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Original Research Article

Comparative Analysis of " Screening vs Modified " APTT based activated protein C resistance (APCR) assay in the diagnosis of Factor V leiden mutation

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ABSTRACT

Introduction: APCR is a hemostatic disorder characterized by increased risk of deep vein thrombosis and pulmonary embolism. Factor V Leiden mutation accounts for 95% of APCR cases & the remainder are due to acquired causes like patients on vitamin k antagonist therapy, direct oral acting anticoagulants, Lupus anticoagulant & oral contraceptive pills. T he main objective of this study is to compare the sensitivity of APTT based APCR test vs Modified APTT with pre -dilution in F actor V-deficient plasma for diagnosis of Factor V leiden mutation & to formulate a systematic diagnostic algorithm for interpretation of APCR tests.

Materials and Methods: The Coagulometer used for APCR test is Sysmex CS-5100. APTT reagent used is Pathrombin SL supplied by seimens. All data were expressed as Mean ± SD. Statistical analysis was done using unpaired students t test & a P value <0.05 is considered as statistical significance.

Results : A total of 150 cases of APCR (100 cases of factor V Leiden mutation confirmed by PCR & 50 non carrier /acquired cases) were studied retrospectively. Sensitivity of screening APTT base d APCR for detection of factor V Leiden mutation is 78% & for non carrier state it is 82%. Sensitivity of modified APTT with pre- dilution in FV-deficient plasmafor detection of factor V Leiden mutation is 93% & for noncarrier state (acquired) is 34%.

Conclusion: Screening APTT test is increased in Activated protein C resistance due to factor V Leiden mutation as well as acquired causes like 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 (1:4) is more sensitive than Screening APTT based APCR test in diagnosis of Factor VLeiden mutation & this test can distinguish homozygous & heterozygous states from normal individuals.

1. Introduction

Activated protein C resistance (APCR) is a hemostatic disorder characterized by increased risk of venous thrombosis, including deep vein thrombosis and pulmonary embolism. APCR can be

1. Hereditary - Factor V Leiden mutation in approximately. 95% of cases

2. Acquired - Vit K antagonists, DOACs, LAC, increased FVIII (pregnancy)[1]

In physiological conditions Activated Protein C degrades Factor Va and VIIIa. This leads to inhibition of Coagulation cascade and prolongs APTT

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

APCR was first reported in 1995 i n approximately 95% of cases due to the Factor V Leiden [FVL] mutation – a G1691a missense mutation at Arginine 506 resulting in its replacement by a glutamine [R506Q] (Figure 1) and the abolition of an APC inactivation cleavage site in Factor Va[1]

The incidence of factor V Leiden mutation in patients with venous thrombosis is approximately 20-40% & it is the mos t common hereditary cause of increased risk of

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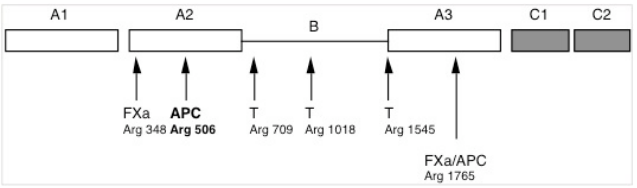

Fig. 1: Missense mutation

venous thrombosis (3-7% of Caucasian). Patients who are heterozygous for factor V Leiden mutation are 5 to 8 times increased risk of venous thrombosis as compared to general population but only 10% of these

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

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

1. Requires a normal baseline APTT
2. There is considerable overlap between healthy individuals and heterozygotes
3. Low pro tein 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 like 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 & specific for factor V leiden mutation.[1,3]

The main objective of this study is to compare the sensitivity of APTT based APCR test Vs Modified APTT with predilution in FV-deficient plasma in diagnosis of factor V Leiden mutation & to formulate a systematic diagnostic algorithm for interpretation of APCR tests

2. Materials and Methods

This is a Retrospective study of 1 year duration (from July 2018 to June 2019) carried out in a tertiary care hospital, medical college & research centre. The Coagulometer used for APCR test is Sysmex CS-5100 with Pathrombin SL APTT reagent supplied by seimens. All data were expressed as Mean ± SD. Statistical analysis was done using unpaired students t - test & a P

Value<0.05 is considered as statistical significance.

2.1. Inclusion criteria

1. APCR positive Homozygous & Heterozygous Factor V Leiden Mutation cases
2. Acquired APCR causes like OCPs, DOAC s, LAC)

2.2. Exclusion criteria

1. Anti-Thrombin III Deficiency
2. Protein S & C deficiency
3. Nephrotic syndrome
4. Pregnancy

2.3. Test Procedure (Summary) – Practical Hemostasis

3. Results

Present study includes a total of 150 APCR positive cases chosen retrospectively.

Of these, 100 cases are of FVleiden mutation confirmed by RT -PCR

50 APCR positive cases (APTT/ modified APTT based) with history of venous thrombosis & non carrier of FV ladeinmutation.

20 LAC cases confirmed by dilute Russell viper venom test DRVVT

20 cases on warfarin 10 cases on OCPs

Overall Sensitivity of Screening APTT based APCR test for detection of carrier FV Leiden mutation is 78%, for Heterozygous state sensitivity is 77% & for Homozygous state it is 80%. Sensitivity of Screening APTT based APCR test for detection of non carrier FVleiden cases like LAC, warfarin & OCPs therapy is 82% (Table 2)

