

Review Article

Laboratory Diagnosis of Congenital Factor V Deficiency, Routine, Specific Coagulation Tests with Molecular Methods

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Abstract

Congenital Factor V (FV) deficiency is a rare bleeding disorder that inherit in autosomal recessive manner. Diagnosis of FV deficiency (FVD) is made by routine coagulation tests, FV activity and molecular analysis. In patients with FVD, routine coagulation tests including activated partial thromboplastin time (APTT), prothrombin time (PT), and even bleeding time (BT) are prolonged while thrombin time (TT) is normal. FV activity assay can use for confirmation of diagnosis as well as for differential diagnosis with acquired forms of disease. Mixing study can be used for screening of inhibitor against FV. In this situation, addition of normal plasma cannot correct prolonged PT and PTT while in congenital FVD prolongation is corrected. Molecular diagnosis of FVD is straight forward but due to large size of FV gene and genetic variability molecular diagnosis is restricted to research laboratory.

Keywords: Factor V deficiency, Diagnosis, rare bleeding disorder

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Introduction

Blood Coagulation factor V (FV) known as a labile factor or proaccelarin first discovered by Paul Owren in 1943 through a study on a woman that affected by hemophilia like syndrome. This protein has an essential role in hemostasis as it acts as a non-enzymatic cofactor in prothrombinase complex. It has vital role in down regulation of factor VIII by increasing the effect of activated protein C. Therefore this coagulation factor has pro and anticoagulant activity (1).

Factor V deficiency

Congenital factor V (FV) deficiency is a rare hemorrhagic disorder with estimated incidence of 1 per 1 million (1). There are more than 200 patients with FVD were diagnosed up to now. Since disorder transmits in an autosomal recessive manner,

distribution of disease in both sex is equal and higher frequency of disorder in any especial area was not reported but such other autosomal recessive disorders, areas with high rate of consanguineous marriage has a higher incidence of disorder (2, 3). FV has a gene with 74 kb length on long arm of chromosome 1 and more than 100 different disease-causing mutations such as nonsense, frame shift, missense, and splice-site mutations were observed throughout the gene. Moreover, approximately 900 polymorphisms were described in FV gene (1, 3). Based on residual FV activity in plasma, FVD divided into three groups of severe with undetectable FV activity, moderate with less than 10% activity and finally, mild FVD with 10% or more than 10% FV activity. No direct correlation was observed between factor activity and severity of bleeding episodes in patients with FVD (4). A wide spectrum of clinical presentations was

Table 1: Results of coagulation tests in patients with factor V deficiency.

Test	Patient	Normal Value
Bleeding Time (BT) (template) (min)	5-20	10>
Thrombin Time (TT) (sec)	10-16	10-16
Prothrombin Time (PT) (sec)	40-50	10-13
Activated Partial Thromboplastin Time (APTT) (sec)	50-60	28-35
Factor V activity (%)		
Mild	10 ≤	70-150
Moderate	< 10	
Severe	Undetectable level	

observed in patients with FVD. This bleeding episodes can be as mild as recurrent epistaxis or can be a life threatening bleeding diathesis such as central nervous system (CNS) bleeding but this kind of bleedings are less common in FVD and are more common in patients with other rare bleeding disorder especially factor XIII deficiency (5, 6). Diagnosis of FVD can be made based on routine coagulation tests including activated partial thromboplastin time (APTT), Prothrombin time (PT) as well as more specific assay such as FV activity and antigen assays and finally by molecular analysis and determination of FVD underline mutation. Therefore, in this study, we presented different required tests for diagnosis of FVD including routine coagulation tests, FV antigen and activity assays as well as FV inhibitor detection and assay and molecular diagnosis of FVD (7).

Diagnosis of factor V deficiency

A set of clinical presentations, family history and laboratory assessment is useful for diagnosis of FVD. Patient with continues bleeding suspected to FVD is examined by routine coagulation tests including Bleeding Time (BT), Thrombin Time (TT) Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), and platelet count(1,

3). Since FV is a part of common pathway, a patient with FVD has a normal TT and platelet count but a prolonged APTT and PT. This situation should be distinguished from acquired form of disease that are result from inhibitor development against coagulation FV or acquired form due to liver diseases. In severe FVD, BT can increase (4, 7). Combined deficiency of factor V-VIII always should be in mind to distinguish from isolated FVD. With complementary tests, all of these situations can be distinguished from FVD (7). Screening test for present of inhibitor can rule out this phenomenon. Liver function tests (LFT) with appropriate additional assessments can determine patients liver situation. FVIII coagulant activity should always be measured in all suspected patients to FVD in order to exclude the combined deficiency of FV and factor VIII (FVIII). In combined FV and FVIII deficiency low levels of both factors (usually 5–20%) is observed (7, 8). Moreover, in patients with combined FV and FVIII deficiency PT and PTT is mild to moderately be increased.

