Case Report

Chromogenic Assay : A Critical Diagnostic Tool for Accurate Diagnosis of Severe Hemophilia A

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ABSTRACT

Haemophilia A is a genetic disorder characterized by the absence or reduced activity of factor VIII (FVIII), a crucial protein for blood clotting. The severity of Haemophilia A is directly related to the level of FVIII activity in the blood. The conventional one-stage clotting assay is the most commonly used diagnostic tool due to its simplicity and widespread availability. However, in cases of mild or moderate Haemophilia A, there can be significant disparities between results obtained from one-stage and two-stage assays. These discrepancies can result in normal FVIII levels being reported by the one-stage assay, potentially leading to a missed diagnosis or underestimation of the patient's bleeding risk. We present a case of a 18-year-old male diagnosed with severe Haemophilia A. The patient's medical history included multiple severe bleeding episodes, including spontaneous haemarthrosis and prolonged bleeding following minor injuries. Despite these symptoms, the initial diagnostic approach using the one-stage assay suggested normal FVIII activity, which could have led to inadequate treatment and management strategies. Findings in Chromogenic Assay were crucial in correctly identifying the severe nature of the disorder, thus underscoring the importance of using multiple diagnostic tools to confirm FVIII activity levels. This case highlights the role of the chromogenic assay in the accurate diagnosis of Haemophilia A, particularly in patients where clinical symptoms do not match with one-stage assay results. This case emphasizes the need for heightened awareness among clinicians regarding the limitations of the one-stage assay and the benefits of incorporating the chromogenic assay into routine diagnostic practice for Haemophilia A.

Key words: Coagulation Disorder, Hemophilia A, Chromogenic Assay

Hemophilia A is a hereditary bleeding disorder caused by a deficiency or dysfunction of coagulation factor VIII (FVIII), impairing the blood's ability to clot and leading to prolonged bleeding episodes ranging from mild to severe. This disorder affects approximately 1 in 5,000 male births and is classified based on FVIII activity levels: mild (>5% to <40% of normal FVIII levels), moderate (1% to 5%), and severe (<1%) [1,2,3]. Diagnosis traditionally relies on the one-stage clotting assay due to its simplicity and accessibility; however, this method may not always accurately reflect FVIII activity, particularly in patients with mild to moderate hemophilia, potentially leading to misdiagnosis or underestimation of disease severity [4,5]. The chromogenic assay, which measures FVIII activity through a different mechanism, has been shown to offer more precise and reliable results in certain cases, making it a valuable tool in the diagnostic process. The presented case of an 18-year-old male with severe Hemophilia A underscores the need for accurate diagnostic methods.

Despite multiple severe bleeding episodes and significant clinical symptoms, the one-stage assay initially suggested normal FVIII activity, which could have resulted in inappropriate management strategies. Findings in Chromogenic Assay were pivotal in correctly diagnosing the severe nature of the disorder, emphasizing its importance in cases where clinical presentation and one-stage assay results do not match. This case highlights the potential pitfalls of relying solely on the one-stage assay for diagnosing

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Hemophilia A and the necessity of incorporating the chromogenic assay, to ensure accurate FVIII activity assessment. By raising awareness about these diagnostic challenges, this case advocates for a better diagnostic approach to improve patient outcomes in Hemophilia A.

CASE REPORT

An 18-year-old male, previously diagnosed with mild Hemophilia A, presented at our hospital for a tooth extraction procedure. To prevent bleeding complications, he received Factor VIII before the dental intervention. However, following the tooth extraction, the patient experienced delayed bleeding, necessitating a week-long treatment with Factor VIII to control the bleeding effectively. His initial diagnosis of Hemophilia A was made in 2018 when he underwent a root canal procedure, during which he exhibited prolonged bleeding. At that time, his Factor VIII levels were found to be 40%.

The patient's medical history revealed recurrent episodes of bleeding from minor wounds since his childhood. Furthermore, he experienced prolonged bleeding following circumcision, which was managed conservatively. Importantly, the patient's familial history was significant. He was born to non-consanguineously married parents. His maternal grandfather's brother's grandson was previously diagnosed with Hemophilia A, indicating a family history of the disorder [Figure 1]. Additionally, his younger brother and several maternal uncles presented with similar bleeding manifestations, although they had not undergone medical evaluation at the time of this report.

