Evaluation of viral antibodies in Iranian multiple sclerosis patients

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ABSTRACT

الأهداف: تقييم حالة الأجسام المضادة لعدد من الفيروسات بين المرضى الإيرانيين المصابين حديثاً بالتصلب العصبي المتعدد.

الطريقة: أُجريت هذه الدراسة المقطعية في قسم أمراض المناعة بجامعة أراك للعلوم الطبية، أراك، إيران، واستمرت خلال الفترة من يناير 2009م إلى مارس 2010م. لقد قُسم المشاركين في الدراسة إلى مجموعتين وهما: مجموعة المرضى المصابين بالتصلب المتعدد وشملت 61 مريضاً (مجموعة الحالة)، ومجموعة المرضى الأصحاء وشملت 60 شخصاً (مجموعة الشاهد). وبعد ذلك جُمعت عينات الدم من كافة المشاركين، وتم تحليل نتائجها بواسطة التحاليل المناعية المرتبطة بالإنزيمات (إليزا) واختبار التصبغ المناعي باستخدام الفلوروسين وذلك بغرض تحليل احتمال ظهور الأجسام المضادة IgG وIgM الموجهة ضد الفيروسات التالية: فيروس ابشتاين–بار، وفيروس الهربس البشري 6، وفيروسات الحصبة، والنكاف، ونظيرة النزلة الوافدة.

النتائج: أسفرت النتائج عن ظهور فروقاً واضحة في نسبة انتشار الأجسام المضادة بين مجموعة الحالة ومجموعة الشاهد وذلك على النحو التالي: الأجسام المضادة IgM الموجهة ضد فيروس الهربس البشري 6 (النسبة الترجيحية=4.3، 95% مدى الأمان الإحصائي=2–9.3) (p=0.001)، والأجسام المضادة IgG الموجهة ضد فيروس الهربس البشري 6 (النسبة الترجيحية=2، 95% مدى الأمان الإحصائي=1–4) (p=0.04)، والأجسام المضادة IgM الموجهة ضد فيروس الحصبة (النسبة الترجيحية=3.2، 95% مدى الأمان الإحصائي=1.5–6.9) (p=0.002)، والأجسام المضادة IgM الموجهة ضد فيروس النكاف (النسبة الترجيحية=4.1، 95% مدى الأمان الإحصائي=1.9–8.8) (p=0.0001)، والأجسام المضادة IgG (النسبة الترجيحية=9.5، 95% مدى الأمان الإحصائي=3–29.6) (p=0.0001). وأظهرت الاختبارات عدم ظهور الأجسام المضادة IgM الموجهة ضد فيروس ابشتاين– بار وفيروس نظيرة النزلة الوافدة بين كافة المشاركين في مجموعة الشاهد ومجموعة المرضى المصابين بالتصلب العصبي المتعدد.

خاتمة: أثبتت النتائج وجود العلاقة بين حدوث مرض التصلب العصبي المتعدد وظهور الأجسام المضادة لفيروسات الهربس البشري 6، والحصبة، والنكاف، كما أنها قد أظهرت مدى تحفيز الاستجابة المناعية الرئيسية (IgM)، وإعادة تنشيط الفيروسات لدى المصابين بهذا المرض. وقد يكون لهذه الفيروسات دوراً هاماً في تطور المرض إذ أنها تعمل كعامل محفز لحدوثه في هذه المنطقة الجغرافية.

Objectives: To evaluate the viral antibodies in new Iranian multiple sclerosis (MS) patients.

Methods: In a cross-sectional study, sera from 61 MS patients and 60 healthy individuals were collected from January 2009 to March 2010 in the Immunology Department of Arak University of Medical Sciences, Arak, Iran, and examined for the presence of the anti-Epstein-Barr virus (EBV), human herpes virus 6 (HHV-6), measles, mumps, and para-influenza viruses IgG and IgM using an enzyme-linked immunosorbent assay or immunofluorescence.

