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# Serum Levels of Glial Fibrillaryacidic Protein in Meningioma

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ABSTRACT

**Background:** Glial fibrillaryacidic protein (GFAP), an intermediate filament protein, is mainly expressed by astrocytes, but some other cells like enteric glia and non-myelinating Schwann cells can also express GFAP. GFAP elevation has been reported in some types of meningioma and malignant brain tumors. In the present study, we analyzed the association between serum levels of GFAP with meningioma.
**Methods:** Sixty-eight newly diagnosed patients with meningioma and 28 healthy individuals (control group) were included. Serum levels of GFAP were measured by ELISA.
**Results:** There was no significant difference in GFAP serum levels between the two groups. Subdivision of the patients also revealed no significant association between GFAP and meningioma.
**Conclusion:** We studied serum levels of GFAP in meningioma in Iranian patients for the first time. We did not observe a significant association between meningioma and GFAP. A larger study including a larger number of different subtypes of meningioma patients may discover a weakly significant difference if it exists.

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## Introduction

Meningiomas are defined as tumors derived from arachnoid cap or meningothelial cells. They comprise approximately 35% of primary central nervous system (CNS) tumors according to the Central Brain Tumor Registry of the United States (CBTRUS) and thus they are considered the most frequently diagnosed primary CNS tumors.[1] Meningiomas are classified into three groups based on the WHO classification system:[2] In grade I, all tumors are benign including meningothelial, fibroblastic, lymphoplasmacyte-rich, transitional, angiomatous, microcystic, secretory, psammomatous, and metaplastic subtypes. Grade II tumors consist of atypical, clear cell, and chordoidmeningiomas. Grade III tumors are anaplastic, papillary, and rhabdoidmeningiomas. Grade II and III meningiomas are significantly more likely to have invasive disease with 30-50, and 50-94% rate of recurrence, respectively.[3,4] Brain-invasiveness in grade II or III tumors is histologically characterized by "irregular, tongue-like protrusions of tumor cells infiltrating underlying parenchyma without an intervening layer of leptomeninges". These changes are accompanied by reactive astrocytosis in the adjacent brain tissue.[5]

Glial fibrillaryacidic protein (GFAP), an intermediate filament protein, is mainly expressed by astrocytes but some other cells, such as enteric glia,[6] non-myelinating Schwann cells,[7] and human fibroblasts[8] in different tissues (i.e. meninges), can also produce GFAP. GFAP is involved in the structure and activities of cytoskeleton, mechanical support of the plasma membrane and maintenance of the shape of the cells.

GFAP expression is increased in injuries to the CNS with reactive gliosis, a process causing an increase in the production of intermediate filaments (GFAP,

vimentin, and nestin). Examples of these injuries are cerebrovascular trauma, stab wounds, and animal models of multiple sclerosis.[9] Moreover, in Alexander disease, a lethal rare neurological disorder, the astrocytes contain unique cytoplasmic inclusions that contain GFAP.[10,11]

Several studies have shown increased level of serum GFAP in astroglial tumors (i.e. astrocytoma and glioblastoma multiforme).[12-14] It was proposed as a glioblastoma multiform diagnostic marker in a study by Jung and colleagues in 2007.[15] Missler and co-workers demonstrated that GFAP level can be used as a serum marker of acute central nervous system damage in patients with traumatic brain injury (TBI).[16] Later, Voset and colleagues[17] and Nyle´n and colleagues[18] studied the use of serum level of GFAP as a prognostic factor in TBI.

Although GFAP is a main part of glial intermediate filaments, its immune-reactivity in meningioma was observed in prior studies.[19-25] A study evaluated serum GFAP level as well as some other immunohistochemical markers (e.g. epithelial membrane antigen and collagen IV) for the histological assessment of brain-invasive growth in meningioma. This study showed that stained sections in human meningioma are adequate for evaluating brain invasion and other immunohistochemical markers did not play significant roles in this issue.[26]

In this study we investigated the serum level of GFAP marker in the sera of patients with meningioma of different grades and also normal subjects. Our aims were to evaluate GFAP protein as a marker of meningioma tumor, and its possible association with the tumor grading.

## Materials and Methods

Sera specimens were collected prospectively from 68 patients diagnosed with meningioma of different grades who had been admitted to Chamran or Nemazee hospitals in Shiraz, prior to any treatment. Their medical records were reviewed for pathology and some other characterizations of the tumor at the admitted hospitals. 68 cases of meningioma consisted of 41 cases of grade I, 7 cases of grade II, 8 cases of grade III and 12 cases with unknown grading. Control samples were obtained from 19 healthy people with no neurological complaints or known disease that had referred to Shiraz branch of Iranian blood transfusion organization. All of them provided informed consent prior to the study participation.

All sera were stored at -80 °C at the biobank of Shiraz institute for cancer research. Serum GFAP levels were calculated blind to the clinical data using a GFAP ELISA kit.

Data were analyzed using SPSS software, version 19. The Chi-square exact test was used and when the expected cell frequency was less than five, the test was replaced by Fischer's exact test. $P<0.05$ was considered as statistically significant.

## Results

In the present study, we investigated serum levels of GFAP in 68 patients with meningioma and compared them to those in 19 healthy controls. The mean serum level of GFAP in patients with meningioma were 2.5±7.2 ng/ml (range: 0-46.122). The mean serum level of GFAP in the control group was 0.427±1.7 ng/ml (range: 0-7.482). Because GFAP serum levels were not normally distributed, the analysis was done by non-parametric Mann-Whitney U test which revealed no significant difference between patients and controls (table 1).

