HERPES SIMPLEX VIRUS IN SALIVA OF PATIENTS WITH BELL'S PALSY

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Abstract- Acute idiopathic peripheral facial paralysis (Bell's palsy) is the most common disorder of the facial nerve. Most patients recover completely, although some have permanent disfiguring facial weakness. Many studies have attempted to identify an infectious etiology for this disease. Although the cause of Bell's palsy remains unknown, recent studies suggest a possible association with Herpes Simplex Virus-1(HSV-1) infection. In this case-control study we investigated the presence of DNA of HSV in the saliva of 26 patients with Bells palsy in first and second weeks of disorder compared to normal population who were matched in sex, age, as well as history of diabetes mellitus, hypertension and labial herpes. In the case group 3 and 7 patients had positive polymerase chain reaction (PCR) for HSV in first and second weeks of disease respectively compared to 4 in controls. It means that there was not any relationship between Bell's palsy and HSV in saliva either in first or in second week. Two and 6 of positive results from the sample of first and second weeks were from patients with severe (grade 4-6) Bell's palsy. Although the positive results were more in second week in patient group and more in severe palsies, but a significant relationship between Bell's palsy or its severity and positive PCR for HSV was not detected (P >0.05).

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INTRODUCTION

Bell's palsy is an acute idiopathic peripheral facial paralysis witch is the most common disorder of the facial nerve. The name of this disease is referred to Charles Bell (1828), although the same disease was described earlier by Nicolas A. Friedrich (1798) and Richard Powell (1813). Its incidence is 20-30 cases per 100,000 per year. It is the cause of 60-75% of cases of unilateral facial paralysis. It may occur at any age but the median age of onset is around 40 years (1).

Most patients recover completely, but some have permanent disfiguring facial weakness which has a great impact on the patient.

Although the exact etiologic agent responsible for this disease has not been identified, a lot of efforts have been put into the understanding of the disease process. It has been suggested that entrapment of the swollen facial nerve at the meatal foramen witch is the narrowest portion of the bony fallopian canal is a critical component in its pathophysiology (2). Intraoperative observations during facial nerve decompression through the middle cranial fossa sometimes demonstrate a swelling of the facial nerve proximal to the meatal foramen (3). Inflammation has been also suggested

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based on the demonstration of a pathologic gadolinium enhancement of the nerve within the labyrinthine segment using magnetic resonance imaging (4). Some histopathologic studies have reported infiltration of inflammatory cells together with marked swelling and edema of the facial nerve proximal to the meatal ganglion of the facial nerve (5).

The cause of initiation of inflammation is not known but autoimmune process, viral infections, and even ischemia seem to be the main causes. Different viruses from the herpes family, such as herpes simplex virus-1 (HSV-1), HSV-2, human herpes virus-6 (HHV-6), and varicella zoster virus (VZV), as well as some other viruses like influenza virus have been considered to play a role in Bell's palsy. HSV has been considered particularly as the etiologic agent in recent studies. In 1972, McCormick first suggested that HSV is responsible for idiopathic facial paralysis (6). Then the role of reactivation of this virus in the geniculate ganglion was suggested by Adour as well (7). In one study in 120 cases of Bell's palsy, although there was no serological evidence of HSV antibody titer rise, in one of 2 patients that a biopsy specimen for virus isolation was obtained during a decompression operation, it was found to contain HSV type I (8). Murakami et al reported that HSV-1 genomic DNA has been amplified from endoneurial fluid and muscle tissue of 11 out of 14 patients with Bell's palsy, but not from control patients with peripheral facial palsy due to other causes such as trauma (9). Mouse model of Bell's palsy has been induced by herpes simplex virus type 1 (10, 11). Abiko et al found HSV-1 genomic DNA in tear and saliva of 5 out of 16 patients (31%) with Bell's palsy. They had collected several specimens from both the affected and unaffected sides from tear fluid and saliva from the parotid and submandibular gland, more than twice during first and second weeks or later from each patient. The detection rate of HSV DNA was 38 of 244 (15.6%) specimens. The high detection (28.5% of patients) was obtained within first and second weeks after onset of disease. No HSV DNA was detected in 36 specimens taken as normal control specimens from 6 healthy subjects (12). In spite of this, the active role of HSV in Bell's palsy is controversially discussed in the literature. In another recent study, viral genomic DNA of HSV-1, HSV-2, human herpes virus 6 A and B (HHV-6A/B), and VZV was not detected by nested polymerase chain reaction (PCR) in facial muscle biopsy specimen in patients with Bell's palsy compared to control patients. Besides HSV-1 genomic DNA was detected in 86% of geniculate ganglion preparations from control specimen and concluded that the sole presence of HSV genomic DNA within the sensory ganglion along the facial nerve does not justify its active role in Bell's palsy (3). Vrabec had a similar finding and concluded that in order to confirm the viral etiology for Bell's palsy demonstration of a significant difference in prevalence of the virus in specimens from afflicted individuals will be necessary (13). Because of this controversy we decided to detect the genomic DNA of HSV in saliva of patients with Bell's palsy in comparison to control group.

