ORIGINAL ARTICLE

POSSIBLE VIRAL ETIOLOGY OF GLIOBLASTOMA MULTIFORME

Glioblastoma Multiforme'nin Muhtemel Viral Etiyolojisi

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Geliş tarihi/Received: 08.09.2021
Kabul tarihi/Accepted: 08.12.2021
DOI: 10.16919/bozoktip.934029

Bozok Tıp Derg 2021;11(4):22-28
Bozok Med J 2021;11(4):22-28

ABSTRACT

Objective: Recently, cytomegalovirus, Epstein barr virus, BK virus and JC virus have been suggested to contribute to glioma genesis, but evidence is largely contradictory. The aim of this study was to test 50 tissue samples from patients with glioblastoma multiforme (GBM) and 21 normal brain tissues obtained from autopsy material of patients without brain tumors in order to investigate the presence of possible oncogenic microorganisms, including EBV, JCV, BKV and Toxoplasma gondii, and to evaluate patient clinical characteristics of patients with respect to microorganism findings.

Material and Methods: Fifty formalin-fixed paraffin-embedded specimens obtained from glioblastoma tissue and 21 normal brain tissues obtained in the autopsy of individuals without brain tumor were retrospectively analyzed. After de-paraffinization of tissue samples, DNA extraction was performed for real-time polymerase chain reaction (RT-PCR) analysis to detect BKV, JCV, EBV and Toxoplasma gondii via commercially available multiplex kits.

Results: Strikingly, viral DNA was detected in 12 specimens (24%) of the GBM group and in none of the non-tumor brain specimens (p=0.014). BKV was detected in 4, EBV was detected in 3 (of these, 2 were alive during the study), Toxoplasma gondii was detected in 5 and JCV genotype was detected in 1 of the total 50 GBM tissue specimens.

Conclusion: Our results suggest that viruses may be associated with the development or progression of GBM. Understanding the role of BKV, JCV, EBV and other oncoviruses in the etiology of gliomas would likely open up new avenues for the treatment and management of this highly fatal central nervous system tumor.

Keywords: *BKV; EBV; Glioblastoma Multiforme; JCV; PCR; Toxoplasma*

ÖZET

Amaç: Son zamanlarda sitomegalovirus, epstein barr virüsü, BK virüs ve JC virüsünün glioma gelişimine katkıda bulunduğu öne sürülmüştür, ancak bu konudaki kanıtlar çelişkilidir. Bu çalışmanın amacı, EBV, JCV, BKV ve Toksoplazma gondi gibi olası onkojenik mikroorganizmaların varlığını araştırmak için Gliyoblastoma Multiforme'li (GBM) hastalardan alınan 50 doku örneğini ve beyin tümörü olmayan hastaların otopsi materyalinden elde edilen 21 normal beyin dokusunu test etmek ve mikroorganizma bulguları açısından hastaların klinik özelliklerini değerlendirmekti.

Gereç ve Yöntemler: Glioblastoma dokusundan elde edilen 50 Formalin ile fikse edilmiş parafine gömülü örnek ve beyin tümörü olmayan bireylerin otopsisinde elde edilen 21 normal beyin dokusu retrospektif olarak incelendi. Doku örneklerinin deparafinizasyonundan sonra, piyasada bulunan multipleks kitler aracılığıyla BKV, JCV, EBV ve Toksoplazma gondi'yi tespit etmek için gerçek zamanlı polimeraz zincir reaksiyonu (RT-PCR) analizi için DNA ekstraksiyonu gerçekleştirildi.

Bulgular: Çarpıcı bir şekilde, viral DNA, GBM grubunun 12 örneğinde (%24) tespit edilirken, tümör olmayan beyin örneklerinin hiçbirinde tespit edilmedi (p = 0,014). Toplam 50 GBM doku örneğinin 4'ünde BKV, 3'ünde EBV (bunlardan 2'si çalışma sırasında hayattaydı), 5'inde Toksoplazma gondi ve 1'inde JCV genotipi tespit edildi.

Sonuç: Sonuçlarımız, virüslerin GBM gelişimi veya progresyonu ile ilişkili olabileceğini göstermektedir. BKV, JCV, EBV ve diğer onkovirüslerin gliomaların etiyolojisindeki rolünü anlamak, bu son derece ölümcül olan merkezi sinir sistemi tümörünün tedavisi ve yönetimi için yeni yollar açacaktır.

