Ki67 AND p53

EXPRESSION IN RELATION TO CLINICOPATHOLOGICAL FEATURES IN PHYLLODES TUMOUR OF THE BREAST

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THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER PATHOLOGY

(ANATOMIC PATHOLOGY)

2020

ACKNOWLEDGEMENT

In the name of Allah, the Most Beneficial, the Most Merciful. I would like to express my utmost gratitude to my supervisor, Dr Wan Faiziah bt Wan Abdul Rahman for her endless support and guidance throughout the journey of my research project. On top of this, to the laboratory technician of Department of Pathology, Hospital Universiti Sains Malaysia and Hospital Sultanah Nur Zahirah, especially to Puan Ummi Atikah Ayub, for the technical and hands-on part.

My appreciation also goes to my beloved mother, S.Meriam Sheh Ali, parents in law and our family members for their constant prayers as well as being a great support system. Next, my special thankful goes to my colleagues: Sarah, Maryam, Zaleha, Balqis, Chua, Nasrun and Poobalan for their ideas and knowledges throughout our days together.

Last but not least, to those whom I owe everything is my husband, Mr (Dr) Mohammad Faizal Nikman Lee and our angelic daughters: Aleesya Nursyafiqah, Dalilee Ismahani and Nailee Sofia for their countless love and caring. Thank you.

Nurul Atiah Mohd Ali (P-UM0190/15)

2020

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LIST OF ABBREVIATIONS

PT	Phyllodes Tumour
DX	Diagnosis
AC	Axillary Clearance
HPE	Histopathological examination
IHC	Immunohistochemistry
Ulc	Ulcerated skin
Bx	Biopsy
WLE	Wide local excision
Mastec	Mastectomy
WC	Well circumscribed margin
Perm	Permeative margin
SH	Stromal hypercellularity
SO	Stromal overgrowth
Y	Yes (Present)
N	No (Absent)
Mild	Negative or mildly stained
Mod	Moderate staining
Mark	Marked staining

ABSTRAK

Pengenalan: Ketumbuhan *Phyllodes* (PT) adalah ketumbuhan payudara jenis *fibroepithelial* yang jarang berlaku. Ciri-ciri yang terdapat dalam tisu patologi merupakan cara utama dalam melakukan pendiagnosan ketumbuhan ini. Ujian lanjutan dengan menggunakan pewarnaan immunohistokimia iaitu Ki67 and p53 jarang digunakan, namun tidak dinafikan ia membantu untuk kes ketumbuhan *phyllodes* jenis *malignant*. Dalam kajian ini, kami berhasrat untuk melihat ekspresi pewarnaan immunohistokimia di dalam tisu ketumbuhan ini dan sekaligus melihat perkaitan antara ekspresi tersebut mengikut klasifikasi ketumbuhan *phyllodes.*

Metodologi: Kajian ini adalah satu kajian keratan rentas yang dijalankan ke atas 57 sampel kaji tisu, bermula tahun 2015 sehingga tahun 2019, diambil dari makmal Hospital USM and Hospital Sultanah Nur Zahirah. Setiap sampel dianalisa melalui kajian tisu patologi. Seterusnya ujian pewarnaan immunohistokimia iaitu Ki67 dan p53 dilakukan ke atas tisu yang telah dikeraskan melalui proses *formalin fixed paraffin embedded (FFPE).*

Keputusan: Dapatan kajian mendapati terdapat kaitan antara data klinikal seperti perubahan pada kulit, saiz melebihi 3 sentimeter, sel *abnormal*, *stromal hypercellularity*, jumlah *mitosis* dan ekspresi pewarnaan immunohistokimia dengan kelas ketumbuhan *phyllodes*. Ekspresi Ki67 dan p53 yang sangat tinggi hadir pada ketumbuhan *phyllodes* jenis *borderline* dan *malignant*. Selain daripada itu, ketumbuhan phyllodes jenis *malignant* juga boleh disyaki dengan kehadiran aktiviti *mitosis* yang tinggi beserta ekspresi Ki67 yang kuat.

