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ORIGINAL ARTICLE

# The importance of the blood levels of homocysteine, folate and vitamin B12 in patients with primary malignant brain tumors

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## Summary

***Purpose:*** *Glioblastoma multiforme and anaplastic astrocytoma represent one of the most frequently occurring primary brain tumors with dismal survival rates. The aim of our study was to investigate whether values of homocysteine, folates and vitamin B12 can be prognostic markers in relapse diagnosis, treatment and monitoring of adult patients with malignant brain tumors.*

***Methods:*** *Twenty-seven patients from the Neurosurgical Clinic, Clinical Center of Serbia with diagnosed malignant brain tumors (anaplastic astrocytoma GR III and glioblastoma multiforme GR IV), were included in the study. The patients were divided in two groups according to the progression of disease, 15 with and 12 without progression.*

***Results:*** *Mean values of homocysteine were significantly higher in the group with progression compared to the group without malignant tumor progression, at the baseline point and after six months. Mean values of folate were similar across groups in all measurements, except in the 3rd month after surgery. Results regarding vitamin B12 were similar to folate, without any significance in group comparisons in the examined time points, as well as in vitamin B12 values change.*

***Conclusions:*** *Our results pointed out that total homocysteine in blood circulation appears to be a tumor marker for monitoring primary malignant brain tumor patients before and after surgery. The association of hyperhomocysteinemia with folate deficiency, also provides strong support for viewing hyperhomocysteinemia as a predictive marker for carcinogenesis. It is hoped that future research will continue to explore the clinical relevance of homocysteine as a tumor marker and a risk factor for astrocytoma and glioblastoma.*

***Key words:*** *brain tumor, folate, homocysteine, hyperhomocysteinemia, vitamin B12*

## Introduction

Glioblastoma multiforme (GBM), a grade (GR) IV glioma, represents the most frequently occurring primary malignancy of the central nervous system (CNS) [1] and one of the most fatal cancers, leading to about 4% of deaths associated with cancer [2]. Regarding this, GBM is of the greatest challenges despite different aggressive pre- and postoperative treatment approaches, including surgical tumor mass removal, chemo- and radiotherapy. The median survival time remains dismal,

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about 14 months after diagnosis, whereby 2-year survival rate is only 3-5% [3].

Anaplastic astrocytoma (AA) is a primary brain tumor, diffusely infiltrating, malignant, astrocytic, most commonly seen in young patients. AA causes 4% of all CNS malignant tumors and 10% of all gliomas. Comparing to GMB, the survival rate is longer, 3 years with conventional treatment and 28% of 5-year survival rates [4].

In recent years, the possible effects of homocysteine (Hcy) and its metabolic compounds on the nervous tissue carcinomas has been shown. High growth rate is typical for malignant cells. Due to increased processes of transmethylation and proteosynthesis, higher methionine (Met) concentrations are claimed. Met is essential for the synthesis of Hcy by transmethylation. Tumor cell proliferation is linked with elevated Hcy followed by inactivation of one-carbon metabolism and folate depletion. Disability of tumor cells to transform Hcy to Met leads to increased Hcy levels. This potentially means that this parameter can be used as a one marker for carcinogenesis [5]. In addition to previous mentioned, a growing interest in Hcy role in neurological disorders has been shown. Furthermore, elevated values of Hcy were associated with lung [6] and colorectal carcinoma [7]. Alerts in the methionine cycle are also present in breast [8], pancreatic [9] and laryngeal cancer [10].

Nevertheless, experimental data obtained from the studies conducted on human glioblastoma cell lines, indicate that these cells die in the case D,L-Hcy application at a concentration of 50 μM. These findings suggest that Hcy in the condition of its elevated values, hyperhomocysteinemia (HHcy), can behave as a gliotoxic agent, capable of inducing the death of human glial cells, but there is a small number of studies that have addressed this issue [11].

The serum Hcy concentration is a sensitive indicator of the folate organism status, while the folate deficiency is often associated with HHcy whereby the folate intake can lower the Hcy level [12]. On the other hand, serum level of vitamin B12 can be used as a prognostic indicator of survival time in patients with metastatic carcinoma of the nervous tissue. The risk is higher due to higher vitamin B12 levels, indicating that the level of vitamin B12 can be used as a diagnostic marker. Furthermore, relation between higher vitamin B12 level and a worse survival outcome was established [13].