Results: There were significant differences between the MS patients and the healthy individuals (controls) in the seroprevalence of anti-HHV-6 IgM (odds ratio [OR]=4.3, 95% confidence interval [CI]=2-9.3, p=0.001); anti-HHV-6 IgG (OR=2, 95% CI=1-4, p=0.04); anti-measles IgM (OR=3.2, 95% CI=1.5-6.9, p=0.002); and the anti-mumps IgM (OR=4.1, 95% CI=1.9-8.8, p=0.0001) and IgG (OR=9.5, 95% CI=3-29.6, p=0.0001). Almost all MS patients and the control individuals were negative to EBV and parainfluenza IgM.

Conclusion: These results confirm an association between the incidence of MS and the antibodies to HHV-6 and the measles and mumps viruses, and show induction of a primary immune response (IgM), or virus reactivation, in MS patients. These viruses may have an important role in development of MS as an initial trigger in this geographical area.

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Multiple sclerosis (MS) is an inflammatory autoimmune disease that affects the brain and CNS.[1] Multiple sclerosis affects more than one million people in the world. The etiology of MS is unknown, but it is well-established that the risk of developing MS is influenced by genetics and environmental factors.[2] Although the mechanisms involved in MS remain elusive, MS is hypothesized to be an autoimmune disease with an initial trigger, which may have viral roots.[3] Many studies have suggested that there is an association between episodes of MS exacerbation and concomitant viral infection. Several viruses may initiate an immune reaction that cross-reacts with CNS antigens, potentially injuring the CNS.[4] The strongest evidence on the viral etiology of MS is based primarily on epidemiological observations and immunopathological characteristics.[5] In addition, viral infections are closely associated with the clinical exacerbation of MS.[6] Numerous studies involved the demonstration of increased antibody titer in response to presence of a particular virus, whereas some described isolation of viruses from MS patients.[7,8] Several studies compared the seroprevalence of common Epstein-Barr virus (EBV) antibodies between MS patients and healthy individuals.[7,9] Ascherio et al[10] showed that the risk of MS in an EBV-negative individual was very low, and that it increased in the same individual after seroconversion. On the other hand, a positive association was observed between other viral infections, such as human herpes virus 6 (HHV-6), and the risk of developing MS.[11] The HHV-6 DNA was also found in over 70% of the brains of both MS patients and controls, however, HHV-6 protein expression was only identified in plaques and non-plaques of MS patients.[11] In contrast, several studies reported a lack of evidence on viral infections in MS patients.[12,13] Within this context, no virus to date has been conclusively shown to be the sole agent responsible for causing MS. Multiple sclerosis is a multifactorial disease that is influenced by geographical location, ethnic background, and environmental factors.[2] Recently, epidemiological and clinical observations have shown that the incidence and prevalence of MS is increasing in Iran, particularly in the middle and north east of Iran.[14,15] In the present study, we evaluate the antibodies to EBV, HHV-6, measles, mumps and parainfluenza viruses in new Iranian MS patients.

Methods.

Patients. The present study was carried out from January 2009 to March 2010. The study sample comprised patients diagnosed with MS according to the McDonald criteria.[16] Selection of MS patients in this study was based on a number of inclusion criteria that included individuals with at least one relapse in the past 2 years; more than 3 lesions on spinal or brain-MRI scans, or both; a score on the baseline Expanded Disability Status Scale (EDSS) within the range of 0-3.5; and age between 15-60 years. Additionally, special consideration was paid to select MS patients who had not previously received any immunosuppressive or corticosteroid treatment. The following main exclusion criteria were adopted: clinically isolated syndrome, drug abuse, and previous therapy with immunomodulatory agents or vaccination for one year before commencement of this study.

Control group. Normal individuals were selected to serve as a control group from the same geographical area and ethnic background. These subjects had no signs of MS and were matched for age, gender, and socioeconomic status.

Ethical approval. This study was approved by the Arak University of Medical Sciences Ethics Committee (AUMSEC-86-22/4), and all patients and healthy controls completed the relevant consent form.