Kesimpulan: Kajian kami mendapati terdapat perkaitan antara ekspresi Ki67 dan p53 didalam klasifikasi kes *borderline* dan *malignant*. Namun begitu dengan aktiviti *mitosis* yang tinggi beserta ekspresi Ki67 yang kuat, ia didapati dalam kes ketumbuhan phyllodes yang *malignant*. Kami mencadangkan kajian yang melibatkan bilangan sampel yang lebih besar dan pembahagian ketumbuhan yang lagi terperinci diperlukan untuk kajian seterusnya bagi memastikan keputusan yang tepat.

ABSTRACT

Introduction: Phyllodes tumour is a rare fibroepithelial neoplasm of the breast. Histopathological features remained the gold standard for the diagnosis. The usage of immunohistochemical markers of Ki67 and p53 acts as a supplement method particularly for the malignant phyllodes tumour. Therefore we aim to study the expression of these markers in phyllodes tumour and to see its relation to the tumour grading.

Methodology: This was a retrospective cross-sectional study conducted on 57 numbers of archival tissue samples from the year 2015 to the year 2018 from the laboratory of Hospital Universiti Sains Malaysia and Hospital Sultanah Nur Zahirah. The histopathological examinations were analysed and further immunohistochemistry markers of Ki67 and p53 protein expression were studied on formalin-fixed paraffin embedded tissue.

Result: The study showed that there was an association between clinical descriptive data of skin changes, lump size of more than 3 cm, cytological atypia, stromal hypercellularity, mitosis and immunohistochemistry with the clinical diagnosis of phyllodes tumour. Both marked expression of Ki67 and p53 are seen in borderline and malignant phyllodes tumour. However the presence of both high mitosis and marked expression of Ki67, on top to other histological features, was merely seen in case of malignant PT.

Conclusion: In our study, there were a significant association of both p53 and Ki67 expression in case of borderline and malignant phyllodes tumour. Nevertheless, with presence of high mitotic rate and strong expression of Ki67 index are exclusively found in malignant phyllodes tumour. Further study of a larger sample size and division into more precise of low-high grade group is recommended for a validated result. On top of that, it is also helpful to predict the prognosis as well as the disease-free-survival of the tumour.

CHAPTER 1: INTRODUCTION

Phyllodes tumour is initially described as cystosarcoma phyllodes at the year 1838 by Johannes Muller due to its biphasic appearance of benign epithelial elements and fibrosarcomatous stroma (Veneti and Manek, 2001; Lee *et al.*, 2007). This fibroepithelial tumour was characterized by stromal hypercellularity altogether with cleft like or cystic spaces lined by epithelium.

The incidence of this disease is 1.5% and in other older study said to be very uncommon in which about 1 in every 100,000 women (MOFFAT *et al.*, 1995; Testori *et al.*, 2015). Nonetheless of the rarity of this neoplasm, in which accounted for less than 1% of all breast neoplasm, it have a malignant-fatal spectrum (Tan *et al.*, 2016). Particular in Asian people, they tend to have this tumour at the younger age and relatively high rate of recurrence as compared to non-Asian (Teo, Cheong and Wong, 2012; Tan *et al.*, 2016).

The histogenesis of this phyllodes tumour is believed to arise de novo, basically from ductal and lobular stroma, involving the interaction between epithelial and stromal component of the breast tissue. Similar to fibroadenoma, the neoplastic fibroblast arise from the specialized stroma, as contrast to non-specialized stroma in pseudoangiomatous stromal hyperplasia (PASH). The neoplastic cells in PT are independent from the glandular epithelial, unlike the fibroadenoma. It has unlimited ability to grow, classified as stromal overgrowth, manifested as large mass that lack of glandular element. Some study said hereditary does play a part in the development in this tumour particularly in TP 53 and MED12 mutation, in a way similar to the fibroadenoma, but with additional genetic aberrations of the tumour suppressor genes in phyllodes tumour.(Tan *et al.*, 2016).