Deficiency of vitamins B9 and B12 is important cause of HHcy since these vitamins are involved in Hcy metabolism [14]. The folate deficiency leads to a reduction in the uracil corporation into DNA, which results in the instability of DNA molecule. Previous data indicates that folate supplementation can be used as an additional therapeutic agent in the glioma treatment in order to limit the level of DNA methylation since it leads to poorer prognosis in patients with GBM [15]. Elevated serum Hcy level and folate deficiency are associated with increased overall risk of cancer [16].

Taking into account all the above data, it can be noticed that the effect of Hcy, folates and vitamin B12 on the formation, development and treatment outcome of the patients with brain cancer is a very intriguing question. The answer requires additional experimental and clinical research. There are insufficient literature data about incidence of elevated Hcy levels in the blood, as well as folates and vitamin B12 disorders in malignant brain tumors. Although the adverse effect of Hcy on neurons is well documented, knowledge about the impact of this amino acid on glial cells is missing. Considering the above mentioned the aim of our study was to investigate whether values of Hcy, folates and vitamin B12 can be prognostic markers in relapse diagnosis, treatment and monitoring of adult patients with malignant brain tumor.

## Methods

*Study population*

Twenty-seven patients from the Neurosurgical Clinic, Clinical Center of Serbia with diagnosed malignant brain tumors (AA GR III and GBM GR IV), were included in the study. The patients were divided in two groups according to the progression of disease (clinical and neurological deterioration due to elevated intracranial pressure, headaches, nausea and vomiting as well as contralateral weakness), 15 with and 12 without progression.

The inclusion criteria were AA GR III and GBM GR IV, radiologically diagnosed (MRI) and pathohistologically confirmed. The exclusion criteria were treatment with chemo- or radiotherapy in the last two years prior to beginning of the study, as well as patients who were on chronic immunosuppressive therapy in any time for any reason. Furthermore, all patients with psychoorganic syndrome were excluded. The degree of cognitive ability and the presence of dementia were established by Mini-Mental State Examination (MMSE).

Illness assessments included a detailed anamnestic data, comorbidities and socio-economic data. All current medications were recorded. Additionally, at the time of blood sampling the patients were free of any medication known to influence level of vitamin B6, B12 or homocysteine.

The study was conducted in accordance to the principles outlined in the Declaration of Helsinki and principles of Good Clinical Practice (GCP). Written informed consent was obtained from all participants, and the study protocol was approved by the Ethics Committee of the Clinical Center of Serbia, prior to the onset of the study.

*Blood sampling*

The blood for the basic group characteristics, regarding blood cell count, lipids, liver function tests, kidney function tests and inflammation parameters, was collected at the beginning of the study, before surgery. The blood for homocysteine, vitamin B12 and folates measurements, was collected in four time points, firstly preoperative and then one, three and six months after surgery.

Following at least a 10-h fasting period, blood was collected in Vacutainer tubes (BD Vacutainer Blood Collection System) in a quiet, air-conditioned, temperature-controlled room (22-24°C).

*Laboratory analyses*

Laboratory analyses of leukocytes, erythrocytes, platelets (Beckman Coulter LH780 analyzer), hemoglobin (Cyanmethemoglobin method, Beckman Coulter LH780 analyzer), lipid profile (cholesterol, triglycerides-Beckman Coulter AU680 analyzer), liver function (alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP)-IFCC method, Beckman Coulter AU680 analyzer), urea (Enzymatic method, Beckman Coulter AU680 analyzer), creatinine (Jaffe method, Beckman Coulter AU680 analyzer), C-reactive protein (CRP) (Turbidimetric method, Beckman Coulter AU680 analyzer), fibrinogen (Clauss method, ACL TOP analyzer by Instrumentation Laboratory), procalcitonin (ECLIA, Cobas e 411, Roche Diagnostics, Mannheim, Germany), homocysteine (HPLC method); vitamin B12 (Chemiluminescent paramagnetic microparticle immunoassay; Access2, Beckman Coulter, USA; folate (Competitive-binding receptor assay Access2, Beckman Coulter, USA), were performed in the Clinical Center of Serbia.

