

UNIVERSITY OF MEDICINE AND PHARMACY OF
CRAIOVA
DOCTORAL SCHOOL

PhD THESIS

ABSTRACT

**THE EVALUATION OF SOME BIOCHEMICAL
MARKERS IN BRAIN TUMORS**

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Craiova 2013

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KEY WORDS:

brain tumors, astrocytoma, glioblastoma, meningioma, brain metastasis,
glycemia, insulin, ferritin

INTRODUCTION

Brain tumors represent one of the most complex part of neurology. The great cell diversity, the evolution in an inextensible region of the body, the invasive character and the presence of oedema are characteristics that individualize these neoplasms.

In the last years, there is a tendency in increasing the survival period of patients diagnosed with brain tumors. Surgery and radiotherapy are the main therapeutic approaches. Chemotherapy represents a secondary choice, the vast majority of studies showing a marginal benefit in increasing the asymptomatic interval and survival period.

GENERAL PART

Recent epidemiologic studies realized on brain tumors come up against some difficulties, mainly because of the uneven access to imagistic investigations, the lack of histopathological diagnosis confirmation and the complexity of histological examination..

Central Brain Tumors Registry (CBTRUS) centralizes and analyzes epidemiological data about brain tumors in the United States. In the latest 2012 CBTRUS report, of the total brain tumors analyzed 35.5% represent meningiomas, 15.8% glioblastomas, 14.1% pituitary tumors, 6.3% astrocytomas, 2.2% CNS lymphoma, 1.8% oligodendrogliomas, 2.0% ependymomas, 1.2% embrionary tumors and 8.3%. nerve sheet tumors. Glial tumors represent 30% of the total, the most frequent of them being glioblastomas (54%).

Tumor markers are biomolecules that have increased values associated with neoplasia. These are key factors in cancer prognosis, diagnosis and treatment. They can be found in blood, urine or various tissues. These molecules can be produced either by the tumor itself or they appear as a response of healthy tissues in the presence of the tumor.

Some tumor markers evaluated in brain tumors were protein p53, protein p16 and protein Rb, 1p/19q deletions, PTEN (phosphatase and tensin homolog), MGMT (O6-methylguanin-methyltransferase), their presence influencing the prognosis and the treatment response.

Tyrosine kinase receptors and their ligands (i.e. growth factors) are involved in the development and progression of the neoplastic process, their inhibition or activity modulation being the target of new antineoplastic drugs. The main growth factor families involved in brain tumors are EGF, PDGF, VEGF and IGF.

The IGF family includes the insulin ligand, the insulin like growth factors (IGF1 and IGF2) and their receptors (IR, IGF-1R and IGF-2R). The main components that are expressed in brain tumors are IGF-1, IGF-2 and IGF-1R. The presence of these family members was found in glioblastoma, astrocytoma, meningioma, meduloblastoma and ependymoma.

Glucose is the main energy source for the brain. The brain is one of the organs with high energy demands, brain metabolism being completely dependent upon blood glucose. Although it represents 2% of total body mass, brain metabolism utilizes 20% of the available oxygen and 25% of the available glucose.

One of the first metabolic changes that appears during oncogenesis is the Warburg effect. Otto Warburg showed that neoplastic cells use predominantly anaerobic glycolysis for energy purposes. This fact also explains the resistance of malignant cells to hypoxia.

Energy production in glial tumors is exclusively dependent on glucose availability. Malignant cells cannot use alternative substrates, such as ketone bodies or glutamine, for energy.

It is well known that insulin stimulates growth, migration and cell division. However, its mitogenic effects are weaker than those of IGF, PDGF, VEGF or EGF.

The association diabetes-cancer is one of the problems brought to discussion in the last years. The vast majority of authors consider that diabetes mellitus (especially type II) is associated with high risk of cancer and high mortality in the presence of cancer.

Ferritin is an intracellular protein that is involved in iron storage, iron transport and release.

