Polish Annals of Medicine

Research paper

Epstein-Barr virus, human cytomegalovirus, human herpesvirus 6 and 7, human adenovirus, John Cunningham virus, and BK virus are not associated with gliomas in humans

Bożena Czarkowska-Pączek[1] ⓘ, *Rafał Górski[2], Aleksandra Wyczałkowska-Tomasik[3], Maciej Bujko[2], Leszek Pączek[3]*

[1] Department of Clinical Nursing, Medical University of Warsaw, Poland
[2] Department of Neurosurgery, John Paul II Western Hospital, Grodzisk Mazowiecki, Poland
[3] Department of Immunology, Transplantology, and Internal Diseases, Medical University of Warsaw, Poland

Article info

Abstract

Introduction: The literature points to several viruses associated with brain carcinogenesis, including gliomas.

Aim: The aim of this study was to assess the presence of several viruses in gliomas and plasma from patients with brain tumor and the possible association of viral positivity with the clinical course.

Material and methods: The study group consisted of 37 patients with gliomas who were subjected to surgery. Mean patient age was 54.59 (SD 15.85) years. The presence of viral DNA was assessed using real-time polymerase chain reaction.

Results and discussion: We did not confirm any BK virus, John Cunningham virus, or human adenovirus-positive gliomas. The percentage of patients with gliomas positive for Epstein-Barr virus (EBV), cytomegalovirus (CMV), and human herpesvirus (HHV) 6 and 7 was 18.9%, 8.1%, 2.7%, and 10.8%, respectively. We did not confirm the co-occurrence of glioma and plasma viral positivity. Fisher's test did not reveal the influence of viral infection on the risk of death. We did not detect any differences regarding sex, WHO classification of gliomas, or the functional impairment evaluated by the Karnofsky scale index at admission to the hospital in the group of patients positive or negative for EBV, CMV, or HHV6.

Conclusions: We cannot confirm an association of the investigated viruses with gliomas.

Corresponding author: Bożena Czarkowska-Pączek, Department of Clinical Nursing, Medical University of Warsaw, E. Ciolka 27, 01-445 Warsaw, Poland. Tel./Fax: +4822 836 09 72.
E-mail address: bozena.czarkowska-paczek@wum.edu.pl.

1. INTRODUCTION

Gliomas account for approximately 60% of all primary central nervous system (CNS) cancers.[1,2] These tumors are typed as astrocytic, oligodendroglial, or rare mixed oligodendroglial-astrocytic, and World Health Organization (WHO) classification grade II (low grade), III (anaplastic), or IV (glioblastoma). Circumscribed gliomas mainly correspond to pilocytic astrocytomas (grade I), and ependymal tumors (WHO grade I, II, or III).[3] Gliomas have poor prognosis depending on tumor morphology, biology, extent of tumor resection, age at diagnosis, and Karnofsky performance status.[1,4] The Karnofsky performance scale index is an 11-point scale for assessing functional impairment. The score ranges from 100 (normal functioning) to 0 (dead).[5] Recently, increasing emphasis has been placed on the association of viral infections and gliomas. Infections are estimated to account for up to 20% of all cancer cases worldwide.[6] Viruses may act by generating genomic instability, increased cell proliferation, induced resistance to apoptosis, modulation of the DNA repair mechanism, or by other alterations in intracellular signaling pathways. They could also compromise the antiviral immune response or induce chronic inflammation. Viruses have been shown to modify the malignant properties of tumors, influencing the prognosis of the disease.[6,7] Previous studies point to several viruses associated with brain carcinogenesis, but results are controversial.[1,7–13]

2. AIM

The aim of the present study was to assess the viral presence of Epstein-Barr virus (EBV), human cytomegalovirus (CMV), human herpesvirus (HHV), BK virus (BKV), John Cunningham virus (JCV), and human adenovirus (HAdV) in gliomas and plasma from patients with brain tumor who were subjected to surgery, and to assess the possible association of viral positivity with the clinical course.

3. MATERIAL AND METHODS

We enrolled 37 patients in this prospective study, 22 men (59.5%) and 15 women (40.5%) with gliomas that were operated between March 2018 and April 2019. During observational period lasted until May 2020, 20 patients died due to cancer progression.