Clinical samples and antibodies detection. Serum samples of 61 new MS patients and 60 normal individuals were collected at the section of Immunology, Arak University of Medical Sciences, Arak, Iran and stored at -80°C awaiting further investigation. Anti-EBV, measles, mumps, and parainfluenza IgG and IgM, and anti-HHV-6 IgG were analyzed by enzyme-linked immunosorbent assay using commercial kits (IBL, Hamburg, Germany) according to the manufacturer's instruction. The levels of the anti-HHV-6 IgM were determined by indirect fluorescence immunoassay (Biotrin, Dublin, Ireland). Patients were considered virus-positive when the serum anti-virus specific antibodies IgG and IgM were detected at levels higher than the corresponding cutoff levels specified by the kits.

Statistical analysis. The Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA), Version 16 was used for statistical analysis. Non-parametric tests were employed since the study variables were non-normally distributed. The Kruskal-Wallis test was used to test for significant differences between group medians in the variables studied. The logistic regression was used to investigate relations between the various study variables and to predict the probability that the incidence of MS will occur as a linear function of one (or more) of the independent variables and to estimate the risk (odds ratio) of the disease. A probability value of less than 0.05 was considered as indicative of statistical significance. The descriptive variables are reported here as mean ± standard deviation. (Mean is measurement of each variable in all patients and compared with the control group).

Results.

Table 1 summarizes the demographic characteristics of the MS patients and controls, and

Table 1 - Demographic characteristics of the Iraqi multiple sclerosis patients and the control group.

Group	N	Gender Male/Female	Mean age ±SD
MS patients	61	9/52	32±17
Control group	60	10/50	35±15

MS - multiple sclerosis, SD - standard deviation

shows that there are no significant differences between the groups. None of the MS patients, or the controls were positive for anti-EBV IgM. The anti-EBV IgG was positive in 80% (49 of the 61 cases) of the MS patients and in 65% of the control group. Furthermore, no significant difference was found in the anti-EBV IgG concentrations between the MS patients and the control group. On the other hand, neither the MS patients, nor the controls were positive for anti-EBV IgM. The prevalence of anti-HHV-6 IgM in the sera of the MS patients was significantly higher than that in the sera of the control group. The IgG antibody to HHV-6 was found in 37 out of the 61 MS patients and in 26 out of the 60 controls (**Table 2**). As also shown in **Table 2**, almost all the MS patients, except 2, and all of control individuals were negative to parainfluenza IgM. The IgG against parainfluenza was found in all of the control and almost all the MS patients (57 out of 61). The prevalence of anti-measles IgM was significantly higher in the MS patients than in the control group. Anti-measles IgG was detectable 85% of MS patients, and in 88% of the controls. Although there was no significant difference in seroprevalence of anti-measles IgG between the 2 groups, the serum anti-measles IgG titers were higher in the MS patients than in the controls. The levels of the anti-mumps IgM were significantly higher in the sera of the MS patients (64%) than in those of the controls (30%). Moreover, the seroprevalence of anti-mumps IgG was significantly higher in the MS patients than the controls (**Table 2**).

Discussion. The genesis and pathogenesis of MS remain under debate. It has been proposed that antigenic cross-reactivity or molecular mimicry between infectious agents and some myelin proteins in the CNS play a role in the development of MS.[4,17] Several studies advocated a correlation between association of the risk for MS and viral infections such as EBV, HHV-6, and the mumps and measles viruses.[5,7,18] Epidemiological and clinical evidence demonstrates a strong association between EBV infection and MS.[19,20] Elevated IgG reactivity to EBV nuclear antigen 1 (EBNA1) has been found in MS patients and correlated with the number of T2 lesions on brain MRI scans and with change in EDSS.[8,21] Serological evidence showed that the risk of acquiring MS is associated with increased levels of EBV antibody titers.[22,23] Banwell et al[24] showed that 86% of the pediatric MS patients were seropositive for EBV compared to 64% of matched controls. Our results showed that anti-EBV IgG levels were positive in 80% of MS patients and in 65% of the control individuals. All the MS patients and controls were negative for anti-EBV IgM. These results suggest that there is no temporal coincidence between EBV reactivation (production of IgM antibody to EBV) and disease activity in MS patients.

