

The Association of Fibroblast Growth Factor-2 (FGF-2) Expression with Benign, Borderline, and Malignant Phyllodes Tumours of the Breast

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Abstract- Phyllodes tumours is a rare biphasic tumours, this is composed of neoplastic stromal cells and evolution containing epithelium. The stromal tumour is more cellular and increased. Phyllodes tumours are far less common than fibroadenoma and de novo arising, and not from previous fibroadenoma tumours. The bad changes are the feared malignant are increased stromal cellularity, anaplasia, high mitotic activity, rapidly increasing tumour size, and infiltrative edges. The incidence of phyllodes is <1% of all breast neoplasm in 0,1-0,5%, with the most incidence occurring at the age of 30- 40 years old.

Objective: This study was aimed to analyze the association of Fibroblast Growth Factor-2(FGF-2) with benign, borderline, and malignant phyllodes tumours of the breast.

Methods: This is an observational analytic study with cross sectional approach, involving 35 paraffin block samples from phyllodes tumours. In this study we found FGF-2 expression in 35 samples, consist of 15 samples of benign phyllodes tumours, 8 sample of borderline tumours, and 12 samples of malignant phyllodes tumours. The specimen of this study were embedded in paraffin and each section was cut from each block. Then FGF-2 staining were done. The positivity for FGF-2 were scored by using the following parameters. FGF-2 counts among 3 groups of cases were analyzed using statistic software.

Result: There were association between FGF-2 expression with benign, borderline, and malignant phyllodes tumours.

Conclusion: The FGF-2 expression in malignant phyllodes tumours has the highest mean value, followed by borderline phyllodes tumours, and benign phyllodes tumours have the lowest FGF-2 expression values.

Index Term: FGF-2, Phyllodes Tumours.

I. INTRODUCTION

Phyllodes tumours is a rare biphasic tumours of the breast. The incidence of phyllodes tumours account for 0,3-0,9% of all primary tumours of the breast.¹ Phyllodes tumours is biphasic neoplasm originally named cystosarcoma phyllodes by Johannes Muler in 1838, a term that is to be avoided because of its malignant connotations. Phyllodes tumours commonly occurs in middle-aged and older woman. Very few patients are younger than 25 years age, which is in striking contrast with the age

distribution of fibroadenoma tumours. However, phyllodes tumours can certainly occur in young adults and even in adolescents, and therefore, the diagnosis can't be excluded on the basis of age.¹⁻³ Phyllodes tumours increased in Asian countries, in Singapore this tumours occurred 6,92% of all malignancies in the breast and occur at a younger ages, 25-30 years old. Although rarely found, there have been reports of this tumours in men. The frequency of these tumours, based on changes in histopathological features (gradations) is 75% benign, 16% borderline, and 9% malignant. Although it has been reported, there is rarely a synchronous or metachronous presence in this tumours.^{4,7} It also difficult to diagnose phyllodes tumours because of subclassification resembling fibroadenomas. The prognostic value of immunohistochemical markers of tumour growth, angiogenesis, and malignant transformation has been confirmed in many tumours, including breast carcinoma. Studies have shown significant differences in FGF-2 expression for benign and malignant phyllodes tumours of the breast.⁹ FGF-2 is a very potent stimulator for proliferation, migration of endothelial cells, and tube formation in vitro and also high angiogenic in a number of tissues in vivo.¹⁰ The existence of FGF can be obtained by two methods, namely: first by conducting immunohistochemical examination of tumour tissue and by examining the Enzyme-Linked Immunosorbent Assay (ELISA) of blood serum, then FGF levels will be obtained. From this examination will be obtained a response from the FGF receptor. Increases in both FGF and FGF receptors have important significance in tumour growth.¹¹ In a previous study, Dacic et al., only examined the expression of FGF-2 in benign and malignant phyllodes breast tumours, and did not evaluate it in phyllodes borderline breast tumours.⁹ In addition, there is still very little research that addresses the problems mentioned above, causing the existing data is also limited. In Indonesia, especially in North Sumatera, there are no studies that analyze the appearance of expression and its relationship with phyllodes tumours.