The histological appearance of leafy architecture and stromal cellularity are the gold standard in diagnosing phyllodes tumour (Tan *et al.,* 2016). Notwithstanding the stromal hypercellularity is the key histological feature to differentiate between a phyllodes tumour and fibroadenoma (Lee *et al.*, 2007; Jara-Lazaro *et al.*, 2010).

There are an overlap features on sonography imaging for both phyllodes tumour and fibroadenoma, as mentioned in many studies, thus necessitate for histopathology examination (Chao *et al.*, 2002; Testori *et al.*, 2015). In fact some of the study said from good fine needle aspiration (FNA) composed of both epithelial and stromal component can yield a better differentiation between this phyllodes tumour and fibroadenoma (Veneti and Manek, 2001; El Hag *et al.*, 2010).

Howbeit, the pathologist still have difficulty to distinguish between benign phyllodes tumour with the cellular fibroadenoma as both carries similar microscopic features except for the presence of minimal stromal cellularity in the former diagnosis (Tan *et al.,* 2016).

With the evidence of necrosis and heterologous element, it aroused an index of suspicion of malignant phyllodes tumour, although it is not counted as a criteria for diagnosis (MOFFAT *et al.*, 1995). According to World Health Organization (WHO), this PT are comprised of group of fibroepithelial neoplasm that resemble fibroadenoma but with other distinctive histological features. Their sarcomatous element may also resemble the pure stromal sarcoma. WHO has further classified this phyllodes tumour into three major category: Benign, Borderline and Malignant, based on 5 major histological features of degree of stromal cellular atypia, stromal hypercellularity, stromal overgrowth, mitotic count and infiltrative or pushing margin.

The recurrent of tumour is defined as similar tumour of histological type that presence at similar quadrant. Tumour that arise from different quadrant or from contralateral breast is said to be a new tumour growth. One of the risk of this recurrence is associated with positive margin or incomplete surgical excision (Teo, Cheong and Wong, 2012). In a lame study yet reliable one indicate that the higher mitotic rate is also associated with the risk of recurrence (MOFFAT *et al.*, 1995).

With the presence of stromal hypercellularity without epithelial element, it is associated with metastatic ability of the tumour. In contrast, the presence of stromal overgrowth, cytological atypia, tumour necrosis and heterologous element does play a role in metastasis capability (MOFFAT *et al.*, 1995).

The usage of immunohistochemistry (IHC) in diagnosing of benign PT is not pronounced as compared to the other tumour. (Korcheva *et al.*, 2011) Nonetheless in the case of malignant PT, it does play a certain role. Multiple studies done to identify histological parameters that predict behaviour have yielded variables results. Thus, there is a need for additional markers of biological potential.

The role of immunohistochemistry (IHC) marker in diagnosing PT is not widely used. Many years, a particular marker of TP53 protein has been studied extensively yet the utility for this marker as a prognostication is remained uncertainty. This protein is ubiquitous in human body and it is a very potent tumour suppressor gene. Therefore it is consider as a beneficial IHC marker as any breakdown in this protein will give rise to the development of neoplasia.

One of the other valuable marker is Ki67. Ki67 is a non-histone nuclear protein that peaks during the G2M phase of the cell cycle (Song and Yoon, 2008).

This Ki67 is strongly correlated with the mitotic index of the tumour, hence it is an important proliferative marker used in immunohistochemistry (IHC) study (Shoker *et al.*, 2001). Some study show that it can be used as a predictive marker as it measure the probability of recurrence particularly in malignant PT (Niezabitowski *et al.*, 2001; Tse, Niu and Shi, 2010). Somehow in a similar study show both IHC marker of Ki67 and P53 as well as evidence of mitotic activity; play as an individual role in a prognostication elements.

