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Research Article

Epstein-Barr virus infection in Multiple sclerosis glioma patients

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Abstract: Aim of study : This study examines the occurrence of EBV infection in glioma tumors that arise in individuals with a history of peripheral multiple sclerosis. the Epstein-Barr virus (EBV) has been implicated in the pathogenesis of glioma, contributing to the formation of tumors. **Patients and methods** we did a retrospective analysis of archival material of 30 individuals who got gliomas as a result of Multiple sclerosis. EBV DNA sequence was examined using real-time polymerase chain reaction (qPCR). The presence of Epstein-Barr virus nuclear antigen leader protein (EBNA-LP) was identified using quantitative polymerase chain reaction (qPCR).recorded were the clinic-pathological characteristics.A statistical analysis was conducted to compare patients who tested positive for EBV with those who tested negative for EBV. **Results** in total, six gliomas tested positive for the Epstein-Barr virus (EBV), which accounted for 20% of the cases. EBV DNA was detected in 6 instances. EBNA-LP was identified in every patient who tested positive for EBV DNA. All patients who tested positive for Epstein-Barr virus (EBV) were diagnosed with glioblastomas multiforme (GBM). EBV-negative patients exhibited superior median overall survival and recurrence-free survival compared to EBV-positive patients. **Conclusion** taken together, these results lend credence to the idea that EBV infection is present in Iraqi GBM. EBV infection also appears to be linked to a poorer prognosis for patients, at least when comparing EBV-positive and negative malignancies. The results are promising, but more advanced molecular research is needed to corroborate them and offer light on the possible function of EBV in these fatal cancers.

Keywords: Epstein-Barr virus, EBV, Multiple sclerosis, glioma..

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1. Introduction

Multiple sclerosis (MS) is the most common chronic inflammatory and neurodegenerative disease of the CNS. Worldwide, MS affects an estimated 2.8 million people or 35.9 out of every 100,000. The prevalence of MS is also rising in children [1] and developing nations [2]. Impaired motor function, visual symptoms, fatigue, eye movement disorders, bladder problems, sensory symptoms, sexual dysfunction, ataxia, deafness, spasticity, dementia, and cognitive impairment [3] are all neurological indications and symptoms of multiple sclerosis. Multiple sclerosis has a complicated and multifaceted etiology due to the interaction between pathogenic agents, a lack of sun exposure and vitamin D, smoking, and obesity, and known genetic risk factors, primarily in genes guiding the immune system [4]. According to the findings of Bahmanyar et al., MS-related persistent brain inflammation may induce carcinogenesis. [5] They also suggest that MS patients who are closely monitored can increase their chances of having brain tumors detected at an early stage. The authors concede that immunosuppressive medication has the potential to promote cancerogenesis, but they argue that this does not account for the varied risk modifications seen, including a reduced overall cancer risk and increased risks only for brain and genitourinary tract malignancies. [5] However, brain lymphomas are strongly linked to immunosuppressive states, and there is also debatable evidence that gliomas are more common in patients with human immunodeficiency virus infection [6].

Malignant brain tumors derived from glial cells are called gliomas, and they come in a wide variety. Gliomas are classified into four histological grades (I through IV) according to WHO standards. Glioblastoma multiforme (GBM), also known as grade IV glioma, is the most common glial tumor in adults and is associated with a poor prognosis and shorter survival times [8]. The possibility that gliomas and infectious agents are linked is a relatively new one [9]. As viruses may play a role in the oncomodulation of gliomas, several investigations have identified correlations with viral infections [10]. Gliomas have been linked to multiple viruses, including Epstein-Barr virus, hepatitis B and C viruses, human adult T-cell leukemia virus type 1, John Cunningham virus, BK virus, simian virus 40, and Kaposi sarcoma-associated herpes virus [11]. This link between persistent viral infection and primary brain cancers, however, was not without its share of debates [12]. Acute cerebellar ataxia, acute encephalitis, and meningitis are all disorders of the CNS that have been linked to EBV [13].

The Epstein-Barr virus (EBV), commonly known as human herpesvirus 4 (HHV4), is a type 4 herpesvirus [14]. Infection with Epstein-Barr virus (EBV), which is spread mostly by saliva and vaginal fluids, can begin as early as infancy and results in a lifelong latent infection that is typically asymptomatic [15]. There are two stages of EBV's life cycle: the active lytic phase and the dormant latent phase. Since B cells contain CR2, the virus's primary cellular receptor, they are the primary targets of EBV during latency [15]. These cells may be running one of three different latency programs (Latency I, II, or III). Different EBV latency programs result in the production of different viral gene products, such as the nuclear oncoproteins (EBNA1, -2, -3A, -3B, -3C, and -LP), the latent membrane proteins (LMP1, -2A, and -2B), and a plethora of EBV non-coding RNAs (ncRNAs), including EBERs and miRNAs [16]. Although Epstein-Barr virus (EBV) is commonly found in primary central nervous system lymphoma identified in immune-compromised people, the link between EBV infection and other brain cancers, and especially gliomas, is still up for debate [17].

