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Study of P53& Ki67 Immunoexpression in Phyllodes Tumors of Breast.

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Abstract: Background: ;*Phyllodes tumors (PTs) are rare biphasic neoplasms. Currently, PTs are classified as benign, borderline, and malignant based on microscopic features, including stromal cellularity, cellular pleomorphism, mitotic activity, margin appearance, and stromal distribution. There is sometimes difficulty in dividing tumors into the three recognized grades, and variability exists regarding the exact histological criteria that should be used to define them. More importantly, they do not always correlate with clinical outcome in terms of predicting recurrence, malignant transformation, metastasis, and overall survival. Ancillary diagnostic tests likeImmunohistochemistry (IHC) will help to identify tumors with potentially aggressive behaviour along with gold standard histopathological examination. Literature data reveals that increasing tumor grade is correlated with the increase of p53 and Ki-67 expressions in the stromal component.*

MATERIALS AND METHODS: *We studied 100 PT cases. 78 benign, 12 borderline and 10malignant PTs were re-evaluated in regards to stromal cellularity mitotic activity,p53/Ki-67 expression rates and the relation between these parameters.*

RESULTS: *Stromal cellularity, mitotic rate, p53 and Ki-67 expression rates were all correlated with benign, borderline and malignant histologic subgroups. Ki-67 and p53expressions were statistically significantly correlated with histologic subgroups, stromal cellularity and mitotic rate (P < 0.005).*

CONCLUSION: *Ki-67 and p53 expression rates were statistically significantly correlated with grade of Phyllodes tumor. Therefore, they can be used in the determination of tumor grade, especially for the differential diagnosis of benign and malignant tumors. It is also useful for sub classification of PT and as an adjunct to morphological diagnosis on the basis of P53 & KI67 immunoexpression.*

Key words: *Phyllodes tumor(PT), benign, borderline, malignant Phyllodes tumors(PT),ki67,p53ihc markers.*

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I. Introduction

phyllodes tumors are well circumscribed biphasic neoplasms, histologically resembling intracanalicular fibroadenomas, characterized by a double layered epithelial component arranged in clefts they are surrounded by a hyper cellular stroma which in leaf-like pattren.[1]Johannes Muller 1838 was the first person to use the term cystosarcoma phyllodes.Rosen[2] sub classified them histologically as benign, borderline, and malignant based on histological features stromal over growth,tumor margins, tumor necrosis, cellular atypia, and number of mitosis per high power field.

There is sometimes difficulty in dividing tumors into the three recognized grades, and variability exists regarding the exact histological criteria that should be used to define them. More importantly they do not always correlate with clinical outcome in terms of predicting recurrence, malignant transformation, metastasis, and overall survival. .It is due to inadequate surgical margins, intra tumoral heterogeneity and tumors may have foci of malignant stroma too small to be identified histologically at first presentation[3].

Benign phyllodes tumors usually do not metastasize but sometimes recur locally, borderline tumors are higher propensity for local recurrence and may rarely metastasize, and malignant tumors are even higher chances of local recurrence as well as distant metastasis[4].

Ancillary diagnostic tools may help to identify tumors with potentially aggressive behaviour. Recently described methods to meet this objective include immuno histological assessment of p53, Ki-67 index[5,6]. Literature data reveals that increasing tumor grade is correlated with the increase of p53 and Ki-67 expressions in the stromal component[7]. It is also useful for sub classification of PT and as an adjunct to morphological diagnosis on the basis of P53 &KI67 immunoexpression[8]

II. Materials And Methods

This study was done in the Department of Pathology at Osmania medical college .Total number of cases are included in this study are100 .Duration of the study is 5 years. PT cases that were diagnosed on the basis of conventional histological parameters were included in this study.The ages of the patients, tumor locations and the relevant details were obtained from the pathology records. The specimens were fixed in 10% neutral buffered formalin. They were examined grossly according to the standard guidelines, with special emphasis on the tumor size and lymph node status of the lesion. The specimens were grossed and sections were taken from representative sites. These sections were then processed in tissue processor and embedded in paraffin wax. Four to five micron thickness sections were prepared from the corresponding paraffin blocks, one on albumin coated slide for Haematoxylin and Eosin (H&E) staining and the other on poly-L-lysine coated slide for immuno-histochemical staining.Immunostaining for Ki 67 and p53 was done using peroxidase-anti peroxidase method according to the protocol described by DACO.Nuclear staining was evaluated as positive for Ki-67 and p53 and counted in five HPF within the stromal and epithelial cells.20% was considered as the threshold value for Ki-67 and p53 positivity.

