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Homocysteine, Genomic DNA Methylation and Cell-free DNA Levels as Biomarkers for Glioblastoma Patient's Outcome

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Abstract

Introduction: Glioblastoma (GBM) is characterized by recurrence (rGBM), resistance to chemotherapy and low life expectancy. The methyl grouping pathway plays a crucial role in macromolecule synthesis, gene expression control and maintenance of cellular redox balance. Under physiological conditions, the clearance pathways of homocysteine (Hcys) do not exist in the brain. This work aimed to determine circulating homocysteine levels according to the location of the tumor lesion (lobar or deep).

Material and methods: Hcys was dosed by enzymatic method in the serum of 61 patients at the time of inclusion in the Phase I/II study protocol (CONEP 9681 no: 25000.009627/2004-25). Medians were compared between the groups according to tumor location and statistical significance by effect size. Moreover, statistical significance by effect size between survival and homocysteine level and significance by effect size between survival and tumor location were calculated.

Results: Cohort included 65.6% men and 34.4% women (age 19-81 years). The mean value of Hcys was 63 times higher than the physiological maximum limit and 8 times higher than in severe hyperhomocysteinemia. Patients with tumor with deep localization had higher Hcys than rGB with lobar tumor. Patients with ≤ 585 μM survived longer than ≥ 585 μM. Patients with right hemisphere tumor localization survived longer than left hemisphere tumor localization.

Conclusions: The results confirm that Hcys may be an indicator of the highly proliferative characteristic and heterogeneity of the methyl group pathway in the different brain microenvironments in rGBM related to distinct microenvironments with marked metabolic demand.

Keywords: Glioblastoma • Homocystein

Introduction

Glioblastoma multiforme (GB) is the most common primary malignancy of the central nervous system [1]. GB is a highly anabolic, proliferative, infiltrative and diffuse primary brain tumor, characterized by recurrence (rGB), resistance to chemotherapy and low life expectancy of 14 months with a survival median of 12 months [2]. Furthermore, GB is associated with different pre and postoperative treatment approaches such as surgery, radiotherapy and chemotherapy with Themozolamide (TMZ), an alkylant agent capable of methylating nucleotide bases and, as a consequence, preventing cell replication. The chemoresistance may be due to vascularization´s difficulty to deliver the drug through the blood brain barrier (BBB) or poor survival with TMZ, which leads to poor prognosis [3,4]. Recently, it has been noticed the relations between high levels of homocysteine (Hcy) and severe pathologies, namely, neurodegenerative disorders [5], lung, colorectal and nervous tissue carcinomas [6]. Methionine (Met) is an aminoacid derived by dietary intake and its methyl grouping pathway plays a crucial role in macromolecule synthesis, gene expression control and maintenance of cellular redox balance. Met is essential for the synthesis of Hcy by transmethylation and its pathway is closely associated with epigenetic processes, including DNA methylation.

Under physiological conditions, the optimal total concentration of Hcy in the plasma is in the range of 5 to 15 μM. Further, when the range of Hcy is between 16 and 30 μMol/L is classified as moderate, 31–100 μMol/L as intermediate and above 100 μMol/L as severe hyperhomocysteinemia (HHcy) [7]. Fluctuations in Hcy levels are related to various diseases, making Hcy an important marker of impaired amino acids and protein homeostasis [5]. Due to tumor cell proliferation, HHcy above 100 μM occurs by inactivation of one-carbon metabolism and folate depletion [8].

DNA methylation is associated with Hcy metabolism through methionine and the generation of S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH). SAM donates the methyl group to the carbon-5 position of cytosine bases, generating 5-methylcytosine and DNA methylation, silencing genes [9]. A lack of essential one-carbon nutrients, including Met, folic acid or choline, significantly reduces SAM and SAM/SAH ratio, besides a decreased global DNA methylation [5]. HHcy and hypomethylation results in a proinflammatory micro ambience in the brain, which leads to ischemia and progression of the disease. Moreover, HHcy is associated with a decreased survival [10].

HHcy is related to many substances such as folate, B12, B6 and methylenetetrahydrofolate reductase (MTHFR). During the remethylation process, homocysteine can return to methionine by the conjunct action of 5-methyltetrahydrofolate, methionine synthase and B12, as a cofactor. To produce 5-methyltetrahydrofolate from 5,10-methylene tetrahydrofolate, MTHFR and B2 behave as co-factors. Folate (B9) is also important in the fabrication of dihydrofolate (DHF) and then in tetrahydrofolate (THF), which originates, using B6 as co-factor, 5,10-methyleneTHF, the same substance that results from methionine synthase´s reaction mentioned before.

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Our study aimed to provide the correlation between HHcy and genomic DNA methylation as biomarkers for glioblastoma's patient's outcomes (Figure 1).

Materials and Methods

The study is a retrospective cohort with 61 patients who had rGB. From those, a database with patients' record was created, including sex, quantity of the patients, tumor location and age, as well as survival weeks, micromolar levels of Hcy and percent of global methylation levels. All patients have tried before other common methods of treatment, such as surgery/radiation/chemotherapy but the outcome weren't effective.

