Detection of Human Herpes Virus-6 in saliva of Patients with Bell's palsy.

Mustafa Hameed Majeed*, *Abdul–Kareem Kadhim Alkhazraji**
Resident of neurology al-Imamein Al-kadhimein Medical City
**** Section of neuromedicine,department of medicine Al-Nahrain Medical College***

Article Info:

Received 22 Nov 2020
Accepted 1 Feb 2021
Published 1 Mar 2021

Corresponding Author email:
Akkkh_neuro@ced.nahrainuniv.edu.iq
orcid: https://orcid.org/0000-0001-6775-5799

Abstract:

Background: Bell's palsy is unilateral peripheral paralysis of the seventh cranial nerve, several mechanisms have been proposed in the pathogenesis of this disease, among the viral infections specially herpes virus's family including roseola viruses that have been detected in patients' saliva.

Method: A case-control study conducted on Bell's palsy patients at al-Imamein Al-kadhimein Medical City throughout the period from March 2019 to November 2019. Saliva samples were taken from 50 patients (18-55) years of age with early stages of Bell's palsy and from 50 apparently healthy and age and sex matched volunteers as control. Viral DNA was extracted from the saliva and then real time PCR for detection and quantification of HHV6 DNA in these patients

Results: Saliva samples taken from 28 patients in the Bell's palsy patients (56%) tested positive for HHV6, as vs. to just 8 (16%) in the healthy group(control) (P= 0.028). There was increase amount of HHV6 load in patients compare to control group (p=0.001). Regarding the demographic values, an important difference in the detection rate for Human herpes virus-6 between male (64%) and females (35%) (P= 0.02). There was significant correlation between age and grading (p =0.01), as increase age correlate with high grading, also between viral load of HH6 and grading as increase viral load of the virus correlate with high grading (p = 0.001).

Conclusion: HHV-6 virus might have a potential role in the pathogenesis of Bell's palsy.

Key words: Bell's palsy, human herpes virus-6 virus, Real-time PCR.

الدور المحتمل لفيروس هربس البشري السادس في مرضى شلل بيلز

مصطفى حميد مجيد *, عبد الكريم كاظم الخزرجي**
***الجملة العصبيه في مدينة الامامين الكاظميين الطبيه**
****الجملة العصبية كلية الطب-جامعة النهرين**

الخلاصة:

الخلفية:
شلل بيلز هو شلل محيطي احادي الجانب للعصب القحفي السابع,وهو غير معروف السبب,بااستخدام تقنية تفاعل البوليمريز المتسلسل أنه من الممكن أخذ عينات من لعاب قابل للوصول وتحديد الدور الممكن لفايروس هربسي بشري السادس.
الغرض من هذه الدراسه هو لتحري علاقة شلل بيلز معه فايروس هربسي بشري السادس بواسطة عينات اللعاب للمرضى.
الهدف:
لتحديد العلاقه الممكنة بين ظهور شلل بيلز مع وجود فايروسهربسي بشري السادس في لعاب المرضى.

الطريقة:

تم اجراء الدراسة (حالات وشواهد) في مدينة الامامين الكاظميين(ع) الطبية من الفترة (أذار 2019 الى كانون الاول 2019)
تضمنت الدراسة 50 مريض تتراوح اعمارهم من (18-55 سنة) مصابين بالمراحل الاولية لشلل بيلز, 50 شخص سليم متوافق جنسيا وعمريا تم اعتبارهم كشواهد في الدراسة.
النتائج:
عينات اللعاب ل 28 مريض مصاب بشلل بيلز (56%) ظهرت ايجابية لفايروس هربسي بشري السادس مقابل 8 مرضى (16%) في مجموعة الشواهد (قيمة ع 0.028)
كان هناك زيادة في حمل فايروس هربسي بشري السادس في المرضى مقارنة بالشواهد (قيمة ع 0.001)
فيما يخص المتغيرات الديموغرافية كان هناك فرق مهم في معدل الاصابة لفايروس عربسي بشري السادس بين الرجال (64%) والنساء (35%) (قيمة ع 0.02)
كان هناك علاقة مهمه بين العمر ونظام التدريج (قيمة ع 0.01), التقدم بالعمر يتناسب طرديا مع التدرج العالي للمرضى, حمل الفايروس يتناسب طرديا مع التدرج العالي للمرضى (قيمة ع 0.001).
الاستنتاجات:
لفايروس هربسي البشري السادس دور مهم في اعتلال العصب السابع لمرضى شلل بيلز.

الكلمات المفتاحية: شلل بيلز, فايروس هربسي بشري السادس, بكر الزمن الحقيقي

Introduction

Bell's palsy, named after the Scottish anatomist physician, Sir Charles Bell, is the most common mono neuropathy, and is the most common diagnosis associated with facial nerve paralysis [1]. The paralysis causes disruptions of facial appearance and affects the normal functions. Bell's palsy affects 11 – 40 people per 100,000 in the peoples per year, commonly in the age groups 15 – 45 years [2].

