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RESEARCH ARTICLE

Human Herpesvirus-6 in Relapsing Remitting Multiple Sclerosis

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Abstract

Background: Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system. HHV-6 has been implicated in the pathology of relapsing Remitting MS. Objective: Study the relation between HHV-6 viremia and relapsing remitting MS. Methods: A case-control study conducted on MS patients. fifty blood samples were collected from relapsing-remitting MS patients-thirty samples from the patients at the time of remission and twenty samples from the patients at the time of relapse and fifty blood samples from apparently healthy controls. Viral DNA extracted from plasma samples, and then, HHV-6 DNA detected and measured by quantitative real-time PCR. Anti HHV-6 IgG and IgM antibodies measured by ELISA. Results: HHV-6 was detected in 22% (11/50) of MS patients, in 45% (9/20) of relapsing patients and 6.77% (2/30) in remission, while none of the controls was positive for the virus, (P=0.0013) (OR=11.45, P=0.045) (RR=2.9, P= 0.0003). IgG was positive in 48% (24/50) of MS patients and 56% (28/50) of the controls, (P=0.42). While IgM was positive in only 4% (2/50) of MS patients in the relapsing group, (P=0.49).Conclusion: HHV-6 could have a role in the pathology of relapses in MS patients and high viral load might be an indicator of relapse.

Keywords: *Relapsing-remitting MS, HHV-6, Real time PCR, IgG, IgM.*

Introduction

Multiple Sclerosis (MS) is a chronic inflammatory demyelinating disease of the Central Nervous System (CNS), which gives rise to focal lesions in the gray and white matter and to diffuse neurodegeneration in the entire brain, is idiopathic, despite of its description over 150 years ago [1]. Throughout the years, long lists of viruses have been associated with multiple sclerosis (MS); however, to date no virus has been definitely implicated in causing of MS.

Many studies during the past years have used a variety of methods to detect possible correlation between HHVs and MS Some Human herpesviruses (HHVs) have been correlated with the development of MS because they neurotropic latent, and ubiquitous [2].Human herpesvirus 6 (HHV6) is a very probable because it is highly neurotropic, characterized by latency and periodic reactivation and the same factors that cause virus reactivation such as the stress have also been associated with MS exacerbation, and the virus is ubiquitous, the primary infection usually occur during the first two years of life [3]. Studies have reported the presence of viral deoxyribonucleic acid (DNA) in the brains [4,5] and Cerebrospinal Fluid (CSF) [6] of MS patients and controls support that HHV-6 is strongly neurotropic.

Other studies reporting higher levels of viral messenger ribonucleic acid (mRNA) in MS brains compared to control brains especially in the demyelinated plaques [7]. Not only studies of the CNS have established an

association between HHV-6 and MS. Other studies focus on early observations of HHV-6 in the serum associated with the detection of immune response to the virus in MS patients with clinically active disease.

Study conducted on Iranian population found greater levels of HHV-6 immunoglobulin M (IgM) and immunoglobulin G (IgG) in MS patients compared to controls, 78.2% of MS patients are positive for HHV-6 specific IgG antibodies in contrast with 76.4% of healthy. The frequency of HHV-6 specific IgM in normal population was 6.5% compared with 34.6% of MS patients. HHV-6 DNA has been detected in serum of 60.2% of MS patients and only 14.6% of healthy [8].

Additionally, studies on the mechanisms of demyelination and oligodendrocyte injury have reinforced the idea that viruses can lead to MS [9]. One such mechanism is molecular mimicry between a pathogen and a self-molecule leads to the generation of an immune response that is cross-reactive between both the pathogen and self. There is a segment of identical amino acids between HHV-6 U24 protein and human myelin basic protein [10].

Recent American study have focused on the role of HHV-6 U94 protein in disruption of human oligodendrocyte progenitor migration [11].This study aimed to measure HHV-6 viral load in plasma samples of patients with relapsing-remitting MS and compare it with that of healthy persons, and to measure antibodies including IgG and IgM against HHV-6 in plasma samples of MS patients and healthy persons.

Subjects and Methods

A case-control study conducted on MS patients from October 2017 to May 2018. Blood samples collected from the MS patients in the multiple sclerosis clinic Baghdad teaching hospital of medical city. Firstly, written informed consent was obtained from all subjects, then fifty blood samples were collected from relapsing-remitting MS patients-thirty samples from the patients at the time of remission and twenty samples from the patients at the time of relapse- and fifty blood samples from apparently healthy age and sex-matched persons as controls.

This study approved by the ethical committee of the College of Medicine-Al-Nahrain University (No.20171009) at the date of 15/10/2017. The study was conducted in the microbiology department at the College of Medicine /Al-Nahrain University. From all relapsing-remitting MS patients and controls, 3 ml of whole blood samples were collected in EDTA tubes. The whole blood samples were centrifuged at 5000 RPM (revolutions per minute) for 5 minutes to get the plasma.