Hcy was measured by enzymatic method in the serum of patients with recurrent GB at the moment of inclusion in the protocol Phase I/II (CONEP 9681 no: 25000.009627/2004-25). They were terminally ill and consented to administer intranasal monoterpene perillyl alcohol (POH) before the protocol (CONEP 9681 no: 25000.009627/2004-25) was executed. Pregnant women, breastfeeding and under 18 years old were not included in this study. While the 61 patients were using perillyl alcohol, none were under any other treatment for GB.

All data were analyzed using SPSS Program (SPSS, v.20) and the results are presented as count (%), mean ± standard deviation or median. Patients were compared using parametric (t-test) and nonparametric tests (Mann-Whitney, Cohen's d). All p values less than 0.05 were considered significant. Statistical significance by effect size between tumor location (right and left hemisphere) and survival were calculated. Patients were divided into two groups according to their Hcy level. They were also divided into two groups according to their survival.

Results

Demographic and clinical characteristics

The study included 61 patients, 65.6% men and 34.4% women (age 19-81 years). The mean value of Hcys before the inclusion in the protocol was 761.58 μM, which is 63 times higher than the physiological maximum limit (12 μM) and eight times higher than the severe HHcy (100 μM). Sixty-one patients had an age mean of 52.07 ± 14.17 years, Hcy level of 806 μM ± 437.53 μM and methylation of 75.52% ± 58.17%; 21 women (34.4%) with an age mean of 50.86 years composed the group and nineteen had lobar tumor location and two had deep location of the tumor; 40 men (65.6%) with an age mean of 52.7 years old and 29 had lobar location and 11 deep location (Table 1).

Distribution of 61 patients according to their Hcy μM level

Two groups were made according to Hcy level (above or lower than 585 μM).

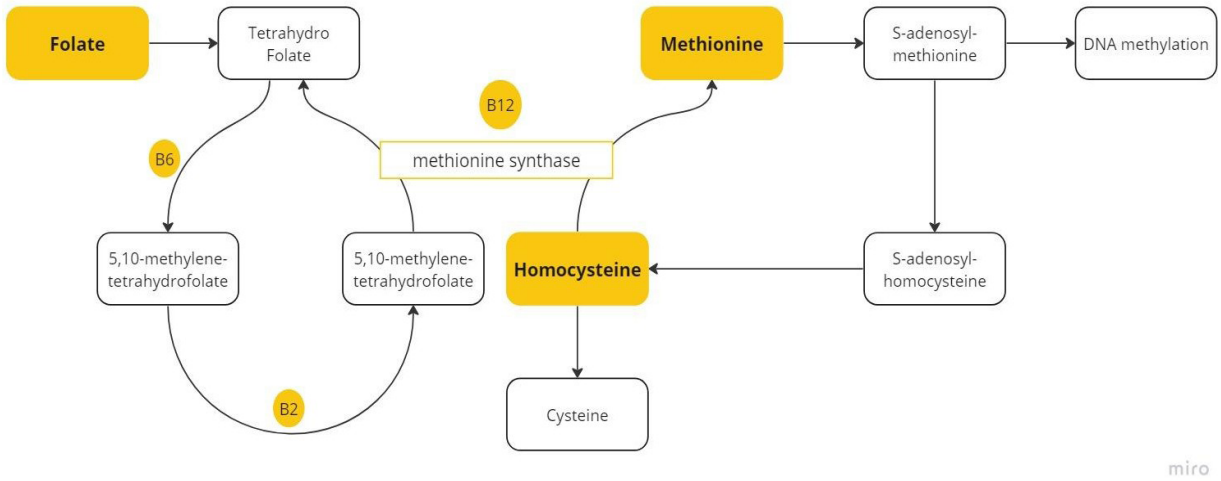

Figure 1. Homocysteíne reactions

Table 1. Demographic characteristics of the 61 patients in this study.

		N (%)	Tumor Localization (N)	Age (mean)
Sex	Female	21 (34.4%)	19 lobar e 2 deep	50.86±14.85
	Male	40 (65.6%)	29 lobar e 11 deep	52.7± 13.98

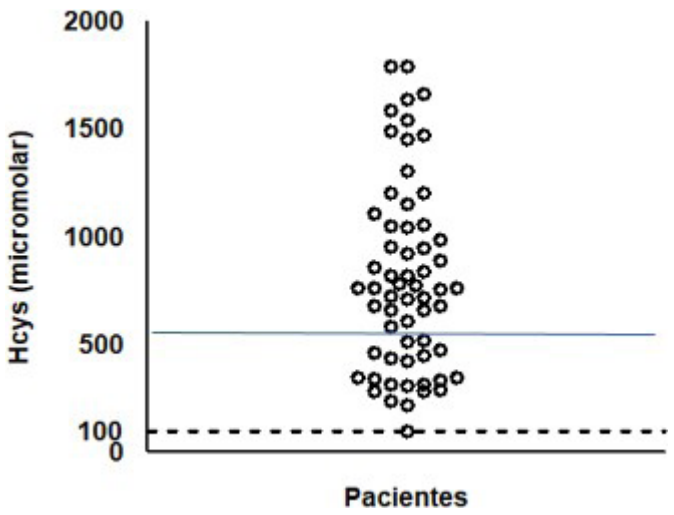

Figure 2. Distribution of patients according to Hcy level (μM). Due to ample distribution, it is important to stratify 2 groups according to Hcys level (585 μM).

