ORIGINAL ARTICLE

Comparative analysis of "APTT vs RVVT" based activated protein C resistance assay in the diagnosis of Factor V Leiden mutation

Mirza Asif Baig

Department of Pathology, BLDE's Shri B.M. Patil Medical College Hospital and Research Centre, Bijapur, Karnataka, India

Address for correspondence:
Dr. Mirza Asif Baig, Former Asstt. Prof. SBMPMC, Bijapur, Karnataka, India. E-mail: drasifbaig@yahoo.com

ABSTRACT

Background: Thrombophilia is a hypercoagulable state characterized by increased venous thrombosis. The most common cause of heritable thrombophilia is Factor V Leiden (FVR506Q) homozygous state, with a relative risk of 10–80 times as compared to normal individuals and Lupus anticoagulant is the most common cause of acquired thrombophilia. The main objective of this study is to compare the sensitivity of activated partial thromboplastin time (APTT) vs dilute Russell viper venom test (DRVVT) based APCR assays with predilution in Factor V-deficient plasma for diagnosis of Factor V Leiden mutation. **Materials And Methods:** The coagulometer used for APCR test was Sysmex CS-5100. APTT reagent used is Pathrombin SL supplied by Siemens. All data were expressed as mean ± SD. Statistical analysis was done using unpaired students *t*-test and a *P* value <0.05 was considered as statistical significance. **Results:** A total of 300 cases of APCR (200 cases of Factor V Leiden mutation was confirmed by PCR and 100 acquired) were studied. The sensitivity of screening APTT-based APCR for detection of Factor V Leiden mutation is 67% and for the noncarrier state, it is 62%. The sensitivity of modified APTT and DRVVT with predilution in FV-deficient plasma for detection of Factor V Leiden mutation is 82% and 84%, respectively and for acquired causes, it is 48% and 86%, respectively. **Conclusion:** Screening APTT test has increased in activated protein C resistance (APCR) due to Factor V Leiden mutation as well as acquired causes such as patients on direct-acting oral anticoagulants, warfarin, lupus anticoagulants, and oral contraceptive pills which are independent risk factors of venous thrombosis. Modified DRVVT with predilution in FV-deficient plasma is more sensitive than screening and modified APTT-based APCR test in the diagnosis of Factor V Leiden mutation and the former test can distinguish homozygous and heterozygous states from normal individuals.

KEY WORDS: Activated protein C resistance, activated partial thromboplastin time, Factor V Leiden mutation, lupus anticoagulant

Access this article online

Website: www.ijpmonline.org

DOI: 10.4103/IJPM.IJPM_899_19

Quick Response Code:

INTRODUCTION

Activated protein C resistance (APCR) is a hemostatic disorder associated with increased risk of deep venous thrombosis and pulmonary embolism. Factor V Leiden mutation accounts for 95% of cases of APCR and remainders 5% are acquired causes such as intake of Vitamin K antagonists, oral contraceptive pills, direct oral acting anticoagulants (DOACs), lupus anticoagulant (LAC,) and conditions associated with an increase in Factor VIII like pregnancy.[1]

Protein C Proteolysis APC -- degrades FVa and FVIIIa - Inhibits coagulation-- prolongs APTT.

In APC-R – no degradation of Factor V – increases coagulation – APTT is not prolonged,[2,3]

APCR was first reported in 1995 in approximately 95% of cases due to the Factor V Leiden [Figure 1].

The incidence of Factor V Leiden mutation in patients with venous thrombosis is approximately 20–40% and it is the most common hereditary cause of the increased risk of venous thrombosis (3–7% of Caucasian). Patients who are heterozygous for Factor V Leiden mutation have 5 to 8 times increased risk of venous thrombosis as compared to the general population but only 10% of these develop thrombosis

How to cite this article: Baig MA. Comparative analysis of "APTT vs RVVT" based activated protein C resistance assay in the diagnosis of Factor V Leiden mutation. Indian J Pathol Microbiol 2020;63:247-50.

