Original Research Article

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

ABSTRACT

Aims: To evaluate the Activated 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 samples (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 assayed by coagulmater machine and fibrinogen level was estimated by colorimetric method.(Clauses Method). Statistical evaluation was performed by SPSS (version 20) using Student's t test and Pearson correlation tests.

Results: A significantly shortened APTT values (26.9±1.3 s) were found in PV patients in comparison to normal control ($p<0.05$). Increased fibrinogen levels were seen IN PV patients (569±79,$p<0.05$).Strong negative correlation between the Fibrinogen level and shortened 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: { Polycythemia vera (PV), myeloproliferative neoplasms (MPNs), Activated Partial Thromboplastin Time(APTT) .hypercoagulable, fibrinogen levels }

1. INTRODUCTION

Polycythemia vera (PV) is the 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 approximately 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 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 laboratories 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.
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.[13] Fibrinogen is acute-phase proteins produced by the liver, stimulus for production is likely to be inflammatory cytokines such as interleukin-1, interleukin-6 and tumour necrosis factor (TNF). [14,15]
The purposes present study, to evaluate and examine the 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 .

Patients

The study population included 19 patients diagnosed with polycythemia vera according to World Health Organization (WHO) 2008 diagnostic criteria(sample size was based on the load of PV cases at hematology clinic during study period). Patients with 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.

Why no mention of controls here? Who were they

Collection of Blood Samples

Under a septic condition 2 ml of venous blood will be collected. 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. APTT and 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 coagulmater machine 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 in 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 g/l.

Reference values: APTT 26.0–36.0s [16]
Fibrinogen 2.0–4.0 g/L [17]

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 among these two groups. A significantly shortened APTT values (26.9±1.3 s) and increased fibrinogen levels (569±79) in Polycythemic group were found. Table1

Table1 Means of APTT, PT and fibrinogen among study groups

Parameter	Control (n=29)	PV (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 g/dl	290±96	569±79 (*P=0.000*)

P-value < 0.05 is considered statistically significant.

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

Delete shotened

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

	Study group (n=50)	
	Fibrinogen level	
Parameter	Correlations (R)	P-value
APTT	-0.766	0.000

DISCUSSION

In our study a significantly shortened APTT values in PV than control subjects were observed. Many studies provided evidence that a shortened APTTs might reflect a hypercoagulable and could be considered as a risk marker for thrombosis. Abdullah WZ. et al [18] revealed that , APTT test is a potential haemostatic marker for hypercoagulable state including in arterial thrombosis. Mina A et al [19]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 hypercoagulant state that could feasibly contribute to thrombotic risk.

Legnani C . et al[20] observed that abnormally short APTT values are associated with a significantly increased risk of venous thromboembolism (VTE) recurrence. In 2015, Lin CH et al [21] 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 [22] 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 than in control. It was reported that increased fibrinogen levels were a strong and independent risk factors for venous and arterial thrombosis.[23-25] An elevated fibrinogen levels in MPNs had been reported by in many studies.[10,11,26] MPNs are accompanied by some degree of chronic inflammation [27,28]. Several inflammatory cytokines and growth factors (IL-6, IL-1, GM-CSF, and TGF-β) are found to be significantly overproduced in all subtypes[29]. 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. A positive Association of APTT and fibrinogen level has been reported in Diabetes Mellitus [30,31] and Hyperthyroidism [32] In our study, there was a statistically significant correlation between shortened APTT, increased fibrinogen levels. Many recent studies have demonstrated coagulation abnormities in MPNs. However, those studies have not evaluate the associated between fibrinogen and APTT.

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 and are significant to 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.