*Statistics*

The results are presented as count (%), mean ± standard deviation or median (25-75$^{th}$ percentile), depending on data type and distribution. Groups were compared using parametric (t-test) and nonparametric (chi-square, Mann-Whitney U test) tests. Linear mix model was used to assess significant correlations of progression and change in the examined parameters. All p values less than 0.05 were considered significant. All data were analyzed using SPSS 20.0 (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.) and R 3.4.2. (R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/).

## Results

*Demographic and clinical characteristics*

The study included 27 patients with AA GR III and GBM GR IV, 12 without progression and 15 with progression. Basic characteristics of groups regarding, age, blood cell count, lipids, liver func-

**Table 1.** Demographic and clinical characteristics in the group of patients with primary malignant brain tumors

| *Characteristics* | *Progression* | | *p value* |
|---|---|---|---|
| | *no* | *yes* | |
| Age (yrs) | 46.2±19.4 | 48.7±9.2 | 0.876[a] |
| Gender | | | |
| Male, n (%) | 6 (42.85) | 8 (57.15) | |
| Female, n (%) | 6 (46.15) | 7 (53.85) | |
| Blood | | | 0.919[b] |
| Leukocytes ($10^9$/L) | 8.18±2.47 | 8.09±2.06 | 0.579[b] |
| Erythrocytes ($10^{12}$/L) | 4.55±0.54 | 4.41±0.76 | 0.199[b] |
| Hemoglobin (g/L) | 138.3±5.3 | 131.9±17.6 | 0.752[b] |
| Platelets (109/L) | 238.1±43.6 | 232.5±45.8 | 0.927[b] |
| Cholesterol (mmol/L) | 6.22±0.88 | 6.25±0.72 | 0.412[b] |
| Triglycerides (mmol/L) | 2.73±0.88 | 3.01±0.83 | 0.045[a] |
| AST (IU/L) | 18.5 (13.5-22.5) | 25.0 (18.0-52.0) | 0.022[a] |
| ALT (IU/L) | 32.0 (23.0-41.0) | 55.0 (38.0-83.0) | 0.671[b] |
| ALP (U/L) | 80.0±22.6 | 75.9±25.8 | 0.608[a] |
| Urea (mmol/L) | 5.65 (4.70-9.05) | 7.30 (5.30-10.20) | 0.179[a] |
| Creatinine (umol/L) | 67.4 (62.3-74.6) | 69.9 (67.5-79.9) | 0.961[a] |
| C-reactive protein (mg/L) | 1.55 (1.10-2.25) | 1.90 (1.10-2.30) | 0.416[b] |
| Fibrinogen (g/L) | 3.38±1.21 | 3.06±0.82 | |
| Procalcitonin (ng/ml) | 1.56±0.35 | 1.57±0.50 | 0.961[b] |

The values are expressed as mean ± SD or median (range). AST: aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: alkaline phosphatase. [a]Mann-Whitney U test; [b]T-test

tion tests, kidney function tests and inflammation parameters are presented in Table 1.

Patients were similar regarding age, blood count, lipids, inflammatory markers and kidney function. Significant difference between groups was in AST and ALT median values. The group of patients with progression of disease had significantly higher median values of these parameters (Table 1). Pathological characteristics of tumors and a number of GBM and AA per group are presented in Table 2.

*Levels of homocysteine, folate and vitamin B12*

The mean values of Hcy were significantly higher in the group with progression compared to the group without malignant tumor progression, at the baseline point and after six months. The mean change of Hcy during the entire follow up period was higher in the group without progression, but without significance regarding the difference between groups (Table 3). Using the linear mix model, significant interaction of progression x time was obtained (p=0.029). But it is obvious that the effect was mostly due to change between baseline and 1 month, as well as 3 months and 6 months, not baseline and the last measurement.

