Original Research Article

Association of Activated Partial ThromboplastinTime and Fibrinogen Level in Patients with Polycythemia Vera

ABSTRACT

Aims: To evaluate theActivated Partial Thromboplastin Time(APTT) and fibrinogen levels in patients with Polycythemia vera (PV).
Study design: Analytical, laboratory, hospital-based, cross sectional study.
Place and Duration of Study: Hematology clinic at Fudail hospital Khartoum State.,Sudan. From April to August 2016.
Methodology: Research protocol was approved by SUMASRI International Review Board (SIRB)at University of Medical Sciences And Technology(UMST),Sudan. A total of 19 patients (14 male ,5 were females; mean age of 59 ±4 years) were obtained from PV patients who came to the hospital during study period and who met inclusion criteria and 29 samples were collected from healthy subject as control . APTT fibrinogen level were assayed.Statistical evaluation was performed by SPSS(version 20) using Student's t test and Pearson correlation tests.
Results: Patients with Polycythemia vera had shortened APTT ((26.9±1.3s vs. 35.4±4.4 s) ; P <0.05) and higher fibrinogen (569±79mg/dl vs. 290±96 mg/dl ; P <0.05) values compared to normal control. Strong negative correlation between the Fibrinogen level and APTT in PV were seen(R -0.766, p<0.05).
Conclusion: This study indicate that, the patients with PV were prone to develop hypercoagulable state. Therefore, routine examinations of APTT and fibrinogen and are significant to assess coagulation abnormality in order to prevent PV-associated thrombosis.

Keywords: { Polycythemiavera (PV), myeloproliferative neoplasms (MPNs), Activated Partial Thromboplastin Time(APTT) .hypercoagulable, fibrinogen levels}

1. INTRODUCTION

Polycythemia vera (PV) is a rare myeloproliferative neoplasms (MPNs) characterized by a clonal expansion of multipotent bone marrow progenitors, which causes in general an increased production of erythrocytes granulocytes and platelets, but most significantly in erythrocytes.[1,2] The median age at presentation about 60 years.[3] Although considered relatively indolent diseases, PV are at lifelong enhanced risk of thrombosis, haemorrhage, and myelofibrotic or leukemic transformation[4-6]. Thrombotic disease in PV patients represents the major cause of morbidity and mortality [5,7]. Patients with PV have a high incidence of thrombosis (12%-39%) as compared to other myeloproliferative disorders.[2] Accordingly, the purpose of prophylactic cytoreduction in managing patients with PV is to reduce the risk of thrombosis.
The pathogenesis of thrombotic state in PV is complicated. However, the most important mechanisms summarize the origin of these disorder are; abnormalities of blood cells arising from the clonal proliferation of hematopoietic progenitor cells which acquire a prothrombotic phenotype[8], host inflammatory response to the cytokines and other mediators by the malignant cells[8] and abnormalities of blood coagulation parameters including high concentrations of plasma markers of blood clotting and vascular endothelium activation resulting in hypercoagulable condition in PV patients.[9-11]
Activated partial thrombin time (APTT) and prothrombin time (PT)test are basic laboratory screening tests for function of the coagulation system.[12] APTT is used to evaluate the intrinsic pathway factors (XI, VIII, IX),contact factors (XII, prekallikrein, and high-molecular-weight kininogen), and common pathway factors (X, V and II and fibrinogen). PT is used to evaluate the extrinsic pathway factors (tissue factor and factor VII) and common pathway factors. Prolonged APTT values have clinical relevance as an indicator of factor deficiency or the presence of coagulation inhibitors.[13] Shortened APTTs are generally considered to be laboratory artifacts associated with difficult venepunctures.[14] However, recent studies have also shown that shortened APTTs may also reflect hypercoaguable state, which is prospectively associated with increased thrombotic risk[13,15,16]. Furthermore, Shortened APTTs may result from an increase of circulating activated coagulation factors in plasma caused by enhanced coagulation activation in vivo.[16,17]Therefore, APTT can be used to estimate the risk of thromboembolic complication associated with diseases.

