Investigation of serum levels and tissue expression of two genes IGFBP-2 and IGFBP-3 act as potential biomarker for predicting the progression and survival in patients with glioblastoma multiforme

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Abstract

Background: Identification of genetic copy number changes in glial tumors is of importance in the context of improved/refined diagnostic, prognostic procedures and therapeutic decision-making. Blood-derived biomarkers, therefore, would be useful as minimally invasive markers that could support diagnosis and enable monitoring of tumour growth and response to treatment.

Objective: The aim of this study was to evaluate the clinical significance of IGFBP-2/3 in glioblastoma multiforme (GBM) and their value as predictors of survival.

Methods: We examined the plasma levels of IGFBP-2 and IGFBP-3 using ELISA in patient suffering from GBM and controls groups. Furthermore, immunohistochemistry method was used to evaluate the expression levels of these markers.

Results: Preoperative plasma levels of IGFBP-2 and IGFBP-3 were markedly higher in glioblastoma patients (mean ± SD: 521.5 ± 164.2 ng/ml; 402.4 ± 126 ng/ml) when compared with healthy controls (301.28 ± 73.12; 244 ± 89.5 ng/ml; p < 0.001). Immunohistochemical results indicated that the median H score for glioblastoma tissues was higher when compared with normal tissues. The mean scores for IGFBP-2 expression in glioblastoma was higher than normal tissues (p < 0.001). Our result showed that the median H score for glioblastoma tissues was higher when compared with normal tissue for IGFBP-3 expression. The mean scores for glioblastoma tissues was higher than normal tissues (p < 0.001). We also evaluated whether plasma IGFBP-2 and IGFBP-3 levels were related to clinical features. The plasma IGFBP-2 level was strongly linked to the patient’s age (R = 0.769, P = 0.001) that were strongly increased in patients with older age (>65), (mean ± SD: 594.36 ± 33.3 ng/ml). On the other hand, plasma IGFBP-3 level was not correlated with age (P = 0.462), sex (P = 0.532), and tumor size (P = 0.245).

Our findings indicated that the tissue IGFBP-2 level was also markedly correlated with the patient’s age (R = 0.612, P = 0.015). On the other hand, tissue IGFBP-3 expression level was not correlated with age (P = 0.472), sex (P = 0.512), and tumor size (P = 0.241). Kaplan-Meier survival and log-rank analysis suggested that patients with high plasma level of IGFBP-2 and tissue expression of IGFBP-2 had shorter overall survival than those with low levels (log-rank test P = 0.027; P = 0.001). Kaplan-Meier survival and log-rank analysis suggested that patients with high plasma level of IGFBP-3 and tissue expression of IGFBP-3 had shorter overall survival than those with low levels groups (log-rank test P = 0.018; P < 0.001).

Conclusion: These data suggest that plasma levels and tissue levels of IGFBP-2 and IGFBP-3 may be as potential biomarkers for predicting the progression and survival in patients with GBM.
1. Introduction

Gliomas are known as the most common primary tumors in brain, [1]. Grade I or II tumors are termed low-grade gliomas and patients with high-grade glioma (WHO grade III) and grade IV glioblastoma multiforme (GBM) have poor survival. It has been reported that that patients suffering from glioblastoma have poor clinical outcome, because of the proliferative and invasive role of GBM [2–6]. Despite multimodal treatment with surgery, radiotherapy, and chemotherapy, these patients cannot be cured [7,8]. The establishment of valuable molecular markers can be beneficial for the prediction of patient prognosis.

The insulin-like growth factor (IGF) signaling pathway has been demonstrated to be involved in the glioma progression [9]. The IGF signaling axis is involved in the complex coordinated actions of IGF-binding proteins (IGFBPs). Among the IGFBPs, the expression level of isoforms-2, 3, and 5 have been investigated in gliomas. Previously, over-expression of IGFBP-2 was reported in GBM, and was found to elevate the invasive potential of GBM cells [10], as well as its expression has been related to poor prognosis of GBM patients [11,12].