Anahtar Kelimeler: *BKV; EBV; Glioblastoma Multiforme; JCV; PCR; Toksoplazma*

INTRODUCTION

Glioblastoma Multiforme (GBM), a fatal grade IV glioma according to WHO (World Health Organization) classification, is the most common glial tumor, accounting for 50–60% of all gliomas. It also has the worst prognosis, with a median survival of 12–15 months (1-3). Although GBM is rare tumor with global incidence of less than 10 per 100,000 people, its aggressive behavior and mortality makes it a critical subject warranting research (4). More than half of all gliomas are either glioblastomas or WHO grade IV astrocytoma (5). Cranial imaging usually shows a single, relatively large, irregular shaped lesion with multifocal hemorrhage, necrosis and cystic areas (2,6). Morphologically, GBM consists of small cells, characterized by polymorphism, increased cellularity, marked nuclear atypia, anaplasia and significant anisokaryosis (7). Depending on the localization and the possible intracranial pressure increase (in relation with clinical stage) the most common signs of GBM include headache, ataxia, dizziness, visual loss and frequent syncope (4,8). Unfortunately, despite substantive investigations into the etiology and pathogenesis of the disease and some advances in treatment options which contribute very little survival time, the outcome of GBM remains appalling.

Similar to other primary brain neoplasms, little is known about the etiology of GBM and exposure to high dose ionizing radiation is the only confirmed risk factor (3,9). Some evidence also indicates that chemical agents or genetic predisposition can increase the risk of GBM (e.g., germline TP53, NF1 and NF2 mutations) (3). Viruses may contribute to oncogenesis and tumor development in humans by causing immunosuppression or modifying host cells–inducing oncoprotein expression (10). Approximately 20% of all cancers have been associated with infectious agents and 12% of all cancers are caused by oncoviruses (11). Viruses such as human papillomavirus (HPV) and human cytomegalovirus (CMV) are strongly linked to the etiology and progression of cervical and colorectal cancers, respectively (12). In addition, several viruses have been associated with brain tumors, such as human herpes virus 6 (HHV-6), CMV, John Cunningham Virus (JCV a polyomavirus), Simian virus 40 (SV40) and EBV (Epstein Barr virus) (13-15). Toxoplasma gondii, an intracellular (obligate) parasite with a tendency to infect the central nervous system (CNS), is also of great importance for its possible oncogenic pathogenicity in humans (16,17).

However, in the case of brain tumors, contemporary evidence is contradictory, and sometimes controversial, with respect to links to these viruses and other microorganisms. Here, we aimed to test 50 tissue samples from patients with GBM and 21 normal brain tissues obtained from autopsy material of patients without brain tumors in order to investigate the presence of possible oncogenic microorganisms, including EBV, JCV, BKV and Toxoplasma gondii, and to evaluate patient clinical characteristics of patients with respect to microorganism findings.

MATERIAL AND METHODS

Patients diagnosed with GBM in the Department of Erciyes University School of Medicine who were undergoing malignant glioma resection surgery using neuro-navigational technology (BrainlabVV2, Brainlab, Germany) were included in the present study. The clinical data of patients were recorded from electronic medical files. Informed consent was obtained from the patients and the legal representatives of deceased individuals (comprising the control group) for study inclusion, and the research protocol was approved by the local ethical committee

The GBM and healthy brain tissue specimens were fixed in 4% buffered formalin and embedded in paraffin. The histological slides of all cases were reviewed by two pathologists and the histopathological diagnoses were performed according to the World Health Organization classification (1).

Paraffin-embedded tissue samples from 50 patients suffering from WHO grade IV GBM were comprised the patient group. This group consisted of 30 males and 20 females, with a median age of 55 years. Additionally, 21 normal brain tissue specimens, from autopsy materials of subjects who had died due to any reason other than brain tumor, were obtained from the Department of Erciyes University School of Medicine.

Cranial Imaging

Diagnosis of brain tumor, including tumor localization, size, peritumoral edema and dimensions, were

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performed using magnetic resonance imaging (MRI) (Gyroscan Intera 1.5T).

