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## Original Article

# Seroprevalence of Herpes Simplex Virus Types 1 & 2 (HSV 1,2) in Subjects with Multiple Sclerosis: A Case Control Study

**Vojdani A[1,2#], Jalali A[1#], Boostani R[3], Saeidi M[3], Amali A[4], Mardani MR[1,2], Taherpoor A[1,2], Gholoobi A[5,6], Hooshyar Chechaklou A[7], Meshkat M[8], Abolbashari S[7*], Meshkat Z[7*]**

1. Student Research Committee, Mashhad University of Medical Sciences, Mashhad, Iran.
2. Department of Microbiology and Virology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
3. Department of Neurology, Ghaem Hospital, Mashhad University of Medical Sciences, Mashhad, Iran.
4. Student Research Committee, Paramedical Department, Mashhad Medical Sciences Branch, Islamic Azad University, Mashhad, Iran.
5. Metabolic Syndrome Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
6. Medical Genetics Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
7. Antimicrobial Resistance Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
8. Mashhad Medical Sciences Branch, Islamic Azad University, Mashhad, Iran.

## Abstract

**Background and Aims:** Multiple sclerosis (MS) is an inflammatory disease of the central nervous system (CNS) in genetically susceptible individuals with various causes. Infections such as viral infections are suggested as potential underlying factors in the development of MS. Many studies have indicated the possible role of Herpes simplex virus (HSV) in the pathogenesis of MS. The current study aimed to evaluate anti-HSV type 1 and type 2 IgG Immunoglobulin of patients with MS.

**Materials and Methods:** In this assessment, serological samples of 38 patients with MS who were referred to the Neurology Department of Ghaem Hospital of Mashhad were compared with the control group; 41 patients from other Departments of Ghaem Hospital who did not have any signs of MS disease. Samples were evaluated for presence of antibody to HSV type 1 and type 2 by the Enzyme-linked immunosorbent assay (ELISA).

**Results:** 29 patients with MS (76.3%) were positive, and 9 patients (23.7%) were negative for anti-HSV IgG antibody. In the control group, 36 samples (87.8%) were positive and 5 (12.2%) were negative for anti-HSV IgG antibody. Therefore, we found no correlation between HSV infection and MS. Although job distribution and MS did show an association in our study (P=0.034).

**Conclusion:** Despite all the studies confirming the connection between HSV and MS disease, in this assessment, no relation between infection with Herpes Simplex Virus and Multiple sclerosis was observed.

**Keywords:** Herpes Simplex Virus, Multiple Sclerosis, IgG antibody

## Introduction

Multiple sclerosis (MS) is a demyelinating inflammatory disease that is characterized by multifocal and temporally scattered damage of the central nervous system (CNS) (1).

Neurological dysfunction is observed in the early course of the disease, which the patient usually recovers from. Over time, extensive and chronic neurodegeneration causes progressive accumulation of disability (2).

MS prevalence has increased worldwide since 2013 (3). The prevalence of MS in Iran was estimated to be around 29.3/ 100,000 (4), which is considered a high number. MS can be presented in different types. In the relapsing–remitting (RRMS) type, multiple episodes of neurological dysfunction occur which are followed by complete or incomplete recovery.

[*] **Corresponding authors:**
Zahra Meshkat,
Email: meshkatz@mums.ac.ir;
Samaneh Abolbashari,
Email: abolbasharis1@mums.ac.ir.
#The first two authors equally contributed to this work.



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The primary progressive MS (PPMS) presents with a gradually progressive disease course from onset. The clinically isolated syndrome (CIS) is a single episode of dysfunction with no previous clinical attacks in a person who does not fulfill the diagnostic criteria for MS (5).

Despite the fact that the pathogenesis of MS is unclear, a number of external factors (e.g., geographical, vitamin D level, infections) serve as potential triggering factors of the auto-immune process that could cause MS, specifically in those who are genetically predisposed (6). There is growing evidence that viruses either play a causal role or may accompany other environmental stimuli (7).

