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ASSOCIATION OF ANTI-HUMAN IGG AND IGM ANTIBODIES WITH THE INCIDENCE OF GLIOMAS

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ABSTRACT

Gliomas are the most common primary of the central nervous system, representing 81% of malignant brain tumors. The exact causes of gliomas are still unclear; however an association of certain risk factors was well documented. The current study aimed to investigate the association of anti-human cytomegalovirus (HCMV)IgG and IgM antibodies with the incidence of glioma. A total of 100 glioma patients from different local hospitals, and 40 apparently healthy control individuals were recruited for this study. Serum samples from each participant were obtained, specific anti-HCMV IgG and IgM antibodies were estimated using ELISA technique. Eighty-five percent of glioma patients gave positive result for anti-HCMV IgG antibodies, compared to 72.5% among control (OR = 2.779, 95% CI = 1.049-7.358, p = 0.04). There are no significant differences in the distribution of these antibodies among different age classes of glioma patients. For IgM, only14% of glioma patients gave positive results compared to 10% in control (OR = 1.737, 95% CI = 0.47-6.421, p = 0.408 Previous infection as indicated by the presence of anti-HCMV IgG antibody may contribute in the initiation of gliomas.

KEYWORDS: Glioma, Anti-HCMV Igg and Igm Antibodies, Human Cytomegalovirus

INTRODUCTION

Gliomas are the most common primary of the central nervous system, representing 81% of malignant brain tumors. Although relatively rare, they cause significant mortality and morbidity (Ostromet al., 2014).In spite of marked progress in characterizing the molecular pathogenesis of gliomas, these tumors remain incurable (Srinivasanet al., 2011). Similar to most other cancers, the exact causes of glioma is beyond current knowledge, however many risk factors such as ionizing radiation (Melissa et al., 2012) and family history (Quinn et al., 2012) are well documented.

In Iraq, brain tumors are the fifth most common tumor in adults and the second most common in children. They represented about 4.77% among males and 3.3% among females (Iraqi Cancer Registry, 2005).

Viral infections are responsible for approximately 15% of cancer world-wide (Young, 1998). Among a few viruses committed, although not well documented, to be associated with many cancers such as brain, breast, and colorectal cancer is HCMV (Hawkins and Croul, 2011; Taheret al., 2013; Tafvizi and Ford, 2014).

Human Cytomegalovirus (HCMV), a widespread beta-herpes virus, infects 70-80% of the world's population with different ages, sex and socioeconomic status (Taheret al., 2013). Although HCMV is not recognized as an oncogenic virus, it may increase the malignancy of infected cell by disrupting cellular pathways involved in cell cycle, apoptosis, angiogenesis, cell invasion and the immune response (Kaveh, 2010).

A simple indication of the association of HCMV with brain tumor can be deduced from the association of anti-HCMV IgG and IgM Abs with the incidence of gliomas. This notion was confirmed recently by Amirianet al. (2013), who found that anti-HCMV Abs levels were associated with glioma risk especially among IgM-positive individuals. However, the previous work by Sjorstromet al. (2011) did not support this concept as they did not found significant association between glioma orglioblastommultiforme (GBM) and anti-HCMV IgG level. The current study aimed to investigate the association of the presence of anti-HCMV IgG and IgM antibodies with the incidence of glioma.

Materials and Methods

Study Subjects

Patients attending five hospitals in Iraq (Neurosurgery Hospital, Neuroscience Hospital, AL-Kadimiya Teaching Hospital, AL- Fallujah Hospital, and Private Nursing Home Hospital) from January 2012 to January 2013 were eligible for this study. Ethical clearance to conduct the research was sought and obtained from these hospitals. Selection of patients was accomplished with the assistance of surgeons.

One hundred patients were selected to be investigated in this study. All had glioma brain tumors of different grade and stages (45 men and 55 women) with a mean age of 40.2 years (range between 4 -70). Data were collected through direct interview with the patient, and by seeking his/her hospital record as well as previous medical reports. Patient's claims were followed as an alternative source of information when his/her previous medical reports were not available.