Tissue samples were collected during surgery. Blood samples were taken before the operation. All tissues removed during the operation were examined by pathologist, who diagnosed them according to WHO classification of gliomas. For each patient, the collected data included sex and age, histomolecular classification of gliomas according to the WHO, and the results of the assessment of functional impairment using the Karnofsky scale.

3.1. Analytical methods

Isolation of DNA from tumor tissue was performed using the NucleoSpin Tissue Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. The method was previously described.[14] Viral nucleic acids were isolated from plasma using EZ1 Virus Mini Kit v2.0 (Qiagen, Hilden, Germany) according to the manufacturer's instructions with a BioRobot EZ1 device (Qiagen, Hilden, Germany). Quality and quantity control was done using an ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware, USA).

The number of EBV, CMV, BKV, JCV, HAdV, HHV-6, and HHV-7 copies in the tissue and the viral copy number in plasma were assessed by the real-time polymerase chain reaction (RT PCR) method using the diagnostic test Gene-Proof PCR Kit (GeneProof, Brno, Czech Republic) according to the manufacturer's instructions. The reaction was run with amplification profile: 2 minutes at 37°C, 10 minutes at 95°C, 45 cycles of 5 s at 95°C and 40 s at 60°C, 20 s at 72°C. The viral load was automatically calculated by the ABI7500 analyzer (Applied Biosystems, Foster City, USA) relative to the standard curves.

3.2. Statistical analysis

Statistical analyses were performed using R statistical package, v. 3.6.0. Descriptive statistics were generated using standard parameters: percentage, mean and standard deviation, and median and range (minimum–maximum). Fisher's exact test was used for categorical variables. Between-group differences were analyzed using the Mann-Whitney U test. Survivability curves were plotted using the Kaplan–Meier estimator. Results were considered significant when $P < 0.05$.

4. RESULTS

The characteristics of the patients are presented in Table 1.

We did not confirm any BKV, JCV, or HAdV-positive gliomas in the investigated group. We identified one HHV6-positive glioma sample, without the cooccurrence of virus in the plasma. HHV7-positive gliomas were confirmed in 4 patients, with co-occurrence in plasma in 1 of them. EBV-positive gliomas were found in 7 patients, with plasma virus positivity in 2 of them. CMV-positive gliomas were confirmed in 3 patients, with co-occurrence in plasma in 2 of them. We did not find any of the investigated viruses in plasma without their presence in the glioma.

The proportion of patients positive and negative for each of the investigated viruses in the glioma and plasma, among the whole group and in survivors vs. deceased, are shown in Table 2. We did not find an influence of viral positivity in glioma or plasma on the risk of death.

Kaplan-Meier survival curves for patients positive or negative for CMV and EBV in glioma are shown in Figure 1 and 2, respectively. Log rank test revealed a lower probability of survival among patients positive for CMV com-
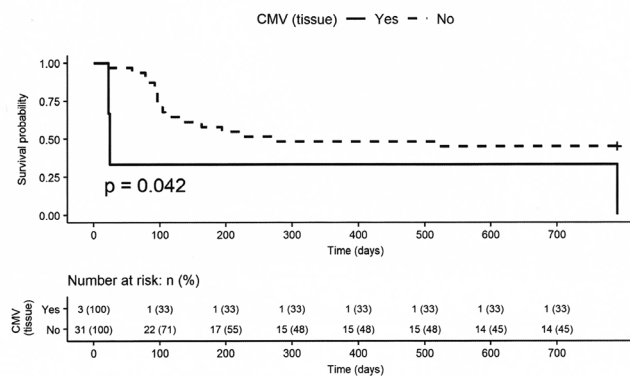
Table 1. Patients characteristics.

