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The frequency of varicella-zoster virus infection in patients with multiple sclerosis receiving fingolimod

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ABSTRACT

Multiple Sclerosis (MS) is thought to be an autoimmune disease of the central nervous system (CNS), in which the immune system becomes activated, cross the blood-brain barrier (BBB), and cause neuroinflammation and neurodegeneration. Fingolimod is considered a disease-modifying therapy (DMT), possessing immunomodulatory effects on the immune system, especially autoreactive T cells being licensed in lymph nodes. Although the fidelity of the drug is undeniable in the management of disease course, various adverse effects have been reported in some patients taking this medication. In this study, 420 MS patients, consisted of 210 patients receiving interferon-beta (IFN-beta) and 210 patients receiving fingolimod therapies. As a control group, 210 age- and sex-matched healthy individuals were recruited in our study. The levels of anti-VZV IgG and IgM were determined using enzyme-linked immunosorbent assay (ELISA). The presence of VZV DNA in peripheral blood mononuclear cells (PBMCs) was also investigated using the PCR method. The percentage of seropositivity for anti-VZV IgG and anti-VZV IgM in MS patients was 94.8% and 0%, respectively in those taking fingolimod therapy. In patients receiving IFN-beta, the rate of seropositivity for anti-VZV IgG and anti-VZV IgM was 93.8% and 0%, respectively. In healthy individuals, the rate of seropositivity for anti-VZV IgG and anti-VZV IgM was 84.3% and 0%, respectively. The PCR results showed that 7.6% of patients receiving fingolimod were positive for VZV DNA, while none of the healthy subjects nor MS patients taking IFN-beta were positive for DNA of VZV. The statistical analysis indicated that the frequency of VZV DNA in patients receiving fingolimod was significantly ($p = .00$) higher than MS patients taking IFN-beta and healthy subjects. It seems that the use of fingolimod should be carefully prescribed as the occurrence of VZV infection/reactivation is increased in comparison to other MS patients who receive different therapy.

1. Introduction

Multiple sclerosis (MS) is allegedly recognized as an autoimmune disease of the central nervous system (CNS) in which immune cells become activated against the myelin components, cross the blood-brain barrier, and cause neuroinflammation (Lassmann, 2018). The etiology of MS remains opaque; however, the epidemiological studies indicated that interaction of environmental factors including smoking, viral infection, and vitamin D deficiency with the genetic background plays an undeniable role in the pathogenesis of MS disease (Handel et al., 2010). To date, dozens of therapeutic options have enriched the armamentarium of available drugs for the treatment of MS such as Interferon-beta (IFN-beta), fingolimod, natalizumab, and dimethyl fumarate, to name a few (Filippini et al., 2016). Among the available medications for management of MS, fingolimod (FTY720) has attracted special attention since it could be orally administered and possesses promising potentials for the alleviation of disease severity in individuals with MS (Chun and Hartung, 2010). Fingolimod has been approved as first-line therapy for the treatment of MS that has immunomodulatory effects on the immune system, especially on T cells (Matloubian et al., 2004). It has been shown that fingolimod is phosphorylated enzymatically to create fingolimod-phosphate, mimicking naturally occurring sphingosine 1-phosphate (S1P), an extracellular lipid mediator whose biological role is mediated via cognate G protein-

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coupled receptors. The phosphorylated form of fingolimod leads to the internalization of S1P receptors, which sequesters lymphocytes in lymph nodes by preventing the egress of reactivated T cells from lymph node and thymus (Pinschewer et al., 2011).

Along with the beneficial effects of fingolimod on the prevention of relapse rate in the disease course, several adverse effects have been so far reported in whom receiving this drug. Studies have indicated that fingolimod can cause a headache, fatigue, bradycardia, and hemorrhaging focal encephalitis. Correspondingly, it was shown that the administration of fingolimod is capable of making some patients prone to develop some viral infections such as a varicella-zoster virus (VZV) and JC virus (Cohen and Chun, 2011). To do so, we hypothesized that fingolimod therapy could be associated with the titers of antibodies secreted against VZV. For this aim, we analyzed the seroprevalence of anti- VZV IgG and IgM antibodies together with the presence of VZV DNA in peripheral mononuclear cells (PBMCs) of patients receiving fingolimod compared with those taking IFN-beta.

