Abstract. Aim: The pathogenic role of Herpes Simplex Virus (HSV) 1 and 2 in Multiple Sclerosis (MS) still remains obscure. The aim of our study was the assessment of HSV1 and 2 DNA prevalence in the cerebrospinal fluid (CSF) of MS patients compared to patients with other neurological disorders (OND). Materials and Methods: HSV1 and HSV2 DNA detection in the CSF of patients was performed by real time polymerase chain reaction (PCR). Results: The genome of HSV1 was present in the CSF of 4.7% of MS patients (4 out of 85), while HSV2 was not detected in any patient. In the sub-group of OND patients, HSV1 was detected in 7.9% of patients (3 out of 38) and HSV2 was detected in 5.3% of patients (2 out of 38). Conclusion: Our data are in accordance with a limited number of previous reports, supporting a prevalence of HSV1 genome in less than 5% of MS patients.

Multiple sclerosis (MS) is a de-myelinating disorder of the central nervous system triggered by both genetic and environmental factors. Degeneration of axons with a progressive course may also occur in certain forms of the disease. However, the pathogenesis of the disorder still remains obscure. Various external stimuli (geographical, vitamin D level, infections) have been characterized as possible underlying causes of the autoimmune process in genetically predisposed individuals (1). The implication of viral infections in the etiology of MS is supported by previous reports (2-4). Many candidate viruses have been examined as putative mediators of central nervous system (CNS) autoimmunity, focusing mainly on long-acting viruses, like herpesviruses. Their relatively high incidence in the general population and their potential to remain silent and reactivate long after the initial infection, renders herpesviruses attractive candidates for a key role in MS initiation and relapses. It has been suggested that viruses might translocate in the CNS through migration of infected immune cells rather than by means of direct crossing of the blood brain barrier (BBB) (5, 6).

Epstein-Barr virus (EBV), varicella- zoster virus (VZV), cytomegalovirus (CMV), human herpesvirus (HHV)-6 and HHV-7 and retroviruses have all been studied as putative MS mediators. A number of former reports favored an impact of herpesviruses on MS etiology (7-11), while others failed to confirm such an interaction (5, 12, 13). Certain herpesviruses (including EBV, CMV, herpes simplex virus (HSV) and VZV) have also been proposed as triggering factors for acute disseminated encephalomyelitis (ADEM) progressing to MS (14, 15). It has been suggested that VZV DNA is encountered more frequently in the cerebrospinal fluid (CSF) of MS patients than in patients with other neurological disorders (6). Notably, a study of Brecht and co-authors (16) provided evidence that the presence of intrathecal antiviral immune response (CSF antibodies against herpesviruses and other viruses like measles and rubella) may serve as a CSF marker in oligoclonal band–negative MS patients. In a recent study of Sotelo and co-authors, VZV DNA has been shown
to be present in the CSF of all MS patients during clinical relapses compared to only 31% of patients in remission. This finding could not be replicated for a number of other herpesviruses (HSV 1 and 2, EBV and HHV-6) (17).

Nevertheless, in spite of many clinical reports focusing on EBV, there is a certain paucity in the literature concerning a possible triggering role of Herpes simplex viruses type 1 and 2 in MS. The aim of the present study was to assess the prevalence of HSV 1 and 2 DNA in the CSF of a cohort of definite MS patients hospitalized in our Clinic during a 2-year period, as compared to measurements in the CSF of patients suffering from other inflammatory and non-inflammatory neurological disorders. We also attempted to evaluate the CSF virus profile of these patients in respect to their clinical and laboratory background.

Patients and Methods

Aegionitosis Hospital MS Clinic is one of the three major reference centers for demyelinating disorders in the region of Attica, central Greece. This area had a population of roughly 4,000,000 inhabitants in December 2011 but a number of patients from nearby islands or continental regions also refer to our Clinic. Patients are followed-up on at least a 6-month basis. On the other hand, Thriassion Hospital represents a local tertiary Hospital in Attica covering a population of roughly 500,000 inhabitants.

We retrospectively reviewed the records of 85 MS patients examined at the Unit of Demyelinating Disorders of the Neurology Department, University of Athens and at the Neurological Clinic of Thriassion Hospital, during the period 2010-2012. The detection of HSV1 and HSV2 DNA in the CSF of patients was performed by real time PCR.

DNA was extracted from 200 μl of patient CSF using the QIAamp DNA Blood Mini kit with CE-IVD approval (Qiagen; Hilden, Germany) according to the manufacturer’s instructions. For ensuring DNA extraction quality and checking for PCR inhibitors presence, a Taqman PCR protocol was used for each DNA preparation, targeting the human RNaseP gene, which is appropriate for CSF clinical specimens. A Taqman PCR protocol targeting a heterologous control was used before DNA extraction (SPUD assay) (18).