Reverse Transcription and Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR) Analysis

Nucleic acid extraction

After de-paraffinization of tissue samples from paraffin blocks, DNA extraction was performed using a fully automated procedure for simultaneous purification of viral DNA with the EZ1 DSP Virus Card V2.0 (Qiagen, Germany) and a DNA extraction kit (EZ1 DSP Virus Mini Kit, Qiagen, Germany) according to standard protocols and the manufacturer's instructions.

Real-time reverse transcription polymerase chain reaction for virus genotyping

To detect EBV, Toxoplasma gondii, JCV, BKV and 16S rRNA genotypes in the RNA-positive tissue samples (identified in the previous step), a commercial multiplex RT-PCR kit (SRL Blood and Body fluids, AUS Diagnostics, Australia) was applied via a standard RT-PCR device (RotorGene-6000, Corbett Research, Australia). After the 70-minute RT-PCR protocol was applied in a routine fashion, the results were analyzed and quantitated automatically via software.

Statistical Analyses

All analyses were performed on SPSS version 15.0 (SPSS Inc. Released 2007. SPSS for Windows, Version 15.0. Chicago, SPSS Inc.). Chi-square tests were used in the analysis of the distributions of categorical data. After identifying the distribution of quantitative variables (normality tests), groups were compared using the non-parametric Kruskal–Wallis test (for three or more groups) or the Mann Whitney U test (between two groups). Spearman rho correlation analysis was used to determine the direction and strength of the relationship between quantitative variables. P-value of <0.05 were considered to demonstrate statistical significance.

RESULTS

The comparison of the two groups in terms of microorganisms determined by RT-PCR is shown in Table 1. None of the analyzed microorganisms were found in the non-tumor control group but 12 specimens in the GBM group had at least one of the microorganisms.

BKV was detected in 4 of the 50 GBM tissue specimens. No significant relationships were observed between BKV presence and the mortality of patients and the radiological features of GBM (localization, edema, necrosis and resection, all p>0.05). Tumors identified to have BKV were mostly localized in the parietal (40%), less frequently in the temporal lobe (10%) and the size of necrosis was found to be more than 50% of the tumor volume in all subjects. In two of 4 patients, edema was smaller than the tumor volume, while it was larger than the tumor volume in the remaining two subjects (Table 2).

JCV genotype was detected in 1 patient, and no relationships were identified (Table 2).

EBV was detected in 3 patients, two of which were alive during the study. There was a significant relationship between EBV presence and two variables: being alive at analysis (p=0.002) and location of lesion (p=0.033). The tumor was localized in the midline-pineal region in all cases with EBV positivity. No significant relationship was observed between BKV presence and radiological features of GBM (localization, edema, necrosis and resection, all p>0.05). Edema was smaller than tumor volume in all three subjects, and necrosis size was 25–50% of the tumor volume in two individuals (Table 2).

Toxoplasma gondii was detected in 5 patients. The presence of Toxoplasma gondii was unassociated with living status. Tumors containing Toxoplasma gondii genetic material were mostly localized in the temporal (40%) lobe, and less frequently in the frontal and occipital lobes (10%).

Table 1. The Presence of Genetic Materials of Microorganisms determined by RT-PCR in GBM specimens compared to non-tumor specimens

	Pathogen		
Group	**Positive n (%)**	**Negative n (%)**	***p***
Glioblastoma Multiforme	12 (24)	38 (76)	*0.014*
Control	0 (0)	21 (100)	

RT-PCR: Real-time polymerase chain reaction

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Table 2. Evaluation of the Presence of BKV, EBV and Toxoplasma gondii in GBM Tissue specimens and relationships with characteristics

	BKV		**EBV**		**Toxoplasma Gondi**	
	negative	**positive**	**negative**	**positive**	**negative**	**positive**
	n(%)	**n (%)**	**n(%)**	**n(%)**	**n(%)**	**n(%)**
Living status						
Alive	2 (100)	0(0)	0 (0)	2 (100)	2 (100)	0 (0)
Dead	44 (92)	4 (8)	47 (98)	1 (2)	43 (90)	5 (10)
p value	*0.014*		***0.002***		*1.000*	
Localization						
Frontal	7(84)	1(16)	8 (100)	0 (0)	7 (88)	1 (12)
Temporal	23(91)	1(9)	23 (100)	0 (0)	21 (88)	3 (12)
Parietal	6 (67)	2(33)	7 (100)	0 (0)	7 (100)	0(0)
Occipital	4 (100)	0(0)	4 (100)	0 (0)	3 (75)	1 (25)
Pineal	6 (100)	0(0)	3 (50)	3 (50)	6 (100)	0(0)
p value	*0.598*		***0.033***		*0.339*	
16 s-RNA						
Absent	22(96)	1(4)	23(100)	0(0)	21(91)	2(9)
Present	24(89)	3(11)	24(89)	3(11)	24(89)	3(11)
p value	*0.614*		*0.240*		*1.000*	

