Association of Fibroblast Growth Factor-2 (FGF-2) Immunoexpression with Histopathology Grade of Meningioma

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Abstract- Meningioma is one of the primary brain tumors, originating from arachnoid cap cells. FGF-2 as a growth factor is a potent stimulator of endothelial proliferation which is very important in the process of angiogenesis. FGF-2 levels in meningiomas have been assessed using the ELISA and qrPCR methods in many previous studies using blood serum. This study used tissue samples of meningioma tumors to assess the association between immunohistochemical expressions of FGF-2 and histopathology grade among meningioma patients. Formalin-fixed paraffin-embedded tissue blocks of 32 meningioma patients were immunohistochemically studied for FGF-2 expression. The basic characteristics of the samples were obtained through medical records or pathology archieves. The association between FGF-2 expression and grade were anylized using SPSS 22 version. FGF-2 was expressed in 15.6% of the meningioma specimens. FGF-2 positive expression was significantly associated with higher histopathological grade ($p < 0.05$). A higher grade has a tendency of 14.3 times to express positive FGF-2. The positive expression of FGF-2 in the higher grade indicates that angiogenesis plays an important role in terms of tumor growth especially in malignant cases. This can be a prognostic indicator and possible target therapy for these neoplasms.

Index Terms-FGF-2, meningioma, grade, immunohistochemistry

I. Introduction

Meningioma is one of the primary brain tumors that most often occurs at athe age of 60-70 years.[1] The classification and grade of histopathology of meningioma currently used is based on the fourth revised edition of the World Health Organization (WHO). WHO grade 1 as benign (75% of cases), WHO grade 2 as atypical (10-15%) and WHO grade 3 as malignant (1-3%).[2] Depending on location and grade, treatment for meningiomas includes surgery and postoperative radiation therapy. Although meningiomas are generally benign tumors but in higher grades, they tend to be progressive and recurrent.[3] Reported recurrence rates for grade 1, 2 and 3 meningiomas are 7-25%, 29-52%, and 50- 94%.[4,5] Many researchers have confirmed the existence of receptors of growth factors in meningiomas such as epidermal growth factor (EGF), platelet-derived growth factors (PDGF), fibroblast growth factor (FGF), and insulin-like factors I and II (IGF-I and IGF-II). Growth factors are a large group of very diverse peptides or proteins that act as cell signals. These factors have a role in tumorigenesis meningioma.[6,7]

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.[8] It has been reported that FGF-2 is produced in more than 90% of glioma tissue and meningioma which shows that both are involved in autonomic cell growth and tumorigenesis as an autocrine growth factor in vivo.[9] In a Japanese study of tumor tissue in the brain including glioma, meningioma, and metastasis tumor, Northern Blot and In Situ Hybridization analyzes were conducted. The expression of mRNA for FGF-2 was found in cases of glioma and meningioma indicating that FGF-2 was produced by these tumor cells.[10] A study was also conducted in analyzing quantitative FGF-2 using the Enzym Linked Immunosorbent Assay (ELISA) method for tissue of several variants of grade 1 meningioma. Of all the meningioma samples that were observed, it was proven that FGF-2 protein could be detected in all meningioma tissue.[11]

The study of FGF-2 levels associated with grade meningioma have been done before in Medan Indonesia but this study used blood samples of patients and not with tissue samples.[12] Therefore, this study aimed to evaluate the immunohistochemical expression of FGF-2 in meningioma from the tumor tissue and analyze its association with histopathology grade of meningioma.

II. Material and Methods

Sample selection

This cross sectional study was conducted in Department of Anatomical Pathology, Universitas Sumatera Utara/ H. Adam Malik General Hospital, Medan and includes 32 cases of meningioma. All samples were obtained through surgical procedure. Inclusion criteria were meningioma 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 location of the tumor. Histological type and grade were determined

independently by researchers through hematoxylin and eosin stained slides examination.

Immunohistochemistry 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. FGF-2 expressions were determined independently by researchers. The expression in cytoplasm was analyzed. Immunostaining of FGF-2 was evaluated in terms of the proportion and staining intensity of tumor cells. The proportion was assessed on the basis of the percentage of immunopositive cells as follows: 0, less than 10%; +1, 10-25%; +2, 26-50%; +3, 51-75%; +4, greater than 75%. Staining intensity was evaluated as negative (0), weak (+1), moderate (+2), and strong (+3). The proportion score (0-4) was multiplied by the intensity score (0-3) and a final score was assigned, 0-4 as negative staining and 5-12 as positive staining.

