DETECTION RATES OF HHV-7 INFECTION AND SINGLE NUCLEOTIDE POLYMORPHISMS IN IL-18 GENE PROMOTER REGION IN A VARIETY OF BRAIN TUMORS: A CONVENTIONAL PCR STUDY IN A GROUP OF IRAQI PATIENTS

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Abstract. Background: HHV-7 infection has been documented to cause CNS complications. The susceptibility to many diseases, including immune dysfunction and cancers, has been linked to SNP in the promoter region of the interleukin-18 (IL-18) gene. Objectives: to explore the rates of both HHV-7 infection and the polymorphisms in the IL-18 -607C/A (rs1946518) promoter region in a group of Iraqi patients with different brain tumors. Patients and methods: one hundred fifteen (115) freshly obtained brain tissue biopsies were enrolled in this study; 85 were from brain cancer cases whereas 30 autopsies were obtained from cases with apparently normal brain tissues as a control group. Conventional PCR was chosen both for the detection of HHV-7 and IL-18 rs1946518 SNP detection as well as sequencing. Results: according to conventional PCR analysis, 34% showed positive results for the HHV-7 genome, while 66% revealed negative results. In various types of brain cancers, HHV-7-PCR detection results were 11.8%, 5.9%, 29.4%, 11.8%, 5.9%, and 11.8% in tissues from patients with Transitional Meningioma, Meningotheliomatous Meningioma, Glioblastoma Multiforme, Diffuse Fibrillary Astrocytoma, Anaplastic Oligodendroglioma, Atypical Meningioma, and Pilocytic Astrocytoma, respectively. The polymorphism distributions according to GG; AA and GA genotypes of IL-18 607C/A (rs1946518) polymorphism were 37.1%; 57.1%, and 5.7%, respectively, in the patients' group and 66.7% and 33.3%, respectively, in the control group. Conclusion: the detected rates of HHV-7 as well as IL-18 607C/A (rs1946518) polymorphism have shed light on the possibility that they might have played or contributed a role in the brain tumors' development.

Keywords: HHV-7, IL-18, brain tumor.

List of Abbreviations
HHV-7 – human herpesvirus-7
IL-18 – interleukin-18
SNP – single nucleotide polymorphism
CNS – central nervous system
CMV – cytomegalovirus
CSF – cerebrospinal fluid

Introduction

The ubiquitous virus, human herpesvirus-7 (HHV-7), belongs to the herpes family of 8 types of viruses (herpesviridae) and is located in the subfamily (beta-herpesvirinae), with HHV-6 which is highly related to HHV-7 on the genetic, epidemiologic and clinical bases. It was reported that people are mostly infected with HHV-7 during the childhood period, via the oral route through saliva, and higher in breast-fed than in bottle-fed children, where > 90% of them are infected by 6 years of age, and initially causing asymptomatic latent infections or clinically presented as nonspecific symptoms, such as a rash disease, febrile seizures or even status epilepticus, also being presented as exanthema subitum, as well (Agut *et al*., 2015; Hasan *et al*., 2023). Subsequently, this HHV-7 infects mainly the lymphocytes (CD4+ T-lymphocytes and to a lesser extent CD8+ and immature T-cells, monocytes, salivary glands, and the CNS, where then is reactivated coincidently with a decreased immunity states) (Hasan *et al*., 2023). Regarding the CNS involvement of this virus, little is known about the way for HHV-7 to enter the blood-brain barrier and invade the CNS. Respecting the clinical presentation of HHV-7 in immunocompetent hosts, it does not typically cause serious symptoms, and the neurological complications are rarely reported in such infections and seem highly heterogeneous, including, cerebellitis,

