group of 41 to 50 years. Most common subtype is bening tumor. As metastasis and local recurrence are more common in malignant tumor this necessitates the use of molecular markers for grading of tumor.

CD10 expression is more in malignant and borderline tumors when compared to benign tumors. In this study CD10 expression is significantly associated with increasing grade of tumor. This concludes the role of CD10 expression in grading of tumor.

A large sample study in future may show the significance of CD10 as a prognostic marker and might pave way for developing targeted therapy.

Bibliography

BIBLIOGRAPHY

1. Fine-Fiks A. Cystosarcoma phyllodes of the mammary gland—Müller's tumor. Virchows Arch [A], 1981; 392: 1–6.
2. Bellocq JP, Magro G. Fibroepitelial Tumours. In: Tavassoli FA, Devile P, editors. Pathology and Genetics: Tumors of the Breast and Female Genital tract. No: 4. Lyon, France: IARC Press; 2003. p. 99-103.
3. Bernstein L, Deapen D, Koss RK. The descriptive epidemiology of malignant cystosarcoma phyllodes tumors of the breast. Cancer 1993; 71: 3020-3024.
4. Parker SJ, Harries SA. Phyllodes tumours. Postgrad Med J.2001; 77: 428-35.
5. Mishra SP, Tiwary SK, Mishra M, Khanna AK. Phyllodes tumor of breast: A review article. ISRN Surgery. 2013; Article ID 361469:10 pages.
6. Barth RJ Jr. Histologic features predict local recurrence after breast conserving therapy of phyllodes tumors. Breast Cancer Res Treat. 1999; 57: 291-295.
7. Moffat CJC, Pinder SE, Dixon AR, et al. Phyllodes tumors of the breast: a clinicopathological review of thirty-two cases. Histopathology 1995; 27: 205-218.
8. Sadler, Thomas W, Langman. Langman’s Medical embryology. 11th ed. New York: Lippincott William Wikins; 2010.p. 431-432.
9. Standring S, ed. Gray’s Anatomy: The Anatomical Basis of Clinical practice (e book).40th ed. Edinburgh: Churchill Livingstone;2008.

10. Mescher AL. Junqueira's Basic Histology: Text & Atlas. 12th ed. New York: Mc Graw Hill; 2010.p. 408-411.
11. Young B, Lowe JS, Stevens A, Heath JW. Wheater's Functional Histology: A Text and Colour Atlas. 5th ed. Edinburgh: Churchill Livingstone; 2010.p.386-391.
12. Rosen PP.Rosen's Breast pathology. 3rd ed. New York: Lippincott William wikins ; 2009.p.1-22.
13. Baddoura FK, Judd RL. Apocrine adenoma of the breast: A report of a case with investigation of lectin binding patterns in apocrine breast lesions. Mod Pathol. 1990; 3: 373–376.
14. Rubin R, Strayer DS. Rubin's Pathology: Clinicopathologic Foundations of Medicine. 6th Ed. New York: Lippincott William wikins ; 2012.p.930-931.
15. Chelius M, Neue Jahrbucher Der Teutschen Medicin and Chirurgie, Naegele und Puchelt, Heidelberg, Germany, 1827.
16. Cooper WG, Ackerman LV; Cystosarcoma phyllodes with a consideration of its more malignant variant. Surg Gynecol Obstet,1943; 77 (16): p. 279.
17. Tse GM, Niu Y, Shi HJ. Phyllodes tumor of the breast: an update. Breast Cancer 2010; 17(1): 29–34.
18. Bartoli C, Zurrida SM, Clemente C: Phyllodes tumor in male patients with bilateral gynaecomastia induced by estrogen therapy for prostatic carcinomas. Eur J Surg Oncol. 1991; 17: 215–217.
19. Grimes MM. Cystosarcoma phyllodes of the breast: histologic features, flow cytometric analysis, and clinical correlations. Mod Pathol. 1992; 5: 232-239.

20. Noguchi S, Motomura K, Inaji H, Imaoka S, Koyama H. Clonal analysis of fibroadenoma and phyllodes tumor of the breast. Cancer Research. 1993; 53(17): 4071–4074.

21. Kuijper A, Burger H, Simon R, Schaferkl, Croonen A, Boecker W, vanderwall E, Vandiest PJ. Analysis of progression of fibroepithelial tumors of the breast by PCR based clonality assay. J Pathol. 2002; 197: 575-581.