MATERIALS AND METHODS

Between 2003 and 2005, all patients of our center with acute idiopathic facial nerve paralysis were informed on the study purpose to identify the possible causative role of HSV in this disease. Based on the observations of Abiko et al. (12), we aimed at identifying viral DNA within the saliva of patients. The diagnosis of Bell's palsy was according to Tavernier's criteria (1959) which outlined the minimum diagnostic criteria for Bell's palsy: 1) paralysis or paresis of all muscle groups of one side of the face, 2) sudden onset, 3) absence of signs of central nervous system (CNS) diseases, and 4) absence of signs of ear or cerebellopontine angle diseases.

Our exclusion criteria were: 1) consumption of corticosteroids in last one month, 2) history of labial herpes in last three months, and 3) immuno-compromised patients. Twenty six cases consented to our study. The severity of disease was detected according to House-Brackmann grading (14) (Table 1). Patients were included in mild (grade 3 or less) and severe (grade 4 or more) paralysis groups. Twenty six healthy volunteers were also selected

who were matched in age, sex, as well as history of hypertension, diabetes mellitus and labial herpes (history of labial herpes in 3 to 12 month ago or more than one year ago). Exclusion criteria for case group were considered for controls as well. This study was approved by the ethics committee at Tehran University of Medical Science and informed consents were obtained from all before entering the study.

The DNA of HSV was evaluated by PCR in saliva of patients twice in first and second weeks of disease. In healthy controls this test was done once. The laboratory was blinded on the samples.

DNA extraction PCR assay

DNA was extracted from all specimens, including negative material as extraction negative controls, using High Pure viral Nucleic Acid Kit (cat # 11 858 874 001, Roche , Germany). Briefly, equal volumes (200 µl) of sample and binding buffer (6 M guanidine-HCl, 10 mM Tris-HCl, 20% Triton X-100, pH 4.4), 0.02 mg Poly (A) and 1 mg proteinase K) were incubated at 72°C for 10 min. Then the mixture was transferred to a high filter tube, centrifuged for 1 min at 1300 rpm and washed with washing buffer two times. To elute the viral nucleic acid, 50 µl elution buffer were added to filter tube and centrifuged for 1 min at 13000 rpm.

Table 1. House- Brackmann grading for Bell's palsy

Grade	Characteristics
I. Normal	Normal facial function in all areas
II. Mild dysfunction	**Gross** Slight weakness noticeable on close inspection. May have very slight synkinesis. At rest, normal symmetry and tone. **Motion** Forehead: moderate-to-good function Eye: complete closure with minimal effort Mouth: slight asymmetry
III. Moderate dysfunction	**Gross** Obvious, but not disfiguring difference between the two sides. Noticeable but not severe synkinesis, contracture, or hemifacial spasm. At rest, normal symmetry and tone. **Motion** Forehead: moderate-to-moderate function Eye: complete closure with effort Mouth: slightly weak with maximum effort
IV. Moderately severe dysfunction	**Gross** Obvious weakness and/or disfiguring asymmetry. At rest, normal symmetry and tone. **Motion** Forehead: none Eye: incomplete closure Mouth: asymmetric with maximum effort
V. Severe dysfunction	**Gross** Only barely perceptible motion. At rest, asymmetry. **Motion** Forehead: none Eye: incomplete closure Mouth: slight movement
VI. Total paralysis	No movement

The target sequence for PCR amplification of a DNA segment from HSV was a 325 base-pair (bp) segment that codes DNA polymerase on the HSV genome. This segment is known to be stable, with which it is possible to amplify both HSV-1 and HSV-2. All PCRs were carried out by Amplisens Herpes Simplex Virus1,2 detection Kit (cat# V8-100-R0.5, Amplisens, Ministry of Health of Russian Federation) according to manufacturer protocol. Briefly, 10 µl of DNA from patient's specimens was added to PCR mixture, all PCRs contained 1X PCR buffer (10 mM Tris–HCl [pH 8.3], 50 mM KCl, 0.01% [wt/vol] gelatin), 1.5 mM MgCl2, 1.25 U Taq DNA polymerase, 200 mM of each dNTP, and 0.2 mM of each primer.

The assays were performed on a multi-channel programmable thermal cycler, Tercyc (DNA technology, Moscow, Russia) using one cycle of 95°C for 5 minutes followed by 42 cycles each of 95°C for 1 minute, 65°C for 1 minute, and 72°C for 1 minute. The amplified PCR products were run on a 2% agarose gel stained with ethidium bromide, and visualized under a UV light transilluminator.

To compare positive results in patients and control groups or in mild and severe palsies, Chi-Square test was used assuming statistical significance at $P < 0.05$.

