Detection of Varicella Zoster Virus in Multiple Sclerosis Patients and its Comparison with a Control Group by Polymerase Chain Reaction

Saeideh Najafi*, Masood Ghane
Department of Microbiology, Tonekabon branch, Islamic Azad University, Tonekabon, Iran
*Email: Saeedeh.najafi@yahoo.com

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Abstract
Multiple sclerosis is one of the chronic diseases of the central nervous system. This disease appears more among young adults and women. Multiple sclerosis (MS) is regarded as a multifactorial disease. One principal and significant probability is that viruses may stimulate the immune system and lead to the occurrence of MS in the persons who are susceptible to the disease. The purpose of the execution of this study is to detect varicella zoster virus (VZV) in the patients afflicted with multiple sclerosis and its comparison with a control group. Blood samples from 83 MS patients and 100 healthy individuals were collected from Northern areas of Iran. DNA extraction was executed from blood by the kit and according to the instruction of the company. In order to make sure of the accuracy of the extracted DNA, the amplification of human beta-globin gene was used. Specific primers produced by TAG Copenhagen (Denmark) were used to amplify the viruses’ gene. At the end, chi-square(x²) statistical test was used to study the frequency of these viruses in the samples of MS patients and the control group and their relationship with each other. The findings of this study showed that 21 individuals (25%) of patients with MS and 9 individuals (9%) of the control group were positive to VZV virus. Statistical analysis represents a significant relationship between frequency of VZV and MS. The role of viral infections including VZV in the affliction with MS has been confirmed for about a decade. A number of studies have been succeeded in identification of genome of this virus in MS patients, but still more researches are required to perceive the relationship between viral infections and MS disease.

Keywords: multiple sclerosis, varicella zoster virus, PCR

Introduction
Multiple sclerosis (MS) is a chronic, progressive degenerative disease of the central nervous system in the brain and spinal cord characterized by the occurrence of small Demyelination. Demyelination means the destruction of myelin. Myelin is a substance made up of fat and protein surrounding the nerve fibers in the brain and spinal cord. Demyelination impairs the transmission of nerve impulse (Sholtis, 2000). MS is One of the most common neurological diseases in human beings and the most disabling disease in adults (Halland and Halper, 2005), and it has different symptoms such as vision loss, numbness and paralysis, spastic ataxia, tremor, disturbance of sphincter control, impotence, crippling, speech impairment, and depression (Nedjast et al., 2006). MS usually begins during adolescence between the ages of 20 to 35 years and is almost twice as common in women as in men (Soltanazadeh, 2004). Nearly 400,000 people in America and 2/2 million people in the world develop MS (Parsa and Sadat Hosseini, 2011). It has been estimated that the prevalence of MS in Europe was 83 per 100 thousand in the past three decades and the annual incidence of the disease on the continent was 3/4 per 100 thousand (Pugliatti et al., 2006). In Iran, nearly 40,000 people are infected (Parsa and Sadat Hosseini, 2011). MS is a pathologically
autoimmune disease (Parsa and Sadat Hosseini., 2011). A series of factors involving MS has been identified and any of these factors may play a role in causing the disease. These factors include genetics, family history, environmental factors, Latitude, socioeconomic status-Migration and infections. Chlamydia pneumoniae, Rickettsia and Borrelia are bacterial infections, and viral infections are EBV, VZV, CMV, HSV, HHV-6, KSHV, CDV, and HERV. (Contini et al., 2008; Fainardi et al., 2008; Djelilovic and Alajbegovic, 2012; Sanadgol et al., 2011). One of the viruses involving MS is human herpes viruses. Herpes virus family includes some of the most important human pathogenic viruses. The remarkable feature of herpes virus is the ability of causing stable infection in the host and reactivation period, and this feature has made them considerable (Jawets, 2001).

VZV, as a member of Alpha-herpes virinae subfamily, causes two different diseases. This virus has the ability of causing the hidden infection by quick growth and cytolytic effect in neurons. It causes chickenpox upon primary infection, which is a very contagious disease and is common among children (Grant et al., 1993). Reactivation of latent virus in ganglion cells causes shingles, which is more common among adults. In addition to these diseases, VZV can cause respiratory, neurological, and skin defects in people with immunodeficiency and even those with healthy immune system (John and Gnann, 2002). Amplification of a small fragment of VZV genome by PCR is the most common technique to detect this virus in clinical samples (Druce et al., 2002).

