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Original Article

Assessment of the Diagnostic Value of GFAP, IGFBP-2, and YKL-40 in Patients with Glioblastoma

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ABSTRACT

Background and Aims: An effective marker search in glioblastoma is precious in controlling and detecting the progression and monitoring of patients with glioblastoma. In this regard, the present study aimed to evaluate the diagnostic and prognostic role of glial fibrillary acidic protein (GFAP), insulin-like growth factor-binding protein -2 (IGFBP-2), and chitinase-3-like protein -1 (YKL-40) tissue and plasma levels in patients with glioblastoma.

Materials and Methods: A total of 22 patients with newly diagnosed glioblastoma (the fourth grade of glioma) who had undergone surgery at the Erfan Hospital were included in the current study. The levels of GFAP, IGFBP-2 were evaluated in 22 tumor tissues, and non-timorous matched adjacent tissue samples of patients with glioblastoma using the enzyme-linked immunosorbent assay . Besides, 22 healthy subjects with no history of glioblastoma served as controls for plasma samples. All analyses were evaluated using the SPSS version 22.0.

Results: The tissue levels of GFAP, IGFBP-2, and YKL-40 were significantly higher in patients with glioblastoma when compared to the healthy controls (p=0.001). Nevertheless, there was no significant difference in comparison to the healthy control group in the plasma samples.

Conclusions: Tissue levels of GFAP, IGFBP-2, and YKL-40 may be potential biomarkers for predicting and the progression in patients with glioblastoma.

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Introduction

Glioma is the most prevalent and offensive type of intracranial brain tumor. It is the major hazardous brain tumor type in adults [1, 2]. For each type of glioma, some neoplasms span a broad spectrum of biological aggressiveness [3]. It can be estimated without treatment that the average life expectancy for patients is only 4–5 months [4]. Hence, the development of suitable strategies and recognition of novel pathways remain in urgent demand for the early diagnosis and treatment of glioma [5]. Based on the World Health Organization (WHO) classification, four grades are considered for gliomas: Grade I and Grade II are known as low-grade, and Grade III and IV are considered high-grade glioma [6]. Glioblastoma multiform (GBM) is the fourth grade of glioma and one of the most invasive brain tumors [7]. Malignant glioma is one of the most common causes of brain tumors and is considered one of the most dangerous cases in the nervous system indicates a relative scare but drastic health burden [8, 9]. On the other hand, although decades of rigorous research is available, the intricate biology of GBM is not entirely understood [10]. This malignant cancer is accompanied by early metastasis and has angiogenesis [11]. Invasive growth tumor, sometimes with no apparent bound areas with normal tissue, causes the removal of the tumor during surgery and results in annual mortality rates [12, 13]. Although recently, researchers tend to discover the genomic pathway of many oncogenic and repressive tumor pathways, proteomics studies indicate the magnitude of proteins in the tumor. Quantitative proteomics, as a useful biologic implement, allows the re-identification of the exact amount of proteins in biological samples [14]. In recent years, according to data from the expression of genes and proteins, researchers have investigated several proteins involved in the development of this pernicious cancer, which has been studied by tumor cells as distinct of clinical substitutes in malignant glioma [15]. Thus, the probable role of markers involved in the development and main cause of the spread of the tumor were examined in this study. One of the markers examined is a glial fibrillary acidic protein (GFAP), which is the main protein organized and almost individually in glial and astrocytes, member of medium-sized intermediate filament type III proteins and exist with the other type III intermediate proteins and some type IV-intermediate filaments in the cytoplasm [16]. It plays a fundamental role in maintaining the shape and motility of astrocytic processes and contributes to white matter architecture myelination.

Their structure is dynamic, active, and integral to muscle cells, the brain, and mesenchyme [16-18]. Another marker is Chitinase 3-like 1 (YKL-40), a secreted glycoprotein with 40 KDa weight, located in an extracellular matrix, has multifunctional activity in aggressive cancers, and is considered as a chitinase-like protein. However, YKL-40 does not have catalytic activity compared to chitinases, which is due to the replacement of the amino acid in the binding site to the active site of the chitinases [19, 20].

