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DOCTORAL THESIS

ABSTRACT

UPDATES IN DIAGNOSIS AND TREATMENT OF CEREBRAL TUMOURS

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Introduction

Observations made by physicians suggest that the number of patients with bra tumors increased in Romanian hospitals but, unfortunately, there is not a National Registry of Brain Cancer and no statistical studies to confirm this.

Our statistics is performed on 262 cases, which is a very small number, but considering the fact that there is no other statistical study in the field so far we hope that this study may represent a model for a larger future study.

I. STATE OF KNOWLEDGE

Despite numerous years of epidemiologic research focusing on determining the environmental risk factors of brain tumors, few causes are still known. Research underlines certain genetic conditions as well as environmental factors that may lead to brain cancer development. In comparison with other cancers, brain tumors have less risk factor. Clear etiology of brain tumors shows rare hereditary syndromes, radiation therapy and immune suppression causing brain lymphomas (1). Proper identification of the etiology and risk factor is extremely important as well as the understanding of genetic base and causes of the disease for primary brain tumors risk assessment.(2)

Recent research shows an inherited susceptibility in genes leading to carcinogenesis, for instance DNA repair and mutagen sensitivity. Other findings challenge referrer to a family history that may impair the possibility of causing certain diseases. Von Hippel-Lindau disease, Li-Fraumeni syndrome, nevoid basal cell carcinoma syndrome, tuberous sclerosis, Turcot syndrome and Neurofibromatosis (NF1 and NF2) are inherited conditions that have been found in families with a history of rare brain tumors. Apart from these findings brain cancer does not run in families.(3)

Still, more data show the relationship between brain tumors and age and gender. Generally, brain cancer develops with age, commonly occurring in individuals aged 65 and older. In spite of these studies, we must focus on the idea that brain tumors are the second type of cancer in terms of frequency, occurring in children under the age of 8

(counting for approximately 15% of all brain tumors). Age is a factor of utmost importance in showing variety regarding the cell type and tumor location. The risk of developing medulloblastomas is very low in adults, but on the other hand they develop gliomas highly commonly. Moreover, adults over age 50 are prone to meningiomas and craniopharyngiomas. As far as gender is concerned, no general rule may be applied to all categories of brain cancer. Women are twice likely to develop certain cancers, like meningiomas, though medulloblastomas occur more frequently in males.

According to certain studies radiation treatment, occupational environment, life behaviour, and exposure to other damaging factors such as nitroso-containing compounds might cause brain tumors. Both exposure to radiation and to certain chemicals or solvents, as well as occupational exposure in oil refining rubber manufacturing and drug manufacturing, especially at a young age, may lead to an increased likelihood of developing gliomas and meningiomas.

Also, some people with compromised immune systems were reported to develop lymphomas of the brain with increased risk. For example, infection with the Epstein-Barr virus (EBV) may increase the risk of central nervous sistem (CNS) lymphoma.(4)

Along with its concomitant diseases (obesity, dislipidemia, hyperglycemia, hypertension), the metabolic syndrome may be an important etiologic factor for the development and progression of several neoplasms.(5)

Until now, there are little data to demonstrate the relationship between metabolic syndrome or components of it and various types of brain tumors. Two important components of metabolic syndrome are hyperglycemia and hyperinsulinemia. Hyperglycemia represents the elevation of blood glucose concentrations above the normal range. Hyperglycemia was associated with increased risk of pancreas neoplasm, malignant melanoma, endometrial and breast cancer.(6-7)

The interrelation between glucose and insulin levels in in metabolic syndrome is well known: high glucose concentration is caused by a decrease in the production of insulin, a decrease in the action of insulin, or a combination of the two abnormalities. Hyperinsulinemia is associated with hypertension, obesity, dyslipidemia, and glucose intolerance which are all parts of metabolic syndrome. This close association between hyperinsulinemia and conditions of metabolic syndrome suggest related or common

mechanisms of pathogenicity. Hyperinsulinemia has also been shown to be linked with breast, endometrial and prostate cancer.(8-10)

II. PERSONAL CONTRIBUTION

1.Specific objectives

The first objective of our study was to estimate the incidence of brain tumors in relation to histology, age, gender, urban or rural areas and geographical area.

So far, few data can demonstrate the correlation between the metabolic syndrome, or its components, and different types of brain tumors. Taking into account the fact that hyperglycemia is an important component of the metabolic syndrome, we considered the hypothesis that hyperglycemia might cause tumor growth. That is why another objective of our study was to evaluate the possible correlation between the glucose levels, insulin levels and various types of brain tumors.