This study intends to investigate the prevalence of VZV virus in blood samples from MS patients. In case of a significant prevalence of the virus in these patients compared with the control group, the results of this study may help to stabilize assumptions related to infectious diseases and MS, so this study was designed and implemented.

Materials and Methods

Sample collection

In this study, 183 blood samples were collected from different regions in the northern areas of Iran, and then were transferred to Genetics Research Laboratory at the University in Tonekabon Branch. Blood samples belong to 83 patients (24 males, 59 females), and 100 blood samples were collected from the control group matched the patient group by age and gender. It should be noted that diagnostic of the disease was done by a neurologist and MRI test, and all the patients in this study were in Relapsing Remitting Multiple Sclerosis (MSRR). The collected blood samples transported in the tubes containing EDTA anticoagulant and they were maintained at the -20°C until the start of the experiment.

DNA extraction

1. Qiagene, Lot No:k92-8 , Cat No:k1014-100). The purity of the extracted DNA was analyzed based on absorbance of the extracted DNA at 230 and 260 nm wavelengths by biophotometer (Eppendorf- Germany).

Human beta-globin gene amplification

Amplification of human beta-globin gene was used in order to make sure of accuracy of the extracted DNA by using the following primers: betaglobin-f:5'-TCCAACATCAACATCTTTGG T-3' and betaglobin-R:5'-TCCCCCAAATTCTAAGCAGA-3' that produced by TAG Copenhagen (Denmark) (Zaravinos et al., 2009). Each reaction was performed in a total volume of 20 μl, which contained 10 μl of primer taq premix(2X), 1 μl of 10 pmol of forward and reverse PCR primers, 3 μl of Distilled water and 5 μl of DNA template. PCR amplification conditions on thermocycler (Biorad-Germany) were as follows: 95°C for 5 min, followed by 35 cycles of 95°C for 45 S, 54°C for 30 S and 72°C for 30 S, with a final extension at 72°C for 5 min. An aliquot of all PCR products was run on a 1.5% (w/v) agarose gels with a 100 bp DNA ladder.

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Polymerase chain reaction for the detection of varicella zoster virus

Specific Primers produced by TAG Copenhagen (Denmark) were used to amplify the VZV gene. The sequences of forward and reverse primers were 5'- ATGTCCGTACAACATCAACT -3' and 5'-CGATTTTCCAAGAGAGACGC -3' (Zaravinos et al., 2009). Each reaction was performed in a total volume of 20 μl, which contained 10 μl of primer taq premix(2X), 1 μl of 10 pmol of forward and reverse PCR primers, 3 μl of Distilled water and 5 μl of DNA template. The negative control tube contained the same PCR reagents as mentioned above but had 5 μl of water substituted for the DNA template. PCR amplification conditions on thermocycler (Biorad-Germany) were as follows: 95°C for 5 min, followed by 40 cycles of 94°C for 45 S, 54.5°C for 40 S and 72°C for 35 S, with a final extension at 72°C for 10 min. An aliquot of all PCR products was run on a 1.5% (w/v) agarose gels with a 100 bp DNA ladder (Fermentas-Russia) and electrophoresed at 75 V for 40 min. The bands were visualized using ethidium bromide staining and photographed after UV treatment by a transilluminator (UV doc, England).

Data analysis

Chi square test was used to determine whether there was any significant difference between the frequency of VZV in the patients with multiple sclerosis and the control group and their demographic characteristics and relationship with each other (SPSS software 17).

Results

In this study, 183 blood samples were examined while 83 samples belonged to the patient group and 100 samples belonged to the control group. Blood samples from the patient group were collected based on factors such as age, gender, geographical areas, a history of viral infection, genetic, medicines and disease duration. The control group samples were collected adjusting the patient group. To describe the samples features, first the collected data were summarized and concluded by descriptive statistics index, then all the results from observing the selected samples were generalized to the society.

The patients who suffered from MS based on age group were: 21 individuals (25.3%) under 30 years old, 55 individuals (66.27%) 30 to 50 years old, 7 individuals (8.43%) 30 to 50 years old, and subsequently the control group were: 31 individuals under 30 years old, 63 individuals 50 to 70 years old, 6 individuals 50 to 70 years old. Based on gender, from the patient group there were 24 men (28.92%), 59 women (71.08%) and from the control group: 30 men, 70 women. Based on a family history of MS and a history of viral infection in the patient group were: 12 individuals (14.45%) with family history of MS and 30 individuals (36.14%) with viral infection. The PCR technique was used to identify the DNA of virus the amplified fragments of human beta-globin gene and Viral DNA were 122 bp and 267 bp, respectively (Figure 1).

