

Prevalence of Herpes Simplex Virus Infection in Patients With Relapsing-Remitting Multiple Sclerosis: A Case-Control Study in the North of Iran

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Abstract

Background: Multiple sclerosis (MS) is a debilitating autoimmune and inflammatory disease of the central nervous system associated with both infectious and non-infectious underlying factors. Many recent studies suggest that infection with herpesviruses has a contributing role in the pathogenesis of MS.

Objectives: The current case-control study aimed to evaluate the prevalence of herpes simplex virus (HSV) in peripheral blood mononuclear cells (PBMCs) of patients with MS compared to those of the healthy controls.

Patients and Methods: PBMC samples of 82 relapsing-remitting patients with MS (23 males, 59 females; mean age 36.9 ± 9.30 years) and 89 subjects in the healthy control group (34 males, 55 females; mean age 34.32 ± 10.56 years), from the North of Iran (2013-2014) were enrolled in a case-control study. The enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) were applied to investigate the frequency of HSV in the participants.

Results: Totally, 63 (76.8%) patients with MS showed a history of HSV exposure by anti-HSV testing compared to 70 (78.7%) subjects in the healthy group (P value = 0.855). The HSV-DNA test was positive in 37 (45.1%) and 3 (3.4%) patients with MS and healthy subjects, respectively (P value < 0.001). Family history of MS was positive in 15 (18.3%) subjects, of whom 3 (8.1%) and 12 (26.7%) were HSV-DNA positive and HSV-DNA negative, respectively (P value = 0.026).

Conclusions: Herpes simplex virus was present in more patients with MS than healthy cases. HSV may be directly or indirectly associated with MS development. Further comprehensive molecular studies are needed to confirm the etiopathologic association between HSV and MS disease.

Keywords: Multiple Sclerosis, Herpes Simplex Virus, Autoimmune Diseases

1. Background

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS), causing progressive neurological deterioration, affecting 2.5 million people worldwide. MS usually begins in early adulthood and is characterized by demyelination of CNS (1, 2). The inflammatory phenotype of MS is defined as a relapsing-remitting period of neurological dysfunction, although the clinical pictures vary between individuals (1). There is a rapid influx of research conducted on different viruses in the etiopathology of MS, although their definite roles are poorly understood. According to the clinical presentation and the histopathological properties of MS lesions, a viral infection could be responsible for the MS development (3). Nowadays, high prevalence of herpesviruses such as Epstein-Barr and varicella-zoster, as well as herpes simplex

virus (HSV) are found in acute MS patients, but absent in the healthy control groups (4-6).

HSV represents a human pathogenic herpes virus belonging to the subfamily of α -herpesvirinae. Herpes simplex type 1 (HSV-1) is probably more constantly present in humans than any other viruses. HSV-1 primary infection occurs early in life and is usually asymptomatic. The highest incidence of HSV-1 infection occurs among children six months to three years of age. By adulthood, 70%-90% of people would have herpes simplex type 1 antibodies. Herpes simplex type 2 (HSV-2) is predominantly spread through genital contact. Recent surveys determined that 17% of adults in the United States harbor HSV-2 antibodies (7). Reactivation and asymptomatic shedding occur both for HSV-1 and HSV-2 post infection (8, 9). Herpesviruses are neurotrophic and have strong ability to remain latent in the nervous system, innervating the site of primary infec-

tion for the life span of the host (8).

The pathogenic role of HSV in MS disease is still incompletely understood. HSV can induce multifocal demyelination in mice determined by preservation of axons and an inflammatory infiltrate (10). HSV is isolated from the cerebrospinal fluid (CSF) during the first MS attack (11) and in patients with MS (12). Previous investigations showed that genome of HSV presents in the CSF of 4.7% to 46% of the patients with MS (13, 14). However, the etiopathogenesis role of HSV in multiple sclerosis still remains ambiguous. Several studies described detection of HSV DNA in different body fluids and other reports gave evidence that HSV DNA is detected in plasma and in peripheral blood mononuclear cells (PBMCs) (15-20). To verify the possible role of human herpes simplex viruses as triggering or exacerbating factors in relapsing-remitting multiple sclerosis (RRMS) clinical acute attack, the prevalence of some herpesviruses in the PBMCs collected from patients with MS during an MS relapse and in a stable phase, indicated that HSV plays an important role in triggering MS relapses (6). The current study reported the prevalence of HSV in patients with MS for the first time in Iranian populations.