Table 2. Patients divided in two groups according to their Hcy μM level.

Group	Patients	Homocysteine μM (mean)	Tumor Localization (deep/lobar)	Tumor Localization (hemisphere)	Survival Weeks (mean)
1(≤585μM)	20	348.83 μM ± 425.49 μM	18 lobar 2 deep	9 RH 3 LH	99.41 ± 87.88
2(≥585μM)	41	1029 μM ± 433.93 μM	30 lobar 11 deep	8 RH 16 LH	46.69 ± 85.82

Table 3. Patients divided in two groups according to tumor localization.

		Survival (weeks) ± std
Tumor localization	Right hemisphere	83.11 ± 85.82
	Left hemisphere	32.94 ± 60.2

Table 4. Patients divided in two groups according to survival period.

Survival	N	Mean Hcy ± std
< 25 weeks	15	918.3 ± 450.7
> 25 weeks	25	684.7 ± 408.7

Patients with ≤ 585 μM had Hcy mean 348.83 μM ± 425.49 μM and survival mean 99.4 ± 87.88 weeks. Patients with ≥ 585 μM had Hcy mean 1029 μM ± 433.93 μM and survival mean 46.69 ± 85.82 weeks. Statistical significance by effect size Cohen d=0.57 (Figure 2 and Table 2).

Tumor localization and survival mean (weeks)

Patients with right hemisphere tumor location had 83.11± 85.82 survivals mean and patients with left hemisphere tumor location had 32.94 ± 60.2 survival mean. Statistical significance by effect size Cohen d=0.85 (Table 3).

Survival and Hcy μM level mean

Twenty-five patients survived more than 25 weeks with 684.7 ± 408.7 μM Hcy mean and fifhteen patients survived less than 25 weeks with 918.3 ± 450.7 μM Hcy mean. Statistical significance by effect size Cohen d=0.55 (Table 4).

Discussion

HHcy is a condition that increases the risk for cardiovascular and neurodegenerative diseases [8]. The mechanism of how Hcys interfirs with the brain´s function is not yet fully understood, but it can be throught blood vessels, modifying the exchange of compounds between the bloodstream and brain parenquima [9]. In our study, we aimed to analyze if Hcy and Met can be used as a biomarker for GB. Survival mean was higher in patients with lower Hcy levels (≤ 585 μM) compared to patients with higher (≥ 585 μM) Hcy levels. Furthermore, when 61 patients were divided according to their survival, those who survived less than 25 weeks had mean Hcy levels of 918.3 ± 450.7 μM. However, those who survived more than 25 weeks had mean Hcy levels of 684.7 ± 408.7 μM. This pottencially means that Hcy values measuring can be used as a biomarker for GB. Previous studies recognised Hcy as a potential tumor biomarker for cancer patients during treatment whereas HHcy can be a predictive risk factor for carcinogenesis [10].

DNA methylation is important for normal genome development and regulation. It is also an essential factor for GB prognosis, whereas mutations in DNA methylation are common in cancer cells and are considered an early event associated with cancer progression. Abnormal methylation of promoters can affect genes connected to tumor suppressors, which results in cancer development [11-13]. Methylation pathway is usually damaged in GB patients, resulting in hypomethylation. Furthermore, regional hypomethylation of DNA sequences is noticed during the early stages of tumorigenesis as well as in hyperplasia and abnormal non-neoplastic tissue [14]. Gene hypomethylation is responsible for overexpression, which becomes a hazard specilly when related to genes that were once silenced. This process is also associated to tumors progression and, in some cases, its degree of malignancy. On the other hand, hypermethylation is linked to transcriptional silencing. In our study, global DNA hypomethylation patients had lower tax of survival in comparison to hypermethylated patients. Therefore, methylation could be an important biomarker for GB and its prognosis.

The gliomas anatomic topographic location influenciates treatment options and prognosis [8]. The scientific literature diverges whether left hemisphere tumors are correlated or not to longer survival. In our study, longer survival was shown in patients with right hemisphere tumors (83.11 ± 85.82 weeks), when compared to the left hemisphere (32.94 ± 60.2 weeks) with statistical significance by effect size Cohen d=0.85. Furthermore, deep location tumors had higher Hcys (1044.69 μM) than rGB with lobar tumor (717.09 μM; d=0.485). Hcy is very cytotoxic for the brain which can indicate lower survival, speciality for deep tumors which a more difficult total resection.

Conclusion

For different brain microenviromens with marked metabolic demand, the results confirm that Hcys may be an indicator of the highly proliferative and heterogeneity characteristics of the methyl group pathway. Administration of Perillyl Alcohol in patients with rGB had better outcome if the tumor was in the right hemisphere, had lower levels of homocysteine (≥ 585 μM) and higher levels of methylation.

Conflict of Interest

None.

Acknowledgement

No potential conflict of interest was reported by the authors.

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