ORIGINAL ARTICLE

Antiphospholipid Antibodies and Stroke in the Young — A Study of Three Cases

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Summary

Cerebral infarction in the young is likely to be non-atheromatous. While in previous studies no cause has been found in 40% to 50% of patients, an increasing role for haemorheological factors is becoming apparent. Among these, an association between antiphospholipid antibodies (aPLs) and ischaemic cerebrovascular disease is now well-recognised. This entity has not been previously reported in Malaysian patients. In a study of 80 patients with stroke below the age of 50 years who were seen at the University Hospital, Kuala Lumpur, between January 1982 and May 1992, 3 patients with ischaemic cerebral infarction were found to have aPLs. aPLs was detected using ELISA method for anticardiolipin antibodies (aCLs), and presence of lupus anticoagulant (LA) was established by kaolin clotting time, thromboplastin inhibition test and platelet neutralisation procedure. Only 1 patient had active systemic lupus erythematous. Cerebrovascular events were recurrent in one of the 2 non-lupus patients. aPL-related stroke should be considered in young patients who have cerebral ischaemia occurring without obvious cause. More cases are likely to emerge in Malaysia with active screening.

Key words: Antiphospholipid antibodies, anticardiolipin, lupus anticoagulant, young stroke.

Introduction

Cerebral infarction in the young is likely to be non-atheromatous. While in previous studies no cause was found in up to 40% to 50% of patients, recent reports suggest an increasing role of haemorheological factors as a cause of stroke. These include antiphospholipid antibodies[1] (aPLs), deficiency of proteins C[2] and S[3], and antithrombin III deficiency for venous infarction[4].

We report here 3 young patients who had stroke associated with aPLs[5]. aPL-related stroke has not been previously documented in Malaysian patients.

Materials and Methods

Three cases of stroke associated with aPLs were identified in a prospective-retrospective study of patients who presented with stroke below the age of 50 years. This study was carried out at the University Hospital, Kuala Lumpur, over the period January 1982 to May 1992. These patients were investigated for risk factors and systemic causes of stroke, and angiographic evidence of cerebral atherosclerosis. Brain CT was done in 38 patients and 22 had cerebral angiography. Search for cardiac disease was made by clinical, ECG and echocardiographic evaluation. When cause of stroke was not apparent, further haematological and immunological studies were performed: blood VDRL, aPL and lupus anticoagulant (LA) tests, and antithrombin III, proteins C and S levels.

Methodology

aPL solid-phase immunoassay

Specific IgG and IgM anticardiolipin (aCL) were detected by an enzyme-linked immunosorbent assay (ELISA)[6]. Cardiolipin (Sigma) (50 ug/ml in ethanol) was coated onto microtitre wells (Nunc-Immunoplate) by overnight evaporation at 4°C. The plate was washed twice with phosphate buffered saline (PBS), pH 7.2. Ten percent adult bovine serum (ABS, Sigma) was added for 2 hours to block non-specific binding sites and the plate washed 3 times in PBS. Test serum (1:50 dilution in PBS/10% ABS) was added for 2 hours. After washing with PBS, alkaline-conjugated goat anti-human IgG or IgM (Sigma) (1:1000 dilution) was added for 1 hour. After washing, binding was visualised by adding the enzyme substrate, p-nitrophenylphosphate disodium hexahydrate (Sigma) 1 mg/ml in diethanolamine buffer, pH 9.6 with 0.1% magnesium chloride. The colorimetric reaction was stopped by adding 3M NaOH. The absorbance was read at 405 nm. All steps in the ELISA were carried out at room temperature. Reaction volume was 50λ/well. IgG and IgM standards (Provided by Dr Graham Hughes, St Thomas's Hospital, London) were included in every assay. Results were expressed in GPL and MPL units for IgG and IgM respectively. The cut-off points for IgG aCL and IgM aCL were 5 GPL and 3 MPL units respectively. All positive results were repeated at least once.

Test for detection of lupus anticoagulant (LA)

All plasma samples were obtained by mixing freshly collected blood into 1/10 final volume of 0.11 M sodium citrate. Platelet-poor plasma (PPP) was obtained by immediate centrifugation at 2,500 g for 15 mins, followed by filtration through 0.22 μm membrane filters (Millipore). Freshly prepared PPP was kept frozen at -70°C until use. Lupus anticoagulant was detected by the following tests:

i. *Kaolin clotting time (KCT)*

The KCT was performed on mixtures of normal and test platelet-poor plasma as previously described[7]. Platelet-poor plasma (200 μl) was incubated with 100 μl kaolin (20 mg/ml) at 37°C for 3 mins. After addition of 200 μl 0.025 M calcium chloride, the clotting time was recorded. The ratios of pooled normal and test platelet-poor plasma were 10:0, 9:1, 8:2, 5:5, 2:8 and 0:10. The KCT Index was calculated according to the formula:

$$KCT = \frac{(PP + NP) - NP}{PP} \times 100$$

A test was considered to be positive when small quantities of test plasma led to prolongation of KCT>15%, and when the mixing curve was convex in the region near the left axis. With borderline increase of 13% to 15%, the test would be repeated in 3 months.