2. Material and method

2.1. Patient and sample collection

A case-control study was designed to detect the titers of anti-VZV (IgM, IgG) and the presence of VZV DNA in PBMCs of MS patients and healthy subjects. The type of MS disease in MS patients was relapsing-remitting multiple sclerosis (RRMS) as both IFN-beta and fingolimod are only prescribed for this type of the disease. RRMS patients were assigned to two groups; the first group (210 patients including 140 women and 70 men) consisted of patients receiving fingolimod at least 7 months prior to enter the study, and the second group constitutes patients (210 patients including 140 women and 70 men) taking IFN-beta for at least 7 months before enrolling in our study. As a control group, 210 age- and sex-matched healthy individuals (140 female and 70 males) were recruited. The demographic characteristics of all participants were listed in Table 1. Before the commencement of sample gathering, informed consent was obtained from all individuals who participated in this study. The plasma sample collection from healthy individuals was based on regular checkups. Patients did not receive any immunosuppressive agents such as corticosteroids at least nine months before the collection of samples. This study was also approved by the ethics committee of the Iran University of Medical Sciences (ECIUMS; ethical code# IR.IUMS.REC 1395. 9378). All patients had the diagnosis of definite MS in accordance with revised McDonald's criteria (Polman et al., 2011). The study was conducted in two major MS centers; Isfahan Multiple Sclerosis Society (IMSS) and Firouzgar Hospital which are affiliated with the Isfahan University of Medical School (located in Isfahan) and Iran University of Medical Sciences (located in Tehran), respectively.

2.2. Serum and PBMC isolation

For each patient, 10 mL of peripheral blood was drawn into sterile EDTA-containing vacutainer tubes. After separation of plasma from whole blood by centrifugation, it was stored at −70 °C usage. The PBMCs of the samples were isolated by a standard procedure of Ficoll-Hypaque (FH) gradient centrifugation (Lymphoprep, Oslo, Norway), following an established protocol (Keyvani et al., 2013). The PBMC pellet was washed more than three times with phosphate-buffered saline (PBS) (pH = 7.3 ± 0.2). The cells were counted and stored at −80 °C until use (Fig. 1).

2.3. DNA extraction from PBMC

DNA from the PBMCs was extracted using the commercial kit according to the manufacturer's instructions (Qiagen, Germany). The purity of the extracted DNA was confirmed based on its absorbance at

Table 1
Demographic and clinical features of patients along with healthy individuals.

Participants	Sex (female/male)	EDSS (mean ± SD)	Age (mean ± SD)	Disease duration (year ± SD)	Lymphocyte number	Duration of therapy based on months (maximum-minimum)
RRMS patients (fingolimod)	(140/70)	1.45 ± 0.52	33 ± 6.5	5.7 ± 2.4	1.5×10^9	7.45 ± 1.2 (6–8)
RRMS patients (IFN-beta)	(140/70)	1.54 ± 0.47	30 ± 5.2	4.5 ± 1.4	2.3×10^9	9.3 ± 3.1 (10−11)
Healthy individuals	(140/70)	NA	29 ± 4.25	NA	2.5×10^9	NA

RRMS; relapsing-remitting MS, IFN-beta; interferon beta, NA; not applicable.

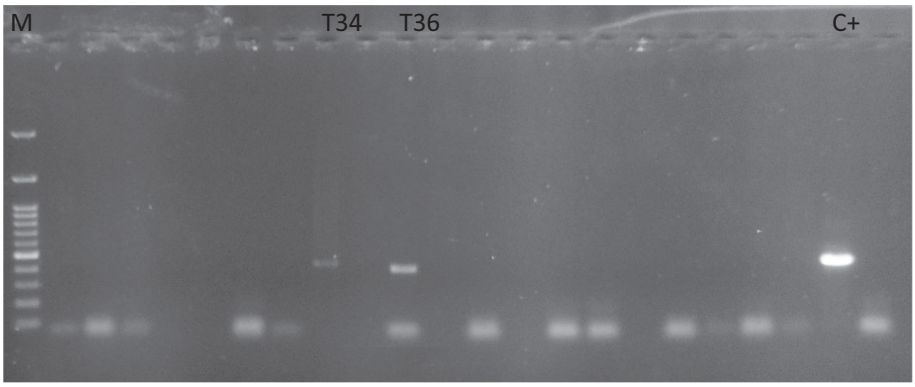

Fig. 1. The PCR product of ORF22 gene in PBMC of MS patients and healthy individuals.
M; marker, T; positive samples for VZV, C+; positive control.