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The last factor is the insulin growth factor binding protein-2 (IGFBP-2). Among all the IGFBPs, IGFBP-2 has been involved in regulating insulin-like growth factor (IGF) activity in the nervous system, peripheral tissue, and organs [21]. The IGFBP-2 roles in regulating insulin activity are one of the growth factors of IGFs. Also, IGFBs inherently have independent features of IGFs [22]. Early studies demonstrated that IGFBP2 mRNA levels were maintained high during embryonic brain development but decreased in the adult brains, also directly or indirectly promotes transcriptional activation of a specific gene [23, 24]. Researchers have focused on several biomarkers based on information from the expression of genes and proteins during the disease [25]. In this regard, determination of serum survivin, hyaluronic acid, and laminin was used as potential tumor markers [26, 27]. The crosstalk between trace elements with DNA damage response, repair, and oxidative stress were reported in cancer [28].

Furthermore, some of the effective factors in disease pathogenesis and the interplay of klotho with signaling pathways in cancers were reported [29, 30]. Regulatory functions of circular RNAs and targeting the mammalian target of rapamycin signaling were reported in cancer [31, 32]. These markers are creating and spreading the tumor with a special role, which is probably due to the use of tumors from them. Besides evaluating these markers, they will clarify the biological status of the brain tumor and help detect the progression of this cancer. Given the devastating nature of GBM, biomarkers are required to assist in diagnosing, prognosis, and predicting treatment outcomes. This study aims to highlight the identity role of tissue and circulate GFAP, IGFBP-2, and YKL-40 for GBM as a combined profile with diagnostic and prognostic value.

Materials and Methods

Study population

A total of 22 patients with newly diagnosed GBM who had undergone surgery at the Erfan Hospital were included in the current study. Tumor size was calculated on preoperative magnetic resonance imaging (MRI). All tumors had been examined histo-pathologically and classified according to the WHO classification. In this study, the levels of GFAP, YKL-40, IGFBP-2 were evaluated in 22 tumor tissues, and non-timorous matched adjacent tissue samples of patients with GBM using the enzyme-linked immunosorbent assay (ELISA). Also, 22 healthy subjects with no history of GBM served as controls for plasma samples.

The inclusion criteria were patients who have been diagnosed with GBM. The exclusion criteria were patients who had a history of using anti-cancer drugs and patients with other malignancy. The patient's written informed consent was obtained before collecting samples from all patients and healthy controls. The study has been approved by the Ethics Committee of Babol University of Medical Sciences (MUBABOL.HRI.REC.1396.197).

Tissue and plasma sample collection

For patients with GBM, blood samples had been taken immediately before surgery. Samples from volunteered subjects were collected in the absence of other illnesses.

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Blood was drawn into tubes with the ethylenediaminetetraacetic acid anticoagulant. The plasma was collected, and an analysis of protein levels by ELISA was done. For tissue samples, 2 ml of phosphate buffered saline (pH = 7.4) were added, and the samples were homogenized thoroughly by a homogenizer. Samples were then centrifuged at 3000 ×g for 20 min. collected the supernatants carefully.

Plasma and tissue GFAP, IGFBP-2, and YKL-40 measurements

Tumor and plasma samples were analyzed for GFAP, IGFBP-2, and YKL-40 using commercially available ELISA kits (Bioassay, China). All samples were tested in duplicate according to the manufacturer's instructions. Plasma samples were diluted 1:10, and tissue samples were diluted 1:100. All standards and test specimens were run in with the volume of 40 µl pipette into each ELISA well. A biotin-labeled anti-GFAP-antibody, anti-YKL-40, and anti-IGFBP-2, specifically recognizing them, was employed as detector antibodies. A reading plate measured the absorbance at 450 nm. Total protein concentrations were determined as absorbance using a spectrophotometer at 450 nanometers (nm). The whole process of this study is demonstrated in Figure 1.

Statistical analysis

All analyses were evaluated using the SPSS version 22.0. The data were reported as the mean ± SD. Students' t-test results were used for all comparisons. The p-value < 0.05 was considered statistically significant. Also, the ROC curve was used to check the diagnostic value of GFAP, YKL-40, and IGFBP-2.

