Original Article

Comparison of serum Epstein - Barr virus antibodies between patients with multiple sclerosis and healthy people in Sanandaj, Iran

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ARTICLE INFO

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ABSTRACT

Introduction: Multiple sclerosis (MS) is one of the chronic inflammatory diseases of the Nervous system. The cause of the disease has not yet been clearly identified. Environmental factors and infections, including the Epstein - Barr virus (EBV), are hypothesized to be the cause of the disease. Our goal was to compare the serum antibody level against EBV in patients with MS and healthy people in Sanandaj, Iran.

Methods: In this case-control study, 100 patients with MS who were registered in the MS Society of Sanandaj and 204 gender matched healthy blood donors from the Sanandaj Blood Transfusion Organization (control group) who signed an inform consent were studied from 2015 to 2016. A 5 ml blood sample was obtained from all subjects and then after isolation of patients' sera, IgG antibodies against EBV-CA and EBNA-1 antigens were measured by ELISA method. Demographic data and the results of the tests were analyzed by SPSS software and Chi-square test.

Results: EBNA-1 antigen was found in 92% of patient group and 91% of control group (P= 0.959). Serum anti EBV positivity was significantly higher among women (p=0.012). The EBV-CA antigen was positive in 95% of the patient group and 90% of controls (p= 0.229). There was no significant gender difference for this test (p=0.115).

Conclusion: There was no significant difference in the results of IgG antibodies against EBV in patients with MS and healthy controls in Sanandaj.

Introduction

Multiple Sclerosis is a progressive autoimmune disease of the central nervous system, in which neuronal myelin sheath is degraded by the host's immune system, which causes permanent disability. Studies show that multiple sclerosis is caused by environmental factors in patients with a genetic predisposition (1-3). The prevalence of multiple sclerosis in Tehran, Iran has increased significantly from 1999 to 2015 (56.22 per 100,000 in 1999 vs 115.94 per 100,000 in 2015) (4-6). The prevalence of multiple sclerosis in the province of Kohkolviyeh and Boyer Ahmad is 60.14 per 100,000 populations (7), and the prevalence of familial multiple sclerosis in Iran is 11.4% (8). Among the strongest risk factors for multiple sclerosis is the presence of Epstein-Barr virus (EBV) antibodies in the blood and the class II MHC antibodies (HLA-DRB1 * 1501) (3,9). The three main mechanisms of EBV pathogens in multiple sclerosis through viral-infection of B

lymphocytes include: EBV relapse in memory B lymphocytes, cross-reaction of anti-EBV antibodies against cell proteins, and facilitating the detection of antigens by B-lymphocytes Banned memory in the central nervous system. Another mechanism is the unintentional identification of human antigens in the central nervous system by molecular mimicry (3) by CD8 + cytotoxic T-lymphocytes that were prepared for EBV antigens. The body of literature regarding EBV identification in the development of multiple sclerosis is increasing day by day (2). Sustained and long-term EBV infection is a risk factor for multiple sclerosis. The EBV virus is a member of lymphocryptovirus family and gammaherpes subfamily viruses. The virus is transmitted through saliva. The virus infects the throat and B lymphocytes; then the virus spreads to the lymphoid tissues infecting B lymphocytes and causes latency in a small number of B lymphocytes. In the developing countries, EBV infection occurs at the onset of life, causing 100% of children to get the infection in the first decade of their life, while most of these infections are undiagnosed. In contrast, in developed countries, EBV infection occurs later in puberty and younger adults, which causes symptomatic infectious mononucleosis in adolescents and young people. The prevalence of EBV in adults in Western countries is 90%. The history of infectious mononucleosis, especially in adults, increases the incidence of multiple sclerosis more than two folds (1). The close connection between the EBV and the immune system has been well known. The EBV pathogenicity is usually caused by the failure of the host immune system against the virus, and the clinical symptoms of EBV infection are caused by the immune response (2). In patients who are EBV-positive, increased IgG Anti-EBNA-1 antibodies increase the risk of multiple sclerosis [3]. Also, this antibody may be a sign of the disease recurrence, because it is accompanied by oligoclonal bands in the spinal fluid (the best prognosis for the disease). In fact, in some patients, anti-EBV antibodies were found in spinal cord oligoclonal bands. These findings provided the first evidence for EBV pathogenicity in multiple sclerosis, as well as an increase in Anti-EBNA-1 antibody in the serum (1). Similarly, a meta-analysis found an association between detection of IgG anti-EBNA-1, anti-complex EBNA, and anti-VCA antibodies, which are indicators for EBV infection, and multiple sclerosis (10). Regarding the importance of multiple sclerosis and the absence of accurate etiology for this disease, our goal was to compare serum anti-EBNA-1 and anti-EBV-CA antibodies in multiple sclerosis patients and healthy individuals in Sanandaj, Iran. Furthermore, the secondary objective of this study