Table 2
Frequency of VZV antibodies in MS patients.

Variable	Number	VZV-IgG positive	VZV-IgM positive	*P* value
RRMS patients (fingolimod)	210	199 (94.8)	0	0
RRMS patients (IFN-beta)	210	197 (93.8)	0	
Healthy Individuals	210	177 (84.3)	0	

260 and 280 nm wavelengths BioPhotometer D30 (Eppendorf, Germany). The extracted DNA was eluted in 50 μL of elution buffer and stored at −80 °C until assayed.

2.4. ELISA for VZV

Antibodies against the varicella-zoster virus in plasma were quantified using Anti-VZV- ELISA kits (Euroimmun, Luebeck, Germany). The procedure was performed in accordance with the manufacturer's instructions.

2.5. Real-Time PCR

The primers used for VZV in this study were previously described by Zerboni et al. (Zerboni et al., 2005). They were 5′-TCTTGTCGAGGAG GCTTCTG-3′ and 5′ TGTGTGTCCACCGGATGAT-3′and specific for the ORF 62 region. Real-time PCR was carried out on the QIAGEN's Real-Time PCR cycler (Rotor-Gene Q 2plex Platform, QIAGEN Co, Germany) instrument. We used the SYBR-Green PCR master mix (Maxima® SYBR Green qPCR Master Mix (2×), Applied Fermentas, EU). The reaction mixture (25 μL), containing 5.0 μL of extracted nucleic acid, 12.5 μ SYBR-Green PCR master mix, 1.0 μM of primers for VZV, and 5.5 mM DDW. PCR was done under the following conditions: an initial cycle at 95C for 10 min; followed by 45 cycles of denaturation at 95°C for 15 s, 60 °C for 60 s, and 72 °C for 30 s and acquiring was in the extension step. Melting curve program (55 °C–95 °C with a heating rate of 2 °C per second and a continuous fluorescence measurement) was used.

2.6. Statistical analysis

The analysis of the data was done using SPSS software (version 24). Patient characteristics were descriptively analyzed. Categorical variables were expressed as percentages, and differences between groups were judged for significance using the chi-squared test or Fisher's exact test. The *p*-values of < 0.05 were considered statistically significant.

3. Results

3.1. Demographic and clinical characteristics

As shown in Table 1, all participants including healthy individuals, patients receiving fingolimod and patients receiving IFN-beta were similar in terms of sex and age as there were no significant differences among them ($P > .05$). Additionally, there was no significant difference between the means of Expanded Disability Status Scale (EDSS) in patients receiving fingolimod and those on the IFN-beta ($P = .61$). Also, there was no considerable difference between the duration of disease in both groups of patients ($P = .66$).

3.2. Detection of VZV-specific IgG and IgM antibody

We examined the seropositivity status of MS patients and controls for VZV-IgG and VZV-IgM (Table 2). When comparing seropositivity of VZV-IgG between MS patients and controls we found MS patients were significantly more likely to be positive as compared to controls (Table 2). On the other hand, there was no statically difference between VZV-IgM among all participant (Table 2).

3.3. PCR results

Real-Time PCR was used to identify VZV DNA in PBMCs of MS patients. As indicated in Table 3, among MS patients and healthy controls, only 16 (7.6%) MS patients taking fingolimod were positive for VZV DNA. The statistical analysis showed that the presence of VZV DNA was significantly different among the three groups ($P = 000$). No significant difference was found between the MS patients and the controls in terms of gender, age, and VZV DNA positivity (P for all >

Table 3
Frequency of VZV DNA in MS patients.