Moreover, it has also been claimed that the long-term use of specific drugs, such as interferon, could possibly increase the function of the virus in the development of MS (8).

The high prevalence of herpes simplex virus deoxyribonucleic acid (HSV-DNA) found in the peripheral blood mononuclear cells of MS patients compared with healthy subjects (8), and the ability of HSVs for reactivation after a long latency period pose them as an appropriate candidate for the onset and recurrence of MS. HSV-1 is mostly located within the trigeminal ganglion and HSV-2 within the sacral ganglion. Additionally, Herpes viruses are neurotropic and might have the tendency to hide inside our nervous system. In patients with MS, specifically for the duration of primary MS attack, HSV has been isolated from the cerebrospinal fluid (CSF). The genome of HSV has been found inside the CSF of 4.7% to 46% of patients with MS, as previous investigations have shown (5,13).

Moreover, in HSV-1 latently-infected CNS, increased levels of the matrix metalloproteinases 2 and 9 (MMP-2 and MMP-9) have been detected. This could have contributions to the degradation of the surrounding cell surface proteins and extracellular matrix, leading to a partial breakdown of the blood-brain barrier (BBB), which has a pivotal role in MS(9, 10). It is possible that low-level expression of viral genes during HSV-1 latency of the CNS could cause this inflammatory response (11) which in turn could promote an inflammatory environment that modulates the onset of neurological disorders.

In a study that investigated the prevalence of IgG and DNA for HSV in clinical samples, prior HSV-1 infection was found to have a possible relation with the onset of pediatric MS, yet it does not manifest any correlation with adult MS. Furthermore, it was observed that prior HSV-2 infection has a correlation with MS(12). In another study, for the purpose of assessing the potential role of Human Herpes Simplex viruses as the causal or aggravating factor in acute clinical attacks in relapsing-remitting MS, the prevalence of specific Herpes viruses in mononuclear cells of peripheral blood in both the recurrent and controlled phases of MS was evaluated, and the results demonstrated that HSV plays a vital role in MS recurrences (8).

HSV infection is typically acquired in young adults by HSV-1 and HSV-2. HSV-1 is transmitted via casual contact in early life, however, HSV-2 is a virus that is mostly transmitted sexually. As of 2016, an estimated 3583.5 million of the global population within 0–49 years of age were infected orally with HSV type 1, and a total of 491.5 million individuals with 15–49 years of age worldwide were living with HSV type 2 infection (13).

In a study conducted in Iran, the prevalence of HSV-1 and HSV-2 and total HSV amongst Iranians were 42.04% (20.9-63.1), 6.5% (4.7-8.2) and 25.7% (8.8-42.5) respectively (14). In a similar study in the US that evaluated individuals with the age of 14-49, HSV-1 prevalence was found to be 48.1%, and HSV-2 prevalence was 11.9% (15).

The current study aimed to evaluate anti-HSV type 1 and type 2 IgG Immunoglobulin of patients with MS using the Enzyme-linked immunosorbent assay (ELISA), which could further elucidate the possible correlation between HSV and MS.

## Methods

This case-control study was conducted from 28/06/2018 to 25/10/2018 at the Microbiology and Virology Research Center of Ghaem hospital, Mashhad. Serum samples of 38



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patients with multiple sclerosis referred to the neurology clinic of Ghaem hospital were used. Results were compared with the control group consisting of 41 serum samples from patients admitted to other wards of Ghaem hospital who did not show any symptoms of multiple sclerosis. All MS subjects had previously been evaluated by a neurologist for the diagnosis of multiple sclerosis. Other variables and patient information including sex, age, job and MS characteristics were completed by the relevant checklist and using the patients' documents. Serum samples were evaluated for anti-herpes simplex virus type 1 & 2 antibodies (IgG) by the Euroimmun Commercial Kit (Medizinische Labordiagnostika AG, Germany; E180315CT) according to the manufacturer instructions. The study protocol was approved by the ethic committee of Mashhad University of Medical Sciences (approval code: IR.MUMS.fm.REC. 1397.253).