The mean values of folate were similar across groups in all measurements, except in the 3rd month after surgery. The mean change of folate from baseline to the end of follow up period was similar in both groups and no significant difference was obtained (Table 3). Using the linear mix model

**Table 2.** Pathological characteristics of tumors type found (% of total), stage/grade

| *Gender* | *Total number* | *Without progression n (%)* | *With progression n (%)* |
|---|---|---|---|
| | Glioblastoma multiforme (GR IV) | | |
| Male | 11 | 5 (45.45) | 6 (54.55) |
| Female | 10 | 4 (30) | 6 (70) |
| | Anaplastic astrocytoma (GR III) | | |
| Male | 3 | 1 (33.33) | 2 (66.67) |
| Female | 3 | 2 (66.67) | 1 (33.33) |

**Table 3.** Comparisons of the levels of **A:** homocysteine, **B:** folate, and **C:** vitamin B12 in patients with primary malignant brain tumors preoperatively 1, 3 and 6 months after operation (groups taken as progression of tumor vs. absence of progression)

| | *Progression* | | *p value* |
|---|---|---|---|
| | *no* | *yes* | |
| **A)** Homocysteine (μmol/l) | | | |
| Preoperative | 10.98±6.49 | 15.60±4.16 | 0.034[b] |
| 1 month | 11.21±3.18 | 13.37±4.47 | 0.170[b] |
| 3 months | 9.91±2.49 | 12.47±4.50 | 0.090[b] |
| 6 months | 7.63±1.45 | 13.80±6.28 | 0.002[b] |
| Δ preop. - 6 months | 3.35±6.50 | 1.80±9.04 | 0.609[b] |
| **B)** Folate (μg/L) | | | |
| Preoperative | 3.56±2.60 | 2.31±0.83 | 0.494[a] |
| 1 month | 5.22±2.56 | 3.84±2.44 | 0.166[a] |
| 3 months | 6.12±1.62 | 4.29±1.85 | 0.012[a] |
| 6 months | 6.60±1.11 | 5.63±2.08 | 0.134[a] |
| Δ preop. - 6 months | 3.04±2.93 | 3.32±2.11 | 0.778[a] |
| **C)** Vitamin B12 (μg/L) | | | |
| Preoperative | 299.3±115.9 | 490.5±398.6 | 0.102[a] |
| 1 month | 401.7±145.4 | 530.0±336.0 | 0.196[a] |
| 3 months | 465.4±131.4 | 557.3±337.6 | 0.807[a] |
| 6 months | 544.1±173.0 | 607.3±308.6 | 0.884[a] |
| Δ preop. - 6 months | 244.8±223.4 | 116.9±261.1 | 0.190[a] |

The values are expressed as mean±SD. [a]Mann-Whitney U test, [b]T-test; Referent values: folate (3.9-26.8 μg/L); vitamin B12 (211-911 μg/L); homocysteine (4-14μmol/L).

with transformed data (logarithmic transformation was used), no significant interaction of progression x time was obtained (p=0.554).

The results regarding vitamin B12 were similar to folate, but without any significance in group comparisons in the examined time points, as well as in vitamin B12 values change (delta) (Table 3). Using the linear mix model, no significance was obtained regarding interaction of progression x time (p=0.531). Data was transformed using logarithmic transformation in order to obtain lower variability.

The examined parameters in four time points are graphically presented in Figure 1.

