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The Potential Role of Human Herpes Virus-6 in Idiopathic Facial Nerve Paralysis

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Abstract

Background Bell's palsy (BP) is an acute, generally unilateral, paralysis or weakness of facial musculature consistent with peripheral facial nerve dysfunction; different factors suspected to contribute to the development of BP including herpesviruses.

Objective To detect human herpes virus 6 (HHV-6) in the saliva samples of patients in the early presentation of BP.

Methods A case-control study included saliva samples taken from 50 patients with BP and 50 apparently healthy controls without any neurological deficit, viral DNA was extracted from the saliva and then subjected for quantitative real time polymerase chain reaction (PCR) for detection of HHV-6 viral load (VL) in saliva.

Results HHV-6 DNA was positive in 22 (44%) out of the 50 patients, and in 12 (24%) of the controls (p=0.028), with a significantly higher mean viral load in patients than control (p=0.002), in addition, 70% of HHV-6 positive patients had severe grades with a significantly higher VL (p=0.007) and (0.015), respectively.

Conclusion HHV-6 could play an important role in the development of BP and HHV-6 might have a neuro-pathological effect in severe cases of BP.

Keywords HHV-6, Bell's palsy, Saliva, real time PCR

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List of abbreviations: • BP = Bell's palsy, HHV-6 = Human Herpes Virus 6, VL = Viral Load

Introduction

Bell's palsy (BP) is the most common condition involving a rapid and unilateral onset of peripheral paresis/paralysis of the seventh cranial nerve. It affects 11.5–53.3 per 100,000 individuals a year across different populations. Bell's palsy is a health issue causing concern and has an extremely negative effect on both patients and their families. Therefore, diagnosis and prompt cause determination are key for early treatment [(1)].

Despite its severe effects, the exact etiology of BP remains unclear. The Guideline Development Group (GDG) [(2)] has identified the diagnosis of BP as one of exclusion, requiring careful clinical elimination of other potential etiologies of facial paralysis/paresis, such as trauma, neoplasms, postsurgical facial paralysis/paresis, or infection by agents including herpes viruses and Lyme disease [(1,3)]. Some studies stressed on the role of Human herpes virus 6 (HHV-6) in the development of Bell's palsy [(4,5)]. HHV-6 (family Herpesviridae, subfamily Beta herpes virinae, genus Roseolo virus, species Human herpes virus 6) is

neurotropic virus that reactivate frequently in immune compromised persons [6,7].

Several studies had been conducted on the possible role of HHV-6 in the etiology of Bell's palsy, it is suggested that the high level of HHV-6 DNA is only a consequence of the patient's immune status, this may be the main predisposing factor for the occurrence of the palsy although the contribution of HHV-6 reactivation/replication cannot be ruled out [4,8,9].

This study aimed to detect the frequency of HHV-6 in the saliva of patients with BP and its association with the grade of the neurological deficit.

Methods

A case-control study was conducted from October 2019 to February 2020. Fifty patients with idiopathic unilateral facial nerve paralysis (Bell's Palsy) were collected from Al-Imamein Al-Kadhimein Medical City and Baghdad Teaching Hospital, and 50 apparently healthy age- and sex-matched volunteers as controls. A written informed consent was taken from each subject enrolled in this study. The study was approved by the Institutional Review Board (IRB) of the College of Medicine-Al-Nahrain University, approval number (269-15-10-2019). The research was conducted in the Microbiology Department at the College of Medicine-Al-Nahrain University.

From all patients and controls about 1-2 ml saliva (without induction) were collected in plane tubes. Saliva samples preserved in deep freeze (-20 °C), and then used for viral DNA extraction. Viral nucleic acid was extracted using (Geneaid/Taiwan) kit, and real time polymerase chain reaction (PCR) amplification kit (Sacace, Italy) was used for quantitative detection of HHV-6 in the saliva samples. Fifteen µl of master mix were added to all PCR tubes and 10 µl of (DNA sample, negative control, positive control or standards) were added to master mix. Real time PCR instrument used in this work was STRATAGENE MX 3005P (Agilent Technologies, USA). For real-time PCR the following amplification protocol was used: 1 cycle at 95 °C for 15 min followed by 5 cycles consisting of 5 s at 95 °C, 20 s at 60 °C, and 15 s at 72 °C, and then 40 cycles consisting of 5 s at 95 °C, 30 s at 60 °C, and 15 s at 72 °C.

Statistical analysis

The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), Version 20 used for statistical analysis. Categorical data formulated as count and percentage. Odd ratio and Chi-square test used to describe the association of these data. Alternatively, Fisher exact test was used if there is 25% of cells less than expected count. Numerical data were described as mean and standard deviation. Independent sample t-test used for comparison between two groups. The lower level of accepted statistically significant difference is equal or less than 0.05.

Results

In this study HHV-6 viral load (VL) was measured in the saliva samples of Bell's Palsy patients and normal controls. HHV-6 DNA was positive in 22 (44%) out of the 50 patients, and in 12 (24%) of the controls (p=0.028), and the mean viral load was also significantly higher in patients than in the controls (p=0.002) (Tables 1 and 2).

