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Original Contribution

CORRELATION BETWEEN ANTICARDIOLIPIN AND ANTI-BETA2 GLYCOPROTEIN I ANTIBODIES IN SCHIZOPHRENIC PATIENTS

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ABSTRACT

Antiphospholipid antibodies are a family of autoantibodies that exhibit a broad range of target specificities and affinities, all recognizing various combinations of phospholipids, phospholipid binding proteins or both. We studied serum levels of antibodies to cardiolipin (aCL) and beta2 glycoprotein I (anti- β2GPI) of IgG and IgM isotype in 23 patients with schizophrenia. Our results showed that aCL and anti-β2GPI of both isotypes had elevated serum levels in this patient population. Positive correlation between aCL and anti- β2GPI of IgM isotype was found. These results suggest that β2-GPI is associated with aCL and may be the actual epitope to which aCL are binding. Thus, aCL in the population studied are more likely β2-GPI-dependent antibodies. On the basis of our results we may conclude that β2-GPI-dependent aCL antibodies and antibodies to β2-GPI are elevated in schizophrenic patients.

Key words: antiphospholipid antibodies, schizophrenia

INTRODUCTION

Altered immune functions and the involvement of autoimmune elements in the aetiology and pathophysiology of schizophrenia have been discussed for decades (1-4) Patients with schizophrenia are shown to possess a range of antibodies including antinuclear, anti-DNA, and antiphospholipid (APL) antibodies (5-9).

The elevation of APL is of interest, as in recent years their association with thrombotic vascular events in autoimmune-related disorders has been highlighted (10). In this regard the most commonly reported antiphospholipids are anticardiolipin antibodies (aCL) (11).

Detailed information on a possible pathogenic role for aCL is still lacking at this time, but the antibodies are known to disrupt a number of cellular and circulatory functions (12). In schizophrenia, alterations in membrane phospholipids of both erythrocytes and platelets have been demonstrated (13), and such abnormalities were also reported in the frontal lobe of drug-naïve first-episode schizophrenic patients (14), raising the possibility that aCL plays some pathogenic role in this disease.

However, data from studies on aCL in schizophrenic patients are still controversial. Chengappa et al. reported a rise in aCL in both treated and never-treated patients. In another study increased levels of aCL were observed in patients and their first-degree relatives (6). In contrast, Sirota et al. demonstrated reduced aCL in first episode and chronic schizophrenia (15). Discrepancies may be due to (i) heterogeneity of schizophrenia, (ii) medication status and (iii) sensitivity and specificity of the techniques used.

One of the most difficult issues in aCL-testing is low specificity for the antigenic target. Clinically significant aCL detected in the aCL ELISA are in fact largely directed to a plasma protein, beta2 glycoprotein I (β2GPI), with the β2GPI itself binding to phospholipid in the assay (16,17). In addition, antibodies from some patients may also be directed to phospholipid itself, or other

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plasma proteins such as prothrombin, annexin V, C and S proteins (18). An increase in specificity can be achieved with a phospholipid-free anti- β2GPI ELISA. Moreover, patients with autoimmune conditions, especially antiphospholipid syndrome, may have a negative aCL result but may be positive for anti- β2GPI in isolation (20).

For the above-mentioned reasons we define the object of our study as to determine and compare the serum levels of aCL and anti- β2GPI in schizophrenic patients.

MATERIALS AND METHODS

Subjects

Serum was obtained from twenty-three patients [10 males and 13 females; age (mean ± SD: 42.38±7.24)] who met the DSM-IV diagnostic criteria for schizophrenia and was admitted to the hospital following exacerbation of psychosis. Twenty sex- and age-matched healthy donors [9 males and 11 females; age (mean ± SD: 42.95±9.67)] were recruited. The study was approved by the Ethics Committee of Psychiatric Hospital "D-r G. Kisjov", Radnevo. The patients gave their written informed consent to participate in the study by signing a consent form that was approved by the same Committee. Other criteria for including in the study were: age between 19 and 55 years, lack of any forms of psychiatric co-morbidity and acute and chronic disorders associated with known alterations in immune function (as determined by the history, physical examination and routine laboratory tests). All patients were drug-free for at least three months before entering the study.

The controls, which are mainly staff members, were free of neuropsychiatric disorders; they had no history of substance abuse or systemic disorders known to be associated with immunological abnormalities; neither the control subjects nor the patients were receiving immunosuppressive drugs.

Serum sample

Ten millilitres of non-heparinised venous blood was drawn and allowed to clot at room temperature. The sera were separated on the same day and stored at -70°C until analysis.