Molecular analysis and determination of underline mutation in FV gene is straight forward but because a wide spectrum of mutation was observed in patients with FVD, molecular diagnosis is not applied in clinical laboratories (8, 9).

Routine coagulation tests in factor V deficiency

At the baseline, each patient with continues bleeding can be screened by routine coagulation tests. Among these routine tests, PT and PTT were more important for coagulation factor deficiency and different coagulation factor deficiency can lead to prolongation of one or both of these tests. Prolongation of each of these tests can be further followed by more specific tests to determined underlined factor deficiency (10).

Prolonged aPTT test is observed in the deficiency of factors that belong to common (X, V, II and fibrinogen) and intrinsic (XI, IX and VIII) pathways of coagulation cascade as well as during medication by heparin. PT test evaluates the coagulation factors involved in the extrinsic (factor VII) and common (factors X, V, II and fibrinogen) pathways of coagulation cascade, and is also used for monitoring of warfarin therapy both of PT and PTT are prolong in patients with FVD but simultaneous prolongation of these tests can be observed in deficiency of each factor in common pathway (factors X, V, II, I) (7, 8, 11). In this situation, addition of normal plasma to patient's plasma can correct prolonged PT and PTT. In FVD, if normal plasma that was absorbed by aluminum hydroxide or barium salts is added to patient's plasma and PT was corrected diagnosis of FVD is suspected. Another interesting finding in FVD is prolongation of BT. Precise, cause of this prolonged BT is not clear but it can attribute to platelet FV (7). Approximately 20% of plasma FV is absorbed in platelet α granules. This platelet FV is a site for activated Factor X (FXa) but the precise mechanism of this prolonged BT with platelet FV is not clear. Inhibitor development is a rare phenomenon in patients with FVD or extremely rare in healthy individuals. This inhibitor against FV led to prolongation of both PT and PTT (7, 8). In contrast to FVD that prolonged PT and PTT was corrected by addition of normal plasma, in cases with inhibitor against FV, This is not corrected in similar situation. It's worth noting, that inhibitor against FV in congenital FVD is IgG that similar to inhibitor against factor VIII is time dependent (12).

Factor V activity and antigen assays

In a suspected patient to FVD with a prolonged PT and PTT next step is performing a FV activity assay. Based on factor activity, patients with FVD is divided to severe (with undetectable level of factor V), moderate ($<10\%$) and mild ($10\leq$) forms. Several methods including manual and automated methods were described that each of them has their own advantages and disadvantages (7, 8).

If a patient suspected to FVD with prolonged PT and PTT, next step to achieve a definitive diagnosis of the disease is to perform a FV activity (7).

FV specific PT assays using known FV deficient plasma can confirm the deficiency and determine the approximate functional factor levels. FV antigen levels (FV: Ag) can be determined by ELISA method. In type I of FVD, both FV activity and antigen are decreased but in type II FV activity is decreased but antigen, level is normal or near the normal (4, 7).

Molecular diagnosis of factor V deficiency

FV protein is encoded by large gene with 74kb length on large arm of chromosome 1 (1q24.2). More than 100 different mutations were observed in patients with FVD. A variety of gene defects including nonsense and missense mutations, insertions, deletions, and splice-site mutations were observed in patients with FVD (13). About 50% of mutations were missense and a large number of nonsense mutations. These different mutations were scattered throughout the FV gene without any recurrent gene defect that can be used for diagnosis of FVD. Since FV gene is large and complex with 25 exons, and diversity of FV gene defects, molecular diagnosis of FVD is not entered in clinical laboratory and is restricted to research laboratory (9, 11).

Conclusion

Taking all aforementioned in to account it can be concluded that diagnosis of FV

deficiency was done by routine coagulation laboratory tests and confirmed by measurement of factor activity. In addition molecular basis can be helpful in diagnosis of disorder. Determination of underline mutation allocated to each region around world can be used as a complementary test in diagnosis and also can be vital in prenatal diagnosis and preventing of distribution of disease.

Acknowledgment

We dedicate this work to all patients with bleeding disorders.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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