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Original Research Article

Detection of Epstein–Barr Virus in Astrocytoma Grade 2 from a Group of Iraqi Patients

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Abstract

Background*:** Astrocytomas are among the most frequent brain tumors affecting both children and young adults. ***Objective*:** To analyze brain tissues with astrocytomas grades II obtained from a group of Iraqi patients for the rate of DNA detection by PCR of oncogenic Epstein- Barr virus. ***Materials and Methods: (75) brain tissue specimens have been enrolled from patients, which were related to patients; among them 50 patients, aged 20 to 77 years, (58 % males and 42% were females) had operations in neurosurgical theatres of Ghazi Al- Hariri Teaching Hospital at The Medical City Complex for astrocytoma (grade 2) and also a number of (25) patients has enrolled, aged 21 to 70 years, (56 % males and 44% were females), whom brain tissues have histopathological examination shown neither in line of benign brain tumors nor brain cancers, and were including as the control group of the present research work. The current method was performed by using the technique of polymerase chain reaction that was done to amplify and localize of the examined DNA sequence of EBV. ***Results*:** 28% (14 out of 50) of the examined brain tissues from astrocytomas grade 2 cases were positive for EBV genome detection. The most infected brain tumor tissues with EBV- DNA are related to the age stratum (20-40 years), which accounted for 14 %, while the age strata of (41-60 years) and (61- 80 years) accounted for 12% and 2 %, respectively. The male patients accounted for 71.4 % while females accounted for 28.6 %, while positive EBV-PCR detection result in brain tissues from control patients without tumors was found in 8% of the examined tissues. ***Conclusion*:** high rate of EBV detection in this percentage of the studied astrocytoma grade 2 tissue samples can possibly considered playing in the induction of these brain tumors.

Keywords: EBV, Brain Tumors, Astrocytoma Grade 2, Brain Control Tissues, PCR.

INTRODUCTION

Gliomas are the most common and lethal primary malignant brain tumors affecting the adults [1], and are the result of glial cellular carcinogenesis in both brain and spinal cord, where many aspects of their etiologies as well as the mechanisms of its tumorigenesis mostly remain unknown. However, several risk factors are recognized by the researchers to be involved in the pathogenesis of gliomas, including both host as well as environmental factors [2].

Among all brain tumors, gliomas and meningiomas are the two most frequent types. Gliomas (glial tumors that are derived from the glial cells) are rare among all tumors in the body (constituting 1.4% - 2%) [3], however, are the most common and diverse malignant brain tumors [4], accounting for 60% of all primary CNS cancers. Gliomas are classified as oligodendrogliomas when have originated from oligodendrocytes, and those have originated from ependymal cells as ependymomas, while those have originated from astrocytes classified as astrocytomas [5]. The reasons behind these brain tumors are yet unknown, urging further research works [6-8].

The research issues of the relation of viral infections with glioma have been recently recognized as one of the most critical aspects in research fields,

however, currently the lack of systematic reviews as well as meta-analyses precluded the evaluation of the effects of viral infections on the prognosis of patients with [1]. Astrocytomas, amongst most frequent brain tumors, and particularly, are affecting children, adolescents, and young adults [9].

According to WHO histological classification, these gliomas distributed into 4 grades (grade I to IV) where the grade IV among them (named as Glioblastoma multiforme [GBM]), is the most frequent, most devastating, highly malignant and aggressive primary brain glial malignancies found in adult patients, with both worst prognosis as well as survival rates [10,11]. Although their etiology still remains unknown, however, researchers had involved the exposure to some chemical agents or ionizing radiation as well as their genetic predisposition in etiology of these malignancies, especially in GBM patients [12].

Recently, the infectious agents in association with gliomas have been taken in consideration, where several viruses have been detected in gliomas, such as infections with human herpetic viruses (CMV, EBV, Kaposi sarcoma-associated herpes virus) , HPV 16 and 18, viral hepatitis agents (HBV, HCV), HTLV1, that belong to the oncovirus subfamily of retroviruses, and also the small, non- enveloped, double-stranded DNA viruses belonging to the Polyomaviridae family (JCV, BK virus and SV40) [13,14]. Extensive controversies, however, have been raised for the correlative association of such chronic viral infections in the tumorigenesis of primary brain tumors [14].