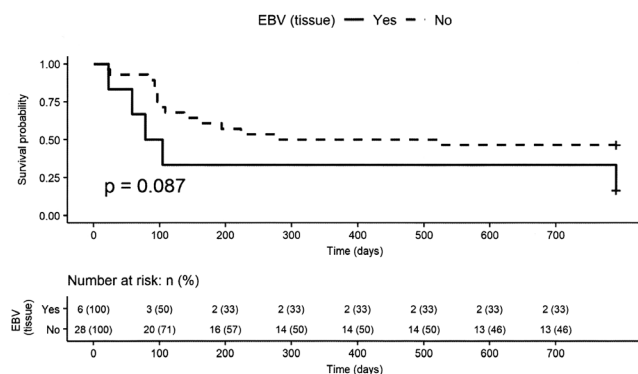| Factor	....
Sex, *n*(%)	
Female	15(40.5)
Men	22(59.5)
Age, y	
N	37
Mean (SD)	54.59 (15.85)
Median (Q1–Q3)	57 (40–67)
Range	29–81
WHO classification, *n*(%)	
G I	1(2.7)
G II	3(8.1)
G III	4(10.8)
G IV	29(78.4)
Karnofsky performance scale index, *n*(%)	
N	34
0	20(58.8)
20	1(2.9)
30	1(2.9)
50	1(2.9)
80	3(8.8)
90	8(23.5)
Median(Q1–Q3)	0(0–80)
Range	0–90

Figure 1. Kaplan-Meier survival curves for patients positive or negative for CMV in the glioma.

researchers revealed that these viruses are rarely found in brain tumors, what is in line with our results.[15–18] However, Delbue et al.[10] showed the presence of JCV DNA and BKV DNA in 40.6% and 9.4% of tested brain tumor samples, respectively. This is the first study examining the presence of HAdV in glioma. HAdV infection could promote the formation of glioma stem cells (GSCs). GSCs contribute to tumor propagation and treatment resistance, and could play a pivotal role in glioma development in humans.[19]

Table 2. Presence or absence of viral load in gliomas and plasma based on patient survival.

Factor copies/mL	Parameter	Whole group* *n* = 37	Survivors* *n* = 14	Died* *n* = 20	*P* value
Gliomas					
EBV	Yes	7(18.9)	1(7.1)	5(25)	0.3636
	No	30(81.1)	13(92.9)	15(75)	
CMV	Yes	3(8.1)	0(0)	3(15)	0.2513
	No	34(91.9)	14(100)	17(85)	
HHV6	Yes	1(2.7)	0(0)	1(5)	1
	No	36(97.3)	14(100)	19(95)	
HHV7	Yes	4(10.8)	3(21.4)	1(5)	0.283
	No	33(89.2)	11(78.6)	19(95)	
Any viral positivity	Yes	12(32.4)	4(28.6)	7(35)	1
	No	25(67.6)	10(71.4)	13(65)	
Plasmas					
EBV	Yes	2(5.4)	0(0)	2(10)	0.5009
	No	35(94.6)	14(100)	18(90)	
CMV	Yes	2(5.4)	0(0)	2(10)	0.5009
	No	35(94.6)	14(100)	18(90)	
HHV6	Yes	0(0)	0(0)	0(0)	1
	No	37(100)	14(100)	20(100)	
HHV7	Yes	1(2.7)	1(7.1)	0(0)	0.4118
	No	36(97.3)	13(92.9)	20(100)	
Any viral positivity	Yes	4(10.8)	1(7.1)	3(15)	0.6272
	No	33(89.2)	13(92.9)	17(85)	

Comments: All *P* values are calculated with the Fisher test; * numbers are given as *n*(%).

pared with the negative (*P* = 0.042). However, it was not confirmed by the Fisher test. None of the patients positive for CMV in the glioma survived the observation period. The probability of survival did not differ significantly between the groups positive and negative for EBV in glioma (log rank test, *P* = 0.087), but the probability of survival in the group positive for EBV in the glioma was lower comparing to the negative group over the observation period.

We did not find any differences regarding sex, WHO classification, or functional impairment when comparing the patients positive or negative for EBV, CMV, or HHV7 in glioma or plasma. HHV6 was not examined due to the low number of positive samples. We also did not find any differences regarding these parameters in the group of patients positive or negative for any virus in the glioma or plasma.