ii. *Tissue thromboplastin inhibition test (TTI)*[8]

To 100 µl of pooled normal plasma (control) was added 100 µl of thromboplastin (Stago) at 1:50 and 1:500 dilutions (where final dilutions were 1:100 and 1:1000). The mixtures were incubated at 37°C for 3 mins. 100 µl of 0.025 M calcium chloride was added. The clotting time was recorded. The procedure was repeated with the patient's plasma. Normal TTI was defined as Patient Time/ Control Time < 1.3.

iii. *Platelet neutralisation procedure (PNP)*[9]

This is based on the ability of platelets to bypass the LA or to significantly correct prolonged coagulation in various test systems. Outdated platelets from the blood bank were used to prepare aliquots of washed platelets which were frozen at -20°C until use. When the platelets were thawed at room temperature, 100 µl was added to 100 µl of patient's plasma together with aPTT reagent. Tris buffered saline was used as a parallel control. The test was positive if there was significant shortening (≤ 5 seconds) of the prolonged activated partial thromboplastin time (aPTT).

LA was considered to be present if both KCT and PNP were positive. PNP, which is based on the correction of prolonged clotting time by added phospholipid, increases specificity for LA[9].

Case Reports

Patient 1

A 13 year old Chinese schoolgirl with systemic lupus erythematosus (SLE) was admitted for tonic-clonic seizures. She also had fever, malar rash, arthritis and oliguria of 10 days duration. There had been a previous episode of lupus cerebritis. On examination, she was febrile but alert, with no focal neurological deficit. The liver was just palpable. Haemoglobin was 106 g/L, white cell count ranged from 2.5 to 14 x 10^9/L, platelets 165 to 522 x 10^9/L, and sedimentation rate 27 to 60 mm/hour. Tests of renal function were normal. Serum albumin was 31 g/L. Serological tests were consistent with active SLE: C_3 13 IU/L, C_4<5 IU/L, positive antinuclear antibody (ANA) titre of 1:64, and positive anti-DNA antibody. CSF was under slight pressure at 21 cm of water, with red cells 6/µL, white cells 2/µL (lymphocytes), sugar 2.7mmol/L and protein 0.33 g/L with no organisms. Initial electroencephalogram (EEG) showed focal slow wave activity over the right temporal and occipital regions.

High dose corticosteroids were given and urinary tract infection was treated. However, despite antiepileptic therapy, seizure activity escalated. She developed right hemiplegia and conjugate gaze palsy, while plantar responses became extensor. Cytoid bodies were noted. Repeat EEG confirmed deterioration, with frequent epileptic discharges localised to the right posterior quadrant. Brain CT showed diffuse cerebral atrophy. Echocardiography was normal. Intensive antiepileptic therapy was given. The illness resolved over 4 months, leaving residual hemiparesis. IgG aCL was positive at 60 GPL units; IgM aCL was negative. Tests for LA were not done. Two years later, the patient was readmitted with status epilepsy, without new focal neurological deficit. She expired within 3 days of admission.

Patient 2

A 15 year old Chinese schoolboy developed repeated episodes of transient left hemiparesis and dysarthria, each lasting 5 to 10 mins. The last episode did not resolve. Past history was unremarkable. He was admitted with blood pressure of 140/90 mmHg and regular pulse of 60/minute. He had left hemiparesis with VIIth nerve palsy. Haemoglobin was 161 g/L, total white cell count 6.7 x 10^9/L and platelet count 356 x 10^9/L. Sedimentation rate was 3 mm/hour. Risk factors for atherosclerosis were absent. Cardiac disease was excluded. ANA was not detected. Brain CT showed infarcts in the posterior limb of the right internal capsule and the basal ganglia. On cerebral angiography, smooth narrowing of the left carotid siphon was

demonstrated. LA was detected, while aCL was negative. Levels of antithrombin III, and proteins C and S were normal. Blood pressure was controlled with nifedipine. He was anticoagulated with warfarin for 3 months and maintained on aspirin 300 mg daily with a single brief episode of left facial paraesthesia over a 13 month follow-up.