II. MATERIAL AND METHODS

This is an observational analytic study, using a cross sectional approach. This study was conducted at the Department of Anatomical Pathology Faculty of Medicine Universitas Sumatera Utara, Department of Anatomical Pathology in H. Adam Malik

Medan General Hospital, and Department of Anatomical Pathology in Pirngadi Medan Hospital. The research was held from July 2019 until November 2019, after approved by the Universitas Sumatera Utara and H. Adam Malik General Hospital Health Research Ethics Committee. All samples were obtained through surgical procedure. Inclusion criteria were phyllodes cases with adequate clinical data, available and undamaged formalin-fixed paraffin embedded tissue block with sufficient tumor tissue. Detailed clinical data were obtained from medical records or pathology archives consisting of age, sex, and size of the tumor. Histological type were determined independently by researchers through hematoxylin and eosin stained slides examination.

Histochemistry protocol and interpretation

The tissue sections were deparaffinized and rehydrated before pretreatment. Endogenous peroxidase was blocked with hydrogen peroxide followed by antigen retrieval. FGF-2 (GTX84502, GeneTex, California, America) mouse monoclonal antibodies was used as primary antibody. Diagnostic BioSystems (Diagnostic BioSystems, Pleasanton, CA, USA) polymer kit was used for detection. The reaction was visualized with diaminobenzidine and counterstained with Mayer's hematoxylin followed by dehydration, clearing, and mounting. Positive control was colon. Expressions of FGF-2 were determined independently by researchers. The expression in cytoplasm was analyzed. FGF-2 expressions were determined independently by researchers. Nuclear and cytoplasmic positivity for FGF-2 by using the following parameters: 0 (no staining) 1+ (1-35% positive cells), 2+ (35-70% positive cells), and 3+ (70-100% positive cells).⁹

Statistical analysis

Statistical analysis was performed using SPSS software package version 22.0 (SPSS Inc., Chicago) with 95% confidence interval and Microsoft Excel 2010. Categorical variables were presented in frequency and percentage. Saphiro-Wilk test was applied to find out the normality continue data. The association between FGF-2 expression with benign, borderline, and malignant phyllodes tumours is assessed by Kruskal-Wallis, and continue with post hoc Dunn test to assessed the differences between the groups. The p-values < 0.05 were considered significant.

III. RESULT

Patients' characteristics

The mean age for phyllodes tumour patients was 43.2 (±14.3) years. The most common in 12-63 years age group, 34 patients (97.1%) were females, only 1 patients (2.9%) were males. The mean size of tumours was 21.85 cm which is 6-25 cm for benign phyllodes, 12-15 cm for borderline phyllodes, and 15-35 cm for malignant phyllodes tumours. The number of the patients benign phyllodes was 15 (42.9%), borderline phyllodes was 8 (22.9%), and malignant phyllodes was 12 (34.2%). The histological subtypes of benign phyllodes was the majority of this case. Clinical basic characteristic of phyllodes patients were summarized in table 1. Representative H&E sections are shown in figure 1.

Table 1. Characteristic of phyllodes patients

Age, mean ± SD, years	43.2 ± 14.3	
Benign 12-63		
Borderline 20-65		
Malignant 17-74		
Tumours size, mean, cm	21.85	
Benign 6-25		
Borderline 12-15		
Malignant 15-35		
Characteristic	N	Percentages(%)
Dagnosis		
Benign phyllodes	15	42.9
Borderline phyllodes	8	22.9
Malignant phyllodes	12	34.2
FGF-2 expression: n (%)		
Mild	10	28.6
Moderate	14	40.0
Strong	11	31.4
TOTAL	35	100