22. Kujiper A, Vos RD, Laagendijk JH, Vanderwall E, Vandiest PJ. Progressive deregulation of cell cycle wit h higher tumor grade in the stroma of breast phyllodes tumors. Am J Clin Pathol. 2005; 123: 690-698.

23. Minkowitz S, Zeichner M, Di Maio V, et al. Cystosarcoma phyllodes: A unique case with multiple unilateral lesions and ipsilateral axillary metastasis. J Pathol Bacteriol 1968; 96: 514–517.

24. Mrad K, Driss M, Maalej M, et al. Bilateral cystosarcoma phyllodes of the breast: a case report of malignant form with contralateral benign form. Ann Diagn Pathol 2000; 4: 370-372.

25. Notley RG, Griffiths HJL. Bilateral malignant cystosarcoma phyllodes. Br J Surg 1965; 52: 360–362.

26. Chulia MT, Paya A, Niveiro M, et al. Phyllodes tumor in ectopic breast tissue of the vulva. Int J Surg Pathol. 2001; 9: 81-83.

27. Saleh HA, Klein LH. Cystosarcoma phyllodes arising synchronously in right breast and bilateral axillary ectopic breast tissue. Arch Pathol Lab Med. 1990; 114: 624-626.

28. Beuglet CC, Soriano R, Kurtz AB. Ultrasound, X-ray mammography, and histopathology of cystosarcoma phylloides. *Radiology* 1983;146(2): 481–486.

29. Liberman L, Bonaccio E, Hamele-Bena D, Abramson AF, Cohen MA, Dershaw DD. Benign and malignant phyllodes tumors: mammographic and sonographic findings. Radiology 1996; 198: 121–124.

30. Chao TC et al. phyllodes tumors of the breast.Eur radiol. 2003; 13: 88-93

31. Cosmacini P, Veronesi P, Zurrida P, Bartoli C, Ferranti C, CoopmansDe Yoldi G. Mammography in the diagnosis of phyllodes tumors of the breast. Analysis of 99 cases. Radiologia Medica 1991; 82(1-2): 52–55.

32. Kinoshita T, Fukutomi T, Kubochi K. Magnetic resonance imaging of benign phyllodes tumors of the breast. Breast Journal. 2004; 10(3): 232–236.

33. Tse GMK, Cheung HS, Pang LM et al. Characterization of lesions of the breast with proton MR spectroscopy: comparison of carcinomas, benign lesions, and phyllodes tumors. American Journal of Roentgenology 2003; 181(5): 1267–1272.

34. Ohta H, Komibuchi T, Nishio T et al. Technetium-99m-sestamibi scintimammography of benign and malignant phyllodes tumors. Annals of Nuclear Medicine. 1997; 11(1): 37–39.

35. Chhieng DC, Cangiarella JF, Waisman J, et al. Fine needle aspiration cytology of spindle cell lesions of the breast. Cancer 1999; 87: 359-371.

36. Deen SA, McKee GT, Kissin MW. Differential cytologic features of fibroepithelial lesions of the breast. Diagnostic Cytopathology 1999; 20: 53-56.

37. Jayaram G, Sthaneshwar P. Fine-needle aspiration cytology of phyllodes tumors. Diagnostic Cytopathology 2002; 26(4): 222–227.

38. Komenaka IK, El-Tamer M, Pile-Spellman E, Hibshoosh H. Core needle biopsy as a diagnostic tool to differentiate phyllodes tumor from fibroadenoma.Archives of Surgery 2003; 138(9): 987–990.

39. Jacklin RK, Ridgway PF, Ziprin P, Healy V, Hadjiminas D, Darzi A: Optimising preoperative diagnosis in phyllodes tumour of the breast. Journal of Clinical Pathology 2006; 59: 454-459.

40. Jacobs TW, Chen YY, Guineeetal DG. Fibro epithelial lesions with cellular stroma on breast coreneedle biopsy: are there predictors of outcome on surgical excision? American Journal of Clinical Pathology. 2005; 124(3): 342–354.

41. Pietruszka M, Barnes L. Cystosarcoma phyllodes: a clinicopathologic analysis of 42 cases . Cancer 1978; 41(5): 1974 – 83.