REFERENCES

1. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. IARC Press: Lyon, France, 2008.
2. Tefferi A.Polycythemia vera and essential thrombocythemia: 2012 update on diagnosis, risk stratification, and management. Am J Hematol 2012 87(3):285–293. doi: 10.1002/ajh.23135.
3. Tefferi A, Rumi E, Finazzi G, et al. Survival and prognosis among 1545 patients with contemporary polycythemia vera: an international study. Leukemia 2013; 27:1874. doi: 10.1038/leu.2013.163.
4. Passamonti F, Rumi E, Pungolino E, et al. Life expectancy and prognostic factors for survival in patients with polycythemia vera and essential thrombocythemia. Am J Med. 2004;117(10): 755-761.
5. Marchioli R, Finazzi G, Landolfi R, et al. Vascular and neoplastic risk in a large cohort of patients with polycythemia vera. J Clin Oncol. 2005;23(10): 2224-2232.
6. Björkholm M, Derolf AR, Hultcrantz M, et al.Treatment-related risk factors for transformation to acute myeloid leukemia and myelodysplastic syndromes in myeloproliferative neoplasms. J Clin Oncol .2011;29:2410–2415. doi: 10.1200/JCO.2011.34.7542.
7. Polednak AP.Recent decline in the U.S. death rate from myeloproliferative neoplasms, 1999-2006. Cancer Epidemiol . 2012;36:133–13. doi: 10.1016/j.canep.2011.05.016.
8. Anna Falanga, Marina Marchetti.Thrombosis in Myeloproliferative Neoplasms. Semin Thromb Hemost 2014;40:348–358. doi: 10.1055/s-0034-1370794.
9. Marchetti M, Falanga A. Leukocytosis, JAK2V617F mutation, and hemostasis in myeloproliferative disorders. Pathophysiol Haemost Thromb 2008;36(3-4):148–159. doi: 10.1159/000175153
10. Gadomska G , Rość D, Stankowska K, Boinska J, Ruszkowska-Ciastek B, Wieczór R. Selected parameters of hemostasis in patients with myeloproliferative neoplasms. Blood Coagul Fibrinolysis. 2014 ;25(5):464-70. doi: 10.1097/MBC.0000000000000088
11. SrySuryani Widjaja, Karmel L Tambunan, Yahwardiah Siregar, Rahajuningsih Dharma, Stephen CL Koh.VEGF, D-Dimer and Coagulation Activation Markers In Indonesian Patients With Polycythemia Vera And Essential Thrombocythaemia and Their Relation with Recurrence Of Thrombosis. International Archives Of Medicine.2015 Vol. 8 No. 157
12. Dacie and Lewis (2011), Practical Haematology, Barbara J Bain, Imelda Bates, Michael A Laffan and S. Mitchell Lewis, Eleventh edition, page: 409.
13. Hermans J, McDonagh J "Fibrin: structure and interactions". Semin. Thromb. Hemost. 1982;8(1):11-24. DOI:10.1055/s-2007-1005039
14. Heinrich PC, Castell TA, Andus T. Interleukin-6 and the acute phase response.Biochem J. 1990;265:621–636.
15. Heinrich PC, Behrmann I, Müller-Newen G, Schaper F, Graeve L. Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. Biochem J. 1998;334:297–314.
16. Dacie and Lewis (2011), Practical Haematology, Barbara J Bain, Imelda Bates, Michael A Laffan and S. Mitchell Lewis, Eleventh edition, page: 411.
17. Dacie and Lewis (2011), Practical Haematology, Barbara J Bain, Imelda Bates, Michael A Laffan and S. Mitchell Lewis, Eleventh edition, page: 415.
18. Abdullah WZ1, Moufak SK, Yusof Z, Mohamad MS, Kamarul IM. Shortened activated partial thromboplastin time, a hemostatic marker for hypercoagulable state during acute coronary event. Transl Res. 2010 Jun;155(6):315-9. doi: 10.1016/j.trsl.2010.02.001
19. Mina A, Favaloro EJ, Mohammed S, Koutts J (2010) A laboratory evaluation into the short activated partial thromboplastin time. Blood Coagul Fibrinolysis 21: 152–157. doi: 10.1097/MBC.0b013e3283365770.
20. Lin CH1, Kuo YW2, Kuo CY1, Huang YC3, Hsu CY1, Hsu HL1, Lin YH1, Wu CY1, Huang YC1, Lee M3, Yang HT1, Pan YT1, Lee JD4. Shortened Activated Partial Thromboplastin Time Is Associated With Acute Ischemic Stroke, Stroke Severity, and Neurological Worsening. J Stroke Cerebrovasc Dis. 2015 ;24(10):2270-6. doi: 10.1016/j.jstrokecerebrovasdis.2015.06.008.
21. Legnani C1, Mattarozzi S, Cini M, Cosmi B, Favaretto E, Palareti G. Abnormally short activated partial thromboplastin time values are associated with increased risk of recurrence of venous thromboembolism after oral anticoagulation withdrawal. Br J Haematol. 2006 ;134(2):227-32. DOI:10.1111/j.1365-2141.2006.06130.x
22. Cihan Ay, Florian Posch, Julia Riedl, Oliver Koenigsbruegge, Peter Quehenberger, Christoph Zielinski, Ingrid Pabinger.Prediction of Venous Thromboembolism in Patients with Cancer By