5. DISCUSSION

Increasing evidence indicates an impact of viral infections on cancer development, but data for gliomas are conflicting. We aimed to examine the presence of selected viruses in gliomas and plasma among patients who underwent surgery due to brain tumor. We did not find BKV, JCV, or HAdV-positive gliomas among the investigated group; therefore we did not further examine their presence in plasma. Other

Figure 2. Kaplan-Meier survival curves for patients positive or negative for EBV in the glioma

The rest of the investigated viruses belong to the family of herpes viruses. The CNS is suggested to be the site of latent HHV6 infection. HHV7 is an 'orphan' virus, with no known diseases attributed to it, but recent evidence suggests that it may be associated with CNS diseases.[20] Some studies have suggested the association of HHV6 with human gliomas, with a positivity rate 40%–50% depending on the histological profile of the glioma.[21] In the study conducted by Crawford et al.[12] the rate of HHV6 positivity was statistically higher than in non-tumor control brain tissue collected at autopsy. But Lin et al.[13] found no significant difference between the positivity of HHV6 rate in gliomas and non-neurological controls, which is in line with our results. This is the first study examining the association between HHV7 and glioma. We found a positivity rate of HHV7 in human gliomas of approximately 10%, which is rather low.[22] Therefore, the association between HHV6 and HHV7 infection and human gliomas is doubtful. We also did not confirm the coexistence of virus positivity in glioma and plasma, the association of HHV7 positivity with WHO classification of gliomas and Karnofsky scores, or the influence of virus positivity on prognosis.

EBV is associated with human proliferative diseases involving lymphoid and epithelial cells.[23] Akhtar et al.[1] analyzed 10 studies on EBV in gliomas. The rate of EBV-positive samples was between 0% and 28% in high grade gliomas. In one study, 55% of low grade glioma sample were EBV-positive. In our study, almost 19% of tissue samples were EBV-positive, which is similar to the results of most of the studies analyzed by Akhtar et al.[1], but we did not find any correlation between EBV positivity and glioma grade. Our results are also in line with the results of Limam et al.,[24] who reported that 24% of gliomas were EBV-positive and found a possible association of EBV infection with the worst patient prognosis. Based on our results, we agree with the conclusion that EBV is present in gliomas, but a conclusive association is not obvious.

Data regarding the association between CMV and gliomas are also conflicting. Farias et al.[25] conducted a meta-analysis to summarize the results on the association between CMV and gliomas. The estimate of combined CMV frequency in patents with gliomas was 63% and there was an association between CMV and gliomas, including viral markers pp65 protein and nucleic acid. Their results were in line with those of Xing and al.,[26] who confirmed the presence of CMV pp65 protein and viral DNA in 65.8% and 54.4% of investigated gliomas, respectively. However, their results differ in terms of the relationship between CMV positivity and the grade of glioma. Farias et al.[25] did not confirm the correlation of CMV positivity with the histological subtype and grade of glioma. Despite these confirmatory reports regarding an association between CMV and gliomas, a growing number of studies, including our data, support the opposite. We found CMV DNA in only 3 patients (8%) in the whole study group. Zavala-Vega et al.[27] confirmed CMV in 4.8% of patients, but they only analyzed patients with glioblastoma multiforme. Other research did not confirm the presence of CMV in gliomas, especially glioblastomas.[13,22,28,29] CMV positivity in gliomas in our study could result from the reactivation of previous latent infection in the patient during the course of the cancer. Lack of a relationship between WHO classification of gliomas, Karnofsky scores, or the absence of CMV DNA in plasma from patients without glioma positivity could possibly support this hypothesis. However, Kaplan-Meier curves, but not the Fisher test, revealed that patients positive for CMV in gliomas had worse prognosis than those who are CMV-negative. The reason could be the reactivation of previous latent infection. In patients with glioma, reactivation of CMV after cancer therapy is associated with neurological deterioration and poor prognosis.[30]

Due to the low number of patients positive for particular viruses, we decided to analyze the impact of positivity for any of the investigated viruses, in both glioma and plasma, on the risk of death. We did not detect any impact. Viral positivity also did not correlate with the glioma grade or Karnofsky score.

6. CONCLUSIONS

(1) There is no association of the investigated viruses with gliomas.
(2) The rate of EBV-positive samples was almost 19%, but it could possibly originate from infiltrating B cells.[21]
(3) CMV or EBV glioma positivity could worsen a patient's prognosis.

Conflict of interest
None.

