Research article

Antiphospholipid antibodies in patients with venous thrombosis at Kenyatta National Hospital

Barasa KA, Mwanda OW, Kitonyi GK, Gontier CS

Department of Human Pathology, School of Medicine, University of Nairobi, P. O. Box 19676 – 00202, Nairobi, Kenya

***Corresponding author**: Dr KA Barasa, Department of Human Pathology, School of Medicine, University of Nairobi, P. O. Box 19676 – 00202, Nairobi, Kenya. Email: annebarasa@gmail.com*

Abstract

Objective: To determine the presence and types of antiphospholipid antibodies in patients with venous thrombosis at Kenyatta National Hospital.

Design: A cross-sectional descriptive study.

Setting: This study was conducted at Kenyatta National Hospital (KNH), a major referral and teaching hospital in Nairobi, Kenya, between January and November 2011. The study areas included the Accident and Emergency department, wards and outpatient clinics, and the Haematology and Immunology laboratories.

Participants: Male and female adult patients diagnosed with venous thrombosis confirmed by Doppler ultrasound or MRI.

Main outcome measures: Age, gender, presence or absence of lupus anticoagulant, and titres of anticardiolipin (ACL) and anti-β_2-GP1 antibodies (anti-β_2GP1).

Materials and Methods: Demographic and clinical information was collected by direct interview of patients. Every patient was examined for clinical manifestations of APA and blood drawn for laboratory tests. A proforma questionnaire was used to collect all the information. The data collected was pooled, screened and entered into SPSS v.19 software for analysis.

Results: A total of 60 patients were studied. Majority of the patients, 52 (86.7%), were females, while males were 8 (13.3%). The mean age was 38.3 years (± 13.7), with a median (IQR) of 52 years (38.8, 58) for males vs. 32.5 years (24.8, 43.5) for females, p value <0.05). The mean APTT value was 38.4 seconds (± 15.1) with 20 patients (33.3%) having prolonged values. Two patients (10%) had a prolonged KCT (RI >0.16; positive for LA), and all 20 (100%) who had a prolonged APTT had a negative DRVVT (NR <1.30; negative for LA). The mean anticardiolipin IgG titre was 107.4 U/mL (SD ± 62.4); 55 patients (91.7%) had a positive ACL result. The media anti-beta-2-glycoprotein IgG titre was 5 G units (IQR= 4.5, 6.5); 55 patients (91.7%) had a negative result. A significant positive correlation existed between APTT and ACL (r=0.39) and between ACL and β_2GP1 (r=0.30).

Conclusions: Antiphospholipid antibodies (LA and anti-β2GP1 IgG antibodies) are present in a very small proportion of patients seen at KNH with venous thrombosis. ACL IgG antibodies may be induced by numerous factors and may not be related to thrombosis. Pathological antiphospholipid antibodies are uncommon in patients seen at KNH with VTE.

Introduction

The Antiphospholipid Antibodies (APA) are a heterogenous family of immunoglobulins directed against anionic phospholipids or protein phospholipid complexes. Lupus Anticoagulant (LA) and Anticardiolipin Antibodies (ACL) are the two best clinically characterized antiphospholipid antibodies[1].

The frequency of APA in the normal population is approximately 3.6%, with most of these antibodies being induced by either infections or drugs. A high prevalence is seen in conditions such as peripheral vascular disease, recurrent foetal loss in women of reproductive age, acquired thrombotic episodes, autoimmune disorders, malignancies and cardiovascular disease, among others[1].

Evidence shows that the presence of antiphospholipid antibodies is associated with the development of venous or arterial vascular thrombosis[2,3]. The most frequent site for venous thrombosis is the lower limb[2]. Other sites that may be involved include retinal, renal and hepatic veins. The most frequent manifestation of arterial thrombosis is ischaemic stroke or transient ischaemic attack. Antiphospholipid antibodies are also associated with a significant risk of recurrent thromboembolism, especially on discontinuation of anticoagulants. Patients with APA-associated venous thrombosis therefore require prolonged or lifelong anticoagulant therapy[4].

This study was designed to determine the presence and types of antiphospholipid antibodies in patients with venous thrombosis at Kenyatta National Hospital. This information provides some measure of the presence of APA in patients with DVT in our setting, as these are patients who would benefit from prolonged anticoagulant prophylaxis.

Materials and Methods

Approval for the study was obtained from Kenyatta National Hospital Ethical and Research Committee prior to commencement of the study. In addition, informed consent was obtained from each study participant.

Consecutive adult patients seen at Kenyatta National Hospital Accident and Emergency department, wards and outpatient clinics with DVT confirmed by Doppler ultrasound or MRI between January and November 2011 were potentially eligible for the study. The exclusion criteria included those on anticoagulant medication and those documented as having a bleeding disorder, which would interfere with the coagulation tests.