2. Objectives

HSV infection is a controversial risk factor in the clinical history of patients with MS. The current study aimed to determine the prevalence of HSV infection in patients with RRMS compared to a healthy control group.

3. Patients and Methods

3.1. Patients

Eighty-two patients with RRMS attending Rasht Poursina hospital (a referral state hospital in Northern Iran) and 89 healthy control subjects were included in a case-control study from 2013 to 2014. All enrolled participants were from North of Iran (Rasht, Ramsar, Tonkabon and Chalus cities). They had no history of diabetes, allergy and autoimmune diseases. Patients were diagnosed according to magnetic resonance imaging (MRI) and McDonald criteria (21). The criteria for the staging of disease were based on expanded disability status scale (EDSS). All patients had received treatment except for 10 who were considered as being 'drug naive'. The study protocol was reviewed and approved by the local ethics committee and confirmed by the ethical guidelines of Islamic Azad University and Rasht Poursina hospital, Tonekabon, Iran. Other ethical issues (including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.)

were completely followed by the authors. All participants signed written informed consent after explaining the study procedures.

3.2. Sample Preparation and DNA Extraction

Five milliliters of peripheral blood was treated with 0.02 mL of EDTA. Serum was separated after centrifugation for 5 minutes at 2000 rpm. Mononuclear cells were separated by gradient centrifugation using lymphocyte separation medium (Ficoll) according to the manufacturer's instructions (Sigma, Germany) and stored in liquid nitrogen until future use.

Commercial kit (Qiagen, Germany) was used to extract DNA from PBMCs. Concentration of the extracted DNA was determined by measuring absorbance at 260 nm by Bio-photometer (Eppendorf, Germany).

3.3. HSV Specific Antibody Detection

Sera from patients with MS and 89 healthy control subjects were examined for specific IgG antibody to HSV using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions (EUROIMMUN, Germany).

3.4. Polymerase Chain Reaction

Test for HSV (general, type 1 and 2) DNA in peripheral blood mononuclear cells was conducted by polymerase chain reaction (PCR) in a blinded fashion for all samples. Specific primers (forward, 5'-CAGTACGGCCCCGAGTTCGTGA-3' and reverse- 5'-GTA-GATGGTGCGGGTGATGTT-3') (TAG Copenhagen, Denmark) were used to amplify the early gene (476 bp product), which is responsible for encoding of DNA polymerase. Thirty amplification cycles were applied in a thermocycler (Biorad, Germany) as follows: initial denaturation of 95°C for 10 minutes followed by 94°C (denaturation), 63°C (annealing), and 72°C (extension) during 60, 30 and 50 seconds, respectively with a final extension of one cycle of 10 minutes at 72°C.

Beta-globin PCR with specific primers was used as indicator of the quality of the extracted DNA from the samples. For β -globin gene, 35 cycles (initial denaturation of 95°C for 5 minutes) were applied under different conditions as follows: denaturation at 95°C for 45 seconds, annealing at 54°C for 30 seconds and extension at 72°C for 30 seconds. The amplification product was followed by a final extension step at 72°C for 10 minutes. Amplified PCR products were visualized in 1.5% agarose gels stained with ethidium bromide. To monitor any contamination, a negative control including distilled water was included in all experiments.

3.5. Statistical Analysis

Statistical analyses were performed using statistical program for social sciences (SPSS-20, SPSS Inc., Chicago, Illinois, USA). Quantitative parameters were evaluated in different groups by the independent T-test. Categorical variables were expressed as percentages, and differences between groups were judged for significance using the Chi-squared test. P values less than 0.05 were considered significant.