The amplification target for both viruses is the HSV glycoprotein B gene and successful amplification would result in a 122 bp product as described previously (19). The assay has been evaluated and monitored for its sensitivity on an annually basis using Quality Control for Molecular Diagnostics external quality assessment panels. Positive control was used in Levey Jennings diagram using a certain detection level (Ct: 32). The assay sensitivity using probit analysis (95% confidence interval (CI)) was for HSV1 105 copies/ml and for HSV2 122 copies/ml. Specificity was determined using a panel of viruses and sequence analysis of the PCR products. The assay sensitivity using probit analysis (95% confidence interval (CI)) was for HSV1 105 copies/ml and for HSV2 122 copies/ml. Specificity was determined using a panel of viruses and sequence analysis of the PCR products. The assay sensitivity using probit analysis (95% confidence interval (CI)) was for HSV1 105 copies/ml and for HSV2 122 copies/ml. Specificity was determined using a panel of viruses and sequence analysis of the PCR products.

Results

Laboratory testing revealed the presence of the genome of HSV1 in the CSF of 4.7% of MS patients (4 out of 85 patients) while HSV2 was not detected in any patient. Focusing on demographic and clinical/laboratory characteristics of HSV1-positive patients, they were female (4 out of 4) with a mean age of 33.3 years. The mean age of HSV1-negative MS patients was 39.4 (32 males/49 females). Brain MRI lesions were present in all four HSV1-positive patients, cervical spinal cord MRI lesions in 75% of patients (3 out of 4) and thoracic spinal cord MRI lesions in 25% of patients (1 out of 4). Positive oligoclonal bands (OBC’s) in the CSF were also detected in every HSV1-positive MS patient. Two of the patients presented with clinical isolated syndrome (CIS), another one with the relapsing remitting form of the disease (RRMS) under natalizumab therapy and the fourth one with secondary progressive MS (SPMS) was hospitalized in order to initiate an escalation therapy (Table I).

Interestingly, in the sub-group of OND patients, HSV1 was detected in 7.9% of patients (3 out of 38) and HSV2 was detected in 5.3% of patients (2 out of 38). No double-CSF-positive patients were detected. HSV-positive cases in this sub-group involved patients with chronic inflammatory demyelinating polynuropathy (CIDP), a patient with cerebrovascular dementia, as well as a patient with probable amyotrophic lateral sclerosis (ALS) (Table II). The prevalence of HSV1 did not differ statistically between MS patients and patients with OND. On the other hand HSV2 was present exclusively in the CSF of a limited number of patients with OND (a patient with acute inflammatory de-
myelinating polyneuropathy (AIDP) and a patient with possible autoimmune encephalitis) and was not detected in the CSF of any MS patient.

**Discussion**

The role of EBV in the pathogenesis of MS is well-established. A probable disease pathway involves the migration of EBV-infected B cells in the CNS where they form ectopic meningeal follicles (3, 21). Alternatively, herpesviruses have been shown to induce human endogenous retroviral proteins resulting in increased immune responses and possibly triggering MS (22, 23). It has been suggested that the extremely low risk of MS among EBV-negative individuals indicates the contribution of EBV infection in the pathogenesis of most MS cases (22).

Nevertheless, the results of former studies, addressing the impact of herpes simplex viruses 1 and 2 in the pathogenesis of MS, appear to be controversial. Laboratory data have shown that both HSV-1 and HSV-2 can cause multifocal CNS de-myelination when administered systematically or intracerebrally, respectively, in rodents (24, 25). This observation may provide an *in vivo* model for a possible involvement of these viruses in clinical MS. Antibodies against HSV and a number of other herpesviruses have been detected in sera of the majority of MS patients in a recent study (9). HSV-2 seropositivity has been found to be higher in a female MS patient population compared to healthy controls (26). Moreover, higher HSV-2 antibody titers in peripheral blood were associated with higher vitamin D levels in pediatric MS patients compared to their control counterparts (27). On the other hand, HSV-1 DNA was traced in peripheral blood mononuclear cells of 13% of acute MS attack patients, whereas it was absent in controls, thus implicating a putative role of this virus in MS relapses (28). Interestingly, in a pediatric MS study, HSV-1 seroprevalence was linked with an increased risk of developing MS in individuals lacking the *HLA-DRB1*15 allele, whereas it was rather protective in carriers of the allele (29). As it is well-established, for years now, the co-existence of EBV DNA and *HLA-DRB1*15 allele, increases decidedly the possibility of MS initiation and progress and the comorbidity with other autoimmune diseases, as we have also shown recently (15). An anti-herpetic medication, acyclovir, has been tried in an experimental protocol of relapsing-remitting multiple sclerosis (30). In any case, the presence of HSV DNA in the CSF of MS patients appears to be low, accounting for less than 5% of MS cases studied (22).