All patients underwent an examination of clinical history and physical examination, and then blood was then collected for the laboratory tests. Seven and a half millilitres of blood was collected and divided into (i) 3.5 ml into a trisodium citrate vacutainer and (ii) 4 ml into a plain vacutainer. Blood collected into trisodium citrate was immediately transported to the haematology laboratory where platelet poor plasma was prepared by centrifugation at 3000 rpm for 10 minutes. The plasma samples were aliquoted and stored at -80°C, awaiting the coagulation tests (PT, APTT, KCT and LA test), which were done after batching of the samples.

The blood collected into plain vacutainers (4 ml) was transported to the Immunology laboratory, and then allowed to clot undisturbed for 1 hour at room temperature. The serum was separated from the clotted blood by centrifugation at 3000 rpm, then aliquoted and stored at -80°C. Analysis for VDRL, TPHA, ACL and anti-β_2-GP1 antibodies was done after batching of the samples.

Frozen specimens were thawed on the bench or in a water bath at room temperature, and then inverted several times to ensure homogeneity before use for a test. The coagulation tests (PT and APTT) were performed at the Haematology laboratory using an automated coagulation analyser (ACL200). Hemosil™ PT-Fibrinogen HS PLUS and APTT Lyophilized Silica kits were used. Samples with prolonged APTT were subjected to LA detection procedures using two different testing methods, as recommended by the International Society of Thrombosis and Haemostasis guidelines[5], KCT[6,7] (LupoTek KCT kits), and DRVVT[8] (LupoTek DetecTin VL and LupoTek CorrecTin VL kits).

The results of the KCT are expressed as a Rosners Index, with a cut-off of >1.30 being positive. The results of the LA DetecTin and CorrecTin tests are expressed as a Normalized Ratio, with a cut-off value of >0.16 considered as positive.

VDRL test was performed in the Immunology laboratory using the Syphilis RPR Test kit[9], followed by a TPHA test on the VDRL positive samples, using the Syphilis TPHA liquid kit. The tests for detection of anticardiolipin and anti-beta$_2$-glycoprotein 1 antibodies were performed using ELISA kits, the IMTEC-Cardiolipin-Antibodies IgG kit and the REAADS® IgG Anti-Beta 2 Glycoprotein 1 Semi-quantitative Test Kit.

There was strict adherence to protocol during sample collection, storage and processing. All tests were performed in accordance with manufacturers' recommendations. Internal quality control materials provided by the manufacturers were included during analysis. Validation of the ELISA tests was done according to the manufacturers' recommendations.

The data collected using a structured questionnaire and pre-designed extraction sheets was entered into Ms Excel computer database, cleaned and verified, then imported into SPSS (v.19) statistical software for analysis. Descriptive statistics on socio-demographic characteristics was presented using percentages and frequencies for categorical or nominal data. Continuous variables were presented using means (standard deviations) if normally distributed and medians (inter-quartile range) for non-normally distributed variables. Tables and appropriate charts were used to display the results. T-test or ranksum tests were used as appropriate to compare difference in means for two groups of continuous variables. Chi-square or Fisher's tests for independence were used to assess association between two nominal or categorical variables. The level of significance was set at 5% with p-values of $\leq$0.05 being considered significant. Correlation analysis to asses for any linear association was done using Pearson correlation coefficient for the continuous variables, and considered significant at 5% level.

Results

A total of 62 eligible participants were enrolled into the study. Two were excluded due to mislabeling of the specimens. The remaining 60 met the inclusion criteria and were evaluated for the requirements of the study. Majority of the patients were females, 52 (86.7%). The mean age was 38.3 years (± 13.7), with a median (IQR) of 32.5 years (24.8, 43.5) for females and 52 years (38.8, 58) for males (Table 1).

Table 1: Demographic characteristics (n=60)

Characteristic	
Age in years, mean (SD)	38.3 (±13.7)
Gender	n (%)
Male	8 (13.3%)
Female	52 (86.7%)

The mean APTT value was 38.4 seconds (± 15.1), (Table 2), with a majority of the patients having either normal 31(51.7%) or prolonged values 20 (33.3%). (Normal control value was 28.3 – 38.3 seconds).

Table 2: APTT results (n=60)

Characteristic	Results
APTT (seconds), mean (SD)	38.4 (± 15.1)
APTT categories	n (%)
Shortened (<28.3 s)	9 (15%)
Normal (28.3 – 38.3 s)	31 (51.7%)
Prolonged (>38.3 s)	20 (33.3%)

KCT was performed on the 20 patients with prolonged APTT, and the results expressed as Rosners Index (RI). The mean RI was 0 (± 0.3), with majority of the patients 18 (90%) having a negative value and 2 (10%) having a positive value (Table 3).

