Dimogerontas et al. Int J Pathol Clin Res 2016, 2:023
Volume 2 | Issue 1
ISSN: 2469-5807

International Journal of
Pathology and Clinical Research

Research Article: Open Access

Glutamate Concentrations in Plasma and CSF in Patients with Glioma and Meningioma

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Abstract

Objectives: Glioma, a malignant intra-axial brain tumor, can release glutamate that facilitates tumor expansion, stimulates tumor-cell proliferation and motility and promotes epileptic activity. Glutamate acid is the major excitatory neurotransmitter in the mammalian Central Nervous System. We explore correlations of glutamate concentrations in blood and cerebrospinal fluid in patients with glioma in comparison to patients with meningioma which is the most common benign cerebral tumor.

Experimental design: Samples from 16 patients with glioma and 13 patients with meningioma were analyzed. Glutamate levels in blood and cerebrospinal fluid were measured by HPLC method.

Principal observations: Mean value ± SD of Glu concentration in CSF of patients with glioma was 64.2 ± 18.7 μM while the same value in serum was 103.0 ± 16.6 μM. In patients with meningioma, mean Glu concentration in CSF was 22.4 ± 7.12 μM while the same concentration in serum was 78.5 ± 11.4 μM. Biostatistical comparison of the two groups of patients revealed significant difference of Glu concentration in CSF compared to Glu concentration in serum but no significant difference was established between CSF concentrations of Glu in glioma and meningioma patients and between serum concentrations of Glu in the same group of patients as well.

Conclusion: Higher blood and CSF Glu concentrations are detected in glioma to meningioma patients. Statistical analysis showed significant difference between Glu concentrations in plasma and CSF in both glioma (p value = 0.01105) and meningioma patients (p value = 0.0012).

The invasive nature of gliomas, enhanced by Glu release, is one of the most important limitations to an effective treatment. CSF or plasma Glu concentrations might consist an easy and cheap biologic marker for tumor expansion, progression and response to therapeutic management.

Keywords

Brain neoplasms, Glioma, Glutamic acid, Meningioma, Glutamate

Introduction

Gliomas originate from glial cells or their progenitors and include astrocytomas, oligodendrogliomas and ependymomas; they comprise 32% of all primary brain tumors with two thirds of them being of high-grade. According to WHO low grade gliomas (grade I-II) are of benign histopathological characteristics while high grade gliomas (grade III-IV) are highly malignant tumors including anaplastic astrocytoma (grade III), glioblastoma multiform (GBM) (grade IV) and the diffuse-intrinsic pons glioma [1]. Glioblastoma the most common type of malignant glioma, accounts for about 17% of all primary cerebral tumors [1,2]. Gliomas are always growing intra-axially and are associated with high incidence of seizures [3].

On the other hand, meningioma is the most common primary, usually slow-growing, brain tumor in adults [4] (approximately 30%) [5] with a female predominance. The vast majority of meningiomas are benign (97.7%) and according to WHO, they are classified into three grades: grade I (typical or classic or benign meningiomas), grade II (atypical meningiomas) accounting for 5-15% and grade III (malignant or anaplastic or sarcomatous meningiomas) accounting for 1-3% of meningiomas [2,6]. Since these tumors are almost always growing extra-axially without invasion of brain parenchyma, they are not so frequently associated with automatic epileptic activity (21.6-39%) [7] as gliomas do [8].

Glutamate (Glu), a nonessential amino acid, is the most abundant free one in the mammalian brain where it is playing the role of the major excitatory neurotransmitter [9]. It is normally restricted to the synaptic and perisynaptic space of glutamatergic synapses where under normal conditions, is released by presynaptic neurons or even by glial cells [10] and mainly removed by astrocytes. In a healthy brain homeostasis keeps glutamate levels stable at harmless micromolar concentrations [11,12]. When brain homeostasis is disturbed the high Glu concentrations, cause excitotoxic death of the exposed neurons leading to irreversible neurological deficits. Furthermore, increased CSF glutamate levels have been linked to regulation of

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Citation: Dimogerontas G, Polissidis A, Karkalousos P, Konstantinidis E, Papadopoulou-Daifoti Z, et al. (2016) Glutamate Concentrations in Plasma and CSF in Patients with Glioma and Meningioma. Int J Pathol Clin Res 2:023

Received: December 02, 2015: **Accepted:** February 04, 2016: **Published:** February 07, 2016

Table 1: Demographic characteristics of study population.

