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Serum GFAP and EGFR as Supportive Diagnostic Biomarker of Glioma Patients: A Single-Center Study

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Abstract

BACKGROUND: High-grade gliomas (HGGs) (World Health Organization Grades III and IV) are aggressive brain tumors with a poor prognosis. Serum concentrations of GFAP and EGFR are theoretically raised in glioma patients, especially primary HGGs.

AIM: This study aims to look at serum levels of GFAP and EGFR in patients with gliomas (low-grade and high-grade glioma) and see if they were related to clinical outcome, MRI parameter, and pathological features.

METHODS: Between 2020 and 2021, pre-operative blood samples were taken from 39 patients with radiologically diagnosed glioma who were performed for tumor excision. The time between blood collection and surgical resection was an average of 10 days. GFAP and EGFR serum were compared in glioma and non-glioma patients.

RESULTS: Glioma patients had average of serum GFAP 747.93 ± 1349.49 pg/ml and average of serum EGFR 9.25 + 3.17 ng/ml. Non-glioma average of GFAP and EGFR, respectively, was 292.91 ± 369.30 pg/ml and 7.81 ± 3.38 ng/ml. From all variables, we performed normality test using the Shapiro–Wilk normality test and all variables were no normally distribution with $p < 0.05$.

CONCLUSION: Circulating GFAP and EGFR are promising method for "supportive" methods for differentiate between glioma and non-glioma patients, especially high-grade glioma.

Introduction

High-grade gliomas (HGGs) (World Health Organization Grades III and IV) are aggressive brain tumors with a poor prognosis. Histologic examination of tumor biopsies is the gold standard for diagnosing HGG. However, it may be limited in its use due to a lack of tissue or intrinsic sampling biases. Moreover, treatment-related alterations might make detecting tumor progression using contrast-enhanced magnetic resonance imaging (MRI) become difficult. A simple blood-based biomarker with diagnostic and prognostic significance might circumvent these restrictions by providing additional information for clinical decision-making [1].

Glial fibrillary acidic protein (GFAP) is a cytoskeleton-associated intermediate filament found mainly in astrocytes. After a stroke or traumatic brain injury, GFAP levels in the blood are known to rise. Serum concentrations of GFAP are likewise raised in primary HGGs before surgical excision, suggesting that serum GFAP is a diagnostically important biomarker. Its prognostic usefulness and connection with recognized prognostic markers such as the IDH1 mutation, however, have not been investigated. Furthermore, the past research has only looked at initial HGGs, with no investigation into the relationship between serum GFAP levels and tumor load in recurrent HGGs [2].

In new or *de novo* glioblastomas, overexpression of the epidermal growth factor receptor (EGFR) is a hallmark, which is commonly linked to EGFR gene amplification. Nearly 40% of initial glioblastomas have EGFR gene amplification, and about half of them have an EGFRvIII mutation, which causes constitutive signaling. As a result, EGFR and EGFRvIII are promising therapeutic targets. Furthermore, EGFR amplification has diagnostic and prognostic significance, with a link to glioblastoma and a worse overall survival rate. EGFR expression and amplification measured

by immunohistochemistry and chromogenic *in situ* hybridization (CISH). Measuring EGFR extracellular domain (ECD) levels in the blood have given researchers more insight into tumor aggressiveness and prognosis in a variety of cancers [3].

Glioblastoma multiforme (GBM) comprises a subset of cancer cells with stem cell properties, such as self-renewal and multipotentiality. The subventricular zone (SVZ) is located between the lateral ventricles and is where neural and cancer stem cells originate. Tumors that come into touch with the SVZ may be more invasive and have a greater ability to recruit migratory progenitor cells. On pre-operative MRI, tumors were categorized as type I, if the contrast-enhancing lesion contacted both the SVZ and cortex, type II, if only the SVZ was involved, type III, if only the cortex was involved, and type IV, if neither the SVZ nor the cortex were contacted. Overall survival and PFS are worse in patients with GBM involving the SVZ, which might have prognostic and therapeutic implications [4].

The goal of this study was to look at serum levels of GFAP and EGFR in patients with glioma (low-grade and high-grade glioma) and see if they were related to clinical outcome, MRI parameter, and pathological features.