The Lupus anticoagulant DetecTin and CorrecTin tests were also performed on samples with prolonged APTT, and the results expressed as a Normalized Ratio (NR). The mean NR was 1.2(± 0.1) with all the samples 20 (100%) testing negative for LA, as shown in Table 3.

Table 3: Lupus anticoagulant detection tests (n=20)

Characteristic	Results
Rosners Index (KCT), mean (SD)	0 (± 0.3)
Rosners Index KCT categories	n (%)
Negative	18 (90%)
Positive	2 (10%)
Normalized Ratio (LA) , mean (SD)	1.2 (± 0.1)
Normalized ratio DRVVT categories	n (%)
Negative	20 (100%)

Fifty-five patients (91.7%) tested negative for VDRL, (Table 4). Out of the 5 who tested positive, 4 (80%) also tested positive for TPHA. The mean anticardiolipin IgG titre was 107.4 U/mL (SD ± 62.4) with almost all 55 (91.7%) having a positive ACL result. With regard to anti-beta-2-glycoprotein IgG, the median was 5 G units (IQR= 4.5, 6.5) with almost all 55 (91.7%) the patients exhibiting a negative result (Figure 1).

Table 4: Immunology and biochemistry test results (n=60)

Characteristic	Results
VDRL n (%)	
Negative	55(91.7%)
Positive	5(8.3%)
TPHA n (%) (n = 5)	
Negative	1(20%)
Positive	4 (80%)
Anticardiolipin IgG (U/mL), mean (SD)	107.4 (± 62.4)
ACL categories n (%)	
Negative	5 (8.3%)
Positive	55 (91.7%)
Antibeta2 glycoprotein IgG (G units), median (IQR)	5 (4.5,6.5)
Antibeta2 glycoprotein IgG categories n (%)	
Negative	55 (91.7%)
Positive	5 (8.3%)

As shown in Table 5, the male patients were significantly older than the females, median age (IQR) 52 (38.8, 58) vs. 32.5 (24.8, 43.5), p value <0.05.

Figure 1: ACL IgG and anti-β_2GP1IgG box plots

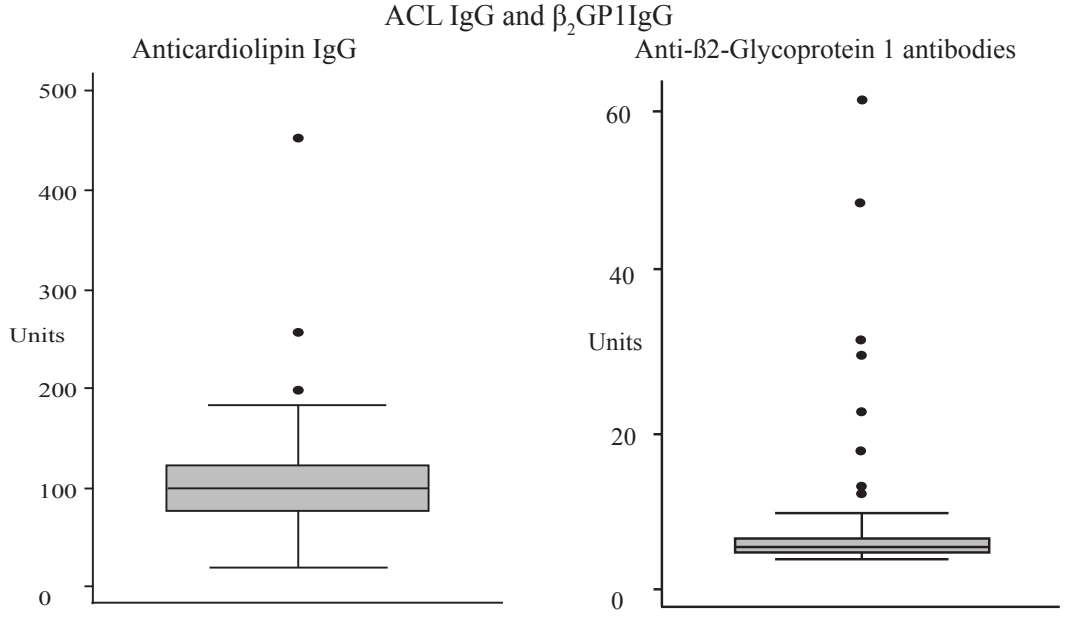
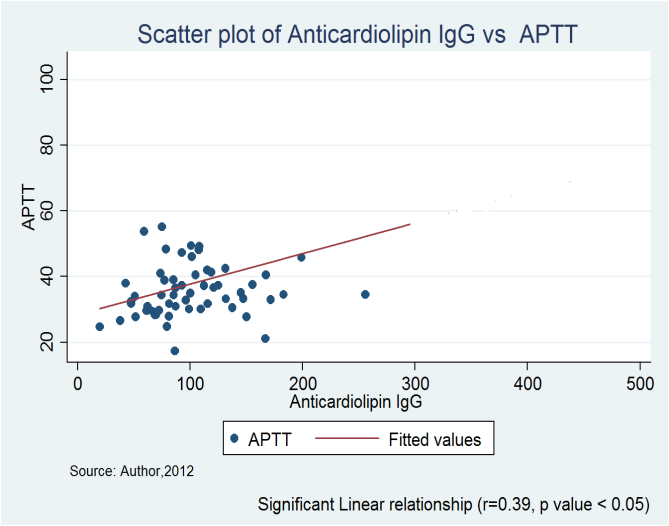