during their lifetime. Individuals who are homozygous have a 30–140-fold risk. Following venous thrombosis, they have a higher risk of rethrombosis than individuals with DVT but normal Factor V.[4]

The first generation assay for screening APCR was initially described by Dahlback (1995) and is based on APTT assay and its prolongation by addition of APC. Second generation tests are modified APTT and modified DRVVT-based APCR assays for differentiating normal individuals from homozygous and heterozygous state of Factor V Leiden mutation.[1]

APTT based screening test for APCR is sensitive for Factor V Leiden mutation but it has certain limitations

- Requires a normal baseline APTT
- There is considerable overlap between healthy individuals and heterozygotes
- Low protein S will also skew the ratio.[5]

Screening APTT test is increased in activated protein C resistance due to Factor V Leiden mutation as well as acquired causes such as patients on direct-acting oral anticoagulants, warfarin, lupus anticoagulants, and oral contraceptive pills which are independent risk factors of venous thrombosis. Modified APTT with predilution in FV-deficient plasma is independent of these confounding factors and specific for factor V Leiden mutation.[1,3] Modified DRVVT with predilution in FV-deficient plasma offers extra-advantage in detecting APCR due to LAC cases.

The main objective of this study is to compare the sensitivity of Screening and modified APTT vs RVVT based APCR test for diagnosis of Factor V Leiden mutation.

MATERIALS AND METHODS

This is a prospective study of 1-year duration (from July 2018 to June 2019) carried out in a tertiary care hospital, medical college and research center. The coagulometer used for APCR test was Sysmex CS-5100 with Pathrombin SL APTT reagent supplied by Siemens. All data were expressed as mean ± SD. Statistical analysis was done using unpaired students *t*-test and a P value < 0.05 is considered as statistical significance.

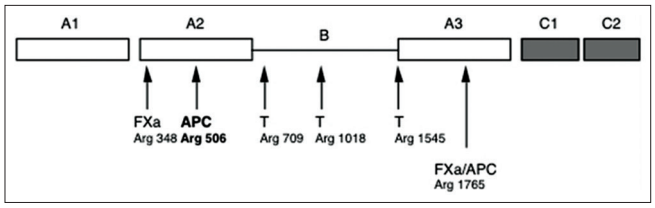

Figure 1: Missense Mutation.[2,3] [FVL] mutation – a G1691a missense mutation at Arginine 506 resulting in its replacement by a glutamine [R506Q] and the abolition of an APC inactivation cleavage site in Factor Va[1]

$$\text{Sensitivity} = \frac{\text{Number of true positive cases}}{\text{Number of true positive} + \text{false - negative cases}}$$

Inclusion criteria

1. APCR positive homozygous and heterozygous Factor V Leiden mutation cases
2. Acquired APCR causes such as OCPs, DOACs, and LAC).

Exclusion criteria

1. Anti-thrombin III Deficiency
2. Protein S and C deficiency

Test	*Explanation*
APTT	Patients plasma sample+Exogenous APC→perform APTT In a plasma sample without APCR, the addition of APC inactivates Factor Va and Factor VIIIa and so prolongs APTT. In contrast, in a sample with the Factor V Leiden mutation, APTT is prolonged but to a lesser extent. A ratio is derived from: [APTT+APC]/[APTT-APC] (APTT in presence of APC÷APTT in absence of APC) A limitation of this test is that it requires a normal APTT in the patient and so cannot be used in cases in which there is a prolongation of the APTT e.g., patients on oral anticoagulants or LAC. Normal ratio>2.2, Heterozygotes~1.7, Homozygotes~1.2 Individuals without the FVL mutation generally have a ratio of>2.0 and individuals who are heterozygous for the FVL mutation have a ratio<2. However, there is considerable overlap between healthy individuals and heterozygotes.
Modified APTT with predilution in FV-deficient plasma.[1,2,6,7]	This is a modification of the original APTT screening test in which a predilution [1:4] of patient plasma with Factor V-deficient plasma is made before the addition of APC. The modified assay reduces the number of exogenous confounding factors that might affect the APTT e.g., high FVIII levels and makes the test specific for mutations within FV. However, the presence of lupus anticoagulants by competing for phospholipid, can prolong PTT measurements in these assays and are a major source of false-positive results if the test is used as a screening test for FVL. (It is important to remember that this modified assay is specific only for mutations within FV whereas the original APTT assay without Factor V–deficient plasma predilution, measures APC resistance from any cause)
Modified Russell Viper Venom Assay with predilution in FV-deficient plasma[6-8]	This test is a modification in dilute Russell Viper Venom assay with a predilution in FV-deficient plasma. The DRVVT is prolonged when a plasma sample is preincubated with a diluted snake venom isolated from *Agkistrodon contortrix contortrix* which activates protein C. The test result is expressed as the ratio between the DRVVT with and without the addition of the venom. DRVVT:- Russell's viper venom is a potent activator of FX. Added to phospholipid, prothrombin and calcium it will clot fibrinogen to fibrinActivates FX so the test is unaffected by deficiencies of FVIII, IX, XI and XII. If an LA is present this will bind to phospholipid and prolong the clotting time Method:-DRVVT+ Venom/ DRVVT-Venom Interpretation > 1.8 = Normal < 1.5 = Heterozygous FV Leiden < 1.05 = Homozygous FV Leiden