myelitis, meningitis, meningoencephalitis, encephalitis, ataxia, coma and facial palsy (Foiadelli *et al*., 2022). These complications are mainly occurring either in children or immunocompromised individuals, whereas in healthy adults, the neurological involvements of HHV-7 infections have been reported in only 4 cases. Herein, most encephalitis cases due to this virus represent reactivation of HHV-7 in transplantation-immunocompromised patients or in those patients with chemotherapy (Foiadelli *et al*., 2022; Aburakawa *et al.*, 2017). Gliomas remain the most common malignancies that originate from the brain, accounting for half of the intracranial tumors, having increasing morbidity rates in young and mid-aged people at annual levels 1. Among all the main classified types of gliomas (astrocytoma, glioblastoma, and ependymoma), glioblastoma is recognized as the most severe type 5 (Mesfin & Al-Dhahir, 2023). Since the current knowledge of glioma pathogenesis remains elusive, therefore, exploring its pathogenesis as well as susceptibility seems of great significance, among them is the genetic polymorphism, which represents a crucial genetic form in its biology (Pandith *et al.*,2 022). Several previous studies have reported that many polymorphisms in certain inflammatory genes have an important expressed role in the development of several diseases. As critical immunity components, the cytokines have been corroborated to influence glioma development, among them, both IL-6 and IL-18 have a crucial regulatory effect on the immune system (Foiadelli *et al.*, 2023; Schmiedel *et al*., 2018). The IL-18 cytokine is a member of the IL-1 superfamily, where its gene expresses a pleiotropic pro-inflammatory cytokine that has been shown to have a role in the inflammatory cascade. The location of the human IL-18 gene is specified to be on chromosome 11 (11q22.2-q23.3. region) (Ihim *et al.*, 2022). Previous studies have shown that two common different single nucleotide polymorphisms (SNPs) located in the promoter region of the IL-18 gene: -607A/C (rs1946518) and -137C/G (rs187238) have confirmed effects on the IL-18 gene activity. The C allele of IL-18-607A/C and G allele of IL-18-137C/G have stimulated higher IL-18 transcription as well as its protein production (Tsai *et al*., 2013; Noha *et al*., 2017). The IL-18 was found to be produced in the brain by a variety of cell types (such as activated microglia and astrocytes). In addition, it was also reported that IL-18 gene polymorphisms might also affect the onset of such gliomas (Yang *et al.*, 2022). Previous studies have also shown that IL-18 gene polymorphisms are associated or correlated with the risk of cardiovascular disease, ischemic stroke, and Alzheimer's disease (Yang *et al*., 2022; Bossù *et al.*, 2007; Jian-xia *et al.*, 2013). The current study aimed to explore the rates of both HHV-7 infection as well as the detection rates of single nucleotide polymorphisms (SNPs) that are located in the promoter region of the IL-18 gene in a group of Iraqi patients with various types of brain tumors.

Materials and Methods

The studied tissues' groups

The studied brain tumor tissues were obtained from those patients aged from 7 to 78 years. The types of brain cancers were 21 cases of brain cancers diagnosed as Transitional Meningioma (24.7%; 11 males and 10 females), 15 cases as Meningotheliomatous Meningioma (17.6%; 8 males and 7 females), 17 cases as Glioblastoma Multiforme (20%; 10 males and 7 females), 13 cases as Diffuse Fibrillary Astrocytoma (15.3%; 7 males and 6 females), 6 cases as Anaplastic Oligodendroglioma (7.1%; 3 males and 3 females), 4 cases as Atypical Meningioma (4.7%; 3 males and 1 female), and 9 cases as Pilocytic Astrocytoma (10.6%; 5 males and 4 females). The control group for this study was obtained from 30 autopsies whose ages ranged from 17 to 63 years and were without any apparent neurological causes and showed normal brain histologies.

PCR analysis for HHV-7

Five hundred nanograms of DNA were obtained from the fresh frozen tumorous tissues that were used for PCR analysis of the DNA-*encoding sequence of VP1* major capsid protein of HHV-7. Great care and precautions were undertaken to avoid the possible contaminations

before, during, and after achieving the PCR reactions for HHV-7. Negative controls were run in all the PCR reactions, too.

Genotyping studies

DNA was extracted from brain tissue samples by using DNeasy blood and tissue kit and according to the manufacturer's instructions (Intron / Korea). The extracted DNA was stored at -20 °C until use. The primer for IL-18 607C/A (rs1946518) was designed in this study using the Primer Quest Tool Software from IDT. The restriction sites in the amplified region were determined by using a restriction mapper and NEB cutter. The SNP of the IL-18 607C/A (rs1946518) gene was analyzed by using primers from previously published literature (25). The detection of IL-18 gene polymorphism was done by using polymerase chain reaction and PCR-products were used to study the genetic polymorphism in this gene.

Primer pairs dilution

The primers were obtained from the IDT / USA source, where the Bioneer' primers are commonly transported in a lyophilized primer state and their units are commonly given as a mass (in Pico moles). For the creation of primers stocks, the need was to reconstitute the primers in a sterile, nuclease-free H_2O. The producing company has supplied us with the required amount of the sterile, nuclease-free H_2O which was added to each primer to obtain a master stock of 100 mol/ul, and this, in turn, was again being used to obtain working stock spinning down the tube before opening the cap, and then adding the desired amount of water and according to the oligos manufacturer instructions so as to obtain a 100 pmol/µl, then was vortexed properly to re-suspend the primers evenly. Then, an amount of 10 µl of the master stock was transferred to a 0.2 ml Eppendorf tube (containing 90 µl of sterile, nuclease-free H_2O, to have a working stock. The master stock as well as working stock were then stored at -20 °C. Finally, the working stock was thawed on ice and vortex before using in PCR, then stored at -20 °C until being used again.

