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ORIGINAL ARTICLE

Cytomegalovirus DNA in non-glioblastoma multiforme brain tumors of infants

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Abstract

Purpose CMV antigens have been detected in some brain tumors specially glioblastoma multiforme (GBM). As brain tumors in the first years of life are among the most aggressive neoplasms with poor prognosis, novel therapeutic options like targeted therapy against virus antigens are demanded. Infantile central nervous system tumors, other than GBM, have not been so far studied for CMV. To our best knowledge, this is the first study in which the presence of CMV-DNA, as a potential viral target for therapy, in non-GBM infantile brain tumors has been investigated.

Methods The paraffin blocks of non-GBM brain neoplasms of 36 infants (age < 24 months) who were operated on between 2006 and 2016 were examined for CMV-DNA, using real-time polymerase chain reaction (PCR). Paraffin blocks of CMV infected lung tissue were used as positive control. Extraction and amplification of β2 microglobulin gene from each tumor tissue were carried as positive internal control. We also assayed 25 paraffin blocks of meningomyelocele for CMV DNA as negative tissue controls.

Results Histopathological diagnoses consisted of 13 glial/neuroglial tumors (36.1%), 8 ependymomas (22.2%), 7 medulloblastomas (19.4%), 3 choroid plexus tumors (8.3%), 2 atypical teratoid rhabdoid tumors (5.6%), 2 embryonal CNS tumors (5.6%), and 1 germ cell tumor (2.8%). We could not detect CMV DNA in all samples examined.

Conclusion Although CMV may be associated with GBM, no role could be proposed for this virus in development of non-GBM infantile brain tumors. Further investigations on larger series of brain tumors should be conducted to confirm or rule out our conclusion.

Keywords Cytomegalovirus · Brain tumor · Infant

Introduction

Cytomegalovirus (CMV) is a common species of the family *Herpesviridae* with 70 to 100% prevalence in various populations [17]. After the initial infection, these viruses remain latent in myeloid cells, becoming periodically active through the life without clinical symptoms. Even though, CMV may have lifelong effects on different organs. For instance, congenital CMV infection is the most common viral cause of birth defects, especially in the central nervous system (CNS). Patients who develop CMV infection in the post-transplant phase are potentially exposed to the risks of organ rejection [5, 11, 22]. Moreover, there is evidence of CMV existence in malignancies arising from numerous tissues. Some of these tumors were positive for specific viral proteins and nucleic acids, while CMV proteins have never been observable in healthy tissues around the tumors [10, 29, 34].

Glioblastoma multiforme (GBM) and medulloblastoma are the most common malignant brain tumors in adults and children, respectively [35]. Standard treatment for these tumors includes surgical resection and radiation therapy with or without chemotherapy, but long-term survival rate of these patients is not promising [32]. In recent decades, molecular

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origins of these tumors and numerous gene mutations have been identified [11, 18, 33, 36]. Hence, different methods of targeted and molecular therapies are under investigation. In some particular tumors, such as GBM, the interaction between specific T cells against CMV and GBM cells has been confirmed, and some in vitro studies have shown that these T cells can increase the death of tumor cells [3, 21]. The presence of the CMV antigen in the GBM was first reported in 2002 by Cobbs et al. [4]. Since then, there have been controversies over the association of CMV with adult or pediatric brain tumors.

Infantile brain tumors are distinct entity among brain neoplasms, in terms of the spectrum of histopathology, aggressiveness, presentation at advanced stages, and poor prognosis [8]. Since novel therapeutic options like targeted therapy against virus antigens are demanded and infantile central nervous system tumors, other than GBM, have not been studied, we investigated the presence of the CMV DNA in non-GBM brain tumors in children aged 0–24 months.

Material and methods

Study design

A retrospective cross-sectional study was conducted at Children's Medical Center. Pathological reports and slides of patients aged 0 to 24 months who had undergone CNS tumor surgery between 2006 and 2016 were reviewed. Those with extensive necrosis in the tissue samples, non-definite diagnoses, mixed tumors (having a combination of more than one pathology), and patients whose clinical histories were impossible to access were excluded. As GBMs are rare in this age group and numerous former studies have investigated the relation of CMV with these tumors in older ages, few samples of GBM were excluded. Thirty-six cases with non-GBM brain tumors were enrolled in the study.

