

Profile of Patients with Thrombosis Evaluated in a Tertiary Care Centre

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Abstract

Background: Venous and Arterial thrombosis are common diseases with an annual incidence of 1 in 1000 per year (1). Several risk factors have been identified like increased levels of coagulation factors –factor VIII,IX,XI,II and fibrinogen which are cumulatively explored by APTT(activated partial thromboplastin time). Elevated factor VIII levels are identified as an independent risk Antiphospholipid antibody syndrome, paroxysmal nocturnal hemoglobinuria, sickle cell disease are also associated with strong tendency for thrombosis.

Objectives: (A) To examine the risk factors involved in the pathogenesis of thrombosis in Indian population.

(B) To investigate the significance of these risk factors in incidence of venous and arterial thrombosis.

Materials: Blood samples were collected from patients presenting with clinical picture of arterial or venous thrombosis for routine tests. The following tests were done on the samples: complete blood count, sickling test, Ham's acidified serum test, Sucrose lysis test, prothrombin time, activated partial thromboplastin time, lupus anticoagulant assay (dRVVT based), Factor VIII:C assay, anti cardiolipin antibody

Results: 310 cases were analysed. 124 patients(40.0%) had presented with proximal and distal venous thrombosis, 68 patients with recurrent abortions (21.9%), 38 CASES(12.7%) ischemic heart disease and 43 cases (14.3%)with cerebrovascular disease, There were 11 cases of vasculitis (3.7%),10 cases of arterial thrombosis and 16 cases of pulmonary thromboembolism (5.3%). Increased factor VIII: c levels was the commonest risk factor identified followed by Antiphospholipid antibody (23.5%) and shortened APTT. No risk factors were identified in 50.6 % of the cases. There was one case each of sickle cell anemia and paroxysmal nocturnal hemoglobinuria who presented with pulmonary embolism and portal vein thrombosis respectively.

Conclusion: The commonest risk factor identified was increased factor VIII: C levels, The presence of antiphospholipid antibodies was found to be linked with ischemic heart disease and venous and arterial thrombosis. There was also a very significant association between the presence of antiphospholipid antibodies, lupus anticoagulant and anticardiolipin antibodies with recurrent abortions. There was a correlation between the shortening of APTT and incidence of venous thromboembolism in this study. This study also showed that APTT is not an optimal screening test for detecting lupus anticoagulants.

| Access this article online | |
|---|---|
| Quick Response Code: | Website: www.innovativepublication.com |
|  | DOI: 10.5958/2394-6792.2016.00016.8 |

Introduction

Venous and Arterial thrombosis are common diseases with an annual incidence of 1 in 1000 per year (1). Several risk factors have been identified like increased levels of coagulation factors –factor VIII,IX,XI,II and fibrinogen which are cumulatively explored by APTT(activated partial thromboplastin time). Elevated factor VIII levels are identified as an independent risk. Antiphospholipid antibody syndrome, Paroxysmal nocturnal hemoglobinuria, sickle cell disease are also associated with strong tendency for thrombosis. Antiphospholipid antibody syndrome is a clinical entity with a strong tendency for thrombosis accompanied by high mortality and morbidity. It is characterized by persistently elevated levels of

antibodies directed against anionic phospholipids. As a result of antibodies homeostatic regulation of blood coagulation is altered. The diagnosis is usually made when arterial or venous thrombosis or recurrent abortions occurs in a patient with a laboratory test persistently positive for an antiphospholipid antibody.

Paroxysmal nocturnal hemoglobinuria is a disease due to clonal disorder of hematopoietic stem cell. This is characterized by increased sensitivity to complement mediated lysis which affects all blood cells including platelets, which results in an increased sensitivity to complement mediated lysis which affects all blood cells including platelets, which results in an increased sensitivity to venous thrombosis.

Sickle cell disease is an inherited disorder characterized by the presence of sickle hemoglobin which results from substitution of glutamic acid by valine at the sixth position of globin chain. Nearly every component of hemostasis, including platelet function, procoagulant, anticoagulant and fibrinolytic system is altered and is associated with an increased risk of venous and arterial thrombosis.