Participants (630)	Number of individuals	VZV DNA positive	P value
RRMS patients (fingolimod)	210	16 (7.6%)	0
RRMS patients (IFN-beta)	210	0	
Healthy individuals	210	0	

0.05).

4. Discussion

Previous studies have indicated that reactivation of latent varicella-zoster virus (VZV) might be associated with relapse (Sotelo and Corona, 2011; Sotelo et al., 2007; Sotelo et al., 2014) and a possible association with progressive disease in patients with multiple sclerosis (MS) (Ordonez et al., 2010), others failed to confirm these findings. Nevertheless, studies have indicated a higher prevalence of VZV seropositivity in patients with MS when compared to the general population (Ross, 1998; Zerboni et al., 2005).

In this study, we observed a higher VZV DNA in MS patients taking fingolimod when compared with MS patients taking IFN-β and healthy individual, respectively. In addition, we showed MS patients were significantly more likely to be positive for specific antibodies against VZV. Our findings are in agreement with a study performed by Arvin et al. that assessed the incidence of VZV-associated infections in relapsing-remitting MS patients (a total of approximately 7500 individuals) treated with fingolimod (0.5 mg/day or 1.25 mg/ day) within Phase II and Phase III clinical trials of the drug, and within uncontrolled extension phases of these trials (Arvin et al., 2015). Although Arvin et al. indicated that the most probable cause of VZV reactivation is associated with the use of fingolimod, it should be taken into account that other immunosuppressive agents are, at least to some extent, capable of reactivating VZV.

Based on reports of adverse events, HZ was documented more often with fingolimod than with other DMTs, such as interferon beta, but the proportion of complicated HZ cases was not higher (Arvin et al., 2015). Symptomatic primary infection or reactivation of latent virus (herpes zoster) may occur more often during selective immunosuppression treatment for immune-mediated diseases such as multiple sclerosis (MS) (Arvin et al., 2015). It is noteworthy that, in contrast to similar research carried out in this context, VZV DNA was only detectable in CSF of MS patients, especially at the relapse phase of the disease. However, we did find VZV DNA even at the remission stage when analyzed in PBMCs of patients with MS. Our results are in line with a study performed by Najafi and colleagues in which they demonstrated 25.6% of VZV positivity in PBMCs of MS patients while all of them were at the remission stage (Najafi et al., 2016).

It is essential to understand whether the increased frequency of VZV infections in patients taking fingolimod compared with controls is biologically plausible and, if so, what might account for it (Tyler, 2015). The CD8+ effector memory T cells are typically considered to play the predominant role in the control of VZV reactivation from latency (Mehling et al., 2008). In the case of VZV, peripheral blood mononuclear cells from patients with MS treated with fingolimod show reductions in both the absolute and relative numbers of CD4+ and CD8+ T cells proliferating in response to ex vivo stimulation with VZV antigen as well as in the total number of interferon γ–producing VZV-specific T cells (Ricklin et al., 2013). Using viral detection in saliva by polymerase chain reaction as a surrogate marker of VZV reactivation, the same authors found salivary reactivation in 4 of 35 fingolimod-treated patients with MS (11%) as compared with none of 28 untreated patients and none of 53 healthy controls (Ricklin et al., 2013).

5. Conclusion

In this study, the anti-VZV IgG and IgM are determined in plasma samples of 420 patients with MS which were divided into two subgroups as the first group received interferon-beta (210 MS patients) and the second fingolimod (210 MS patients), and the presence of VZV DNA was also determined by PCR. The results showed that the seropositivity of VZV-IgG in MS patients was significantly positive as compared to healthy controls. Correspondingly, 16 (7/6%) patients undergone fingolimod were positive for VZV DNA among the MS patients and control groups. Statistical analysis showed that the frequency of VZV DNA in the MS patients taking fingolimod was significantly higher than MS were on IFN-β and healthy controls. In Conclusion, this study highlights the occurrence of VZV infection/reactivation should be revisited in MS patients undergone fingolimod therapy.

Conflicts of interest

The authors report no conflicts of interest.

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