Termination of blood coagulation is controlled by conversion of fibrinogen to fibrin, an insoluble polymer that gives structural stability, strength, and adhesive surfaces to growing clots.[18] Fibrinogen is acute-phase proteins produced by the liver, stimulus for production is likely to be inflammatory cytokines such as interleukin-6, interleukin-1 and tumour necrosis factor (TNF). [19,20] The reference range for the fibrinogen is 200-400 mg/dL [21]. Normal fibrinogen level usually reflect normal blood clotting capability. Elevated fibrinogen levels can be seen in conditions of acute or chronic inflammatory illnesses, tissue damage, infection, cancer,pulmonary embolism, acute coronary syndrome, Pregnancy or estrogen therapy and strokes.[22,23]Increased fibrinogen levels are a strong and independent cardiovascular risk factor.[24-26] Decreased fibrinogen levels (< 100 mg/dl) are associated with afibrinogenemia, hypofibrinogenemia, end-stage liver disease, severe malnutrition, disseminated intravascular coagulation (DIC), abnormal fibrinolysis and large-volume blood transfusions.[27,28]

The purposes present study, wasto evaluate and investigatethe relationship between the activated partial thromboplastin time (APTT) and fibrinogen values in patients with Polycythemia vera (PV).

2. MATERIAL AND METHODS

Study design and duration:
The study was cross sectional study conducted from April to August 2016 on the PV patients attending the hematology clinic of Fedail private hospital,Sudan .

Study Participants:
A total of 48 subjects were participated in this study; 19were diagnosed with polycythemia vera(Cases) according to World Health Organization (WHO) 2008 diagnostic criteria [1](sample size was based on the load of PV cases at hematology clinic during study period), 29 apparently healthy subjects served as controls.Subjectswith liver disease, renal disease , pregnancy, lactation, diagnosed haemostatic disorder,any systemic infection or chronic disease likely to affect haemostasis or patients who on warfarin or heparin or any other anticoagulation therapy which might affect APTT and fibrinogen were excluded for the study.

Collection of Blood Samples
Under aseptic condition 2 ml of venous blood wascollected. Then 1.8ml of the collected blood were placed in 3.2% trisodium citrate vial and mixed properly. This makes a dilution of 1:9. Platelet-poor plasma was isolated from citrated blood by centrifugation for 15 min at 3000rpm and stored at –80℃ until testing. APTTand fibrinogen were assayed on venous blood sample of the patients.

Assays

Procedure for APTT determination
Firstly 100 µL of PPP plasma was warmed at 37℃ for 3 minutes. At the same time the APTT reagent and CaCl2 were also simultaneously incubated. Then 100 µL APTT reagent was added to the warmed plasma and mixed and again incubated at 37℃ for exactly 3 minutes (activation time). After that, 100 µL pre-warmed CaCl2 was added. Then the analyzer read the clotting time of APTT and displayed the result in seconds.

Fibrinogen Assay colorimetric method.(Clauses Method)
First of all the test plasma was diluted with Owren's buffer to give a dilution of 1:10. Then 200 µL of diluted plasma was warmed for 2-5 minutes at 37℃. Then 100 µL of thrombin solution (prewarmed at 37C°) was added. Simultaneously, stop watch was started and clot was observed carefully, the watch was stopped at the appearance of the first visible fibrin web. Then clotting time obtained in seconds was plotted on the calibration curve and fibrinogen concentration was quantified in mg/dl.

Reference values: APTT 26.0–36.0s [29]
Fibrinogen 2.0–4.0 g/L [21]

Statistical analysis:

Results obtained were analyzed using SPSS software (version 20). Results were expressed as mean and standard deviation. Student's t test was used to determine the level of significance.
Associations between Fibrinogen levels and APTT values were examined using Pearson correlation coefficients.