III. Observation And Results

A Total number of 100 cases were studied. All the patients were 25 to 65 years age group and mean age 48 years.PT in right breast 68% and left breast 32%involved ,right breast was more commonly involved .Grosely tumor size is minimum size 2 cm and maximum size is 23cm.Cut section shows well circumscribed tumor grey white in colour and fleshy and focal necrosis seen in some cases .

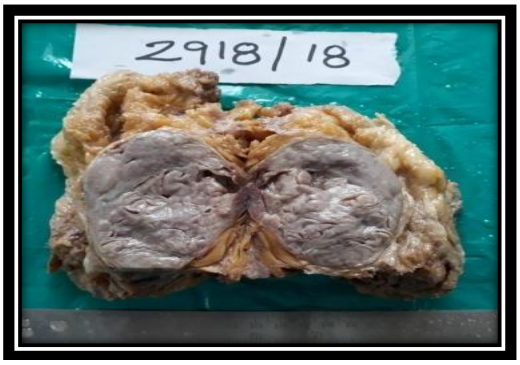


Fig 1 gross- Phyllodes tumor

Cytology phyllodes tumor shows moderate cellularity with clusters branching sheets of ductal epithelial cell bare nuclei, plump spindle shaped stromal cell against hemorrhagic background.

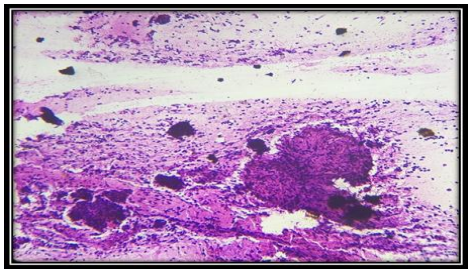
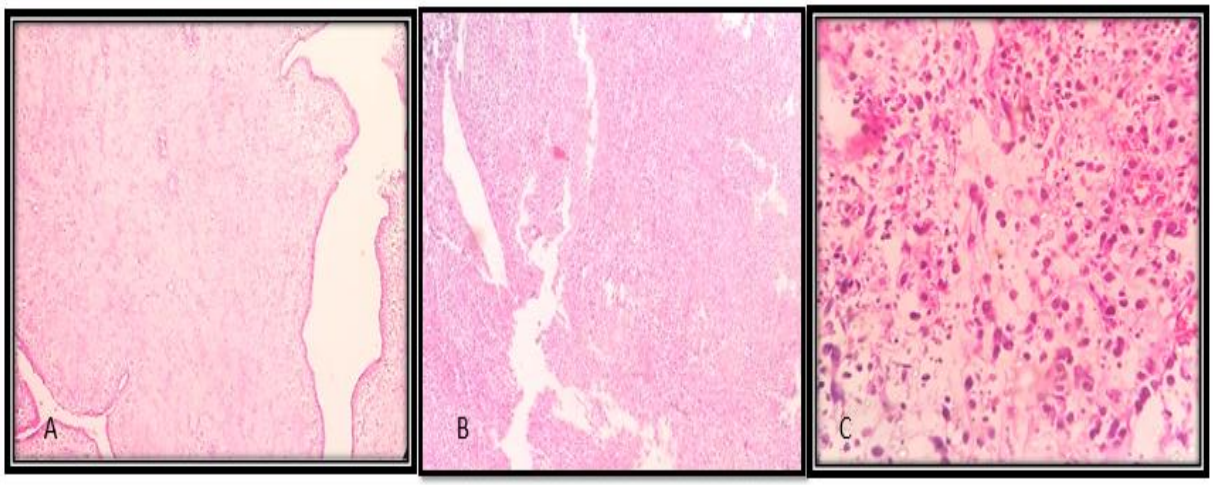


Fig 2cytology- phyllodes tumor(10x)

