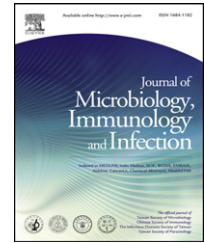




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ORIGINAL ARTICLE

Human herpesvirus-6 viral load and antibody titer in serum samples of patients with multiple sclerosis

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KEYWORDS

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HHV-6;
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Background: Despite the number of cases with definite diagnosis of multiple sclerosis (MS) being on increase, the role of human herpesvirus-6 (HHV-6) infection as a trigger for MS disease still is deliberated. Based on antibody detection and quantitative HHV-6 polymerase chain reaction assay, this study was achieved to find out the possible association between infection with HHV-6 and clinical progression of MS disease.

Methods: A total of 108 serum samples were obtained from 30 MS patients followed prospectively for a 6-month period. These samples were analyzed for the presence of HHV-6 DNA by nested polymerase chain reaction enzyme-linked immunosorbent assay and for anti-HHV-6 IgG titer. Activation of the disease was determined by either magnetic resonance imaging or by clinical status of the patients. Control groups were also included.

Results: The average antibody index for the MS patients in the first sample collection was higher than both control groups ($p = 0.001$). HHV-6 DNA was detected in the serum samples of 10 of 30 MS patients. The mean HHV-6 viral load in patients with relapsing-remitting multiple sclerosis (RRMS) with and without relapse was 973 and 714, respectively. Seven patients showed an exacerbation during the study period. Of those, four patients had HHV-6 DNA in their collected samples. The prevalence of HHV-6 DNA was significantly higher in patients with MS as compared with control groups ($p = 0.001$).

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Conclusions: The results indicate that HHV-6 is implicated somehow in MS disease. Over time, rising HHV-6 IgG antibody titers together with an exacerbation and detection of HHV-6 DNA in serum samples of some MS patients suggests possible association between the reactivation of the virus and disease progression.

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Introduction

Multiple sclerosis (MS) is the most common demyelinating disease of the central nervous system (CNS).^{1–3} Several types of studies over the past decade support the hypothesis that human herpesvirus-6 (HHV-6), which is associated with a common childhood illness, roseola, is a strong candidate to play a role in the course of MS, either as a causal agent or as a cofactor. In recent years, HHV-6 has been investigated as a possible causative agent for MS. These studies have examined anti-HHV-6-specific antibody responses, HHV-6 viral DNA, or HHV-6 presence in the CNS tissue in both MS patients and controls.^{4–11} However, there are still discrepancies between the results obtained from studies concerning how the presence of HHV-6 is linked with clinical activity in MS patients. Active HHV-6 viral infection in some MS patients may fluctuate over time during the progression of the disease,¹² causing variation in viral detection.

The prevalence of MS, according to World Health Organization reports, should be about 4 in 100,000. However, a recent study has shown that Iran could be considered as an area with a medium to high risk of MS.¹³

Despite the number of cases with definite diagnosis of MS being on the increase in this region, the role of HHV-6 active infection in this group of patients has not yet been studied. Therefore, based on IgG antibody titer and quantitative HHV-6 polymerase chain reaction (PCR) assay, this study was performed to determine the possible association between infection with HHV-6 and clinical parameters of MS disease progression. The results were then compared with HHV-6 infection in normal subjects and in a group of patients with other neurological diseases (OND).

Methods

Patients and samples

Thirty MS patients (21 females and 9 males) with clinically definite MS were randomly selected from those attending the Neurology Clinic at the Namazi Hospital, Shiraz University of Medical Sciences, Shiraz, Iran during February 2008 and January 2009. The mean duration of the MS disease was 4 years (2–20 years). The mean age of the patients with MS was 33 years (18–45 years). Twenty-two patients had relapsing-remitting MS (RRMS), seven had secondary progressive MS (SPMS), and one patient had primary progressive MS (PPMS). These patients suffered from various symptoms and clinical manifestations. Activation of the disease (exacerbation) was determined by either gadolinium-enhancing magnetic

resonance imaging (MRI) or clinical status according to the criteria of Poser et al.¹⁴

The control patients included 20 individuals (11 females and 9 males) with OND including motor neuron disease, dizziness, cerebrovascular disease, migraine, meningitis, and febrile seizure, and 20 randomly selected healthy blood donors (10 females and 10 males). The mean age of the control groups were 32 years (18–48 years) and 28 years (18–35 years), respectively. All the participants gave informed consent before the examination.

A total of 108 serum samples were collected from 30 MS patients. Two to four serum samples were collected from each patient during the 6 months follow-up of the study. Surveillance serum samples were collected at first admission and then every 4–6 weeks for 6 months. MRI was performed for the entire patients on the day of admission and for 18 subjects at the end of the study. During the study period, 15 MS patients were under treatment of Interferon beta-1a (Avonex, Biogen Idec Brazil, Brazil). Serum samples were also collected from the patients with OND and for the normal subjects as well. All the serum samples were frozen at –70°C to be used for DNA detection by PCR and enzyme-linked immunosorbent assay (ELISA) for detection of specific antibodies against HHV-6.

Extraction and amplification of viral DNA

DNA extraction from serum samples was performed by the methods detailed in Kramvis et al.,¹⁵ with some modifications. Serum (100 µL) was extracted with a proteinase K-sodium dodecyl sulfate (SDS) extraction mix (267 µg of proteinase K per mL, 1% sodium dodecyl sulfate, 2.5 mM EDTA, 25 mM sodium acetate) by incubation at 70°C for 2 hours. DNA was then purified by phenol-chloroform extraction and ethanol precipitation. The resulting DNA pellet was resuspended in 20 µL of sterile water and incubated at 95°C for 10 minutes to reduce or inactivate PCR inhibitory factors. DNA extracts were then allowed to cool and were amplified immediately.

DNA–enzyme-linked immunosorbent assay (DELISA) was designed for quantifying HHV-6 DNA molecules in patient serum samples. The nested primer set complementary to the gene coding the major capsid protein (MCP) was used with the aim of recognizing both HHV-6A and HHV-6B variants. The nucleotide sequence of the primers 5'-GCTA GAACGTATTTGCTG-3' and 5'-ACAAGTCTGACTGGCA-3' (outer pairs); 5'-TCACGCACATCGGTATAT-3' and 5'-CTCAA GATCAACAAGTTG-3' (inner pairs) were used for the first and second round of the PCR assay, respectively. The inner primers amplify a 124-bp fragment of HHV-6. Each PCR contained 5 µL of extracted DNA in a final volume of 50 µL

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