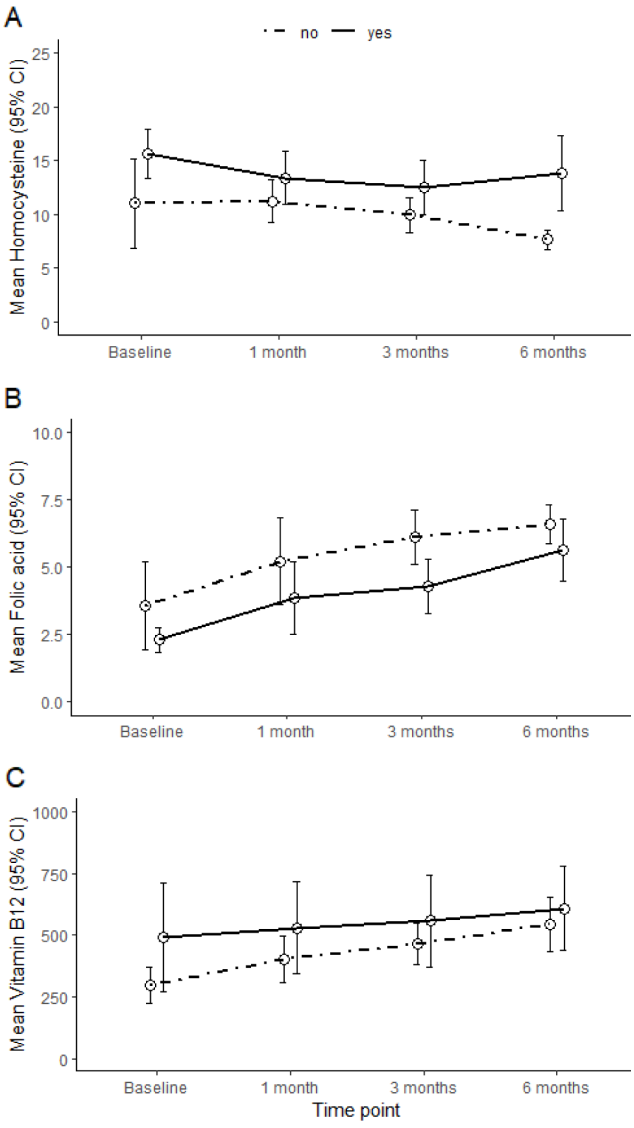


**Figure 1.** Levels of homocysteine **(A)**, folic acid **(B)**, and vitamin B12 **(C)** in patients with primary malignant brain tumors preoperatively and 1, 3 and 6 months after operation (groups taken as progression of tumor vs absence of progression). The results are expressed as mean value (95% confidence interval/CI). P values between the followed groups are presented in Table 3.

## Discussion

Hyperhomocysteinemia is a condition known as state of evaluated concentration of L-homocysteine, above 15μM, whereby even mild HHcy (10-30μM) increases the risk for cardiovascular diseases, stroke and neurodegenerative diseases [11,17]. HHcy represents an imbalance in molecules and processes involved in the Hcy metabolism such as genetic abnormalities; folate, vitamin B6 and vitamin B12 nutritional deficiencies; methionine rich diet, and renal function impairment [18].

In our study, we aimed to examine whether Hcy can be used as a prognostic and predictive marker in patients with diagnosed primary brain tumors, AA and GBM. Preoperative levels of Hcy were higher in patients with disease progression compared to patients without progression, wherein the values of Hcy in the group with progression were above physiological range. Those results could be related with the previously mentioned fact that tumor cells have a disability to transform Hcy to methionine, leading to increased Hcy levels [4]. This state is supported by decreasing Hcy levels in these patients after surgery. However, the levels of the Hcy remain significantly higher in the patients with progression compared to patients without progression, even six months after surgery, but in the referent values. This potentially means that Hcy values measuring can be used as a marker for carcinogenesis and progression of disease in AA and GBM. Previous studies recognized Hcy as a potential tumor biomarker for cancer patients during treatment monitoring, whereas HHcy can be a predictive risk factor for carcinogenesis [19]. Furthermore, according to previous data, disturbed Hcy metabolism can be associated with the presence of cancer [20]. Elevated and significant concentration of total Hcy have been found in many different types of carcinomas, showing a very important prognostic role of this molecule in carcinogenesis [7, 21-24].

Moreover, in these patients, total concentration of Hcy can be predictive marker of cardiovascular disorders considering the fact that Hcy has been recognized as a significant risk factor for atherosclerosis, coronary artery disease, cerebrovascular disease, peripheral vascular disease, myocardial infarction, and cardiovascular-related morbidity and mortality [25]. In that manner, this should be considered as a possible marker for cardiovascular disorders prevention in this population of patients. Furthermore, intake of folate and vitamin B6 through diet or supplementation can reduce the risk for cardiovascular diseases [25], given the fact that folic acid and vitamin B complex have a role in the Hcy metabolism, and that Hcy level can

increase due to minor or major deficiency of these vitamins [26,27].

Therefore, there is an inverse relation between plasma Hcy and folate. In our study, deficiency of folate was noticed. Values of this parameter were decreased in both groups before, with increased values after surgery as well as higher values in the group without disease progression, during the entire monitoring period. The lowest value of folate and the highest value of Hcy were observed preoperatively in the group with disease progression. Deficiency of folate additionally occurs in mild to moderate HHcy, associated with a variety of disorders [18], including induced and accelerated carcinogenesis [28]. Folate deficiency and elevated serum Hcy level are associated with increased overall risk of cancer [16].