Results

The result showed that the mean levels of GFAP, YKL-40, and IGFBP-2 in tissue samples of timorous of the patients with GBM were higher (0.61 ± 0.09,1.84 ± 0.73 and 48.01 ± 13.8, µg/ml respectively) when compared with non-tumorous brain tissue (0.30 ± 0.08, 0.86 ± 0.35, and 29.47 ± 13.80, µg/ml respectively). Nevertheless, the results showed that the mean levels of GFAP, YKL-40, and IGFBP-2 in plasma samples of the patients with GBM were lower (0.35 ± 0.32, 1.18 ± 2.08, and 2.30 ± 1.47, µg/ml respectively) when compared to healthy control (0.34 ± 0.31, 2.70 ± 6.25, and 23.24 ± 20.58, µg/ml respectively). Characteristics of the study population were demonstrated in Table 1. Clinical-pathological characteristics of the patient group were shown in Table 2. Levels of GFAP, IGFBP-2, and YKL-40 in tissue samples of tumorous and non-tumorous brain tissue of the patients with GBM were also indicated. As indicated in Table 3, levels of GFAP, IGFBP-2, and YKL-40 in tissue samples of tumors were markedly higher when compared with non-tumorous brain tissue of the patients with GBM (p=0.001).

However, the plasma levels of GFAP, IGFBP-2, and YKL-40 were not significantly different between the two groups (Table 4). Table 5 demonstrated the correlation between tissue levels of GFAP, IGFBP-2, and YKL-40. The diagnostic value of GFAP, IGFBP-2, and YKL-40 for differentiation of patients with GMB in tissue samples was also shown (Table 6). The diagnostic value of GFAP, IGFBP-2, and YKL-40 for the differentiation of patients with GBM

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in plasma samples was shown in Table 7. ROC curve for differentiating patients with GBM from healthy subjects in a tissue sample (Fig. 2). ROC curve for determining patients with GBM from healthy subjects in a tissue sample. ROC curve for determining patients with GBM from healthy subjects in a plasma sample (Fig. 3). Furthermore, ROC analysis in calculating the diagnostic accuracy for an optimized specificity obtained the cut-off point of 0.47, 1.24, and 37.44 µg/mg for GFAP, YKL-40, and IGFBp-2, respectively.

Table 1. Demographic characteristics of the Study Population

Variables	Healthy control group	Patient group
Male	14	14
Female	8	8
Age (year)	48.55±12.33	50.64±14.32

Table 2. Pathological characteristics of the patient group

Characteristics/ Involved side	Right	Left
Forehead	66.7%	33.3%
Parietal	33.3%	66.7%
Temporal	0	100%
Occipital	50%	50%

Table 3. Levels of GFAP, YKL-40 andIGFBP-2in tissue samples of tumorous and non-tumorous brain tissue of the patients with GBM

Variables(µg/ml)	Non-tumorous	Tumor tissue	p-value
GFAP	0.30±0.08	0.61 ± 0.09	0.001
YKL-40	0.86±0.35	1.84±0.73	0.001
IGFBP-2	29.47± 13.80	48.01±13.8	0.001

Data are presented at mean±SD

Table 4. Plasma levels of GFAP, IGFBP-2 and YKL-40 in patients with GBM and healthy controls

Variables (µg/ml)	Healthy Control	Patients with GBM	p-value
GFAP	0.34±0.31	0.35 ± 0.32	0.89
YKL-40	2.70±6.25	1.18±2.08	0.29
IGFBP-2	23.24± 20.58	2.30±1.47	0.55

Data are presented at mean±SD

Table 5. The correlation between YKL-40,GFAP,IGFBP-2in patients with GBM Parameters Pearson correlation coefficient(r) p-value

Variables	YKL-40	IGFBP-2	GFAP
Pearson correlation coefficient	-0.21	0.22	-0.17
Statistical significant	0.92	0.33	0. 94

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Table 6. Diagnostic value of IGFBP-2, GFAP, YKL-40 for differentiation of patients with GBM in tissue samples