Previous studies showed that high expression of IGFBP-2 might be correlated with development and progression of different kinds of cancer [13–16]. Increased serum IGFBP-2 levels have been found in different kinds of malignant diseases such as gastro-colorectal [17], ovary [18], prostatic cancers [19,20]. It has been indicated that there was a strong association between high expression of IGFBP-2 and advanced histological grade, indicating that IGFBP-2 may have important role in progression of glioma [21]. A study indicated that increased plasma levels of IGFBP-2 can be correlated with adverse prognosis in patients with high-grade glioma [22]. A recent study indicated that plasma levels of IGFBP-2 can predict prognosis in elderly glioblastoma patients after combined chemotherapy and radiotherapy [23]. IGFBP-2 was frequently overexpressed in GBM [24,25]. IGFBP-2 can control the cellular functions including cell proliferation, invasion and angiogenesis [10,26–28]. There are emerging information about the some IGFBP isoforms in gliomas, that were indicated the association of three IGFBP isoforms (IGFBP-2, -3, and -5) with the malignant progression of astrocytoma. We aimed clinical significance of IGFBP-2/3 in GBM and their value as predictors of survival in patients.

2. Materials and methods

2.1. Patient

We evaluated 28 patients with GBM, who underwent surgery between January 2008 and December 2013. Moreover, the patients with other acute infection, ischemia, diabetes, were excluded from the study. Surgical operation was conducted by using similar operational techniques. Tumor tissues were confirmed according to the World Health Organization (WHO) Classification by two pathologists. Samples were stored at 80 °C after resection until use. Tumor size was calculated based on preoperative MRI. Normal brain tissue samples (anterior temporal lobe) obtained during surgery for intractable epilepsy were used as control samples. The mean age of the patients was 56.1 years (range 27–84 years). Overall survival of patients was determined between surgery and death of the patient due to disease. The median follow-up of patients was calculated to be 13.25 months (range, 0.8 to 36.2 months). The clinical features were as falso: age, sex, tumor size.

2.2. Plasma samples

The blood samples were collected preoperatively from all diagnosed patients with glioblastoma, in addition to 40 healthy controls. The blood samples were left for 60 min at room temperature and were centrifuged at 3500 rpm for 15 min. The supernatant was aliquoted and then stored at −80 °C until use.

2.3. ELISA method

The Elisa method was used to measure the IGFBP-3 and IGFBP-2 levels in plasma according to the manufacturer's protocol. Examination of each collected sample was repeatedly conducted for three times, and average plasma levels of IGFBP-2 and IGFBP-3 were determined and used for statistical analysis.

2.4. Immunohistochemical analysis

Immunohistochemistry was done using 4-μm formalin-fixed paraffin embedded tissue sections, and dewaxed in xylene, rinsed in graded ethanol, and then rehydration was done using distilled water. After that, incubation of the selected sections was done in 3% hydrogen peroxide to block endogenous peroxidase activity. Slides preparations were performed by steaming in sodium citrate buffer (10 mM sodium citrate, pH 6.0) for 15 min at 100 °C. Then, the samples were washed with phosphate-buffered saline for 3 min, and then immunostaining with mouse anti-human IGFBP-2 and mouse anti-human IGFBP-3 were conducted at 1:400 dilution with standard avidin-biotin peroxidase. The IGFBP-2 and IGFBP-3 expressions were evaluated by using H score.

2.5. Statistical analysis

The differences of plasma levels of IGFBP-2 and IGFBP-3 between the patients with glioblastoma and healthy controls were determined using t-test. The plasma levels of IGFBP-2 and IGFBP-3 compared between glioblastoma tissues and normal tissues by χ² test. The associations of plasma levels of IGFBP-2 and IGFBP-3 with clinicopathological features in patients were determined using one-way ANOVA. Differences were significant at p < 0.05. All analyzes were evaluated using the SPSS 16.0 (Chicago, IL, USA). Survival evaluation was performed using the log-rank test and Kaplan-Meier method.

