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CELLULAR LOCALIZATION OF EXPRESSIONAL IMMEDIATE EARLY AND EARLY ANTIGENS OF HUMAN CYTOMEGALOVIRUS AND DETECTION OF NEUROTROPIC HPV-18 DNA IN PRIMARY CNS TUMORS: A RETROSPECTIVE STUDY IN A SET OF IRAQI PATIENTS

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Abstract
Human Papillomavirus (HPV) and Human Cytomegalovirus (HCMV) have been associated with CNS tumours and may influence brain tumor development. This study aims to evaluate the prevalence of HPV genotype 18 and HCMV infections in Iraqi patients who underwent surgery for various types of primary CNS tumors. Materials and Methods Seventy-nine CNS tissue specimens were analyzed from patients with various tumors, including 22 Diffuse Fibrillary Astrocytomas, 14 Pilocytic Astrocytomas, 13 Anaplastic Oligodendrogliomas, 11 Glioblastoma Multiforme, 6 Craniopharyngiomas, 5 Medulloblastomas, and 4 each of Anaplastic Astrocytoma and Chordomas. Additionally, 50 control brain tissues were assessed. Conventional PCR identified HPV genotype 18, while IHC for HCMV antigens was conducted on different primary CNS tumors.Results: The study analyzed the detection rates of HPV 18 DNA in central nervous system (CNS) tumor tissues, finding a prevalence of 16.5% (13 out of 79 samples), while all 50 control samples were negative. The highest rates of HPV 18 positivity were observed in Diffuse Fibrillary Astrocytoma at 38.5%, followed by Anaplastic Oligodendroglioma and Glioblastoma Multiforme, each at 23.1%, and lower percentages in Pilocytic Astrocytoma, Anaplastic Astrocytoma, and Craniopharyngioma. Notably, Chordomas and Medulloblastoma tissues exhibited no HPV 18 positivity. Furthermore, HCMV antigens were detected in 35.4% of CNS tumours compared to only 4% in the control tissues, with the highest rates found in Diffuse Fibrillary Astrocytoma (46.4%). Other tumors such as Pilocytic Astrocytoma, Glioblastoma Multiforme, and Anaplastic Oligodendroglioma, followed with 14.3% positivity. Statistical analysis indicated significant discrepancies in detection rates of both HPV 18 and HCMV across tumour types and in comparison to control samples. **Conclusion**: This preliminary study highlights the potential role of HPV 18 and HCMV in the pathogenesis of CNS tumours, suggesting they might function as co-factors or directly contribute to tumour development.

KEYWORDS: HPV 18; HCMV; CNS tumours; IHC; Diffuse Fibrillary Astrocytomas; Pilocytic Astrocytoma; Anaplastic Oligodendrogliomas; Glioblastoma Multiforme; Craniopharyngiomas; Medulloblastomas ; Chordomas; Anaplastic Astrocytoma.

INTRODUCTION

To date, head and neck cancers are the seventh most common type of cancer worldwide, having association both with different environmental factors and oncogenic viral infections (1).In the past 2 decades, the research studies intended for multiple viral oncogenesis topics, have included more than 120 different histological types of primary CNS tumors to unravel whether these viral infections have implication or roles as tumor- viral causation in the neural etiopathogenetic mechanisms, or rather as mere random viral association or coincidence with these primary CNS tumors (2), (3), (4-10). In the realm of cancer research, various types of Herpes viruses have been linked to the development of diverse types of cancer (11). Human herpes virus type 5 (HHV 5), i.e. HCMV, often has broad tropism for cellular targets, that act as viral reservoirs (12). HCMV infects both human neural stem cells and astrocytes and this virus has links to generate and release of Transforming Growth Factor-β (TGF- β) by the infected astrocytes (13).

Several previous studies have searched for a human herpes virus type 5 (HHV 5), i.e. HCMV, in brain tumors from both adults and children patients, particularly in glioblastoma multiforme (14, 15). A high prevalence of

HCMV infection in glioblastomas, as represented by HCMV proteins and nucleic acids, was first reported in 2002. The significance of this finding remains debated; However, This has created an area of considerable interest for further researches for linking HCMV to human cancers, which necessitating a better understanding of the potential role of this virus in tumorigenesis, aiming for the development of many related diagnostic, prophylactic, and therapeutic measures (16, 17).

Brain gliomas are the most common primary tumors affecting CNS, comprising 4 most prevalent malignant types (glioblastomas, astrocytomas, oligodendrogliomas and ependymomas) (18). Their etiology has been linked to genetic predisposition, chemical and radiation exposure, and, more recently, viral agents (19, 20).