The astrocytic proliferation is usually reported to be the result of different types of brain tissue aggression, including viral infection, particularly, Epstein-Barr virus (EBV) that have suggested for a playing role in the pathogenesis of astrocytomas [9].

Epstein–Barr virus which is a DNA virus among viral members in the Herpes Viridae and officially has named as human herpes virus 4. Epstein–Barr virus infecting individuals early in childhood so as to establish a lifelong asymptomatic latent infectious state and expresses three possible latency programs, Latency I, II or III, in these cells. Globally, EBV infection infects almost 90% of the population [15, 16]. Epstein–Barr virus is infecting and then persisting within the principal targets of EBV (B cells, the epithelial cells and some T as well as Natural Killer cells) which are expressing the major cellular receptor type 2 (CR2, CD21) which is expressed on these cells [17].

The researchers revealed that this (CR2) receptor of EBV also being present on the astrocytes [15]. The latency of EBV involves turning off most of viral genes when this virus switched to the latent phase and this Virus may remain invisible to immune defense mechanisms, expressing three possible latency programs, Latency I, II or III, in these cells and where it was found that EBV-associated cancers have revealed differential expression of the latency genes of EBV [18]. Epstein–Barr virus (EBV) was recognized as the first and novel human oncovirus and one of the important risk factors for human cancers that originated from the lymphocytes, epithelial cells, and mesenchymal cells [19]. Wide- ranged linkages have been established by Epstein–Barr virus with both lymphoproliferative disorders as well as the solid tumors [14].

The designated aim of the present research work was to unravel the neuropersistance rate of cephalic infection with Epstein–Barr virus in a group of brain tissues from Iraqi patients affected with astrocytomas grade II.

MATERIALS AND METHODS

Studied Groups

The studied tissues of astrocytoma grade 2 brain tumors were obtained from patients whom their ages have a range of 20 years to 77 years, while the collected control tissues were obtained from patients with non-tumorous neurological causes and have shown a normal brain histology and who have an age range of 21 years to 70 years.

DNA Extraction and PCR:

Using Viral DNA/RNA Extraction Kit (Intron / Korea) to extract Epstein– Barr Viral Nucleic Acid from brain tissue specimens obtained from patients with astrocytoma as well as their non- tumorous neurological caused control brain tissues. The DNA samples were then subjected to PCR analysis to determine presence of Epstein–Barr Viral infection. Five microliters of DNA from each sample was amplified in a 50-µL reaction mixture containing PCR buffer and primers.

EBV Primers Design:

F- 5’-CCAGTGCTGTGATCGAGCATCT-3’
R- 5’-CTGCTGACAAACTGCTGCATTC-3’

The PCR analysis for EBV

Five hundred Nano-gram of DNA from fresh frozen tumors were used for PCR of DNA. All the proposed precautions and care to avoid the possible contamination were undertaken during achieving each extraction as well as during preparation of the PCR reactions for EBV. Negative controls were also run in all the PCR reactions.

Thermal Cycles Conditions

The master mix solutions were placed then in a thermal cycler (Biometra- Germany) that had been preheated to 94°C and before the hand set up of the desired cyclic conditions. The target regions of EBV was amplified using specific primers according to mentioned conditions in table (1A).

Table 1A: The study conditions both for amplification EBV gene

Gene	Initial denaturation	Denaturation	Annealing	Extension	Final extension	No. of cycles
EBV	94°C /5 min	94°C / 1 min	60 °C/45Sec	72 °C/ 2min	72 °C/5min	40

Statistical Analysis:

To detect the significance between the variables in this study, Chi –square test was used, and all the statistical analyses were done by using SPSS (Version–23) and the P value was considered significant when p <0.05.

RESULTS

Demographic Descriptive Preview of the Studied Groups:

1. Age Distribution of Studied Groups:

The obtained tissue samples were related to those patients with brain tumors that on histopathological examination diagnosed as astrocytoma grade 2 whom ages ranged from 20 to 77 years with a mean age of 43.7+ 12.6 years, as compared to the ages range of 41.6 + 13.8 years of their control counterpart. However, between these age groups, no significant variations were detected (> p 0.05) (Table).