This study is aimed at identifying the acquired risk factors of thrombosis like antiphospholipid antibody

syndrome, elevated factor VIII levels, shortened activated partial thromboplastin time, paroxysmal nocturnal hemoglobinuria and sickle cell disease so that the patient at risk can be identified and prevented from the occurrence of thrombosis.

Materials and Method

Blood samples were collected from patients presenting with clinical picture of arterial or venous thrombosis. The following tests were done on the samples:

Complete blood count, Sickling test, Ham's acidified serum test, Sucrose lysis test, Prothrombin time, Activated partial thromboplastin time, Lupus anticoagulant assay (dRVVT based), Factor VIII; c assay (one stage APTT based), Anticardiolipin antibody

Complete blood count was done using the patients EDTA blood sample on a Beckman coulter which is a 26 parameter fully automated hematology analyzer with five part leukocyte differential count.

Sickling test was done using the Sicklevue test kit manufactured by Tulip diagnostics. This test is based on the solubility difference between HbS and HbA in concentrated buffer solution. 100 µl of EDTA anticoagulated blood sample from the patient is added to 2 ml of solubility test reagent. This is mixed and allowed to stand for ten minutes. The tubes are then centrifuged at 1200 g for 5 minutes. The pattern formed in the reaction tubes is observed. A control is also run simultaneously by using a normal subject's sample. In normal peoples the lower layer will be clear and dark red in colour and upper layer will have a grey precipitate. In sickle cell anemia (Hb-SS), the lower layer will be colorless and upper layer will have red precipitate. In Sickle cell trait (Hb-AS), the lower layer will be clear and light red to pink in colour while the upper layer shows red precipitate

Sucrose lysis test: An isoosmotic solution of sucrose is required (92.4 g/l). Two test tubes are set up, one containing 0.05 ml of fresh normal ABO compatible serum diluted in 0.85 ml of sucrose solution and the other containing 0.05 ml of fresh normal serum diluted in 0.85 ml of saline. To each test tube 0.1 ml of 50 % suspension of washed red cells is added. The tubes are incubated at 37° Celsius for 30 minutes, centrifuged and observed for lysis. The quantum of lysis is measured using a spectrophotometer. In a case of paroxysmal nocturnal hemoglobinuria 10-80% lysis is observed

Ham's acidified serum test: 0.5ml samples of fresh normal AB group serum or serum that is ABO compatible with patient's blood are delivered into six test tubes. Two of the tubes are placed at 56°C for 10-30 minutes in order to inactivate complement. The other two pairs are kept at room temperature and 0.05 ml of 0.2 ml/l hydrochloric acid is added. Similar

volumes of acid are added to the inactivated serum samples. All the tubes are incubated at 37°C.

50 % suspensions of washed patients and control red cells are added to the tubes containing un acidified fresh serum, acidified fresh serum and acidified inactivated serum. These tubes are then incubated at 37°C for 1 hour. The tubes are then centrifuged. In a patient with PNH, lysis is seen in the tube containing acidified serum. There is no lysis in the tube containing non acidified or heat inactivated serum.

PT and APTT are done on STAGO coagulation analyzer with plasma samples collected from patient and control. When the APTT is prolonged, correction studies are done to differentiate whether the prolongation is due to an inhibitor or a factor deficiency. This is done by mixing equal volumes of test plasma and control pool plasma and repeating the test. When inhibitors are present the prolonged timing will not correct.

Lupus anticoagulant assay (Diluted Russell's Viper venom time based): This test is performed at 37°C , mixing one part of platelet poor plasma obtained from patients sample as well as controls sample to one part of lyophilized preparation of Russell's viper venom and phospholipids (Tulip diagnostics) and one part of 0.025m calcium chloride (Tulip diagnostics)and timing the plasma clot formation .This the' screen time ' which is normally 28-45 seconds .When the screen time is more than 45 seconds, The test is repeated using lupus anticoagulant confirm kit(Tulip diagnostics) which contains excess of phospholipids. Ratios of mean screen time/mean confirm time <1.0 is normal. If it is more than one it is lupus anticoagulant positive.

Platelet poor plasma is prepared by centrifuging blood sample collected by venepuncture into a tube containing 3.2 % trisodium citrate in a ratio of nine parts blood to one part citrate. The samples were centrifuged at 1500g for 15 minutes to obtain platelet poor plasma.