The recent progress recorded in the molecular neural-oncology field demonstrated that the receptors of the growth factors presented major alterations in brain tumors. Therefore, another objective of the present study was to investigate the therapeutic potential of an EGFR inhibitor as a single therapeutic method and in combination with temozolomide in the treatment of glioblastomas.

2. Patients and Methods

2.1. Patients

In our study incidence rates have been calculated based upon cancer cases registered in a brain cancer bank established in collaboration with Bagdasar Arseni Hospital, Bucharest, during the period 2006-2012. The patients included in the study were being subjected to surgery with total or partial resection of the tumor. Brain tumors were defined as tumors of the brain indicated by clinical features and/or CT / IRM examination and verified histopathologically after surgery. The histopathology grading was performed in accordance with the most recent World Health Organization (WHO) classification.

2.2. Methods

Blood samples were drawn from overnight-fasting brain tumor patients. Plasma to determine glycemia and insulin was separated within one hour of sampling and stored at -150ºC until assayed.

2.3. Statistical analysis

Statistical analysis was performed by the Biostatistics Department of the University of Medicine and Pharmacy of Craiova, Romania, using Microsoft Excel (Microsoft Corp., Redmond, WA, USA), together with the XLSTAT add-on for MS Excel (Addinsoft SARL, Paris, France). To test the normality of the data we used the Anderson-Darling and Shapiro-Wilk tests.

2.4. Cell Cultures

GB1B cell line was taken from brain tumors of grade IV at the Emergency Hospital Bagdasar Arseni, Bucharest, according to standard procedures.

The cells were cultivated in cell culture containers, in Minimum Essential Modified environment (MEM), supplemented with 10% fetal bovine serum (FBS), 2 mM of glutamine and antibiotics (100UI/ml penicillin and 100UI/ml streptomycin), humidified incubator at 37 °C, 95% O2 and 5% CO2.

3.Results

The distribution of the brain cancer between several regions is different.. There are three regions where the incidence of brain tumors are very low (in Crisana and Banat the distribution is 0.38%; in Maramures the distribution is 0.76%), one region where the incidence of brain tumors is low (in Bucovina the distribution is 2.29%), three regions where the incidence of brain tumors is high (in Oltenia and Dobrogea the distribution is Moldva, 12.21%; in Moldova the distribution is 12.98%) and two regions where the incidence of brain tumors is very high (in Bucharest and Muntenia the distribution is 29.39). Cases included in the category “others” were represented by: adenoma, anaplastic oligodendroglioma, angiosarcoma, condrom condroid, disembryoplastic neuroepithelial tumor, fibrosarcom, ganglioneuroblastoma, grawitz tumor, liposarcom myxoid, myxoid condrosarcoma, primary neuroectodermal tumor (PNET), subependinom and miopericitoma.

The distribution of glioblastomas and schwannomas in rural areas were 65.79% and 66.67% respectively. The remaining types of tumor were mainly encountered in urban areas. Gliosarcoamas, hemangioblastomas, hemangiopericytomas, neurocitomas and oligodendrogliomas recorded a 100% value in urban areas, which definitely accounted for a lot.

Regarding the repartition of cases according to gender, we found a slight increase of the number of cases in male gender (135 cases/51.53%) compared to female gender (127 cases/48.47%). Distribution according to the area of origin showed a higher percentage in urban areas (184 cases/69.17%) than rural (77 cases/28.95).

We can notice a predominance of males for gliosarcomas and meningiosarcomas (100%) and brain metastases (70%). For astrocytomas, hemangioblastoamas, hemangiopericytomas and neurocitomas the distribution of cases was equal for both sexes. In women meningiomas (55.1%) and schwannoma (66.67%) predominated.

Maximum incidence of cases is recorded in the age group 55-64 years (31.68%), followed by age group 45-54 years (20.61%).

First , we determined the serum glycemia and we found that 47.94% of the 262 studied patients that were diagnosed with had high values of the plasma glucose.

Next, we considered important to determine the correlations between the brain tumors types and the glycemia. The corresponding p values resulted from computing the tests were both greater than 0.05.

Therefore we decided to determine the correlations between the grade of the brain tumors and glycemia and we found that the corresponding p values were similar with those found when we correlated glycemia with brain tumors types ($p>0.05$).