PCR experiments

PCR amplification was done using a conventional thermal cycler (Biometra – Germany) where (2 µl) of the template DNA was added into a PCR master mix tubes, then 1 µl of each of the forward and reverse primers were added into this PCR master mix tubes. Distilled water was added to PCR premixed tubes to a total volume of 25 µl, and as described in Table 1.

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 HHV-7 and IL-18 polymorphism were amplified using specific primers according to the conditions mentioned in Table 2.

Statistical Analysis

To detect the statistical analytic significance between the studied variables in this study, Chi – square test was applied, where all these statistical analyses were done by using the Version 24 SPSS program where the $p < 0.05$ value was considered as significant one.

Ethical Approval

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients' verbal and analytical approval before the sample was taken. The study protocol, the subject information and the consent form were reviewed and approved by a local ethics committee according to document number 7/17/1336 (including the number M220904 and the date 28/9/2022) to get this approval.

Results

1. Distribution of the age of studied patients with brain cancers

The tissue samples enrolled in this investigative study were from brain cancer patients aged 7 to 78 years. These patients had a mean age of 51.3 + 11.7 years, who were compared to a mean age of 49.5 + 13.2 years of their apparently healthy counterparts (AHC) revealed no significant variations ($p = 0.06$) (Table 3).

Table 1

Recommended volumes and concentrations for applying PCR into AccuPower® PCR tubes

No.	Contents of PCR reaction mixture	Volume / µl
1	Master mix	10 µl
2	Forward primers (each one of snps)	4 µl
3	Reverse primers (each one of snps)	4 µl
4	Template DNA	2 µl
5	Nuclease free water	5 µl
Total		25 µl

Table 2

The study conditions both for amplification HHV-7 and IL-18 genes

Gene	Initial denaturation	Denaturation	Annealing	Extension	Final extension	No. of cycles
HHV-7	95 °C / 5 min	95 °C / 1 min	58 °C / 45 sec	72 °C / 2 min	72 °C / 5 min	40
IL-18	95 °C / 5 min	95 °C / 1 min	60 °C / 1 min	72 °C / 2 min	72 °C / 5 min	40

Table 3

The age distribution of the studied groups of patients with brain cancers

Studied Groups	N	Mean age	S.D	S.E	Min	Max
Patients with brain cancers	85	51.3	11.7	1.9	7	**78**
Control group (AHC)	30	49.5	13.2	2.9	17	**63**
Statistical analysis	Non-significant (P = 0.06)					

2. Gender distribution of studied patients with brain cancers:

Males accounted for 47 (55.3%) of brain cancer patients, while females accounted for 38 (44.7%) of these cancer patients. The ratio of males to females was 1.2:1. While in the presumably healthy control (autopsied) group, males had a gender distribution of 19 (63.3%) while females constituted 11 (36.7%), among the control group. The comparison between brain cancers and control groups showed a significant difference (P = less than 0.001) on their statistical analysis (Table 4).

3. Distribution of studied patient groups according to their age stratum and genders

In terms of age of studied patients, 11.7% of cases were between the ages of 7 and 17 years (6 men and 4 women), 15.3% of cases were between the ages of 18 and 34 years (7 men and 6 women), 21.2% of cases were between the age of 35 and 51 years (10 men and 8 women), 27.1% of cases were between the age of 52 and 68 years (13 men and 10 women), and 24.7% of them were between the age of 69 and 83 years (11 men and 10 women). The highest male frequency (13) was found in the 52–68 year age group, whereas the highest female frequency (10) was found in both age groups of 52–68 years and 69–78 years (Table 5).

4. Histopathological characteristics of brain cancers

4.1. WHO Grading of brain cancer cases

In this study, grade I was found in 40 (47.1%) of brain cancers cases (22 men and 18 women), while grade II was found in 18 cancers cases (21.2%) (10 males and 8 females), grade III was recognized in 14 cases which constituted (16.5%) (8 males and 6 females), finally, grade IV was seen in 13 cases (15.3%) in brain cancer group (7 males and 6 females) (Table 6). There were statistically significant differences ($P < 0.05$) between groups of brain tumors based on their grade comparison.