Forty controls were selected randomly from apparently healthy individuals (17 men 13 women) with a mean age of 33.9 years (range between 21- 55). Individuals who have been previously diagnosed with cancer at the time of enrolment were excluded from this group.

Samples

Three milliliter of blood was taken from patients and controls in plain tube. Serum was obtained and preserved at -20^{o}C until be used.

HCMV ELISA IgM and IgG

Anti-CMV IgG and IgM ELISA test kits (Biokit, Barcelona, Spain) were used for qualitative detection of serum anti-CMV IgG and IgM antibodies according to the manufacturer's instructions. Briefly, monoclonal mouse anti-human IgG and IgM were used. After the wells of microplate were washed with the washing solution, 100 µl of the conjugate were added to each well, incubated for 1h at 37^{o}C, washed, 100 µl of substrate-TMB added to each well, incubated at room temperature for 30min, and finally, the reaction was stopped by adding 100µl of stopping solution. The absorbance was read at 450 nm. The cut-off values include three values: Positive, ratio absorbance ≥1. 1; negative: ratio absorbance < 0.9; equivocal: ratio observance ≥ 0.9 <1.1.

Statistical Analysis

The Statistical Package for the Social Sciences (SPSS, version 14) was used for statistical analysis. Logistic regression was used to calculate the association of seropositivity for anti-HCMV IgG and IgM antibodies with the incidence of glioma. A p-value < 0.05was considered statistically significant.

Results

Seropositivity for anti-HCMV IgG Antibodies

Out of 100 glioma patients, 85 (85%) gave positive result for anti-HCMV IgG antibodies, compared to 29 (72.5%) among control. Logistic regression revealed significant association between seropositivity for anti-HCMV IgG antibodies and glioma (OR = 2.779, 95% CI = 1.049-7.358, p = 0.04), which means that individuals who have previous infection with HCMV are 2.779- fold more likely to get glioma compared with individuals with no such infection.

Table (1) shows the distribution of seropositivity for anti-HCMV IgG antibodies among different age classes in glioma patients and control group. Overall, there are no significant differences in the distribution of these antibodies among different age classes of glioma patients. However, age class >50 years has the highest percentage (88.89%), while the lowest percentage was among the youngest age class (≤ 20 years) which was 81.81%. Among control group, age class 21-30 years has higher seropositive percentage (91.67%) than other classes with significant differences (p = 0.037).

Comparison of the age classes between glioma patients and control revealed significant differences in the seropositivity to the advantage of glioma patients in all classes except 21-30 years class which is higher in control group (91.67%) than that of glioma patients (85%).

Table (1): The distribution of seropositivity for anti-HCMV IgG antibodies among different age classes in glioma patients and control group.

Glioma patients						Control				
Age classes (years)	No	Seropositive		Seronegative		No	Seropositive		Seronegative	
		No	%	No	%		No	%	No	%
≤20	22	18	81.81	4	18.19	9	6	66.67	3	33.33
21-30	20	17	85	3	15	12	11	91.67	1	8.33
31-40	19	16	84.21	3	15.79	10	7	70	3	30
41-50	21	18	85.71	3	14.29	7	4	57.14	3	42.86
>50	18	16	88.89	2	11.11	2	1	50	1	50

Seropositivity for Anti-HCMV IgM Antibodies

Result of ELISA test for the detection of anti-HCMV IgM antibodies in glioma patient and controls showed insignificant association between these antibodies and the incidence of glioma. Out of 100 glioma patients, only 14 (14%) gave positive results compared to 4 (10%) in control (OR = 1.737, 95% CI = 0.47-6.421, p = 0.408).

The distribution of seropositivity for anti-HCMV IgM antibodies among different age classes in glioma patients and control group is shown in table (2). From the table, it can be recognized the low distribution of such antibodies among both patients and control. Nevertheless, in glioma patients, the age class 41-50 years had higher seropositivity percentage (19.05%) than other classes with significant differences, while three age classes among control group had zero percentage, and only the class 31-40 years old (30%) was higher than its counterpart age class in glioma patients (15.79%) with significant differences (p = 0.0141).

