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Multipl Sklerozda Sistemik Otoantikor Pozitifliğinin Oligoklonal Band Varlığı ve Özürlülük ile İlişkisi

Association of Systemic Autoantibody Positivity with the Presence of Oligoclonal Bands and Disability in Multiple Sclerosis

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Özet

Amaç: Rölapsing remiting multipl skleroz (RRMS) hastalarında sık gözlenen sistemik otoantikorların sıklığının ve oligoklonal band (OKB) ve hastalık şiddeti ile ilişkilerinin belirlenmesi amaçlandı. **Gereç ve Yöntem:** Otoimmün hastalığı olmayan toplam 150 kesin RRMS hastası ve 150 sağlıklı kontrol çalışmaya alındı ve serumları otoantikor tayini için incelendi. **Bulgular:** RRMS olgularında anti-tiroid peroksidaz antikoru (anti-TPO) (%20), anti-kardiolipin (AKA) IgG (%22) ve AKA IgM (%24) antikorları sağlıklı kontrollerden belirgin derecede yüksekti ($p<0.001$). Yaş, cinsiyet, OKB pozitifliği ve ortalama "expanded disability status scale" (EDSS) skorları açısından otoantikorlu ve otoantikorsuz RRMS olguları arasında fark yoktu. **Sonuç:** Bulgularımız serum otoantikorlarının hastalık şiddeti ve OKB oluşumu ile ilişkili olmadıklarını ve sadece MS hastalarında otoimmün hastalığa yakalanma riskindeki artışın bir göstergesi olduklarını düşündürmektedir.

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Abstract

Objective: We aimed to determine the frequency of common systemic autoantibodies in patients with relapsing remitting multiple sclerosis (RRMS) and their association with the presence of oligoclonal bands (OCB) and disease severity. **Materials and Methods:** A total of 150 definite RRMS patients and 150 healthy controls with no autoimmune disease were enrolled and their sera were examined for the presence of autoantibodies. **Results:** The positivity rates for anti-thyroid peroxidase antibody (anti-TPO) (20%), anti-cardiolipin (ACA) IgG (22%) and ACA IgM (24%) antibodies were significantly higher than those of the healthy individuals ($p<0.001$). Age, gender, OCB positivity rates and average expanded disability status scale (EDSS) scores were comparable among RRMS patients with and without autoantibodies. **Conclusion:** Our findings suggest that serum autoantibodies are not significantly associated with disease severity or OCB formation and they are merely indicators of an increased susceptibility to autoimmune disease induction in MS patients.

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INTRODUCTION

Multiple sclerosis (MS) is a predominantly T cell mediated autoimmune demyelinating disease. The course of the disease, site of involvement, prognosis and treatment responses are well known to be highly heterogeneous (3-5, 11). However, despite this heterogeneity, most MS patients display similar immunological abnormalities. As an example, in approximately 90% of MS patients, an abnormal increase is observed in the cerebrospinal fluid (CSF) oligoclonal IgGs, which generally do not react with specific targets. Nevertheless, the presence of these intrathecal oligoclonal bands (OCB) in the CSF suggest that the humoral immune system plays a role in MS pathogenesis (10, 16, 17).

On the other hand, the association of MS with non-neurological autoimmune diseases and systemic autoantibodies are much less well-characterized. Several immunological abnormalities that have long been reported in MS patients and recent reports on the association of autoantibodies with MS and other accompanying autoimmune diseases (3, 4, 8, 16, 18) suggest that circulating autoantibodies could be related with MS pathogenesis and influence the disease outcome.

For instance, antiphospholipid antibodies that have been associated with epilepsy, idiopathic intracranial hypertension, migraine and chorea have also been linked to MS, optic neuritis and transverse myelitis (6). Although some studies have shown an association between white matter lesions and the presence of antiphospholipid antibodies, many others have disputed this relationship (9, 20). In the present study, we aimed to investigate the frequency of a wide array of commonly observed systemic autoantibodies in MS patients and their association with the presence of OCB and disability.

MATERIALS AND METHODS

A total of 150 consecutive patients followed in Department of Neurology of Haydarpasa Numune Training and Research Hospital with a diagnosis of definite relapsing remitting MS (RRMS) according to the McDonald's criteria and 150 gender and age-matched healthy controls were included. Patients with a systemic autoimmune disorder other than MS and controls with any autoimmune disorder were excluded. Blood samples were obtained from all patients and healthy controls. None of the patients or controls were under steroid or immunomodulating drug treatment during sampling. Samples were immediately frozen and stored at -80°C until investigation. EDSS scores of MS patients were evaluated during blood sampling. Magnetic resonance imaging (MRI) data were not available for all patients and are not reported here. Informed consents were obtained from all patients and controls. The study was approved by the Institutional Review Board of Haydarpasa Numune Training and Research Hospital.