Materials and Methods

Between 2020 and 2021, pre-operative blood samples were taken from 39 patients with radiologically diagnosed glioma who were performed for tumor excision. The time between blood collection and surgical resection was an average of 10 days. All procedures performed in studies involving human participants were in accordance with the ethical standards of the Medical Faculty, Universitas Diponegoro and Kariadi Hospital, Ethical Research Committee and with the 1964 Helsinki Declaration standards. This study was approved by the Joint Ethics Committee of the Kariadi General Hospital No. 606/EC/KEPK-RSDK/2020. Written informed consent was obtained from all patients prior the surgery. For patients under the age of 18 years, informed consent was obtained from a parent and/or legal guardian.

Serum GFAP and EGFR levels were measured using enzyme-linked immunosorbent assay (ELISA) kits. Both tests were carried out according to the manufacturer's instructions. The absorbance of GFAP and EGFR was determined by reading the plate at 450 nm and 650 nm. All readings below this detection limit were assigned a value of 0 ng/ml, which was likewise used when the other absorbance measurement of a duplicate fell below the detection limit.

The data are displayed as mean ± standard deviation. The Kruskal–Wallis test and Mann–Whitney U-test with Bonferroni correction, or one-way ANOVA, were used to compare blood protein levels across groups. The Mann–Whitney U-test or independent samples t-test was used to compare blood protein levels according to MRI parameter, pathological features, and clinical outcome. The efficacy of serum GFAP and EGFR levels to distinguish glioblastoma from low-grade glioma was assessed using ROC curve analysis (if satisfied the statistic requirement). ROC curve analysis was used to generate a GFAP cutoff value. SPSS 21 was used for statistical analysis.

Results

From 39 patients radiologically diagnosed glioma, 24 patients with pathologically confirmed with glioma were obtained. From the WHO grading, two patients were Grade I WHO, nine patients were Grade II WHO, five patients were Grade III WHO, and Grade IV WHO were eight patients.

Table 1: Grade WHO of glioma patients

Grade WHO	Frequency	%
1	2	8.33
2	9	37.5
3	5	20.8
4	8	33.3
Total	24	100

Diffuse astrocytoma and glioblastoma multiforme were majorly found in sample, with eight patients each group. Five patients were anaplastic astrocytoma and the rest were oligodendroglioma, subependymal giant cell astrocytoma, and gemistocytic astrocytoma with one patient each group (Tables 1 and 2).

Table 2: Type of glioma pathology

Type of pathology	Frequency	%
Anaplastic astrocytoma	5	20.83
Diffuse astrocytoma	8	33.33
Glioblastoma multiforme	8	33.33
Oligodendroglioma	1	4.16
Subependymal giant cell astrocytoma	1	4.16
Gemistocytic astrocytoma	1	4.16
Total	24	100

Serum GFAP and EGFR were obtained at average 10 days pre-operative. We compare glioma and non-glioma patients of GFAP and EGFR serum value. Glioma patients had average of serum GFAP 747.93 ± 1349.49 pg/ml and average of serum EGFR 9.25 ± 3.17 ng/ml. Non-glioma average of GFAP and EGFR, respectively, was 292.91 ± 369.30 pg/ml and 7.81 ± 3.38 ng/ml (Table 3). From all variables, we performed normality test using Shapiro–Wilk normality

Table 3: Distribution of numeric data

Variable	Non-glioma		Glioma	
	Mean	SD	Mean	SD
GFAP (pg/ml)	292.91	369.30	747.93	1349.49
EGFR (ng/ml)	7.81	3.38	9.25	3.17

test and all variables were no normally distribution with p<0.05.

Mann–Whitney U-test was used to asses difference of GFAP and EGFR serum level each variable. Between glioma and non-glioma patients, GFAP and EGFR serum were found higher in glioma patient with p <0.05 (Table 4). Between high- and low-grade glioma, GFAP and EGFR serum were found higher in high-grade glioma patients with p < 0.05 (Table 5).