Plasma samples were separated, divided into two halves, and preserved in deep freeze (-20 ºC), one half was used for viral DNA extraction and the other for ELISA. Viral nucleic acid was extracted using (Gene aid/Taiwan) kit. Sacace (Italy) real time amplification kit was used for quantitative detection of HHV-6 in the plasma samples. 15 µl of master mix was 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 95ºC for 15 min followed by 5 cycles consisting of 5 s at 95 ºC, 20 s at 60 ºC, and 15 s at 72 ºC, and then 40 cycles consisting of 5 s at 95 ºC, 30 s at 60 ºC, and 15 s at 72 ºC. Fluorescence is detected at the 2nd step of Cycling 2 stage (60°C) in JOE/Yellow and FAM/Green fluorescence channels for viral and internal control DNA respectively.

The ELISAs kits (VIDITEST/ Czech Republic) for anti-HHV-6 IgG and IgM antibodies measurement depended on the binding of antibodies in the sample with HHV-6 antigen that coat the wells of ELISA plate. For semi-quantitative evaluation, the cut-off value was calculated by multiplying the two calibrators mean with a correction factor (0.21) for IgG and (0.52) for IgM, and the results were processed according to the following formula to obtain sample positivity index:

$$\text{Sample Positivity Index} = \frac{\text{Sample absorbance}}{\text{Cut-off value}}$$

The results were evaluated according to the following Tables (1) and (2) For IgG and IgM results respectively.

Table 1: Evaluation of HHV-6 IgG Ab	
Index value	**Interpretation**
< 0.90	**Negative**
0.90-1.10	**+/-**
1.11-2.00	**+**
2.01-4.00	**++**
>4.00	**+++**

Table 2: Evaluation of HHV-6 IgM Ab	
Index value	**Interpretation**
< 0.90	**Negative**
0.90-1.10	**+/-**
1.11-2.00	**+**
2.01-3.00	**++**
>3.00	**+++**

Statistical analysis was performed with the statistical package for social sciences (SPSS), version 21. The chi-square or two-tailed Fisher's exact test was used to test differences in categorical variables. Mann-Whitney test (two-tailed) was used to compare the mean of numerical variables. The result was considered statistically significant when P≤0.05.

Results

Among the 50 MS patients; 33 (66%) were females, and 17 (34%) were males. The mean age of MS patients was 33.84 ± 9.57 years, ranging between 17 and 58 years. Statistically, there was no significant difference (P=0.29) between the mean of the RTRs and control group indicating that they were of a comparable age. By q-RT-PCR, HHV-6 was detected in 22% (11/50) of MS patients, but none of control group was positive for the virus. HHV-6 viremia was positive in 45% (9/20) of relapsing patients and 6.67% (2/30) of the patients at the time of remission, which was significantly higher in relapsing patients (P=0.0013), and with significant odds ratio (OR=11.45, P=0.045) and relative risk value (RR=2.9, P= 0.0003).

Table 3: Clinical and demographic characteristics of MS patients in relation with HHV-6 positivity by q-PCR

		Virus positivity No. (%)			P value	OR*	RR*
		Positive	Negative	Total			
Age groups	11-25 26-40 41-55 >55	 2 (20) 4 (14.82) 3 (30) 2 (66.6)	8 (80) 23 (85.18) 7 (70) 1 (33.3)	10 27 10 3	0.2		
Sex	Male Female	3 (17.65) 8 (24.24)	14 (82.35) 25 (75.76)	 17 33	0.6		
Duration of disease	0-5 6-10 >10	4 (16) 4 (26.67) 3 (30)	21 (84) 11 (73.33) 7 (70)	 25 15 10	0.58		
Number of relapses	 0-5 6-10 >10	 9 (28.12) 2 (15.38) 0	 23 (71.88) 11 (84.62) 5 (100)	 32 13 5	0.3		
Treatment	 B-feron 1a B-feron 1b Natalizumab Fingolimod	 1 (25) 7 (25.93) 3 (23.08) 0	 3 (75) 20 (74.07) 10 (76.92) 6 (100)	 4 27 13 6	0.625		
IgG	Positive	8 (33.33)	16 (66.67)	24	**0.06**	**3.83 P=0.07**	
	Negative	3 (11.54)	23 (88.46)	26			
IgM	Positive	2 (100)	0	2	**0.045**	**20.78 P=0.05**	
	Negative	9 (18.75)	39 (81.25)	48			
MS patients groups	 Relapsing Remission	 9 (45) 2 (6.67)	 11 (55) 28 (93.33)	20 30	**0.0013**	**11.45 P=0.045**	**2.9 P=0.0003**

OR (Odds Ratio)

RR (Relative Risk)

IgG was positive in 48% (24/50) of MS patients and 56% (28/50) of the controls. While IgM was positive in only 4% (2/50) of MS patients in the relapsing group, however, these results were statistically not significant (P=0.42) for IgG and (P=0.49) for IgM. The average index of IgG antibody for MS patients were 1.75 ± 1.54 and control group 1.88 ± 1.58, while the IgM were 0.25 ± 0.28 in MS patients and 0.26 ± 0.18 in controls 0.26 ± 0.18. According to Mann-Whitney test, these differences were not significant and there were no significant difference in level of anti- HHV-6 IgG and IgM antibodies between MS patients and the control group.