Previous studies have shown that the pathological development or neural oncogenesis of different CNS tumors, among them, gliomas carcinogenesis have been linked to varied degrees of certainty to a wide variety of infectious agents, including viral agents, as HPV 16, HPV 18, EBV, CMV, HHV8, HBV, HCV, HTLV1, and particularly infections with certain human polyoma viruses like JCV, BK virus, and SV40 where the presence of viral DNA, mRNA, and oncoprotein expression within brain tissue regarded essential evidences to raise and/or confirm viral oncogenic involvement (19, 21-25). Molecular technologies have identified over 450 HPV genotypes; among them, at least 25 types are high-risk and are linked to various cancers. HPV 16 and 18 cause about 70% of cervical cancers, and detection rates are rising in head and neck cancers, especially oropharyngeal cancers (70-80%) and in glioblastoma cases, HPV has also possibly been suggested to have a role, potentially worsening and impacting their prognosis (26-38). Some Initial research studies indicated a higher prevalence of transcriptionally active high –oncogenic risk genotypes of Human Papillomaviruses (HPV) in invasive pituitary adenomas compared to non-invasive ones, and raised the suggestion for a potential link between HPV and pituitary adenomas. However, these findings aren't enough conclusive evidence to establish a direct causal relationship and need further confirmation through larger, more diverse studies (39). Craniopharyngiomas are rare, slow-growing, benign intracranial epithelial tumours that develop from Rathke's pouch remnants, predominantly located in the sellar as well as parasellar regions, near the pituitary and hypothalamus, and account for 1-3% of adult and 5-10% of childhood intracranial tumours. While these tumours are primarily considered to be caused during embryonic development rather than directly by a virus, some researchers have explored a role for viruses in the initiation and inflammation of such tumours (40-43).

Medulloblastoma is the most common malignant (World Health Organization [WHO] grade IV) embryonic neuro-epithelial tumor arises in the cerebellum, accounting for up to 25% of primary CNS neoplasms in children (44). Medulloblastoma may be linked to certain viral infections that contribute to tumor development. Additionally, engineered oncolytic viruses like Measles, Parvovirus H-1, Myxoma, reovirus, and others are being studied as promising, less toxic alternatives to traditional treatments like radiation and chemotherapy (45, 46).Given that HPV genotype 18 is the most commonly recognized human oncogenic virus and Human CMV, as common "oncomodulation" virus that enhancing the malignant processes, and by using rigorous scientific laboratory methods, the current research intend to examine to the rates of HPV 18 and Human CMV infections in a cohort of Iraqi patients operated for different histological brain and other CNS tumors and to shed light on the possible link between these infections with the development of these selected variety of tumors, therefore adding an important new information to the growing body of knowledge in this area.

Materials and Methods:

Study Population :

A total of 79 different primary human brain and other CNS tumours and/or cancers tissue samples were obtained from individuals aged (2 to 79) who had been diagnosed with different brain tumours. The control group consisted of 50 archived tissue specimens from the brains of adults aged 18 to 67 and operated for other non- tumorous pathologies in their brains. The established histological criteria were applied for the tumour categorisation process. Institutional review board and local ethics committee permission was obtained for the project.

2. Viral genome Extraction:

Viral genome was extracted from specimens of brain tissues from patients whom were already have operated for brain tumors as well as other non- tumorous pathologies in their brains by using Patho Gene-spinTM DNA/RNA Extraction kit (iNtRON Biotechnology Co., Korea) which is designed to isolate high-quality nucleic acids from a variety of pathogen and specimen using low elution volumes that allow sensitive downstream analysis. Viral genome was extracted which then stored until used at (-20˚C).

3**. Primer selection:**

Primer sets were used in this study to detect the HPV18 forward primer sequence was 5-TCTAAACCTGCCAAGCGTGT -3; and for HPV18 reverse primer sequence, was *R'* 5-AAGGGTAGACAGAATGTTGGACA -3." While the size of PCR product was(517bp).

4. PCR Technique:

The process of polymerase chain reaction was conducted using conventional thermal cycler (Biometra-Germany), PCR reaction mixture done with the total volume of 25 microliters which consist of: master mix (12.5µl), forward and reverse primers (1µl of each one), completed with nuclease free water (5.5µl) , as well as added extracted DNA (5µl) , and as shown in table 1.

Table (1): Recommended PCR mixture contents and concentration

PCR Reaction Mixture Contents	Volume per µl
1- Master mix	12.5 µl
2- Forward and Reverse primer	1 µl of each one
3- Extracted DNA	5 µl
4- Nuclease free water	5.5 µl
Total	25 µl

5. PCR condition:

The reaction mixture contents were ultimately put into the thermal cycler (Biometra / Germany), which had been pre-heated to 94°C and set up with the proper cycle settings as described in table (2). The target region of HPV18 was amplified using specific primers. Following that, the PCR products were electrophoresed used 1.5% of an agarose gel, which can then be seen utilizing the gel documentation system.

Table (2): The thermal conditions used for genome amplification

Genes	No. of cycles	Initial denaturation	Denaturation	Annealing	Extension	Final extension
HPV18	35	95˚C/5 min	95˚C/1 min	64˚C/45 sec	72˚C/2 min	72˚C/5 min

6. Preparation of Reagents for the IHC Test.

This Immunohistochemistry (IHC) technique detects HCMV immediate early and early nuclear antigens using a specific cocktail of mouse anti-human monoclonal antibodies (DDG9 and CCH2 which bind to a specific epitope in the unclearly-targeted proteins of gene expression (the 72-kDa immediate early 1 -IE1 as well as 43 KD early antigens) encoded by human cytomegalovirus (HCMV) in malignant and normal cells. The bound antibodies are visualized via a peroxidase-labeled polymer system with DAB (diaminobenzidine) chromogen, producing a brown precipitate at the antigenic site within the tested tissue.