**Statistical Analysis:** Student-T-test was used for the analysis of our data. Chi-square test was performed to analyze the data with nominal scale. Additionally, Fisher's Exact Test was used in cases which more than 20% of the expected frequencies of the tables were less than 5 (Cochran). SPSS v.20 was used for statistical analysis, and the significance level of the tests was considered less than 5%.

## Results

**Age Distribution:** In the case group, the youngest person was 16 and the eldest was 52 years old and the mean age was 33.8±8.9 years. The youngest person in the control group was 27 years of age and the eldest was 41 years old and the mean age was 32.1±3.0 years. Therefore, no significant difference was observed between the two groups regarding their age (*P*=0.256).

**Sex Distribution:** In the case group, 9 individuals were male (23.7%) and 29 were female (76.3%), and in the control group, 14 individuals were male (34.1%) and 27 were female (65.9%). Considering all subjects, 23 were male (29.1%), and 56 were female (70.9%). There was no statistically significant difference between the two groups regarding their sex (*P*=0.305).

**Job Distribution:** In the case group, the number of students was 2 (5.3%), the number of employed individuals was 13 (34.2%), and the number of housewives was 23 (60.5%). In the control group, 2 people were students (4.9%), 25 were employed (61%), and 14 were housewives (34.1%). Considering all subjects, 4 were students (5.1%), 38 were employed (48.2%), and 37 were housewives (46.8%). The difference in the case and control groups regarding the individuals' job was not statistically significant (*P*=0.053).

**Distribution of the Type of MS:** During this study, the number of individuals diagnosed with relapsing-remitting multiple sclerosis (RRMS) was 30 (78.9%), and the number of individuals diagnosed with progressive multiple sclerosis (PMS) was 8 (21.1%) (Fig. 1).

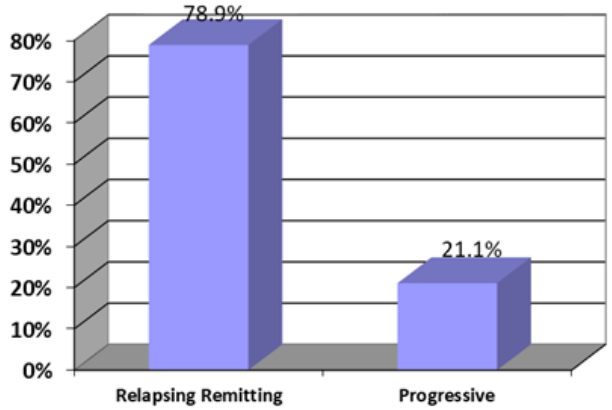



**Fig. 1.** The distribution of the type of MS.

**Distribution of the First Symptom of the Disease:** The most common symptom of MS among the subjects of this study was walking difficulty that was observed in 13 individuals (34.2%), and the least common was urinary disorder that was not observed in any of our subjects.

Other symptoms in order of their occurrence were: Simultaneous walking difficulty and visual impairment observed in 10 people (26.3%), visual impairment observed in 5 people (13.2%), paresis in the upper limbs observed in 4 people (10.5%), simultaneous walking and urinary disorder observed in 2 people (5.3%),



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simultaneous walking difficulty and paresis observed in 2 people (5.3%), and simultaneous visual impairment and paresis observed in 2 people (5.3%) (Fig. 2).