Although 47.94% of the 262 studied patients had values of the serum glycemia over 105mg/dl we found no statistical difference between the mean values of serum glycemia in the various types (brain metastasis, astrocitoma, glioblastoma, meningioma) ($p>0.05$).

We also determined the insulin level in these patients. Unexpectedly we discovered that 57.68% of the 262 patients diagnosed with brain tumors that we studied had high levels of plasma insulin.

Therefore, next we considered important to correlate the type of the studied brain tumors and the values of plasma insulin.We observed that the mean values of insulin were the highest in metastatic brain tumors (43.36) followed by astrocitoma (42.76) , while meningioma (31.75) and glioblastoma (31.49) patients had smaller plasma levels of insulin. Our results show that there is a statistically significant difference between the mean values of the insulin levels found for each tumor type ($p<0.05$). On the other hand, we found that there is no significant difference between the ranks of those values, so the studied samples are part of the same statistical population.

Therefore we can conclude that brain metastasis and astrocitomas have higher plasma insulin levels compared with meningiomas and glioblastomas. There is a significant statistical difference between the mean values of serum insulin in metastasis compared with glioblastomas or meningiomas. We obtained a similar result when we compared astrocitomas with glioblastomas and meningiomas while between metastasis and astrocitomas or between meningiomas and glioblastomas we found no significant statistical difference of the mean values of plasma insulin.

Interaction between AG556 and temozolomide was analyzed by multiplicative mathematical method. The treatment with AG556 (EGFR inhibitor) and temozolomide produced a significant inhibition of glioblastoma cell growth in a dose and time dependent manner.

4.Discussions

Although Romania has faced with a certain decrease in the population it is still a large country in the European Union, the data gathered in the 2011 census reporting a number of 19 million inhabitants. At the moment, in Romania, the general health figures seem to be in the same line with those reported in developed countries.

A study run in Bagdasar Arseni Hospital, regarding Intramedullary Tumors - Clinical, Radiological and Histological Correlations, within 2006- 2009 on a number of 36 patients (19 females/17 males) determined two maximum values in age distribution, at 20-29 yrs and 40-59 yrs, with a mean age of diagnosis of 40.5 yrs (ranging between 18 and 72). In terms of gender, they did not determined any gender dominance: 42% of the patients diagnosed had ependymoma and 22% astrocytoma. However, the high incidence of ependymoma in this study (42%) was caused by the exclusion of pediatric patients

from the studied group, where the astrocytoma is the most frequent intramedullary tumor. The rest of 36% patients presented cavernoma, hamartoma, oligodendroglioma, ganglioglioma, hemangioblastoma, and metastasis (11).

In our study, analysis of histological types of tumors indicated that the most common brain tumors are meningiomas (33.21%), glioblastomas (29.01%) astrocytomas (9,92%) and metastasis (8,78%) and the least common ones schwannoma (2,29%), gliosarcoma (1,15%), meduloblastomas (1,91%), hemangioblastomas (0,76%), hemangiopericytomas (0,76%), neurocitomas (0,76%) and oligodendrogliomas (0,76%).

Regarding the case distribution by gender, we determined a slight increase in the number of cases in males (135 cases/51.53%) in comparison with females (127 cases/48.47%). Case distribution according to the area of origin showed a higher percentage in urban areas (184 cases/69.17%) than in rural areas (77 cases/28.95%).

In terms of geographical distribution related to the histological type of tumor, we must underline that the most affected regions were Muntenia (29.39%) and Bucharest, including Ilfov (24.34%), Moldova (12.98%), Oltenia (12.21%) and Dobrogea (12.21%) registering average rates. The lowest incidence was recorded in the Western and Northern regions (Transilvania, Bucovina, Maramures, Crisana, Banat).

It is already well known that carcinogenesis is a very complex multifactorial process which in part is influenced by the metabolic disturbances.(12) An important component of the metabolic syndrome is hyperglycemia. Glucose is the major substrate for brain metabolism. Under normal physiological conditions the adult brain develops his energy from the aerobic oxidation of the glucose. When the blood glucose levels are reduced the mature brain will metabolize ketone bodies for energy. In contrast, brain tumors are dependent only on glucose for energy, and high-grade brain tumors consume even more glucose.(13) Some studies describe correlations between glucose level and the neoplastic transformation of the cells however most of the results of the studies are inconclusive.(14) Therefore we considered important the hypothesis that maybe hyperglycemia itself promotes tumor growth. Although 47.94% of the 262 studied patients had values of the serum glycemia over 105mg/dl , in our study we didn’t asses correlations between brain tumor types or grades and the values of serum glycemia. We can conclude that in our group of patients glycemia doesn’t necessarily mediates the

relationship with the brain tumors.It is important to remark that 47.94% of the patients had levels of serum glucose over 105mg/dl which may display an abnormal activity of insulin. It can be expressed either through abnormal glucose tolereance or insulin resistance (followed by compensatory hyperinsulinemia), or less insulin secretion.(15) As it is known that insulin is involved in the differentiation of the cells, an abnormal activity of the insulin may indicate a greater risk of undifferentiated growth of the cancer cells.(16)