Gliomas							
No	**SEX***	**Age (yo)**	**Type of glioma and grade**	**Seizures**	**Volume (cm^3)**	**Mass effect**	**Treatment**
1	2	68	Grade III	NO	33.493	YES	Depakine
2	2	63	GBM	YES	14.13	NO	Epanutin
2	1	74	Grade III	YES		YES	Keppra
4	2	53	GBM	YES	115.5	YES	Epanutin
5	1	39	Grade II	YES	14.13	NO	Depakine, Epanutin
6	2	74	Grade III	YES		YES	Depakine, Epanutin
7	1	71	GBM	YES	20.46	YES	Depakine, Keppra
8	2	71	GBM	NO	20	YES	Epanutin
9	1	56	GBM	NO	24	YES	Epanutin
10	1	62	GBM	NO		YES	Epanutin
11	1	71	GBM	NO	49.281	YES	Epanutin
12	2	74	GBM	YES	17.968	NO	Epanutin
13	2	71	GBM	NO	48	YES	Depakine
14	2	66	GBM	NO	35.28	YES	Epanutin
15	1	66	GBM	NO	14.534	YES	Epanutin
16	1	62	GBM	NO	23.68	YES	Depakine
Meningiomas							
No	**SEX***	**Age (yo)**	**Type of meninigioma and grade**	**Seizures**	**Volume (cm^3)**	**Mass effect**	**Treatment**
1	1	78	Meningotheliomatous meningioma Grade I	NO	85.425	YES	Epanutin
2	2	65	Psammomatous meningioma (Atypical or Grade II)	YES		YES	Depakine
3	1	70	Meningotheliomatous meningioma Grade I	NO	15.994	YES	Depakine
4	1	65	Fibroblastic meningioma (Atypical or Grade II)	NO	48.125	YES	Depakine
5	2	57	Meningotheliomatous meningioma Grade I	NO	4.576	YES	NO
6	2	79	Meningotheliomatous meningioma Grade I	NO	38.772	YES	Epanutin
7	2	49	Meningotheliomatous meningioma Grade I	NO	5.057	NO	Epanutin
8	2	67	Meningotheliomatous meningioma Grade I	YES	7.084	NO	Depakine
9	2	65	Transitional meningioma Grade I	NO	44.579	YES	NO
10	2	66	Transitional meningioma Grade I	NO	50.625	YES	Epanutin
11	2	61	Meningotheliomatous meningioma Grade I	YES		YES	Epanutin
12	2	73	Psammomatous meningioma Grade I	NO	66.555	YES	Epanutin
13	2	59	Meningotheliomatous meningioma Grade I	YES	6	NO	Epanutin, Keppra

*Number 1 in sex column means male and number 2 means female.

proliferation, differentiation and survival of several neuronal tumors, including medulloblastoma and high grade glioma [13,14].

Unfortunately the huge amount of evidences for the role of Glu in brain tumors mostly refer to animal studies or cultured cell lines [15-17] and despite the considerable body of in vitro experience clinical studies regarding Glu levels either in blood or cerebrospinal fluid (CSF) are very limited.

The aim of the present study was to determine Glu concentrations in blood and CSF in patients with glioma, a malignant and epileptogenic cerebral tumor and in patients with meningioma, a benign and less epileptogenic tumor and establish possible correlations.

Patients and methods

Patients

Twenty nine patients, 16 patients with glioma and 13 patients with meningioma, were included in the study. All patients (11 men and 18 women) were admitted at the Neurosurgery Department of a State Hospital, during four consecutive years and underwent craniotomy and brain tumor resection. The patients participated in the study after informed consent (Table 1).

Data about demographic (age, gender etc.) and clinical characteristics (seizures, time of operation from first symptom, survival etc.) of study participants were recorded (Table 1 and Table 2). Tumor volume was calculated from cerebral MRI. Grade and Ki67 labeling index were obtained from pathology reports.

Twenty six out of twenty nine cases (3 patients were lost) were followed up for a period of at least one year as follows: ten out of eleven grade I meningiomas, both cases of atypical meningiomas and one case with grade II astrocytoma (grade 2), all three cases of anaplastic astrocytomas (grade 3) and ten out of twelve cases of grade 4 astrocytomas (GBM) (Table 3).

The study protocol was approved by the Research Ethics Committee of "Asclepeion" General Hospital, Voula, Athens, Greece.

Methods

Glu concentrations were measured in peripheral blood samples of 15 glioma and 13 meningioma patients as well as in CSF samples of 6 glioma and 7 meningioma patients by using High-Performance Liquid Chromatography (HPLC). All subjects abstained from tobacco, coffee and alcohol for at least 12 hours prior to blood sampling.

Sample preparation

Blood (2.5 cc) was obtained by venous puncture and collected in glass anticoagulated tubes with EDTA, immediately before the operation; it was centrifuged at 3000 g for 15 minutes and the supernatant was conserved at -80°C until it was processed.

CSF was obtained by direct aspiration from the operation field or through external lumbar drainage, which was preoperatively placed when a tight parenchyma was expected. CSF was centrifuged at 2000 g for 10 minutes and the supernatant was stored at -80°C until it was processed for the assessment of Glu levels [18].