Variables	Confidence intervals	Negative predictive value	Positive predictive value	Specify	Sensitivity	AUC
GFAP	(0.94-1.00)	0.91	0.95	0.95	0.90	0.97
YKL-40	(0.83-0.99)	0.82	0.82	0.81	0.81	0.91
IGFBP-2	(0.73-0.96)	0.82	0.86	0.85	0.81	0.85

Table 7. Diagnostic value of IGFBP-2, GFAP, YKL-40 for differentiation of patients with GBM in plasma samples

Variables	Confidence intervals	Negative predictive value	Positive predictive value	Specify	Sensitivity	AUC
GFAP	(0.32-0.68)	0.44	0.49	0.18	0.77	0.5
YKL-40	(0.19-0.53)	0.45	0.48	0.23	0.73	0.36
IGFBP-2	(0.44-0.79)	0.64	0.51	0.57	0.73	0.62

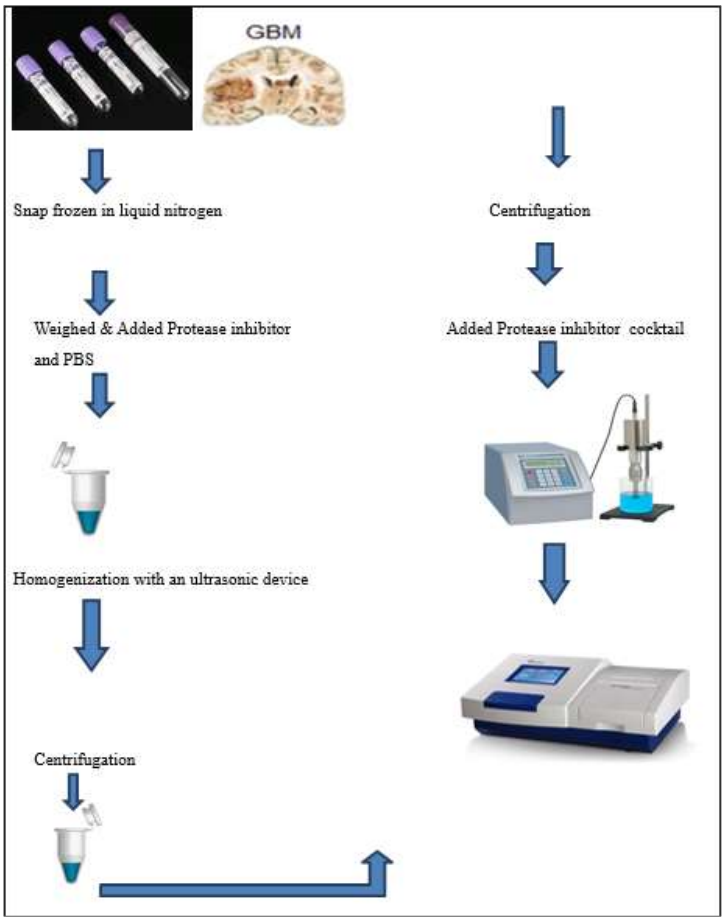

Fig. 1. The whole process in this stydy

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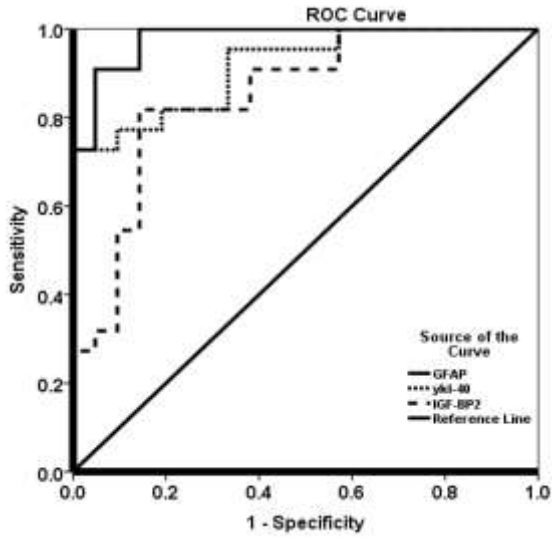