Decreased plasma folate levels are also associated with altered DNA methylation, which is present in many diseases, including cancer. Appropriate DNA methylation is important for normal genome development and regulation [29]. In many types of cancer, such as metastatic prostate cancer, chronic lymphocytic tumors, and hepatocellular carcinoma, global genomic hypomethylation has been associated with an increased risk of cancer [30]. Furthermore, regional hypomethylation of DNA sequences is noticed during the early stages of tumorigenesis as well as in hyperplasia and abnormal non-neoplastic tissue [30]. Folate deficiency has been implicated in the pathogenesis of different carcinomas such as colorectal, breast, ovary, pancreas, brain, lung and cervix cancer [6,28,31]. However, in cancer patients, lower concentrations of folic acid are expected. The main reason for folate deficiency is because tumor cells need folate for *de novo* purine synthesis, whereby they have to draw folate from the blood [20].

The folates deficiency combined with HHcy can be predictive and prognostic indicator for poorer prognosis and disease progression in patients with malignant brain tumors. Moreover, those values can be markers for the effectiveness of surgical treatment due to the fact that values of these parameters return to physiological range after surgery.

The third examined parameter assumed to be a prognostic marker in patients with AA and GBM, was vitamin B12. In our study, there were no any difference in vitamin B12 values between the observed groups, whereby values of this parameter were in physiological range. Previous data indicates that cancer patients with elevated vitamin B12 levels have higher mortality compared to patients with normal levels of this vitamin [32]. These findings may have clinical significance for evaluating the prognosis of cancer patients [32], whereas a previous study found that in patients with the metastatic cancer, including neurological cancer, the serum vitamin B12 level can be used to predict survival time [13]. Furthermore, high vitamin B12 levels can be an unspecific marker for cancer [33,34]. On the other hand, there are different conditions which indicate deficiency of vitamin B12, primarily neurological or haematological abnormalities, as well as poor dietary intake in malnourished patients, such as elderly and alcohol abusers [35].

Thus, it can be assumed that folates can be more specific prognostic biomarkers in patients with malignant brain tumors, especially for those with progressive form of disease. Additionally, previous studies suggest that the serum B12 level might not be good tumor marker for other types of cancer such as hepatocellular carcinoma and it is not specific for this condition [36] as well as lung cancer [6], while other studies indicated correlation between vitamin B12 and hepatocellular carcinoma [37]. Those results can characterize an unspecific role of vitamin B12 in carcinogenesis due to dissenting opinions about the possible role of vitamin B12 as a predictor of clinical characteristics in patients with carcinoma. A previous study [38] on patients with neuroblastoma showed that neuroblastoma patients had slightly but not significantly lower serum vitamin B12 levels compared to healthy subjects, but none of them had serum vitamin B12 less than 150pg/ml, which represents deficiency. These findings indicated that serum vitamin B12 levels were within normal limits and there was no evidence of vitamin B12 deficiency in the group of patients with neuroblastoma. Therefore, these results correlate with our study, where vitamin B12 levels were in physiological range in patients with or without AA and GBM progression [38]. Besides, the fact that the level of vitamin B12 didn't change in patients with malignant brain tumors, a previous study showed that even in patients with normal serum level, treatment with vitamin B12 can correct defects and have beneficial effects in patients with malignancy [39].

## Conclusion

In summary, our results pointed out that total Hcy in blood circulation appears to be a tumor marker for monitoring primary malignant brain tumor patients before and after surgery. It also had a potential to be an early marker for carcinogenesis and progression of disease. The association of HHcy with folate deficiency also provides strong support for viewing HHcy as a predictive marker for carcinogenesis. It is hoped that future research will

continue to explore the clinical relevance of Hcy as a tumor marker and a risk factor for AA and GBM.

In addition, significantly higher Hcy and lower folate levels were found in plasma of patients with disease progression before surgery, compared to patients without progression, whereas there was no difference in B12 levels between the two groups. In order to evaluate the value of plasma Hcy and folate levels in malignant brain tumors as markers, further studies with larger number of patients are required.