The procedure involved deparaffinizing 6µm sections, antigen retrieval, and incubation, with results validated against positive and negative controls under light microscopy. The chromogen solution contains DAB (diaminobenzidine), and when a positive reaction occurs, a brown-colored precipitate forms at the antigen site within the tested tissue and were evaluated under light microscopy at magnifications of X100, X400, and X1000. Immunohistochemistry (IHC) staining intensity was scored from 0 (negative) to 3 (high). Positive cells identified via immunohistochemistry were counted in ten fields per sample, and the average was calculated. Tissues have correspondingly assigned for a score based on this average and as follows: Score 0 (Negative): No stained cells; Score 1 (+): Stained cells constitute 10% of total cells; Score 2 (++): > 10% & up to 30%; and Score 3 (+++): > 30% & up to 50% of total cells (47).

7.Ethical certification:

A local ethics commission reviewed and approved the study protocol, consent form, and subject information on September 10, 2024, under project number M240903.

8. Statistical analysis:

In order to assess the significance of the variables examined in this study, the Chi-square test was utilized. All statistical analyses were conducted using the SPSS program, Version 24. A $p < 0.05$ value deemed to indicate statistical significance.

RESULTS

1. General description of patients who received surgical managements for different CNS pathologies according to their age & gender:

The characteristics of the 79 patients with CNS tumors and 50 control groups are summarized in table 1. The mean age of CNS tumor patients and control group was 48.7±12.35 years and 46.5 ± 13.44 years, respectively. According to their age, there were no significant differences between patients and control groups.

This study has included 46 males and 33 females among the 79 CNS tumor patients, while out of 50 control group, 27 males and 23 females were enrolled. Statistically, significant differences were detected between patients and control groups (p=0.03).

Table 1: Some Demographical description of patients with different CNS pathologies

Study Group	No.	Mean Age (Years)	Maximum	Minimum	S.E	S.D
Patients (CNS tumors)	79	48.7	79	2	2.12	12.35
Control Patients (Non-tumors brain pathologies)	50	46.5	68	17	1.73	13.44

Statistical analysis		Non-significant differences (p=0.6)				
Variables		Patients (CNS tumors)		Control Patients (Non-tumors brain pathologies)		*p*
		No.	%	No.	%	
Sex	Male	46	58.2%	27	54%	0.03*
	Female	33	41.8%	23	46%	

Regarding the studied CNS tumor patients and according to their age stratification, 10.1 % of patients were in the age stratum of 2 to 18 years, 21.5 % in the age stratum of 19 to 35 years, 25.3 % in the age stratum of 36 to 52 years, 22.8 % in the age stratum of 53 to 69 years, and 20.3 % of them were in the age stratum of 70 to 85 years. Significant differences were found according to different age strata of CNS tumour patients (p=0.04) (Table 2).

Table 2: Rates of CNS tumour patients according to their age stratification

Ages \ years	CNS Tumour Patients		P value
	No.	Percentage	
2-18	8	10.1	0. 04*
19-35	17	21.5	
36-52	20	25.3	
53-69	18	22.8	
70-85	16	20.3	
Total	79	100%	

* Statistically significant

2. Co-Distribution of CNS tumors according to their histological typing and gender of the patients:
Among to the studied CNS tumors (table 3), 14 cases were Pilocytic Astrocytoma (8 male and 6 female), 4 cases of Chardomas (2 male and 2 female), 6 cases have Craniopharyngioma (4 male and 2 female), 22 cases with Diffuse Fibrillary Astrocytoma (13 male and 9 female), 17 cases have Anaplastic Oligodendroglioma (7 male and 6 female), 4 cases with Anaplastic Astrocytoma (3 male and 1 female), cases 11 cases with Glioblastoma Multiforme (6 male and 5 female), and finally 5 cases were Medulloblastoma (3 male and 2 female).

Table 3: Distribution of brain tumors according to their histological typing and gender of the patients

CNS Tumours Type	**No.**	**%**	**Male**	**%**	**Female**	**%**
Pilocytic Astrocytomas	**14**	**17.7**	**8**	**57.1**	**6**	**42.9**
Chardomas	**4**	**5.1**	**2**	**50.0**	**2**	**50.0**
Craniopharyngiomas	**6**	**7.6**	**4**	**66.7**	**2**	**33.3**
Diffuse Fibrillary Astrocytomas	**22**	**27.8**	**13**	**59.1**	**9**	**40.9**
Anaplastic Oligodendrogliomas	**13**	**16.5**	**7**	**53.8**	**6**	**46.2**
Anaplastic Astrocytoma	**4**	**5.1**	**3**	**50.0**	**1**	**50.0**
Glioblastoma Multiforme	**11**	**13.9**	**6**	**54.5**	**5**	**45.5**
Medulloblastomas	**5**	**6.3**	**3**	**60.0**	**2**	**40.0**
Total	**79**	**100.0**	**49**	**58.2**	**33**	**41.8**

3. Detection of Human Papilllomavirus-18 (HPV18) in the CNS tumours using the PCR technique:
3.1. Distribution of HPV-18 –DNA detection results in the total group of CNS tumour tissues:
The PCR results have shown positivity for HPV-18 in 16.5% (13 out of 79 CNS tumour cases), while 83.5% (66 out of 79 cases) have revealed negative results, as shown in Table (4). While, no positive PCR results for HPV-

18 have been shown in the CNS control tissues group. There was significant difference (p = 0.03) between the patients and control groups.