Plasma and CSF samples were deproteinized with 3% sulfosalicylic acid (SSA.=.1.5 g/50 ml dd H_2O) by adding 50 μl of plasma to 450 μl SSA solution and leaved for 30 minutes at 4°C. Then they were centrifuged for 30 minutes at 9500 g and supernatants were stored at -80°C until the analysis.

Table 2: Statistical analysis of demographic characteristics of study population.

CLINICAL FEATURES	Glioma N.16	Meningioma N.13	Glioma *Vs* Meningioma P value < 0.05
Age			
mean ± SD years	65.06 ± 9.384	65.69 ± 8.290	P = 0.7750 [a]
range	(39-74)	(49-79)	
Gender			
-Male N (%)	8 (50%)	3 (23.07%)	
-Female N (%)	8 (50%)	10 (76.92%)	
Grade (WHO) N (%)			
I	0	11 (84.61%)	
II	1 (6.25%)	2 (15.38%)	
III	3 (18.75%)	0	
IV	12 (75%)		
Ki67			
I mean ± SD %	ns [b]	3.5 ± 1.78	
II mean ± SD %	ns [b]	7.333 ± 2.082	
III mean ± SD %	12.33 ± 6.429	ns [b]	
IV mean ± SD **%**	16.60 ± 12.55	ns [b]	
	(p = 0.7988)	(p = 0.0392)	
Tumor volume			
Mean ± SD cm^3	33.11 ± 27.5	31.85 ± 27.64	p = 06833 [a]
range	(14.13-115.5)	(4.576-85.43)	
Mean time of operation from first symptom			
Mean ± SE days	115.4 ± 38.87	269.7 ± 87.93	p = 0.2296 [a]
range	(2-540)	(4-720)	
Seizures			
-Yes N (%)	7 (43.75%)	5 (38.46%)	
-No N (%)	9 (56.25%)	8 (61.53%)	
Recurrent disease			
-Yes N (%)	1(6.25%)	2 (15.38%)	
-No N (%)	15(93.75%)	11 (84.61%)	
Survival			
Mean ± SE months	14.29 ± 3.569	23.92 ± 4.680	p = 0.1283 [a]
range	(1-46)	(1-46)	

[a]Mann-Whitney U test

[b]Not specified

High-performance liquid chromatography (HPLC) analysis

Following deproteinization of the samples, Glu concentrations were measured by HPLC, using specific software (Chromatography Station for Windows; Data Apex, Prague, Czech Republic), as described previously, [19,20] with minor modifications. The detection limit of HPLC was 0.05 μM. The mobile phase consisted of 5% acetonitrile (Merck, Darmstadt, Germany) - 0.1M phosphate buffer ($Na_2HPO_4 \cdot 2H_2O$ 8.8995 g/500 ml), pH 4.9-5.1, containing 50 μM EDTA (9.305 mg/500 ml). The sensitivity of the assay was tested for each series of samples, using external standards. Standard reactions were prepared as follows. Before analysis, samples were diluted with dd H_2O 1:5 (40 μl plasma + 160 μl dd H_2O) and 40 μl of sample (standard solution or sample) was added to 1-ml Eppendorf tubes along with 40 μl of 0.1M Borax buffer (Sigma-Aldrich, St. Louis, MO), pH 9.6, and 2 μl of the derivitizing reagent OPA (ortho-phthaldialdehyde; Sigma-Aldrich) and left at room temperature to react for 10 minutes. Final values were expressed in micromoles per liter (μmol/L or μM).

Ki67 (MIB-1) labeling index

The Ki67 labeling index was calculated as a percentage of labeled nuclei per 1000 cells. One thousand tumor cells were counted in several areas of tissue where positively stained nuclei were evenly distributed. In cases with uneven distribution of positive nuclei, the tumor cells were counted in the areas with highest density of positive nuclei by visual analysis [21].

Statistical analysis

Since the values of each variable were not many, non-parametrical statistical methods were used. Specifically, for each comparison of two different groups, the Mann-Whitney test was used and for each comparison of more than two different groups the Kruskal-Wallis test. For the statistical analysis and the majority of the diagrams the statistical packages S.P.S.S. and Minitab were used (academic licenses). For some diagrams we used MS Excel. Values are presented as the mean ± SD or mean ± SE; P values less than 0.05 were considered significant.

Results

Mean age of the 16 patients with glioma was 65.06 ± 9.38 years; (age range 39 to 74 years) and of the 13 patients with meningioma was 65.69 ± 8.29 years (age range 49 to 79 years). Eleven cases (38%) were in males, while eighteen (62%) were in females (Table 2).

Table 3: Follow up ratios and mortality of tumour subgroups at the end of first post-operative year.