The HHV-6 has been associated with several diseases such as meningeo-encephalitis, autoimmune diseases, and MS. High levels of HHV-6 DNA have been detected in the CNS, sera, and CSF of MS patients.[18] Results of earlier studies indicated that MS patients had increased titers of serum antibodies to HHV-6 and that 50-70% of them were positive for HHV-6 IgM.[25] The results of this study showed that the frequencies and concentrations of anti-HHV-6 IgM and IgG in

Table 2 - Seroprevalence of antibodies to Epstein-Barr virus (EBV), human herpes virus 6 (HHV-6), parainfluenza (PI) virus, measles, and mumps viruses in Iranian MS patients and controls.

Anti-viral antibodies	MS patients n=61	Controls n=60	Odds ratio	*P*-value
	SP/SN (mean ± SD)			
Anti-EBV IgM ≥22 U/mL	0/61 (2.8±0.83)	0/60 (2.7±0.9)		NS
Anti-EBV IgG ≥22 U/mL	49/12 (166±114)	39/21 (156±146)		NS
Anti-HHV-6 IgM ≥2 positive	46/15	25/35	4.3 (2-9.3)	0.001
Anti-HHV-6 IgG ≥0.9 OD	37/24 (1.2±0.65)	26/34 (1.02±0.7)	2(1-4)	0.04
Anti-PI IgM ≥12 U/mL	2/59 (4±7)	0/60 (2.6±2.2)		NS
Anti-PI IgG ≥12 U/mL	57/4 (107±54)	60/0 (96±48)		NS
Anti-measles IgM ≥0.45 OD	33/28 (0.53±0.3)	16/44 (0.4±0.2)	3.2 (1.5-6.9)	0.002
Anti-measles IgG ≥12 U/mL	51/10 (405±450)	53/7 (168±230)		NS
Anti-mumps IgM ≥ 0.31 OD	39/22 (0.54±0.2)	18/42 (0.4±0.17)	4.1 (1.9-8.8)	0.0001
Anti-mumps IgG ≥ 0.47 OD	57/4 (1.3±0.47)	36/24 (1.2±0.46)	9.5 (3-29.6)	0.0001

MS - multiple sclerosis, SP/SN - seropositive/seronegative, OD - optical density, NS - not significant

the MS patients were significantly higher than those in the control group. Accordingly, these findings confirm an association between HHV-6 antibodies and the incidence of MS, and illustrate a correlation between HHV-6 reactivation and eruption of activity of the MS disease. Almost all related earlier studies have failed to detect evidence for an association between HHV-6 and MS. For example, a study of MS patients in Kuwait failed to find evidence of HHV-6 DNA in the blood of a small sample of people with MS.[26] Enbom et al[27] detected anti-HHV-6 IgM in only one of 55 MS patients. In fact, none of these studies detected these HHV-6 markers in all the subjects with MS, whereas all were found in some of the control subjects.

Several studies have found indicators that measles or mumps viruses exist in significantly higher concentrations in MS patients than in control subjects.[28,29] Adams and Imagawa[30] were the first to propose a possible link between measles virus infections and MS. Our results showed that anti-measles IgM was significantly higher in the MS patients (54%) than in the control group (27%), while there was no difference in prevalence of anti-measles IgG between the 2 groups. The increased levels of anti-measles IgM in the MS patients suggest a reactivation or primary immune response in these patients. Our results also showed that the frequencies of detectable anti-mumps IgM and IgG in the MS patients were significantly higher than those observed in the control group. Hence, our results reassert a strong association between MS and mumps virus infection in this geographic area. Many earlier studies affirmed such results, though some studies did not. These discrepancies may be due to differences in geographical locations and/or differences between patients in genetic backgrounds. The increase in specific antibody titers of HHV-6, and the measles and mumps viruses may reflect a different course of these infections or a genetically determined difference in the immune response of those individuals susceptible to MS.

In conclusion, this study found association between the incidence of MS and the antibodies to HHV-6 and the measles and mumps viruses, however, MS is a multifactorial disease and a viral association may not be seen in all patients. The increase in MS incidence in recent years may be related to national programs of vaccination against measles, mumps, and rubella viruses. Large epidemiological studies are needed to obtain more reliable data to further confirm the above-mentioned associations.

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