Serum levels of EBVNA-IgG and EBVCA-igG in patients with multiple sclerosis

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Abstract
The present case-control study includes 50 patients with multiple sclerosis, diagnosed according to McDonald criteria, 35 women and 15 men, median age 40.8 years (21-61). Twenty of them had familial history with disease transmitted from the mother. Relapsing-remitting course was noted in 42 of them, relapsing-progressive in 4 and secondary progressive in 4. Statistically significant association with multiple sclerosis was found only for the highest serum levels of EBVCA IgG > 750 U/ml (17 vs. 8, p<0.05) and EBVNA IgG > 1,000 U/ml (23 vs. 3, p<0.05). The subgroup analysis by gender and family history did not reveal significant difference between cases and controls. Two of the sporadic cases were EBVCA IgG and EBVNA IgG negative. In both groups there was identical age-depended increase of the serum levels. The analysis of HLA-G 14bp ins/del polymorphism did not reveal a significant difference between the patients with the EBVCA >750 U/ml and EBVNA > 1,000 U/ml, the entire cohort and controls. In contrast to the literature, we found no convincing evidence for the role of EBV in MS. Except a true association, the highest serum levels may reflect a synergistic influence of other genetic or environmental factors or may be just a secondary phenomenon. However, the possible role of EBV at the early stages of MS pathogenesis could not be excluded and future and larger studies with a proper design are justified.

INTRODUCTION
Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of CNS with an unknown etiology. The genetic epidemiology clearly demonstrates that MS is a result of complex interactions between genetic and environmental factors acting at a population level. The epidemiology of the Epstein-Barr virus (EBV) infection is very similar to MS. In addition, several studies have suggested its possible role in MS [1, 2].

The aim of the present study was to determine a possible association between the serum levels of EBV-nuclear antigen (EBVNA) and EBV-capsid antigen (EBVCA) IgG and MS in the Bulgarian population.

MATERIALS AND METHODS
This is a case-control study, which includes 50 cases with multiple sclerosis and 50 healthy individuals. Only patients with definite MS according to McDonald and examined personally by the authors were included in the study. All of them have been treated and followed-up in the same clinic. The clinical course was assessed according to the classification of Lublin and Reingold. Fifty randomly assigned healthy individuals, matched by gender and age, were used as controls. The enzyme-linked immunosorbent assay was used to detect the IgG-antibody to EBV in the peripheral blood samples. All analyses were performed blind in a referent laboratory. The statistical analysis was performed by Pearson’s chi-square test using SPSS, version 21.0 (Chicago, IL, USA). The study was approved by the
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Ethical Committee of the Medical University, Sofia.

RESULTS

Demographic characteristic.
The mean age of the entire cohort was 40.8% (21-61), 15 men and 35 women. Familial MS was found in 20/50 (40%), 13 women and 7 men. The disease was transmitted by the mother in all of them.

Clinical characteristic.
Relapsing-remitting (RR) course was noted in 41 cases, relapsing-progressive in 6, secondary progressive after initial RR course in three. The mean age at the first symptoms and diagnosis was 30.4 (19-46) и 33.6 years (18-51), respectively. The mean duration of the disease was 10.3 years (1-35) with mean EDSS 3.68 (1.5-6.5). Smoking was noted in 15/51 (29.4%), accompanying autoimmune diseases in 4/51 (7.8%) (Graves’s disease, Hashimoto’s thyreoiditis, scleroderma and psoriasis).

Statistical analysis.
Statistically significant association with multiple sclerosis was found only for the highest serum levels of EBVCA-IgG > 750 U/ml (17 vs. 8, p < 0.05) and EBVNA-IgG > 1.000 U/ml (23 vs. 3, p < 0.05), (Table 1). There were no difference between familial and sporadic cases 8/9 and 12/11, respectively. The subgroup analysis by gender and family history did not reveal a significant difference between cases and controls. Two of the sporadic cases were EBVCA-IgG and EBVNA-IgG negative. Both groups had identical age-depended increase of the serum levels (Figs. 1, 2).

DISCUSSION

EBV is a member of Herpes virus family with ubiquitous distribution, which affects over 90% of the world population. The infection in the early childhood is usually symptomless, whereas 30-50% of the adolescents and young people develop infectious mononucleosis (IM). After the recovery, EBV may remain in a dormant state in B-lymphocytes with a potential for reactivation [3]. There are 4 types latent infection, which include a complex interaction between EBV and the host immunity. This may lead to different lymphoproliferative and autoimmune diseases and neoplasms [4]. EBVNA-1 has a key role in the virus replication, in the maintenance of the latent infection and in the blast transformation of the cells. EBVCA is accountable only for the active replication of EBV. [2].

Table 1. Distribution of the serum levels of EBVCA-IgG и EBVNA-IgG.

<table>
<thead>
<tr>
<th>EBVCA-IgG</th>
<th>EBVNA-IgG</th>
</tr>
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<tbody>
<tr>
<td>U/ml</td>
<td>Cases N</td>
</tr>
<tr>
<td>&lt; 45</td>
<td>2</td>
</tr>
<tr>
<td>&gt;55</td>
<td>1</td>
</tr>
<tr>
<td>&gt;106</td>
<td>30</td>
</tr>
<tr>
<td>&gt;750</td>
<td>17</td>
</tr>
</tbody>
</table>

Fig. 1 Age-depended distribution of EBVCA-IgG.