4. Results

4.1. Overview of the Patients

Table 1 shows the details of general characteristic for all participants. Overall, 82 patients (23 males, 59 females; mean age 36.9 ± 9.30 years) with MS and 89 subjects in the healthy group (34 males, 55 females; mean age 34.32 ± 10.56 years) were recruited. Of the total 82 patients with MS, 15 (18.3%) had a family history of MS disease. No significant association was found between demographic features (gender and age) in RRMS and healthy groups (P value > 0.05).

4.2. Serological Assay

The total number of anti-HSV positive subjects was 133 (77%). In patients with RRMS and healthy group, 63 (76.8%) and 70 (78.7%) subjects presented positive anti-HSV, respectively (P value > 0.05) (Table 1). However, no significant association was found between the presence of anti-HSV and MS disease (P value = 0.855). No significant associations were also found comparing patients with MS based on gender, drug receiving status, age group and the type of drug treatment (P value for all > 0.05) (Table 2).

4.3. PCR Finding

The characteristics of HSV-DNA positive cases are shown in Table 3. HSV-DNA was found positive in 40 subjects including 37 (45.1%) patients with MS and 3 (3.4%) subjects in the control group. Statistical methodology showed strong associations between HSV-DNA status of patients with MS and healthy groups (P value < 0.001) (Table 1).

A family history of MS was positive in 15 (18.3%) subjects of which 3 (8.1%) and 12 (26.7%) were in HSV-DNA positive and HSV-DNA negative, respectively (P value = 0.026). No significant differences were found comparing patients with MS based on gender, treatment status and the type of drug treatment and age groups (P value for all > 0.05).

5. Discussion

The epidemiology of multiple sclerosis together with its immune disorders and the presence of viral foot-print infection that result in demyelination, support a viral etiopathology for MS. Despite all data, no such established role is clearly and definitely confirmed for viruses causing MS. Observational and experimental evidence support the hypothesis that viral infections might lead to brain dysfunction, including demyelination of CNS (22). Despite the fact that no single virus is consistently related with MS and considering MS as an autoimmune response against oligodendrocytes, it would be possible to consider at least a temporary viral infection role for such inflammatory processes (23).

Herpes simplex viruses are ubiquitous human pathogens displayed by two different serotypes: HSV-1 and HSV-2. In the general population, adult seropositivity rate is approximately 90% and 20%-25% for HSV-1 and HSV-2, respectively. HSV is a common neurotropic virus that is capable of inducing protract latency. It can account for focal demyelination in animals (24). Previous study indicated that the human HSV play and crucial role in triggering or aggravating RRMS medical severe attack (6). A total of 82 patients with RRMS and 89 healthy subjects were included in this study; 45.1% of patients with relapsing-remitting MS had HSV-DNA, which were remarkably higher than the rate for healthy subjects in the control group (3.4%). Detection of HSV-specific DNA may indicate infection with herpes simplex virus. Previous studies indicate that HSV-1 reactivate in the peripheral blood of patients with MS during clinical acute attack and probably play a role in the triggering of MS relapses (6).

In the current study, seropositivity to anti-HSV IgG was different between the patient with MS and subjects in the control groups (76.8% and 78.7%, respectively), but this difference was not significant and did not indicate association between seropositivity to anti-HSV IgG and MS disease. In the Italian cooperative MS case-control investigation, there was a significantly higher frequency of HSV-2 antibodies in the MS group compared with the controls, but levels of HSV-1, Epstein-Barr virus and human T-lymphocytic virus-III were not different (25). Also, based on the largest analysis of HSV-2 antibodies in patients with MS, a significant increase in HSV-2 seropositivity for patients with MS is suggested (26).