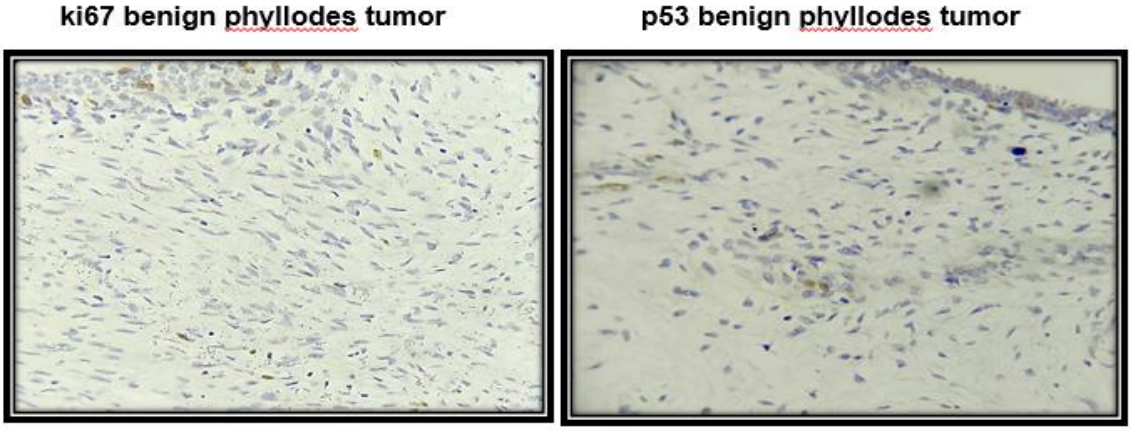
Based on histological features of the 100 PT cases, 78 were classified as benign, 12 borderline and 10 malignant Phyllodes tumor based on histomorphological features .All the benign Phyllodes tumors showing glands and stroma with leaf-like projections stroma showing hyper cellularity below epithelium, mitotic rate <4/HPF, with mild nuclear atypia present. And borderline tumors showing moderate stromal cellularity, hyperplasia and mitotic rate in between 4-10/HPF. Malignant Phyllodes tumors showing severe stromal cellularity, hyperplasia, marked atypia and mitotic rate >10/HPF with infiltrating borders.

Fig 3-A) benign B) borderlineC)malignant phyllodes tumor(10x)



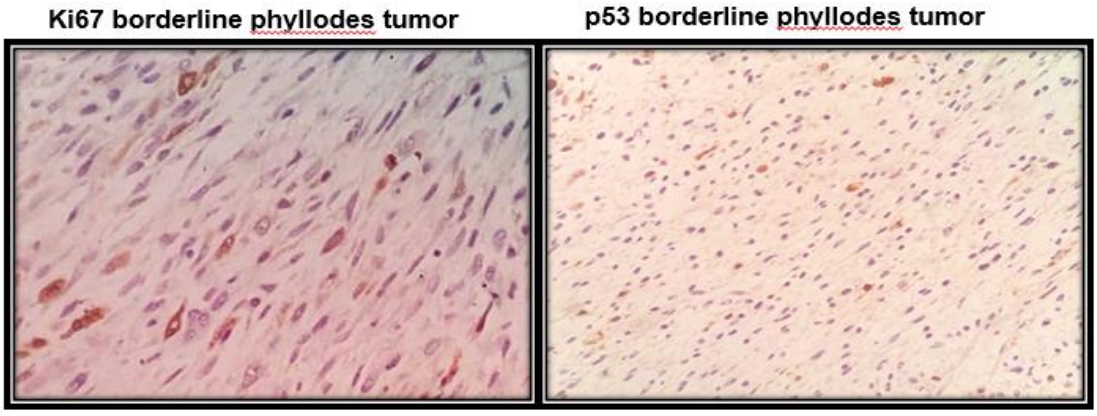
In present study 79 (79%) cases are showing ki67 index less than 20% belongs to low grade, 13 cases showing 20-40% belongs to intermediate grade, and 8 cases showing more than 40% belongs to high grade phyllodes tumor.78 cases showing P53 immuno expression less than 20% belongs to low grade,14 cases showing 20-40% belongs to intermediate grade,and 8 cases showing more than 40% belongs to high grade phyllodes tumors.