Table 4: Difference between glioma versus non-glioma patients

	Glioma_Non-glioma	N	Mean rank	Sum of ranks	p
GFAP	Glioma	24	21.00	516.00	0.00*
	Non_Glioma	15	17.00	264.00	
EGFR	Glioma	24	21.00	520.00	0.00*

From MRI zone parameter, patients with SVZ and cortical involvement tend to be lower GFAP than cortical only involvement. EGFR was found higher in cortical only involvement than cortical ± SVZ with p < 0.05. Because area under curve was <50%, we did not perform ROC analysis. (Table 6).

Table 5: Difference between low-grade and high-grade glioma

	Low_High_Glioma	N	Mean rank	Sum of ranks	p
GFAP	Low grade	10	10.00	105.00	0.00*
	High grade	13	13.00	171.00	
	Total	23			
EGFR	Low grade	10	12.00	124.00	0.00*
	High grade	13	11.00	151.00	
	Total	23			

Discussion

Since GFAP and EGFR serum are considerably greater in glioma patients, especially in high-grade glioma, GFAP and EGFR serum are possible biomarkers for supplemental diagnosis of glioblastoma and to discriminate between high-grade and low-grade glioma in this study. Serum GFAP may thus be useful in the follow-up of individuals with HGG who frequently have MRI results that are inconclusive. Previous investigations have confirmed the diagnostic utility of serum GFAP in distinguishing GBM from lower-grade gliomas. However, a longer-term investigation with a bigger patient population is needed to investigate the efficacy of serum GFAP to detect HGGs at an early stage [2], [5], [6].

The higher serum GFAP levels linked with bulky tumors might thus be explained in part by tumor necrosis. The rise in serum GFAP levels followed by surgical tumor removal is similar to a recent research in which plasma GFAP levels were enhanced 24–48 h after surgery in both low-grade and high-grade gliomas. Furthermore, in our sample, pre-operative blood GFAP levels were greater in glioma patients, particularly in high-grade glioma. These findings suggest that GFAP levels in the blood before surgery are a marker of tumor entity as well as brain damages but not caused by the surgery. As a result, serum GFAP might be a useful tool in the follow-up of HGG patients [2], [5], [6].

Table 6: Difference between MRI zone and GFAP and EGFR serum

	MRI_Zone	N	Mean rank	Sum of ranks	p
GFAP	Cortical ± SVZ	10	9.00	92.00	0.00*
	Cortical	10	11.00	118.00	
	Total	20			
EGFR	Cortical ± SVZ	10	11.00	111.00	0.00*
	Cortical	10	9.00	98.00	
	Total	20			

Both astrocytes and malignant gliomas produce large amounts of GFAP. GBM is usually linked to tumor cell necrosis and disruption of the blood–brain barrier, which explains why GFAP is released into the bloodstream. Previous research in various disorders has shown that GFAP is released from the brain into the bloodstream when there is extensive astroglial loss, such as in the event of an expanding intracerebral hemorrhage or traumatic brain injury. Patients with most other neurological illnesses, such as MS, neurodegenerative entities, and epilepsy, did not have GFAP in their blood. GBM is the only “non hyperacute” condition in which a significant amount of GFAP is released into the bloodstream. This is most likely due to GFAP expression and subsequent release in cases of necrosis and blood–brain barrier failure, as described above [3], [7], [8], [9].

One of the genetic hallmarks of GBM is EGFR gene amplification. Even when the histologic criteria for GBM are not met because of the absence of necrosis and microvascular proliferation in the biopsy, identification of neoplastic astrocytes with EGFR amplification by fluorescent or chromogenic *in situ* hybridization constitutes strong evidence that the tumor is a GBM, or at least should be treated as a GBM, in diagnostic neuropathology practice. In underdeveloped countries, circulating EGFR might be utilized to diagnose a suspected grade of glioma and provide a reliable follow-up strategy for patients [3].

We regard GFAP and EGFR to be a clinically significant indication of GBM, despite the fact that it cannot be employed as a diagnostic “stand-alone” tool due to limits in diagnostic sensitivity caused by tumor features. Future research is needed to find correlation between pre- and post-operative circulating GFAP and EGFR with larger cohort study.

Conclusion

Circulating GFAP and EGFR are promising method for “supportive” methods for differentiate between glioma and non-glioma patients, especially high-grade glioma. Routine radiology examination, clinical assessment, and pathological analysis are mandatory needed to confirm the diagnosis of glioma.

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