The study was approved by the institutional ethical committee. Informed consents of parents had been taken at admission time to use parts of the samples for investigational purposes.

Laboratory molecular method

Paraffin blocks of the tumor specimens were studied for CMV-DNA using real-time polymerase chain reaction (PCR). Nucleic acids were extracted from two or three 5-μm-thick slices of the paraffin blocks using the instruction of a commercial DNA extraction kit (QIA amp DNA FFPE Tissue kit). The extracted DNA was stored at a temperature of − 20 °C until the test was carried out. CMV qualitative PCR was performed by a commercial kit (Gene proof, Czechoslovakia). The PCR took place in the final volume of 20 μL. Each microtube, contained 5 μL of the tumors' DNA mixed with 15 μL of 25x Master Mix Solution (Gene proof, Czechoslovakia), was placed in a thermocycler (Rotor-Gene 6000). The procedure steps consisted of initial denaturation at 95 °C for 5 min and then 50 cycles of 15 sec at 95 °C, 25 sec at 60 °C, and 30 sec at 72 °C. To evaluate the correctness of isolation procedure and to check for possible PCR inhibition, β2 microglobulin gene was used as a positive internal control. For negative control, a 25 μL reaction microtube containing deionized water and master mix was used without DNA template under similar conditions. For external positive control, samples of CMV-infected lung tissue were used. Additionally, 25 paraffin-embedded samples of myelomeningocele were used as negative external control.

Statistics

Data analyses were performed using SPSS statistical software. Description of qualitative data was given in terms of frequency percentage and quantitative data in mean (standard deviation). To determine the relationship between subgroups of patients and CMV positivity, chi-square test was planned. The significance level of the tests was set to 0.05.

Results

Patients' demographic data

A total of 36 infantile brain tumors were assessed. The mean age of the affected children was 13.81 ± 7.56 months, ranging from 45 days to 24 months. Twenty-four cases (66.7%) were boys, and 12 cases (33.7%) were girls, with a male to female ratio of 2:1.

Characteristics of tumors

The most commonly diagnosed tumors were glial/neuroglial tumors in 13 cases (36.1%), followed by 8 ependymomas (22.2%), 7 medulloblastomas (19.4%), 3 choroid plexus tumors (8.3%), 2 atypical teratoid/rhabdoid tumors (5.6%), 2 CNS embryonal tumors (5.6%), and a single case of germ cell tumor (2.8%). WHO grade I, II, III, and IV tumors were diagnosed in 14 (38.9%), 6 (16.7%), 3 (8.3%), and 13 (36.1%) cases, respectively. Tumors' location and subtypes are summarized in Table 1. CMV-DNA was not found in any tumor sample in this series. At the same time, positive and negative controls showed expected positive and negative results, respectively.

Table 1 Characteristics of 36 infantile brain tumors

	Characteristics		Total
Age (month)	13.8 ± 7.56		
Gender	24 males (66.7%)	12 females (33/3%)	36 (100%)
Tumor location	20 supratentorial (55.6%)	16 infratentorial (44.4%)	36 (100%)
Relation to ventricular system	19 intraventricular (52.8%)	17 extraventricular (47.2%)	36 (100%)
Histopathological diagnosis	13 glial/neuroglial tumors* (36.1%) 8 ependymomas (22.2%) 7 medulloblastomas (19.4%) 3 choroid plexus tumors (8.3%) 2 atypical teratoid rhabdoid tumors (5.6%) 2 CNS embryonal tumors (5.6%) 1 germ cell tumor (2.8%)		36 (100%)
Tumor WHO grade	Grade I:14 (38.9%) Grade II: 6 (16.7%) Grade II: 3 (8.3%) Grade IV: 13 (36.1%)		36 (100%)

*Other than glioblastoma multiforme

Discussion

Viral infection is a potential environmental key factor in carcinogenesis, and there is evidence that parts of the virus genome are present in some tumors. The role of CMV proteins in some types of malignant CNS tumors like GBM has been discussed in the literature. CMV is not typically an oncogenic virus, but CMV proteins are supposed to activate mechanisms that stimulate tumor-related biological mechanisms.