Fig 4-Ki67&p53 benign phyllodes tumor



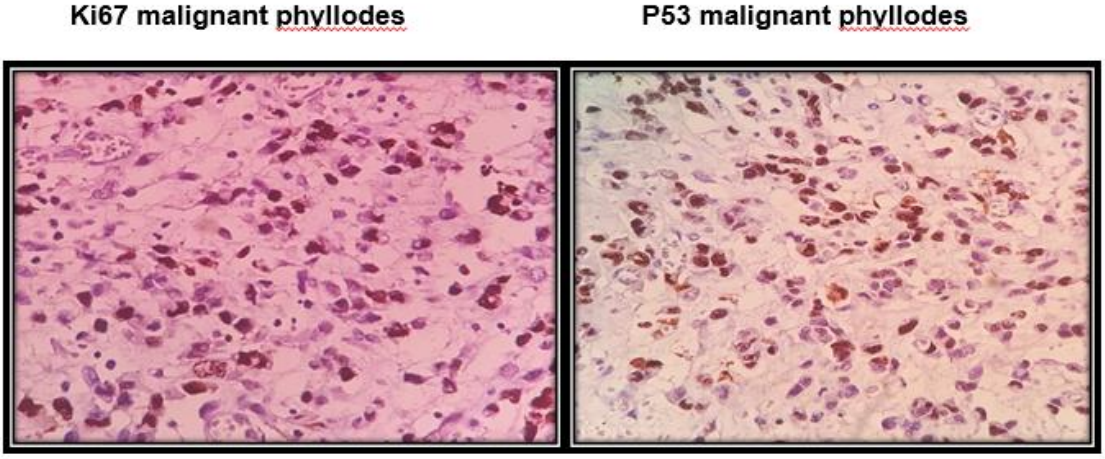
Ki 67&p53-40x showing low expression in benign phyllodes tumor

Fig 5-Ki67&p53 borderline phyllodes tumor



Ki67&p53-40x showing intermediate expression in borderline phyllodes tumor

Fig 6-Ki67&p53-malignant phyllodes tumor



Ki67 &p53-40x showing high expression in malignant Phyllodes tumor

Correlation between histological grade and ki67 immunoexpression in benign phyllodes tumor 77/78(98%) showing >20%, only one case (2%) showing 20-40% ki67 immunoexpression, borderline phyllodes tumors 9/12(75%) showing 20-40%, 2/12(16%) showing <20%, and 1 /12(9%) showing >40% ki67Immunoexpression. malignant phyllodes tumors 7/10(70%) showing >40% and 3/10(30%) cases showing 20-40%ki67 immunoexpression.Ki-67 expression statistically significantly correlated with histological grading of phyllodes tumor, p value is <0.05.And 75/78 (96%) of benign tumors showing p53 immunoexpression<20%, and 3 Benign Phyllodes tumors showing 20-40% p53 immunoexpression,borderline phyllodes tumors 8/12 (68%) showing 20-40%, 2/12 showing <20%, and 2/12 showing >40% p53 immunoexpression and malignant phyllodes tumors 6/10(60%) showing >40%, 1/10(10%) showing >20%, 3/10(30%) showing 20-40% p53 immunoexpression P53 expression statistically significantly correlated with histological grading of phyllodes tumor, p value is <0.05.P value is calculated from chi square test.

IV. Discussion

Phyllodes tumours are rare biphasic neoplasm, that account for less than 1% of all breast neoplasms[9,10,11]. Displaying a broad range of clinical and pathological behaviour, phyllodes tumours should be regarded as a spectrum of biphasic neoplasms rather than a single disease entity.

Malignant phyllodes tumours, if inadequately treated, have a propensity for rapid growth and metastatic spread,benign phyllodes tumours on clinical, radiological, and cytological examination are often indistinguishable from fibroadenomas and it can be cured by local surgery. With the non-operative management of fibroadenomas widely adopted, the importance of phyllodes tumours today lies in the need to differentiate them from other benign breast lesions[12].Triple assessment by clinical, radiological, and cytological or histological examination forms the fundamental basis for the evaluation of all breast lumps. In patients with Phyllodes tumours, all three aspects individually have a low sensitivity and, even in combination, the diagnostic accuracy is often poor[13,14].

Radiological imaging

As with fibroadenomas, phyllodes tumours are mammographically well defined with a smooth and occasionally lobulated border. A radiolucent halo may be seen around the lesion, due to compression of the surrounding breast stroma. Coarse microcalcification has been reported with in both fibroadenomas and PTs.No mammographic indicators have been identified that allow differentiation between benign and malignant tumours.

Cytology

An accurate cytological diagnosis of Phyllodes tumors by fine needle aspiration can be difficult cytologically, it is often easier to differentiate benign from malignant phyllodes tumors than to separate benign phyllodes tumors from fibroadenomas.In the correct clinical setting, the presence of both epithelial and stromal elements within the cytological smear supports the diagnosis.epithelial cells may, however, be absent from specimens taken from malignant lesions. The presence of cohesive stromal cells, isolated mesenchymal cells, clusters of hyperplastic duct cells, foreign body giant cells, stromal fragments, and bipolar naked nuclei and the

absence of apocrine metaplasia are highly suggestive of a phyllodes tumor. And the value of FNAC in the diagnosis of Phyllodes tumor remains controversial, with an overall accuracy of about 63% [23,24].