There are a number of hypotheses about the role of iron and ferritin in carcinogenesis. Free iron was reported to be toxic for the cells and it was also demonstrated to be a catalyst in Fenton reaction between ferrous iron (Fe^{2+}) and hydrogen peroxide. Ferritin appears as a defense mechanism that transforms ferrous iron in ferric iron and deposits it in a non-toxic manner. Iron was also indicated as a factor

involved in cell proliferation, being required in the transition from G1 to S phase of cell cycle.

High ferritin synthesis in glial malignant cells could result in high ferritin concentrations in plasma or CSF. High CSF levels were found in gliomas, CNS lymphoma and meningeal carcinomatosis. High plasma levels were found in grade II, III and IV astrocytomas. Some studies reported decreases in plasma ferritin levels after surgical resection for astrocytoma.

PERSONAL STUDY

1. OBJECTIVES

Knowing the great diversity of brain tumors, the first objective was to identify the frequency of various histologic types, to analyze the distribution of tumor grades and the tumoral distribution according to the tissue of origin. Then, we analyzed the influence of sex and age on histologic type, tumor grade or tissue of origin of the studied tumors and we compared our results with other published data.

Another objective was to determine the glucose plasmatic levels of our patients in order to analyze the distribution of its values according to tumor type, tumor grade or tissue of origin. Other investigated variables were insulin and ferritin. We determined their plasmatic levels and statistically analyzed the data.

For each of these three parameters we studied a possible influence of the distribution of abnormal high values in a certain histologic type, tumor grade or tissue of origin.

2. MATERIAL AND METHOD

We conducted a prospective study during a 6-year period in the 3rd Neurosurgery Clinic of Bagdasar-Arseni Hospital, Bucharest. We followed 267 consecutive patients diagnosed with brain tumors during December 2006- February 2012.

We excluded from the study the patients who received corticotherapy before biological samples were collected.

We used an enzymatic colorimetric method to measure plasma glucose concentration. Immunoenzymatic colorimetric methods were used for quantitative assay of plasma insulin and ferritin.

To test data distribution we used Anderson-Darling and Shapiro-Walk tests. Non-parametric Kruskal-Wallis test and ANOVA test were used especially to determine possible semnificative differences between pairs or groups. The results were confirmed by post-hoc analysis (Tuckey HSD and Fisher LSD test). To establish differences between the distribution of normal and abnormal values of our variables we used chi square test.

3. REZULTS

The studied lot included 267 brain tumour patients, ages between 15 and 83 years old. Average age for our group was 54.41 (SD 13,36) years. The most frequent tumors were meningiomas (32.96%), glioblastomas (29.21%), cerebral metastasis (10.49%) and astrocytomas (8.24%).

We classified brain tumors according to grading and we considered a separate group of metastatic tumors. Of the 267 tumors studied, 32.58% were grade I tumors, 13.48% grade II, 8.61% grade III and 34.83% grade IV tumors.

We observed that average age was 38.82 (SD 13.37) years for astrocytomas, 56.18 (SD 11.04) years for glioblastomas, 54.99 (SD 13.08) years for meningiomas and 60.68 (SD 10.99) years for cerebral metastasis. Kruskal-Wallis and ANOVA tests showed high semnificative statistical correlations ($p < 0.0001$) between age and histologic tumor type.

According to tumoral grading, average age was 46.57 for grade III, 47.89 for grade II, 51.80 for grade I, 53.54 for grade IV and 60.68 for metastatic tumors. Kruskal-Wallis and ANOVA tests showed high semnificative statistical correlations ($p < 0.01$) between age and tumor grading.

In our group of 267 brain tumour patients, 128 (47.94%) have high abnormal values of plasma glucose. According to tumor type, average values of glycemia were 105.64 mg/dl (SD 25.55) for astrocytomas, 119.18 mg/dl (SD 42) for glioblastomas, 123.25 mg/dl (SD 50.48) for meningiomas and 126.93 mg/dl (SD 82.08) for brain metastasis.