Ethics

The Bioethics Committee of Warsaw Medical University approved the study (No. KB/14/A/2018 dated 14 03 2018). Each patient provided written informed consent to participate in the study.

References

1. Akhtar S, Vranic S, Cyprian FS, Al Moustafa AE. Epstein-Barr Virus in Gliomas: Cause, Association, or Artifact? *Front Oncol.* 2018;8:123. https://doi.org/10.3389/fonc.2018.00123.

2. Mudassar F, Shen H, O'Neill G, Hau E. Targeting tumor hypoxia and mitochondrial metabolism with anti-parasitic drugs to improve radiation response in high-grade gliomas. *J Exp Clin Cancer Res.* 2020;39(1):208. https://doi.org/10.1186/s13046-020-01724-6.

3. Perry A, Wesseling P. Histologic classification of gliomas. *Handb Clin Neurol.* 2016;134:71–95. https://doi.org/10.1016/b978-0-12-802997-8.00005-0.

4. Ostrom QT, Bauchet L, Davis FG, et al. The epidemiology of glioma in adults: a 'state of the science' review. *Neuro Oncol.* 2014;16(7):896–913. https://doi.org/10.1093/neuonc/nou087.

5. Péus D, Newcomb N, Hofer S. Appraisal of the Karnofsky Performance Status and proposal of a simple algorithmic system for its evaluation. *BMC Med Inform Decis Mak.* 2013;13:72. https://doi.org/10.1186/1472-6947-13-72.

6. Morales-Sánchez A, Fuentes-Pananá EM. Human viruses and cancer. *Viruses.* 2014;6(10):4047–79. https://dx.doi.org/10.3390%2Fv6104047.

7. Rahbar A, Orrego A, Peredo I, et al. Human cytomegalovirus infection levels in glioblastoma multiforme are of prognostic value for survival. *J Clin Virol.* 2013;57(1):36–42. https://doi.org/10.1016/j.jcv.2012.12.018.

8. Zavala-Vega S, Palma-Lara I, Ortega-Soto E, et al. Role of Epstein-Barr Virus in Glioblastoma. *Crit Rev Oncog.* 2019;24(4):307–338. https://doi.org/10.1615/critrevoncog.2019032655.

9. Tognon M, Casalone R, Martini F, et al. Large T antigen coding sequences of two DNA tumor viruses, BK and SV40, and nonrandom chromosome changes in two glioblastoma cell lines. *Cancer Genet Cytogenet.* 1996;90(1):17–23.

10. Delbue S, Pagani E, Guerini FR, et al. Distribution, characterization and significance of polyomavirus genomic sequences in tumors of the brain and its covering. *J Med Virol.* 2005;77(3):447–454. https://doi.org/10.1002/jmv.20474.

11. Boldorini R, Pagani E, Car PG, et al. Molecular characterisation of JC virus strains detected in human brain tumours. *Pathology.* 2003;35(3):248–253. https://doi.org/10.1080/0031302031000123245.

12. Crawford JR, Santi MR, Thorarinsdottir HK, et al. Detection of human herpesvirus-6 variants in pediatric brain tumors: association of viral antigen in low grade gliomas. *J Clin Virol.* 2009;46(1):37–42. https://dx.doi.org/10.1016%2Fj.jcv.2009.05.011.

13. Lin CT, Leibovitch EC, Almira-Suarez MI, Jacobson S. Human herpesvirus multiplex ddPCR detection in brain tissue from low- and high-grade astrocytoma cases and controls. *Infect Agent Cancer.* 2016;26;11:32. https://doi.org/10.1186/s13027-016-0081-x.

14. Kryst P, Poletajew S, Wyczałkowska-Tomasik A, et al. Epstein-Barr Virus and Human Adenovirus Viremia in Renal Tumors Is Associated with Histological Features of Malignancy. *J Clin Med.* 2020;9:3195. https://doi.org/10.3390/jcm9103195.

15. Rollison DE, Utaipat U, Ryschkewitsch C, et al. Investigation of human brain tumors for the presence of polyomavirus genome sequences by two independent laboratories. *Int J Cancer.* 2005;113(5):769–774. https://doi.org/10.1002/ijc.20641.

