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Annexin V antibodies in multiple sclerosis and SLE/APS

Research Article

Marta Baleva[1]*, Detelina Stoilova[2], Petko Shotekov[2], Krasimir Nikolov[1]

1 Department of Clinical Immunology, University Hospital Alexandrovska, 1.G. Sofiiski Str., Sofia 1431, Bulgaria

2 Clinic of Neurology, University Hospital "St Ivan Rilski", Sofia, Bulgaria

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Abstract: Multiple sclerosis (MS) is an autoimmune disease with unclear etiopathogenesis. Some MS patients have anticardiolipin (ACL), anti-beta-2-glycoprotein-I (B2GPI) and anti-annexin V (AnV) antibodies. These antibodies can also be found in systemic lupus erythematosus with antiphospholipid syndrome (SLE/APS). The aim of our study was to compare the levels of ACL, B2GPI and AnV antibodies in MS and SLE/APS. Materials and methods: We investigated serum levels of IgG and IgM ACL, B2GPI and AnV in 21 MS patients, 30 SLE/APS patients and 30 controls using ELISA. Results: Mean levels of IgM and IgG ACL and B2GPI in MS were comparable with controls and lower than SLE/APS ($p<0.05$). Mean levels of IgM AnV in MS were higher compared to SLE/APS and controls ($p<0.05$); mean levels of IgG AnV in MS were higher than normal but similar to SLE/APS ($p>0.05$). Discussion: The results show that MS with negative "classic" autoantibodies (ACL and B2GPI) and without clinical data for antiphospholipid syndrome may have other positive antiphospholipid antibodies, such as AnV. Larger studies are needed to clarify whether AnV are epiphenomenon of the vascular and organ damage or they play a pathogenic role in the development of MS.

Keywords: *Multiple sclerosis • SLE/APS • Antibodies to annexin V*

1. Introduction

Until now, there have been no systematic investigations on the prevalence of anti-annexin antibodies (AnV) in MS patients. The data concerning the significance of AnV and antophospholipid antibodies (APL) for the development of thrombophilia in other diseases and conditions remain controversial. Anti-annexin V antibodies have a high prevalence in patients with systemic lupus erythemasus (SLE) [1-3] and rheumatoid arthritis (RA) [4] but currently there is no consensus on whether they are associated with certain disease features [5,6]. Some authors suggest that the presence of different antibodies, including AnV, in antiphospholipid syndrome (APS) could explain the differences in the clinical manifestations in this disease [7]. Rodrigues-Garcia et al. [5] suggests that the extracellular annexin V could play the role of antigenic stimulus for the production of AnV. These autoantibodies could participate in the pathogenesis of RA by inhibiting annexin V, the binding of type II collagen, the inhibition of phospholipase A2and the Fc receptor activity. The attempts to better characterize these autoantibodies in APS (with the APS being a heterogenous disease) have suggested that some plasma co-factors, such as beta-2-glycoprotein I, could be important for the action of ACL [8,9]. Similarly, prothrombin [10,11], beta-2-glycoprotein I [12], annexin V [13] appear to be important for the lupus anticoagulant (LA).

In the medical literature, there is a wide number of publications on the diagnosis of MS and its differentiation from other diseases and conditions, especially the neuro-psychiatric manifestations of systemic lupus erythematosus with antiphospholipid syndrome (SLE/APS) [14]. The underlying mechanisms of the MS-like manifestations in APS patients include molecular

* *E-mail: marta_baleva@yahoo.com*

mimicry with myelin and other antigens in the central nervous system (CNS), microvascular thrombosis and autoimmune CNS vasculitis similar to that in SLE/APS. The APL shows cross-reactivity to myelin, myelin-binding proteins and cerebral phospholipids kephaline and sphingomyelin [15,16].

APL has been found in variable number of MS patients. Some authors suggest that the detection of APL could have diagnostic and prognostic significance; however the data from the literature is very heterogenic. Some authors found positive anticardiolipin antibodies (ACL) in 5.3% [17] and 6.2% [18] of the studied MS patients. Sugyama and Yamamoto [19] detected IgM ACL in 44% and IgG ACL in 9% of MS patients. Bidot et al. [20] studied the prevalence of a wide spectrum of APL (antibodies against cardiolipin, beta-2-glycoprotein I, factor VIIa, phosphatidylcholine, phosphatidylserine and phosphatidyl ethanolamine) in MS and found only IgM but not IgG APL in a large number of patients – both during remission and during disease exacerbation. The authors found at least one IgM APL in 88 % of the patients during MS flair, and during remission this prevalence was lower – 57%. Ijdo et al. [21] also detected high prevalence of APL, mainly of IgM class (88%) in MS patients. Roussel et al. [22] suggest that despite anti-beta-2-glycoprotein I antibodies are found in 32% of the studied MS patients, they show no correlation with the disease manifestations and progression. Heinzief et al. [23] found positive ACL in 15% of 285 MS patients, mainly of IgM class, and these antibodies showed no correlation with the disease manifestations and evolution. Gard et al. [24] found APL in 55% of MS patients, mainly of IgM. The studies of Liedorp et al. [25] revealed no such correlations. The authors found positive ACL in 5.6% of a group of MS patients and despite the persistence of the detected ACL for a period of 12 weeks, none of the patients fulfilled the diagnostic criteria for APS.