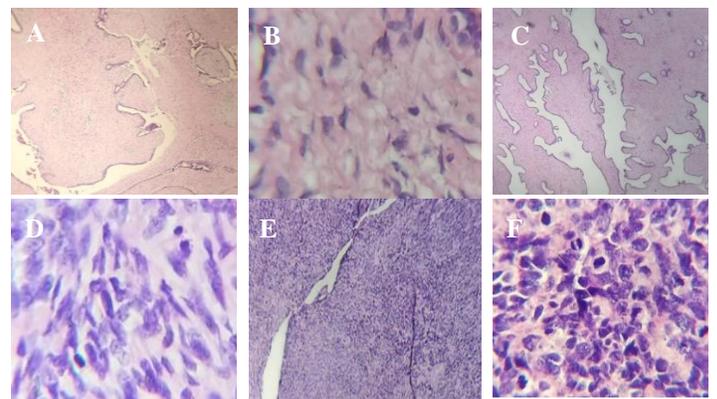
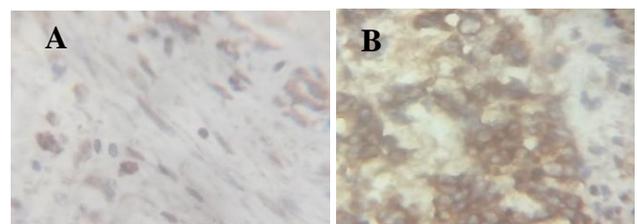


Figure 1. Histological type. A, Benign phyllodes (HE, 40x). B, Benign phyllodes (HE, 400x). C, Borderline phyllodes (HE, 40x). D, Borderline phyllodes (HE, 400x). E, Malignant phyllodes (HE, 40x). F, Malignant phyllodes (HE, 400x).

FGF-2 expression

The intensity of FGF-2 expression in nuclear and cytoplasm are shown in figure 2.



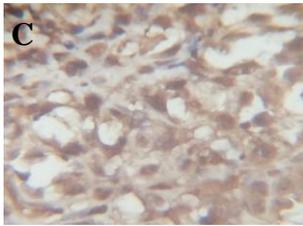


Figure 2. Immunohistochemistry FGF-2 expression. A, Mild intensity B, Moderate intensity. C. Strong intensity.

Association between FGF-2 expression in benign, borderline, and malignant phyllodes tumours

The number of cases for positive FGF-2 expression was found more in malignant phyllodes tumour. In this study, a statistical test was performed to determined the relationship between FGF-2 expression and benign, borderline, and malignant phyllodes breast tumours, the a statistical test was performed to determined it.

Tabel 2. Association between FGF-2 expression in benign, borderline, and malignan phyllodes tumours

Diagnosis	FGF-2 expression						p-value*
	Mild		Moderate		Strong		
	n	%	n	%	n	%	
Benign	9	60.0	5	33.3	1	6.7	0.0008
Borderline	0	0	6	75.0	2	25.0	
Malignant	1	8.3	3	25.0	8	66.7	

*Kruskal-Wallis test

In this study, we found that the test p-value 0.0008 was obtained, it can be concluded that there is a relationship between FGF-2 expression and phyllodes tumours.

Table 3. The differences FGF-2 expressions between group lesions of benign, borderline, and malignant phyllodes tumours

The differences of FGF-2 expression	p-value*
Benign vs Borderline	0.012
Benign vs Malignant	0.0001
Borderline vs Malignant	0.169

There were differences in the FGF-2 values between groups of benign phyllodes tumour lesions with malignant phyllodes tumour lesions with $p < 0.001$, while between groups of benign tumour lesions with borderline lesion with malignant there are no differences in values with p-values of 0.012 and 0.169, respectively.

IV. DISCUSSION

The incidence of breast phyllodes tumours is very rare, with the most incidence occurring at the age of 30 to 40 years, even in one study reported phyllodes tumours occurred at an older age, ie 45-54 years. In a research, Tan et al. reported that phyllodes tumours were 6.92% of all malignancies in the breast and

occurred at a younger age of 25-30 years.¹ In some previous study found that phyllodes tumours occurs at a younger age of 19-66 years, with a mean \pm sd 40.11 ± 12.06 and a median of 38.9, according to the young age in the study conducted by Ben et al., with a mean of 39,5 (14-71) and Nurhayati's study with a mean of 42 (16-78).¹⁹ In this study the mean age of the sufferers was 43.2 ± 14.3 years with the youngest age is 12 years, and the oldest age is 74 years. And most occur at the age of 12-63 years.