was to determine the seroepidemiological status of the disease and EBV infection.

Methods

Sampling: In this case-control study, 100 volunteer patients were selected from the list of registered multiple sclerosis patients in the MS Association in Kurdistan province, from 2012 to 2013. Patients were randomly selected and signed a written informed consent (case group). A total 200 healthy individuals who were matched with the patient group in terms of the age, gender, occupation and place of living, were selected from eligible blood donors who referred to the Sanandaj Blood Transfusion Organization. Data collection lasted for about one and a half years. Blood samples were collected from the patient and control groups. No re-sampling was needed. Demographic data (age, gender, place of residence and occupation) were collected from patients and control group. In both groups, patient information was kept confidential. Blood samples were centrifuged and the extracted serum was stored in test tubes at -20 ° C until ELISA tests were performed. ELISA test was performed on serum using anti-EBV IgG antibody, IgG antibody, IgG antibody kits (Anti-EBNA-1 IgG, ELISA Kit, EUROIMMUN, Germany) and IgG Antibody Anti-EBV- CA IgG ELISA Kit, (EUROIMMUN, Germany). According to the manufacturer's instructions, the upper threshold for detection of EBV infection is more than 20 relative units (Relative Unit: RU) per ml of serum for both antibodies (cut-off = 20 RU / ml). Statistical analysis: The results of ELISA tests were entered into the statistical package for social sciences (SPSS) software, version 21. The Chi-square test was used to analyze the data. The p value smaller than 0.05 was considered statistically significant. The study protocol was approved by the University Ethics Committee (Proposed Code: MUK.REC.1394.202).

Results

A total of 300 subjects participated in this study. Overall, 96 (32%) subjects were male and 204 (68%) subjects were female. Among the 300 subjects, 100 (32 males and 64 females) were in the patient group (case group) and 200 (64 males and 128 females) were in the control group. The age of patients was between 20 and 40 years and the control group was also matched accordingly. The place of residence of all patients and all the control group was urban. In

this study, IgG antibodies were tested against EBNA-1 and EBV-CA antigens. IgG Anti-EBNA-1Antibody: Out of 300 samples, anti-EBNA-1 antibody was positive in 274 cases (91.3%). The anti-EBNA-1 antibody was positive in 92 cases (92%) in the patient group and 91% of the control group. There was no significant difference between the patients and the control group in terms of anti-EBNA-1 antibody. But there was a significant difference in gender distribution of anti-EBNA-1 antibody (p = 0.122) (Table 1). IgG Anti-EBVCA Antibody: Of all the 300 ELISA tests, 275 (91.7%) were positive for IgG anti-EBVCA antibody. Of the 100 patients, 95 (95%) were positive, but anti-EBVCA antibody was positive in 90% of the case group. There was no significant difference in the distribution of IgG anti-EBVCA antibody between the patients and the control group. Similarly, there was no significant gender difference in terms of anti-EBVCA antibody positivity (Table 2).