First, we descoverd that 57.68 % of the 262 brain tumors patients that we studied had high levels of serum insulin (over 25 μlU/ml) Therefore we determined the correlations between brain tumors types and the values of serum insulin.. We found that brain metastasis and astrocitomas have higher serum insulin levels compared with meningiomas and glioblastomas. Our results showed statistical significant differences between the mean values of serum insulin in metastasis compared with glioblastoma or meningioma and between insulin expression in astrocitoma compared with glioblastoma or meningioma.

Our findings rise the question whether the management of hyperglycemia and hyperinsulinemia would lead to an improvement in the survival, quality of life and treatment options in patients with brain tumors.

In this study, we found no correlation between the serum glycemia and brain tumor types. We also found that the mean values of plasma insulin were higher in brain metastasis and astrocitomas and the results were statistically significant when compared with other types of brain tumors.

As far as the interaction between the GB1B cell line and the treatment with AG556 and temozolomide is concerned, the results of this study led to a synergy effect in 93% of the combinations used: AG556 1μM + TMZ 1μM, AG556 1μM + TMZ 5μM, AG556 5μM + TMZ 1μM, AG556 5μM + TMZ 5μM, AG556 10μM + TMZ 1μM, AG556 10μM + TMZ 5μM,

5.Conclusions

1. The study emphasized the influence of the environment in the etiology of brain tumors so as certain geographical regions were of major risk in brain tumours incidence, the highest incidence being recorded in Muntenia (29.39%), Bucharest-Ilfov (24.34%)

and Moldovia (12.98%). The regions of Oltenia (12.21%) and Dobrogea (12.21%) had an average risk factor, while in the western and northern parts of the country (Transylvania, Bucovina, Maramures, Crişana Banat) there was recorded a low brain tumours incidence.

2. The histological type of brain tumor is influenced by age. The peak incidence of the cases was recorded in the age group of 55-64 years (31.68%), followed by the age group of 45-54 years (20.61%). In our study we noticed a tendency of brain tumors occurrence at younger ages than those reported in the literature.

3. The analysis of brain tumors histology showed a maximum incidence of meningiomas (33.21%), glioblastomas (29,01%), astrocytomas (9.92%) and metastasis (8.78%). There was recorded a less incidence of schwannomas (2.29%), hemangioblastomas (0.76%), hemangiopericytomas (0.76%), oligodendrogliomas (0.76%), neurocytomas (0.76%), medulloblastomas (1, 91%) and gliosarcomas (1.15%); these results being in accordance with the literature data.

4. There was a slighter increase of brain tumours in males (135 cases/51.53%) than in females (127 cases/48.47%).

5. The distribution of brain tumour cases by the area of origin indicated a higher percentage in the urban areas (184 cases/69.17%) than in the rural ones (77 cases/28.95%).

6. 47.94% of the patients studied had serum glucose values above 105mg/dl, but we could find no correlations between the histology of the brain tumors and the values of serum glycemia. We conclude that in our group of patients glycemia does not necessarily mediate the correlation with the brain tumors. This can be explained either by an abnormal glucose tolerance or insulin resistance (hyperinsulinemia, followed by compensation), or secretion of less insulin.

7. 57.68% out of the 262 patients with brain tumors had high levels of serum insulin. Consequently, there is a correlation between the increased level of insulin and the incidence of brain tumors.

8. Our study identified correlations between the histology of the brain tumors and the values of serum insulin. The brain metastasis and the astrocytomas had elevated insulin levels in plasma comparatively with meningiomas and glioblastomas.

9. The treatment with AG556 (EGFR activity inhibitor) and TMZ produced a significant inhibition in the growth of GB1B glioblastoma cells, depending on the dose and the administration period of the treatment.

10. Our results showed that in GB1B cell line the interaction between the treatment with AG556 and TMZ treatment was synergistic in 93% of the subjects studied.

6.References

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