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. Fisher's Exact test was applied to find out the association between FGF-2 expressions with histopathology grade of meningioma. The p-values < 0.05 were considered significant.

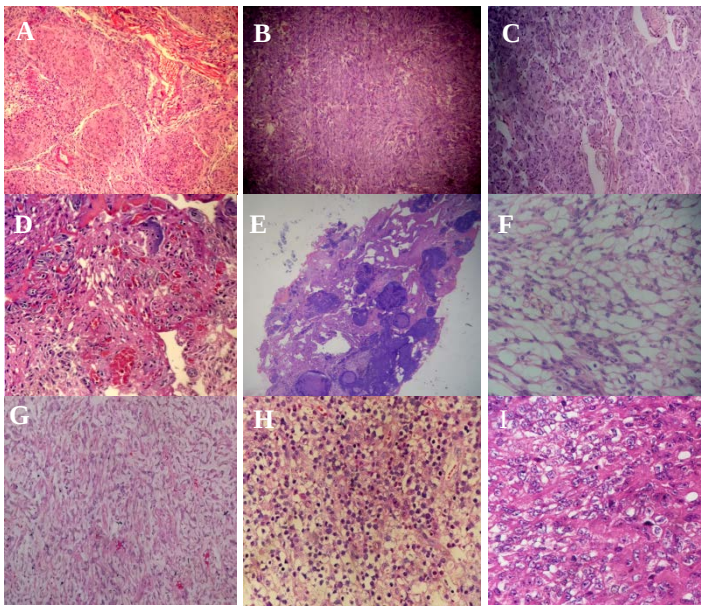
III. RESULT

Patients' characteristics

The mean age for meningioma patients was 43.97 (±11.47) years. The most common in 41-50 years age group. Twenty-three patients (71.9%) were females, only 9 patients (28.1%) were males. All the tumors were located in intracranial where convexity was the predominance. The number of the patients with WHO Grade 1 meningioma was 25 (78.1%), with Grade 2 meningioma was 5 (15.6%), and with Grade 3 meningioma was 2 (6.3%). The histological subtypes of meningioma varied and meningothelial meningioma was the majority of this case. Clinical basic characteristic of meningioma patients were summarized in table 1. Representative H&E sections are shown in figure 1.

Table 1. Characteristic of meningioma patients

Characteristics	Number of cases	Percentage (%)
Age, mean ± SD, years	43.97 ± 11.47	
< 20 years	1	3.1
21-30 years	1	3.1
31-40 years	10	31.3
41-50 years	12	37.5
51-60 years	6	18.8
61-70 years	2	6.3
Sex		
Female	23	71.9
Male	9	28.1
Location		
Supratentorial	28	87.5
Convexity	23	82.1
Parietal	8	34.8
Frontal	4	17.4
Temporal	1	4.3
Temporoparietal	6	26.1
Frontoparietal	3	13
Parietoccipital	1	4.4
Sphenoid	2	7.1
Parasagital	1	3.6
Suprasella, parasella	2	7.1
Infratentorial	4	12.5
Cerebellum	1	25
Foramen Magnum	1	25
CPA	2	50
Subtype		
Meningothelial	11	34.4
Fibroblastic	4	12.5
Transtitional	5	15.6
Psammomatous	2	6.3
Angiomatous	1	3.1
Microcyst	2	6.3
Chordoid	1	3.1
Clear cell	1	3.1
Atypical	3	9.4
Anaplastic	2	6.3
Histological grade		
Grade 1	25	78.1
Grade 2	5	15.6
Grade 3	2	6.3



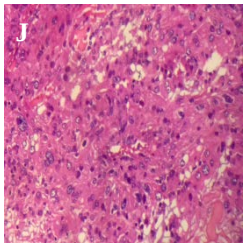


Figure 1. Histological type. A, Meningothelial meningioma. B, Fibroblastic meningioma. C, Transtitional meningioma. D, Angiomatous meningioma. E, Psammomatous meningioma. F, Microcyst meningioma. G, Chordoid meningioma. H, Clear cell meningioma. I, Atypical meningioma. J, Anaplastic meningioma.

FGF-2 expression

Twenty-seven out of 32 (84.4%) meningioma cases were negative while positive expression was seen in five cases (15.6%) (table 2). The intensity of FGF-2 expression in cytoplasm are shown in figure 2.