BKV: BK Virus, EBV: Epstein Barr Virus

DISCUSSION

In the current study, in order to assess the presence of BKV, EBV, JCV and Toxoplasma gondii in GBM samples, DNA extracted from tumor samples was analyzed by PCR and at least one microorganism was identified in 12 of the 50 cases of GBM. Detection of viral nucleic acid or proteins in GBM samples and research focusing on their effects on cellular regulatory pathways have illustrated the mechanisms by which they can transform cells. The results of such studies led to the hypothesis that microorganisms may play a role in central nervous system (CNS) tumorigenesis.

In the current study, four specimens were positive for BKV and 1 for JCV. The BKV and JCV viruses are ubiquitous human polyomaviruses with varied clinical presentation (18). Both have been associated with human tumors (14). The specific association of BKV with human tumors may be associated with the lifelong latent infection which develops following primary infection with BKV (19). From data provided by epidemiological, in vitro and animal studies, the review by Tognon at al., demonstrated that BKV can be latent in many human tissues and may disrupt normal cell growth –with particular effects leading to oncogenicity due to BKV transformation (20). Therefore, it appears that BKV has the potential to be a factor inducing or contributing to the progression of human tumors in many tissues and organs. The oncogenic potential of human BKV infection, in relation with the integration of the viral genome into cellular DNA, has recently been shown (21). While the tumorigenic potential of BKV in humans is still a matter of debate, our data support the notion that this virus can be related to the etiology or progression of GBM in humans.

JCV is a human neurotropic polyomavirus causing progressive multifocal leukoencephalopathy (PML), and the tumorigenic role of JCV has been well documented in animal studies (22). Del Valle at al. examined 85 samples of glial tumors for the presence of JCV DNA and confirmed that 57% to 83% of tumors were positive for JCV (23). We were also able to detect JCV, albeit in only one GBM specimen, which indicates that future studies should include JCV analysis into GBM studies. That is, in order for this finding to gain

recognition as a non-anecdotal relationship, further research is needed to investigate and understand the association (if any) of JCV with the development of various types of brain tumors.

EBV, also known as HHV-4, belongs to a group of gamma-herpes viruses and is present in more than 90% of the human adult population who largely remain asymptomatic (24). Although the role of EBV in B-cell lymphomas and nasopharyngeal carcinomas is well defined, its role in gliomas is only recently being explored (15).

Our results are in accordance with the literature of recent years. Fonseca et al. analyzed 75 frozen glioma tissues using conventional PCR and reported 14.7% prevalence of EBV DNA in WHO III (2/11) and WHO IV (1/11) gliomas (25). Similarly, in the present study, we found EBV DNA in three out of the 50 GBM specimens. Similar to our study, Strojnik et al. (14) studied the presence of EBV in 45 high-grade gliomas and found that only three of the 45 patients were positive for EBV. Several other studies have reported on the complete absence of EBV in gliomas (26,27). In a review of the clinical literature, Akhtar at al. (15) suggested that the reasons for conflicting findings may lie within population-based or geographic differences, individual variabilities in genetics, the heterogeneity of investigated tumors, variations in tumor localization, differences in viral DNA primers, and the sensitivity and precision of methodology. Further molecular and epidemiological studies are needed to establish the possible role played by EBV in the tumorigenesis of gliomas. Toxoplasma gondii is a protozoan parasite capable of infecting most animals, including humans, and it is one of the few non-viral pathogens shown to be associated with the occurrence of brain tumors (28). Previous studies have shown that Toxoplasma gondii could cause glioma in experimental animals (29). In a particularly interesting research conducted in France by Vittecoq et al., it was shown that mortality rates due to brain cancer correlated positively with the local seroprevalence of Toxoplasma gondii (30). In the current study, Toxoplasma gondii was detected in 5 of the 50 individuals with GBM. Thus, according to this study and prior results, it is evident that Toxoplasma gondii should be investigated as a possible oncogenic pathogen in humans. Despite this evidence, it is unclear how the infection might cause GBM in humans. There are case studies in the literature that demonstrate patients with Toxoplasma gondii infection masquerading as GBM with respect to clinical findings (31,32). Such studies demonstrate the rationale for biopsy and pathologic diagnosis prior to the initiation of treatment for malignant brain tumors. In the current study, no such cases were observed.