Tumour type and grade	N of patients in the study	N of patients with available follow up at the end of first postoperative year	N of alive patients at the end of the first post-operative year (%)	Mortality N(%)
Grade I meningiomas	11	10 (90.9%)	8 (80%)	2 (20%)
Grade II meningiomas (atypical)	2	2 (100%)	1 (50%)	1 (50%)
Grade II astrocytomas	1	1 (100%)	1 (100%)	0
Anaplastic astrocytomas	3	3 (100%)	2 (66.6%)	1 (33.3%)
Glioblastoma multiforme (GBM)	12	10 (83.3%)	4 (40%)	6 (60%)
Total	**29**	**26 (89.6%)**	**16 (61.5%)**	**10 (38.4%)**

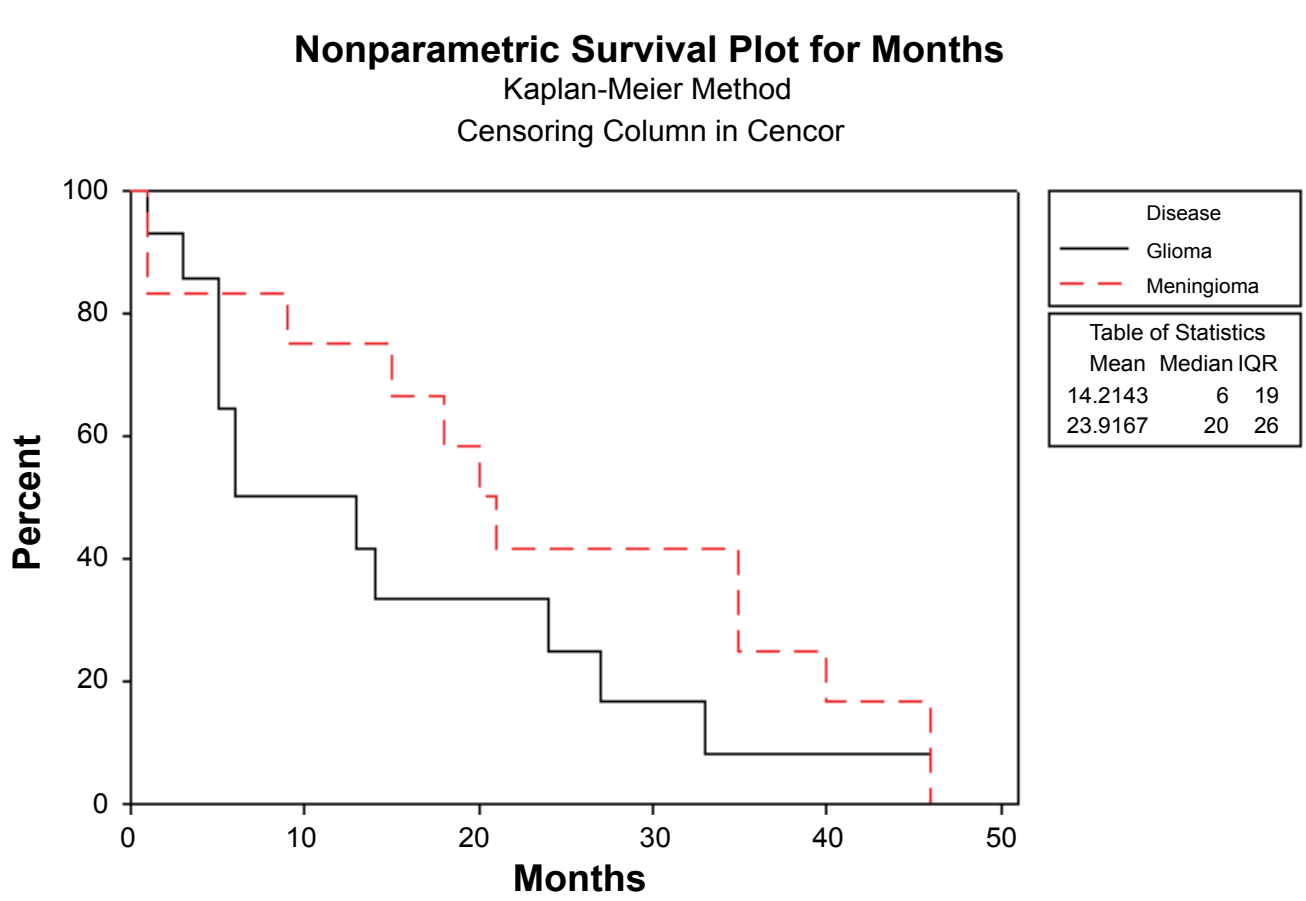

Figure 1: Nonparametric Survival Plot for patients suffering from Glioma and Meningioma showing that only 50% and 75% of them, respectively, survived at the end of first postoperative year.

Glioblastoma multiform (WHO Grade IV) was the most common histologic type (12 cases; 75%) of glial neoplasms followed by anaplastic glioma (3 cases; 18.75%) (WHO Grade III). Meningotheliomatous meningioma (WHO Grade I) was the most common histologic type (8 cases; 61.53%) of meningeal neoplasms.