Table 4

Gender distribution of the studied patients

Studied gender	Brain cancers group		Control group (AHC)		P-value
	No.	%	No.	%	
Male	47	55.3	19	63.3	0.003*
Female	38	44.7	11	36.7	
Total	85	100	30	100	

Note: * Significant difference (P = less than 0.001) on their statistical analysis

Table 5

Patients with brain cancers classification according to their age and gender

Age stratum (years)	Gender of patients with brain cancers		Total	
	Male	Female		
	No.	No.	No.	%
7-17	6	4	10	11.7
18-34	7	6	13	15.3
35-51	10	8	18	21.2
52-68	13	10	23	27.1
69-78	11	10	21	24.7
Total brain cancer patients	47	38	85	100

Table 6

The distribution of brain cancers on the basis of their grading

WHO grades of brain cancers	Patient gender		Total		P-value
	Male	Female			
	No.	No.	No.	%	
I	22	18	40	47.1	0.04*
II	10	8	18	21.2	
III	8	6	14	16.5	
IV	7	6	13	15.3	
Total brain cancer	47	38	85	100	

Note: * Significant difference (< P 0.05) on their statistical analysis

4.2. Frequency of studied brain cancer cases according to their types

Table 7 shows the types of brain cancers cases where 21 cases of brain cancers were diagnosed as Transitional Meningioma (24.7%) (11 males and 10 females), 15 cases as Meningotheliomatous Meningioma (17.6%) (8 males and 7 females), 17 cases as Glioblastoma Multiforme (20%) (10 males and 7 females), 13 cases as Diffuse Fibrillary Astrocytoma (15.3%) (7 males and 6 females), 6 cases as Anaplastic Oligodendroglioma (7.1%) (3 males and 3 females), 4 cases as Atypical Meningioma (4.7%) (3 males and 1 female), and 9 cases as Pilocytic Astrocytoma (10.6%) (5 males and 4 females). There were significant differences (P < 0.05) between the types of brain cancers (Table 7, Fig. 1).

Table 7

Frequency of studied brain cancer cases according to their types

Type of cancers	Gender		Total		
	Male	Female			
	No.	No.	No.	%	
Transitional Meningioma	11	10	21	24.7	
Meningotheliomatous Meningioma	8	7	15	17.6	
Glioblastoma Multiforme	10	7	17	20	**P-value 0.03**
Diffuse Fibrillary Astrocytoma	7	6	13	15.3	
Anaplastic Oligodendroglioma	3	3	6	7.1	
Atypical Meningioma	3	1	4	4.7	
Pilocytic Astrocytoma	5	4	9	10.6	
Total brain cancer	47	38	85	100	