Table 4: PCR results for detection of HPV-18 –DNA in tissue samples from patients with surgeries for different CNS tumors type

The PCR results of HPV-18 detection	Non-Tumorous Brain Pathology Patients [Control Tissues Group] (N=50)		CNS tumors (n=79)		P Value
	N	%	N	%	
Negative	50 / 50	100	66 / 79	83.5	P= 0.04
Positive	0 / 0	0	13 / 79	16.5	

3.2. Distribution of PCR results for detection of HPV-18 –DNA in the CNS tumors according to patient's gender:

Out of 79 patients, 9 CNS tumor tissues infected with HPV18 were from males and 4 from females, while the remaining CNS tumor tissues from 37 males and 29 females have showed no HPV18-DNA detection. According to the statistical analysis of correlation of HPV18 infection in the CNS tumor tissues with patients gender, there was significant difference (*P*=0.04) (Table 5).

Table 5: Distribution of *HPV18* infection in patients with CNS tumors according to sex

Patients Gender		PCR For HPV18 DNA		P value
		Positive	Negative	
Male	No.	9	37	P=0.04
	%	69.2%	56.1%	
Female	No.	4	29	
	%	30.8%	43.9%	
Total	No.	13	66	
	%	16.5%	73.5%	

3.3.The distribution of PCR- detection results for Human Papilllomavirus-18 according to age strata of patients with CNS tumors:

In patients with CNS tumor group, the most commonly infected age group with HPV-18 was (53-69 years) (constituted 38.5% ; 57 out of 13 patients), while in the age groups (2-18 years) ; (19-35 years) ; (36-52 years) and (70-85 years) HPV-18 was constituted 7.7%;15.4%; 30.8% and 7.7%,respectively . Statistically, significant differences were revealed among the studied age groups ($p < 0.05$) (Table 6).

Table 6: HPV18 –PCR detection frequencies among CNS tumor patients depending on age stratum

Age Stratum		**HPV18**			***P* value**
		positive	**negative**		
	No.	**%**	**No.**	**%**	
2-18	**1**	**7.7%**	**7**	**10.6%**	**0.03***
19-35	**2**	**15.4%**	**15**	**22.7%**	
36-52	**4**	**30.8%**	**16**	**24.24%**	
53-69	**5**	**38.5%**	**13**	**19.7%**	

70-85	**1**	**7.7%**	**15**	**22.7%**	
Total	**13**	**26.6%**	**66**	**73.4%**	

3.4. The distribution of PCR results for Human Papillomavirus-18 detection according to the histological type of CNS tumours:

Table (7) shows positive HPV18 PCR detection results from patients with various forms of CNS tumors, which were 7.7%, 4.8%, 38.5%, 23.1%, 7.7% ;23.1%, and 16.5%, of Pilocytic Astrocytoma; Craniopharyngioma, Diffuse Fibrillary Astrocytoma ; Anaplastic Oligodendroglioma, Anaplastic Astrocytoma, and Glioblastoma Multiforme, respectively, showed positive PCR results for HP18 detection, while none of Chardomas and Medulloblastoma tissues have revealed positive HPV18 PCR detection results. The statistical analysis of HPV18-positive results and as presented in different types of CNS tumors, have revealed significant differences ($p \leq 0.05$).

Table 7: Frequency of HPV18- positive PCR results according to the histological type of CNS tumors

Histological Type of CNS Tumours	No.	With HPV18 Infection	%	Without HPV18 Infection	%	*P* value
Pilocytic Astrocytoma	**14**	**1**	**7.7%**	**13**	**19.7%**	**0.03**
Chardomas	**4**	**0**	**0.00%**	**4**	**6.1%**	
Craniopharyngioma	**6**	**0**	**4.8%**	**6**	**9.1%**	
Diffuse Fibrillary Astrocytoma	**22**	**5**	**38.5%**	**17**	**25.8%**	
Anaplastic Oligodendroglioma	**13**	**3**	**23.1%**	**10**	**15.2%**	
Anaplastic Astrocytoma	**4**	**1**	**7.7%**	**3**	**4.5%**	
Glioblastoma Multiforme	**11**	**3**	**23.1%**	**8**	**12.1%**	
Medulloblastoma	**5**	**0**	**0.00%**	**5**	**7.6%**	
Total	**79**	**13**	**16.5%**	**66**	**73.4%**	

4. Detection of HCMV- 76 KD as well as 43 KD proteins in different histological groups of the studied CNS tumours using the immunohistochemical (IHC) technique:

Table (8) shows the positive -IHC detection results of HCMV- immediate early and early antigens (76 KD as well as 43 KD proteins), where 35.4 % (28 out of 79 cases) from CNS tumors group showed positive signals, including 60.7 % (17 out of 28 cases) as low score (I) followed by 32.1 % (9 out of 28 cases) and 7.2 % (2 out of 28 cases)as moderate score (II) and high score (III), respectively. Statistically, significant differences were found among signal scorings of IHC ($P<0.05$).

Table (8) also shows the positive -IHC detection results of HCMV- 76 KD as well as 43 KD proteins where 35.4 % (28 out of 79 cases) from CNS tumors group showed positive signals included 64.3 % (18 out of 28 cases) as weak intensity (I) followed by 25 % (7 out of 28 cases) and 10.7 % (3 out of 28 cases) as moderate intensity (II) and strong intensity (III), respectively.

Statistically, significant differences were noticed between negative, weak, moderate and strong intensities of tissues at 5 percent level ($P<0.05$) in CNS tumors group.

