Anticardiolipin antibodies in stroke patients in Sudan

Appraisal of their significance in a region of high burden of endemic infections

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ABSTRACT

Objectives: To screen and evaluate the significance of anticardiolipin seroprevalence in patients with acute ischemic stroke, in patients with infectious disease, and in healthy subjects resident in Sudan, a tropical country endemic for several infectious diseases.

Methods: We conducted the study in Khartoum Teaching Hospital in Khartoum, Sudan between July 2003 and January 2005. We included 89 stroke cases, 30 infectious disease patients, and 30 asymptomatic healthy subjects. We estimated IgG and IgM anticardiolipin antibody titers in serum samples from all subjects in the 3 study categories at the time of hospital admission.

Results: We found a significantly higher prevalence of anticardiolipin antibodies in the stroke and infection groups compared to the healthy subjects. However, there was no significant difference in anticardiolipin seroprevalence between patients with stroke and patients with infectious disease.

Conclusions: Caution is necessary when interpreting the presence of antiphospholipid antibodies as a stroke risk in patients harboring infection or living in places with high endemicity of infectious diseases.

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Antiphospholipids antibodies (aPL) are a heterogeneous group of circulating immunoglobulins that react with several negatively charged phospholipids and are capable of promoting abnormal coagulation that predisposes to arterial or venous thrombosis. Anticardiolipin (aCL) is the most commonly investigated aPL in relation to stroke and transient ischemic attacks. Although several well-conducted studies have demonstrated the association of aCL with a prothrombotic state and stroke,[1] the pathogenesis and the risk prediction in ischemic stroke are still debatable.[2,3] Several reports have clearly shown that several viral, bacterial, and parasitic infections are associated with generation of aPL.[4-8] A high seroprevalence of aPL was observed in inhabitants of regions such as tropical Africa, where there is high endemicity of infectious diseases.[4,6] Although these infections can induce aPL expression, the increase in antibodies is not often accompanied by any manifestation of the antiphospholipid syndrome (APS) such as thrombosis.[9] A study on patients with leprosy found that the generated aPL differed from those characterizing the APS with respect to immunoglobulin subclass, avidity and epitope specificity suggesting a distinct pathophysiological significance.[6] In this context we aimed to investigate, in a hospital-based prospective study, the prevalence of aCL in patients admitted with acute ischemic stroke and in other patients admitted during the same period of time with common endemic infections in the region, and compared the results with those in asymptomatic subjects.

Methods. *Study area.* We conducted the study in Khartoum Teaching Hospital, the largest hospital in the region, located in Khartoum the capital city of Sudan in Central Africa between July 2003 and January 2005. The hospital provides health services to the town dwellers and villagers in the surrounding suburban region, a population of over 5 million. The region has a

sub-Saharan hot climate with seasonal rainfall. The study area is endemic for several parasitic, bacterial, and viral diseases. Common infectious diseases include malaria, bilharziasis, tuberculosis, brucellosis, and hepatitis.

Patients. We randomly selected and recruited 89 consecutive patients, aging between 20-65 years (mean age 53.8 years), admitted to the hospital with acute ischemic stroke between July 2003 and January 2005. We interviewed the patients particularly for stroke risk factors and one of the study collaborates physically examined them. We confirmed the ischemic infarction by cranial CT (n= 89), MRI (n=12), or both. The stroke work-up included carotid Doppler, ECG, echocardiography, total cholesterol, triglycerides, prothrombin time, and activated partial thromboplastin time. We also determined aCL in another 30 non-stroke patients who had no recent or history of transient ischemic attack (TIA) or ischemic stroke, admitted to the hospital with different infectious diseases (namely, tuberculosis 9, hepatitis B 6, pneumonia 5, pyelonephritis 4, bilharzia 3, and mixed infections 3) during the same time. We confirmed tuberculosis and bilharzia by light microscopy identification of acid-fast bacilli and Schistosoma eggs, and hepatitis B by the presence of e-antigen. We included 36 healthy volunteers, residing in the same area as a control group for comparison. The non-stroke groups were age- and sex-matched with the stroke group. Other laboratory tests carried out on all subjects included white blood cell counts, hemoglobin, hematocrit, erythrocyte sedimentation rate, blood glucose, urea, electrolytes, and venereal disease research laboratory (VDRL). In the stroke group, we excluded patients who had a stroke older than 2 days, overt concurrent infection or a possible etiology for stroke other than ischemia. In the infection and control groups, we did not include subjects with a previous history of TIA or ischemic stroke. The study was approved by the ethical committee of the Graduate Medical Studies Board of the Faculty of Medicine of the University of Khartoum.