42. Azzopardi JG. Problems in Breast Pathology, vol. 11. Philadelphia: WB Saunders , 1979 .

43. Hawkins RE, Schofield JB, Fisher C, Wiltshaw E, McKinna A. The clinical and histologic criteria that predict metastases from cystosarcoma phyllodes. Cancer 1992; 69: 141–7.

44. Gisser SD, Toker C. Chondroblastic sarcoma of the breast. Mt Sinai J Med 1975; 42: 232-235.

45. Anani PA, Baumann RP. Osteosarcoma of the breast. Virchows Arch [A] 1972; 357: 213-218.

46. Qizilbash AH. Cystosarcoma phyllodes with liposarcomatous stroma. Am J Clin Pathol 1976; 65: 321-327.

47. Belkacemi Y, Bousquet G, Marsiglia H, et al . Phyllodes tumor of thebreast . Int J Radiat Oncol Biol Phys 2008; 70(2): 492–500.

48. Chaney AW, Pollack A, McNeese MD, et al. Primary treatment of cystosarcoma phyllodes of the breast. Cancer 2000; 89(7): 1502 – 11.

49. Guillot E, Couturaud B, Reyal F, et al. Management of phyllodes breast tumors. Breast J 2011; 17(2): 129 – 37.

50. Barth RJ Jr. Histologic features predict local recurrence after breast conserving therapy of phyllodes tumors. Breast Cancer Res Treat 1999; 57(3): 291–5.

51. Chaney AW, Pollack A, McNeese MD, Zagars GK. Adjuvant radiotherapy for phyllodes tumor of breast. Radiat Oncol Invest 1998; 6(6): 264–7.

52. Macdonald OK , Lee CM , Tward JD , et al . Malignant phyllodes tumor of the female breast: association of primary therapy with cause-specific survival from the Surveillance, Epidemiology, and End Results (SEER) program. Cancer. 2006; 107(9): 2127–33.

53. McGowan TS, Cummings BJ, O ’Sullivan B, et al. An analysis of 78 breast sarcoma patients without distant metastases at presentation. Int J Radiat Oncol Biol Phys 2000; 46(2): 383–90.

54. Barth RJ Jr, Wells WA, Mitchell SE, Cole BF. A prospective, multiinstitutional study of adjuvant radiotherapy after resection of malignant phyllodes tumors. Ann Surg Oncol. 2009; 16(8): 2288–94.

55. Hawkins RE, Schofield JB, Wiltshaw E, et al. Ifosfamide is an active drug for chemotherapy of metastatic cystosarcoma phyllodes. Cancer 1992; 69: 2271–2275.

56. Burton GV, Hart LL, Leight Jr GS, et al. Cystosarcoma phyllodes. Effective therapy with cisplatin and etoposide chemotherapy. Cancer 1989; 63: 2088–2092.

57. Tse GM, Putti TC, Kung FY, Scolyer RA, Law BK, Lau TS, et al. Increased p53 protein expression in malignant mammary phyllodes tumors. Mod Pathol. 2002; 15: 734–40.

58. Yonemori K, Hasegawa T, Shimizu C, Shibata T, Matsumoto K, Kouno T, et al. Correlation of p53 and MIB-1 expression with both the systemic recurrence and survival in cases of phyllodes tumors of the breast. Pathol Res Pract. 2006; 202(10): 705–12.

59. Chan YJ, Chen BF, Chang CL, Yang TL, Fan CC. Expression of p53 protein and Ki-67 antigen in phyllodes tumor of the breast. J Chin Med Assoc. 2004; 67: 3–8.

60. Kocova L, Skalova A, Fakan F, Rousarova M. Phyllodes tumour of the breast: immunohistochemical study of 37 tumors using MIB1 antibody. Pathol Res Pract. 1998; 194: 97–104.

61. Tan PH, Jayabaskar T, Yip G, Tan Y, Hilmy M, Selvarajan S, et al. p53 and c-kit (CD117) protein expression as prognostic indicators in breast phyllodes tumors: a tissue microarray study. Mod Pathol. 2005; 18: 1527–34.

62. Tse GM, Putti TC, Lui PC, Lo AW, Scolyer RA, Law BK, et al. Increased c-kit (CD117) expression in malignant mammary phyllodes tumors. Mod Pathol. 2004; 17: 827–31.

63. Sapino A, Bosco M, Cassoni P, Castellano I, Arisio R, Cserni G, et al. Estrogen receptor-beta is expressed in stromal cells of fibroadenoma and phyllodes tumors of the breast. Mod Pathol. 2006; 19: 599–606.