Autoantibody measurements

Blood tests for systemic autoantibodies including anti-nuclear antibody (ANA), anti-double stranded DNA (anti-dsDNA), anti-neuronal nuclear antibody (ANNA), anti-neutrophil cytoplasmic antibody (ANCA), IgG/IgM anti-cardiolipin antibodies (ACA) and anti-thyroid peroxidase (TPO) antibodies were performed. Anti-TPO was detected by a chemiluminescence immunoassay using Roche Elecsys 1010/2010 (Roche Diagnostics, Mennheim, Germany) immunoassay analyzer. Values over 35 IU/mL were considered as positive. ANA, anti-dsDNA, ANNA and ANCA were analyzed via an indirect immunoflorescence assay (IFA) method. ACA IgG and IgM were detected by enzyme linked immunosorbent assay (ELISA) and the test was considered positive when ACA IgG was over 15 U GPL or ACA IgM was over 10 U MPL.

OCB detection

In all MS patients, the CSF samples were collected by routine lumbar puncture as a part of the standard diagnostic procedures. OCBs were determined in both CSF and sera of MS patients using agarose gel electrophoresis and isoelectric focusing. pH gradient strips were subjected to focusing steps with samples in focusing buffer. Strips were then press-blotted onto a nitrocellulose membrane under a 2-kg weight for 1 hour at room temperature. The bands were visualized by direct silver staining. Type 2 (oligoclonal IgG bands in CSF, not in the serum sample) and Type 3 (oligoclonal IgG bands in CSF and additional identical oligoclonal bands in CSF and the serum sample) patterns of OCBs were considered as positive.

Statistical analysis

Age, gender, OCB findings and EDSS scores of RRMS patients and their concordance with the presence of autoantibodies were statistically investigated. Statistical analysis was performed using Fisher's exact test for antibody seropositivity, OCB status and gender parameters, by Student's t-test for the age parameter and antibody levels and by Mann-Whitney U test for the EDSS parameter. The significance level was considered as $p<0.05$.

RESULTS

RRMS patients versus healthy controls

One hundred fifty RRMS patients (105 women; mean age, 38.1 ± 11.91) and 150 gender and age-matched healthy controls (101 women; mean age, 39.4 ± 16.47) were included. OCB were detected in 80% of RRMS patients (Table 1). Among 150 RRMS patients, 20%, 22%, 24%, 4%, 2%, 1%

and 1% were seropositive for anti-TPO, ACA IgG, ACA IgM, ANA, dsDNA, ANNA and ANCA antibodies, respectively. Anti-TPO and ACA IgM antibodies were detected in 2% and 4% of the healthy controls, respectively. All of the other autoantibodies were negative in the control group (Table 1). The positivity rates for TPO, ACA IgG and ACA IgM antibodies were significantly higher in RRMS patients as compared to healthy individuals ($p<0.001$ by Fisher's exact test, Table 2), whereas seropositivity for other antibodies was not significantly higher in the RRMS group.

Anti-TPO in RRMS patients

The mean anti-TPO values in the RRMS and control groups were 122.68 ± 202.62 IU/mL and 10.14 ± 5.43 IU/mL, respectively and this difference was statistically significant ($p<0.001$ by Student's t-test). No significant differences were found beween anti-TPO positive and negative RRMS patients regarding OCB status, gender, age and EDSS scores ($p=0.722$, $p=0.351$, $p=0.253$ and $p=0.166$; Table 2).

ACA IgG and ACA IgM in RRMS patients

The mean ACA IgG and ACA IgM values in the RRMS group were 16.77±4.93 GPL U/mL and 12.05±4.78 MPL U/mL, respectively, in the control group the values were 4.09±2.01 GPL U/mL and 3.55±3.08 MPL U/mL, respectively and these differences were statistically significant ($p<0.001$ by Student's t-test). There were no significant differences between ACA IgG positive and negative RRMS patients by means of OCB status, gender, age and EDSS scores ($p=0.197$, $p=0.468$, $p=0.398$ and $p=0.182$; Table 2). Likewise, no significant differences were found between ACA IgM positive and negative RRMS patients regarding OCB status, gender, age and EDSS scores ($p=0.416$, $p=0.304$, $p=0.484$ and $p=0.283$; Table 2).

CONCLUSION

Over the past few decades, the pathogenesis of MS has been recognized to be far more complicated than previously predicted. While T-cell mediated immune mechanisms were once considered to be predominantly responsible for MS induction, the B-cell mediated mechanisms are now known to have a significant influence on MS pathogenesis (11). The presence of immunoglobulin abnormalities such as CSF OCB and serum autoantibodies are among the factors implying that B cell-related immune processes might participate in MS pathogenesis (1, 10, 16, 17). Although some reports have demonstrated that the presence of OCB does not evidently influence the MS course and disability (10, 19), the significance of the systemic autoantibodies and accompanying autoimmune diseases in MS pathogenesis is not clearly understood.