Determination of aCL. We collected and immediately centrifuged the blood samples on admission. We separated the sera and stored it at -70°C until the aCL assay. We determined the IgG and IgM class antibodies to aCL using a standardized indirect solid phase enzyme-linked immunosorbent assay. We briefly coated the microplates with 50µl/well of highly purified cardiolipin solution (Sigma-Aldrich, Gillingham, Dorset, UK) and allowed them to air-dry overnight. We saturated the microplates with β2 glycoprotein I (β2GPI; co-factor required for binding of autoantibodies). We diluted the serum samples 1:100 and placed 10 µl in the microplate wells, incubated them for 60 minutes at room temperature and then washed them. Using a kit with cardiolipin as a detecting antigen, we assayed anti-β2GPI-dependent aCL IgG and IgM and detected the complexes by a chromogen substrate. Levels of aCL were expressed in phospholipid (GPL and MPL) units. One GPL is defined as the cardiolipin binding activity of 1 µg/ml of an affinity-purified IgG aCL preparation from a standard serum. The MPL unit is similarly defined. According to manufacturer instruction, an arbitrary cut of 10 units was used to define positivity. We considered a value >10 GPL or GPM units a positive result for aCL.

Statistical analysis. We carried out statistical analysis using the software Statistical Package for Social Sciences (SPSS/PC). We used Chi-square test (X^2) to determine the statistical significance ($p<0.05$) in difference between frequencies.

Table 1 - Demographic and clinical characteristic in patients with acute ischemic stroke.

Characteristic	aCL-positive (n = 31)	aCL-negative (n = 58)
Mean age (year ± SD)	54.7±19.2	53.3±12.7
Female gender (%)	19 (61.3)	29 (50)
Female:Male ratio	1.6:1	1:1
Stroke risk factors (%)		
Hypertension	8 (25.8)	16 (27.5)
Diabetes mellitus	7 (22.6)	9 (15.5)
History of TIA	2 (6.4)	5 (8.6)
Cardiac disease	3 (9.7)	6 (10.3)
High cholesterol	4 (12.9)	26 (44.8)
Prolonged aPTT	5 (16.1)	1 (1.7)
Positive ANF	4 (12.9)	1 (1.7)
Positive VDRL	5 (16.1)	-
Thrombocytopenia	4 (12.9)	-

aCL - anticardiolipin, aPTT - activated partial thromboplastin time, ANF - antinuclear factor, VDRL - venereal disease research laboratory

Results. ***Stroke patients.*** Table 1 summarizes the demographic characteristics and risk factors in stroke patients. We found elevated aCL in 31 (34.8%) of the stroke patients with a slight female predominance (F: M 1.6:1). The distribution of seropositivity (34.8%)

Table 2 - Mean age and the frequency pattern of anticardiolipin (aCL) among the 3 study groups.

Study group	No. of subjects	Mean age year ± SD	aCL-positive n (%)	aCL-negative n (%)
Stroke	89	53.8±15.1	31 (34.8)	58 (65.2)
Infection	30	52.4±17.1	10 (33.3)	20 (66.7)
Healthy	36	56.7±10.0	6 (16.7)	30 (83.3)

was significantly higher ($x^2 = 4.06$, $p<0.05$) in ischemic stroke compared to that in healthy control subjects (16.7%) (**Table 2**). There was no difference in mean age between aCL-positive and aCL-negative stroke patients. The status of conventional stroke risk factors in the subsets of patients did not show a difference in the prevalence of hypertension, history of cardiac disease, and TIA, however, the presence of diabetes mellitus was slightly more in aCL-positive patients, and cholesterol was more often elevated in aCL-negative patients. We encountered prolonged aPTT, thrombocytopenia, high antinuclear factor titer, and positive VDRL test in some of the aCL-positive patients, but these were absent or rare in aCL-negative patients.