It is possible that technical factors and the stage of disease at sampling limited detection in the remaining 38 GBM specimens. Also, detailed patient history and immunological characteristics would be required to obtain more accurate results with respect to the temporal relationship between disease development or progression and microorganism isolation. An infectious etiology would suggest that the cancerous process of GBM might be mediated by the immune system, and therefore, the timing of infection and/or its spread must be elucidated. It is also possible that prior exposure to stress and/or immunodeficiency induced by therapies may actually predispose patients to viral infection. Therefore, the relationships determined herein could be associated with other factors, and the results should be confirmed in future studies.

It must be re-iterated that the identification of viral genetic material in tissue samples does not directly prove that these microorganisms may be related to the etiology of GBM. Further studies and techniques, such as assessment of viral integration into the cancer genome, are required to confirm such relationships. Although current data appears to be promising in terms of identifying a relationship between microorganisms and GBM, we conclude that further research is required to ascertain whether causal relationships exist, or whether these findings only indicate associations in this malignancy.

CONCLUSION

The etiology of GBM has been a subject of numerous comprehensive studies. Our results suggest that viruses may play a critical role in the initiation and/or progression of GBM and other types of brain carcinogenesis; however, there is virtually no evidence to suggest a causal relationship at this time. Understanding the role of BKV, JCV, EBV and other oncoviruses, in addition to Toxoplasma gondii, in gliomas

would likely open up new avenues for the treatment and management of these highly fatal CNS tumors.

Acknowledgements

The authors declare no conflicts of interest in association with the study. The authors declared that this study has received no financial support.

REFERENCES

1. Banan R, Hartmann C. The new WHO 2016 classification of brain tumors—what neurosurgeons need to know. Acta Neurochir (Wien). 2017;159:403-18.

2. Alifieris C, Trafalis DT. Glioblastoma multiforme: Pathogenesis and treatment. Pharmacol Ther. 2015;152:63-82.

3. Hanif F, Muzaffar K, Perveen K, Malhi SM, Simjee Sh U. Glioblastoma Multiforme: A Review of its Epidemiology and Pathogenesis through Clinical Presentation and Treatment. Asian Pac J Cancer Prev. 2017;18:3-9.

4. Shea A, Harish V, Afzal Z, Chijioke J, Kedir H, Dusmatova S, et al. MicroRNAs in glioblastoma multiforme pathogenesis and therapeutics. Cancer Med. 2016;5:1917-46.

5. Louis DN, Perry A, Reifenberger G, Von Deimling A, Figarella-Branger D, Cavenee WK, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol. 2016;131:803-20.

6. Agnihotri S, Burrell KE, Wolf A, Jalali S, Hawkins C, Rutka JT, et al. Glioblastoma, a Brief Review of History, Molecular Genetics, Animal Models and Novel Therapeutic Strategies. Arch Immunol Ther Exp (Warsz). 2013;61:25-41.

7. Urbanska K, Sokolowska J, Szmidt M, Sysa P. Glioblastoma multiforme - an overview. Contemp Oncol (Pozn). 2014;18:307-12.

8. Lakhan SE, Harle L. Difficult diagnosis of brainstem glioblastoma multiforme in a woman: a case report and review of the literature. J Med Case Rep. 2009;3:87.

9. Barnholtz-Sloan JS, Ostrom QT, Cote D. Epidemiology of Brain Tumors. Neurol Clin. 2018;36:395-419.

10. White MK, Pagano JS, Khalili K. Viruses and human cancers: a long road of discovery of molecular paradigms. Clin Microbiol Rev. 2014;27:463-81.