16. Ahye N, Bellizzi A, May D, Wollebo HS. The Role of the JC Virus in Central Nervous System Tumorigenesis. *Int J Mol Sci.* 2020;21(17):6236. https://doi.org/10.3390/ijms21176236.

17. Kim JY, Koralnik IJ, LeFave M, Segal RA, Pfister LA, Pomeroy SL. Medulloblastomas and primitive neuroectodermal tumors rarely contain polyomavirus DNA sequences. *Neuro Oncol.* 2002;4(3):165–170. https://doi.org/10.1093/neuonc/4.3.165.

18. Weggen S, Bayer TA, von Deimling A, et al. Low frequency of SV40, JC and BK polyomavirus sequences in human medulloblastomas, meningiomas and ependymomas. *Brain Pathol.* 2000;10(1):85–92. https://doi.org/10.1111/j.1750-3639.2000.tb00245.x.

19. Zang J, Zheng MH, Cao XL, et al. Adenovirus infection promotes the formation of glioma stem cells from glioblastoma cells through the TLR9/NEAT1/STAT3 pathway. *Cell Commun Signa.* 2020;18(1):135. https://doi.org/10.1186/s12964-020-00598-7.

20. Actor JK. Clinical Virology. *Elsevier's Integrated Review Immunology and Microbiology.* 2012;129–138. https://dx.doi.org/10.1016%2FB978-0-323-07447-6.00014-4.

21. Chi J, Gu B, Zhang C, et al. Human herpesvirus 6 latent infection in patients with glioma. *J Infect Dis.* 2012;206(9):1394–1398. https://doi.org/10.1093/infdis/jis513.

22. Strong MJ, Blanchard E 4th, Lin Z, et al. A comprehensive next generation sequencing-based virome assessment in brain tissue suggests no major virus – tumor association. *Acta Neuropathol Commun.* 2016;4(1):71. https://doi.org/10.1186/s40478-016-0338-z.

23. Elgui de Oliveira D, Müller-Coan BG, Pagano JS. Viral Carcinogenesis Beyond Malignant Transformation: EBV in the Progression of Human Cancers. *Trends Microbiol.* 2016;24(8):649–664. https://doi.org/10.1016/j.tim.2016.03.008.

24. Limam S, Missaoui N, Mestiri S, et al. Epstein-Barr virus infection in gliomas. *Curr Res Transl Med.* 2019;67(4):129–133. doi: 10.1016/j.retram.2019.06.002.

25. Farias KPRA, Moreli ML, Floriano VG, da Costa VG. Evidence based on a meta-analysis of human cytomegalovirus infection in glioma. *Arch Virol.* 2019;164(5):1249–1257. https://doi.org/10.1007/s00705-019-04206-z.

26   Xing Y, Wang Y, Wang S, et al. Human cytomegalovirus infection contributes to glioma disease progression via upregulating endocan expression. *Transl Res*. 2016;177: 113–126. https://doi.org/10.1016/j.trsl.2016.06.008.

27   Zavala-Vega S, Castro-Escarpulli G, Hernández-Santos H, et al. An overview of the infection of CMV, HSV 1/2 and EBV in Mexican patients with glioblastoma multiforme. *Pathol Res Pract*. 2017;213(3):271–276. https://doi.org/10.1016/j.prp.2016.12.006.

28   Cosset É, Petty TJ, Dutoit V, et al. Comprehensive metagenomic analysis of glioblastoma reveals absence of known virus despite antiviral-like type I interferon gene response. *Int J Cancer*. 2014;135(6):1381–9. https://doi.org/10.1002/ijc.28670.

29   Hashida Y, Taniguchi A, Yawata T, et al. Prevalence of human cytomegalovirus, polyomaviruses, and oncogenic viruses in glioblastoma among Japanese subjects. *Infect Agent Cancer*. 2015;10:3. https://doi.org/10.1186/1750-9378-10-3.

30   Goerig N, Semrau S, Frey B, et al. Clinically significant CMV (re)activation during or after radiotherapy/chemotherapy of the brain: Correlation with neurological deterioration and improvement upon antiviral treatment. *Strahlenther Onkol*. 2016;192(7):489–97. https://doi.org/10.1007/s00066-016-0987-7.