Determination of aCL

For the detection of aCL from IgG and IgM classes, quantitative commercial kits were used (*The Binding Site, UK*). Microwells were pre-coated with bovine cardiolipin and human β2-GPI as a cofactor. The assays were calibrated against Louisville diagnostic standards – LAPL-GM-100 for IgG-aCL and IgM-aCL. A serum dilution of 1:100 was used in all assays. The test samples were run in duplicates along with five standards (6.25; 12.5; 25; 50; 100 U/ml). Peroxidase labelled anti-human IgG and IgM, respectively, were used as second antibodies. The reaction was visualized with 3,3', 5,5' TMB as a substrate and read at 450 nm. Following manufacturer's instructions, values exceeding 11 U/ml for IgG-aCL and 10 U/ml for IgM-aCL were regarded positive.

Determination of anti- β2GPI

For the detection of anti- β2GPI from IgG and IgM classes, quantitative commercial kits were used (*The Binding Site, UK*). Microwells were pre-coated with human β2-GPI as an antigen. The assays are calibrated in U/ml against an internal arbitrary reference calibrator for IgG- anti-β2GPI and IgM- anti-β2GPI. The test samples were run in duplicates along with five standards (3.7; 11.1; 33.3; 100; 300 U/ml). Peroxidase labelled anti-human IgG and IgM, respectively, were used as second antibodies. The reaction was visualized with 3,3', 5,5' TMB as a substrate and read at 450 nm. Following manufacturer's instructions, values exceeding 20 U/ml for IgG-aCL and 10 U/ml for IgM-aCL were regarded positive.

Data analyses

The results are expressed as mean ± SE. To establish the statistical significance of observed differences, the Fisher's exact test (frequencies) and Mann-Whitney U test (mean ranks) were used. Spearman's correlation coefficients were used to demonstrate the correlations between serum levels of aCL and anti- β2GPI. A value of $p<0.05$ was considered as indicating a significant difference.

RESULTS

There were no significant differences in age or sex distribution between study groups.

Anticardiolipin antibody levels of both isotypes (IgM and IgG) tested were significantly higher in patients (18.43±4.85 for IgM-aCL and 7,61±1,79 for IgG-aCL; mean ±SE) than in controls (5,59±0,64 for IgM-aCL and 2,31±0.29 for IgG-aCL; mean ±SE) (Mann-Whitney U-test: p=0.014 and p=0.0001 respectively) **(Figure 1)**.

The similar result with higher levels in patients (9,26±4,45 for IgM- anti-β2GPI and 3,25±0,51 for IgG- anti-β2GPI; mean ±SE) compared to controls (5,59±0,64 for IgM-anti-β2GPI and 1,55±0,21 for IgG- anti-β2GPI; mean ±SE) (Mann-Whitney U-test: p=0.001 and p=0.001 respectively) was observed for serum levels of anti-β2GPI antibodies (**Figure 2**).

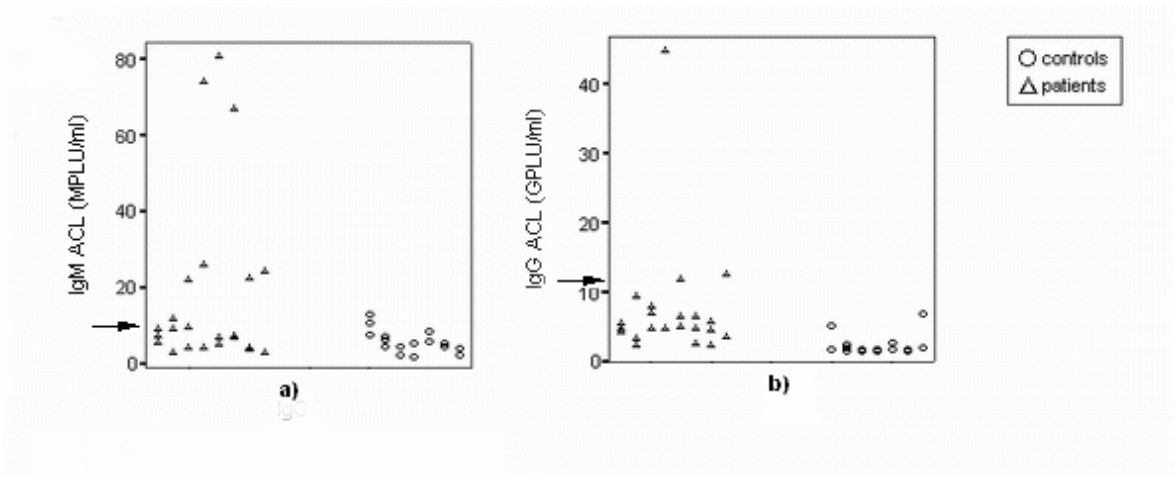

Figure 1. *Serum levels of IgM (a) and IgG (b) anticardiolipin antibodies (aCL) in patients and controls. Cut-off values are marked by arrows. p=0.014 for IgM-aCL and p=0.0001 for IgG-aCL compared to controls*