Table 1: Distribution of Astrocytoma grade 2 Brain Tumors Patients patient and AHC according to their Ages

Study Griuos	No.	Mean Age (Years)	S.D	S.E	Range(years)	
					Minimum	Maximum
Astrocytoma grade 2 Brain Tumors Patients	50	43.7	12.6	2.8	20	77
Apparently Healthy Control (AHC)	25	41.6	13.8	3.1	21	70
Total	**75**	***P-value* = 0.3 No. sign. (*p*<0.05)**				

2. Sex Distribution of Studied Groups:

Males accounted for 58 % (29 out of 50) of astrocytoma grade 2 cases, while females accounted for 42 % (21 out of 50). The ratio of males to females of astrocytoma grade 2 cases was 1.4:1, while, the control group had a sex distribution of 14 / 25 (56%) for males and 11 / 25 (44%) for females. The astrocytoma grade 2 group on comparison to control group had shown a significant difference (P 0.05) on statistical analysis (Table 2).

Table 2: Distribution of the studied astrocytoma grade 2 brain tumors group according to their sex

Gender	Astrocytoma grade 2		Control		P-value
	No.	%	No.	%	
Male	29	58	14	55	0.03*
Female	21	42	11	45	
Total	**50**	**100**	**25**	**100**	

3. Distribution of Patients with Astrocytoma Grade 2 Group According to their Age Strata and Gender

Regarding the age strata and sex of astrocytoma grade 2 brain tumors patients, the age stratum of 36 percent of cases are between 20 and 40 years (10 men and 8 women), the age stratum of 38 percent are between 41 and 60 years (12 men and 7 women), and the age stratum of 26 percent are between 61 and 80 years (7 men and 6 women) (Table 3).

Table 3: Patients with astrocytoma grade 2 brain tumors according to their age strata and gender

Age	Gender		Total	
	Male	Female		
	No.	No.	No.	%
20-40 years	10	8	18	36
41-60 years	12	7	19	38
61-80 years	7	6	13	26
Total Astrocytoma grade 2	**29**	**21**	**50**	**100**

4. Epstein–Barr Viral Genome Detection in Astrocytoma Grade 2 Samples Using Conventional PCR

According to Conventional PCR results, 28% (14 out of 50) of astrocytoma grade 2 samples were positive for EBV genome detection, while 72% (36 out of 50) of astrocytoma grade 2 samples have revealed negative detection results for EBV genome, and as illustrated in table 4 and figure 1. In the control group, 8% (2 out of 25) of the control cases were found to have positive detection for EBV EBV genome. The differences between the astrocytoma grade 2 and control groups were statistically significant (P = 0.04).

Table 4: The Conventional - PCR results of Epstein–Barr Viral Genome Detection in of astrocytoma grade 2 tissue samples

Conventional - PCR Results of EBV Genome	Astrocytoma grade 2 Samples Group N%	Apparently Healthy Control Group N%
Positive	14 (28%)	2 (8%)
Negative	26 (72%)	23(92%)
Total	**50 (100%)**	**25 (100%)**

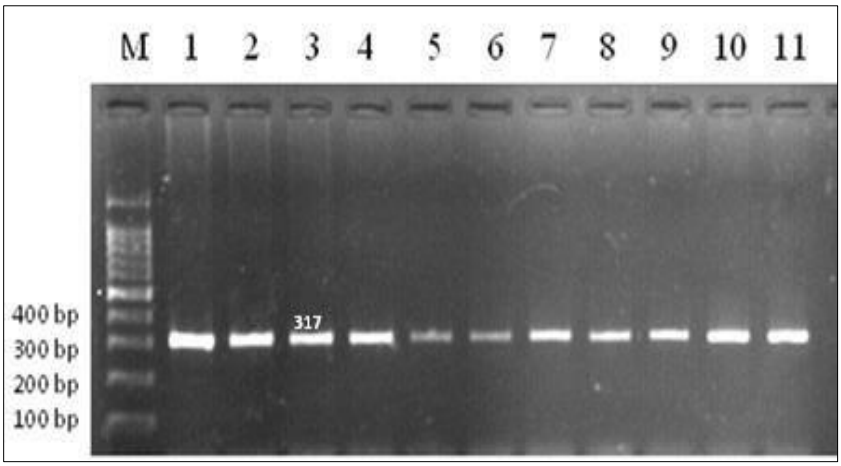

Figure 1: The electrophoresis pattern of EBV DNA (317-bp) detection in tissues from patients with astrocytoma grade II by PCR. Lanes 1-11refer to EBV DNA; Electrophoresis conditions, 1% agarose, 75 V, 20 mA for 1h (5 µl in each well), stained with a red safe solution