Figure1(A-D) are revealing the final semi-quantified scoring of positive immunohistochemical staining results of detection of HCMV- 76 KD & 43 KD proteins in the examined CNS tumors tissues, where Figure (A) shows a final strong semi-quantified Score 6 (score III and intensity III) (40x); Figure B: Final strong semi-quantified Score 5 (score III and moderate intensity) (20x); Figures C and Figure D, (10x) and (40x), respectively: Final moderate semi-quantified Score 4 (score II and moderate intensity).

Table 8. Immunohistochemistry (IHC) results of HCMV- 76 KD as well as 43 KD proteins according to the IHC- signal scoring among studied groups.

IHC for HCMV- 76 KD and 43 KD proteins detection		Non-Tumorous Brain Pathology Patients [Control Tissues Group] (N=50)		CNS tumors (n=79)		P Value
		N	%	N	%	
Negative		**23/50**	**96**	**51 / 79**	**64.6**	**P< 0.03 is significant**
Positive		**2/ 50**	**4**	**28 / 79**	**35.4**	
IHC- signal SCORING	**I**	**1**	**50**	**17**	**60.7**	
	II	**1**	**50**	**9**	**32.1**	
	III	**0**	**0.00**	**2**	**7.2**	

IHC-signal INTENSITY	**I**	**2**	**100**	**18**	**64.3**	**P< 0. 04 significant**
	II	**0**	**0.00**	**7**	**25**	
	III	**0**	**0.00**	**3**	**10.7**	
Mean Rank		**92.5**		**95.3**		