Discussion

A total of 300 subjects participated in this study, of which 100 were in the patient group (case) and 200 in the control group (control). A total of 96 (32%) subjects were male and 204 (68%) were female. IgG antibodies against EBNA-1 antigen were positive in 274 of the subjects (91.3%). IgG Anti-EBVCA antibody was positive in 275 (91.7%). There was no statistically significant difference between the two tests in the patients and the control group. In a prospective study on EBV infection as a risk factor for multiple sclerosis in the United States military revealed that seroconversion in younger adults may produce multiple sclerosis in the next decade of life (a mean lag of 3.8 years, range from 1.7 to 1.7 Year) (12). The relative risk of multiple sclerosis in carriers of EBV was more than 2-3-fold compared to healthy individuals (13).

However, most EBV infections occur in childhood (14), but the onset of multiple sclerosis later in life is usually seen at the beginning of adolescence (15), which indicates the need for other environmental factors to produce multiple sclerosis. In Iranian population (Tehran), EBV antibodies were measured in 60 patients and 50 healthy individuals in a case-control study using chemiluminescence method. All patients with multiple sclerosis were positive for EBV antibodies, but EBV antibodies were positive inly in 82% of the control group (p = 0.0006). Multiple sclerosis, as with other populations in the developed countries, had a significant association with EBV infection (16). In a study in Gilan province, Iran, EBV infection was assessed in 46 patients and 46 controls using ELISA method. In that study serum IgG, anti-EBNA-1, anti-EBVCA and anti-EBV-EA antibodies were assessed. The study revealed that serum IgG anti-EBNA-1 antibodies were present in 92.9% and 99.4% of case and controls respectively, while IgG, anti-EBVCA antibodies were present in 95.2% and 99.3% of cases and controls respectively. There was no significant difference in terms of EBV antibody positivity between the two groups. Anti-EBV-EA-D antibody was negative in all patients and in 95.3% of the control group. Anti-EBNA-1 and anti-EBVCA antibodies, that indicate a positive history of infection, did not significantly correlate with multiple sclerosis. The authors concluded that both IgG, anti-EBNA-1 and anti-EBVCA antibodies had no association with multiple sclerosis (17). The sensitivity and specificity of the test used in the measurement of antibodies were previously found to be effective in the association between multiple sclerosis and EBV (11).

Table 1. Evaluation of the association between multiple sclerosis and the presence of IgG antibody EBNA-1

Variable		Test Result		Degrees of freedom	Test statistic	P value
		Negative	Positive			
Sex	Male	14 (53.8%)	82 (29.9%)	1	6.224	0.012
	Female	12 (46.2%)	192 (70.1%)			
Group	Case	8 (30.8%)	92 (33.6%)	1	0.084	0.772
	Control	18 (69.2%)	182 (66.4%)			

Table 2. Evaluation of the association between multiple sclerosis and the presence of anti-EBVCA IgG antibody

Variable		Test Result		Degrees of freedom	Test statistic	P value
		Negative	Positive			
Sex	Male	12 (48%)	84 (30.5%)	1	3.209	0.115
	Female	13 (52%)	191 (69.5%)			
Group	Case	5 (20%)	95 (34.5%)	1	2.45	0.295
	Control	20 (80%)	180 (65.5%)			

Conclusion

The results of this study indicate that there was no significant difference in the presence of IgG antibodies for EBV between patients with multiple sclerosis and healthy controls in Sanandaj. The result of our study in some cases was consistent with the results of previous studies. However, in some studies, no relationship was found between EBV antibodies and multiple sclerosis. Furthermore, due to the high prevalence of EBV infection in our country since childhood and the presence of immune memory in the population, it is recommended for further researchers to conduct studies to compare serum IgM antibodies against EBVCA and EBNA-1 antigens and viral activity, based on molecular studies, between multiple sclerosis patients and healthy controls.

Ethical disclosure

Before performing the research, it was explained to the participants. An informed consent was obtained from all participants included in the study.

Acknowledgements

Authors' contribution

All authors contributed equally in planning and carrying out this project.

Conflict of interest

There is no conflict of interest in this study.

Funding/Support

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