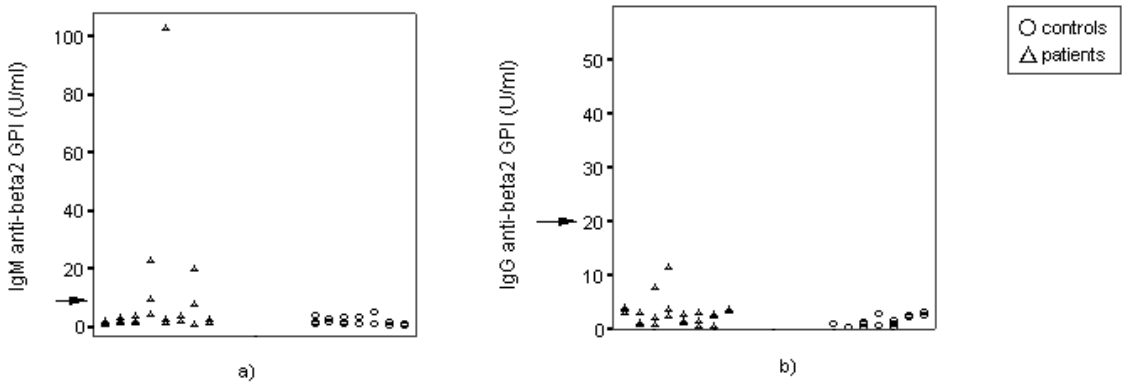

Figure 2. *Serum levels of IgM (a) and IgG (b) anti-beta2-glycoprotein I antibodies (anti-β2 GPI) in patients and controls. Cut-off values are marked by arrows. p=0.001 for IgM- anti-β2GPI and p=0.001 for IgG-anti-β2GPI compared to controls*

IgM-aCL were significantly more frequent in patients (9/23) than in controls (2/20) (Fisher's exact p=0.039). There were no significant differences in number of positive sera for IgG-aCL observed in patients (3/23) and controls (0/20). Frequency of IgM anti-β2GPI did not differ significantly in patients (4/23) and controls (0/20). We did not find any positive for IgG anti-β2GPI sera in either the patients or the controls.

A significant positive correlation between aCL and anti-β2GPI IgM levels (R=0.582 and p=0.004) in patients was found.

DISCUSSION

This study has detected higher levels of aCL of both IgG and IgM isotypes in chronic schizophrenic patients in acute exacerbation of the disease as compared to healthy individuals. This observation was supported by the results from previous reports for elevated aCL levels in this mental disease. Canoso et al. (20) found a higher incidence of these antibodies in patients treated with neuroleptics. However, other authors demonstrated elevated levels of aCL in drug-naïve and drug-free schizophrenic individuals (5,6). Our patients were also drug-free (for at least 3 months before admission) and, furthermore, they were free of any clinical and or laboratory evidence of either acute or chronic disease known to influence immune functions or to be a result of immune dysfunction. Thus, we are prone to accept that the aCL measured in this population are not drug-mediated, although such a possibility cannot be fully excluded.

The levels of anti-β2GPI in our patients are also elevated. These autoantibodies are most characteristic for antiphospholipid syndrome and show a well-marked association with thrombosis. They are thought to have a direct pathogenetic role in the development of this symptom (21). According to their levels in schizophrenic patients the review of the literature failed to present any previous results. To our knowledge this is the first report on the association of anti- β2GPI antibodies (IgG, IgM type) with schizophrenia.

In previous years growing interest has turned to the significance of β_2GPI in the binding of aCL to cardiolipin. Anticardiolipin antibodies can be detected by solid-phase immunoassay using cardiolipin as antigen, but binding of aCL antibodies depends on the presence of β2GPI (22-24). β_2GPI is a phospholipid binding plasma protein which acts as a natural anticoagulant (25,26) and also participates in immune clearance (27, 28). Hypothetical mechanisms of its significance for the detection of aCL include: a) the aCL recognizes a cardiolipin/β_2GPI complex, b) the β_2GPI is the target for aCL and c) the actual epitope is present on the protein proper not on the cardiolipin (24,29). Thus, aCL can be classified as β_2GPI independent aCL antibodies ('true' aCL, binding to native cardiolipin), β_2GPI-dependent aCL antibodies (requiring the protein as cofactor) and, a third group of directed to β_2GP-I antibodies (in the absence of phospholipids).

For the detection of aCL in samples of schizophrenic patients, we used for the first time ELISA kits where microwells were pre-coated with bovine cardiolipin and human β2-GPI as a cofactor.

Our study revealed a positive correlation between aCL and anti-β2-GPI antibodies of IgM isotype in schizophrenic patients. These results suggest that β2-GPI has been associated with aCL and may be the actual epitope to which aCL are binding. Thus, aCL in the population studied are more likely β2-GPI-dependent antibodies.

On the basis of our results we may conclude that β2-GPI-dependent aCL antibodies and antibodies to β2-GPI are elevated in schizophrenic patients.

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