Consistent with the current study, Sanders et al. reported the presence of HSV DNA in patients with MS more than the subjects in the control group which was found in more active plaques than inactive plaques of brain tissue biopsies (14). Franciotta et al. examined the presence of HSV DNA in serum and CSF of patients with MS. The results

Table 1. Demographic, Serologic and HSV DNA Characteristics of Study Participants

Variable	RRMS (n = 82)	Healthy (n = 89)	P Value
Gender, n (%)			0.194
Male	23 (28)	34 (38.2)	
Female	59 (72)	55 (61.8)	
Age (Year), mean ± SD	36.9 ± 9.30	34.32 ± 10.56	0.096
Disease onset age, mean ± SD	27.46 ± 7.9	-	-
Disease definite recognition age, mean ± SD	30.04 ± 9.53	-	-
Family history, n (%)		-	-
Yes	15 (18.3)		
No	65 (79.3)		
Unavailable	2 (2.4)		
HSV antibody, n (%)			0.855
Positive	63 (76.8)	70 (78.7)	
Negative	19 (23.2)	19 (21.3)	
HSV DNA, n (%)			< 0.001
Positive	37 (45.1)	3 (3.4)	
Negative	45 (54.9)	86 (97.6)	

Abbreviations: HSV, herpes simplex virus; RRMS, relapsing-remitting multiple sclerosis.

Table 2. Frequency of HSV Antibody in Patients With RRMS

Variable	HSV positive (n = 63)	HSV Negative (n = 19)	P Value
Gender			0.773
Male	17 (27)	6 (31.6)	
Female	46 (73)	13 (68.4)	
Age (Year), mean ± SD	38.08 ± 9.37	33.10 ± 8.21	0.060
Family history, n (%)			0.850
Yes	11 (17.5)	4 (21.1)	
No	50 (79.4)	15 (78.9)	
Unavailable	2 (3.2)	0 (0.0)	

Abbreviations: HSV, herpes simplex virus; RRMS, relapsing-remitting multiple sclerosis.

did not support the role of herpesviruses in the etiopathogenesis of MS disease (27). Also, Koros et al. indicated that the prevalence of HSV genome was positive in less than 5% of patients with MS (13). Feng et al. showed that there was no statistically significant difference in the positive rates of IgM antibody against HSV-1 between the two RRMS and control groups (28). However, Sanders et al. reported that 46 % of the MS cases were positive for HSV-DNA (14).

Several characteristics of human herpesvirus make them popular candidates as the triggers for MS: they cause latent infections and are capable of reactivation, which

could play a role in the relapsing-remitting MS development. Contribution to MS pathogenesis could appear via an immune attack to the virus (29). Ruprecht et al. showed that human endogenous retrovirus family W (HERV-W) Gag and Env proteins were advocated by HSV-1 in neuronal and brain endothelial cells, however in vitro conditions. The activation of HERV-W proteins by HSV-1 could induce their potential oligodendrotoxic and immunopathogenesis effects, indicating a mechanism by which HSV-1 and possibly other herpesviruses related to MS, may be associated with pathogenesis of the disease (30).

Table 3. Frequency of HSV DNA in Patients With MS

Variable	HSV DNA positive (n = 37)	HSV DNA negative (n = 45)	P value
Gender			0.466
Male	12 (32.4)	11 (24.4)	
Female	25 (67.6)	34 (75.6)	
Age (Year), mean \pm SD	36.38 \pm 7.94	37.31 \pm 10.36	0.660
Family history, n (%)			0.026
Yes	3 (8.1)	12 (26.7)	
No	32 (86.5)	33 (73.3)	
Unavailable	2 (5.4)	0 (0.0)	

Abbreviations: HSV, herpes simplex virus; MS, multiple sclerosis.

In conclusion, the results of the current study support the hypothesis for an association between HSV and MS disease. Herpes simplex virus was present in more relapsing-remitting MS cases than healthy control groups. Despite the large sample size of the study, the findings should be accepted with caution. Further comprehensive molecular studies including the recent high throughput platforms of next generation sequencing are needed to compare patients with MS with controls who are both positive for HSV DNA.

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Footnotes

Authors' Contribution: All authors contributed in the process of sampling, and serological and molecular analysis as well as results interpretation equally.

Conflicts of Interest: Authors have no conflicts of interest.

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