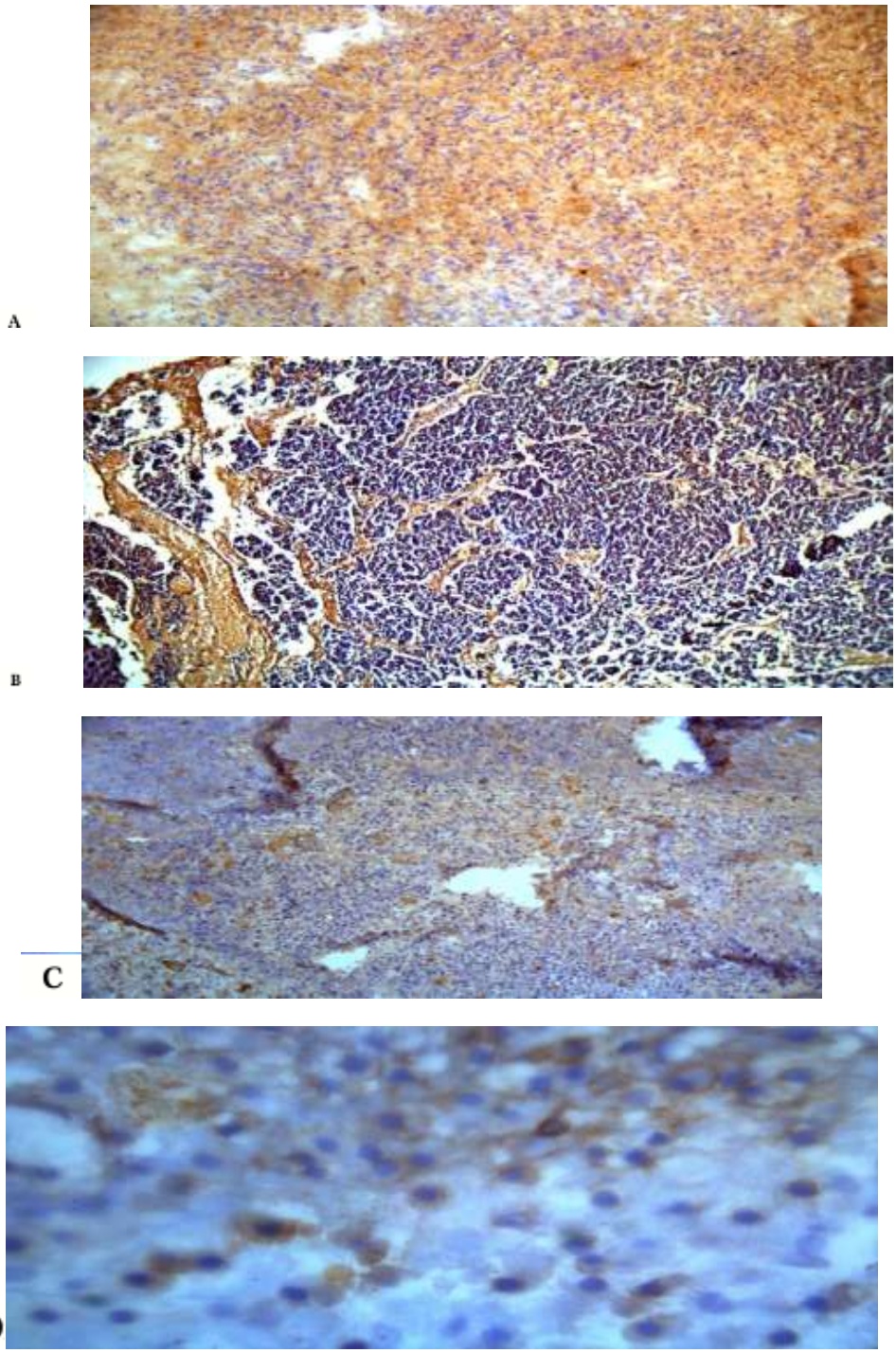

Figure 1 (A-D): The immunohistochemical process (IHC) results of detection of HCMV- 76 KD and 43 KD proteins in CNS tumors Tissue Samples using specific primary mouse anti-human monoclonal antibodies, composed of DDG9 isotype IgG2a, Kappa and CCH2 isotype IgG1a, Kappa, which binds for a specific epitope in these nuclear-targeted proteins. The chromogen solution contains DAB (diaminobenzidine), and when a positive reaction occurs, a brown-colored precipitate forms at the antigen site within the tested tissues.
Figure A. Final strong semi-quantified Score 6- positive immunohistochemical staining result of HCMV- 76 KD & 43 KD proteins (score III and intensity III) (40x).
Figure B. Final strong semi-quantified Score 5- positive immunohistochemical staining result of HCMV- 76 KD & 43 KD proteins (score III and moderate intensity) (20x).

Figure C. Final moderate semi-quantified Score 4- positive immunohistochemical staining result of HCMV- 76 KD & 43 KD proteins (score II and moderate intensity) (10x).
Figure D. Final moderate semi-quantified Score 4- positive immunohistochemical staining result of HCMV- 76 KD & 43 KD proteins (score II and moderate intensity) (40x).

4.1. The distribution of IHC results of HCMV-76 KD & HCMV- 43 KD proteins expression according to age strata of patients with different CNS tumours:

The most highly percentage of infected CNS tumor tissues with *HCMV* was related to the patients in the age stratum (53-69 years), accounted for 35.7 % (10 out of 28 tissues), while in the age strata (2-18 years), (19-35 years), (36-52 years) and (70-85 years) were accounted for 7.1 % (2 out of 28 tissues); 25 % (7 out of 28 tissues); 21.4 % (6 out of 28 tissues)and 10.7 % (3 out of 28 tissues), respectively. Statistically significant differences ($P<0.05$) were observed when comparing these age strata (Table 9).

Table 9: The frequency of IHC results for HCMV detection among the total CNS tumour tissues group according to age strata

Age Stratum (Years)	IHC Results for HCMV Detection				*P* value
	Positive		Negative		
	No.	%	No.	%	
2-18	2	7.1%	6	11.8%	0.03*
19-35	7	25%	10	19.6%	
36-52	6	21.4%	14	27.4%	
53-69	10	35.7%	8	15.7%	
70-85	3	10.7%	13	25.5%	
Total	28	35.4%	51	64.6%	

4.2. The distribution of IHC results for HMCV detection according to the gender of patients with CNS tumours:

Table (10) illustrates the proportion of CNS tumour tissues exhibiting positive *HMCV*-IHC results categorised by patient sex, with men representing 53.6% (15 out of 28 samples) and females constituting 46.4% (13out of 28 samples). The statistical analysis of the CNS tumours cohort indicated a significant sex difference concerning positive- *HMCV* IHC results ($P<0.05$).

Table 10: HMCV infection rates in CNS tumor patients based on their gender

CNS Tumor Patients	Positive IHC Results for HMCV Detection	
Gender		
	No.	%
Male	15	53.6%
Female	13	46.4%
Total	28	100%
The Statistical Analysis	(P<0.05)= 0.04*	

*** Statistically Significant**

4.3. The distribution of IHC results for HMCV detection in the patients with CNS tumors according to their types:

Table (11) shows positive- HMCV-IHC detection results in tissues from patients with various CNS tumors, where 14.3%, 3.6%, 3.6%, 46.4%, 14.3%, 3.6%, 14.3%, and 3.6% of Pilocytic Astrocytoma; Chardomas; Craniopharyngioma, Diffuse Fibrillary Astrocytoma, Anaplastic Oligodendroglioma, Anaplastic Astrocytoma, Glioblastoma Multiforme and Medulloblastoma, respectively, have showed positive- IHC results for HMCV detection. The statistical analysis of different types of CNS tumours with HMCV-positive tumours presented significant differences ($p \leq 0.05$).

Table 11: Frequency of CNS tumours with HMCV- positive IHC results according to their types

CNS Tumors Type		Positive-HMCV Infected Tissues	%	Non-HMCV Infected Tissues	%	*P* value
Pilocytic Astrocytoma	**14**	**4**	**14.3%**	**10**	**19.6%**	**0.04**
Chardomas	**4**	**1**	**3.6%**	**3**	**5.8%**	
Craniopharyngioma	**6**	**1**	**3.6%**	**5**	**9.8%**	
Diffuse Fibrillary Astrocytoma	**22**	**12**	**46.4%**	**10**	**19.6%**	
Anaplastic Oligodendroglioma	**13**	**4**	**14.3%**	**9**	**17.6%**	
Anaplastic Astrocytoma	**4**	**1**	**3.6%**	**3**	**5.8%**	
Glioblastoma Multiforme	**11**	**4**	**14.3%**	**7**	**13.7%**	
Medulloblastoma	**5**	**1**	**3.6%**	**4**	**7.8%**	
Total	**79**	**28**	**35.4%**	**51**	**64.6%**	

5. The distribution of co-infection results for HPV18 and HMCV detection in the patients with CNS tumors according to their types:

Table (12) shows distribution of co-infection results for HPV18 and HMCV detection in the patients with CNS tumors, where 22.4%, 0.00%, 0.00%, 22.7%, 15.4%, 0.00%, 18.2%, and 0.00% of Pilocytic Astrocytoma; Chardomas; Craniopharyngioma, Diffuse Fibrillary Astrocytoma, Anaplastic Oligodendroglioma, Anaplastic Astrocytoma, Glioblastoma Multiforme and Medulloblastoma, respectively, have showed positive- co-infection results for HPV-18 and HMCV detection.