Table (3) demonstrated no significant association between HHV-6 positivity with patients' age, sex, side of the weakness or duration of BP, however there was a significant association between HHV-6 and the severity of BP, in which 70% of HHV-6 positive cases were in the severe grades (p=0.007), also the mean HHV-6 VL is significantly higher in the severe grades (p=0.015).

Table 1. Comparison of the number of HHV-6 DNA positive cases between patients and controls

		Patients	Controls
HHV-6	Positive	22	12
	%	44.0	24.0
	Negative	28	38
	%	56.0	76.0
Total		50	50
%		100.0%	100.0%
p value		0.028	
Odds Ratio (Confidence interval)		2.488 (1.06-5.85)	

Table 2. Comparison of the mean Viral Load (VL) of HHV-6 between patients and controls

HHV-6	Patients	Control
Mean VL (Copies/ml)	4026.00	117.04
Standard Deviation	9912.57	252.71
Median	0.00	0.00
Percentile 25	0.00	0.00
Percentile 75	3000.00	0.00
P value	0.002	

Table 3. The associations between HHV-6 DNA positivity and viral load (VL) with patients' clinical and demographic data

		HHV6		P value	Mean VL Copies/ml	Standard Deviation	P value
		Positive	Negative				
Age groups	≤25 years	9 (45.0%)	11 (55%)	0.907	6640.00	14686.42	0.125
	>25 years	13 (43.3%)	17 (56.7%)		2283.33	4105.51	
Sex	Female	8 (66.7%)	4 (33.3%)	0.073	6608.33	13971.04	0.305
	Male	14 (36.8%)	24 (63.2%)		3210.53	8321.77	
Side	Left	9 (39.1%)	14 (60.9%)	0.577	2265.22	4514.58	0.250
	Right	13 (48.1%)	14 (51.9%)		5525.93	12761.50	
Severity	Moderate	10 (30.3%)	23 (69.7%)	0.007	630.30	1072.52	0.015
	Severe	12 (70.6%)	5 (29.4%)		10617.65	15121.85	
Duration days	1	4 (40%)	6 (60%)	0.522	1000.00	1333.33	0.253
	2	3 (30%)	7 (70%)		1330.00	2921.21	
	3	15 (50%)	15 (50%)		5933.33	12385.35	

Discussion

HHV-6 is a common neurotropic virus which has been associated with conditions such as febrile convulsions, encephalitis, and multiple sclerosis and is another candidate in Bell's palsy. HHV-6 has been directly or indirectly associated with several neurological diseases [8,10,11], in cases of primary infection in immune-competent young children, reactivation in

healthy adults [12], or in immunosuppressed patients [13].

The present study found that both the number of HHV-6 positivity and its mean VL in saliva were significantly higher in patients than in the controls, which is supported by the study of Turriziani et al. in 2014 [4] which showed that the value of HHV-6 DNA copies was significantly higher than that detected in healthy subjects. In addition, patients with the highest levels of HHV-6 DNA showed no change in facial palsy HB grade (The House-Brackmann FacialNerve Grading System) or even an increase of at least one HB grade at the first visit. This suggests that the high level of HHV-6 DNA is only a consequence of the patient's immune status: this may be the main predisposing factor for the occurrence of the palsy although the contribution of HHV-6 reactivation/replication cannot be ruled out. These finding also agrees with results of the current study in which 70% of HHV-6 positive cases were in the severe grades (p=0.007), also the mean HHV-6 VL is significantly higher in the severe grades (p=0.015).

One study searched for HHV-6 in the tear fluid of patients with BP, finding a significantly higher detection rate of HHV-6 than that in healthy controls [8]. In addition, recently a study of HHV-6 in saliva of children and adolescents with BP found even a higher frequency of HHV-6 in patients than the current result [14]. However, it is still uncertain whether HHV-6 is involved in the development of the disease or that the underlying disease mechanism might predispose patients to HHV-6 reactivation. Perhaps the major limitations of this study are that the species of HHV-6 detected in samples were not characterized, and absence of follow up VL levels in these patients.

Turriziani et al. in 2014 [4] suggested that co-infection with this HHV-6 is possible, and could be more implicated in the pathogenesis of BP. The combined role of viruses also could be explained by the autoimmune theory in which viral replication may provoke an autoimmune reaction against peripheral nerve myelin components, leading to the demyelination of cranial nerves, especially the facial nerve [15]. This is supported by studies on multiple sclerosis which could be caused by autoimmune reaction to multiple herpesviruses interactions especially antibodies against HHV-6 that cross react with myeline basic protein [16].

Recently Ptaszynska-Sarosiek et al. in 2019 [9] have revealed a common presence of the herpesviruses (including HHV-6 and EBV) in trigeminal and facial nerve ganglia post-mortem among a random group of Polish population and demonstrated simultaneous infection of the ganglia with different herpesviruses.

In conclusions, the significantly high frequency and viral load of HHV-6 in Bell's palsy make it possible player in the pathogenesis of this disease. The high HHV-6-frequency and viral load in severe HB grades of BP strengthens its role in this neurological condition.

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Author contribution

Rhaif did the sampling and run the real time PCR. Dr. Al-Obaidi put the research plan and writing the manuscript. Dr. Al-Khazrajee did the selection of patients and grading of BP.

Conflict of interest

Authors declare no conflict of interest.

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