3. Nephrotic syndrome
4. Pregnancy.

Test Procedure (Summary) – Practical Hemostasis

Factor V Leiden (FVR506Q) mutation was detected by Real-time-polymerase chain reaction (RT-PCR). PCR technique amplify genomic DNA followed by identification of presence or absence of the missense mutation e.g., restriction enzyme digestion and mapping. Homozygous and heterozygous state of Factor V Leiden mutation were determined. All steps of PCR like amplification, heating were employed.

RESULTS

The present study includes a total of 300 APCR positive cases Of these, 200 cases are of FV Leiden mutation confirmed by PT-PCR 100 APCR positive cases (APTT/modified APTT based) with history of venous thrombosis and noncarrier of FV Leiden mutation.

- 70 LAC + Cases confirmed dilute Russell viper venom test (DRVVT)
- 20 cases on warfarin
- 10 cases on OCPs.

Modified DRVVT with predilution in FV-deficient plasma (1:4) is more sensitive than Screening and modified APTT-based APCR test in the diagnosis of Factor V Leiden mutation and the former test can distinguish homozygous and heterozygous states from normal individuals [Table 3].

However, in contrast to modified test, screening APTT is increased in APCR due to Factor V Leiden mutation as well as acquired causes such as patients on DOACs, LAC, and OCPs which are independent risk factors of venous thrombosis.

DISCUSSION

APCR is a hemostatic disorder characterized by an increased risk of venous thrombosis, including deep vein thrombosis and pulmonary embolism. FV Leiden mutation accounts for 95% of APCR cases and the remainder are acquired like Vitamin K antagonists, DOACs, LAC, and OCPs.[1]

APCR can be tested by

1. APTT based screening test
2. Modified APTT with predilution in FV-deficient plasma
3. DRVVT based
4. Chromogenic assay.

In this study, 3 types of assays were performed

1. Routine APTT test
2. Modified APTT
3. Modified DRVVT test.

The Gold standard for diagnosis of Factor V Leiden mutation is by PCR technique but this is not cost-effective. The cost evaluated per test is $36.38 for Modified APCR test and $83.77 for RT-PCR (Mayo special coagulation lab).[8-10]

Overall Sensitivity of Screening APTT-based APCR test for detection of FV Leiden mutation is 67%, for heterozygous state sensitivity is 69%, and for the homozygous state, it is 64%. The sensitivity of screening APTT-based APCR test for the detection of acquired causes such as LAC, DOACs, and OCPs therapy is 62% [Table 1].

Studies by Elizabeth *et al.*[11] also concludes that APC-R assays that dilute patient plasma into Factor V-deficient plasma are much more accurate for detecting FV Leiden than other assays.