Approximate 3% of normal breast epithelial cells show positivity for Ki67 staining. Expectantly it will be higher in the invasive breast carcinoma particularly hormonal negative tumours. (Shoker *et al.*, 2001). The expression of Ki67 staining in malignant PT is fluctuating between 15-100% whilst it is less than 25% in a case of benign PT (Tse, Niu and Shi, 2010). In the case of malignant PT with presence of osteosarcomatous differentiation exhibiting higher Ki67 index. In fact in the cases of morphologically benign PT displaying more than 10% staining of Ki67 are risky for malignant changes (Ribeiro-Silva, Ramalho and Zucoloto, 2006). On top of it, other markers that can be used for PT are CD117 (ckit), ER, PR, CD 31, CD10 (CALLA), EGFR and undoubting increasing trend is a molecular study.

The mainstay treatment remains the surgical procedure (El Hag *et al.*, 2010; Mishra *et al.*, 2013). The choice of procedure is depending on the classification of this tumour, however the breast-conserving therapy is most preferable. In benign phyllodes, tumour excision with additional 1cm margin of normal breast tissue is sufficed. Fretful, the risk of local recurrence is as high as 27% in case of malignant phyllodes tumour (Teo, Cheong and Wong, 2012). On top of it, it have a possibility of metastasis as well as fatal risk.

2.0 OBJECTIVES

2.1 General Objectives

To study the Ki67 and p53 proteins expression in phyllodes tumour of the breast in relation to clinicopathological features.

2.2 Specific Objectives

1. To determine the proportion of phyllodes tumour
2. To determine the Ki67 protein expression in phyllodes tumour
3. To determine the p53 protein expression in phyllodes tumour
4. To determine the association of Ki67 and p53 with clinical descriptive data and histopathological features of the phyllodes tumour.

3.0 MANUSCRIPT

3.1 Title: Ki67 AND p53 EXPRESSION IN RELATION TO CLINICOPATHOLOGICAL FEATURES IN PHYLLODES TUMOUR OF THE BREAST.

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Running Title:

The Expression of p53 and Ki67 of Phyllodes Tumour in HUSM and HSNZ.

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Statement Conflicts of Interest: There is no conflict of interest.

3.2 Abstract

Objective: Phyllodes tumour is a rare fibroepithelial neoplasm of the breast that carry a risk of malignancy. The gold standard for diagnosis remained the histopathological findings. The usage of immunohistochemical markers of Ki67 and p53 act as a supplement method particularly for the malignant phyllodes tumour. Therefore we aim to study the expression of these markers in phyllodes tumour and to see its relation to the tumour grading.

Methodology: This was a retrospective cross sectional study conducted on 57 archival tissue samples of phyllodes tumour from the year 2015 to the year 2018, from Hospital Universiti Sains Malaysia and Hospital Sultanah Nur Zahirah. The histopathological examination was analyzed and further immunohistochemistry markers of Ki67 and p53 proteins expression were studied on formalin-fixed paraffin-embedded tissue.

Result: There was an association between clinical descriptive data of skin changes, lump size of more than 3 cm, cytological atypia, stromal hypercellularity, mitosis and immunohistochemistry with the clinical diagnosis of phyllodes tumour. Both marked expression of Ki67 and p53 were seen in borderline and malignant phyllodes tumour. However the presence of both high mitosis and marked expression of Ki67, on top to other histological features, was merely seen in case of malignant PT.

Conclusion: In our study, there was significant association of both p53 and Ki67 expression in the case of borderline and malignant phyllodes tumour. Nevertheless with the presence of high mitotic rate and strong expression of Ki67 index are exclusively found in malignant phyllodes tumour. Further study of a larger sample size and division into more precise of low-high grade group is recommended for a validated result. On top of that, it is also helpful to predict the prognosis as well as the disease-free-survival of the tumour.

Key words: Ki67, p53, Phyllodes tumour, breast tumour, immunohistochemistry

3.3 Introduction

Phyllodes tumour (PT) was initially described as cystosarcoma phyllodes in the year 1838 by Johannes Muller due to its biphasic appearance of benign epithelial elements and fibrosarcomatous stroma (Veneti and Manek, 2001; Lee *et al.*, 2007). This fibroepithelial tumour was characterized by stromal hypercellularity altogether with cleft like or cystic spaces lined by epithelium.