RESULTS

Twenty six patients with Bell's palsy (9 women and 17 men) and the same number of control subjects were studied. The mean age of patients was 45.5 ± 17.1 years (range: 19-84). Table 2 displays the clinical characteristics of patients.

In the case group, 3 patients had positive PCR for HSV in first week of paralysis. One of these patients had mild (grade 3) and two had severe (grade 4 and 5) type of disease. In one of the patients with positive result in first week, the result of PCR was positive in second week as well. Besides this one, six more patients had positive result in second week. Among these seven patients one had mild (grade3) and other six had severe (three grade 4, two grade 5 and one grade 6) type of disease. Also four of controls had positive PCR for HSV.

There was not any relationship between Bell's palsy and HSV in saliva in first week. Although the positive results were more in second week in patient group and more in severe palsies, a significant relationship between Bell's palsy or its severity and positive PCR for HSV was not detected ($P > 0.05$) (Table 3 and 4).

Table 2. Clinical characteristics of 26 patients in our study.

Characteristics	patients	controls
Male: female	1.8 : 1	1.8 : 1
History of diabetes mellitus	4(15.4%)	4(15.4%)
History of hypertension	6(23.1%)	6(23.1%)
History of labial herpes(>3month ago)	11(42.3%)	11(42.3%)
Grade:		
<=3	8(30.8%)	-
>3	18(69.2%)	-

Table 3. Positive PCR in 1st and 2nd week compared to controls.

Positive PCR in compared groups		Significance (*P* value)
Samples of patients in1st week	controls	NS
3	4	
Samples of patients in 2nd week	controls	NS (p= 0.308)
7	4	

NS: Not significant

Table 4. Positive PCR in 1st and 2nd week in severe compared to mild Bell's palsy.

Sample time	PCR	Severe BP	Mild BP	Total	Significance (*P* value)
1st week	Positive PCR	2	1	3	NS (P=0.919)
	Negative PCR	16	7	23	
2nd week	Positive PCR	6	1	7	NS (P=0.269)
	Negative PCR	12	7	19	

NS: Not significant

BP: Bell's palsy

DISCUSSION

Bell's palsy is the most common cause of acute facial paralysis. Despite many attempts that have been made to understand its etiology, it still remains unknown. It has been suggested that this disease might be caused by reactivation of latent HSV inside the geniculate ganglion, but to explain a viral reactivation theory, the viral presence has to be determined. In one study HSV-1 genomic DNA was identified in 86% of human geniculate ganglion samples at autopsy of control patients without any previous history of facial palsy (3). Takasu et al have detected HSV-1 DNA in autopsy of 16 of 17 (94%) trigeminal ganglia and in 15 of 17 (88%) geniculate ganglia of adults. (15) So the sole presence of viral genomic DNA within the geniculate ganglion in most of the normal human beings does not explain that only 20 to 30 per 100,000 people develop unilateral Bell's palsy. Despite some papers in favor of seropositivity of HSV in Bell's palsy (16, 17) most studies could not find any definite association between antibody titers and Bell's palsy. To confirm the active role of HSV, its presence during acute Bell's palsy needs to be shown in comparison with healthy controls. Viral shedding could theoretically be expected in the periphery within saliva because of the innervation of submandibular and sublingual glands by facial nerve. In our study, we analyzed the presence of HSV genomic DNA in saliva samples from patients with Bell's palsy and controls. According to our results there was not any relationship between Bell's palsy and HSV in saliva in first week. The positive results were more in second week in patient group, but in our study a significant relationship between Bell's palsy and positive PCR for HSV was not detected (P value >0.05). Our findings do not support a causative role of these viruses in Bell's palsy. Contrary to our results, Abiko found HSV-1 genomic DNA in 5 out of 16 patients (31%) with Bell's palsy and none of healthy controls (12). We finally tried to find a relationship between severity of disease and positive PCR in samples. Again although the positive results were more in severe palsies, but a significant relationship was not detected (p >0.05). Although the discrepancy may be explained by technical differences in the PCR assay used in our laboratory compared with the assay utilized by Abiko, this is unlikely, because PCR is a highly sensitive method. Another explanation may be that there are different types of Bell's palsy, one dependent on HSV, while others are not. It should be noted that Abiko was unable to detect HSV genomic DNA in all the patients with acute Bell's palsy. Finally, we can consider that the presence of a virus in Bell's palsy patients could be a mere coincidence. Besides, if Bell's palsy is to be caused by HSV, the proper treatment using antiviral agents should be effective. Although there are some studies showing a significant better outcome with these drugs (18), more available studies do not support this effectiveness (19).

In fact antiviral agents are advised in Bell's palsy according to the evidences that support only a level "C" recommendation (20).

So we agree that until proven otherwise, Bell's palsy should still be considered idiopathic rather than viral or herpetic facial paralysis.

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