Table 1: Sensitivity of Screening APTT-based APCR

Screening APTT	*FV Leiden mutation (200) (RT-PCR)*			*Acquired (100)*		
	Overall Sensitivity	*<1.7 Heterozygous (130 cases)*	*<1.2 Homozygous (70 cases)*	*LAC (70)*	*Warfarin (20)*	*OCPs (10)*
Sensitivity - % Screening APTT	135 (67%)	90 (69%)	45 (64%)	40 (57%)	14 (70%)	08 (80%)

APCR ratio of >2.2 is taken as Normal. Overall Sensitivity of Screening APTT based APCR test for detection of carrier FV Leiden mutation is 67%, for heterozygous state sensitivity is 69% and for homozygous state, it is 64%. The sensitivity of Screening APTT based APCR test for the detection of acquired causes like LAC, warfarin, and OCPs therapy is 62%

Table 2: Sensitivity of Modified APTT-based APCR

Modified APTT with predilution in FV-deficient plasma (1:4)	*FV Leiden mutation (200) (RT-PCR)*			*Acquired (100)*		
	Overall sensitivity	*<1.7 Heterozygous (130 cases)*	*<1.2 Homozygous (70 cases)*	*LAC (70)*	*Warfarin (20)*	*OCPs (10)*
Sensitivity -Modified APTT	164 (82%)	110 (84%)	54 (77%)	30 (42%)	12 (60%)	06 (60%)

>2.2=Normal. Overall Sensitivity of Modified APTT with predilution in FV-deficient plasma (1:4), based APCR test for detection of FV Leiden mutation is 82%, for heterozygous state sensitivity is 84% and for homozygous state, it is 77%. The sensitivity of modified APTT-based APCR test for detection of acquired causes like LAC, warfarin, and OCPs therapy is 48%

Table 3: Sensitivity of Modified DRVVT-based APCR test

Modified APTT with predilution in FV-deficient plasma (1:4)	*FV Leiden mutation (200) (RT-PCR)*			*Acquired (100)*		
	Overall sensitivity	*<1.5 Heterozygous (130 cases)*	*<1.05 Homozygous (70 cases)*	*LAC (70)*	*Warfarin (20)*	*OCPs (10)*
Sensitivity -Modified APTT	168 (84%)	112 (86%)	56 (80%)	62 (88%)	18 (90%)	6 (60%)

>1.8=Normal. Overall Sensitivity of Modified DRVVT with predilution in FV-deficient plasma (1:4), based APCR test for detection of FV Leiden mutation is 84%, for heterozygous state sensitivity is 86% and for homozygous state, it is 80%. The sensitivity of modified DRVVT-based APCR test for detection of acquired causes like LAC, warfarin, and OCPs therapy is 86%

Table 4: Comparative Analysis of various studies in FV Leiden mutation

Test	*Mayo et al.*[9]	*Optum lab database*[9]	*Present study*
APCR	1256	5395	150
FV Leiden mutation	268	78525	100
APCR/FV Leiden	1 : 0.2	1:15	1.5:1

The discrepancy in APCR/FV Leiden ratio in Mayo *et al*. and the present study is because the former study was conducted on a large population and the duration of the study was longer

APC-R assays are advantageous as they are easily automated, cost-effective, and may detect rare causes of APC resistance other than Factor V Leiden[4] [Table 4].

Devreese *et al*. showed the effect of DOACs such as Dabigatran, Rivaroxaban, and Apixaban on APCR tests and the results are in correlation with present studies.

Results of the present study suggest that modified APTT with predilution in Factor V-deficient plasma is more sensitive than screening APTT-based test for diagnosis of Factor V Leiden mutation. Studies conducted by Stephan *et al*.,[4] Taylor and Fristma *et al*.,[8] Juliana *et al*.,[9] and Pruller *et al*.[10] shows similar results.

The overall sensitivity of modified APTT with predilution in FV-deficient plasma (1:4), based APCR test for detection of FV Leiden mutation is 82%, for heterozygous state sensitivity is 84% and for the homozygous state is 77%. The sensitivity of modified APTT-based APCR test for the detection of acquired causes such as LAC, warfarin, and OCPs therapy is 48% [Table 2].