Table 4: Comparison of HHV-6 IgG index values between MS patients and controls

Study groups	N	Mean	Std. Deviation
Controls	50	1.88	1.58
MS patients	50	1.75	1.54
Mann-Whitney test: P= 0.725			

Table 5: Comparison of HHV-6 IgM index values between MS patients and controls

Study groups	N	Mean	Std. Deviation
Controls	50	0.26	0.18
MS patients	50	0.25	0.28
Mann-Whitney test: P= 0.086			

The difference in the mean of IgG and IgM for the MS patients (relapsing versus remission groups), was not significant for IgG (P= 0.23) in contrast to IgM which was significantly higher in relapsing patients (P= 0.022).

Table 6: Comparison of HHV-6 IgG index values between relapsing and remission groups of multiple sclerosis patients

	Relapsing-MS	Remission-MS
Median	2.05	0.47
Mean	2.007	1.36
Standard deviation	1.53	1.46
Mann-Whitney test: P= 0.23		

Table 7: Comparison of HHV-6 IgM index values between relapsing and remission groups of multiple sclerosis patients

	Relapsing-MS	Remission-MS
Median	0.17	0.13
Mean	0.37	0.16
Standard deviation	0.52	0.07
Mann-Whitney test: P= 0.022 (significant)		

There was significant dependency between positivity of IgG/IgM with positive detection of virus by q-PCR, for the IgG (p=0.06, borderline significant), and for the IgM (P= 0.045) (OR=20.78, P= 0.05).

Discussion

MS is a growing global problem, until this date the cause of disease is undefined [1], but, like other autoimmune disease, it may be triggered by an infectious agent [12, 14]. Since 1993, HHV-6 has been suggested in the pathogenesis of MS [15], after that, many studies proposed the association between HHV-6 and MS 16, 18] however, the nature of this association is not proved. To the best of our knowledge, there are no previous studies on the prevalence of this virus in Iraq, only two recent studies [19, 20].

One of them has been conducted on HHV-6 association with certain hematological malignancies, in which the rate of occurrence of this virus in the plasma using polymerase chain reaction (PCR) technique was 27.3% in patients, comparing with control 0%, and the results of indirect fluorescence assays test (IFAT) was 81.8% in patients, comparing with control 61.0 %, [19].

The other study has been detect, actively increasing viral load in the blood samples of 16.3% of renal transplants, all of them were

symptomatic, and 75% of them with renal allograft rejection [20].

This study support the association between HHV-6 and MS, by the detection of HHV-6 in the plasma samples of 22% (11/50) of RRMS patients, especially in the relapsing patients, while, the virus was not detected in the control samples. This is similar to what was reported by [21], and other studies that reported higher levels of HHV-6 in samples of MS patients than controls [8,22].

In RRMS patients groups; 45% (9/20) of relapsing patients have HHV-6 in their samples, in comparison with 6.67% (2/30) in remission group, which is significantly higher in relapsing patients (P=0.0013) with significant odds ratio (OR=11.45, P=0.045), and relative risk factor (RR=2.9, P=0.0003).

This gives the attention that the virus may be associated with relapse or with progression of the disease, these results are in agreement with that of other studies [8,23, 25]. In this study, the prevalence of IgG was 48% (24/50) in MS patients and 56% (28/50) in control group there is no any official report about the prevalence of HHV-6 infection in Iraq.

The average index of IgG antibody for MS patients was 1.75 ± 1.54 and control group 1.88 ± 1.58, this difference was not significant, and this finding agrees with other studies which showed no relation between the IgG level and MS [8,21]. The low number of relapsing MS patients may explain the insignificant results of IgM study, the low number of relapsing MS patients was the main obstacle faced in this study because of the shortage of supply and expensive methyl prednisolone that is used during relapses. In conclusion, HHV-6 may have an important role in the relapses of MS patients and could be an indicator of relapse or an aggravating factor, while HHV-6 ELISA study is inconclusive and unhelpful for detecting HHV-6 whether in acute, or reactivation of previous infections.

However, if HHV-6 is involved in relapses or not the nature of this involvement remains obscure. Does the virus play an important role in initiating or potentiating the inflammation that is associated with the relapse, or is it a marker of the disease activity or it is activated from latency, as a result of the surrounding inflammation.

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List of abbreviations: CNS= central nervous system, CSF= cerebrospinal fluid, DNA= deoxyribonucleic acid, ELISA= enzyme linked immunosorbent assay, HHVs= human herpesviruses,HHV-6= human herpesvirus 6, IFAT= indirect fluorescence assays test, mRNA= messenger ribonucleic acid, MS= multiple sclerosis q-PCR = quantitative polymerase chain reaction,