5. The EBV-PCR Results in Patients with astrocytoma grade 2 According to Their Age Strata

The highest % of astrocytoma grade 2 brain tumor tissues with DNA-EBV are related to the age stratum (20-40 years), which accounted for 14 percent (7 out of 50 tissues), followed by the age stratum (40- 60 years) and (61-80 years) accounted for 12%, and 2%, respectively. Significant differences ((P<0.05)) were found when these age strata are compared statistically (Table 5).

Table 5: Frequency of EBV-PCR results among astrocytoma grade 2 tissues according to their age strata

Age Stratum	Years	EBV-PCR Results			P value
		No.	Positive	Negative	
	20-40	18	7	11	Anova test P=0.0 (P<0.05)
		17.5%	14%	22%	
	41-60	19	6	13	
		20%	12%	26%	
	61-80	13	1	12	
		30%	2%	24%	
Total		**50**	**14**	**36**	
		100%	**28%**	**72%**	

6. The EBV Results in Astrocytoma Grade 2 Brain Tumors Tissues According to the Gender of the Patients

Table (6) shows the percentage of astrocytoma grade 2 brain tumor tissues that have positive EBV -PCR results based on the gender of patients, where the males accounting for 71.4% (10 out of 14 cases) and females accounting for 28.6% (4 out of 14 cases). In the astrocytoma grade 2 brain tumors group, statistical analysis have revealed significant differences in gender of patients in relation to the positive EBV- PCR results (P<0.05).

Table 6: Positive EBV percentage in astrocytoma grade 2 brain tumor patients based on their sex

Gender of patients with Astrocytoma grade 2 brain tumors	Astrocytoma grade 2 brain tumor tissues showed EBV- genome sequence %	
	Positive	%
Men	10	71.4
Women	4	28.6
The analysis Statistical	(P<0.05) = 0.03	

DISCUSSION

Using different molecularly- designed techniques have abled the researchers to find linkages between viral agents and brain tumorigenesis and / or their progression [20].

The EBV roles in following tumors / cancers: Burkitt lymphoma (aggressive non- Hodgkin B-cell lymphoma), Hodgkin's lymphoma, peripheral T-cell lymphoma, nasopharyngeal carcinoma, gastric carcinoma and thymoma are well-defined in the past [21, 22], yet, Zhang and associates in 2022 [23], stated that EBV infection is albeit not strictly related to cancer development. Further, researchers found that Epstein-Barr virus infections of the central nervous system have a causal association with a number of non- tumorous CNS disorders, such as, acute cerebellar ataxia, demyelinating disease, myelitis, meningitis, acute encephalitis and other CNS neuropathies, even though, only since the last two decades the attention of investigations have focusing to explore EBV role involvement in gliomagenesis [21-24].

The current Iraqi case- control research study was designed to unravel as well as document the detection rate of EBV in a group of brain tissues obtained from an Iraqi patients diagnosed to have astrocytic glioma grade II.

Table (1) in the present study shows that the age of the enrolled patients with astrocytic glioma grade II was ranged from 20 to 77 years with a mean age of 43.7+ 12.6 years. Males accounted for 58 % (29 out of 50) of astrocytoma grade II cases, while females accounted for 42 % (21 out of 50). The ratio of male to female with astrocytoma grade II cases was 1.4:1 (Table 2).

Sugita and Associates in 2016 reported that primary central nervous system lymphoma cases are frequently being EBV-positive. In addition, and regarding gliomas cases, several studies from North America [in USA], South America, Europe, and Japan, and recently in Slovenia, Mexico and Brazil have reported a positive association with EBV [25, 26].