64. Tse GM, Lui PC, Scolyer RA, Putti TC, Kung FY, Law BK, et al. Tumor angiogenesis and p53 protein expression in mammary phyllodes tumors. Mod Pathol. 2003; 16: 1007–13.

65. Charles L, White III, Bancroft JD, Marilyn Gamble (Ed), Theory and practice of histological techniques, Churchill Livingstone 2002.p.493-517.

66. Bosman FT. Some recent developments in immunocytochemistry.The Histochemical Journal. 1983; 15(3):189-200

67. Chevalier J, Yi J, Michel O. Biotin and Digoxigenin as Labels for Light and Electron Microscopy in Situ Hybridization Probes: Where Do We Stand? J Histochem Cytochem. 1997; 45(4): 481-491.

68. D'Amico F, Skarmoutsou E, Stivala F. State of the art in antigen retrieval for immunohistochemistry. Journal of Immunological Methods. 2009; 341 (1-2): 1-18.

69. Krenacs L, Krenacs T, Stelkovics E, Heat-induced antigen retrieval for immunohistochemical reactions in routinely processed paraffin sections.Mol Biol. 2010; 588: 103-119.

70. Miller K, Auld J, Jessup E, Rhodes A, Antigen unmarking by pressure cooker method. A comparison with microwave oven heating and traditional methods, Advances of anatomical pathology. 2: 60-64.

71. Lerwill MF, Current practical applications of diagnostic immunohistochemistry in breast pathology. Am J Surg Pathol. 2004; 28(8): 1076-1091.

72. Iwaya K, Ogawa H, Izumi M, et al. Stromal expression of CD10 in invasive breast carcinoma: a new predictor of clinical outcome. Virchows Arch. 2002; 440: 589–93.

73. Ogawa H, Iwaya K, Izumi M, et al. Expression of CD10 by stromal cells during colorectal tumor development. Hum Pathol. 2002; 33: 806–11.

74. Tse GM, Tsang AK, Putti TC, Scolyer RA, Lui PC, Law BK,et al. Stromal CD10 expression in mammary fibroadenomas and phyllodes tumors. J Clin Pathol. 2005; 58: 185–9.

75. Tsai WC, Jin JS, Yu JC, Sheu LF. CD10, actin, and vimentin expression in breast phyllodes tumors correlates with tumor grades of the WHO grading system. Int J Surg Pathol. 2006; 14: 127–31.

76. Ibrahim w.s. comparison of strmal Cd10 expression in benign, borderline and malignant phyllodes tumors among Egyptian female patients. Indian J Pathol Microbiol. 2011; 54: 741-4.

77. Masri MA,Darwazeh G, Sawalhi S, et al. Phyllodes tumor of the breast: Role of CD10 in predicting metastasis. Ann surg Oncol 2012 ; 19: 1181-1184.

78. Kucuk U, Bayol U, Pala EE, Cumurcu S. Importance of p53, Ki-67 expression in the differential diagnosis of benign/ malignant phyllodes tumors of the breast. Indian J Pathol Microbiol 2013; 56: 129-134.

79. Onkendi EO, Jimenez RE, et al. Surgical treatment of borderline and malignant phyllodes tumors: The effect of the extent of resection and tumor characteristics on patient outcome. Ann surg Oncol 2014; 21(10): 3304-9.

80. Zissis C, Apostolikas N, et al. The extent of surgery and prognosis of patients with phyllodes tumor of the breast. Breast cancer research and treatment. 1998; 48: 205-210.

81. Ho Sk, Thike AA, Cheok PY, Tse GMK, Tan PH. Phyllodes tumors of the breast: The role of CD34, vascular endothelial growth factor and β Catenin in histological grading and clinical outcome. Histopathology 2013; 63:393-406.

Annexures

ANNEXURE-I

PROFORMA

Case number : Name :

HPE number : Age :

IP number : Sex :

Clinical diagnosis :

Complaint :

Radioimaging :

Side of breast : Right/Left

Specimen : Trucut biopsy/Incision biopsy/

Lumpectomy/Wide local excision/Mastectomy/others.