2. Material and methods

Extraction of Virus DNA Following the manufacturer's instructions, viral DNA was isolated using a Viral Nucleic Acid Extraction Kit (gSYNC TM DNA extraction kit, Junaid, Lot No. FA30411-GS, USA).

Real-time polymerase chain reaction (PCR) The qPCR experiment was conducted using a specific set of primers to amplify a 237 bp fragment of the Epstein-Barr virus (EBV). Forward primer sequence: 5'-GCCAAACACCTCCAGTCCTA-3'; Reverse primer sequence: 5'-TCTGCGGGGTCTATAGATGG-3'.

The gene amplification technique was employed to detect the EBNA-LP gene of the Epstein-Barr virus in all blood samples examined. This was achieved using a real-time kit (Cat, Lot 71301, Addbio, Korea; Cat, Lot 2001A, ABM, Canada). The mixture was prepared with a final volume of 20 μl by mixing all ingredients indicated in (Table 1), The PCR conditions were described in (Table 2), and method was performed in the Alamin Center for Advanced Research and Biotechnology by using (Analytik Jena3G) instrument.

Table 1. The Components of Real-time (RT-qPCR) mixture for EBNA-LP of EBV

Item	Volume
BrightGreen 2X qPCR MasterMix	10 μl
Forward Primer	2 μl
Reverse Primer	2μl
Template DNA	5 μl
Nuclease-free water	1 μl
Total reaction volume	20 μl

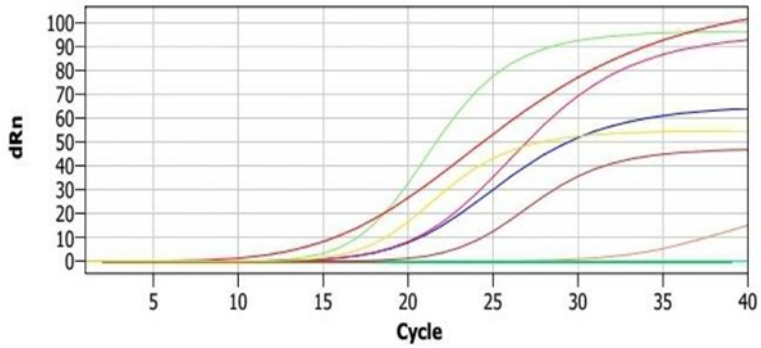

Figure 1. Detection EBNA-LP of EBV DNA by qPCR in Multiple Sclerosis developed glioma.

Table 2. The thermo-cycler conditions for the amplification of EBNA-LP of EBV

Step	Condition	No. of Cycle
Pre-Denaturation	95 °C / 2 min	1
Denaturation	95 °C/30sec	40
Annealing	57.2°C / 30sec	
Extension	72 °C /50.0sec	
Final extension	72 °C / 5 min	1
Hold	4 °C	Forever

3. Results

The ages of our patients ranged from 20 up to 80, with a mean age of 45. We had 18 males and 12 females in total. The frontal lobe (23.33%), temporal lobe (13.33%), parietal lobe (16.67%), occipital lobe (10%), and mixed localizations involving more than one lobe (16.67%) accounted for the majority of gliomas caused by Multiple Sclerosis. In the remaining 20% of gliomas, pathology reports did not identify a specific tumor location. All of the glioma samples from MS patients were tested for EBV infection. As shown in (Fig. 1), EBV DNA was found in 6 of 30 cases (20%) using EBV-specific qPCR.

Table 3

Type of Glioma	N0. of samples
frontal lobe	23.33%
temporal lobe	13.33%
parietal lobe	16.67%
occipital lobe	10%
mixed localizations	16.67%

4. Discussion

When it comes to primary brain tumors, GBM is the most dangerous kind. The typical survival time for individuals with GBM is less than 1 year, and just 2% of patients live for 3 years or more [19]. It is prevalent in secondary glioblastomas but is essentially lacking in main glioblastomas [18], which explains this patient's excellent result, as he is still living 5 years after his diagnosis of GBM. Even in individuals with negative CSF JC Virus PCR, a brain biopsy should be undertaken if new lesions appear following treatment because they may be caused by glioma. In 2004, Isidori et al. published a report describing 40 patients who had progressed

from MS to glioma over the course of their disease. If GBMs in MS patients were secondary tumors rather than primary ones, as we hypothesized, [20].