Limam *et al*., study in 2019 [27], conducted a retrospective study on Mexican patients with gliomas enrolling archival materials from 112 gliomas and reported 21.4% (24 / 112) EBV-positive in these studied tissues, among these cases, the EBV DNA was identified in all EBV-positive tissues, while both LMP1 and EBER were detected in only 4 out of 24 (16.7%)EBV DNA-positive cases, where all EBV-positive cases were diagnosed as glioma grade IV (glioblastoma multiforme, GBM).

A Fonesca *et al*., [28], studied primary glioma cases in Brazil to detect EBV in 75 fresh frozen tissues from tumor samples, by using PCR then have confirmation by direct sequencing, they found 14.7% positivity for EBV gliomas (11/75), of these 54.5% positivity being low-grade gliomas, 18.2% being grade III gliomas, and only 9.1% being grade IV gliomas (GBM) and none in other CNS tumors including non-HL that where tumors known to have EBV association as previously reported.

In the present study, the detection rate of EBV by polymerase chain reaction technique (PCR) was found to be 28 % (14 out of the total number of 50 patients) with astrocytic glioma grade II while the detection rate of Epstein–Barr viral nucleic acid in the control tissues was 8% (2 out of the total number of 25 specimens) (Table 4). The highest detection rate of EBV in astrocytic glioma grade II cases was in the age stratum (20-40 years), which accounted for 14% (7 out of 50 tissues), followed by the age stratum (40- 60 years) and (61-80 years) accounted for 12%, and 2%, respectively.

A retrospective Mexican study by Zavala-Vega *et al*., [12], in 2017 on 21 brain tissues with high-grade astrocytoma (GBM) whom age was ranged from 23 to 83 years, has indicated the presence EBV infection in 28.6% of brain astrocytoma tissues by using both immunohistochemistry for LMP-1 by and in situ hybridization for EBER expression.

A study by Leibovitch and Team in 2016 (27), and by using multiplex droplet digital PCR (ddPCR), have found 8.9% EBV- detection rate in the total tissue samples from brain astrocytoma cases with low-and high-grades. Lin *et al*., [28], in 2016, also used multiplex droplet digital PCR, have showed positivity of EBV in 4 out of 19 tissues (21.1%) of formalin-fixed paraffin-embedded tissue samples from GBM patients while no EBV detection revealed in any control tissue samples. Another study by Cosset and Associates in 2014, have used comprehensive metagenomic analysis of gliomatous tissue samples, have found absence of EBV in low-grade brain astrocytoma tissues despite presence of antiviral-like type I interferon gene response [29].

Another study in (2008) on cerebellar pilocytic astrocytoma tissue samples from patients with an average age of 15.5 years was analyzed for quantitative analysis of human EBV by PCR and detected EBV in 30% (9 out of 35) of pilocytic astrocytoma tissues while none of these samples were positive for EBV - LMP1 by IHC [30].

Karimzadeh *et al*., [31], in their study indicated that EBV-DNA had the highest prevalence (44.4% and 33.3%) in astrocytoma and glioblastoma multiforme, respectively.

Freixo and co-researchers in their 2019 [8], systematic review article concluded from the summarized results of EBV infection in relation to astrocytoma pathophysiology that EBV play a role in the

development of a subset of these tumors and not in total lot.

In the current as well as in these previous studies, and because PCR analyses and viral-specific IHC assays were biased since they only are investigating targeted genes or proteins of EBV in tumors, and the next-generation sequencing (NGS) is one technologies now used for rather unbiased approach and in this respect, recently, NGS studies have been used to study EBV sequences in gliomas [32].

CONCLUSION

We concluded that the apparently documented moderate percentage rate of EBV DNA detection in the studied subset of astrocytoma tumorous grade 2 tissues can possibly shedding lights on the considered role in the development and / or induction of these brain this subset of tumors by this virus. We recommend the need to other additional (with more detailed and comprehensive) studies using advanced molecular techniques (such as next-generation sequencing) to fully implicate EBV in having a direct association in gliomagenesis and on comodulation in this studied subset of astrocytoma grade 2 tumors.