Much etiology has been going to the possibility that Bell's palsy caused by herpes viral etiology, and the most obvious etiological viral cause of facial palsy is varicella-zoster virus (VZV) infection, known as the Ramsey-hunt syndrome. The last data suggests that herpes simplex virus type1 (HSV-1) may be an important obvious cause agent in Bell's palsy [3], human herpes virus-1 was found in the endoneural fluid aspirated during decompression operation from the facial nerve tract of Bell's palsy patients [4]. Mostly, in a primary infection, herpes simplex virus type 1, that is often dormant in the geniculate ganglion, may be reinfect the ganglia after surgical stress [5].

Human herpes virus 6 (HHV-6) is a common virus that affect the nervous system, which has been conducted with illness like febrile seizure, central nervous system infection and multiple sclerosis [6].

The cause of Bell's palsy is difficult to identified directly, since it is difficult to gain samples through facial nerve tract.

Human herpes virus-6 multiplication in the salivary glands, as human herpes virus-1, these two viruses mostly founds in the saliva of healthy people [7, 8].

Human herpes virus 6 (HHV- 6) is a human invaded pathogen of developing clinical important[9], Salahuddin et al., (1986) were the first to isolate HHV-6, using peripheral blood lymphocytes (PBL) obtained from patients with lymphoproliferative disease. Cell tropism is the greatest for T lymphocytes [10], Two genetically distinct variants of the virus exist, HHV-6 A and -6 B [11].

Activation of latent human HerpesVirus-6 has been associated with infection of facial nerve in the studies. However, there was no correlation between detection of Human Herpes Virus- 6 and the facial nerve palsy has been established. One research found human Herpes Virus- 6 DNA gene in the eye fluid of facial nerve palsy patients, while other study found viral DNA gene in the cerebrospinal fluid(csf) of one patient with facial nerve palsy [16,17]. The objective from the research is to identify the possible relation between the development and

severity of Bell's Palsy and the presence of HHV-6 in patients' saliva.

Patients and methods

Design and settings:

A case-control study conducted on Bell's palsy patients from March 2019 to November 2019. The study enrolled 50 patients (18-55) years of age with early stages of Bell's palsy. Saliva samples was collected from the Bell's palsy patients in the neurology consultation clinic at al-Imamein Al-kadhimein Medical City, 50 healthy employees of the same age and gender from Al-Nahrain University Faculty of Medicine were participated as volunteers to work as controls. Patients and controls were informed regarding the nature of the research.

Inclusion criteria: Eligible Patients with idiopathic lower motor neuron unilateral facial palsy within the first 72 hours of facial palsy.

Exclusion criteria:

1.Otogenic causes of the facial nerve palsy.
2.Central nerves system causes.
3.Finding of ear vesicles.
4.Patients with hypertension, diabetes, pregnancy and any neurological problem that causes bilateral facial nerve paralysis.
5.Patients taking antiviral therapy (acyclovir), or any steroid therapy.
6.Patients admitted after 72 hours after the facial nerve eliminated from the research.

All patients graded according House–Brackmann (HB) facial grading scale,which is scale use to grade the severity of the bells palsy. Saliva was subjected for viral DNA extraction, DNA samples were used for real time PCR analysis for HHV6, Using Real Time PCR kit for detection of Human Herpes Virus 6 (HHV6 RealTM Quant) (Sacace-Italy)), HHV6 Real-TM Quant kit is an in vitro Real Time amplification test for detection of HHV 6 in the biological materials. DNA is extracted from saliva samples, amplified using real time amplification with fluorescent reporter dye probes specific to pol-gene of HHV6 and Internal Control (IC). Test contains an IC (b-globine gene) which act as an amplification control for each person processed specimen and to identify possible reaction inhibition.

Statistical analysis:

Statistical analysis was performed with the statistical package for social sciences (SPSS), version 21. s. Categorical data presented by frequencies and percentages. Pearson's Chi–square test was used. The result was considered statistically significant when($P \leq 0.05$).

Results

Patients' mean age was (32.18±12.23), while mean age of control was (32.36±12.03). Frequency of male 38(76%) female 12(24%) in bell's palsy group, frequency of males was 28(56%) and females 22(44%) in control group. Saliva collected from 28 patients in the facial nerve palsy group (56%) found positive to HHV6, and only 8 tested positive (16%) in the health group ($P = 0.028$), (table 1) and (table 2) respectively. Regarding the frequency of involved site, right side involved in 26(56%) while left site in 24(48%). And frequency of involvement according to each grade, (table 3).

Table (1): The frequency of HHV6 in Bells in patients.