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3.4 Material and Methodology

A retrospective cross sectional study were conducted in Hospital Universiti Sains (HUSM) Malaysia and Hospital Sultanah Nur Zahirah (HSNZ), Kuala Terengganu, diagnosed from 1st January 2015 until 31st December 2018. The study was approved by the Human Ethics Committee of the School of Medical Sciences, Universiti Sains Malaysia (USM/JEPem/18030185) and Medical Research and Ethics Committee (MREC) Ministry of Health, Malaysia (NMRR-19-2255-49034).

Patients' clinicopathological data were retrieved from the medical record unit and laboratory information system (LIS). A total 57 participants were selected. The sample size was determined via the first objective, based on Moffat *et.al* and Testori *et.al*. The Power and Sample Size Calculation software was used with dropout of 20%, producing a corrected sample size of 29. The second and third objectives involved a descriptive statistical analysis. For the objective 4, univariable analysis of Pearson Chi Square test was used to see the association between the clinical aspects with histopathological and immunohistochemistry markers of the tumour.

Proforma form was used for data collections and a table for the histopathological examination (HPE) and immunohistochemistry (IHC) scoring of the tumour tissue. The previous Haematoxylin & Eosin (H&E) stained slides were retrieved for HPE scoring. Cases that fulfilled the criteria will be selected and their formalin fixed paraffin embedded (FFPE) blocks will be further stained with IHC study of Ki67 and p53.

Ki67 and p53 are a common IHC at the laboratory yet its usage to diagnose PT is not routinely done. It is specially used for this study. Positive and negative controls were stain and optimal dilution of 1 in 100 were selected for both antibodies. The internal control for both Ki67 and p53 are using a lymphocytes and normal colon tissue.

Semiquantitative scoring analysis is used by two observers. The presence of nuclear staining for both Ki67 and p53 is considered as positive. Based on Vani *et.al* the measurement of Ki67 is defined as any detectable brown staining of the nucleus. It is categorised into 3 aspects: Mild (0-10%), Moderate (11-30%) and Marked (more than 30%). Marked expression as depicted in Figure 1.

Whereas for the p53 protein expression, based on A Niezabitowski *et.al* and Remmele *et.al*, the assessment will be graded into 3 similar categories: Mild (0-50%), Moderate (51-80%) and Marked (more than 80%). The example of marked expression is displayed in Figure 2.

The data was analysed using a software IBM SPSS version 24. The association between clinicopathological data and protein expression of Ki67 and p53 were analysed using Pearson Chi Square test.

3.5 Result

A total number of 57 participants were recruited for this study based on patients diagnosed with Phyllodes Tumour (PT) at HUSM and HSNZ. The clinicopathological data of the participants were presented in table 1. All of the participants were Malay (94.7%) except for one Chinese. The result from the data analysis show that the majority of the patient's age group were more than 20 years (n=54, 94.7%). All of them presented with a palpable lump, in which all patients with malignant PT will have a lump size of more than 3 cm. About 17.5% (n=10) of the patient presented with skin changes, predominantly attributed by a malignant PT.

From the histopathological aspect; more than half of the patients show evidence of cellular atypia 68.4% (n=39) and presence of stromal overgrowth (n=44, 77.2%), as compared to the slightly lower amount of the stromal hypercellularity (n=27, 47.4%). In many cases, it shows mitosis of less than 5 (n=44, 77.2%). Based on Table 1, the proportion of benign phyllodes tumor were 73.7%. In majority of the Ki67 (89.5%) and p53 (59.5%) protein expression were graded in mild group.

Table 2 show the association between the clinicopathological data with the protein expression of Ki67 and p53. There were significant association between clinical descriptive data (skin changes ($p=0.004$), histopathological features (atypia status ($p<0.001$), stromal hypercellularity ($p=0.012$), mitosis ($p<0.001$)) and immunohistochemistry p53 $p<0.001$, Ki67 ($p<0.001$) with the diagnosis of PT.