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## Conflict of interests

The authors declare no conflict of interests.

## References

1. Wilson TA, Karajannis MA, Harter DH. Glioblastoma multiforme: State of the art and future therapeutics. Surg Neurol Int 2014;5:64.
2. Karim R, Palazzo C, Evrard B, Piel G. Nanocarriers for the treatment of glioblastoma multiforme: Current state-of-the-art. J Control Release 2016;227:23-37.
3. Adamson C, Kanu OO, Mehta AI et al. Glioblastoma multiforme: a review of where we have been and where we are going. Expert Opin Investig Drugs 2009;18:1061-83.
4. Grimm SA, Chamberlain MC. Anaplastic astrocytoma. CNS Oncol. 2016;5:145-57.
5. Škovierová H, Vidomanová E, Mahmood S et al. The Molecular and Cellular Effect of Homocysteine Metabolism Imbalance on Human Health. Int J Mol Sci 2016;17. pii:E1733. Review.
6. Tastekin D, Erturk K, Bozbey HU et al. Plasma homocysteine, folate and vitamin B12 levels in patients with lung cancer. Exp Oncol 2015;37:218-22.
7. Keshteli AH, Baracos VE, Madsen KL. Hyperhomocysteinemia as a potential contributor of colorectal cancer development in inflammatory bowel diseases: a review. World J Gastroenterol 2015;21:1081-90.
8. Cave DD, Desiderio V, Mosca L et al. S-Adenosylmethionine-mediated apoptosis is potentiated by autophagy inhibition induced by chloroquine in human breast cancer cells. J Cell Physiol 2018;233:1370-83.
9. Benavides MA, Bosland MC, da Silva CP et al. L-Methionine inhibits growth of human pancreatic cancer cells. Anticancer Drugs 2014;25:200-3.
10. Nacci A, Dallan I, Bruschini L et al. Plasma homocysteine, folate, and vitamin B12 levels in patients with laryngeal cancer. Arch Otolaryngol Head Neck Surg 2008;134:1328-33.
11. Škovierová H, Mahmood S, Blahovcová E, Hatok J, Lehotský J, Murín R. Effect of homocysteine on survival of human glial cells. Physiol Res 2015;64:747-54.
12. Murín R, Vidomanová E, Kowtharapu BS, Hatok J, Dobrota D. Role of S-adenosylmethionine cycle in carcinogenesis. Gen Physiol Biophys 2017;36:513-20.
13. Oh HK, Lee JY, Eo WK et al. Elevated Serum Vitamin B(12) Levels as a Prognostic Factor for Survival Time in Metastatic Cancer Patients: A Retrospective Study. Nutr Cancer 2018;70:37-44.
14. Shevchuk SV, Postovitenko KP, Iliuk IA et al. The relationship between homocysteine level and vitamins B12, B9 and B6 status in patients with chronic kidney disease. Wiad Lek 2019;72:532-8.
15. Hervouet E, Debien E, Campion L et al. Folate supplementation limits the aggressiveness of glioma via the remethylation of DNA repeats element and genes governing apoptosis and proliferation. Clin Cancer Res 2009;15:3519-29.
16. Zhang D, Wen X, Wu W, Guo Y, Cui W. Elevated homocysteine level and folate deficiency associated with increased overall risk of carcinogenesis: meta-analysis of 83 case-control studies involving 35,758 individuals. PLoS One 2015;10:e0123423.
17. Petras M, Tatarkova Z, Kovalska M et al. Hyperhomocysteinemia as a risk factor for the neuronal system disorders. J Physiol Pharmacol 2014;65:15-23. Review.
18. Djuric D, Jakovljevic V, Zivkovic V, Srejovic I. Homocysteine and homocysteine-related compounds: an overview of the roles in the pathology of the cardiovascular and nervous systems. Can J Physiol Pharmacol 2018;96:991-1003.
19. Wu LL, Wu JT. Hyperhomocysteinemia is a risk factor for cancer and a new potential tumor marker. Clin Chim Acta 2002;322:21-8. Review.
20. Hasan T, Arora R, Bansal AK, Bhattacharya R, Sharma GS, Singh LR. Disturbed homocysteine metabolism is associated with cancer. Exp Mol Med 2019;51:21. Review.
21. Aleksic D, Djokic D, Golubicic I, Jakovljevic V, Djuric D. The importance of the blood levels of homocysteine, folic acid and vitamin B12 in children with malignant diseases. JBUON 2013;18:1019-25.
22. Gatt A, Makris A, Cladd H et al. Hyperhomocysteinemia in women with advanced breast cancer. Int J Lab Hematol 2007;29:421-5. Erratum in: Int J Lab Hematol 2008;30:87.
23. Santotoribio JD, Del Valle-Vazquez L, García-de la Torre A, Del Castillo-Otero D, Lopez-Saez JB, Sanchez Del Pino MJ. The diagnostic value of pleural fluid homocysteine in malignant pleural effusion. PLoS One 2019;14:e0222616.
24. Varela Almanza KM, Puebla-Pérez AM, Delgado-Saucedo JI et al. Increased homocysteine plasma levels in