At the end of the first post-operative year only sixteen of the twenty six patients (61.53%) were alive as follows (respective mortality as percentage): eight out of ten patients with grade I meningioma (20%), one out of two patients with atypical meningioma (50%), the single patient with grade II astrocytoma (0%), two out of three cases with anaplastic astrocytoma (33.3%) and four out of ten cases with GBM (60%) (Figure 1). Mortality rate at the end of first post-operative year is shown at Table 3. Of the fourteen glioma suffering patients in whom follow up was available, ten suffered from GBM (71.4 %) and of the twelve meningioma suffering patients in whom follow up was available, 10 had a benign meningioma (WHO Grade I) (83.3%).

Mean value ± SE of Glu concentrations in CSF of patients with glioma was 64.2 ± 18.7 μM while in plasma it was 103.0 ± 16.6 μM. In patients with meningioma, mean ± SE Glu concentrations in CSF was 22.4 ± 7.12 μM while in plasma was 78.5 ± 11.4 μM (Table 4). Comparison between Glu concentrations in plasma and CSF either in patients with glioma or in patients with meningioma, using Mann-Whitney t-test, revealed a statistical significant difference between them (p value a/b = 0.01105, and p value c/d= 0.0012 for gliomas and meningiomas respectively) (Table 4 and Figure 2).

Table 4: A. Mean values ± SE of Glu concentration in blood and CSF in patients suffer from cerebral glioma (**A**) or meningioma (**B**). Values are expressed in μM. Comparison made using Mann-Whitney t-test. Statistical significance between the groups is as follows: **A**: Serum Glu and CSF Glu (p value a/b = 0.01105, **B**: Serum Glu and CSF Glu(p value: c/d = 0.0012).

A	**Glu concentration**
Gliomas	Mean ± SE μM/ml
Plasma	103.0 ± 16.6[a]
CSF	64.2 ± 18.7[b]
B	**Glu concentration**
Meningiomas	Mean ± SE μM/ml
Plasma	78.5 ± 11.4[c]
CSF	22.4 ± 7.1[2]d

Mean Glu CSF or blood concentrations in patients with glioma compared to patients with meningioma, using the non-parametric Mann-Whitney t-test, revealed no significant difference in none of the compared groups (p value = 0.9431 and p value = 0.3334 for CSF and blood respectively) (Table 5 and Table 6).

We also seek if there was any correlation between Glu levels either in blood or CSF in the various subgroups, using Mann-Whitney t-test or 1-Single Wilcoxon test, but no statistical significant relationships was found except for the subgroups of patients with meningioma

with and without epileptic spasms, in which paradoxically mean Glu concentrations in plasma were significantly higher in those without spasms (p value = 0.025 < 0.05) (Table 6) and in the subgroup of glioma patients with mass effect, that had Glu blood levels significantly higher than in the subgroup with no mass effect (p value = 0.0253 < 0.05) (Table 6).

Detailed survival analysis between GBM patients revealed that patients with Glu blood concentration below 40 μM survived over the first post-operative year in a statistical significant manner (p = 0.0188) in contrast to those with Glu blood concentration over 40 μM, although two patients with very high Glu blood concentration (mean ± SD= 149.7 ± 65.87 mM accomplished to survive over twelve months) but in a non-significant manner (p = 0.4492) when compared with those patients who didn't accomplish it (mean ± SD = 91.83 ± 35.95 μM).

In patients with grade I meningioma with Glu blood concentration between 70 μM and 130 μM (mean ± SD = 87.65 ± 21.79) survived less than two years in a statistical significant manner (p = 0.0197) in contrast to those with Glu blood concentration below 70 μM (mean ± SD = 44.96 ± 16.63) or over 130 μM (150 ± 25.01) who survived over two years.

No correlation between Glu CSF concentration and survival either in glioma neither in meningioma patients was established.

Discussion

In the present study Glu levels in blood and CSF were studied by HPLC in patients with a common brain tumor (glioma or meningioma). We demonstrated higher blood and CSF Glu concentrations in glioma than in meningioma patients.

A plethora of evidences in the literature indicate the tight correlations between gliomas and Glu [22-24] while such a relation of Glu with benign cerebral tumors in humans has not been confirmed. Glutamate, under physiological conditions at micromolar concentration, is of great importance for the homeostasis in the brain extracellular fluids and crucial for the proper physiological functioning of the brain, a fact accomplished through the protective mechanisms of Na^+-dependent, excitatory amino acid transporters [(EAATs): EAAT1= glial glutamate/aspartate amino acid transporter or GLAST, EAAT2 = glutamate transporter-1 or GLT-1 and EAAT 3] [11].