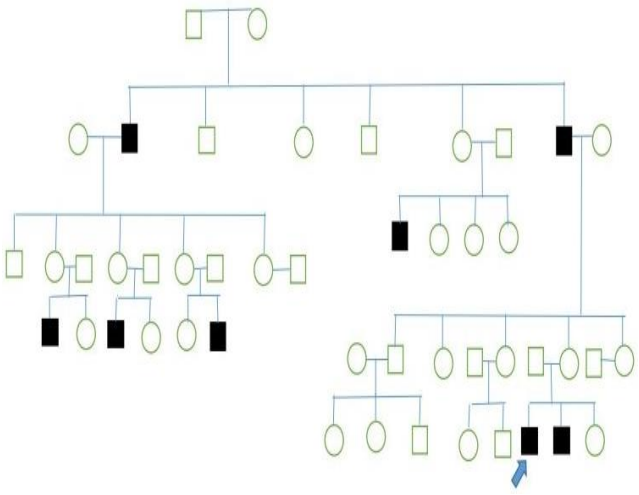

Figure.1. Pedigree Analysis of the Family

Routine blood tests, including bleeding time, clotting time, platelet count, and platelet morphology, did not reveal any abnormalities. Prothrombin time and activated partial thromboplastin time (APTT) were within the normal range. However, Factor VIII activity assays yielded varying results. The one-stage Factor VIII assay demonstrated 85% activity, while the chromogenic Factor VIII assay indicated a lower Factor VIII level of 31%.

DISCUSSION

Hemophilia A is the most prevalent hereditary clotting factor deficiency, affecting approximately 30 to 100 cases per million in the population. This condition results from mutations in the FVIII gene, responsible for encoding the FVIII protein, which plays a vital role in the intrinsic blood coagulation cascade. Severe hemophilia is characterized by significant bleeding following trauma and frequent spontaneous bleeding in patients not receiving regular prophylactic treatment.

Treatment for Hemophilia A typically involves replacement therapy with FVIII concentrates, either plasma-derived or recombinant, to prevent or control bleeding episodes. Prophylactic treatment aims to maintain FVIII levels sufficient to prevent spontaneous bleeding, especially in severe cases. Differential diagnoses for bleeding disorders include von Willebrand disease, platelet function disorders, and other rare factor deficiencies such as Hemophilia B (Factor IX deficiency).

Accurate measurement of factor VIII (FVIII) activity is essential for a precise diagnosis of hemophilia and for categorizing the disease's severity [6]. This measurement can be conducted through one-stage clotting assays, two-stage clotting assays, or chromogenic assays. Factor activity assays are also instrumental in monitoring post-infusion responses to factor replacement products, including both plasma-derived and recombinant options, as well as for detecting FVIII inhibitors.

The one-stage clotting assay, initially described in 1953 and later modified into the activated partial thromboplastin time (APTT) assay, remains the most commonly used method [7]. It assesses to what extent a plasma sample corrects the prolonged coagulation time of FVIII-deficient plasma in an APTT-based test. However, this assay's results can be influenced by various factors, including other coagulation factors within the intrinsic coagulation system, lupus anticoagulants, and heparins, potentially leading to misleading results.

To overcome some of the limitations of the one-stage assay, the two-stage coagulation assay was developed. In this approach, FXa is produced during the first incubation stage, with FVIII activity acting as the rate-limiting factor. The quantity of FXa is then estimated in a second clotting stage. This advancement eventually evolved into the chromogenic assay, a two-stage test that involves purified coagulation factors in the first stage and an FXa-specific chromogenic substrate in the second stage. Chromogenic assays are

generally more sensitive to mildly reduced factor activity levels and offer increased specificity compared to one-stage assays, primarily due to the high dilution factor applied to the sample [8].

In a case reported by Oldenburg et al. (2016), a patient with moderate Hemophilia A presented with recurrent bleeding episodes, yet initial one-stage assay results indicated normal FVIII levels. Subsequent evaluation using a chromogenic assay revealed significantly lower FVIII activity, leading to a change in the treatment plan [9]. This case mirrors the challenges observed in our 18-year-old male patient, where reliance on the one-stage assay could have led to inadequate management and an underestimation of bleeding risk.

CONCLUSION

Accurate factor activity assays are indispensable for ensuring the correct diagnosis and for implementing effective therapeutic interventions in patients with hemophilia. Increasing awareness of the use of appropriate diagnostic approaches will be crucial in optimizing treatment in individuals living with hemophilia.

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