We can also see that in the presence of very high mitosis (more than 9) as well as marked protein of expression of Ki67 (more than 30%) in a case of malignant PT. Meanwhile other relevant important findings of the presence of skin changes, lump size of more than 3cm, evident of cytological atypia altogether with the presence of marked p53 protein expression (more than 80%) shall raise the suspicion of at least borderline PT.

Pearson Chi Square test was used to examine the association between the clinicopathological data and protein expression of Ki67 and p53 in phyllodes tumour.

Based on table 3, there was a significant association between distribution group of Ki67 and p53 in relation to the diagnosis (both $p<0.001$). It was shown that most of the negative/mild Ki67 and p53 protein expression fall into the benign group while marked expression of both Ki67 and p53 had fall into the malignant group.

In case of the borderline PT, our study showed a marked staining for p53 marker only and negative/mild staining for Ki67. Meanwhile for the malignant PT its shows the presence of marked expression for dual immunohistochemistry marker of Ki67 and p53, counted 7% and 5.3% respectively. Therefore Ki67 protein expression is beneficial for the case of malignant PT, on top of other histopathological and clinical suspicion.

3.6 Discussion

Phyllodes tumour (PT) is defined as biphasic tumour of epithelial component within a neoplastic spindle cell stroma. Based on WHO classification, it is categorized into benign, borderline and malignant, as distinctive by their histological appearance. Due to its intratumoral heterogeneity the features might be intermingled, hence in the presence of suspicious foci of heterologous component, the diagnosis should be upgraded (Zhang and Kleer, 2016).

We find that in a case of benign PT, patients usually presented with innocuous small breast lump. However in the present of larger size and ulceration, the malignant tumour is prime as most of them usually manifest late in their disease course. It is rare to have positive nodes yet it prompt to malignant PT (Testori *et al.*, 2015). Contrary in our study, the size of the lump and skin changes does not represent the tumour grading.

Based on our study, amongst the 5 key histological features, the mitosis play a vital role to aid the diagnosis of highest tumour grade of malignant PT; in which the diagnosis of malignant PT shall be considered in the presence of mitosis of more than 9. Other microscopic findings of cytological atypia, permeative margins, stromal hypercellularity and stromal overgrowth is needed to make the diagnosis but not for tumour grading. In spite of that, the presence of these features increase the risk of local recurrence and metastasis (Chao *et al.*, 2002).

On top of its ultrasonographic features, needle biopsy is superior to cytological analysis to differentiate it from a commoner diagnosis of cellular fibroadenoma as well as malignant sarcoma in the presence of larger stroma fragment and numerous stromal bare nuclei (Veneti and Manek, 2001; Chao *et al.*, 2002; El Hag *et al.*, 2010; Berner and Sauer, 2011; Tsang *et al.*, 2011; Tan *et al.*, 2016). The key histological features will be a stromal hypercellularity that presence immediately beneath the subepithelial region (Jara-Lazaro *et al.*, 2010; Tsang *et al.*, 2011). Hence this appearances support the hypothesis that this PT are arise from the periductal areas instead of intralobular stroma cells (Kleer *et al.*, 2001; Tse, Niu and Shi, 2010).

Histologically, to diagnose malignant PT, the differential of sarcoma should be lingered as it shows a stromal differentiation of fibrosarcomatous type. Having negative expression of basal keratin and beta catenin, the expression of Ki67 index together with stromal positivity of CD34 may aid in the diagnosis of PT over the sarcoma (Lee, 2008). Genetic study is helpful since this PT has lack of oncogenic mutation as compared to other carcinoma, sarcoma and melanoma (Korcheva *et al.*, 2011).

Some studies conclude that the size do not correlate with local recurrence except for the stromal overgrowth (Aydoğan, Tasçi and Sagara, 2016). The risk of local recurrence are also higher with higher grade. Therefore bringing along the risk of local recurrence and metastatic property, hence proper pre-operative diagnosis and post-operative management are crucial. (Mishra *et al.*, 2013; Lu *et al.*, 2019). A wide local excision with 1 cm of free margin act as primary treatment intraoperatively. Lymph node resection is not recommended initially and the need for further adjuvant relies on subsequent follow up of histopathological exam (Chen *et al.*, 2005; Telli *et al.*, 2007).