Table (2): The distribution of seropositivity for anti-HCMV IgM antibodies among different age classes in glioma patients and control group.

Glioma patients						Control				
Age classes (years)	No	Seropositive		Seronegative		No	Seropositive		Seronegative	
		No	%	No	%		No	%	No	%
≤20	22	3	13.67	19	86.36	9	0	0	9	100
21-30	20	3	15	17	85	12	1	8.33	11	91.67
31-40	19	3	15.79	16	84.21	10	3	30	7	70
41-50	21	4	19.05	17	80.95	7	0	0	7	100
>50	18	1	5.56	17	94.44	2	0	0	2	100

Discussion

Many viral infections have been well documented as causative agents for cancer such as human papillomavirus which appears to be necessary factor in the development of almost all cases of cervical cancer (Kumeret al., 2007), and human T-cell lymphotropic virus (HTLV) as a cause for adult T-cell leukemia/lymphoma (Banerjee et al., 2010). In many other cases, there is obvious association between the viral infection and the incidence of certain cancer, such as the association between Epstein Barr Virus and lymphoma (Friiset al., 2013), but the exact mechanism by which these viruses could induce the cancer is not fully illustrated. What can be called as the third category for the relationship between cancer and viral infection is that the overall association is a matter of debate. HCMV lies within this category. Although the vast majority of literature indicated an association between HCMV infection and various types of human cancer such as breast, prostate, colon, lung and brain, (Cobbset al., 2002; Cobbset al., 2007; Giuliani et al., 2007; Scheureret al., 2008; Harkins et al., 2010). Other reports indicated no obvious association (Utrera-Barillas et al., 2013; Huang et al., 2014) in breast and thyroid cancer respectively.

According to the results of the current study, previous infection with HCMV, as it is indicated by positive anti-HCMV IgG antibodies, can be involved in the susceptibility to glioma (those who have previous infection with this virus are 2.779-fold more likely to develop glioma compared to individuals with no such infection). This result agreed with many previous works (Sjorstromet al., 2011; Amirianet al., 2013).

In the context of linking such association, many researchers have investigated the protein and nucleic acid of the virus in the glioma cells. The expression of HCMV proteins and oligonucleotides in a high percentage of glioma was first reported by Cobbset al. (2002), while HCMV genomic DNA was detected in 94% glioblastoma samples using combined PCR-DNA sequencing methodology (Dziurzynskiet al., 2012). Since it is clear that the virus

has no direct oncogenic effect, the mechanism that the virus employed to facilitate tumerigenesis in the infected cell is an argument issue.

It is not easy to say that exposure to HCMV may confer susceptibility to glioma, because most individual can expose to such infection in their life time. Rather it appears that some type of recurrent infection (recurrent reactivation of the virus) can be involved in such susceptibility. This recurrent reactivation increases the level of anti-HCMV IgG antibodies. Unfortunately, it was not possible to estimate the levels of such antibodies. Studies examining antibody response to HCMV in relation to glioma yielded relatively equivocal results (Wrenschet al., 2001; Wrenschet al., 2005; Dziuryndkiet al., 2012). A study by Sjorstromet al. (2011) examined serology data from 197 adult glioma cases and 394 controls from Sweden and Denmark. They did not find a significant association between glioma or glioblastoma risk and anti-HCMV IgG levels. However, high anti-HCMV IgG levels have been associated with longer survival among glioblastoma patients (Pandery, 2011).

Anti-HCMV IgM antibodies indicate a current infection with the virus, and it is well known that tumoregenesis process (if the virus can be involved in it) takes relatively long time. Therefore, it is not expected that positive cases for such antibodies are associated with increased risk of glioma. However, recurrent infection can initiate an activation of latent infection which induces anamnestic effects represented by increase IgG titer, which showed association with the risk of glioma although without estimation of antibodies levels. Collectively, these data indicate that previous infection with HCMV can contribute in the initiation of gliomas.

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