Fig. 2. ROC curve for differentiating patient with GBM from healthy subjects in a tissue sample

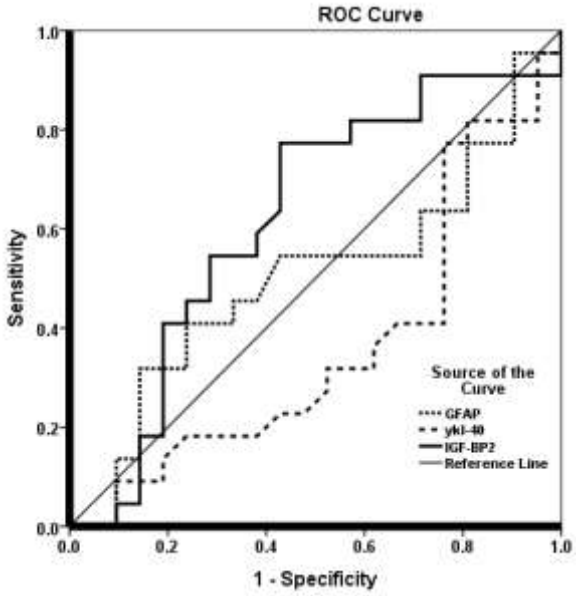

Fig. 3. ROC curve for differentiating patient with GBM from healthy subjects in plasma sample

Discussion

The gold standard method for GBM detection is neuro-imaging, but there are limitations to use this method, such as its high cost. Therefore, having the potential of markers for early detection is still the primary goal. This information stimulates us to find markers responsive to the earlier stages of cancer without any difficulty in detecting sera. In this regard, to overcome these shortcomings, circulating biochemical markers might be associated with GBM and could be evaluated as potential diagnostic biomarkers in patients with GBM since most current biomarkers cannot reach a certain level of sensitivities or specificities. Therefore, in the current study, GFAP, IGFBP-2, and YKL-40, which can be

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sampled non-invasively and cost-effectively, were evaluated as ideal biomarkers for patients with GBM.

The most important finding of the current study was a difference in GFAP level between tissue samples of tumorous and non-tumorous brain tissue of the patients with GBM. As expected, due to necrosis and the spread of brain cancer, the level of GFAP increased in patients, which is consistent with the results reported by researchers. It reported a significantly higher rate of this factor in brain tissue samples of patients with GBM [22]. Furthermore, ROC curve analysis confirmed that tissue GFAP acts as discriminatory to differentiate patients with GBM from non-tumorous brain tissue. According to the results of this study, the analysis of the ROC curve, the sensitivity, and specify of this biomarker was determined as 90% and 95% , respectively.

Contrary to our research, some researchers study on the brain tissue sample reported that the expression of this factor in the gradient IV glioma is reduced [33]. The results of this study showed that GFAP in plasma was unrelated to the study of researchers who reported GFAP as markers below 0.05 µg/L with 76% sensitivity and 100% specificity. It should be noted that in this study, the sample size of patients with GBM cancer was 50 [34]. One of the reasons for this research's difference from other research is its small sample size and the different behavior of this marker in plasma and the tissue. Other researchers compared GFAP serum levels between various gliomas and non-glioma cases [35]. Also, the relationship between tumor size and GFAP was not found in this study, which was consistent with other studies [36].

Another finding of the present study was the existence of a difference in the YKL-40 between tissue samples of timorous and non-tumorous brain tissue of the patients with GBM. Researchers expected it to increase, which is in line with previous studies.

Other studies have shown that YKL40 -40 is an influential factor in angiogenesis [37, 38]. The ROC curve was analyzed, and the sensitivity and specificity of this biomarker were estimated at 81%, 81%, and the area under the curve was 0.916. Therefore, the YKL40 is suggested as an appropriate marker for tissue samples in patients with GBM. Nevertheless, there was no significant difference between the plasma YKL-40 levels in the patients with GBM compared to the healthy control group. This research suggested increased plasma YKL-40 values in patients with GBM patients [15]. Various effective factors in this area can be mentioned for these dereferences, including sample size, racial group, clinical and individual characteristics, and analysis methods or the technical platform.