In recent years, there were extensive studies of evaluating the usage of immunohistochemistry (IHC) markers to aid in the diagnosis the PT. The usual markers that had been commonly used were the proliferative marker of Ki67 and a tumour suppressor gene of p53. Other markers were hormonal receptor, angiogenesis group of marker (ie CD31), epidermal growth factor, CD117, CD10, pH3, Actin, BCL2 and Cyclin D1 (Kleer *et al.*, 2001; Niezabitowski *et al.*, 2001; Shoker *et al.*, 2001; Esposito *et al.*, 2006; Lee, 2008; Song and Yoon, 2008; Bose *et al.*, 2010; Tse, Niu and Shi, 2010; Jara-Lazaro *et al.*, 2010; Korcheva *et al.*, 2011; Noronha *et al.*, 2011; Wang *et al.*, 2017).

The p53 protein of tumour suppressor gene is found ubiquitous in our body, readily function as a regulator in cell cycle, cell differentiation and DNA cell repair. This is the initial marker that had been studied as it is extensively altered in breast cancer as well as other neoplastic tumour (Tse, Niu and Shi, 2010). In our study the expression of this marker presence in all cases of PT with different intensity. The benign cases are expressed up to moderate intensity only, whereas for malignant PT the expression is equal between moderate and marked staining. Concluded by our study, the marked expression of p53 are seen equally in both cases of borderline and malignant PT.

The cell cycle proliferative marker of Ki67 is a well-known immunohistochemical protein that had been helpful to distinguish many types of actively proliferating tumour mass in our body part. Based on our study, the malignant PT show moderate to marked intensity for Ki67 staining.

However in a case of benign and borderline tumour, this Ki67 expressed in less than 10% of the nuclear staining, thus fall into the negative/mild group. We also find that there is significant correlation between Ki67 index and tumour histological type particularly of high mitosis (more than 9/10 HPF), on top of other microscopic features.

Therefore the expression of Ki67 can be helpful in a difficult case of malignant PT. Combination with p53 positivity can act as an added value. Nevertheless both p53 and Ki67 expression do not reliable to predict the rate of recurrence (Kleer *et al.*, 2001; Niezabitowski *et al.*, 2001).

In many research they found that this Ki67 index plays an important prognostic factor in PT. In similar study, Feakins et al displays that high expression of this Ki67 index carry a worst outcome in term of local recurrence and metastatic rate. However in one study emphasizes that the usage of these biomarkers does not significantly improved the prognostic outcome, therefore they emphasize that the histological features remained a predictor factor of the biological-behavior of the PT (Tan *et al.*, 2016).

Phyllodes tumour shows an expression of cyclin D1 albeit it is not associated with the tumour grading. The tumour also show significant staining of p53 and CD117 expression in a higher tumour grade (Korcheva *et al.*, 2011). Similarly in other study suggest that the combination between p53, Ki67 and CD117 expression can aid to distinguish the tumour grade (Noronha *et al.*, 2011; Tse, Niu and Shi, 2010)

Undoubtedly, the tumour behaviour is predicted by surgical-margin status (Esposito *et al.*, 2006). As opposed to many other research, the expression of Cyclin D1 is non conclusive since it also express in normal as well as cancerous breast tissue (Shoker *et al.*, 2001; Noronha *et al.*, 2011).

Therefore we suggest of further research in dividing this Ki67 index into low-and high grade of more precise group, as it may be helpful to predict the prognosis as well as the disease-free-survival of the tumour. Till date, the clinical use of molecular genetic is still under investigation. Few studies involving the PDGFRA and the KIT mutation show no pathogenetic role in breast PT (Bose *et al.*, 2010; Korcheva *et al.*, 2011).

3.7 References

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