REFERENCES

1. Cai, Z., Yang, S., Li, X., Chen, F., & Li, W. (2020). Viral infection and glioma: a meta-analysis of prognosis. *BMC cancer*, *20*, 1-9.
2. Zavala-Vega, S., Palma-Lara, I., Ortega-Soto, E., Trejo-Solis, C., de Arellano, I. T. R., Ucharima-Corona, L. E., ... & Arellano-Galindo, J. (2019). Role of Epstein-Barr virus in glioblastoma. *Critical Reviews™ in Oncogenesis*, *24*(4).
3. Gladson, C. L., Prayson, R. A., & Liu, W. M. (2010). The pathobiology of glioma tumors. *Annual Review of Pathology: Mechanisms of Disease*, *5*(1), 33-50.
4. Siegel, R. L., Miller, K. D., & Jemal, A. (2018). Cancer statistics, 2018. *CA: a cancer journal for clinicians*, *68*(1), 7-30.
5. Wen, P. Y., & Kesari, S. (2008). Malignant gliomas in adults. *New England Journal of Medicine*, *359*(5), 492-507.
6. Bergo, E., Lombardi, G., Pambuku, A., Della Puppa, A., Bellu, L., D'avella, D., & Zagonel, V. (2016). Cognitive rehabilitation in patients with gliomas and other brain tumors: state of the art. *BioMed research international*, *2016*(1), 3041824.
7. Langen, K. J., Stoffels, G., Filss, C., Heinzel, A., Stegmayr, C., Lohmann, P., ... & Galldiks, N. (2017). Imaging of amino acid transport in brain tumours: positron emission tomography with O-(2-[18F] fluoroethyl)-L-tyrosine (FET). *Methods*, *130*, 124-134.
8. Freixo, C., Hermouet, S., & Neves, A. M. (2019). Epstein-Barr Virus and Astrocytoma. *Critical Reviews™ in Oncogenesis*, *24*(4).
9. Manikandan, C., Kaushik, A., & Sen, D. (2020). Viral vector: potential therapeutic for glioblastoma multiforme. *Cancer Gene Therapy*, *27*(5), 270-279.
10. Hanif, F., Muzaffar, K., Perveen, K., Malhi, S. M., & Simjee, S. U. (2017). Glioblastoma multiforme: a review of its epidemiology and pathogenesis through clinical presentation and treatment. *Asian Pacific journal of cancer prevention: APJCP*, *18*(1), 3.
11. Stupp, R., Taillibert, S., Kanner, A. A., Kesari, S., Steinberg, D. M., Toms, S. A., ... & Ram, Z. (2015). Maintenance therapy with tumor-treating fields plus temozolomide vs temozolomide alone for glioblastoma: a randomized clinical trial. *Jama*, *314*(23), 2535-2543.
12. Zavala-Vega, S., Castro-Escarpulli, G., Hernández-Santos, H., Salinas-Lara, C., Palma, I., Mejía-Aranguré, J. M., ... & Arellano-Galindo, J. (2017). An overview of the infection of CMV, HSV 1/2 and EBV in Mexican patients with glioblastoma multiforme. *Pathology-Research and Practice*, *213*(3), 271-276.
13. de Oliveira, D. E., Müller-Coan, B. G., & Pagano, J. S. (2016). Viral carcinogenesis beyond malignant transformation: EBV in the progression of human cancers. *Trends in microbiology*, *24*(8), 649-664.
14. Mui, U. N., Haley, C. T., & Tyring, S. K. (2017). Viral oncology: molecular biology and pathogenesis. *Journal of clinical medicine*, *6*(12), 111.
15. Odumade, O. A., Hogquist, K. A., & Balfour Jr, H. H. (2011). Progress and problems in understanding and managing primary Epstein-Barr virus infections. *Clinical microbiology reviews*, *24*(1), 193-209.
16. Erdei, A., Kovács, K. G., Nagy-Baló, Z., Lukácsi, S., Mácsik-Valent, B., Kurucz, I., & Bajtay, Z. (2021). New aspects in the regulation of human B cell functions by complement receptors CR1, CR2, CR3 and CR4. *Immunology Letters*, *237*, 42-57.
17. Dawson, C. W., Port, R. J., & Young, L. S. (2012, April). The role of the EBV-encoded latent membrane proteins LMP1 and LMP2 in the pathogenesis of nasopharyngeal carcinoma (NPC). In *Seminars in cancer biology* (Vol. 22, No. 2, pp. 144-153). Academic Press.
18. Moghoofei, M., Mostafaei, S., Nesaei, A., Etemadi, A., Sadri Nahand, J., Mirzaei, H., ... & Khodabandehlou, N. (2019). Epstein–Barr virus and thyroid cancer: The role of viral expressed proteins. *Journal of cellular physiology*, *234*(4), 3790-3799.
19. Akhtar, S., Vranic, S., Cyprian, F. S., & Al Moustafa, A. E. (2018). Epstein–Barr virus in gliomas: cause, association, or artifact?. *Frontiers in oncology*, *8*, 123.
20. Khalil, M., Enzinger, C., Wallner-Blazek, M., Scarpatetti, M., Barth, A., Horn, S., & Reiter, G. (2008). Epstein-Barr virus encephalitis presenting with a tumor-like lesion in an immunosuppressed transplant recipient. *Journal of neurovirology*, *14*(6), 574-578.