The present study's finding demonstrated a difference in the IGFBP-2 level between tissue samples of tumorous and non-tumorous brain tissue of the patients with GBM. Our results were consistent with the study by other researchers, which showed an increase of IGFBP-2 in a fourth grade glioma tissue specimen compared to healthy control [39]. Besides, by analyzing the ROC curve, the sensitivity and specificity of this biomarker in our study were 81% and 85%, respectively, and

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area under the curve was 0.85. Our results demonstrated that this marker is an appropriate marker for tissue samples in patients with GBM. In the present study, any significant correlation between tumor volume and IGFBP-2 level was not observed. A study conducted by other researchers showed a significant correlation between serum IGFBP and tumor size [22]. Some researchers reported a correlation between IGFBP-2 and tumor volume and GBM development [40]. Also, in the present study, there was no significant correlation between plasma IGFBP-2 between two groups of patients with GBM and healthy control subjects, which contrasted with the study by other researchers, who had found a significant increase in IGFBP-2 factor in patients with GBM [39]. These differences can be explained by different factors, such as sample size, racial group, clinical and individual characteristics, and analysis methods or the technical platform. More importantly, a combined three-marker panel consisting of GFAP, IGFBP-2, and YKL-40 had a higher discriminatory power. Therefore, multiple panel-based markers could overcome shortcomings and provide better judgment. Further studies on larger groups of patients are required to confirm the results obtained.

Conclusion

In the current study, the tumor levels of all three tested proteins were significantly higher in patients with GBM compared with healthy controls, and all three tested proteins allowed for the differentiation of patients with GBM from the healthy controls. These results suggest that a combined profile of GFAP, IGFBP-2, and YKL-40 tissue levels might constitute a diagnostic approach to patients with GBM. Nevertheless, no difference has been found between the plasma levels of all three tested proteins in patients with GBM compared with healthy controls. Future studies should investigate whether plasma gap, IGFBP-2, and YKL-40 could also be used to detect the GBM. We hope that our data will provide some insights into the detection of GBM.

Conflict of Interest

There are no competing interests to be disclosed.

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References

[1]. Goradel NH, Asghari MH, Moloudizargari M, Negahdari B, Haghi-Aminjan H, Abdollahi M. Melatonin as an angiogenesis inhibitor to combat cancer: Mechanistic evidence. Toxicology and App Pharmacol. 2017; 335: 56-63.

[2]. Kaluzova M, Bouras A, MachaidzeR, Hadjipanayis CG. Targeted therapy of glioblastoma stem-like cells and tumor non-stem cells using cetuximab-conjugated iron-oxide nanoparticles. Oncotarget 2015; 6(11): 8788-806.

[3]. Scott JG, Suh JH, Elson P, Barnett GH, Vogelbaum MA, Peereboom DM, et al . Aggressive treatment is appropriate for glioblastoma multiforme patients 70 years old or older: a retrospective review of 206 cases. Neuro-oncology 2011; 13(4): 428-36.

[4]. Farina P, Lombardi G, Bergo E, Roma A, Zagonel V. Treatment of malignant gliomas in

[DOI: 10.18502/ijml.v8i2.6274]