Anticardiolipin antibody test:

Reagents required –

Calibrators – containing diluted human serum and sodium azide as preservative (tulip diagnostics) conjugates – 15 ml IgG, 15 ml IgM (anti human immunoglobulin conjugated to horse peroxidases), TMB substrate -15 ml(stabilized hydrogen peroxide), stop solution - 1M hydrochloric acid. Sample buffer-containing trisodium chloride, sodiujm azide, BSA, wash buffer containing tris, sodium chloride, tween and sodium azide (Tulip diagnostics).

Method – Antigen precoated microplate wells are incubated with calibrators, controls and serum specimens. During the incubation, antibody present in the test sample binds to coated wells. The wells are then washed to remove unbound antibodies and horse radish peroxidase labeled antihuman Ig is incubated into the wells. Chromogen is added and antibodies are measured using a spectrophotometer plate reader using a 450 nm

filter. Calibration curves are made with absorbance values at 450/630 nm on the Y axis and cardiolipin class specific Ig on X axis. The concentration of anticardiolipin class specific antibody is read from the calibration curve. Values >15MPL/ml for IgM and >15 GPL/ml for IgG was labeled as positive.

Factor VIII assay: One part of test plasma is mixed with one part of factor VIII deficient plasma (Stago) and one part of APTT reagent (CK PREST, STAGO), incubated at 37°C, one part of calcium chloride is added and the time taken to clot is noted. Abnormal system control plasma with a factor VIII level of 32-46% and normal system controls with factor VIII level of 87-121 % are subjected to the test simultaneously with patient's sample. Normal levels of factor VIII are 50-150 %.

Results

In this study 310 cases were analysed to identify the risk factors of thrombosis. 124 patients (40.0%) had presented with proximal and distal venous thrombosis, 68 patients with recurrent abortions (21.9%), 38 CASES (12.7%) ischemic heart disease and 43 cases (14.3%) with cerebrovascular disease, there were 11 cases of vasculitis (3.7%), 10 cases of arterial thrombosis and 16 cases of pulmonary thromboembolism (5.3%). Increased factor VIII: c was the commonest risk factor identified (32.3 %), followed by antiphospholipid antibody (23.5%) and shortened APTT. No risk factors were identified in 50.6 % of the cases. There was one case each of sickle cell anemia and paroxysmal nocturnal hemoglobinuria who presented with pulmonary embolism and portal vein thrombosis respectively.

Analysis of risk factors in different thrombotic diseases:

1. **Venous thrombosis:** 124 patients had distal and proximal venous thrombosis (41.3%). Majority of the patients had deep vein thrombosis of posterior tibial vein (29 %). 8 % patients had multiple deep vein thrombosis. Multiple deep vein thrombosis was more common in intra abdominal veins. Antiphospholipid antibodies was positive in 24 cases (19%). Increased factor VIII levels were observed in 37 cases (29.9%). 18 %cases showed elevated factor VIII levels as well as antiphospholipid antibodies. Shortened APTT was present in 13 cases (10.5 %). All risk factors were negative in 60 cases (54.8%). A 37 year old male had multiple deep vein thrombosis of lower limbs , all tests were normal except for a shortened APTT of 24 seconds . A male of 54 years had come with intermittent episodes of hemoglobinuria, Ultrasound abdomen showed portal vein thrombosis. He had all tests normal except for positive Ham's test and sucrose lysis test. That was the only case with paroxysmal nocturnal hemoglobinuria in this entire study.