In the present study overall sensitivity of modified DRVVT with predilution in FV-deficient plasma (1:4), based APCR test for detection of FV Leiden mutation is 84%, for heterozygous state sensitivity is 86%, and for Homozygous state, it is 80%. The sensitivity of modified DRVVT-based APCR test for detection of acquired causes like LAC, warfarin, and OCPs therapy is 86%.

In a similar study conducted by Aboud MR *et al*.[11] both modified assays (APTT and DRVVT) demonstrated a sensitivity and specificity of 100% for FV Leiden, thrombophilic patients and patients on oral anticoagulants, with the modified RVVT-based assay giving better separation between normals and FV Leiden. Inhibition of phospholipid-dependent coagulation by LAC antibodies rendered the APTT-based system less suitable than the phospholipid-rich RVVT-based one, and as nine of the 20 LAC-positive patients were on warfarin, showed only the modified RVVT assay to be a reliable predictor of factor V Leiden in this patient group.[11]

The present study showed that Modified DRVVT is more sensitive than screening and modified APTT-based APCR assay in the diagnosis of Factor V Leiden mutation patients presenting with features of venous thrombosis and pulmonary embolism as well as in asymptomatic individuals. Modified DRVVT offers extra-advantage of identifying LAC cases (sensitivity = 90%), patients on warfarin and is independent of factor VIII, IX, XI, and XII levels.

CONCLUSION

Screening APTT detects APC-R due to Factor V Leiden mutation and acquired causes like LAC cases, patients on OCPs and DOAC cases, presenting with features of venous thrombosis. Modified APTT with predilution in FV-deficient plasma is independent of these confounding factors and specific for Factor V Leiden mutation.

Modified DRVVT is more sensitive than screening and modified APTT based APCR assay in the diagnosis of Factor V Leiden mutation and offers extra-advantage of identifying LAC cases and can distinguish normal cases from homozygous and heterozygous state of Factor V Leiden mutation.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

REFERENCES

1. Dahlback B. Inherited deficiency of Activated protein C, A major cause of venous thrombosis is due to mutation in factor V gene. Hemostasis 1994;24:139-51.
2. Mohammed S, Favaloro EJ. Laboratory testing for activated protein C resistance (APCR). Methods Mol Biol 2017;1646:137-43.
3. Jorquera HI, Fernandez MA. Modified test for APCR. Lancet 1994;344:1162-3.
4. Kadauke S, Khor B, Van Cott EM. Activated protein C resistance testing for factor V Leiden. Am J Hematol 2014;89:1147-50.
5. Rosendaal FR, Koster T, Vandenbroucke JP, Reitsma PH. High risk of thrombosis in patients homozygous for factor V Leiden (activated protein C resistance). Blood 1995;85:1504-8.
6. Ronde H, Bertina RM. Laboratory diagnosis of APC-resistance: A critical evaluation of the test and the development of diagnostic criteria. Thromb Haemost 1994;72:880-6.
7. Brandt G, Gruppo R, Glueck CJ, Stroop D, Becker A, Pillow A, *et al*. Sensitivity, specificity and predictive value of modified assays for activated protein C resistance in children. Thromb Haemost 1998;79:567-70.
8. Taylor LJ, Oster RA, Fritsma GA, Tichenor PH, Reed CE, Eiland BM, *et al*. Screening with the activated protein C resistance assay yields significant savings in a patient population with low prevalence of factor V Leiden. Am J Clin Pathol 2008;129:494-9.
9. Perez Botero J, Majerus JA, Strege AK, Johnson RD, Chen D, Pruthi RK. Diagnostic testing approaches for APCR and factor V Leiden. A comparison of Institutional and national provider practices. Am J Clin Pathol 2017;147:604-10.
10. Prüller F, Weiss EC, Raggam RB, Cervar-Zivkovic M, Renner W, Wagner J, *et al*. Activated protein C resistance assay and factor V Leiden. N Engl J Med 2014;371:685-6.
11. Aboud MR, Ma DD. A comparison between two activated protein C resistance methods as routine diagnostic tests for factor V Leiden mutation. Br J Haematol 1997;97:798-803.