Inflammatory microenvironment and brain tumors

Some brain tumors like GBM and medulloblastoma are associated with increased cyclooxygenase (COX-2) expression, which is involved in the conversion of arachidonic acid to pre-inflammatory prostaglandins [1]. COX-2 expression levels are associated with tumor grade, and non-steroidal anti-inflammatory drugs (NSAIDs) could reduce the growth of tumor cells by inhibiting COX-2 [6]. Accordingly, the inflammatory microenvironment seems necessary for tumor cell proliferation and augmentation of angiogenesis, metastasis, and suppression of the immune system. Such an inflammatory environment can be triggered by a variety of oncogenes or pathogens, leading to activation and secretion of cytokines and chemokines which affect tumor growth. Co-infection by some viruses, autoimmune diseases, and even certain foods may further affect the activity of immune system and the inflammatory environment which play roles in the development and spread of tumors [1, 15, 26]. Based on the available evidence, the presence of CMV in brain tumors such as gliomas and peripheral solid tumors as neuroblastomas was indicated.

CMV genes and proposed tumorigenesis

The genome associated with CMV encodes more than 180 proteins, of which only 45 are associated with virus replication. The reminders are involved in some of the virus activities, such as its aggressive nature. Therefore, the expression of these proteins in tumors is supposed to play roles in the proliferation and transformation of the tumor cells [30]. CMV proteins are proposed to control the cycle of GBM tumor cells, inducing telomerase activity, inhibiting apoptosis, inducing angiogenesis, and the migration of cancer cells [16]. It is suggested that CMV proteins may control the expression of some oncogenes and non-expression of some tumor inhibitors through p53 mutations [20]. Other CMV genes and proteins are also proposed to have role in tumorigenesis. An area in the virus genome called mtrII with a 980 kb sequence is associated with the transformation of fibroblasts [9, 23]. The expression of IE72 and IE86 proteins from the virus has led to the transformation of fibroblasts in nerve cells [27]. The virus US28 protein may play a major role in the oncogenic properties through triggering the expression of COX-2 and production of VEGF oncogenes [28, 31]. Despite all the above-mentioned evidence, the role of CMV proteins in GBM and other CNS tumors has remained controversial.

CMV and brain tumors

In the current study, the aim was to assess CMV involvement in the brain tumors of children aged 0 to 2 years. GBM tumors and samples containing only necrotic tissues were excluded, and subsequently, the relationship between CMV and infantile

non-GBM tumors was investigated. None of the samples was detected to have any sign of CMV genome.

The absence of CMV in these samples should be interpreted with caution. The first and simplest assumption is that CMV is not involved in these tumors, so it is not a good target for antiviral therapy in non-GBM infantile brain tumors. Second, the negative results could be due to the fact that tumors may harbor only part of the CMV genome which could not be targeted by the assays used in our investigations. Third, the occurrence of infantile brain tumors is relatively rare, and therefore, the sample size was small and heterogeneous. Although all eligible cases for 10 consecutive years were evaluated, only 36 tumors could be confirmed and traced. This small sample could not mirror the pattern of viral involvement of such tumors in the whole society. Fourth, the difference in genomic expression and behavior of CMV in different populations may yield dissimilar virus expression in brain tumors among different communities. Examples of the dissimilar relationships between viruses and malignancies in different geographical areas can be given in some cases. The Epstein–Barr virus (EBV) and human papillomavirus (HPV) are known instances of these phenomena. About the behavior of EBV in different geographical areas, it can be pointed out to its relationship with Burkitt's lymphoma which is 100% EBV positive in endemic tumors, though in sporadic cases there are varying degrees of EBV involvement [13]. The same is true for the association of various types of HPV and some malignancies such as invasive cervical carcinoma in different areas [14].