Note: * Significant difference (< P 0.05) on their statistical analysis

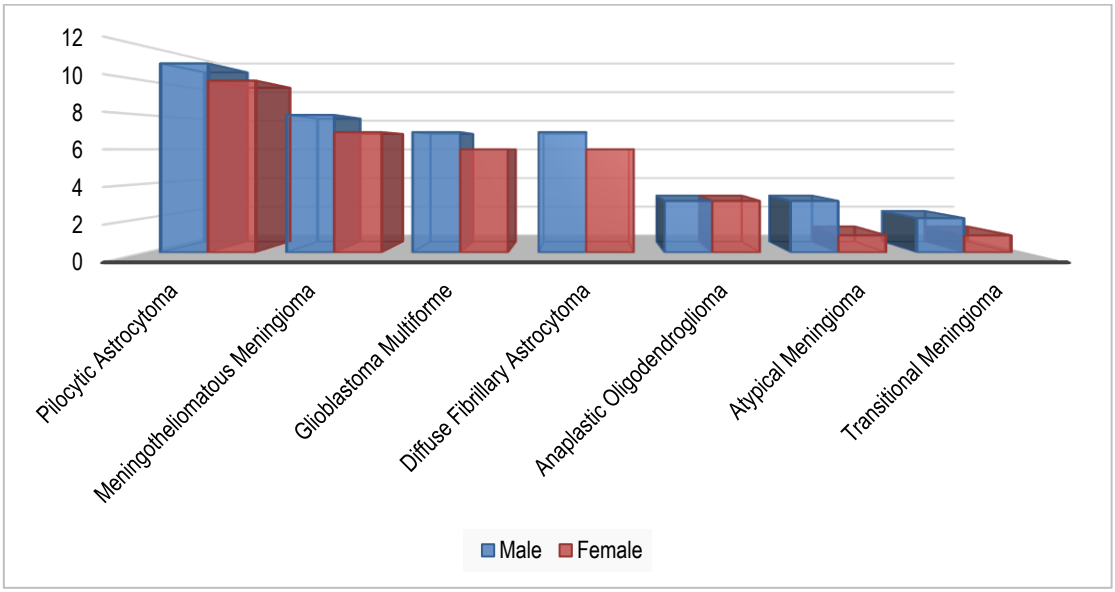

Fig. 1. Rating of brain tumors according to their typing

5. Frequency of the studied Human Herpes Virus-7 (HHV-7) by Polymerase Chain Reaction (PCR) Detection

5.1. Frequency of studied extracted nucleic acid using a viral DNA/RNA extraction kit

Fifty (50) patients without a viral genome were detected in this study's 85 brain cancer specimens, accounting for 41.17% of the total number of patients. No viral nucleic acid was found in all the 25 control group of apparently healthy postmortem specimens. The results showed statistically significant differences (p = 0.02) (Table 8).

5.2. Frequency of studied HHV-7 genome detection using conventional PCR

According to conventional PCR detection, 34% (17 out of 50) of the specimens have an HHV-7 genome, while 66% (33 out of 50 specimens) have negative results for the HHV-7 genome, as indicated in Table 9 and Figure 2. The differences between the groups of patients were statistically significant (< P 0.05).

5.3. The HHV-7 results by age strata of patients with brain cancers

The most HHV-7 infected patients with brain cancers in this study were those within

the age stratum of 18–34 years, which accounted for 7.1% (6 out of 13 patients), while the age strata 7–17 years, 35–51 years, 52-68 years, and 69–78 years accounted for 0.07%, 4.7%, 3.5%, and 3.5%, respectively (Table 10). Significant differences (P < < 0.05) found when these age strata were compared statistically.

Table 8

Viral genome extraction percentage in brain cancer patients and AHC groups

Viral genome		Study groups
		Brain cancers No. (85)
Positive	N	50
	%	58.8%
Negative	N	35
	%	41.2%
Total	N	85
	%	100%
P-value		0.02

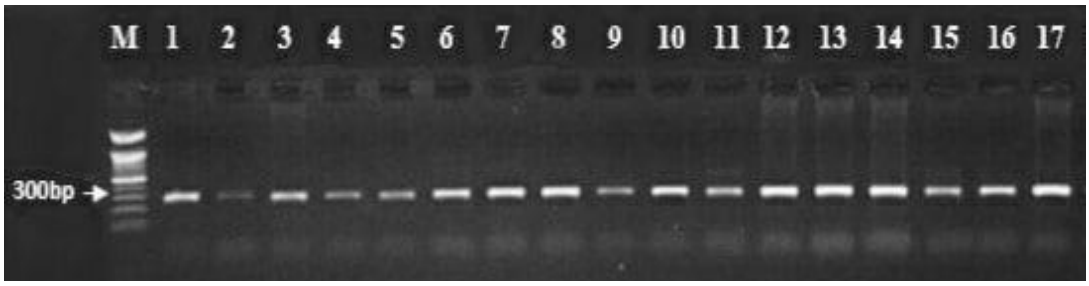

Fig. 2. The PCR patterns detection of HHV-7 DNA (300bp) in patients with brain tumors: the lanes from lane 1 to lane 9 refer to HHV-7 DNA samples. The electrophoresis conditions: agarose of 1, 75 V, 20 m Amp for 1 h (5 µl in each well), and later on stained with a red safe solution

Table 9

Percentage of HHV-7 positive signals by using PCR technique in specimens from patients with brain cancers

	No.	%	P-value
Positive	17	34	0.04
Negative	33	66	
Total	50	100	

Note: * Significant difference (< P 0.05) on their statistical analysis

Table 10

HHV-7-PCR frequency results among patients with brain cancers according to age strata

Age stratum	Years	HHV-7			P-value
		No.	Positive	Negative	
	7–17	10	1	9	P = 0.04 (P < 0.05)
		6.7%	0.07%	10.6%	
	18–34	13	6	7	
		14.7%	7.1 %	8.2%	

End of table. 10

	Years	HHV-7			P-value
		No.	Positive	Negative	
Age stratum	35–51	18	4	14	
		22.6%	4.7%	16.5%	
	52–68	23	3	20	
		28%	3.5%	23.5%	
	69–78	21	3	18	
		28%	3.5%	21.2%	
		85	17	68	
		100%	20%	80%	

5.4. The HHV-7 results in patients with brain cancers by their gender

Table 11 shows the percentage of patients with brain cancers who had positive HHV-7-PCR results based on their gender, with males accounting for 70.6% (12 out of 17 patients) and females accounting for 29.4% (5 out of 17 patients). The statistical analysis revealed significant differences in gender of brain cancer group with positive HHV-7-PCR ($P < 0.05$).