Histology

A wide range of histological features are seen within phyllodes tumours with heterogeneity existing within the same lesion[12]. Predicting the biological behaviour of PTs remains a challenge in pathology. Various grading systems have been proposed, but none is universally accepted.The threshold for number of mitoses required for classification into each subgroup of PTs varies from one grading system to another.Stromal over growth has been defined as marked stromal proliferation to the point where the epithelial component is absent in at least 1low-power field. Infiltration into adjacent tissue is a feature of malignant PTs, whereas benign PTs tend to having pushing rather than infiltrative margins.

Adequate sampling is important, with at least 1 block for every 1 cm of maximal tumor dimension. The grading should be based on the areas of highest cellular activity and most florid architectural pattern. Follow-up studies to determine the behaviour of PTs have demonstrated the inadequacy of histological criteria alone in predicting biological behavior[15]. All these are lead to various immunohistochemical markers being evaluated to more reliably predict patient outcome[8].Biological markers have often been used as adjuncts to morphology in predicting behaviour of tumors. p53 and ki67 most widely studied in neoplastic processes.

Ki 67

Antigen KI-67 also known as Ki-67 or MKI67 is a protein that in humans is encoded by the MKI67 gene antigen identified by monoclonal antibody Ki-67Antigen[16,17,18] . Antigen KI-67 is a nuclear protein that is associated with cellular proliferation and ribosomal RNA transcription[18.] Inactivation of antigen KI-67 leads to inhibition of ribosomal RNA synthesis[19.]

The expression of Ki-67 occurs during all phases of the cell cycle except the G0 phase and the early G1 phase. The expression level increases as cell proliferation progresses, especially in the S phase, with peaks in the G2 and M phases. It is used to assess the growth fraction of neoplastic cell population.In samples from normal breast tissue, ki 67 is expressed at low levels.

p53

p53 is probably the most extensively studied marker. p53 has been described as the guardian of the genome because of its role in conserving stability by preventing genome mutation[21]. Hence p53 is classified as a tumor suppressor gene the p53 gene is located on the short arm of chromosome 17 (17p13.1).PTs, expression of p53 is shown to be associated with tumor grade, nuclear atypia, stromal overgrowth, mitotic rate, stromal nuclear pleomorphism and infiltrative tumor margins[1,22].

Kucuk, et al(2013)[59] studied 26 PT cases Ki-67 and p53 expression rates Stromal cellularity, mitotic rate, p53 and Ki-67 expression rates were all correlated with benign and malignant histologic subgroups (p= 0.000-0.001).Noronha et al (2014)[17] total number of cases are 33 phyllodes tumor studied, most of belong to benign grade 21(21/33 : 64%),borderline 6(6/33 :18%) , malignant group 6(6/33 :18%) based on histological features. The Ki-67 expression in BPT <2% in the BLPTs ranged from 1% to15% , and it was between 3 and 25% IN MPT.A the tumor grade increasing ki67 expression also increasing.

Molecular mechanisms of PTs are a paradigm of epithelial–stromal cross-talk, with the epithelium influencing stromal growth, via the Wnt signalling pathway, upregulation of transcriptionally active beta-catenin and downstream effectors such as cyclin D1. The stroma in turn is able to influence the epithelium, via IGFandIGFR1[25,26]

V. Conclusion

Phyllodes tumor (PTs) is a benign biphasic breast tumor that is composed of cellular spindle stroma with epithelial elements.The neoplastic component of the tumor is the stroma, which determines its behaviour. There is sometimes difficulty in dividing tumors into the three recognized grades benign, borderline, and malignant PTs based on histological features.Ki67&p53 are differentially expressed in benign, borderline and malignant PTs. These markers are useful for sub classification o f PTs.Most of the benign tumors showing low ki67, p53 expressions, borderline showing intermediate and malignant cases showing high ki67, p53 expression. So they can be used in the determination of tumor grade, especially for the diagnosis of borderline and malignant tumors. Study of ki67 & p53 expression in phyllodes tumor will helpful for further accurate treatment protocols for borderline and malignant Phyllodes tumor.

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