HHV6		Frequency	%	Cumulative Percent
	positive	28	56.0	56.0
	negative	22	44.0	100.0
	Total	50	100.0	

Table (2): The frequency of HHV6 in the control group.

HHV6		Frequency	Percent	Cumulative Percent
	positive	8	16.0	16.0
	negative	42	84.0	100.0
	Total	50	100.0	

Table (3): The frequency Bell's palsy according to grade.

Grade		Frequency	Percent	Cumulative Percent
	2.00	2	4.0	4.0
	3.00	17	34.0	38.0
	4.00	14	28.0	66.0
	5.00	3	6.0	72.0
	6.00	14	28.0	100.0
	Total	50	100.0	

There was important difference in the finding percent for Human herpes virus-6 between males (64%) and females (35%) (P = 0.02). Important positive correlation between age and grading (p =0.001), as increase age correlate with high grading of Bells' palsy, also significant correlation between viral load of HHV6 and grading as increase viral load correlates with high grading (p = 0.001), (table 4).

Table (4): Correlations among patients' demographic and HHV6 viral load

		grade	Viral Load	gender
frequency in patients	Pearson Correlation	-.331*	-.408**	-.309*
	Pvalue	.019	.003	**.029**
	N	50	50	50
Age	Pearson Correlation	.447**	-.098	-.090
	P value	**.001**	.499	.535
	N	50	50	50
Grade	Pearson Correlation	1	.543**	.133
	P value		**.000**	.356
	N	50	50	50

There was increase amount of HHV-6 load in patients compare to control group (p=0.001), (table 5).

Table (5): Mean HHV6 viral load among patients and controls

		Paired Differences				
				95% Confidence Interval of the Difference		
		Mean	Std. Deviation	Lower	Upper	P value
Pair 1	Viral load in patient – Viral load in control	5304.0000 390.33	11801.1926 120.234	1950.1381 77	8657.86183 2044.144	.003

Discussion

In the current study, the frequency of HHV-6 in Bell's palsy is (56%), and (16%) among control group (P = 0.028). Pitkäranta et al., study the tear fluid and result was (35%), as opposed to (5%) in the control group (P = 0.044) [16]. Pitkäranta et al., studied the cerebrospinal fluid (CSF) and the result was (3%) in bell's palsy patients and (0%) in the healthy group [17]. Linder et al., study the tear fluid and the result was 40% of control patients and 30% of Bell's palsy patients [18]. Pereira et al., found HHV-6 prevalence of (9.8%) among control group, while (16%) in our control group [19].

This high prevalence in our study explained by human herpes virus-6 infected the salivary glands and is found mostly in the saliva of healthy individuals, and different age group and the site that sample chosen. It is known that human herpes virus-6 is common in young children more than adults.

The detection of human herpes virus-6 by our study have an important higher viral load of human Herpes Virus -6 between patients of bell's palsy compare to control group, this finding also reported by Pitkäranta et al., and Turriziani et al., [20, 21].

In the current study, the frequency of human Herpes Virus-6 in facial nerve palsy in the saliva are (56%), and (16%) among control group (P = 0.028). Turriziani et al., found a human herpes Virus -6 in the saliva of bell's palsy and healthy volunteer (61% vs 50%)[21], Genizi et al., also study the saliva and report (71%), as opposed to only (37%) in the healthy group (P = 0.001)[22], this high result of Genizi et al., explained that most of patients was below age of 18.

The highest level (viral loads) of human herpes Virus -6 DNA was associated with high grade in House Brackmann grading system as reported in our study compared to Turriziani et al., that found no important change in facial palsy grade [21].Genizi et al., do not correlate between viral load and facial palsy grade[22].

In the current study there was significant incidence among males compared to females (p =0.02) while same finding reported by Genizi et al., [22]. Rowhani-Rahbar found an increase of facial nerve palsy in female gender versus male gender, this higher rate of infection in females may be due to hormonal disturbance during puberty and menarche [23].

This study has several limitations, including difficulty in getting samples at early stage of Bell's palsy and that the difficulty to specify the type of human herpes Virus -6.