5.5. Association of HHV-7 infection according to the types of brain cancers

Table 12 shows positive HHV-7-PCR detection results from patients with various types of brain cancers, with 11.8%, 5.9%, 29.4%, 11.8%, 5.9% and 11.8% were in the Transitional Meningioma, Meningotheliomatous Meningioma, Glioblastoma Multiforme, Diffuse Fibrillary Astrocytoma, Anaplastic Oligodendroglioma, Atypical Meningioma, and Pilocytic Astrocytoma, respectively. The statistical analysis of different types of the brain cancers that were HHV-7 positive showed significant differences ($p < 0.05$) (Table 12).

6. IL-18-607C/A (rs1946518) gene polymorphism among studied groups

The results of this study showed that the DNA polymorphism distributions according to GA; AA and GG genotypes of IL-18 607C/A (rs1946518) polymorphism were 37.1% (13 out of 35 cases); 57.1% (20 out of 35 cases) and 5.7% (2 out of 35 cases), respectively, in the patients group and 20% (6 out of 30 cases); 53.3% (20 out of 30 cases) and 26.7% (8 out of 30 cases), respectively, in the control group (Table 13).

We have new recording in IL-18 SNPs in GENE BANK and NCBI.

7. Spearman's rho statistical testing of age, brain cancer types, HHV-7, SNPs of IL-18 (rs1946518) to evaluate the studied markers in the studied population groups

A strong positive relationship and also a correlation with high significance was found between HHV-7 and SNP IL-18 rs1946518 in brain cancers ($r = 0.968$, $P = 0.007$). In addition, a strong positive relationship as well as a correlation with high significance was found between SNPs of IL-18 rs1946518 according to the age of the patients who have brain cancers ($r = 0.855$, $P = 0.001$). However, there were no significant correlations among HHV-7 and SNPs of IL-18 rs1946518 according to the type of brain cancers in the current study (Table 14).

Discussion

The tissue samples enrolled in this study were obtained from brain cancer patients whose ages ranged from 7 to 78 with a mean age of 51.3 + 11.7 years. According to WHO mortality rate rankings, malignant glioma is reported as both among major life-threatening brain tumors and ranked the second leading cause of death among tumor patients < 34 years of age (Dan *et al*., 2023; Fan *et al.*, 2022). In this study, the types of brain carcinomas where among them we enrolled Transitional Meningioma (24.7%), Meningotheliomatous Meningioma (17.6%), Glioblastoma Multiformi (20%),

Table 11

HHV-7 percentage in brain cancer patients based on their gender

Brain cancer patients	HHV-7 infection	
	+	%
Men	12	**70.6**
Women	5	**29.6**
The statistical analysis	**(P < 0.05) = P = 0.02**	

Table 12

Frequency of cases of brain cancer according to their typing and viral genome detection

Type of cancers	Brain cancers		HHV-7 positive		P-value
	With viral genome	Without viral genome			
	No.	No.	No.	%	
Transitional Meningioma	10	11	2	11.8	**0.03**
Meningotheliomatous Meningioma	8	7	1	5.9	
Glioblastoma Multiforme	13	4	5	29.4	
Diffuse Fibrillary Astrocytoma	8	5	2	11.8	
Anaplastic Oligodendroglioma	4	2	1	5.9	
Atypical Meningioma	2	2	2	11.8	
Pilocytic Astrocytoma	7	2	4	23.5	
Total	50	35	17	20	

Table 13

Genotyping of IL-18 -607C/A (rs1946518) gene (528 bp) in patients with brain tumors and control groups

Zygosity status	Brain tumors No. (%)	Control No. (%)	OR (95%)	SNP type	Sig.
G/A	13 37.1%	6 20%	1.26 (0.46–3.43)	Missense variant	0.03
A/A	20 57.1%	16 53.3%	1.53 (0.55–4.18)		0.04
G/G	2 5.7%	8 26.7%			0.56
Totals	35	30			

Table 14

Spearman's rho statistical testing of age, brain tumor types, HHV-7, SNPs of IL-18 rs1946518 to evaluate the studied markers in the studied population groups