elderly patients: a concise overview of the literature. BioMed Res Int. 2014; 2014: 734281.
[5]. Lin TK, Chang CN, Tsai CS, Huang YC, Lu YJ, Chen WJ, et al. The long non-coding RNA LOC441204 enhances cell growth in human glioma. Scientific Rep. 2017; 7(1): 5603.
[6]. Bleeker FE, Molenaar RJ, Leenstra S. Recent advances in the molecular understanding of glioblastoma. Journal of Neuro-oncology 2012; 108(1): 11-27.
[7]. Najafi M, Soltanian-Zadeh H, Jafari Khouzani K, Scarpace L, Mikkelsen T. Prediction of glioblastoma multiform response to bevaci-zumab treatment using multi-parametric MRI. PloS One. 2012; 7(1): 29945.
[8]. Tano K, Akimitsu N. Long non-coding RNAs incancer progression. Frontiers in genetics 2012; 3(3): 219.
[9]. Ho VK, Reijneveld JC, Enting RH, Bienfait HP, Robe P, Baumert BG, et al. Changing incidence and improved survival of gliomas. European Journal of Cancer 2014; 50(13): 2309-318.
[10]. Noroxe DS, Poulsen HS, Lassen U. Hallmarks of glioblastoma: a systematic review. ESMO open. 2016; 1(6): 144.
[11]. Zhang W, Bi Y, Li J, Peng F, Li H, Li C, et al. Long noncoding RNA FTX is upregulated in gliomas and promotes proliferation and invasion of glioma cells by negatively regulating miR-342-3p. Laboratory Investigation 2017; 97(4): 447-57.
[12]. Ding H, Shen J, Yang Y, Che Y. Saw palmetto extract inhibits metastasis and antiangiogenesis through STAT3 signal pathway in glioma cell. Evid Based Complement Alternat Med. 2015; 2015: 926946.
[13]. Mattick JS, Makunin IV. Non-coding RNA. Human molecular genetics. 2006; 15(1): 17-29.
[14]. Bi B, Li F, Guo J, Li C, Jing R, Lv X ,et al. Label-free quantitative proteomics unravels the importance of RNA processing in glioma malignancy. Neuroscience 2017; 351: 84-95.
[15]. Gallego Perez-Larraya J, Paris S, Idbaih A, Dehais C, Laigle-Donadey F, Navarro S, et al. Diagnostic and prognosticvalue of preoperative combined GFAP, IGFBP-2, and YKL-40 plasma levels in patients with glioblastoma. Cancer 2014; 120(24): 3972-980.
[16]. Hol EM, Capetanaki Y. Type III intermediate filaments desmin, glial fibrillary acidic protein (GFAP), vimentin, and peripherin. Cold Spring Harbor Perspec Biol. 2017; 9(1): 12-22.
[17]. Kiviniemi A, Gardberg M, Frantzen J, Parkkola R, Vuorinen V, Pesola M, et al. Serum levels of GFAP and EGFR in primary and recurrent high-grade gliomas: correlation to tumor volume, molecular markers, and progression-free survival. Journal of Neuro-oncology 2015; 124(2): 237-45.
[18]. Mayer CA, Brunkhorst R, Niessner M, Pfeilschifter W, Steinmetz H, Foerch C. Blood levels of glial fibrillary acidic protein (GFAP) in patients with neurological diseases. PloS one 2013; 8(4): 62101.
[19]. Qin G, Li X, Chen Z, Liao G, Su Y, Chen Y, et al. Prognostic Value of YKL-40 in Patients with glioblastoma: a systematic review and meta-analysis. Mol Neurobiol. 2017; 54(5): 3264-370.
[20]. Ku BM, Lee YK ,Ryu J, Jeong JY, Choi J, Eun KM, et al. CHI3L1 (YKL-40) is expressed in human gliomas and regulates the invasion, growth and survival of glioma cells. International Journal of Cancer 2011; 128(6): 1316-326.
[21]. Russo VC, Azar WJ, Yau SW, Sabin MA, Werther GA. IGFBP-2: The dark horse in metabolism and cancer. Cytokine & Growth Factor Reviews 2015; 26(3): 329-46.
[22]. Abdolhoseinpour H, Mehrabi F, Shahraki K, Khoshnood RJ, Masoumi B, Yahaghi E, et al. Investigation of serum levels and tissue expression of twogenes IGFBP-2 and IGFBP-3 act as potential biomarker for predicting the progression and survival in patients with glioblastoma multiforme. Journal of the Neurological Sciences 2016; 366: 202-206.
[23]. Shen F, Song C, Liu Y, Zhang J, Wei Song S. IGFBP2 promotes neural stem cell maintenance and proliferation differentially associated with glioblastoma subtypes. Brain Research 2019; 1704: 174-86.
[24]. Yau SW, Azar WJ, Sabin MA, Werther GA, Russo VC. IGFBP-2 - taking the lead in growth, metabolism and cancer. Journal of Cell Communication and Signaling 2015; 9(2): 125-42.
[25]. Chiorean R, Berindan-Neagoe I, Braicu C, Florian IS, Leucuta D, Crisan D, et al. Quantitative expression of serum biomarkers involved in angiogenesis and inflammation, in patients with glioblastoma multiforme: correlations with clinical data. Cancer biomarkers 2014; 14(2-3): 185-94.
[26]. Wilhelmsson U, Eliasson C, Bjerkvig R, Pekny M. Loss of GFAP expression in high-grade astrocytomas does not contribute to tumor development or progression. Oncogene 2003; 22(22): 3407-411.
[27]. Jung CS, Foerch C, Schanzer A, Heck A, Plate KH, Seifert V, et al. Serum GFAP is a diagnostic marker for glioblastoma multiforme. Brain: a journal of neurology. 2007;130(Pt 12):3336-341.
[28].Wei P, Zhang W, Yang LS, Zhang HS, Xu XE, Jiang YH, et al. Serum GFAP autoantibody as an ELISA-detectable glioma marker. Tumour biol. 2013; 34(4): 2283-292.
[29]. Baumgarten P, Quick-Weller J, Gessler F, Wagner M, Tichy J, Forster MT, et al. Pre- and early postoperative GFAP serum levels in