the Activated Partial Thromboplastin Time: Results from the Vienna Cancer and Thrombosis Study.Blood 2015 126:653. doi: 10.1182/blood-2010-02-270116.

23. Yu Shi, MD, Yihua Wu, MD, PhD, Chang Bian, MD, PhD, Wanjun Zhang, MD, Jun Yang, PhD, andGeng Xu, MD, PhD.Predictive Value of Plasma Fibrinogen Levels in Patients Admitted for Acute Coronary Syndrome. Tex Heart Inst J. 2010; 37(2): 178–183.
24. Rehana S. Lovely,Steven C. Kazmierczak, Joseph M. Massaro,Ralph B. D'Agostino, Sr.,Christopher J. O'Donnell, and David H. Farrell.γ' Fibrinogen: Evaluation of a New Assay for Study of Associations with Cardiovascular Disease. Clin Chem. 2010 May; 56(5): 781–788. doi: 10.1373/clinchem.2009.138347
25. Tochi M. Okwuosaa, Oana Kleinb, Cheeling Chanc ,Nancy Swords Jennyd, Pamela Schreiner, David Green, Kiang Liuc .13-year long-term associations between changes in traditional cardiovascular risk factors and changes in fibrinogen levels: The Coronary Artery Risk Development in Young Adults (CARDIA) study. Atherosclerosis. 2013 Jan; 226(1): 214–219. doi: 10.1016/j.atherosclerosis.2012.10.043
26. Sokołowska B1, Nowaczyńska A, Bykowska K, Chocholska S, Wejksza K, Walter-Croneck A, Gromek T,Kowalska AM, Kandefer-Szerszeń M, Dmoszyńska A.JAK2 mutation status, haemostatic risk factors and thrombophilic factors in essential thrombocythaemia (ET) patients. Folia Histochem Cytobiol. 2011;49(2):267-71.
27. Mantovani, P. Allavena, A. Sica, and F. Balkwill, “Cancer-related inflammation,” Nature. 2008 Jul 24;454(7203):436-44. doi: 10.1038/nature07205.
28. S. Yaqub and E. M. Aandahl, “Inflammation versus adaptive immunity in cancer pathogenesis,”Critical Review Oncology,2009;15(1-2):43-63.
29. Vaidya R., Gangat N., Jimma T., et al. Plasma cytokines in polycythemia vera: phenotypic correlates, prognostic relevance, and comparison with myelofibrosis.*American Journal of Hematology*. 2012;87(11):1003–1005. doi: 10.1002/ajh.23295.
30. Ying Zhao, Jie Zhang, Juanwen Zhang,and Jianping Wu.Diabetes Mellitus Is Associated with Shortened Activated Partial Thromboplastin Time and Increased Fibrinogen Values. PLoS One. 2011; 6(1): e16470.. doi: 10.1371/journal.pone.0016470.
31. Binaya Sapkota,Saroj Kumar Shrestha and Sunil Poudel Association of activated partial thromboplastin time and fibrinogen level in patients with type II diabetes mellitusBMC Research Notes.2013;6:485. doi: 10.1186/1756-0500-6-485.
32. Giuseppe Lippi,Massimo Franchini,Giovanni Targher,Martina Montagnana,Gian Luca Salvagno, Gian Cesare Guidi and Emmanuel J. Favaloro.Hyperthyroidism is associated with shortened APTT and increased fibrinogen values in a general population of unselected outpatients.Journal of Thrombosis and Thrombolysis October 2009, 28:362. doi:10.1007/s11239-008-0269-z

ABBREVIATIONS

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