Spearman's rho		Age groups (years)	IL-18 rs1946518	Brain cancer types	HHV-7
HHV-7	R	0.855**	**0.986	0.175	
	P	0.001	0.007	0.8	
IL-18 rs1946518	R	0.788**			
	P	0.009			

End of table. 14

Spearman's rho		Age groups (years)	IL-18 rs1946518	Brain cancer types	HHV-7
Brain tumor types	R	0.739**			
	P	0.004			
Age groups (years)	R	0.166	-0.749	0.123	0.145
	P	0.249	0.077	0.512	0.034

Diffuse Fibrillary Astrocytoma (15.3%), Anaplastic Oligodendroglioma (7.1%), Atypical Meningioma (4.7 %) and Pilocytic Astrocytoma (10.6%). In addition, in the current study, grade I was found in 47.1% of brain cancer cases, while grade II was found in 21.2%, grade III was seen in 16.5%, and grade IV was seen in 15.3% of brain cancer group. Changes in genetic characters are one of the most common as well as important causes of malignant glioma (Hanif *et al.*, 2017). Additionally, studies have currently suggested that environmental factors, such as using mobile phones and exposing to ionizing radiation, are the probable contributors in glioma. In addition, both neurofibromatosis and tuberous sclerosis might be susceptibility factors in this issue (Karipidis *et al.*, 2018; Yang *et al*., 2017).

Many Herpes family viral agents, similar to other neurotropic viruses, have a highly neurotropic predilection, causing both central as well as peripheral neurological symptoms, however, it remains largely unknown whether are caused by a direct viral effect on cellular levels, or by the effects of the inflammatory reaction in the affected CNS places due to the activated immune response (Ahmad *et al.*, 2021). The HHV-7 member of Herpesviridae has been both poorly as well as very recently investigated in respect to its involvement in CNS localizations. The CMV, HHV-6, and HHV-7 are 3 herpes viruses, classified under the â-herpesvirinae subfamily, share a high degree of genomic homology and thus might share a similar oncogenic potential (Handous *et al*., 2020).

According to PCR, in this study, it was found that 17 out of 50 of the specimens (34%) have HHV-7 genome, while 66% (33 out of 50 specimens) are negative, and the differences between these groups of patients were statistically significant. Few researchers reported neurological complications of HHV-7 in those patients with HIV infection or in those recipients with transplantations, however, the neurological complications were very rarely reported in immunocompetent adults with HHV-7 infection (Erin *et al.*, 2011). Researchers in a previous study using the PCR method had investigated Human Herpes viral load in brain tissues according to its different regions and had revealed non-uniform viral distribution that varies by region and they concluded that all areas should be examined (Karipidis *et al*., 2018; Erin *et al*., 2011). In that study, as in this study, this could be the reason for the non-detection of HHV-7 DNA from brain tissues in some cases. The HHV-7 DNA in patient's CSF was first reported in an immunocompetent encephalitis Japanese adult with significantly high re-activated levels of serum HHV-7 IgG titers, while such a case was ranked fifth worldwide (Karipidis *et al.*, 2018). Schwartz and colleagues have lately conducted the largest retrospective study and identified HHV-7-positive patients with CNS symptoms, where they mostly referring them to be the result of delayed HHV-7 primary infection (Schwartz *et al*., 2014). It was exclusively considered that the neurologic issues of delayed HHV-7 primary infection were usually both more serious and aggressive when the patient grows up. This has been referred to a more aggressive inflammatory response due to a more mature immune system, similarly to those noticed with varicella, rubella and measles, for instance (Chan *et al.*, 2002). In a previous report (Schwartz *et al.*, 2014), a dead case suggestively diagnosed with viral encephalitis due to HHV-7 (since HHV-7 DNA detected in CSF and also on basis of the brain histopathological examination) showing characteristic intracellular inclusion bodies, perivascular lymphocytic infiltration, necrosis,

and gliosis as well as glial nodules, Human herpes virus 6 and 7 are lymphotropic viruses that often transmitted in the first months of life, where HHV-7 is commonly infecting > 90% of persons by age of six years via oral salivary route resulting in roseola infantum and febrile seizers (Hasan *et al.*, 2023).