Taken together, it seems that the role of CMV infection in the development, progression, or metastasis of infantile CNS tumors in our population remains unclear. Larger sample size should be studied to confirm or rule out this relationship. A review of literature from different populations showed a significant difference in the prevalence of CMV involvement among different tumor samples. In a study conducted in Brazil in 2012 on patients with glioma, CMV was observed in peripheral blood of 75% of the affected population, and 36% of biopsy samples were positive for the virus DNA [6]. In contrary to the mentioned studies, in an investigation by Yamashita et al. in Japan, none of the 10 samples studied was positive for CMV DNA [37]. In a study performed in Taiwan by Yang and colleagues on cases of adult GBM, all in situ hybridization (ISH) and immunohistochemistry (IHC) tests were reported negative for CMV. Therefore, similar to Yamashita et al., they concluded that CMV is less possible to play a crucial role in the pathogenesis of such tumors [38]. In a study conducted by Garcia-Martinez in Spain, from 122 samples of glioma patients between 4 and 81 years who were examined for CMV, no single case was positive [7]. In 2017, John Hopkins University conducted a large-scale study by Holdhoff et al. which used six advanced high-sensitivity techniques including Chromogenic ISH (CISH), IHC, and microarray for different types of tissue and blood samples from GBM patients. There was no evidence of CMV in the samples [12]. Few studies have been done on tissue samples obtained from pediatric populations. In Sardi's study, which is closely similar to our work, 27 children with brain tumors who had positive serological results of CMV were selected, and their tumor tissue samples were examined for CMV using PCR and IHC. All tumor samples were negative, and the authors concluded that CMV is not associated with central nervous system tumors in children [25]. Hence, the occurrence of this virus in different types of central nervous system tumors in different populations has a completely different face and therefore will have a different value in predicting the occurrence or progression of tumors.

Controversies and different methods of investigations

Studies on the carcinogenic role of CMV in recent decades have been the subject of many controversies. The studies have been varied in terms of sample types and the diagnostic methods of detecting CMV, which is itself a potential cause of the variations in results. The methods of detection in these studies include serology, cell culture, antigen, PCR, IHC, nucleic acid sequencing, and hybrid capture, the last four of which can be used to study the virus in paraffin blocks. In serologic studies of blood samples, as was to be expected, more positive results have been reported, because seropositivity of the virus in healthy adult population is usually up to 40 to 60% [2]. The preferred sensitive and functional method for the diagnosis of CMV is PCR. This method is the gold standard, and false positives and negatives are very rare. False positives may occur via contamination in the field, reagent, or samples, and false negatives may occur in case of reagent deterioration, DNA loss, and improper priming. Immunohistochemical methods have a high specificity but low sensitivity because in cases of non-uniform distribution of the virus in the tissue, there is a high probability of false diagnosis. Hybrid capture method has also a low sensitivity [24]. Various studies have compared these methods. For example, in the study of Persons et al., CMV was measured by PCR and ISH methods in 34 autopsy samples of immunodeficiency patients. Using PCR and ISH methods, the virus was positive in 25% and 15% of the samples, respectively, which indicates greater sensitivity of the PCR method [19]. In the current study, PCR method was used to detect virus DNA in tumor samples. To minimize false-positive and false-negative results, deionized water and Beta2 microglobulin gene were used for negative and positive internal control and myelomeningocele and CMV-infected lung tissue for negative and positive external controls. Using real-time PCR, none of the samples of brain tumors studied in this work showed CMV infection. It can be concluded from this investigation

that in our community, CMV infection is less likely to play a role in the occurrence and progression of these tumors. Even though, it should be noted that the significance of results is not yet confirmed or ruled out. Either case stands in need for further investigations.

Limitations

This study is limited by its retrospective nature, small sample size, dissimilar histopathology of tumors, and inevitable potential technical errors. The lack of serologic tests due to retrospective design of study was another limitation, although Iranian populations are mostly seropositive. Moreover, infantile brain tumor is a relatively rare entity, consisting of heterogeneous tumors, which further limits sampling. The patients of this series were not from all geographical regions of Iran, and there may be other patterns of relationship between CMV and pediatric CNS tumors in other geographical areas which requires more extensive studies with larger sample size from different areas and different ethnical groups.

Conclusion

Although CMV may have roles in progression of GBM in some populations, similar roles could not be proposed for non-GBM infantile brain tumors in Iranian children.

Compliance with ethical standards

Conflict of interest The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

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