Average value for glycemia in the whole group was 118.07 mg/dl (SD 49.58). The lowest average value was registered for grade III tumors (106.74 mg/dl, SD 22.93), followed by grade I tumors (115.69 mg/dl, SD 47.18), grade II tumors (119.56 mg/dl, SD 43.38), grade IV tumors (119.87 mg/dl, SD 46.27) and brain metastasis (126.93mg/dl, SD 82.08).

Another parameter analysed in our study was the plasmatic insulin. More than a half of our patients (57.68%) were found with abnormal high insulin values.

The highest average value was in brain metastasis (42.10 μ UI/ml, SD 30.86) and astrocytomas (42.76 μ UI/ml, SD 25.97) while meningiomas (31.75 μ UI/ml, SD 17.39) and glioblastomas (31.49 μ UI/ml, SD 19.04) had lower average insulin levels. ANOVA test ($p=0,013$) showed the possibility of statistical difference. Fisher LSD indicate statistic differences between brain metastasis, respectively astrocytoma, and meningioma or glioblastoma.

According to grading, the highest insulin levels were found in brain metastasis (average 42.10 μ UI/ml, SD 30.86), followed by grade III tumors (average 35.30 μ UI/ml, SD 23.45). Grade I, IV and II brain tumors have lower average levels (30.25 μ UI/ml-SD 20.36; 29.06 μ UI/ml-SD 18.68 and 27.07 μ UI/ml-SD 11.97). Average plasma insulin concentration for the entire lot was 31.08 μ UI/ml (SD 20.83). Fischer LSD test showed statistical differences between metastatic tumors and grade I, II or IV tumors.

Chi square test showed that the distribution of abnormal high values of insulin is dependent on tumor type and tissue of origin.

We found the highest levels of plasma ferritin in brain metastasis (average 296.02 ng/ml, SD 152.07), followed by astrocytomas (212.22 ng/ml, SD 136.50), meningiomas (202 ng/ml, SD 167.09) and glioblastomas (173.71 ng/ml, SD 126.79). Only the average value found in glioblastomas was comparable with the average found in general

population (175 ng/ml). ANOVA ($p=0,007$) and Kruskal-Wallis ($p=0,011$) tests showed statistical difference between tumor types.

According to grading, we found high levels of plasma ferritin in grade II (252.65 ng/ml, SD 186.99) and grade III (218.72 ng/ml, SD 164.76) tumors. For grade I and IV we found average levels comparable with those of general population (180.29 respectively 187.47 ng/ml). ANOVA and Kruskal-Wallis tests showed statistical difference between the means and between the ranks of values ($p=0,002$ respectively $0,007$). Post-hoc analysis showed differences between metastasis and grade I or IV tumors. There are also statistical differences between grade II tumors and grade I and IV tumors.

4. DISCUSSION

In the last years, several research studies correlated the presence of diabetes mellitus or metabolic syndrome with increased risk for cancer diseases. A number of physiopathological specific-disease changes in diabetes mellitus were indicated as factors involved in transformation and growth of malignant cells. Two of the serum molecules with modified levels both in diabetes mellitus and metabolic syndrome are glucose and insulin. Ferritin is another protein associated with insulin resistance, metabolic syndrome and cancer.

Compared to data from published articles we observed that our population have similar characteristics regarding the distribution of meningiomas and astrocytomas. Glioblastomas frequency was higher in our group of patients.

Age median for the entire lot was 55 years. According to the tumor type, the **median age** at diagnosis was 37,5 years for astrocytomas, 56 years for glioblastomas, 57 years for meningiomas and 63 years for brain metastasis. The last CBTRUS analysis reports an median age of 59 years for the 311.202 followed patients. For astrocytoma they reported a median between 48 and 54 years depending on various histologic characteristics, for glioblastomas 64 years and for meningiomas 65 years. Regarding these data we observed that the median age in CBTRUS report are 4 to 10 years higher than those in our study. We concluded that in our lot there is a tendency of brain tumors to affect younger patients.