Karim et al., reported that although it was rarely found, phyllodes tumours had been reported report in men.¹² This was in line with this study where of the 35 samples studied, there was only one male samples (2.9%), while 34 other samples were female (97.1%). The frequency of these tumour events based on changes in histopathological features is 75% benign, 16% borderline, and 9% malignant.¹² Tan et al., reported that the relative proportions of benign phyllodes tumours were 60-70%, borderline 15-20%, and malignant at 10-20%.¹ Xiaofang et al., in their study reported from 52 patients there were 64% benign phyllodes tumours 25% borderline phyllodes tumours, and 6% malignant phyllodes tumours.¹³ From this study we found 15 benign phyllodes tumours (42.9%), 8 borderline phyllodes tumours (22.9%), and 12 malignant phyllodes tuomors (34.2%). The FGF-2 expression in this study was found to be mild in 10 samples (28.6%), moderate expression in 14 samples (40%), and in strong expression there were 11 samples (31.4%).

Phyllodes tumours can be clearly seen if it rapidly enlarges. Rapid enlargement does not always indicates malignancy. Looks shiny with stretched skin surface accompanied by widening of the skin surface veins. In some cases that are nor handled properly, skin ulcers can occur due to tissue ischemia. Although proper skin changes in breast tumours always show signs of malignancy, but not phyllodes tumours, ulcer on the skin can occur in benign, borderline, or malignant lesions. Nipple retraction is not common. Ulceration indicates tissue necrosis due to large tumour suppression. Large size of tumours can also cause necrosis with bleeding.^{14,15,16} In one study it was reported that changes in clonality in stromal cells lead to benign phyllodes tumours and progression to monoclonal progression in epithelial and stromal cells in borderline and malignant.⁷ Approximately 10-40% of tumours of this type have a risk of local recurrence and spread systemically.⁵ This corroborates the results of the study why delay in handling from patients and tumour recurrence can cause the percentage of malignancy to increase. Research on 8.567 breast tumour patients in 1969-1993 only found 31 cases of phyllodes tumours (0.37%). Overall, 2.1 cases per million women, very rare in men. Most phyllodes tumour cases occur in the 4th decade, rarely in adolescents, can occur at any age. Tumours are usually benign but local recurrence can occur and sometimes can spread systematically; rarely bilateral (either synchronous or metachronous).²⁰⁻²² Several previous studies found that tumours to be less than 5 cm in size, therefore, the diagnosis cannot be made based solely on tumour size. Prolonged gaps (leaf-like appearance) in the across section are typical signs of phyllodes tumours.¹⁷ In this study, the average tumour size was 21.85 cm, with a range of 6-35 cm. In benign phyllodes tumours the tumour size ranges from 6-25 cm, borderline 12-15 cm, and malignant phyllodes tumours 15-35 cm. Calhoun et al., in their study found that the phyllodes tumour size ranged from 1-40 cm. This research indicate that most phyllodes tumours are

large, however, there are also small-sized phyllodes tumours. In a previous study conducted by Flynn et al., and Calhoun et al., it was reported that phyllodes tumours were generally large (>2-3 cm).^{15,16,18} This is suitable with the results of this study where it was found that phyllodes tumours size ranged from 6-35 cm.

This study used the WHO classification breast tumours involving 35 samples of phyllodes tumour consisting of 15 samples of benign phyllodes tumours, 8 samples of borderline phyllodes, and 12 samples for malignant phyllodes tumours.