2. **Pulmonary venous thromboembolism:** 16 cases of pulmonary thromboembolism were analysed. None of them showed positive antiphospholipid antibodies. Increased factor VIII levels were observed in 8 cases (50%). Shortened APTT was observed in 3 cases (18.75%). Increased factor VIII and shortened APTT was seen in 2 cases (12.5 %). Sickie vue test was positive in a case (6.25 %) – A 22 yr old lady had presented with pulmonary embolism. she had moderate anemia, peripheral smear showed a mild hemolytic picture with occasional sickle cells. sicklevue test was positive (heterozygous). The serum electrophoresis and sickling test was also confirmatory of this test. This was the only case of sickle cell anemia with thrombosis in the entire study.
3. **Arterial thrombosis:** 10 cases of arterial thrombosis were studied. The cases were of superior mesenteric artery thrombosis, subclavian artery thrombosis and pulmonary artery thrombosis. Antiphospholipid antibodies were positive in 3 cases (30 %). Increased factor VIII levels were seen in 2 cases. All risk factors were negative in 5 cases (50%).
A 4 year old girl had idiopathic pulmonary hypertension and pulmonary arterial thrombus. On analysis lupus anticoagulant and anticardiolipin antibodies were positive. Rest all tests were normal. This patient was the youngest patient in the study.
4. **Ischemic heart disease:** 38 cases of ischemic heart disease were analysed in this study. There were cases of myocardial infarction and acute coronary syndrome. Antiphospholipid antibodies were positive in 11 cases (28.6 %). Increases factor VIII: c was seen in 9 patients (23.7 %). All risk factors were negative in rest of 18 cases (71.4%).
5. **Cerebrovascular diseases:** There were 43 patients with cerebrovascular diseases like superior sagittal sinus thrombosis as well as venous and arterial infarcts in the brain parenchyma. 16 patients (37.2%) had antiphospholipid antibodies. Increased factor VIII levels were seen in 22 cases (50%). Shortened APTT was seen in 11 cases. All these cases had antiphospholipid antibody positivity as well .All risk factors were negative in 10(23.3%) patients.
6. **Recurrent abortions:** Women referred to obstetric department with a history of early recurrent abortion (atleast three pregnancy losses before 13 weeks of gestation) were eligible to be included in this study. Exclusion criteria were endocrine, immunological or anatomical causes of embryo demise. 68 cases of recurrent abortion were included in this study. 19 cases (27.3 %)

were positive for antiphospholipid antibodies. Increased factor VIII levels were seen in 25 cases (36.4%). 12 cases (18%) showed positivity for antiphospholipid antibodies as well as elevated factor VIII levels. All risk factors were negative in 36 cases (52.9%).

7. **Vasculitis:** 11 cases of vasculitis were included in the study. 6 patients had elevated factor VIII levels (54.5 %). All risk factors were negative in rest of 5 cases (45.5 %).

Discussion

Recent discoveries of impaired down regulation of procoagulant system like activated protein C resistance, factor V leiden mutation and increased plasma concentration of procoagulant factors such as factors I,II, VIII, IX, and XI(2,3) have added a new dimension to the list of inherited or acquired disorders predisposing to thrombosis. The identification of risk factors for thrombosis is a controversial topic. However, such studies should help to identify a vulnerable population and help target prophylaxis to those who benefit the most and ultimately reduce the occurrence of venous thromboembolism. With the above objectives in mind, 310 patients with symptomatic venous or arterial thrombosis were evaluated to identify the possible etiology of thrombosis. Patients were classified according to their underlying disease syndrome. The investigations done included PT, APTT, factor VIII:C assay (one stage APTT based), lupus anticoagulant (dRVVT based), anticardiolipin antibodies, Ham's test, sucrose lysis test and sickling test.

Increased factor VIII:C was the commonest risk factor identified, (32.3%), followed by antiphospholipid antibody (23.5%) and shortened APTT (8.7 %). No risk factors were identified in 50.6 % of the cases. There was one case each of sickle cell anemia and paroxysmal nocturnal hemoglobinuria who presented with pulmonary embolism and portal vein thrombosis respectively. 15.5% cases had multiple risk factors positive – positive APL with increased factor VIII:C, increased factor VIII:C and shortened APTT and in 12.5 % of the patients who presented with cerebrovascular symptoms had a combination of APL positivity together with increased factor VIII:C levels and shortened APTT.

Literature shows that antiphospholipid antibodies are positive in young patients <45 years (4,5,6). Most of the studies showed antiphospholipid antibodies in age group between 20-55 years. In our study the youngest patients was a 4 year old girl with pulmonary arterial thrombosis. This study has shown positivity for lupus anticoagulant and anticardiolipin antibodies in other clinical situations like arterial thrombosis, ischemic heart disease and in patients with recurrent abortions. 34 % of the patients with positive antiphospholipid antibodies had arterial thrombosis.