Several research reports speculate that hyperglycemia could be one of the promoters of tumoral growth. To verify this hypothesis, we analysed plasma glucose concentration in our group of brain tumour patients. Although 47.94% of the 267 patients have a plasmaic levels of glucose higher than 105 mg/dl, we couldn't find any statistical correlations between tumor type or tumor grading and the values of glycemia.

A large number of published articles also evaluate the risk generated by diabetes mellitus for the development of some neoplasms. As for the presence of diabetes mellitus in cancer patients, the already published data suggests that the highest prevalence is found in pancreatic cancer, diabetes affecting 68% of patients. Other cancers with high prevalence of diabetes are lung cancer (19.6%), breast cancer (19.4%), colon cancer (20.7%) and prostate cancer (14.8%).

It is a known fact that insulin is involved in cell differentiation and abnormal activity of insulin could increase the risk for the development of undifferentiated malignant cells. We also found that 57.68% of our patients have abnormally high levels of insulin ($> 25 \mu\text{UI/ml}$). Post-hoc analysis of insulin concentration related to tumor type showed statistic difference between brain metastasis and glioblastomas or meningiomas, respectively between astrocytomas and glioblastomas or meningiomas. Thus, we concluded that metastatic tumors and astrocytomas have hgher levels of plasma insulin than meningiomas or glioblastomas.

When we analyzed insulin levels according to grading, we found that brain metastasis have higher levels of plasmatic insulin than grade I, II and IV tumors, the differences was statistically signifiant ($p < 0,05$).

Ferritin is a plasmatic marker wich has abnormal high levels in various systemic neoplasms. In our group of patients, the average level of plasmatic ferritin was 207.99 ng/ml. This value is higher than the average reported in general population (105 ng/ml for women and 175 ng/ml for men).

The statistical analysis showed significant differences between the histologic types studied ($p\text{ANOVA}=0.007$ and $p\text{K-W}=0.011$). Post hoc analysis showed that only metastatic brain tumors have higher ferritin levels than primary brain tumors.

Regarding tumor grading, we observed similar patterns in distribution of ferritin concentrations for grade I and grade IV tumors, respectively for grade II and grade III

tumors. The statistical analysis showed significant differences ($p < 0,05$) between metastasis and grade II brain tumors when they are compared with grade I or grade IV tumors.

Other published articles have associated abnormally high levels of serum ferritin with grade II, III and IV astrocytomas. Following the dynamics of ferritin concentrations, these studies showed normal values of ferritin after surgical resection and the authors concluded that ferritin concentration was correlated with the presence of malignant cells.

Our results indicate that ferritin could have biomarker properties for brain metastasis but further studies are required to determine its role in primary brain tumors.

5. CONCLUSIONS

1. The histologic type of brain tumors is influenced by patient's age. Low grade astrocytoma appear with greater frequency in the 4th decade of life. Glioblastoma, meningioma and brain metastasis are more frequent in 6th and 7th decade of life.
2. In our study we observed a tendency of brain tumors to appear at younger age than in United States statistics (the largest epidemiological report available at this moment).
3. Although glycemia is one of the parameters which has frequently high levels in patients with brain tumors, we could't correlate its values with the histologic type, tumor grading or tissue of tumoral origin.
4. Plasma insulin levels were correlated with histologic types of brain tumors. Brain metastasis and astrocytomas have statistically significant higher insulin levels when compared with glioblastomas and meningiomas patients. The presence of abnormally high insulin levels is dependent of tumor type and tissue of tumoral origin.
6. Ferritin plasma levels are the highest in brain metastasis. Grade II brain tumor patients have statistically significant higher plasma ferritin levels, when compared to grade I and IV tumors.

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