ORIGINAL ARTICLE

Anticardiolipin Antibody Isotype Profile in Lupus Nephritis - A Cross Sectional Survey

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Summary

Thirty-six patients with lupus nephritis (LN) attending the Nephrology Clinic, Hospital Kuala Lumpur were studied for the prevalence of anticardiolipin antibody (ACA) isotypes (IgG and IgM) and other associated antibodies, antinuclear antibody (ANA) and anti-ds DNA antibody and to determine the possible association between serological and clinical parameters. The study population consisted of 20 (55.6%) Malays, 15 (41.7%) Chinese and 1 (2.8%) Indian with a mean age of 31.4 ± 11.3 years, range 14 to 60 years. The female to male ratio was 11:1. The average time between diagnosis and blood sampling was 4.4 years (range 0.25 to 15 years). Increased ACA levels were found in 20 (55.6%) patients where raised IgG ACA and IgM ACA were observed in 20 (55.6%) and 2 (5.6%) cases respectively. ANA and anti-ds DNA antibodies were detected in 22 (61.1%) and 4 (11.1%) individuals respectively, with the majority (82%) showing a speckled pattern of nuclear staining. However, neither the IgM ACA nor IgG ACA showed any significant association with thrombosis or any other clinical parametres. Our preliminary study indicates that ACA is a frequent finding in lupus nephritis and that the IgG isotype is more prevalent.

Key Words: SLE, Lupus nephritis, Anticardiolipin antibody

Introduction

Many studies have demonstrated the presence of circulating anticardiolipin antibodies (ACAs) in patients with systemic lupus erythematosus (SLE)[1,2] but may also occur in patients without SLE or other connective tissue diseases[3,4]. These antibodies can either be detected by the lupus anticoagulant or the enzyme-linked immunosorbent assay (ELISA) ACA test. The latter is more generally accepted since it is more sensitive, easier to perform and has recently been standardised. Recent investigations have shown that the ACAs are present in about 40% of patients with SLE but reported frequencies range from 23 to 82%[5-10]. SLE presents with a wide spectrum of clinical and laboratory manifestations. A variable portion of these patients present with the antiphospholipid syndrome which is now recognized as a separate clinical entity [11,12]. It is characterized by recurrent arterial or venous thrombosis[13], thrombocytopenia,[14] and recurrent spontaneous abortions[15]. In Malaysia, SLE is not an uncommon disease with a reported prevalence of ACA to be 16.5%,[16] among these patients. However, in this study we only investigated the prevalence of IgG and IgM ACA isotypes in a group of SLE patients with nephritis and determined whether any correlation existed between clinical and laboratory findings.

Materials and Methods

Subjects in this study consisted of 36 patients attending the Nephrology Clinic at the Institute of Nephrology, Hospital Kuala Lumpur and who satisfied at least four

of the American Rheumatism Association (ARA) Revised Criteria for the classification of SLE[17] They were patients who clinically had urinary abnormalities or biopsy-proven lupus nephritis. Five ml of venous blood was drawn from each patient and left to clot. Samples were centrifuged and sera stored at -20°C until the assays were performed.

A clinical interview was carried out with the aid of a prepared questionnaire followed by physical examination of each recruited patient.

IgG and IgM ACA isotypes were measured using an enzyme-linked immunosorbent assay kit obtained commercially (Medical Innovations, Australia). Briefly, test samples, standards and controls were diluted 1/20 with diluent before 50ul of test samples and controls were added to cardiolipin-coated polystrene microtiter plates. It was left to incubate for 30 minutes at room temperature. Plates were washed with 3 changes of phosphate-buffered saline (PBS) and 50 ul of sheep anti-human IgG (Fc) peroxidase conjugate or IgM conjugate diluted 1/500 in PBS-10%Neonatal calf serum was added to each well. This was again left to incubate for a further 45 minutes at room temperature. Plates were again washed before adding 50 ul per well of substrate and left to react at room temperature. Reaction was stopped after 15 minutes by the addition of a stopping solution (2.5% sodium fluoride) and read with an ELISA reader at a single wavelength of 405 nm. This kit provided a negative (samples absorbed with cardiolipin) and a positive control, and 6 samples with known ACA levels from which a standard curve was constructed, plotting mean optical density versus ACA concentration. Test sample values were then interpolated. Standards provided have been calibrated to the international reference standards. The cut-off points were determined by quantitating ACA levels of 182 blood donors. Values of ≥7.125U/ml and ≥6.995 U/ml were taken to be positive for IgG and IgM ACA respectively.

ANA and anti-ds DNA antibodies were determined by indirect immunofluorescence (IIF) technique using rat liver and *Crithidia Lucille* as substrates respectively. A serum titre of ≥1:20 for ANA was considered positive. Antimitochondrial (AMA), antismooth muscle (ASMA) and antiparietal cell antibodies (APA) were also determined by IIF.

Statistical Analysis

The association of ACAs with different clinical and laboratory parameters were statistically tested by chi-square analysis and Fischer's exact test where appropriate. Significance level was set at $p<0.05$.

Results

A total of 36 patients were enrolled into the study. They comprised of 20 (55.6%) Malays, 15 (41.7%) Chinese and 1(2.8%) Indian with a mean disease duration of 2.5 years. There were 33 (91.7%) females and 3 (8.3%) males giving a sex ratio of 11:1. The median age was 29.5 years (range 14-60 years). The demographic data of the patients are shown in Table I.