Table 12: Frequency of CNS tumors with HPV18 and HMCV- co-positive results according to their histological types

CNS Tumor Type		HPV18 / HMCV Co-Infected CNS Tumorous Tissues	%
Pilocytic Astrocytoma	**14**	**3**	**22.4%**
Chardomas	**4**	**0**	**0.00%**
Craniopharyngioma	**6**	**0**	**0.00%**
Diffuse Fibrillary Astrocytoma	**22**	**5**	**22.7%**
Anaplastic Oligodendroglioma	**13**	**2**	**15.4%**
Anaplastic Astrocytoma	**4**	**0**	**0.00%**
Glioblastoma Multiforme	**11**	**2**	**18.2%**
Medulloblastoma	**5**	**0**	**0.00%**
Total	**79**	**9**	**11.4%**

DISCUSSION

About 12% of human malignancies are linked to viral infections. Cancers typically characterized to be developed years after the original infection and chronic inflammation (48, 49). Viruses alone are not sufficient cause in viral oncogenesis but are necessary cancer contributors. By altering cellular metabolic pathways, viruses in an oncomodulatory manner promote malignant transformation (50, 51).

Previous studies have shown that the etiology of many primary CNS tumors has been linked to genetic predisposition, chemical and radiation exposure, and, more recently, viral agents (19, 20).

The pathological development or neural oncogenesis of different CNS tumors, among them, gliomas carcinogenesis have been linked to varied degrees of certainty to a wide variety of viral agents, as HPV 16, HPV 18, EBV, CMV, HHV8, HBV, HCV, HTLV1, JCV, BK virus, and SV40 (21-52).

In the present study, we assessed a total cohort of 123 tissue blocks (73 samples from patients with different histological kinds of primary brain and CNS tumors and / or cancers and 50 samples from patients with non-tumorous histological changes) as represented by their archived formalin-fixed paraffin-embedded tissue blocks. Given that HPV genotype 18 is the most commonly recognized human oncogenic virus and Human CMV, as common "oncomodulation" virus that enhancing the malignant processes, the current research may aiming to examine to the rates of HPV 16 and Human CMV occurrence in the resected tissue samples from a cohort of Iraqi patients operated for different histopathological brain and CNS tumors, and to be compared to their counterpart control brain tissues obtained from patients whom sustained operations for non-tumorous brain lesions.

Using rigorous scientific methods (to explore the percentage of the expressed HCMV proteins, by IHC analysis, and HPV 18, by conventional PCR) intend to shed light on the possible link of HPV 18 and / or HCMV infections with the development of a variety of brain tumors and / or malignancies, therefore adding an important new information to the growing body of knowledge in this area.

IHC method was a preferred method in our study over other methods since it documents the active expression of viral genes as compared to the per se presence of the viral genome in these tissues, denying a possible suggestive relation to viral roles in the pathogenesis or oncogenesis.

Like previous studies (59-60). in their standardization of the working steps during the IHC procedures. The present study has followed careful application and standardizations of the steps of IHC according the manufacturing company, including we chosed 6μm brain tumor tissue sections (to avoid non-specific excessive binding [for less-thickened sections] or non- proper staining [for more-thickened sections], then deparaffinizing them via microwave heating followed by xylene treatment and using (1:50) working dilution of antibodies concentration.

Positive- IHC reactions for HCMV antigens in the total group of CNS tumors and / or cancers were found to be 35.4 %, where the highest % in Diffuse Fibrillary Astrocytoma (46.4%), then Pilocytic Astrocytoma, Glioblastoma Multiforme and Anaplastic Oligodendroglioma (each14.3 %), and lastly Anaplastic Astrocytoma, Craniopharyngioma, Chardomas and Medulloblastoma (each 3.6%).

Previous studies, including 191 studies, have identified HCMV in 36% of the examined 2529 tumor samples, including glioblastomas, while other researchers did not detect HCMV DNA and proteins in glioma samples (37, 53-55).

An active area of studies in line with the concept of "oncomodulation" where a virus is not being the sole cause but enhancing the malignant processes (56).

HCMV DNA and antigens have been found in GBM samples, yet the viral contribution to oncogenesis or its direct oncogenic role in GBM remains unclear (57).

However, previous studies on glioblastoma tumor tissues have reported that HCMV infection and expression of viral antigens that have an oncogenic as well as suppressing immunomodulatory properties in glioblastoma tumor tissues, aiding GBM tumors in evading immune surveillance, thereby promoting virus's role in tumor progression (58).

The results of a survey by Ranganathan (14) detected as many as 20 different regions / loci of HCMV genome in only a minority of GBM cells than in specimens from other brain tumor types, , defining the virus as onco-accessory for such tumors.

According to a study by (61), HCMV may actively contribute to the etiology of gliomas because it is widely expressed in almost all GBM samples but not in normal brain tissue or other benign tumors.