In general, this virus establishes a latent infection in several cellular sites, as in lymphocytes, salivary glands, and CNS. HHV-7 is carried after the infection to the CNS via the bloodstream and its super-infection with CMV results commonly in CNS symptoms (Kei *et al.*, 2022). HHV-7 is a T-lymphotropic virus where this viral infection of the primary CD4+ T lymphocytes as well as SupT1 lymphoblastoid T-cells contributes to cancer development for the accumulation of HHV7- infected cells in G2/M phase and polyploidy as well as increasing the cellular size, and for the cell-cycle regulation, cdc2 is then activated, leading in the prevention of cytotoxic T-cells activity and altering the immune response (Alibek *et al.*, 2014). However, the HHV7 transforming role has not been shown; although being proposed for its cofactor roles in T-cell and B-cell lymphomas and also it might potentiate pathogenic roles of other herpes viruses (Morales-Sánchez *et al.*, 2014). Interleukins are considerably important vital components that have effects to regulate the functions of immunocytes, including their proliferation and differentiation, among them, IL-18, that both can facilitate Th1 cellular proliferation as well as stimulate the secretion of many other cytokines by different immunocytes (Nakanishi, 2018). IL-18, as an inflammatory factor, has a wide range of immune regulatory effects which were recognized in its participation in inflammation, immune as well as other pathophysiologic processes and in tumorigenesis. IL-18 in addition to IL-12 works for exerting a synergistic induction IFN-γ production. Both IL-18 and IL-6 gene polymorphisms were found by previous studies to affect the development as well as progression of various diseases. IL-18 SNP was related to gram-negative bacteria and fungal infections and also has been associated both with cardiovascular and cerebrovascular diseases (Yasuda *et al.*, 2019).

The current results showed that DNA polymorphism distribution of IL-18 (rs1800629) according to GG; AA and GA genotypes of IL-18 (rs) polymorphism were respectively 37.1% (13 out of 35 cases); 57.1% (20 out of 35 cases) and 5.7% (2 out of 35 cases) in the patient group and 71.4% (5 out of 7 cases); and 28.6% (2 out of 7 cases) in the control group, respectively. In a study by Yang et al. (2022), they detected polymorphisms in the IL-18 gene, and found that the allele distributions of IL-18 gene polymorphisms of rs371411440 ($p = 0.041$) and rs371828055 ($p = 0.002$) in the nucleated RBCs of 200 glioma patients (disease group) were significantly different from their 200 healthy counterpart people (control group). In addition, these researchers (Yang *et al.*, 2022) also observed statistically significant differences between the disease group and its control counterpart in the genotypic distributions in relation to IL-18 gene polymorphisms (namely, rs371828055 and rs201211345 and rs201439472), among them, genotype GG of IL-18 gene polymorphism rs371828055 in the disease group has exhibited significantly higher frequencies than in their control group. Moreover, and according to the haplotypes as well as polymorphisms analytical findings, it was found by those researchers a significant difference in the recessive haplotypes distribution of IL-18 gene polymorphism rs371828055 (GT+TT) in disease group from what they found in the control group (Yang *et al.*, 2022). Also, statistical significant differences between disease group and its control counterparts were reported both in the distributions of haplotypes CC and GT of IL-18 gene polymorphisms rs371411440 and rs371828055. The results indicated that genotypes of IL-18 gene polymorphism rs371828055 were evidently correlated with the gene expression of IL-18 ($p = 0.000$), and patients with genotype GT had a distinctly lower expression level of IL-18 (Yang *et al.*, 2022).

The current results have necessitated further in-depth analysis to unravel the importance of mechanisms of IL-18 dysregulation and / or activation in different tumors of glioma types and reaching to useful and applicable bioinformat-

ics from this study. These results have also indicated that the studied genotypes of IL-18 gene polymorphism rs371828055 have an evident correlation with IL-18 gene expression while among them, patients having genotype GT had lower IL-18 expressional level (Yang *et al*., 2022). HHV-6A (similarly, HHV-7) affect the immunomodulatory cytokines because they possess both immunosuppressive properties as well as pro-inflammatory properties, inducing the receptors of both IL-18 and IFNγ , suppressing the apoptotic response-associated IL-6 and TNF-α cytokines, converting T cells to a Th1 phenotype, and promoting the IL-6, IL-8, and TGF-β production astrocytes cultures (Chi *et al*., 2012; Reynaud & Horvat, 2013). Moreover, proteins U22, U51 and U83 of HHV-6 and HHV-7 resemble human chemokines, bind to CCR receptors on leukocytes, and promote their infiltration (Zou *et al*., 1999; Atedzoé *et al*., 1999). In the current study, we inferred as well as concluded from these results (as what researchers in Yang *et al*., 2022) that the obtained results of both IL-18 gene polymorphisms [rs371411440 and rs371828055] could truly imply that both those gene polymorphisms have served as important susceptibility factors and were able to affect glioma onset, notably not only by a single gene polymorphism.

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