To determine whether the systemic autoimmune processes trigger or somehow influence the OCB formation and progression of disability, we investigated several autoantibodies in the sera of MS patients and healthy controls. While OCB positivity of our cohort (80%) was relatively lower than many previously published studies, it was compatible with a recent report demonstrating reduced OCB positivity in Turkish MS patients (7). Additionally, we found significantly increased anti-TPO and anti-cardiolipin antibody positivity in MS patients, as previously reported (2, 6, 14).

Abnormal thyroid function results have been reported in 8%-33% of MS patients and a high frequency of anti-TPO antibodies has been shown in various MS cohorts (2, 12, 13, 15). Also, thyroid antibody positivity has been associated with reduced EDSS scores in two studies (2, 14). While our anti-TPO positive RRMS patients also showed trends towards displaying reduced EDSS scores, this difference did not attain statistical significance. To our knowledge, the association between thyroid antibodies and OCB has never been investigated. Our study showed that, in a similar manner to EDSS scores, anti-TPO positive and negative patients had comparable OCB positivity rates.

Previous studies have revealed a relationship between ACA positivity and MS, optic neuritis and transverse myelitis and the reported rates for ACA positivity in different MS cohorts range between 5% and 33% (6). However, conflicting results have been reported on the association between ACA and clinical or demographic features of MS. While a single study suggests that ACA positivity is associated with a benign MS course (9), two other reports (6, 20) and our results contradict this finding. Also, in contrast with two reports indicating an association between ACA positivity and the absence of OCB (9, 20), in our study, most ACA positive MS patients displayed OCB and ACA positivity was relatively higher in OCB positive MS patients than OCB negative ones. Our results are in keeping with those of Heinzlef et al., who have not determined a correlation between ACA and age, gender, clinical symptoms, MS type, clinical course or MRI findings (6). These conflicting results might be caused by patient selection criteria and the influence of immunosuppressive treatments. In our study, we investigated a homogeneous cohort composed of RRMS patients who were not under treatment during blood sampling and thus avoided the interference of these factors.

In conclusion, our results suggest that the positivity of systemic autoantibodies in MS is a non-specific finding reflecting a general autoimmune hyperactivity or dysregulation and has no appreciable clinical significance.

Table 1. The positivity rates of the autoantibodies in multiple sclerosis patients and healthy individuals.

Antibodies	Multiple sclerosis patients n (%)	Healthy individuals n (%)
OCB	120 (80)	NA
ANNA	1 (0.7)	0 (0)
ANCA	1 (0.7)	0 (0)
ANA	6 (4)	0 (0)
Anti-dsDNA	2 (1.3)	0 (0)
Anti-TPO	30 (20)	3 (2)
ACA IgG	33 (22)	0 (0)
ACA IgM	36 (24)	6 (4)

OCB: Oligoclonal bands; NA: not applicable; ANNA: Anti-neuronal nuclear antibodies; ANCA: Anti-neutrophil cytoplasmic antibodies; ANA: Anti-nuclear antibody; Anti-dsDNA: anti-double stranded DNA; Anti-TPO: Anti thyroid-peroxidase antibodies; ACA: Anti-cardiolipin antibodies

Table 2. Comparison of clinical and demographic features of autoantibody positive and negative subjects.

	Anti-TPO			Anti-cardiolipin IgG			Anti-cardiolipin IgM		
	Negative	**Positive**	**p**	**Negative**	**Positive**	**p**	**Negative**	**Positive**	**p**
MS patients (n=150) n (%)	120 (80)	30 (20)	**<0.001**	117 (78)	33 (22)	**<0.001**	114 (76)	36 (24)	**<0.001**
Healthy controls (n=150) n (%)	147 (98)	3 (2)		150 (100)	0 (0)		144 (96)	6 (4)	
OCB negative MS patients (n=30) n (%)	21 (70)	9 (30)	0.722	18 (60)	12 (40)	0.197	27 (90)	3 (10)	0.416
OCB positive MS patients (n=120) n (%)	99 (83)	21 (17)		99 (83)	21 (17)		87 (72)	33 (27)	
Female MS patients (n=105) n (%)	83 (79)	22 (21)	0.351	78 (74)	27 (26)	0.468	75 (71)	30 (29)	0.304
Male MS patients (n=45) n (%)	37 (82)	8 (18)		39 (87)	6 (13)		39 (87)	6 (13)	
Age of MS patients (mean ± SD)	37.8 ± 11.7	39.4 ± 11.6	0.253	38.2 ± 9.8	37.6 ± 12.5	0.398	38.1 ± 10.4	38.1 ± 11.7	0.484
EDSS of MS patients (mean ± SD)	3.8 ± 2.4	3.3 ± 2.6	0.166	3.2 ± 2.2	3.7 ± 2.9	0.182	3.5 ± 2.2	3.9 ± 2.8	0.283

Anti-TPO: anti thyroid-peroxidase antibodies, MS: multiple sclerosis, OCB: Oligoclonal bands, SD: standard deviation, EDSS: Expanded Disability Status Scale.

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