GROSS

Specimen size : Skin :

Tumor size : Tumor margin:

Appearance :

Resected margins :

(In WLE and mastectomy)

MICROSCOPY : Histological diagnosis

IHC

CD10 : Intensity of staining :

Percentage of cells Stained :

Positive / Negative :

ANNEXURE II

Mammogram

Normal breast appears dark grey to black on mammogram due to radioluscent fat which provides excellent background.

Breast Imaging And Reporting Data System:

It is modified from American College of radiology.

Category	Assessment	Recommendation
1	Negative	
2	Benign finding	
3	Probably benign finding	Short term follow up recommended
4	Suspicious looking abnormality	Biopsy should be considered
5	Highly suggestive of malignancy	Appropriate action to be taken
6	Known cancer	Appropriate action to be taken

MASTER CHART

S.NO	HPE NO.	AGE	SIDE	T/L	C/F	SIZE	BIRADS	P/D	G/MARGIN	CELLULARITY	ATYPIA	O. GROWTH	MITOSIS	MARGIN	TYPE	CD 10 INT	% OF CELLS	CD 10 +/-
1	6920/12	51	RT	UOQ	LUMP	8	IV	WLE	1	3	3	3	3	1	M	3	2	P
2	8563/12	54	RT	AQ	LUMP, PAIN	19	V	SM	1	3	3	3	3	1	M	2	2	P
3	987/13	60	RT	LOQ	LUMP	7	III	SM	1	3	3	3	3	1	M	3	2	P
4	2560/13	51	RT	CQ	LUMP	7	IV	WLE	1	3	3	3	3	1	M	3	2	P
5	5025/14	56	LT	AQ	LUMP, PAIN, ND	14	V	IN	0	2	3	3	3	0	M	3	2	P
6	6119/14	52	RT	AQ	LUMP, PAIN	10	V	SM	1	3	3	2	3	2	M	2	2	N
7	5695/12	60	LT	AQ	LUMP	9	III	SM	1	3	2	2	2	1	I	1	2	N
8	9524/12	35	LT	AQ	LUMP ,PAIN	14	IV	SM	1	2	2	1	2	1	I	2	1	N
9	7358/13	44	LT	UIQ	LUMP	5	III	TR	0	1	2	2	2	0	I	0	0	N
10	8626/13	42	LT	CQ	LUMP, PAIN	8	IV	SM	1	2	2	2	2	2	I	3	2	P
11	238/14	54	RT	AQ	LUMP	9	IV	TR	0	2	2	2	2	0	I	3	2	P
12	6829/14	52	LT	UOQ	LUMP	6	IV	WLE	1	2	2	2	2	2	I	3	2	P
13	8303/12	42	RT	UOQ	LUMP	5	IV	WLE	2	1	0	1	1	2	B	1	2	N
14	587/13	50	RT	UOQ	LUMP	5	II	IN	0	2	0	1	1	0	B	1	1	N
15	1079/13	38	RT	LOQ	LUMP	3.5	II	WLE	2	1	1	1	1	2	B	0	0	N
16	1680/13	35	RT	UOQ	LUMP	3	III	LU	0	1	1	1	1	0	B	2	1	N
17	2002/13	40	LT	UIQ	LUMP	3	II	WLE	2	1	0	1	1	2	B	0	0	N
18	2076/13	45	LT	UOQ	LUMP	4	IV	TR	0	1	0	1	1	0	B	0	0	N
19	2543/13	45	RT	CQ	LUMP	5	IV	SM	2	1	1	1	1	1	B	0	0	N
20	3583/13	37	RT	LOQ	LUMP	4	III	WLE	2	1	0	1	1	2	B	2	1	N
21	3881/13	42	RT	UOQ	LUMP	3	III	TR	0	1	1	1	1	0	B	1	1	N
22	4936/13	34	RT	CQ	LUMP	6	IV	WLE	2	1	1	1	1	2	B	1	1	N
23	5786/13	40	LT	UOQ	LUMP	4	III	WLE	2	1	1	1	1	2	B	0	0	N
24	6352/13	17	B/L	UIQ	LUMP	4	II	WLE	2	1	1	1	1	2	B	1	2	N
25	6533/13	50	RT	CQ	LUMP	5	III	TR	0	1	0	1	1	0	B	1	1	N
26	6743/13	52	RT	LOQ	LUMP	4	IV	WLE	2	1	0	1	1	1	B	1	1	N
27	7158/13	18	RT	CQ	LUMP	5	III	WLE	2	1	0	1	1	2	B	0	0	N
28	7580/13	28	RT	UOQ	LUMP	6	IV	TR	0	1	0	1	1	0	B	0	0	N
29	7917/13	35	RT	UOQ	LUMP	4	III	WLE	2	1	0	1	1	2	B	0	0	N
30	8117/13	42	LT	CQ	LUMP	5	IV	IN	0	1	1	2	1	0	B	2	1	N
31	8251/13	35	RT	UOQ	LUMP	3.5	IV	WLE	2	1	0	1	1	2	B	0	0	N