In gliomas, Glu can be produced and released from their cells facilitating their expansion by destroying the surrounding brain tissue [16], by stimulating tumor-cell proliferation and motility in an autocrine or paracrine manner and by promoting epileptic activity from surrounding neuronal cells [25]. Over 50% of Glu produced by glioma cell lines has been found to be released as an obligatory byproduct of the x_c^- specific transporter [16,26], a transporter that mediates the exchange of intracellular Glu for extracellular cystine which is required by glioma cells for glutathione-based protection against reactive oxygen radicals [27]. Such a transporter has not been identified in meningiomas. Although the role of Glu as signal mediator in neuronal tumors, such as glioma [22-24], is well known, in the last decade evidence has emerged about the involvement of Glu in non-neuronal tumors [27] such as colorectal cancer [28], gastric cancer [29], oral squamous cell carcinoma [30], prostate cancer [31], melanoma [32], osteosarcoma [33] suggesting a more complex role of glutamate than simply the death of the exposed neurons and the entailing irreversible neurological deficits.

Blood Glu concentrations (μM) both in patients with glioma or meningioma, were significantly higher compared to CSF concentrations (3.5 times higher in meningioma patients and 1.6 times higher in glioma patients). Several studies have tried to establish the physiological glutamate levels either in plasma or in CSF [34-37] but the considerable variability noted was probably due, among others, to the methodology used. The two compartments, blood/plasma and CSF, are in equilibrium with the blood-brain barrier at the interface, regulating exchanges of several constituents but correlations of Glu concentrations in blood and CSF in cerebral tumor patients lack. Since in neurological disorders the blood-brain barrier can be damaged, implying alterations of the physiologic crosstalk between central and peripheral tissues [38,39]; the increased CSF glutamate levels are probably due to glial and neuronal cell necrosis. Although it is difficult to determine the exact source of plasma glutamate, based on the ratio between Glu plasma and CSF levels, it can be postulated that plasma levels derive, at least partially, from the brain through the damaged blood-brain barrier. The relationship between glutamate release from glioma tissue and its clearance dynamics into and from the CSF is not fully elucidated according to our knowledge.

Although theoretically, malignant tumors ought to produce Glu in massive amounts in contrast to benign tumors, brain Glu

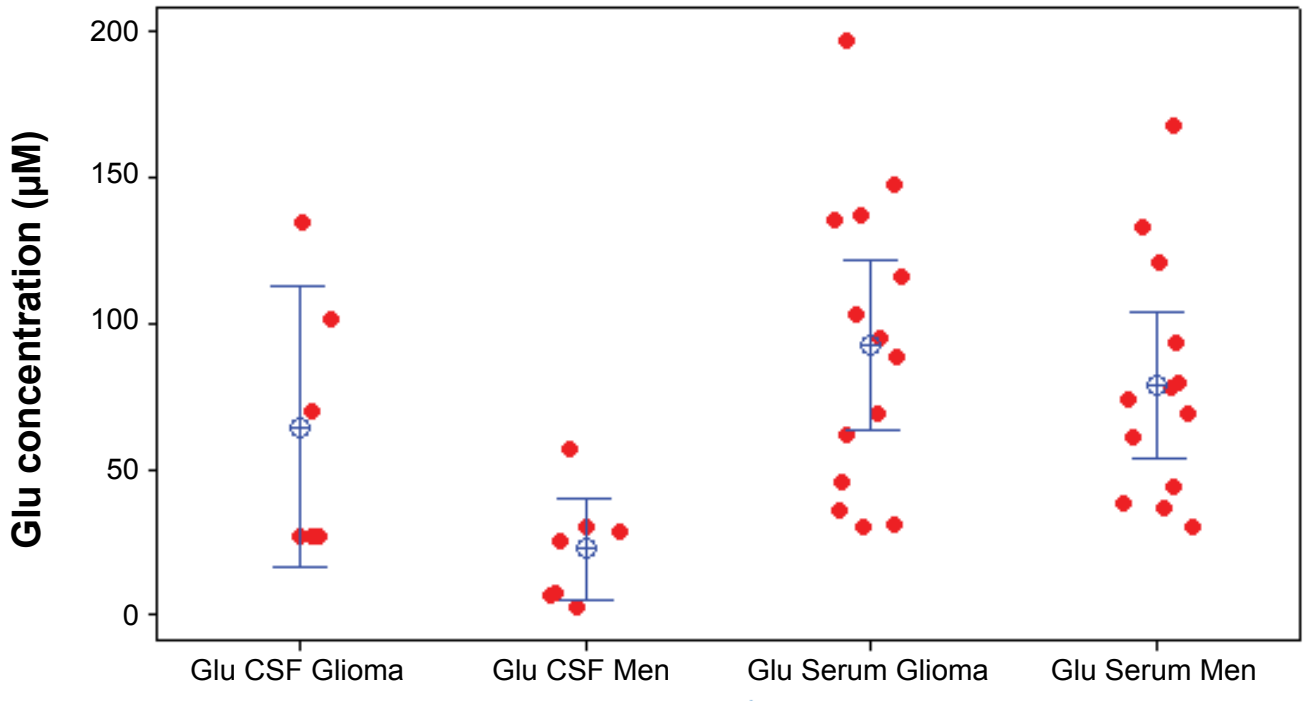

Figure 2: Dot plot of Glutamate concentrations in blood and CSF for patients with Glioma and Meningioma.