Briefly, the frequency distribution of VZV virus based on age group of MS patients included: 4 individuals (19.05%) under 30 years old, 14 individuals (25.45%) 30 to 50 years old, 3 individuals (86.42%) 50 to 70 years old,; and the control group were: 3 individuals (68.9%) under 30 years old and 6 individuals (52.9%) 30 to 50 years were infected with the virus. The frequency distribution of VZV based on gender from the patient group ,4 men (16.67%) and 17 women (28.81%),and from the control group, 3 men (10 %)and 6 women (57.8%) were infected with the virus. 3 individuals (25%) based on family history of MS and 10 individuals (33%) based on history of viral infection were infected with the virus from the patient group (table 1&2).
Figure 1. VZV amplification products analyses in a 1.5% agarose gel stained with ethidium bromide. Lane M: molecular marker 100bp, lane 1 viral DNA, lane 2 human beta-globin gene and lane 3 negative control

Table 1. Relative and absolute frequency of VZV infection in patients with MS

<table>
<thead>
<tr>
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<th>-VZV</th>
<th>p-value</th>
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<tbody>
<tr>
<td>Age</td>
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<td>17</td>
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<tr>
<td></td>
<td>30-50</td>
<td>55</td>
<td>14</td>
<td>41</td>
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<tr>
<td></td>
<td>50-70</td>
<td>7</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
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<td>24</td>
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<td></td>
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<td>59</td>
<td>17</td>
<td>42</td>
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<tr>
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<td>3</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>ü</td>
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<td>History of viral infection</td>
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<td>30</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>ü</td>
<td>53</td>
<td>11</td>
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</table>

Table 2. Relative and absolute frequency of VZV infection in the control group

<table>
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<td>30-50</td>
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<tr>
<td>Gender</td>
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<td>3</td>
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<td>Female</td>
<td>70</td>
<td>6</td>
<td>64</td>
</tr>
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</table>

Virtually, the frequency distribution of VZV virus in the patient and control groups was: 21 individuals (25%) from patients and 9 individuals (9%) from controls were viral infected. P-Value was reported (0.004), which showed there was a significant relationship between the frequency of VZV virus and the MS patient group.
Discussion

The exact cause of MS is still unknown (Barnaun et al., 2005). MS is a multifactorial disease which means more than one factor needs to interact each other and cause the disease (Ramagopalan et al., 2007). There is a main probable that viruses may stimulate the immune system and cause MS in people who are genetically susceptible to MS (Shaygannejad and Sadrameli., 2009).

For nearly a decade, studies have confirmed that viral infections play an important role for developing MS (Kurtzke et al., 1993; Larner, 1986). Then, a wide list of viruses related to MS has been offered. Many clinical studies have suggested that viral or bacterial infections can be one of the causes of MS (Gilden., 2005; Buljevac et al., 2002). In these studies the role of some viruses such as EBV, HSV, CMV in MS patients were examined (Sandgol et al., 2011; Djelilovic and Alajbegovic., 2012; Wandinger et al., 2000). The findings of researchers have represented a significant prevalence of these viruses in patients with MS. Although the investigation of the noted viruses has not been the purpose of this study, the results have showed that the viral infections have an important role in causing MS. Since there are a few studies about the role of VZV virus in causing MS, the present study has investigated the prevalence of VZV virus in MS patients and compared it with the control group by PCR technique. The other purpose of this study has been the comparison of the VZV prevalence between the MS patients and control group based on age, gender and epidemiological factors. The findings have showed there is a significant relationship in MS patients compared with the control group. Similar to this research, some researchers such as Ordonez and his colleague in 2004 and 2010 (Ordonez et al., 2010; Ordonez et al., 2004) could find out a significant relationship between VZV and MS. But, there are some researchers such as Franciotta and his colleague in 1996 and 2009 (Franciotta et al., 2009) reported lack of a significant relationship between VZV and MS just unlike the present research. Currently, we have been characterizing the genomic profile of VZV found in the blood of MS patients.

Conclusion

Considering the prevalence of edipathological disease in different areas in the world and in Iran, among young adults, and also the stable effect of this disease on life style of patients, and according to the available evidence and hypothesis about the relationship of infection with MS, it is necessary to perform foundational studies based on etiology by the view of the probable role of viruses. Thus, this study has investigated the prevalence of VZV virus in blood samples of MS patients and control group. The results have represented a significant relationship between VZV and MS. Further studies are still required to figure out the relationship between viral infections in causing MS.

References