11. De Martel C, Ferlay J, Franceschi S, Vignat J, Bray F, Forman D, et al. Global burden of cancers attributable to infections in 2008: a review and synthetic analysis. Lancet Oncol. 2012;13:607-15.

12. Mui UN, Haley CT, Tyring SK. Viral Oncology: Molecular Biology and Pathogenesis. J Clin Med. 2017;6(12):111.

13. Kofman A, Marcinkiewicz L, Dupart E, Lyshchev A, Martynov B, Ryndin A, et al. The roles of viruses in brain tumor initiation and oncomodulation. J Neurooncol. 2011;105:451-66.

14. Strojnik T, Duh D, Lah TT. Prevalence of Neurotropic Viruses in Malignant Glioma and Their Onco-Modulatory Potential. In Vivo. 2017;31:221-9.

15. Akhtar S, Vranic S, Cyprian FS, Al Moustafa AE. Epstein-Barr Virus in Gliomas: Cause, Association, or Artifact? Front Oncol. 2018;8:123.

16. Ali MI, Abd El Wahab WM, Hamdy DA, Hassan A. Toxoplasma gondii in cancer patients receiving chemotherapy: seroprevalence and interferon gamma level. J Parasit Dis. 2019;43:464-71.

17. Thirugnanam S, Rout N, Gnanasekar M. Possible role of Toxoplasma gondii in brain cancer through modulation of host microRNAs. Infect Agent Cancer. 2013;8(1):8.

18. Pinto M, Dobson S. BK and JC virus: a review. J Infect. 2014;68 Suppl 1:S2-8.

19. Reploeg MD, Storch GA, Clifford DB. BK Virus: A Clinical Review. Clin Infect Dis. 2001;33(2):191-202.

20. Tognon M, Corallini A, Martini F, Negrini M, Barbanti-Brodano G. Oncogenic transformation by BK virus and association with human tumors. Oncogene. 2003;22:5192-200.

21. Kenan DJ, Mieczkowski PA, Burger-Calderon R, Singh HK, Nickeleit V. The oncogenic potential of BK-polyomavirus is linked to viral integration into the human genome. J Pathol. 2015;237(3):379-89.

22. 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: 6236.

23. Del Valle L, Gordon J, Assimakopoulou M, Enam S, Geddes JF, Varakis JN, et al. Detection of JC virus DNA sequences and expression of the viral regulatory protein T-antigen in tumors of the central nervous system. Cancer Res. 2001;61:4287-93.

24. Niedobitek G, Meru N, Delecluse HJ. Epstein-Barr virus infection and human malignancies. Int J Exp Pathol. 2001;82:149-70.

25. Fonseca RF, Rosas SLB, Oliveira JA, Teixeira A, Alves G, Carvalho MDGC. Frequency of Epstein-Barr virus DNA sequences in human gliomas. Sao Paulo Med J. 2015;133:51-4.

26. Cosset E, Petty TJ, Dutoit V, Cordey S, Padioleau I, Otten-Hernandez P, et al. Comprehensive metagenomic analysis of glioblastoma reveals absence of known virus despite antiviral-like type I interferon gene response. Int J Cancer. 2014;135:1381-9.

27. Khoury JD, Tannir NM, Williams MD, Chen Y, Yao H, Zhang J, et al. Landscape of DNA Virus Associations across Human Malignant Cancers: Analysis of 3,775 Cases Using RNA-Seq. 2013;87:8916-26.

28. Robert-Gangneux F, Dardé M-L. Epidemiology of and Diagnostic Strategies for Toxoplasmosis. 2012;25:264-96.

29. Wrensch M, Bondy ML, Wiencke J, Yost M. Environmental risk factors for primary malignant brain tumors: A review. J Neurooncol. 1993;17:47-64.

30. Vittecoq M, Elguero E, Lafferty KD, Roche B, Brodeur J, Gauthier-

Clerc M, et al. Brain cancer mortality rates increase with Toxoplasma gondii seroprevalence in France. Infect Genet Evol. 2012;12:496-8.

31. Staller A. Presumed Glioblastoma Multiforme: A Case for Biopsy Prior to Treatment. Clin J Oncol Nurs. 2016;20:95-7.

32. León Ruiz M. A Novel Case of Solitary Cerebral Toxoplasmosis Mimicking Glioblastoma as the First Presentation of HIV. J Clin Neurol. 2016;12:248-50