The aim of our study was to investigate the prevalence of AnV in MS patients in comparison with SLE/APS patients and controls from the Bulgarian population.

2. Materials and methods

We investigated 21 Caucasian patients – 10 women (mean age 26.4 ± 8.6) and 11 men (mean age 33.1 ± 8.7) with relapsing-remitting MS without clinical or immunological symptoms of APS. The Expanded Disability Status Scale (EDSS) level of MS patients was ranging from 2.5 to 5. The mean duration of disease was 3.4 years. The study included also 30 Caucasian patients with SLE/APS (28 – women, 2 men, mean age 25 ± 6) and 30 Caucasian controls. All studied persons were investigated for IgG and IgM antibodies to: annexin V, cardiolipin, beta-2-glycoprotein I using ELISA (Orgentec, Germany). The data was processed using variation analysis (SPSS 13.0).

3. Results

High levels of AnV were detected in 11 MS patients (52%) – 5 of them with high IgM, and 6 with high IgM + IgG levels. MS patients had the highest levels of AnV and were significantly higher for the IgM class ($p<0.05$) when compared with SLE/APS and controls (Table 1).

Table 1. Levels of AnV in patients with multiple sclerosis, in systemic lupus erythematosus and controls.

	IgG (U/ml)	IgM (U/ml)
MS	6.68 ± 8.02	7.29 ± 8,1
SLE/APS	4.5 ± 3.59	2.72 ± 1.92
Controls	3.62 ± 1.95	1.74 ± 1.33

ACL levels were normal in all the studied MS patients and only 2 had elevated IgM anti-beta-2-glycoprotein I (B2GPI) antibodies. The mean levels of IgM and IgG ACL and B2GPI in MS patients were similar to these in controls ($p>0.05$) (Table 2).

Table 2. Levels of anti-cardiolipin (ACL) and anti-beta-2-glycoprotein I (B2GPI) antibodies in patient with multiple sclerosis (MS) and controls.

	ACL IgG (U/ml)	ACL IgM (U/ml)	B2GPI IgG (U/ml)	B2GPI IgM (U/ml)
Controls	11.38 ± 4.86	7.24 ± 4.81	3.62 ± 7.1	1.94 ± 1.31
MS	4.14 ± 3.39	1.56 ± 2.63	5.83 ± 2.66	0.81 ± 1.01

4. Discussion

The results of our study are in accordance with the investigations of Tournah et al. [18] and Liedorp et al. [25] who found low prevalence of ACL and B2GPI in MS patients, mainly IgM class. Many authors have suggested that the clinical features of MS could mimic APS. Cuadrado et al. [26] emphasize that the differentiation between APS and MS could be very difficult, especially when positive antinuclear (ANA) and/or APL are found. In such cases, thorough patient's history should be taken, especially concerning the development of miscarriages and thromboses in the past, along with careful evaluation of the localization of CNS lesions on MRI. Karussis et al. [27] suggest that the combination of MS and positive ACL could represent a different disease

entity, especially in the presence of atypical symptoms, such as headache, and in the absence of oligoclonal binding in the cerebro-spinal fluid (CSF oligoclonal binding). The authors found lower rates of deterioration of the myelopathic/spinocerebellar syndrome compared to these in the "classical" MS and suggested introduction of anti-platelet and anticoagulant agent in patients with positive ACL. Ferreira et al. [14] also emphasize that many SLE/APS patients often happen to be misdiagnosed in having MS, especially the atypical cases. Our previous studies [28] in 10 such patients (female patients with MS and livedo reticularis) showed that 7/10 patients had history of recurrent miscarriages and/or deep vein thromboses [DVT]. In 2 patients the presence of DVT was confirmed by Doppler-ultrasound examination, 4 had headache, 7 had different echocardiographic changes – mainly of the mitral valve, and 5 patients had high ACL levels. These findings made us investigate a wider group of MS patients without clinical signs of APS and add the AnV to the study panel. The small number of patients does not allow us to make general conclusion regarding the exact diagnostic and prognostic value of AnV in MS.

The diagnostic significance of AnV in autoimmune diseases and SLE/APS remains unclear. The majority of the investigations on AnV are in spontaneous abortions and the results are controversial. According to some authors AnV could represent a single risk factor for recurrent miscarriages [29]. Anti-annexin V antibodies could be important for the development of cerebral vascular endothelial damage in MS [30], having in mind that the research group led by Schoenfeld [31,32] has revealed the neurotropic effects of APL in animal models. Our literature search did not find any articles discussing the parallel investigation of AnV in MS and SLE/APS patients.

Our results show that MS patients without clinical and laboratory (negative ACL and B2GPI) findings for APS are AnV positive. Moreover these antibodies are higher in MS than SLE/APS. Further studies are needed to determine whether these autoantibodies take part in the development of MS or are merely an epiphenomenon.

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