Fig. 2 Age-depended distribution of EBVNA-IgG.
Meta-analysis of 11 case-control and 3 cohort studies found an increased risk for MS in the cases with past history for IM. According to the model proposed by the authors the risk for is minimal for the persons without infection, intermediate for those with an early and symptomless infection and high for those with past IM history. Several studies have been reported increased serum levels of the antibodies against EBV, detected several years before the clinical onset of MS [5-8]. Anti-EBV nuclear antigen IgG (EBVNA-complex in EBVNA-1) were found as most important predictors for MS. These findings showed that EBV infection might be an early event rather than a consequence of MS.

In contrast to the controls, Munch et al., found one and same EBV strain in all MS patients [9]. Alotabi et al., found past EBV-infection in 83% of the children with MS in comparison to 42% in those with other neurological diseases (OR = 7.04) and 42% in the healthy controls (OR = 8.7) [10]. Serafini et al., found ectopic lymph follicles and a presence of EBV in nearly 100% of the B-lymphocytes in contrast to the lesions in other inflammatory neurological diseases [11].

An indirect evidence for a possible role of EBV in MS is the beneficial effect of Rituximab (anti-CD 20 antibody) on the clinical and MRI activity of the disease. This hypothesis was supported by the effectiveness of Rituximab in EBV-related lymphoproliferative diseases (Burkitt’s and Hodgkin’s lymphomas and extra-nodular NK/T cells lymphoma), probably through an elimination of the EBV-infected B-cells [12].

Molecular mimicry is a plausible explanation of the role of EBV in the pathogenesis of MS. There is an evidence for a clonal expansion of cross-reacting with myelin EBVNA-1-specific CD4+Th1 lymphocytes in patients with MS [13]. This subpopulation produced γ-interferon and IL-2 in a higher extent than lymphocytes not reacting with myelin. Lang et al., found that T-cell receptor (TCR) of these T-lymphocytes cross-reacted with a part of EBV-antigen on the background of a lack of an obvious similarity and presentation by different HLA II molecules [14, 15].

According to another hypothesis, EBV may induce an autoimmune reaction in non-organ specific way [16]. The ability to escape the host immunity is a characteristic feature of EBV, which is a consequence of the blockage of the proteasomal processing of EBVNA-1. The underlying mechanism is the unique glycine-alanine repeats in its structure and this process is also facilitated by the lytic gene products during an active replication.

On other hand, there are several studies not supporting the above-cited findings. Willis et al., found neither EBV in the MS lesions, nor ectopic follicles [17]. This was confirmed by a large study of 642 CNS samples from 94 died patients with MS [18] and by other authors [19]. Five of the cases of Alotabi et al. (17%) were EBV-negative as two cases in our series [10]. In a case-control study, Castellazzi et al., reported a significantly higher frequency of the specific antibodies in the serum and CSF of the patients with other inflammatory and non-inflammatory neurological diseases than in MS. An intrathecal synthesis was found in only 1.3-6.3% of the MS patients [20]. The wide screening for the presence of DNA herpes viruses in the serum and CSF of 35 MS cases found no evidences, except 1 [21].

The present study also did not find a significant association with MS, except for the highest serum levels of EBVCA-IgG >750 U/ml (17 vs. 8, p=0.038) and > 1.000 U/ml for EBNA-IgG (23 vs. 3, p<0.001). In this light, there are several problems concerning the role of EBV in MS that should be discussed. These include the absence of EBV in CNS, the absence of EBV in a small subset of MS patients. Other unanswered questions are whether the EBV infection is a primary or secondary phenomenon in MS, what is the exact autoimmune mechanism (molecular mimicry, activation of super-antigens, bystander activation from non-specific product of the inflammation, what is the exact residing place of the infected B lymphocytes [22, 23].

Our results should be interpret with a caution due to the small sample size, the retrospective design and the lack of a reliable information about the serum levels of EBVCA-IgG and EBNA-IgG prior to MS onset respectively. An influence of the immunomodulatory treatment on the EBV-infection is also possible. Despite the contradictory results in the literature, EBV still remains a plausible candidate and its role in MS could not be excluded. Other environmental factors such as vitamin D, co-infection with other viruses or bacteria and acquired immune disturbances may act as modifiers of its role to increase or decrease the risk for MS. At last, but not least, a synergetic influence of genetic factors is also possible. Nielsen et al., found 7-fold increased risk for MS in HLA-DRB1*15 carriers with a past history for IM in comparison to two-fold in the cases without IM [24].

**CONCLUSION**

In the present study we found no significant association between the serum levels of EBVCA-IgG and EBNA-IgG and MS. Except for a true relation, the reported association with the highest serum level may reflect a synergistic influence of genetic or other environmental factors.
factors, or it is due to a chance. Despite the contradictory results from the literature, the role of EBV at the early stages of MS pathogenesis could not be excluded and future investigations are justified.

DISCLOSURE
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