Similarly with the aforementioned data, we were able to detect the HSV1 genome in the CSF of 4.8% of MS patients, a prevalence consistent with previous reports (22). In contrast, the HSV2 genome was not present in the CSF of MS patients in our study. This observation could either be a coincidental finding or reflect a selective contribution of HSV1 in triggering MS onset and relapses. A higher prevalence of HSV1 in younger aged individuals in the

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### Table I. Patients with multiple sclerosis (MS) who have been tested positive for HSV1 or 2 in the CSF by real-time PCR.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Neurological disorder</th>
<th>Age</th>
<th>Gender</th>
<th>OCB</th>
<th>Brain MRI</th>
<th>Cervical MRI</th>
<th>Thoracic MRI</th>
<th>CSF positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>MS (CIS)</td>
<td>40</td>
<td>F</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>HSV1(+)</td>
</tr>
<tr>
<td>Patient 2</td>
<td>MS (CIS)</td>
<td>28</td>
<td>F</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>HSV1(+)</td>
</tr>
<tr>
<td>Patient 3</td>
<td>SPMS</td>
<td>32</td>
<td>F</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>HSV1(+)</td>
</tr>
<tr>
<td>Patient 4</td>
<td>RRMS</td>
<td>33</td>
<td>F</td>
<td>Positive</td>
<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>HSV1(+)</td>
</tr>
</tbody>
</table>

CIS, Clinical isolated syndrome; SPMS, secondary progressive MS; RRMS, relapsing remitting form of MS; OCB, oligoclonal bands; MRI, magnetic resonance imaging; CSF, cerebrospinal fluid.

### Table II. Patients with other neurological disorders (OND) who have been tested positive for HSV1 or 2 in the CSF by real-time PCR.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Neurological disorder (OND)</th>
<th>Age</th>
<th>Gender</th>
<th>CSF positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>Oculomotor nerve palsy</td>
<td>65</td>
<td>F</td>
<td>HSV1(+)</td>
</tr>
<tr>
<td>Patient 2</td>
<td>CIDP</td>
<td>38</td>
<td>F</td>
<td>HSV1(+)</td>
</tr>
<tr>
<td>Patient 3</td>
<td>Probable ALS</td>
<td>53</td>
<td>M</td>
<td>HSV1(+)</td>
</tr>
<tr>
<td>Patient 4</td>
<td>GBS</td>
<td>41</td>
<td>M</td>
<td>HSV2(+)</td>
</tr>
<tr>
<td>Patient 5</td>
<td>Epilepsy</td>
<td>17</td>
<td>F</td>
<td>HSV2(+)</td>
</tr>
</tbody>
</table>

OND, Other neurological disorders; HSV, Herpes Simplex Virus; CIDP, Chronic Inflammatory Demyelinating Polyneuropathy; ALS, Amyotrophic Lateral Sclerosis; GBS, Guillain Barré Syndrome; CSF, cerebrospinal fluid.
general population might account for the involvement of this virus in MS pathogenesis in young patients. At this point we should highlight the study of Franciotta and co-authors (13) who screened the CSF of MS patients with OND and healthy controls for a number of herpesviruses (including HSV1 and 2) and were able to detect only EBV-positive samples.

We also detected HSV1 and 2 DNA in the CSF of approximately 6% of patients with other neurological disorders. According to literature, HSV has been shown to participate as an antecedent infection in the pathogenesis of Guillain Barré syndrome or Bickerstaff’s brainstem encephalitis, although infrequently comparing to Campylobacter jejuni or EBV (31-33). There is also limited evidence that a former herpesvirus infection might predispose to neurodegenerative disorders like ALS (34). In any case, the prevalence of HSV1 in our study does not differ statistically between MS patients and patients with OND. On the other hand, HSV2 was detected exclusively in the CSF of a very small sub-group of OND patients.

**Conclusion**

The novelty of our report lies on the fact that we assessed the presence of HSV1 and 2 genome in the CSF of patients with MS and OND, by means of real-time PCR. In contrast, most previous studies, with the exception of those of Alvarez-Lafuente and co-authors and Franciotta and co-authors (13, 22), have focused on the detection of either DNA or antibodies of HSV and other herpesviruses in peripheral blood samples of MS patients that do not necessarily reflect CNS involvement (8, 9, 26). Hoping that our initial results will pave the way for further research on the role of HSV in the complex pathogenesis of MS, we propose that HSV1 and 2 viruses should be included in the basic viral laboratorial profile of MS patients.

**Conflicts of Interests**

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**References**


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