3. Results

As shown in Figs. 1 and 2, our result showed that preoperative plasma levels of IGFBP-2 and IGFBP-3 were significantly higher in patients with glioblastoma (mean ± SD: 521.5 ± 164.2 ng/ml; 402.4 ± 126 ng/ml) when compared with healthy controls (301.28 ± 73.12; 244 ± 89.5 ng/ml; p < 0.001). Seventy-three percent of GBM patients had higher levels of IGFBP-2 than the mean level of healthy controls, while 65% of patients had IGFBP-3 levels above the mean level of healthy controls.

![Fig. 1. The mean plasma level of IGFBP-2 in glioblastoma patients and control group.](image-url)
3.1. Immunohistochemical findings

3.1.1. IGFBP-2

We found that the median H score for glioblastoma tissues was 90 when compared with normal tissues (33). The mean scores for IGFBP-2 expression in glioblastoma was higher than normal tissues (Patients: 91.74; Normal: 35.83; \( p < 0.001 \)). We categorized the patients with glioblastoma into low and high expression groups of IGFBP-2 expression based on the H scores (Low expression: \( H \leq 100 \); High expression: \( H > 100 \)). 21 cases of patients were assigned to the high-expression group, the remaining 7 cases with low expression.

3.1.2. IGFBP-3

Our result showed that the median H score for IGFBP-3 expression in glioblastoma tissues was higher (72) when compared with normal tissues (29). The mean scores for glioblastoma tissues was higher than normal tissues (80.23; 30.19; \( p < 0.001 \)). Based on H scores, 18 cases of patients were assigned to the high-expression group of IGFBP-3 expression, the remaining 10 cases with low-expression.

3.2. Clinical correlation of plasma IGFBP-2 and IGFBP-3 levels

We also evaluated whether plasma IGFBP-2 and IGFBP-3 levels were associated with clinical features. The plasma IGFBP-2 level was significantly correlated with the patient’s age (\( R = 0.769, P = 0.001 \)) that were strongly higher in patients older than 60 (mean ± SD: 594.36 ± 33.3 ng/ml). Plasma IGFBP-2 level was not significantly correlated with sex (\( P = 0.17 \)) and tumor size. On the other hand, plasma IGFBP-3 level was not correlated with age (\( P = 0.462 \)), sex (\( P = 0.532 \)), and tumor size (\( P = 0.245 \)).

3.2.1. Clinical correlation of tissue IGFBP-2 and IGFBP-3 expressions

Our findings indicated that the tissue IGFBP-2 level was also markedly correlated with the patient’s age (\( R = 0.612, P = 0.015 \)), but no significant correlation was determined with other clinical features including sex (\( p = 0.641 \)) and tumor size (\( p = 0.019 \)). On the other hand, tissue IGFBP-3 expression level was not correlated with age (\( P = 0.472 \)), sex (\( P = 0.512 \)), and tumor size (\( P = 0.241 \)).

Kaplan-Meier survival and log-rank analysis suggested that patients with high plasma level of IGFBP-2 and tissue expression of IGFBP-2 had shorter overall survival than those with low levels (log-rank test \( P = 0.027; P < 0.001 \), Figs. 3 and 5). Kaplan-Meier survival and log-rank analysis suggested that patients with high plasma level of IGFBP-3 and tissue expression of IGFBP-3 had shorter overall survival than those with low levels groups (log-rank test \( P = 0.018; P < 0.001 \), Figs. 4 and 6).

4. Discussion

This family of IGFBPs has six members that act as regulator of the IGFs functions including, IGFBP-1 to IGFBP-6. IGFBP-2 is as a modulator of the mitogenic roles of IGFs, indicating that IGFBP-2 act as regulator of tumor growth and invasion [29]. Decreased expression of IGFBP-2 has been determined in GBM, and its role in prompting the invasive potential of GBM cells has been defined [10–12]. Moreover, increased expression of IGFBP-2 may be linked to the developing and progression of different kinds of cancer [13–16]. On the other hand, elevated serum levels of IGFBP-2 have been detected in different kinds of malignant diseases such as gastro-colorectal, ovarian, prostatic cancers [17–20].