breast cancer patients of a Mexican population. Exp Oncol 2018;40:114-8.

25. Fanapour PC, Yug B, Kochar MS. Hyperhomocysteinemia: an additional cardiovascular risk factor. WMJ 1999;98:51-4. Review.
26. Mahalle N, Kulkarni MV, Garg MK, Naik SS. Vitamin B12 deficiency and hyperhomocysteinemia as correlates of cardiovascular risk factors in Indian subjects with coronary artery disease. J Cardiol 2013;61:289-94.
27. Molina-López J, Molina JM, Chirosa LJ, Florea DI, Sáez L, Planells E. Effect of folic acid supplementation on homocysteine concentration and association with training in handball players. J Int Soc Sports Nutr 2013;10:10.
28. Duthie SJ. Folate and cancer: how DNA damage, repair and methylation impact on colon carcinogenesis. J Inherit Metab Dis 2011;34:101-9.
29. Crider KS, Yang TP, Berry RJ, Bailey LB. Folate and DNA methylation: a review of molecular mechanisms and the evidence for folate's role. Adv Nutr 2012;3:21-38. Review.
30. Ehrlich M. DNA hypomethylation, cancer, the immunodeficiency, centromeric region instability, facial anomalies syndrome and chromosomal rearrangements. J Nutr 2002;132:2424S-29S.
31. Phelip JM, Ducros V, Faucheron JL, Flourie B, Roblin X. Association of hyperhomocysteinemia and folate deficiency with colon tumors in patients with inflammatory bowel disease. Inflamm Bowel Dis 2008;14:242-8.
32. Arendt JF, Farkas DK, Pedersen L, Nexo E, Sørensen HT. Elevated plasma vitamin B12 levels and cancer prognosis: A population-based cohort study. Cancer Epidemiol 2016;40:158-65.
33. Arendt JF, Nexo E. Unexpected high plasma cobalamin: proposal for a diagnostic strategy. Clin Chem Lab Med 2013;51:489-96.
34. Arendt JF, Pedersen L, Nexo E, Sørensen HT. Elevated plasma vitamin B12 levels as a marker for cancer: a population-based cohort study. J Natl Cancer Inst 2013;105:1799-805.
35. Shipton MJ, Thachil J. Vitamin B12 deficiency - A 21st century perspective. Clin Med (Lond) 2015;15:145-50.
36. Buamah PK, James OF, Skillen AW, Harris AL. Serum vitamin B12 levels in patients with primary hepatocellular carcinoma during treatment with CB3717. J Surg Oncol 1987;34:100-03.
37. Cui LH, Quan ZY, Piao JM et al. Plasma Folate and Vitamin B12 Levels in Patients with Hepatocellular Carcinoma. Int J Mol Sci 2016;17. pii: E1032.
38. Areekul S, Hathirat P, Churdchu K. Folic acid, vitamin B12 and vitamin B12 binding proteins in patients with neuroblastoma. Southeast Asian J Trop Med Public Health 1986;17:184-8.
39. Volkov I. The master key effect of vitamin B12 in treatment of malignancy-a potential therapy? Med Hypotheses 2008;70:324-8.