Previous studies (62, 63] have reported that high percentages of primary GBM tumors have expressed HCMV immediate-early and late proteins (92% & 73%, respectively). HCMV DNA and proteins are also frequently detected (>90% & 99%) in GBM tumor tissue and peripheral blood of GBM patients, respectively. In these studies, the high prevalence of HCMV DNA and proteins in GBM and astrocytoma, but not in non- tumorous control tissues, suggests a potential role for the virus in glioma carcinogenesis.

A Study in Iran revealed HCMV DNA in 7.1% of 42 GBM samples (64), while in Pakistan a study revealed HCMV in 0.9% among 112 GBM biopsies (65) and a Mexican study has revealed HCMV in 4.8%among 21 GBM samples (52).

The lifelong latency of these HCMV products within tumor cells supports a role for this virus in tumor development, where can down regulate tumor immunogenicity through various mechanisms. Furthermore, treatment of HCMV-positive tumors can induce viral reactivation, leading to increased infection of both tumors and normal adjacent cells, and further immunosuppression. These represent a vicious circle leading one to another in deteriorating the outcomes of patients with such tumors as well as such viral infections (13).

These findings are supported by the results of a previous study (3). on glioblastoma patients who received anti-HCMV drugs and have evidenced a significant reduction in the growth of HCMV-positive glioma tumors and increased of survivals (61).

However, other researchers (2) concluded that CMV infection has no significant effect on the prognosis of glioma patients.

World -Globocan 2020 as well as Iraq- Globocan 2020 (66, 67) have reported an increased incidence of brain cancers during 2020 (10.74 & 10.18 per 100 000, respectively).The most common primary brain tumors are the Gliomas, which are the carcinogenetic sequels of glial cells in brain as well as these in spinal cord (68).

Of importance to disclose the roles of rating Human Papilloma Viral 18 in relation to the studied gliomas grading as well as other brain and CNS tumors/cancers, whether at early or late events in the process of the current brain tumorigenesis.

In the current study, HPV 18- DNA in the overall kinds of examined tissues from CNS tumors were found to be 16.5 %, where the highest % of HPV 18 is related to Diffuse Fibrillary Astrocytoma (38.5%), then Anaplastic Oligodendroglioma and Glioblastoma Multiforme (each 23.1%), Pilocytic Astrocytoma and Anaplastic Astrocytoma (each 7.7%), Craniopharyngioma (4.8%) while none of Chardomas and Medulloblastoma tissues revealed HPV 16- positivity reactions.

A previous nested- PCR study, and confirmed CISH and IHC by (Vidone et al, 2014) (69) has reported an active ongoing human papilloma viral protein production process from the HPV 16 genome in in 25% of glioblastoma multiforme cancer tissue samples

Other previous studies have revealed that histological cancer types are relevantly dependent on HPV types, where HPV 16 infections predominantly found in squamous cell carcinoma type, while the adenocarcinomas are more often to have HPV 18- type infection (70, 71). In addition, another study (72) has shown that HPV 18 genome, as compared to HPV 16, is more likely to integrate into host cellular genome. This viral integration in cellular genetic materials has effectively contributed both to the persistence process and deregulated expression of HPV E6 and E7 oncogenes of HPV, led to inactivation of p53 and pRB, that ultimately increasing cellular proliferation, in line with viral roles in oncogenesis and / or carcinogenesis (38, 73-75).

Higher prevalence of transcriptionally active high –oncogenic risk genotypes of Human Papillomaviruses in invasive pituitary adenomas compared to non-invasive ones were also revealed in another research work 39).Our results on HPV in glioma and glioblastoma parallel those reported by other research groups of Vidone and collaborators (38), Arsene et al., 2022 (5), Hashida et al., 2015(37),Adnan Ali et al., 2019 (65), and Limam et al., 2020 (76).Regarding the study of viral oncogenesis in tumors of the central nervous system by Arsene et al., 2022 (5), HPV was detected in 20.78% of 154 analyzed samples; specifically, HPV was detected in 25% of meningioma cases, 25.86% of glioma cases and 7.5% of control samples (14.3% in grade II and III astrocytoma and 29.5% in glioblastoma). Studies of Hashida et al., 2015(37) and Adnan Ali et al., 2019 (65) have revealed 25– 28% HPV-positive cases in patients with glioblastoma, emphasising the possible role of HPV in the pathophysiological mechanism of this tumour. Limam and collaborators (2020) (76) recently found a higher % of HPV positivity in glioblastoma cases (39.3%).

CONCLUSION

Although the present retrospective research study has investigated a small set of archived- tumour tissues from Iraqi patients with different primary CNS tumours, it can be concluded from the current results of the assessment that the high detection rates of HPV 18 DNA suggest a significant association with the development and carcinogenesis of these tumors. These findings indicate a potential role of HPV infection in brain tumor formation. Further larger and more detailed studies are needed to assess the relationship between HPV infection and glioma grading. Additional research is crucial to evaluate early or late events of HPV -temporal relationship in brain tumorigenesis, prevalence of such important high-risk HPV infections in the general population, as well as whether HPV infection is persistent or merely as transient infections in these patients.Despite these observations, the alone detection of HCMV in tumor tissues is insufficient to confirm causation role in brain- gliomagenesis and CNS tumor formation. However, a significantly increased expression of HCMV proteins in different gliomas and other CNS tumor tissues may suggest a possible association between HCMV infection and development, pathogenesis or progression of other CNS tumors.

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