References:

1- Peitersen E. Bell's palsy: the spontaneous course of 2,500 peripheral facial nerve palsies of

different etiologies. ActaOtolaryngol Suppl. 2002(549):4-30.
2- Yanagihara, N. (1988).” Incidence of Bell’s palsy.”The Annals of otology, rhinology & laryngology. Supplement 137: 3-4.
3- Murakami, S., M. Mizobuchi, Y. Nakashiro, T. Doi, N. Hato, and N. Yanagihara. 1996. Bell palsy and herpes simplex virus: identification of viral DNA in endoneural fluid and muscle. Ann. Intern. Med. 124:27–30.
4- Murakami, S., M. Mizobuchi, Y. Nakashiro, T. Doi, N. Hato, and N. Yanagihara. 1996. Bell palsy and herpes simplex virus: identification of viral DNA in endoneural fluid and muscle. Ann. Intern. Med. 124:27–30.
5- Pazin, G. J., M. Ho, P. J. Janetta. 1978. Reactivation of herpes simplex virus after decompression of the trigeminal nerve root. J. Infect. Dis. 138:405–409
6- Kimberlin, D. W., and R. J. Whitley. 1998. Human herpesvirus-6: neurologic implications of a newly-described viral pathogen. J. Neurovirol. 4:474–485.
7- Furuta, Y., S. Fukuda, E. Chida, T. Takasu, F. Ohtani, Y. Inuyama, and K. Nagashima. 1998. Reactivation of herpes simplex virus type 1 in patients with Bell palsy. J. Med. Virol. 54:62–66.
8- Kimberlin, D. W., and R. J. Whitley. 1998. Human herpesvirus-6: neurologic implications of a newly-described viral pathogen. J. Neurovirol. 4:474–485.
9- Hall, C. B. (1997). Human herpesviruses at sixes, sevens, and more. Ann Intern Med 127, 481–483.
10- Lusso, P., Markham, P. D., Tschachler, E., di Marzo Veronese, F., Salahuddin, S. Z., Ablashi, D. V., Pahwa, S., Krohn, K. & Gallo, R. C. (1988). In vitro cellular tropism of human B-lymphotropic virus (human herpesvirus-6). J Exp Med 167, 1659–1670.
11- Schirmer, E. C., Wyatt, L. S., Yamanashi, K., Rodriguez, W. J. &Frenkel, N. (1991). Differentiation between two distinct classes of viruses now classified as human herpesvirus 6. ProcNatlAcadSci U S A 88, 5922–5926.
12- Yamanishi K, Okuno T, Shiraki K, et al. Identification of human herpesvirus-6 as a causal agent for exanthemsubitum. Lancet 1988; 1: 1065–7
13- Berneman, Z. N., Ablashi, D. V., Li, G., Eger-Fletcher, M., Reitz, M. S., Jr, Hung, C. L., Brus, I., Komaroff, A. L. & Gallo, R. C. (1992). Human herpesvirus 7 is a T-lymphotropic virus and is related to, but significantly different from, human herpesvirus 6 and human cytomegalovirus. ProcNatlAcadSci U S A 89, 10552–10556
14- Campadelli-Fiume G, Mirandola P, Menotti L. Human herpesvirus 6:an emerging pathogen. Emerg Infect Dis 1999; 5:353–66.
15- Braun DK, Dominguez G, Pellett PE. Human herpesvirus 6. ClinMicrobiol Rev 1997; 10:521–67.
16- Pitka¨ranta A, Piiparinen H, Mannonen L, Vesaluoma M, Vaheri A. 2000. Detection of human herpesvirus 6 and varicella-zoster virus in tear fluid of patients with Bell’s palsy by PCR. J ClinMicrobiol 38:2753–2755.
17- Kanerva M, Ja¨a¨skela¨inen AJ, Suvela M, Piiparinen H, Vaheri A, Pitka¨ranta A. 2008. Human herpesvirus-6 and -7 DNA in cerebrospinal fluid of facial palsy patients.ActaOtolaryngol 128:460–464.
18- Linder T, Bossart W, Bodmer D. 2005. Bell’s palsy and herpes simplex virus: Fact or mystery? OtolNeurotol 26:109–113.
19- Pereira CM, Gasparetto PF, Correˆa ME, Costa FF, de Almeida OP, Barjas-Castro ML. 2004. Human

herpesvirus 6 in oral fluids from healthy individuals. Arch Oral Biol 49:1043–1046.

20- Joséphine M. Reynaud and BrankaHorvat.Human Herpesvirus 6 and Neuroinflammation. Hindawi Publishing Corporation ISRN Virology.2013:5-7.

21- Turriziani O, Falasca F, Maida P, et al. Early collection of saliva specimens from Bell's palsy patients: quantitative analysis of HHV-6, HSV-1, and VZV. J Med Virol.2014; 86:1752–1758.

22- Jacob Genizi, MD, Orit Golan-Shany, PhD,TanyaTarazov, MD, SarelPechter, MD, NuritAssaf, MD, Idan Segal, MD, Isaac Srugo, MD, and Ellen Bamberger, MD. Does Herpes 6 Infection Have a Role in Bell's palsy Among Children and Adolescents? The Pediatric Infectious Disease Journal.2019.38 (5):482.

23- Rowhani-Rahbar A, Baxter R, Rasgon B, et al. Epidemiologic and clinical features of Bell's palsy among children in Northern California. Neuroepidemiology. 2012; 38:252–258