In the present study, we determined that preoperative plasma levels of IGFBP-2 was markedly higher in patients with glioblastoma in comparison with healthy controls, which is consistent with previous reports of IGFBP-2 expression in glioblastoma. Seventy-three percent of glioblastoma patients had IGFBP-2 levels above the mean level of healthy controls levels, while 67% of patients had IGFBP-3 levels above the mean level of healthy controls levels. The mean scores for IGFBP-2
expression glioblastoma were higher than normal based on immunohistochemical method. Our result suggested that the plasma IGFBP-2 level was significantly correlated with the patient’s age that were strongly higher in patients older than 60.

It has been previously reported that there was a strong association between high expression of IGFBP-2 and histologic grade, indicating that IGFBP-2 may have important role in progression of glioma [21]. A study indicated that high plasma levels of IGFBP-2 can be correlated with high prognosis in patients with high-grade glioma [22], and preoperative plasma IGFBP-2 level was found to be correlated with GBM patient prognosis. It has been indicated that preoperative plasma IGFBP-2 levels are linked to recurrence and disease-free survival in patients with GBM [22]. IGFBP-2 levels have been detected to be higher in GBM than in grade III glioma tissues [12,24,25]. IGFBP-2 likely has its oncogenic role through inhibition of the cellular functions including cell proliferation, invasion and angiogenesis [10,26,27]. Moreover, differential IGFBP-2 and -5 expression levels in astrocytoma tissues has been previously detected [30]. Kaplan-Meier survival and log-rank analysis suggested that patients with high plasma level of IGFBP-2 and tissue expression of IGFBP-2 had shorter overall survival than those with low expression.

It has been characterized that IGFBP-2 has prognostic value [11,22]. Moreover, it was indicated that IGFBP-2 is as a prognostic biomarker in high-grade astrocytic neoplasms [12]. It has been showed that the plasma level of IGFBP-2 is linked to progression-free survival and recurrence in gliomas patients with high-grade [22]. Furthermore, a previous study indicated that IGFBP-2 is an important marker for poor prognosis prediction in patients suffering from glioblastoma [31]. Moreover, we found that preoperative plasma level IGFBP-3 was significantly higher in patients with glioblastoma when compared with healthy controls, and 67% of patients had IGFBP-3 levels above the mean level of healthy controls levels. Furthermore, the mean scores for IGFBP-3 expressions in glioblastoma tissues were higher than normal tissues. We found that plasma IGFBP-3 level was not linked to age, sex, and tumor size. On the other hand, tissue IGFBP-3 expression level was not related to age, sex, and tumor size. There are few studies that focused on the role IGFBP-3 and 5 in gliomas [10,32]. Santosh et al. [31] reported that IGFBP-3 were markedly increased in GBM as compared to diffuse astrocytoma, anaplastic astrocytoma, and controls group. They determined that increased expression of IGFBP-3 protein is markedly related to shorter survival time in human glioblastomas. It has been detected that IGFBP-3 overexpression be correlated with poor prognosis breast cancer patients [33]. Kaplan-Meier survival and log-rank analysis suggested that patients with high plasma level of IGFBP-3 and tissue expression of IGFBP-3 had shorter overall survival than those with low expression. In vitro studies have demonstrated that the mitogenicity of IGFBP-3 was mediated in breast epithelial cells via its interactions with RAS-p44/42 mitogen-activated protein kinase signaling and EGFR [34]. Further investigations are required to evaluate the clinical significance of IGFBP-3 and its molecular mechanisms in GBM.

5. Conclusions

In conclusion, the result of this study showed that plasma levels and tissue levels of IGFBP-2 and IGFBP-3 may be as potential biomarker for predicting the progression and survival in patients with GBM.

Competing interests

The authors declare that they have no competing interests.

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