Table 2. Meningioma cases based on FGF-2 expression

FGF-2 expression	Number of cases	Percentage (%)
Negative	27	84.4
Positive	5	15.6

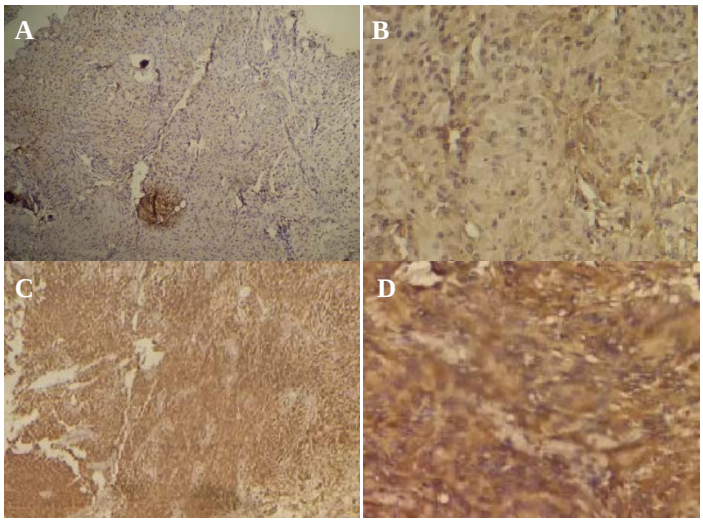


Figure 2. Immunohistochemical FGF-2 expression. A, Negative intensity. B, Mild intensity. C, Moderate intensity. D, Strong intensity.

Association between FGF-2 expression and grade

The number of cases for positive FGF-2 expression was found more in higher grade meningioma (57.1%) while negative expression was found more in grade 1 (96%) compared to higher grade (42.9%). This difference was significant (p=0.004) with prevalence ratio was 14.3 (table 3).

Table 3. Association of FGF-2 expressions with histopathology grade

Grade	Negative		Positive		p	Prevalence ratio (CI=95%)
	n	%	n	%		
Grade 1	24	96	1	4	0.004*	14.3 (1.88-108.19)
Grade 2 and 3	3	42.9	4	57.1		

IV. DISCUSSION

Data in the United States (2002-2006) reported that meningioma occupies 33.8% of all cases of primary brain and central nervous system tumors. The prevalence rate in the United States confirmed by pathology examination of 97.5 per 100,000 populations.[13]

Significant levels of FGF-2 and its receptors, FGFR1 and FGFR2 have been detected in meningioma. FGF becomes mitogen as well as differentiation and angiogenic agents that can affect tumor development as an autocrine growth factor.[14] FGF exerts extensive mitogenic activity by stimulating the growth of fibroblasts, endothelium, and cancer cells. FGF-2 is an important regulator of cell growth and differentiation under physiological and pathological conditions.[15] FGF-2 is present in any part of the body and has a strong affinity for glycosaminoglycans such as heparan sulfate. FGF-2 is stored by binding to glycosaminoglycans on the cell surface or extracellular matrix. When FGF-2 is actually needed, cells secrete various enzymes such as heparinase which release FGF-2 from glycosaminoglycans so that they can reach the target cell.[16] Previous studies have suggested the role of FGF-2 as a prognostic marker for various types of malignancy.[15]

The expression of FGF-2 in the cancer surgery section was evaluated using immunohistochemical techniques, Western blot, and qRT-PCR. The latest diagnostics and antibodies allow proper detection and quantification of FGF-2. Immunohistochemical and immunofluorescence studies have shown that FGF-2 staining is heterogeneous and increases significantly in malignant tissue compared to benign or normal tissue.[15]

This study used an immunohistochemical technique FGF-2 in meningioma cases. Denizot et al. also analyzed FGF-2 levels through tumor tissue samples, but used the quantitative ELISA method. Based on the results obtained, there was no significant difference in FGF-2 levels between meningioma subtypes grade 1.[11] The current study, the authors only tried to classify FGF-2 expressions based on grade and did not evaluate further for each subtype due to no all subtypes can be found as well as the number of cases that are not the same between each of the existing meningioma subtypes.