Table 5: Subgroups of patients with brain tumours and the corresponding Glu concentrations in CSF. Values are expressed in μM

CSF		Glioma Glu concentration (mean ± SE μM)	Meningioma Glu concentration (mean ± SE μM)
	Glu Concentration (N of patients)	64.2 ± 18.7 (N = 6) 22.4 ± 7.12 (N = 7) (p = 09431)	
Age	≤ 65 years old > 65 years old (N of patients)	80.35 ± 54.00 (N = 2) 56.13 ± 17.99 (N = 4)	25.73 ± 9.150 (N = 5) 61.72 ± 48.16 (N = 2)
Sex	Female Male (p)	87.4 ± 31.7 (N = 3) 41.0 ± 14.3 (N = 3) (0.1904)	16.7 ± 5.1 (N = 6) 56.45 (N = 1) **(0.036)**
Grade (WHO 2007) (p)		**II** 26.35 (N = 1) **III** 51.6 ± 24.6 (N = 3) **IV** 102 ± 32.4 (N=2) (III vs II : 0.3865)	**I** 16.70 ± 5.07 (N = 6) **II** 56.45 ± 0 (N = 1) **(0.036)**
Tumor volume ≤ 25 cc > 25 cc		 76.78 ± 31.38 (N = 5) 100.875 ± 0.0 (N = 1)	 21.67 ± 7.497 (N = 3) 49.76 ± 28.46 (N = 4)
Mass Effect Yes No (p)		 56.1 ± 18.0 (N = 4) 80.4 ± 54.0 (N = 2) (1.0)	 24.3 ± 9.6 (N = 5) 17.7 ± 11.0 (N = 2) (0.8465)
Mean time of operation from first symptom ≤ 90 days > 90 days		 26.70 ± 0.35 (N = 2) 65.83 ± 21.43 (N = 3)	 80.77 ± 39.02 (N = 2) 10.47 ± 5.045 (N = 4)
Seizures Yes No (p)		 56.9 ± 21.1 (N = 5) 100.9 ± 0.0 (N = 1) (0.584)	 6.7 ± 0.0 (N = 1) 24.9 ± 7.8 (N = 6) **(0.025)**
Ki67 (p)		**≤ 15%** 56.0 ± 18.1 (N = 4) **> 15%** 80.7 ± 53.7 (N = 2) (0.817)	**≤ 5%** 55.7 ± 34.2 (N = 4) **> 5%** 22.8 ± 12.3 (N = 3) (0.3123)
Survival		**≤ 1 year** 85.26 ± 15.61 (N = 2) **> 1 year** 26.78 ± 0.2186 (N = 3)	**≤ 2 years** 4.695 ± (N = 2) **> 2 years** 35.02 ± 7.204 (N = 4)

concentrations in glioma patients remain controversial; some of the studies report elevated Glu in peritumoral brain [40] others report unchanged or even lower in the tumor [41]. Two studies in which microdialysis probes were placed into the brain of ambulatory patients allowing a continuous sampling of extracellular Glu showed elevated Glu concentrations [42,43]. In another study Glu concentrations were in excess of 100 μM at the tumor margin in all nine patients examined; yet, neither non-malignant brain tumor nor acute brain trauma have shown sustained elevation in Glu [43]. In our study, CSF Glu levels were approximately 3 times higher in glioma patients compared to meningioma ones while plasma Glu concentrations showed a smaller difference. However, the interpretation of our results must be carefully evaluated since we could not study the profile of CSF glutamate levels at repeated intervals (due to ethical reasons).

Stratification of both groups of patients according to age, sex, grade of tumor, tumor volume, mean time of operation from first symptom appearance and postoperative survival did not reveal any significant correlation between these subgroups, in accordance to other studies. Although Glu is an epileptogenic neurotransmitter, unexpectedly, mean concentrations of Glu in the plasma of non epileptic patients with meningioma were significantly higher than the corresponding concentrations in epileptic patients, but the underlying pathophysiologic mechanisms of tumor related seizures is more complex, involving: different structural changes (such as neuronal loss in the CA1 and CA3a/b regions of hippocampus as in mesial temporal sclerosis) [44], biochemical (like pH) changes in peritumoral brain tissue [45], decreased extracellular [Mg^{2+}] [46], and increased peritumoral extracellular [Fe^{3+}] [47] and histologic alterations (such as increased expression of connexin (CX) 43 protein in surrounding of brain tumor tissue etc.) [48,49]. This phenomenon must be studied in depth.