Table 5: Bivariate analysis by gender

Variable	Male	Female	Test statistic	p value
Age in years Median(IQR)	52 (38.8,58)	32.5 (24.8,43.5)	Ranksum test	0.013*

* Significant result (p <0.05)

Figure 2: Correlation between ACL and APTT



Correlations were done using Pearson correlations coefficients and statistical significance determined at 5% level. A significant positive correlation existed between APTT and ACL (r=0.39; p value <0.05), (Figure 2), and between ACL and β_2GP1 (r=0.30; p value <0.05).

Discussion

This study evaluated 60 patients, most of who were females (86.7%). The male participants were significantly older than the females, with a median age of 52 years and 32.5 years respectively.

The APTT was slightly prolonged in a third of the patients evaluated in this study. The causes of the prolonged APTT in these patients' samples may have been due to the presence of heparin, LA, or a coagulation factor deficiency. Thrombin test would have been useful to confirm for the presence of heparin in these samples, as it would cause prolongation of test. This was a limitation of this study, as the thrombin test was not performed. The DRVVT reagent used in this study contained a heparin neutralizer, but unfractionated heparin in excess of therapeutic levels may not be completely neutralized by these neutralizers, and this would interfere with the LA results. Few patients had a shortened APTT, which may have been due to difficulties during sample collection, with partial clotting of the specimens before analysis.

LA was positive in two patients by the KCT test, while DRVVT was negative for all. However, one of the patients with a positive KCT test had syphillis, which may have interfered with the test results to give a false positive LA. The incidence of LA positivity in this study is low (1.6%), compared to those from the West, and is similar to the incidence found in the general healthy population (1%)[10]. Simioni *et al*[11] found five LA positive patients among 59 unselected patients with DVT (8.5%), while Ginsberg *et al*[12], found nine LA positive patients out of 65 (14%).

VDRL test was negative in most patients. Out of the five with a positive VDRL, only one was a false-positive result, as evidenced by a negative TPHA. The other four were confirmed to have syphilitic infection by the TPHA test. Almost all the patients recruited into this study were positive for IgG anticardiolipin antibodies. Several factors may explain this; firstly, the ACL antibodies were measured in the blood after the thrombosis. An assumption made by this study was that the APA measured after the thrombotic event reflects the antibody status before the event. Transiently elevated ACL antibody levels are found in many patients after a venous thrombosis, suggesting that the antibodies may be a result, rather than a cause of thrombosis in these patients[13].

Secondly, analytic issues in the ACL assays may also contribute to false-positive results. Even when a β_2-dependent ACL assay is used, the recommended dilutions during testing enable other endogenous proteins in the serum, apart from β_2-GP1, to be present in a sufficiently high concentration that allows binding in a non-β_2GP1-dependent fashion, thus reducing the specificity of the test. Thirdly, the cut-off values used in this study for interpretation of the ACL assay were those recommended by the manufacturer of the reagent used. It is recommended that local cut-off values be used whenever possible[14]; however, local reference ranges for ACL have not been established for this population.

Five patients (8.3%) were positive for anti-β_2-glycoprotein IgG antibodies. This finding is similar to that of a study by Zanon *et al*[15], who found a prevalence of 8.4% of anti-β_2GP1 antibodies in patients with acute thromboembolic events.

In conclusion, this study shows that antiphospholipid antibodies (LA and anti-β2GP1 IgG antibodies) are present in a minority of patients seen at KNH with venous thrombosis, with the positive tests for anti-β2GP1 being more common than for LA. ACL IgG antibodies may be induced by numerous factors and may not be related to thrombosis. Screening for antiphospholipid antibodies in patients with venous thrombosis at KNH should therefore be limited to relatively young patients with unprovoked thrombosis or recurrent thrombosis. An LA assay together with an anti-β_2-GP1 assay may be more useful than an ACL antibody assay, unless local cut-off values for ACL are established.

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