Baritaki et al. also evaluated FGF-2 from tissue samples through qRT-PCR examination of brain tumors including glioma, meningioma, and metastasis. All cases of meningioma show higher expression than normal brain tissue. However, unfortunately in that study all cases of meningioma were included in the benign meningioma group, so there was no comparison with cases of higher grade meningioma.[9]

Beside immunohistochemical techniques, FGF-2 examination can be done through blood serum. Significant correlation

between serum FGF-2 level and tumor stage, size, and metastasis has been reported in several cases of malignancy.[15]

Granato et al. reported that patients with breast cancer had serum FGF-2 levels that were significantly higher than serum VEGF levels, in comparison with healthy individuals, and they suggested serum FGF-2 levels might serve as a tool for diagnosing breast cancer. While Ueno et al. also measured serum FGF-2 levels in patients with lung cancer types of adenocarcinoma, squamous cell carcinoma, and small cell carcinoma.[16]

Vesely et al. reported that patients with thyroid cancer had significantly higher serum FGF-2 levels ($p < 0.01$) than serum FGF-2 levels in healthy individuals. Landriscina et al. reported that patients with colon cancer had significantly higher serum FGF-2 levels ($p < 0.04$) than serum FGF-2 healthy individuals. However, they also reported that serum FGF-2 levels were not associated with cancer stage.[16] Different things were revealed by Akl et al. through additional studies in the tissue section that high intratumoral FGF-2 levels were associated with advanced bladder tumor, glioma , head and neck, liver, and prostate.[15] Takei et al. compared serum FGF-2 serum levels before and after surgery for breast cancer and found significantly lower postoperative compared with before surgery. Takei et al. also noted the potential presence of FGF-2 secreted by tumor cells.[16]

Hanzhiev et al. also conducted a serum FGF-2 study combined with VEGF by taking samples from brain tumor patients such as meningioma, low grade astrocytoma, and high grade astrocytoma (glioblastoma). The results indicate that FGF-2 levels have much higher levels in the case of glioblastoma, while meningiomas in this study were not grouped into grade 1, 2, or 3 eventhough it was proven that all cases of meningioma detected FGF-2 protein.[17]

While for cases of brain tumors in Indonesia, Risfandy et al. also examined the relationship between serum FGF-2 levels and histopathological grade of meningioma. From the test analysis obtained it was found that there was no significant relationship between the two things.[12] In contrast to the immunohistochemical study in this study found a significant difference between the expression of FGF-2 and grade meningioma. This may be due to the number and distribution of different samples. Risfandy et al. only classified meningioma grades into 1 and 2, while the authors divided grade 1 and combined grades 2 and 3 based on tumor biological behavior. Although it was considered not significantly significant, Risfandy et al. revealed that serum FGF-2 levels were found to have higher levels in higher grade meningiomas. This can show that FGF-2 as a growth factor, plays a role greater in cell proliferation and angiogenesis in higher grade meningiomas. Angiogenesis itself is controlled by the balance of promoters and inhibitors. The Vascular Endothelial Growth Factor (VEGF) and angiopoietin (Ang) are key roles in the balance of angiogenic growth factors. FGF-2 has also received attention as another very potent angiogenic growth factor. Although the detailed mechanism of the relationship between FGF signals and the onset of malignant tumors is still unclear, however, at this time, mutations in the FGF receptor are assumed to activate the FGF signal constantly and ultimately promote tumor cell growth and metastasis.[16]

Although in this study it did not take samples of normal brain tissue for comparison, there was a significant comparison of FGF-2 expression between grade 1 or benign meningioma with grades 2 and 3 as atypical and malignant meningioma. Many literatures have explained that grade 2 and 3 do have a higher level of recurrence and a more aggressive nature. Angiogenesis which will play an important role in this recurrence rate. However, it does not mean that grade 1 meningioma cannot occur in recurrence, only the possibility is smaller than the higher grade.

It is also important to think that in most studies, serum and intratumoral levels of FGF-2 were associated with decreased survival. High intratumoral and serum FGF-2 levels are also associated with recurrence of various types of cancers such as lung, bladder, breast, esophagus and Hodgkin Lymphoma.[15,18] Donnem et al. explained in their study that high expression of FGF-2 in NSCLC cases has 37% for 5 years of survival, while for low FGF-2 expression, the number increased to 59%.[18]

Apart from several contradictory findings, FGF-2 is considered a significant tumor biomarker and potential target therapy. Current and future clinical trials can guarantee to determine whether FGF-2 can be included in the prognosis of cancer and whether FGF target therapy has a beneficial effect in cases of recurrent tumors and cases of malignancy with high mortality.

V. CONCLUSION

There is significant association between FGF-2 expression and histopatholgy grade of meningioma.

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, Universitas Sumatera Utara, Medan, Indonesia approved this study.

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