[DOI: 10.18502/ijml.v8i2.6274]

glioma and brain metastases. Journal of neuro-oncology 2018; 139(3): 541-46.
[30]. Francescone RA, Scully S, Faibish M, Taylor SL, Oh D, Moral L, et al. Role of YKL-40 in the angiogenesis, radioresistance, and progression of glioblastoma. The Journal of biological chemistry 2011; 286(17): 15332-5343.
[31]. Tanwar MK, Gilbert MR, Holland EC. Gene expression microarray analysis reveals YKL-40 to be apotential serum marker for malignant character in human glioma. Cancer Research 2002; 62(15): 4364-368.
[32]. Lin Y, Jiang T, Zhou K, Xu L, Chen B, Li G, et al. Plasma IGFBP-2 levels predict clinical outcomes of patients with high-grade gliomas. Neuro-oncology 2009; 11(5): 468-76.
[33]. Fukushima T, Kataoka H. Roles of insulin-like growth factor binding protein-2 (IGFBP-2) in glioblastoma. Anti-cancer Res. 2007; 27(6): 3685-692.
[34]. Mahmoudi A, Qujeq D, Daneshdoust D, Karimi M. Determination of serum survivin for prognostic role in esophageal cancer. Int J Res Appl Basic Med Sci. 2020; 6(1): 9-13.
[35]. Aghcheli K, Parsian H, Qujeq D, TalebiM, Mosapour A, Khalilipour E, et al. Serum hyaluronic acid and laminin as potential tumor markers for upper gastrointestinal cancers. Eur J Internal Med. 2012; 23(1): 58-64.
[36]. Samavarchi Tehrani S, Mahmoodzadeh Hosseini H, Yousefi T, Abolghasemi M, Qujeq D, Maniati M, et al. The crosstalk between trace elements with DNA damage response, repair, and oxidative stress in cancer. J Cell Biochem. 2018; 120(2): 1080-105.
[37]. Nejat Pish-Kenari F, Qujeq D, Maghsoudi H. Some of the effective factors in the pathogenesis of gastro-oesophageal reflux disease.Journal of Cellular and Molecular Medicine 2018; 22 (12): 6401-404.
[38]. Abolghasemi M, Yousefi T, Maniati M, Qujeq D. The interplay of Klotho with signaling pathway and microRNAs in cancers. Journal of Cellular Biochemistry 2019;120:14306-4317.
[39]. Tamddoni A, Mohammadi E, Sedaghat F, Qujeq D.As'Habi A. Anticancer effects of curcumin via targeting the mammalian target of rapamycin complex 1 (mTORC1) signaling pathway. Pharmacol Res. 2020; 156: 104798.
[40]. Soghli N, Qujeq D, Yousefi T, Soghli N. The regulatory functions of circular RNAs in osteosarcoma. Genomics 2020; 112(4):2845-856.

[DOI: 10.18502/ijml.v8i2.6274]