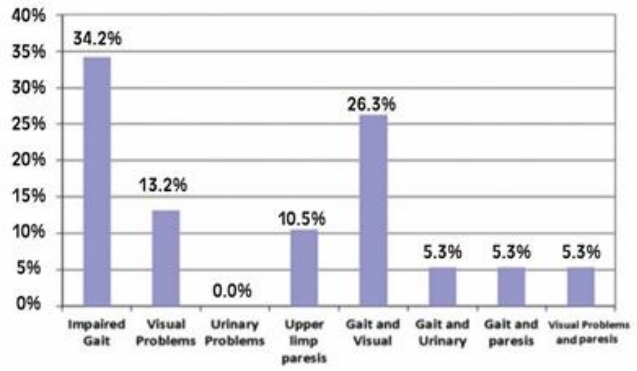



**Fig. 2.** Distribution of the first symptom of the disease

**HSV distribution:** In the control group, 36 individuals (87.8%) had anti-HSV IgG antibodies, and 5 individuals (12.2%) did not.
In the case group, 29 individuals (76.3%) had anti-HSV IgG antibodies, and 9 individuals (23.7%) did not have it (Fig. 3). Therefore, no significant differences regarding HSV-antibodies was observed between the two groups ($P$=0.242).

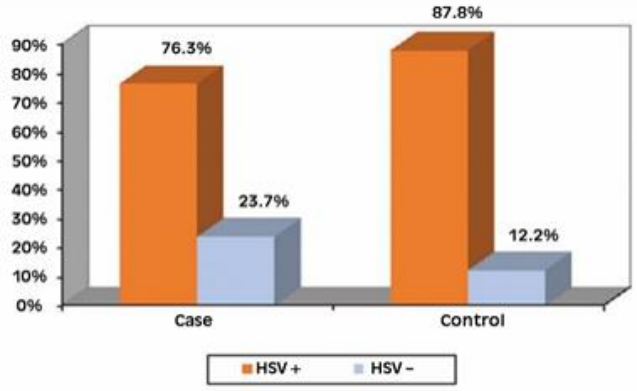



**Fig. 3.** HSV IgG distribution.

## Discussion

The unknown pathogenesis of MS, along with the epidemiologic data that have been found in previous researches, suggest a potential correlation between MS and viral infections. Therefore, we aimed to evaluate the correlation between MS and HSV. Our findings suggest that there is no significant difference between the control group and the case group when age, sex, and duration of illness were compared.
Furthermore, regarding the presence of anti-HSV antibodies, 87.8% of the control group subjects and 76.3% of the case group subjects had the aforementioned antibodies. Therefore, no correlation was found between HSV and MS. In a study conducted by Sanders et al., 37 MS cases were screened for the presence of HSV in the white and gray matter, and they were unable to determine HSV as an etiologic factor for MS (16). In another study, Martin et al. evaluated the CSF and serum of MS patients for the presence of a number of viruses, including HSV-1 and HSV-2, using the PCR method. Their results demonstrated no sign of virus DNA in the samples (17). As a result, they argued against the continuous disseminated herpes virus infection in MS. Also, in studies carried out by Franciotta et al., Koros et al., and Feng et al., no correlation was found between HSV and MS (18, 19).
Contrary to our findings, Najafi et al. evaluated HSV prevalence in peripheral blood mononuclear cells (PBMCs) of patients with MS and found that HSV-DNA was present in more patients with MS than healthy cases (20).
They only assessed patients with relapsing-remitting MS using the PCR and ELISA method. Moreover, in a study conducted by Nourbakhsh et al., susceptibility to pediatric MS due to herpes virus infection was assessed (21). This specific research revealed that EBV seropositivity is associated with pediatric MS, as is HSV-1 seropositivity in subjects negative for HLA-DRB1*15:01.
The number of our subjects was limited, and we suggest further investigations done in larger populations. Novel approaches are suggested to be further evaluated; such as the approach in the study conducted by Hirose et al. in which HSV-IL-2-induced CNS demyelination in a mouse model of multiple sclerosis was investigated and it was found that ILC2s contribute to MS pathogenesis (22).
We also suggest that the relationship between HSV infection and MS be evaluated in



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different MS types which will only be possible in studies with larger samples. The prevalence of HSV-1 and HSV-2 was not measured individually in this study.

## Conclusion

In this study, no significant relationship was found between HSV 1,2 infection and multiple sclerosis. Studies with larger samples are necessary to confirm this result.

## Acknowledgment

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## Conflict of interest

No conflict of interest is declared.

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