Serum GFAP level was detectable in 21 (30%) out of 68 meningioma patients, and 3 (15.7%) out of 19 people from the control group. The chi-square test also showed no significant difference in the number of patients with a positive value compared to the number of the controls with a positive value of GFAP (P=0.193).

Tumor grading was available in 56 of the cases, of which, 41, 7, and 8 had WHO grades I, II, and III, respectively. As it is indicated in table 2, grading of the tumor was not associated with GFAP serum levels (Kruskal–Wallis test, P=0.12). In grades I to III, 11, 5, and 2 patients had detectable values of GFAP, respectively. The number of the patients with a positive value was not significantly different according to the tumor grade as calculated using the chi-square test (P=0.15).

The location of meningiomas with positive serum GFAP were as follows: 5 tumors in sphenoid wing, 4 as a frontal mass, 3 tumor in falxcerebri, 2 in parasagittal area, 2 in posterior fossa, 2 convex meningioma, 2 in tuberculum sellae and another tumor with unmentioned site in the archived file. No significant association was also found between GFAP serum levels and tumor site.

## Discussion

GFAP as the main protein of intermediate filament network in mature astrocytes was first detected in the

**Table 1:** Serum levels of Glial fibrillary acidic protein in meningioma patients and controls

| Group | N | Mean±SD (pg/ml) | P value |
|---|---|---|---|
| CXCR4 patients | 68 | 2.585±7.2 | 0.15 |
| Controls | 19 | 684.1±123.7 | |

**Table 2:** Glial fibrillary acidic protein serum levels based on tumor grade in meningioma

| | N | Mean±SD (pg/ml) | Std. Deviation | Minimum | Maximum |
|---|---|---|---|---|---|
| 1 | 41 | 2.8±8.4 | 8.416315 | 0.000 | 46.122 |
| 2 | 7 | 1.3±2.0 | 2.021615 | 0.000 | 5.711 |
| 3 | 8 | 0.56238±1.2 | 1.225123 | 0.000 | 3.457 |
| Total | 56 | 2.3±7.2 | 7.277253 | 0.000 | 46.122 |

P value was 0.129 and calculated using The Kruskal–Wallis test.

plaques of multiple sclerosis patients studied by Enget and colleagues.[9] Different types of GFAP proteins alongside vimentin, nestin, and synemin (other members of intermediate filament network) have been found in different subsets of astrocytes.[27,28]

In 1995 and 1996, four laboratories produced GFAP knocked out mice, of which, three reported the same number of neurons and astrocytes in these mice compared to wild types.[29-31] However, Liedtke and co-workers detected some decreased myelination in certain part of the brain and some tissue architecture differences in optic nerve and spinal cord.[32] All four studies showed that even in the absence of GFAP, reactive gliosis could be induced and vimentin would still be expressed. To learn more about this process, Eliasson and colleagues conducted another study in GFAP and vimentin knocked out mice. They showed cytoplasmic intermediate filaments in reactive astrocytes were not formed. GFAP and vimentin deficiency also caused a decreased reactive gliosis, scar formation and vulnerability to ischemia.[33] On the other hand, some other studies have shown better regenerative potentials such as better synaptic regeneration in the hippocampus in the presence of GFAP.[34,35] A role for GFAP in regulation of vascular flow has also been proposed; as in a study the brain infarction volume was higher after transient occlusion of carotid artery in GFAP null mice.[36] GFAP is also involved in cell motility,[37] cell division,[38] synaptic formation,[39] and maintenance of myelination of the CNS.[40]

GFAP overexpression has also been studied. Messing and colleagues conducted a study that used a human GFAP transgene to increase GFAP expression in astrocytes. 15 to 20-fold aggregation of GFAP proteins higher than controls was lethal. This study proved that GFAP mutations were the major cause of Alexander disease.[41]

In the process of maturation of astrocytes, GFAP substitutes vimentin as the major intermediate filament network protein and in some astrocytes vimentin expression decreases to undetectable amounts. GFAP expression continues to increase as aging occurs (probably as a consequence of aggregation of oxidative damaged proteins in the brain).[27,28] Rosengren and colleagues in 25 neurological healthy individuals detected an age-dependent increase in GFAP.[42] This change of GFAP serum levels was an indicator of changes in astrocyte functions as aging occurs.[42]

GFAP has been known as a diagnostic marker in glioma tumors. There has been some reports of positive serum GFAP in patients with meningioma in some previous studies that in most cases they had rhabdoid morphology or papillary variant (both type are aggressive). It was explained by a heterogeneous expression of GFAP in rhabdoid subtype of meningioma. Another possibility is that human fibroblasts in the meninges may express GFAP.[43] Two other studies have described a 'whorling-sclerosing' histological variant that the cases were positive for serum GFAP.[22,23]

In our study, some of the patients were positive for serum GFAP levels; however, it did not significantly differ from the control group. There was an atypical meningioma with focal rhabdoid feature that was not positive for serum GFAP level. We also had two cases of papillary meningioma who were negative for serum GFAP. No meningioma with "whorling-sclerosing" pathology was present in our cases.

There are some limitations to our study that requires more research. First our sample size was limited. Second, a number of the archived files were incomplete in some tumor features like tumor volume, staging, site, and type of the tumor that made more evaluation impossible.

## Conclusion

We investigated serum levels of GFAP in patients with meningioma and compared them to those in the healthy control group. The mean serum level of GFAP in patients with meningioma compared to those in the control group was increased but did not reach a statistical significance. A larger study including a larger number of different subtypes of meningioma patients will help to discover any possible association.

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**Conflict of Interest:** None declared.

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