32	8616/13	27	RT	UOQ	LUMP	4.5	III	SM	2	1	0	1	1	2	B	0	0	N
33	8665/13	30	LT	UOQ	LUMP	3	III	WLE	2	1	1	1	1	2	B	0	0	N
34	8843/13	48	LT	LOQ	LUMP, PAIN	6	IV	WLE	2	1	0	1	1	2	B	1	2	N
35	9144/13	37	LT	LOQ	LUMP	3	III	WLE	2	2	0	1	1	2	B	1	1	N
36	9816/13	27	RT	UOQ	LUMP	6	III	WLE	2	1	0	1	1	2	B	0	0	N
37	9844/13	26	LT	UIQ	LUMP	8	IV	TR	0	1	0	1	1	0	B	0	0	N
38	10624/13	40	RT	UOQ	LUMP, PAIN	15	IV	SM	2	1	0	1	1	2	B	0	0	N
39	345/14	36	RT	CQ	LUMP	7	III	SM	2	1	0	1	1	2	B	1	1	N
40	729/14	42	RT	LOQ	LUMP	6	III	WLE	2	1	0	1	1	2	B	1	1	N
41	2339/14	59	RT	AQ	LUMP, PAIN	11	IV	SM	2	2	0	1	1	2	B	0	0	N
42	2914/14	45	LT	UOQ	LUMP	7	V	TR	0	1	0	1	1	0	B	3	2	P
43	3287/14	43	RT	UIQ	LUMP	6	III	TR	0	1	1	1	1	0	B	0	0	N
44	3639/14	45	LT	CQ	LUMP	7	III	SM	2	1	0	1	1	1	B	1	1	N
45	3728/14	50	RT	AQ	LUMP, PAIN	8	IV	SM	2	1	0	1	1	2	B	1	1	N
46	3809/14	29	RT	UOQ	LUMP	6	IV	SM	2	1	0	1	1	2	B	0	0	N
47	4233/14	45	RT	UOQ	LUMP	4	IV	SM	2	2	1	1	1	2	B	2	2	P
48	5054/14	48	RT	CQ	LUMP	8	IV	SM	2	1	0	2	1	2	B	0	0	N
49	5292/14	50	RT	UOQ	LUMP	6	III	SM	2	1	0	1	1	2	B	2	2	p
50	5368/14	57	LT	CQ	LUMP	9	IV	SM	2	2	1	1	1	2	B	1	2	N

KEY TO MASTER CHART

SIDE:

Rt - Right

Lt - Left

T/L - Tumor location

AQ - All quadrant

CQ - Central quadrant

UOQ - Upper outer quadrant

UIQ - Upper inner quadrant

LOQ - Lower outer quadrant

C/F - Clinical features

ND - Nipple discharge

P/D - Procedure done

IN - Incision biopsy

TR - Trucut biopsy

WLE - Wide local excision

SM - Simple mastectomy

LU - Lumpectomy

G/margin- gross margin

0 - Margin not known

1 - Infiltrative

2 - Pushing

Cellularity

1 - Minimal

2 - Moderate

3 - Marked

Atypia

0 - None

1 - Minimal

2 - Moderate

3 - Marked

O.Growth- Stromal over growth

1 - Minimal

2 - Moderate

3 - Marked

Mitosis

1 - 0-4/Hpf

2 - 5-9/Hpf

3 - >10/Hpf

Margin

0 - Margin not known

1 - Involved

2 - Uninvolved

Type of tumor

B - Benign phyllodes

I - Intermediate phyllodes

M - Malignant phyllodes

CD10 INT-CD10 intensity of stain

0 - No staining

1 - Weak

2 - Moderate

3 - Strong

% OF CELLS- Percentage of cells taken stain

0 - Nil

1 - <20% cells

2 - >20% cells

CD10+/- - CD10 positive/Negative

N - Negative

P - Positive