In a research conducted by Parker, found that phyllodes tumour are more often in the upper lateral quadrant with the same tendency between the right and left breast.²³ In this study also found that of the 35 samples examined, there were 28 (80%) patients with tumour location in the upper lateral quadrant and 7 (20%) patients with tumour location in the lower lateral quadrant. It is not yet known exactly why the location of this phyllodes tumour is predominantly in the upper lateral quadrant, suggesting that it may be caused by more glands in the upper lateral quadrant of the breast.

In this study the authors performed FGF-2 staining on benign, borderline, and malignant phyllodes breast tumour sample with the aim of improving the accuracy of diagnosis so that management can be carried out appropriately. Dacic et al., in their study stated that of the 23 samples studied, there were 14 samples of benign phyllodes tumours and 9 samples of malignant phyllodes tumours. In that study it was stated that there were differences in the expression of FGF-2 between benign phyllodes breast tumour and malignant phyllodes tumours. FGF-2 expressions are more expressed in the stroma than in the epithelium in both benign and malignant phyllodes tumours.⁹ FGF-2 is expressed in benign breast tumours and malignant breast tumours. FGF-2 enhances tumour growth through the proliferation effect of tumour cells undergoing the degeneration of the surrounding matrix, or the formation of new blood vessels.^{9,24}

In their study, dacic et al., showed that FGF-2 is expressed in the cytoplasm in the stroma and epithelial components in most tumours. FGF-2 has also been detected in normal breast tissue (myoepithelial cells and extracellular perivascular matrix) and in malignant breast tumours. This shows that mitogenic polypeptide which stimulates plasminogen activator and has been detected in tumours that have invaded. It was further said that there was a significant correlation between FGF-2 expression and benign and malignant phyllodes breast tumours. In addition, FGF-2 is also involved in the regulation of angiogenesis and synthesis of various proteases.⁹

Granato et al., in their study stated that the percentage of FGF-2 immunopositive breast tumours varies greatly in different tumours, from negative to positive in all tumour areas. In the majority of cases (84%) were positively expressed in the cytoplasm, and only a small proportion (16%) were expressed in the cell nucleus and cytoplasm of tumour cells.²⁴ In line with the research conducted by Granato et al., in this study was found that the expression of FGF-2 in benign, borderline, and malignant phyllodes tumours varied in value, and performed in the cytoplasm and nucleus of tumour and stromal cells.

In this study, for FGF-2 expression, from the 15 benign phyllodes tumour samples, 9 (60%) samples were mild expressed, 5 (33.3%) samples were moderately expressed, and 1

(6,7%) sample was strongly expressed. For borderline phyllodes tumours, from 8 samples, there were no sample that were mildly expressed, 6 (75%) samples were moderately expressed, and 2 (25%) samples were strongly expressed. Whereas in malignant phyllodes tumours, from 12 samples, there were 1 (8.3%) samples that were mildly expressed, 3 (25%) samples were moderately expressed, and 8 (66.7%) samples were strongly expressed. From the results of this study it was also found that there were differences in values between the groups of benign phyllodes tumour lesions with malignant phyllodes with $p < 0.001$, while between groups of benign tumour lesions with borderline and groups of borderline lesions with malignant there were no differences in values of 0.012 and respectively 0.169.

V. CONCLUSION

For FGF-2 expression, from the 15 benign phyllodes tumour samples, 9 (60%) samples were mild expressed, 5 (33.3%) samples were moderately expressed, and 1 (6,7%) sample was strongly expressed. For borderline phyllodes tumours, from 8 samples, there were no sample that were mildly expressed, 6 (75%) samples were moderately expressed, and 2 (25%) samples were strongly expressed. Whereas in malignant phyllodes tumours, from 12 samples, there were 1 (8.3%) samples that were mildly expressed, 3 (25%) samples were moderately expressed, and 8 (66.7%) samples were strongly expressed.

COMPETING INTERESTS

The authors have no relevant financial interest in the products or companies described in this article.

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ETHICAL APPROVAL

Health Research Ethical Committee, University of Sumatera Utara, Medan, Indonesia approved this study.

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