B-cell lymphomas and nasopharyngeal carcinomas have a well-defined relationship with EBV [21]. Recent studies [22] have looked into the possibility of EBV infection in gliomas. EBV infection in gliomas in Africa and the Arab world: a first report [23].

Thirty gliomas from Iraq were tested using qPCR to see if they harbored the Epstein-Barr virus. EBV infection was linked to 6 of the cases. Previous research has also shown EBV in glioma samples, albeit the results varied greatly among studies, demographics, and methods [22]. Lin et al. studied 112 gliomas from preserved tissues for the presence of LMP1 sequence using multi-plex droplet digital PCR; only 4 GBM cases were linked to EBV (21.1

All low-grade gliomas and the matched controls tested negative for EBV [24]. However, no evidence of EBV was observed in any of the grade III gliomas examined by Strojnik et al. [22], who used RT-PCR and nucleotide sequencing to examine 33 cases of GBM in Slovenian patients. We found no viral DNA in gliomas of grades I–III, which suggests that EBV is more commonly linked to the worst glial tumors. Contrary to these results, Fonseca et al. screened 75 specimens of various histological subtypes for EBV DNA by PCR and direct sequencing and found that EBV DNA was present in 11 gliomas (6 low-grade gliomas, 2 grade III gliomas, 1 oligoastrocytoma, 1 ependymoma, and only 1 GBM) [25].

Studies utilizing EBV-specific PCR, EBV DNA, showed cytoplasmic staining of the EBV LMP1 in tumor cells [26], suggesting that the sample type was glioma tissues. Extraction and identification of viral DNA in tissue by real-time PCR for the EBNA gene [22], after initial histological diagnosis given during surgery. Additionally, the study included EBV testing for various astrocytoma grades. During the study, samples were taken. Genomic DNA was extracted from the samples, and then a Multiplex digital droplet PCR assay based on highly conserved genomic sequences [24] was used to test for various human EBV for LMP-1.

These scientists used fresh frozen gliomas from a Brazilian cancer hospital to conduct a PCR and direct sequencing screen of 75 specimens representing a variety of histopathological subtypes. Gliomas were found to have EBV DNA, low-grade gliomas, two grade III gliomas, one oligoastrocytoma, one ependymoma, and only one GBM [25] were subjected to PCRs utilizing primers for amplification of the EBV BamM region. Neves et al. (2012) found that PCR [27] detection of human Epstein-Barr virus was more common in WHO grade I pilocytic astrocytoma.

Our results suggested that EBV infection in gliomas in MS patients may have a predictive role. Overall, those who tested negative for Epstein-Barr virus had a higher median survival rate than those who tested positive. Furthermore, EBV-positive individuals had a markedly lower recurrence-free survival compared to EBV-negative subjects. By stratifying GBM patients by age, Vidone et al.'s earlier work [28] showed for the first time that HPV is associated with a poorer prognosis.

Strojnik et al. [22] recently identified no EBV in the plasma of 45 patients with high-grade gliomas, casting doubt on the validity of prior investigations that investigated EBV serology in afflicted patients and yielded conflicting results. However, previous studies found that EBV antibodies were present in 89% of individuals with glial tumors [16], including GBM patients.

However, several investigations have ignored the possibility of EBV in gliomas [29]. Using semi-quantitative PCR, Cosset et al. [30] were unable to find EBV DNA in any of the 20 GBM tissues or the matching patient serum. This was also the case for the three low-grade gliomas, one oligodendroglioma, two meningiomas, one ependymoma, and one oligoastrocytoma. Similarly, Khoury et al. [31] found no DNA viral transcripts in either low- or high-grade gliomas. Hashida et al. [32] performed real-time PCR tests on GBM from Japanese patients and found no evidence of the LMP1 gene.

Population/geographic variances, individual genetic diversity, the inherent variety of gliomas, disparities in the actual viral genes probed, and technique sensitivity and precision may all contribute to the observed discrepancies in EBV results in gliomas [33]. Inconsistent findings on EBV infection may also be a result of discrepancies in sampling, processing, or preparation of specimens, or problems with archival tissues [34,35].

5. Conclusion

Our results provide more evidence for the presence of EBV. Since gliomas originated in MS tissue samples, the latter likely play a role in the aggressive tumors' oncogenesis and development. In addition, it appears that EBV infection is linked to a worse prognosis when compared to EBV-negative data.

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Conflicts of Interest: Write conflict of interests or write "The authors declare that they do not have any conflict of interests."

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