3. RESULTS

The study included 19 patients, out of which14 were male and 5 were females with a mean age of 59 ±4 years. The analysis of haemostatic parameters (PT, APTT and fibrinogen) were as follows-there were no significant differences in PT between cases and controls groups. A significantly shortened APTT values (26.9±1.3 s) and increased fibrinogen levels (569±79) were found in PV cases compared with controls. Table1

Table1Mean of APTT, PT and fibrinogen among study groups

Parameter	Control (n=29)	Cases (n=19)
Age (Years)	47± 8	59 ±4
Male/Female	17/12	14/5
PT(sec)	11.4±1.1	11.5±0.7 (*P = 0.72*)
APTT(sec)	35.4±4.4	26.9±1.3 (*P= 0.000*)
Fibrinogen mg/dl	290±96	569±79 (*P=0.000*)

Analysis of correlations indicates that there was a significant negative correlation between Fibrinogen level and APTT in PV cases (R=-0.766, P=0.000).Table2

Table 2 Correlation of APTT with Fibrinogen level in patients with Polycythemia Vera

	Casesgroup (n=19)	
	Fibrinogen level	
Parameter	**Correlations (R)**	**P-value**
APTT	-0.766	0.000

DISCUSSION

In our study a significantly shortened APTT values in PV**Cases** than controls were observed. Many studies provided evidence that a shortened APTTs might reflect a hypercoagulable and could be considered as a risk marker for thrombosis.[17,30-33]Mina A et al [17]in 2010, they prospectively evaluated the phenomenon of short APTTs in 113 consecutive samples compared with an equal number of age and sex-matched normal APTT samples. They found plasma from patients presenting with short APTTs is reflective of a complex hypercoagulantstate that could feasibly contribute to thrombotic risk.Abdullah WZ. et al [30] revealed that , APTT test is a potential haemostatic marker for hypercoagulable state including in arterial thrombosis. Legnani C .et al[31] observed that abnormally short APTT values are associated with a significantly increased risk of venous thromboembolism (VTE) recurrence. In 2015, Lin CH et al [32] provided evidence that a shortened APTT is a prevalent and independent risk factor for ischemic stroke, stroke severity, and neurological worsening after acute stroke. Cihan Ay et al [33] also observed an impressive and highly significant association between a shorter APTT and an increased risk of VTE.

The present study showed that, fibrinogen levels have been significantly elevated in PV**Cases** than in controls. It was reported that increased fibrinogen levels were a strong and independent risk factors for venous and arterial thrombosis.[24-26] An elevated fibrinogen levels in MPNs had been reported by in many studies.[10,11,34] MPNs are accompanied by some degree of chronic inflammation [35,36]. Several inflammatory cytokines and growth factors (IL-6, IL-1, GM-CSF, and TGF-β) are found to be significantly overproduced in all subtypes[37]. This may be may possibly cause of elevated fibrinogen levels in MPNs.

The recent scientific literature supported the theoretical association between shortened APTT, increased fibrinogen levels and the risk of venous thrombosis.[38-41] A significant association of APTT and fibrinogen level has been reported in Diabetes Mellitus [38,39] and Hyperthyroidism.[40,41] In our study, there was a statistically significant correlation between shortened APTT and increased fibrinogen levels in PV **Cases**. Many recent studies have confirmed coagulation abnormities in MPNs.[10,11,34] However, none of those studies haveinvestigatedthe relationship between the activated partial thromboplastintime (APTT) and fibrinogen values in MPNs.

In conclusions, results shown in this study indicate that, the patients with PV were prone to develop hypercoagulable state. Therefore, routine examinations of APTT and fibrinogen are significantto assess coagulation abnormality in PV in order to prevent PV-associated thrombosis.

ETHICAL CONSIDERATIONS

The research protocol was approved by the SUMASRI International Review Board (SIRB)at University Of Medical Sciences And Technology(UMST),Sudan. The purpose and objectives of the study was explained to the patients. Written informed consent was obtained from the patient at the time of enrollment. A copy of the written consent is available for review by the editorial office/chief editor/editorial board members of this journal.

STUDY LIMITATION

The study was limited to a single hospital only. The sample size might not be the exact representatives of the whole case so as to generalize the findings of the study. Further studies are needed to confirm these findings.

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ABBREVIATIONS

APTT	Activated Partial Thrombin Time
MPNS	Myeloproliferative Neoplasms
PT	Prothrombin Time
PV	Polycythemia Vera
TNF	Tumour Necrosis Factor
WHO	World Health Organization