Ki67, a non-histone protein, expressed in the proliferative phase of the cell cycle, is the most widely used immunohistochemical marker to measure cell proliferation (the net growth rate of a tumor is a balance between cell proliferation and apoptosis); Ki67 labeling index (LI) correlates well with increasing histological grade of meningiomas [50,51] and gliomas [52,53], poor prognosis of higher grades, reduced interval of recurrence [54,55] and decreased survival [56]. A cut off value of 4.2 for Ki67 has been suggested as indicative of high tumor proliferation activity and as a predictor of meningiomas recurrence [57]. Glu concentrations in plasma and CSF didn't differ significantly between glioma patients with Ki67 over or less than 15%.

In accordance with the literature, Ki67 LI was statistically different between groups of grade I and grade II meningiomas, but Glu concentrations in plasma and CSF didn't differ significantly in any subgroup of these patients either with Ki67 < 5% or Ki67 > 5%.

Our findings strongly suggest that Glu release has to be considered a common attribute of malignant gliomas and important in considering the various ways in which Glu affects

Table 6: Subgroups of patients with brain tumours and the corresponding Glu concentrations in peripheral blood. Values are expressed in μM.

PLASMA		Glioma Glu concentration Mean ± SE μM	Meningioma Glu concentration Mean ± SE μM
Glu Concentration (N of patients) (p)		103.0 ± 16.6 (N = 15) 78.5 ± 11.4 (N = 13) (0.3334)	
Age	≤ 65 years old > 65 years old	99.74 ± 20.57 (N = 6) 105.2 ± 25.04 (N = 9)	60.01 ± 9.123 (N = 7) 100.0 ± 19.65 (N = 6)
Sex	Female Male (p)	109.6 ± 27.8 (N = 7) 97.2 ± 21.1 (N = 8) (0.7723)	67.50 ± 12.57 (N = 10) 115.0 ± 11.73 (N = 3) (0.0519)
Grade (WHO) (p)		**II** 137.05 ± 0.0 (N = 1) **III** 74.7 ± 13.1 (N = 2) **IV** 104.9 ± 20.4 (N = 12) (III vs II:0.6481)	**I** 78.8 ± 13.40 (N = 11) **II** 76.8 ± 15.9 (N = 2) (0.9214)
Tumor volume	≤ 25cc > 25cc	107.1 ± 26.88 (N = 8) 101.0 ± 34.59 (N = 4)	71.72 ± 14.78 (N = 5) 95.12 ± 19.43 (N = 6)
Mass Effect (p)	Yes No	83.7 ±14.3 (N = 12) 180.1 ± 38.0 (N = 3) **(0.0253** < 0.05**)**	86.1 ± 13.7 (N = 10) 53.1 ± 12.4 (N = 3) (0.3525)
Mean time of operation from first symptom	≤ 90 days > 90 days	95.03 ± 20.31 (N = 8) 121.4 ± 35.64 (N = 5)	100.0 ± 23.56 (N = 5) 51.93 ± 7.931 (N = 5)
Seizures (p)	Yes No	129.7 ± 23.7 (N = 7) 79.6 ± 21.1 (N = 8) (0.093)	43.1 ± 6.7 (N = 4) 94.2 ± 13.0 (N = 9) (**0.025** < 0.05)
Ki67 (p)		**≤ 15%** 94.34 ± 19.00 (N = 8) **> 15%** 86.25 ± 24.86 (N = 5) (0.7144)	**≤ 5%** 80.54 ± 16.39 (N = 6) **> 5%** 61.53 ± 9.098 (N = 6) (0.3785)
Survival		**≤ 1 year** 106.5 ± 28.60 (N = 7) **> 1 year** 102.7 ± 23.83 (N = 6)	**≤ 2 years** 77.26 ± 19.81(N = 7) **> 2 years** 88.65 ± 8.50 (N = 5)

the tumor biology. The determination of Glu signaling is feasible by functional imaging techniques or by microdialysis, but these techniques are invasive and expensive, limitating the large scale applicability of these methods [58]. Thus we strongly believe that CSF, or even better, plasma concentrations of Glu might consist an easy and cheap biologic marker indicating the role of excitotoxicity in CNS tumor expansion and their progression or their response to therapeutic management; several studies have shown that Glu receptor antagonists [15,59,60] and silencing of selected receptor subunits [61] inhibit proliferation of cancer cells [62,63] while potent blockers of the x_c^- system such as sulfasalazine and (S)-4-carboxyphenylglycine [64] can inhibit the autocrine/paracrine signal which promotes glioma cell invasion [65,66]. In our study the individual variability in glutamate values does not permit to clearly identify a cut-off value of diagnostic or prognostic usefulness thus larger studies are needed.

Disclosure

No conflict of interest

Acknowledgement

This study was supported by a scholarship of "Stella Tsaga - Letta Despotidou" Foundation.

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