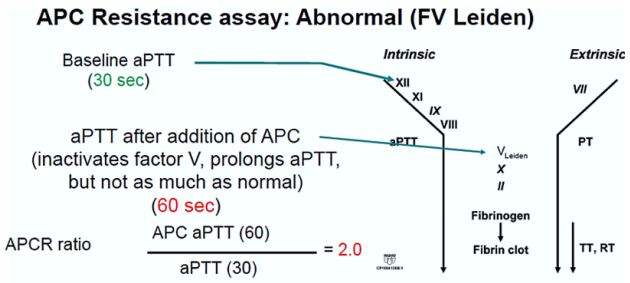

Fig. 2: Coagulation Cascade. Mechanism of APCR[6,7]

3.1. If the APCR normalized ratio is <2 then What test next?

Individuals with a low APC ratio should confirm F5 gene for the factor V Leiden mutation by PCR. However, it should be remembered that although most cases of APC resistance are due to the factor V Leiden mutation, testing with the original APTT-based APC resistance assay may be useful in detecting independent risk factors for venous thrombosis including pregnancy, oral contraceptive, LAC.[8,9]

The Gold standard for diagnosis of F actor 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 78%, for

Table 1: 2.3. Test Procedure (Summary) – Practical Hemostasis

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 imitation 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.
Normalised APTT	The normalised APTT was established to try and improve the discrimination between FVL heterozygotes and normal persons. The normalised ratio is derived by first dividing the APTT in the presence of APC by the APTT in the absence of APC (same as above). This ratio is then normalised against a reference pool to obtain an APC sensitivity ratio. [**APTT+APC]/[APTT-APC] of patient sample [APTT+APC]/[APTT-APC] of a normal reference plasma poo**l 80% of patients with an APC sensitivity ratio <0.84 and 100% of patients with an APC sensitivity ratio <0.70 were heterozygous or homozygous respectively for the FVL mutation.
Modified APTT with predilution in FV-deficient plasma	This is a modification of the original APTT screening test in which a pre-dilution [1 : 4] of patient plasma with factor V-deficient plasma is made before the addition of APC and calcium. 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 pre-dilution, measures APC resistance from any cause.*}

Table 2: Sensitivity of Screening APTT based APCR

Screening APTT	**FV leiden mutation (100) (RT-PCR)**			**Non Carrier FV leiden (50)**		
	<1.7 Heterozygous (65 cases)	<1.2 Homozygous (35 cases)	Overall sensitivity	LAC (20)	Warfarin (20)	OCPs (10)
Sensitivity - % Screening APTT	50(77%)	28(80%)	78%	16(80%)	17(85%)	08(80%)
Sensitivity Normalized APTT	52 (80%)	30(85%)	82%	16(80%)	15(75%)	08(80%)

APCR ratio of > 2.2 is taken as Normal

Table 3: Sensitivity of Modified APTT based APCR

Modified APTTwith predilution in FV-deficient plasma (1:4)	**FV leiden mutation (100) (RT-PCR)**			**Non FV Leiden (50)**		
	<**1.7** Heterozygous (65 cases)	<1.2 Homozygous (35 cases)	Overall sensitivity	LAC (20)	Warfarin (20)	OCPs (10)
Sensitivity - Modified APTT	61(94%)	32 (92%)	93%	06	09	02

heterozygous state sensitivity is 77% & for homozygous state it is 80%.

Sensitivity of Screening APTT based APCR test for de tection of non carrier FV leiden cases like LAC, DOACs & OCPs therapy is 82%.

Studies by Elizabeth et al also concludes that APC-R assays that dilute patient plasma into factor V-deficient plasmaare much more accurate for detecting FV Leiden than other assays.

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

Overall Sensitivity of Modified APTT with predilution in FV-deficient plasma (1:4), based APCR test for detection of F V Leiden mutation is 93%, for Heterozygous state sensitivity is 94% & for Homozygous state it is 92%.Sensitivity of Screening APTT based APCR test for detection of non carrier FV ladein cases like LAC, warfarin & OCPs therapy is 34%.

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

These results shows that Modified APTT with predilution in FV-deficient plasma (1:4) is more sensitive than Screening APTT based APCR test in diagnosis of Factor VLeiden mutation& this test can distinguish homozygous & 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 like patients on DOACs, LAC & OCPs which are independent risk factors of venous thrombosis.

4. Discussion

APCR is a hemostatic disorder characterized by increased risk of venous thrombosis, including deep vein thrombosis and pulmonary embolism. Factor V Leiden mutation accounts for 95% of APCR cases & the remainder are due to acquired causes like intake of warfarin,OCPs, DOACs & LAC.[1]

4.1. 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

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

Devreese et al showed the effect of DOACs like Dabigatran, Rivaroxaban, Apixaban on APCR tests & the results are in correlation with present studies

Results of present study suggests that Modified APTT with pre-dilution 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 & Fristma et al,[8] Juliana et al,[9] Pruller et al,[10] shows similar results.

APTT based Screening test For APCR

If Normalized ratio = < 1.7

↓

Carrier FVL + LAC, OCPs, warfarin

↓

Modified APTT – F V deficient plasma (1:4)

↓

APTT ↑ suggests FVL mutation

(Confirmed by PCR)

5. Conclusion

1. Screening APTT based APC-R test is increased in Factor V Leiden mutation & in LAC, OCPs & DOAC intake.
2. Modified APTT with predilution in FV-deficient plasma (1:4) is more sensitive than Screening APTT test in diagnosis of Factor V Leiden mutation & can distinguish homozygous & heterozygous states from normal individuals.

5.1. Abbreviations

DOACs - direct acting oral anticoagulants, LAC - lupus anticoagulant, OCPs -oral contraceptive pills,

FVLeiden - Factor V Leiden, APCR - Activated protein C resistance. DOACs - direct acting oral anticoagulants, LAC - lupus anticoagulant, OCPs -oral contraceptive pills,

OCPs -oral contraceptive pills

6. Source of funding

None.

7. Conflict of interest

None.

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