Patient 3

A 23 year old Malay male factory worker was admitted for sudden right-sided weakness which progressed. It was preceded by right-sided headache with dizziness and vomiting. There was no significant past history. Blood pressure was 140/80 mmHg. Haemoglobin was 182 g/L (172 g/L when repeated the next day), PCV 52%, white cell count 11.5 x 10^9/L, and platelet count 243 x 10^9/L. Sedimentation rate was 1 mm/hour. Glucose and lipids were normal. Brain CT was normal on 2 occasions. CSF examination showed red cells 0, white cells 6/μl (lymphocytes), organisms negative, glucose 3.1 nmol/L and protein 0.54 g/L. ECG and 2D-echocardiography were normal. Blood and CSF VDRL tests were negative. ANA was not detected. aCL IgG antibody was positive at 9 GPL units; IgM antibody was not detected. On further study 6 months later, LA was detected. Antithrombin III and proteins C and S were normal. He was maintained on aspirin 300 mg b.d. with no recurrent thrombosis at 3 years' follow-up.

Discussion

An association between aPLs and ischaemic cerebrovascular disease is now well-recognised. aPLs have also been implicated in a wide variety of neurologic disorders of putative vascular origin, including ocular ischaemia, migraine, chorea and seizures[10]. A minority of patients have the 'primary' antiphospholipid syndrome[1], in which thrombocytopaenia, foetal loss and systemic venous and arterial thrombosis are associated with high levels of aPLs, usually of IgG isotype.

aPLs are heterogenous, being cross-reactive with most anionic phospholipids and occasionally DNA as well. They may be detected by VDRL, solid-phase aPL immunoassays and LA tests. Low sensitivity of the VDRL test (1 in 5 aPL-related stroke) precludes its use as a diagnostic test. Solid-phase aPL immunoassays measure antibody concentration while the LA test is a functional measurement of aPL activity. There is partial overlap of the spectrum of antibodies detected by either test. LA and aPL positivity have independently been associated with stroke.

Twenty percent to 40% of patients with SLE have aPLs. The prevalence of aPLs is 2% to 8% in young healthy people without a history of thrombosis, and 18% to 50% in healthy people over the age of 60 years. Thus, pathogenic aPLs need to be distinguished from autoantibodies which appear with increasing age.

aPLs as a risk factor for premature and recurrent stroke has been documented by both retrospective[11] and prospective[12] studies. The incidence of aPL in unselected patients of any age with a first stroke is about 5%. In young patients, the incidence varies widely between studies, and approaches 46%[13]. Such variability probably reflects differences in patient selection.

Patients with aPL-related stroke tend to be young, with a slight female preponderance in those studies which include a higher proportion of SLE[14]. Cerebral ischaemia may present as cerebral infarction (over 75%), transient ischaemic attacks (almost 15%) and rarely cerebral venous thrombosis.

To date, we have detected 3 patients with aPLs in a series of strokes in the young. While early studies focused on the association of aPLs with stroke in SLE, it has subsequently been shown that only a minority have SLE. Patient 1, who had both cerebritis and focal ischaemia, fulfilled criteria for SLE. Features of the antiphospholipid syndrome such as thrombocytopaenia, amaurosis fugax, migraine, chorea and skin

lesions were not seen in the other 2 patients. It is therefore likely that, consonant with experience elsewhere, aPL-related stroke in Malaysia is not uncommon in non-SLE patients.

About two-thirds of cerebral angiograms are abnormal in aPL-related stroke, with occlusive lesions tending to be intracranial, affecting usually the middle cerebral artery. Vasculitis is rare. Mitral valve lesions are frequent, suggesting cardioembolic mechanism of stroke. In patients such as ours, where cardiac evaluation is normal, *in situ* thrombosis may be an alternative mechanism. In Patient 2, the intracranial portion (siphon) of the carotid artery was narrowed.

aPL-related strokes should be identified as they tend to recur. Following the index cerebrovascular event, risk for stroke is 18.7% per year, and for TIA, 15.2% per year[15]. Patient 2 had recurrent ischaemic events. Multiple infarctions may lead to premature dementia[16].

Treatment of aPL-related stroke still needs careful evaluation. Corticosteroids, immunosuppresants, plasma exchange, immunoglobins, antiplatelet agents and anticoagulation have all been used with variable results. A multicentre, controlled trial of anticoagulation versus aspirin is being planned (Antiphospholipid Antibodies in Stroke Study Group, personal communication). In the absence of clear guidelines, treatment remains empiric. Aspirin is generally prescribed, together with rigorous treatment of associated risk factors. For patients with cardiac embolic source, anticoagulation would be considered.

Conclusion

In young patients who have stroke without obvious cause, aPLs should be considered. This entity is important to recognise since recurrent thrombosis is likely. Further studies are needed to determine whether the incidence of aPL-related stroke in our population is as high as has been reported elsewhere. More cases on non-lupus aPL-related stroke may emerge with more intensive screening, particularly of younger patients.

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