21. Fujimoto, H., Asaoka, K., Imaizumi, T., Ayabe, M., Shoji, H., & Kaji, M. (2003). Epstein-Barr virus infections of the central nervous system. *Internal medicine*, *42*(1), 33-40.
22. Zhang, N., Zuo, Y., Jiang, L., Peng, Y., Huang, X., & Zuo, L. (2022). Epstein-Barr virus and neurological diseases. *Frontiers in Molecular Biosciences*, *8*, 816098.
23. Sugita, Y., Muta, H., Ohshima, K., Morioka, M., Tsukamoto, Y., Takahashi, H., & Kakita, A. (2016). Primary central nervous system lymphomas and related diseases: Pathological characteristics and discussion of the differential diagnosis. *Neuropathology*, *36*(4), 313-324.
24. Fonseca, R. F., Rosas, S. L. B., Oliveira, J. A., Teixeira, A., Alves, G., & Carvalho, M. D. G. C. (2015). Frequency of Epstein-Barr virus DNA sequences in human gliomas. *Sao Paulo medical journal*, *133*(1), 51-54.
25. Limam, S., Missaoui, N., Mestiri, S., Yacoubi, M. T., Krifa, H., Selmi, B., & Mokni, M. (2019). Epstein-Barr virus infection in gliomas. *Current research in translational medicine*, *67*(4), 129-133.
26. Lin, C. T. M., Leibovitch, E. C., Almira-Suarez, M. I., & Jacobson, S. (2016). Human herpesvirus multiplex ddPCR detection in brain tissue from low- and high-grade astrocytoma cases and controls. *Infectious agents and cancer*, *11*, 1-10.
27. Lin, C. T. M., Leibovitch, E. C., Almira-Suarez, M. I., & Jacobson, S. (2016). Human herpesvirus multiplex ddPCR detection in brain tissue from low- and high-grade astrocytoma cases and controls. *Infectious agents and cancer*, *11*, 1-10.
28. Cosset, É., Petty, T. J., Dutoit, V., Cordey, S., Padioleau, I., Otten-Hernandez, P., ... & Preynat-Seauve, O. (2014). Comprehensive metagenomic analysis of glioblastoma reveals absence of known virus despite antiviral-like type I interferon gene response. *International journal of cancer*, *135*(6), 1381-1389.
29. Neves, A. M., Thompson, G., Carvalheira, J., Trindade, J. C., Rueff, J., Caetano, J. M., ... & Hermouet, S. (2008). Detection and quantitative analysis of human herpesvirus in pilocytic astrocytoma. *Brain research*, *1221*, 108-114.
30. Karimzadeh, M., Tabibzadeh, A., Moghoofei, M., Abbasi, S., Nahand, J. S., Sadeghi, F., ... & Esghaei, M. (2021). As Evidence-Based Tumorigenic Role of Epstein-Barr Virus miR-BART1-3p in Neurological Tumors. *Asian Pacific journal of cancer prevention: APJCP*, *22*(1), 257.
31. Goodwin, S., McPherson, J. D., & McCombie, W. R. (2016). Coming of age: ten years of next-generation sequencing technologies. *Nature reviews genetics*, *17*(6), 333-351.
32. Yohe, S., & Thyagarajan, B. (2017). Review of clinical next-generation sequencing. *Archives of pathology & laboratory medicine*, *141*(11), 1544-1557.