Anticardiolipin antibodies (ACA) are not such strong risk factors for thrombosis as lupus anticoagulants. Separate analysis of different types of thrombosis show that anticardiolipin antibodies ACA are more associated with cerebral stroke and myocardial infarction and not with DVT(7). In the present study it was observed that 28.6 % and 37.2 % respectively of cases of ischemic heart disease and cerebrovascular disease were associated with positive anticardiolipin antibodies. 19.6 % of the cases with venous thrombosis were positive for anticardiolipin antibodies.

This study did not reveal any statistically significant difference in the antiphospholipid antibody positivity for the following groups- patients with ischemic heart disease and recurrent abortions, patients with venous thrombosis and cerebrovascular disease and patients with pulmonary venous thromboembolism and vasculitis. In fact, there were no positive antiphospholipid antibody case in pulmonary venous thromboembolism and vasculitis.

However the investigation brought out a significant difference in the contribution of antiphospholipid antibodies as a risk factor for venous thrombosis when compared to pulmonary thromboembolism ($p < 0.05$). The antiphospholipid antibody positivity seen in venous thrombosis was more than what was observed in pulmonary venous thromboembolism. Similarly, there is a statistically significant difference in the contribution of antiphospholipid antibody for pulmonary venous thromboembolism and ischemic heart disease ($p = 0.003$) as well.

In the present study it was observed that 30 patients were positive only for lupus anticoagulant, 43 patients for anticardiolipin antibody and 22 patients showed positivity for both lupus anticoagulant and anticardiolipin antibody. However there are 237 patients in this study who presented with thrombosis and were negative for both tests. Laboratory testing for lupus anticoagulant is not standardized and various combinations of screening, confirmatory and integrated test systems can be used. THE CONVENTIONAL TESTS THAT WERE USED- APTT and dRVVT may be insensitive to the presence of lupus anticoagulants, necessitating the use of multiple assays before reporting a patient sample as negative for lupus anticoagulant.

The APTT is considered to be a readily available and inexpensive screening test for lupus anticoagulant. The present study did not show prolonged APTT in any of the cases that tested positive for lupus anticoagulant. Literature suggest that APTT cannot be considered an optimal screening test for lupus anticoagulant.(8).

A growing literature has suggested that elevated levels of factor VIII:C may represent a risk factor for venous thromboembolism (9,10). Relatively less is known about the relation between factor VIII and cardiovascular disease at risk. This study shows a

significant association between elevated factor VIII:C levels and myocardial disease (23.7 %).

The present study revealed a high association between elevated factor VIII:c levels and patients with early recurrent abortions (36.4%). This finding suggest a possible association between this thrombophilic condition and early reproductive failure.

Many acquired variables, above all acute phase reactions may increase the factor VIII:C activity . In order to avoid inflammatory response as a confounding factor we should have ideally included measurement of C reactive proteins levels in our patients.

The APTT is a simple coagulation test that has been in routine laboratory use for decades. 8.7 % cases had shortened APTT. Of these 27 patients, 16 patients had venous thrombosis including pulmonary venous thrombosis. Two of these patients had in addition elevated factor VIII:c levels, 27 had (25 %) had shortened activated partial thromboplastin time. 11 patients who had cerebrovascular diseases had multiple risk factors – shortened APTT and positive antiphospholipid antibody and five of these patients , in addition had increased factor VIII levels. This indicates that the cause for the low APTT is more complex than jus increased factor VIII levels.

It was observed that there was a statistically significant difference in the presence of shortened APTT in cerebrovascular disease compared to IHD. The literature survey revealed only a single study of a case of fatal dural sinus thrombosis associated with a shortened APTT (11).

Only one patient each for sickle cell disease and paroxysmal nocturnal hemoglobinuria presented with venous thrombosis. Austin et al observed 6% of cases with sickle cell disease and venous thrombosis (12).

The present study was limited to the acquired risk factors since facilities for genetic studies were not available at our institution. The confirmation of antiphospholipid antibodies as risk factors can be done only if repeat testing after a period of 6 weeks is performed. However their results were encouraging since all of them continued to be positive for lupus anticoagulant and anticardiolipin antibodies

Therefore, although this study had significant observations and correlations of risk factors for thrombosis, more detailed studies will have to be performed.

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