Frequency of ACA

Using the 95th centile of ACA levels in 182 healthy blood donors as cut-off points, 20 (55.6%) were found to have increased levels of IgG and 2 (5.6%) with increased levels of IgM. Two (5.6%) had both isotypes increased.

Table I
Demographic data of the patients studied

Data	N=36	Percentage
Male	3	8.3
Female	33	91.7
Median age (years)	29.5	
Race: Malay	20	55.6
Chinese	15	41.7
Indian	1	2.8
MANIFESTATION:		
Cutaneous	27	75
Musculo-skeletal	22	66.1
Cardio-respiratory	3	8.3
Neurological	11	30.6

Correlation of ACA and Clinical Manifestations

The disease duration did not correlate with the presence of ACA. The following variables were tested for association with ACA: age, sex, race, onset, mucocutaneous symptoms (malar rash, photosensitivity, bullous LE, maculopapular rash, discoid LE, vasculitis, livedo reticularis, oral ulcer, alopecia, and Raynaud's phenomenon), musculoskeletal (arthralgia, arthritis, nodules, deforming arthritis and myositis), cardio-respiratory (myocarditis, pleural/cardial effusion and pulmonary hypertension) and neuro-psychiatry (psychosis, impaired cognition, seizure, cerebro-vascular accident and headache), (as shown in Table II). No significant correlation was found between the presence of ACA and the manifestations described above.

Thrombosis and Thrombocytopenia

Three (8.3%) subjects had a reported event of cerebro-vascular accident and another 3 (8.3%) had demonstrated periods of thrombocytopenia (platelet count below 120,000 x 10^9/L). Two patients had raised IgG and one of them had an associated raised level of IgM. However, we did not find any correlation between raised ACA levels and thrombosis or thrombocytopenia.

Abortion

From the 33 women, only 5 had history of a cumulative number of 7 pregnancies and 2 of them had experience one episode of abortion each. Thus we were not able to perform any statistical analysis. However, while one was positive for both isotypes, the other one was negative.

Correlation of ACA and Laboratory Findings

The majority of samples positive for ANA (75%) exhibited a speckled pattern. No correlation was found between ACA and serological parameter (Table III).

Discussion

Anticardiolipin antibodies have been known to occur in a subset of patients with SLE manifesting as recurrent arterial or venous thrombosis, thrombocytopenia and recurrent abortion. This clinical entity has been termed the antiphospholipid syndrome. Initial studies reported highly variable prevalence of circulation ACA in these patients. The reported prevalence of ACA in Malaysian SLE patients was 16.5% [16].

We investigated the prevalence of IgG ACA and IgM ACA isotypes in patients with lupus nephritis and

Table II
ACA Correlation with clinical findings

Manifestation	ACA positive		ACA Negative	
	N=20	Percentage	N=16	Percentage
Cutaneous	17	85	11	68.8
Musculo-skeletal	11	55	6	37.5
Cardio-pulmonary	0	0	3	18.75
Neurological	6	30	5	31.25

determined the association of these antibody isotypes with clinical and laboratory features. The prevalence of elevated IgG ACA in 20 (55.6%) and 2 (5.6%) for IgM ACA is in accordance with that observed in other studies [18,19]. However our study population was more selective, comprising of patients with a relatively more active disease as opposed to a non-selective SLE group studied earlier[16]. McHugh et al[20] suggested that IgG ACA was associated with renal disease, while another study found an increased association with renal disease probably due to a possible decrease in the antiphospholipid titres as a result of treatment[21].

The prevalence of ACAs from the University Hospital Kuala Lumpur study group was 16.5%, with 13% having raised IgG ACA and 2.5% with raised IgM isotype[16]. The ACA in this study group was low with rare association with thrombosis. A study among the Indian SLE population in India[22] also reported this finding which was thought to be related to genetic and environmental factors. The circulating ACA may not be a serological marker but only appear as a part of the immune process. It may also not be an independent risk factor for thrombosis or recurrent abortions[6,2,23].

Discrepancies in results have been due to a number of factors. The sensitivity of various assay techniques have undoubtedly contributed to the wide range of reported frequencies. Investigators have used different techniques of interpreting positive cases. Cut-off points have been analysed differently. The definition of a cut-off point for seropositivity is yet to be determined. The ELISA technique has been widely used but the specificity for different clinical associations appear to be different[5,9,10,14,15]. Recent efforts were directed at introducing standardized assays for ACA detection. An international workshop was held with the aim of minimizing the problem of discrepancies in results obtained.

In this study we found no association between the ACA positivity and thrombosis but numbers are too small to draw further conclusions especially since lupus nephritis has not been reported to be associated with thrombosis[24]. We had three cases who experienced cerebro-vascular accident, where two of them had raised IgG ACA with one having associated raised level of IgM ACA. It has been observed that IgM ACA have a low damaging capacity while IgG ACA have high damaging potential[25]. Thus ACA predominantly of the IgG isotypes may be of pathogenetic relevance.

Changes in ACA concentrations are a common phenomenon in patients with SLE [26,27] where variability and fluctuation in ACA titres occur in the course of the disease[28]. We did not classify our patients according to their disease activity. Blood samples are taken only once. A prospective study would be more accurate to determine the relationship between ACA levels and disease activity as IgG ACA have been reported to be significantly associated with disease activity whereas IgM ACA was much less influenced[28]. In conclusion, this study shows that the rate of 55.6% ACA positivity is in accordance with those found by investigators elsewhere and that the IgG ACA is the predominant isotype.

Acknowledgement

The authors would like to thank the Director of the Institute for Medical Research for permission to publish this paper.

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