Infection group. We detected a positive aCL titer in 10 (33.3%) of the 30 patients admitted with infectious disease. This frequency rate is approximately similar to that observed in patients who developed ischemic stroke (34.8%) and there is no statistical difference between the 2 values ($x^2 = 0.02$, $p=0.88$).

Discussion. The association between the presence of aPL and ischemic stroke has long been recognized, but its precise role and mechanism in thrombogenesis remains debatable.[1-3] The prevalence of aPL in healthy and in ischemic stroke patients have widely varied in different study designs. In a healthy population, the prevalence of aCL is estimated to be from 2-5%, however, in subjects older than 65 years, rates higher than 20% have been reported.[10,11] Controversies among well-designed epidemiological studies reflect the magnitude of subject complexity. A recent Framingham cohort and offspring study has shown that aCL, independent of other cardiovascular risk factors, significantly predicts the risk of future ischemic stroke in women but not in men.[12] However, some studies have suggested the presence of independent risk for stroke in men.[13,14] A limited number of studies have even refuted any significant role for aCL as a risk predictor.[2,15] Our data conforms to observations suggesting that aCL seropositivity is increased in stroke patients compared with apparently healthy non-stroke patients.

There is evidence that several endemic bacterial and parasitic infections in Africa are associated with production of aPL and other features of the APS.[4-8] In Sudan, it has been shown that a proportion of malaria patients living in an endemic area produce aPL during infection and that titers of these antibodies are associated with persistence and severity of disease.[5,7] It has been advocated that aPL in viral and other microbial infections are produced by mechanisms involving molecular mimicry.[16] In autoimmune disorders such as APS, binding of aPL to phospholipids is enhanced by a co-factor, β2 glycoprotein I (β2GPI), that has anticoagulant properties. It was initially believed that infection-related aPLs are co-factor independent and hence non-thrombogenic, however, this distinction has subsequently been found not to be absolute. Expression of β2GPI dependent aCL had been confirmed in leprosy and B19 parvovirus infections.[8,9,17]

Infections, on their own merits, as well as systemic markers of inflammation, such as leukocyte count, C-reactive protein and fibrinogen, have long been recognized as stroke risk factors.[18] Nevertheless, the nature of the association is nonspecific and the link so far is lacking adequate final proof. It is noteworthy that 5 of our patients with stroke and increased aCL also had positive serological tests for syphilis. Compared with stroke patients without infection, patients with infection associated with cerebral infarction tend to have increased cardiolipin immunoreactivity, increased fibrin generation and hyperfibrinogenemia.[19,20] Some of the inflammatory mediators, such as interleukins and tumor necrosis factor-α that have a procoagulant effect (for example, by upregulation of intercellular adhesion molecule-1) and the hyperfibrinogenemia can contribute to the pathogenesis of cerebral vascular thrombosis.

Our data showed that elevated aPL is as common in ischemic stroke patients as in non-stroke patients admitted to hospital with infection. We have also observed that the frequency of aCL seropositivity in our healthy control subjects was slightly higher than the average reported. This observation can be explained by the fact that healthy subjects living in disease-endemic areas are subject to repeated exposure to relatively low levels of the infectious microbial agents, and they may develop sub-clinical and sub-patent infections that are not detectable by conventional means.[4,21]

It is important to realize that, beside conventional risk factors, several other host and environmental interrelated mechanisms, including immunity, infection,[7,18] and seasonal variability,[22] may influence the phospholipids status and stroke tendency. Therefore, special caution should be undertaken when interpreting the presence of aPL as a stroke risk, particularly in patients with infection or subjects residing in places with high prevalence of infectious endemic diseases.

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