Utility of Mixing Studies for Screening Haemophilia in a Resource Limited Hospital Setting

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Abstract:
Background: Mixing studies are initial screening tests done in cases of suspected bleeding disorders. In cases with abnormal coagulation test, they help differentiate if the prolongation in time is due to deficiency of Factor or due to presence of an inhibitor. The principle is that the normal plasma contributes a sufficient concentration of clotting factors to correct for a factor deficiency. Factor deficient plasma are commercially available but are very expensive. However the reagents for mixing studies can be prepared in the laboratory, thereby making it cost effective. Method: The study was conducted in the Department of Pathology, Assam Medical College & Hospital, Dibrugarh. The duration of our study was from June 2017 to July 2018. The clinical details were collected from the patients. The patients were tested for bleeding time, platelet count, Prothrombin time, Activated partial thromboplastin time and mixing studies. Results: Total number of cases in the study was 36, of which 31 were male patients and 5 female patients. Hemophilia A was found in 14 cases, haemophilia B in 2 cases, inhibitor was detected in 3 cases and 17 cases had normal values. Conclusion: Mixing studies are cost effective diagnostic screening tests for evaluation of abnormal coagulation tests.
Keywords: Mixing studies, Prothrombin time, Activated partial thromboplastin time, Hemophilia, Factor deficiency, inhibitor.

Introduction

Haemophilias are rare bleeding disorders, usually inherited as an X-linked recessive trait. Haemophilia A occurs due to deficiency of Factor VIII, with an incidence of 1 per 10,000 live births. Hemophilia B occurs due to deficiency of Factor IX, with an incidence of 1 per 50,000 live births. The vast majority of affected patients in haemophilia are males, but very rarely it can also occur in females.[1] Bleeding into muscles and joints is the hallmark of haemophilia. The residual levels of Factor VIII or Factor IX is inversely correlated with the frequency and severity of bleeding. In Haemophilia A, patients with Factor VIII level < 1% have severe disease, those with 2 to 5 % of Factor VIII level have moderate disease, and those with 6 to 50 % of Factor VIII level have mild disease.[2]

Patients with haemophilia usually have a prolonged activated partial thromboplastin time and normal prothombin time, platelet count and bleeding time. Mixing studies are tests performed on blood plasma of patients to distinguish factor deficiencies from factor inhibitors. The basic purpose of mixing study is to determine the cause of prolongation of Prothrombin Time (PT) and Partial Thromboplastin Time. Here, we mix equal volumes of test plasma and control plasma and repeat the test. If mixing corrects the prolonged time, a factor deficiency can be suspected.[3]

The study was conducted to screen Haemophilia patients in a resource limited hospital setting.

Methodology

The study was conducted in the Department of Pathology, Assam Medical College & Hospital, Dibrugarh. The duration of our study was from June 2017 to July 2018. Informed consent was taken from the patients before conducting the study.

Inclusion criteria: All patients suspected of having a bleeding disorder during the study period and willing to participate were included in the study

Exclusion criteria: Patients not giving consent were excluded from the study.

Clinical history and physical examination of the patients were done. The patients were tested for bleeding time, platelet count, prothrombin time, Activated Partial Thromboplastin Time, and mixing studies.

The reagents needed for mixing studies are, Pooled normal plasma, Adsorbed plasma and Aged serum. However, commercially available Factor VIII deficient plasma and Factor IX deficient plasma can also be used instead of adsorbed plasma and aged serum. The reagents can be prepared in house. To prepare pooled normal plasma we collect plasma from atleast 20 healthy donor male and females. The collected plasma is immediately frozen at -80 ℃. It is then thawed at 37℃ and pooled in a plastic container. The pooled plasma is then centrifuged at 4000 rpm for 10 minutes to obtain platelet poor plasma and then aliquot in vials and stored at -80 ℃. Adsorbed plasma can be prepared from the pooled normal plasma by adding Barium sulphate. We weigh Barium sulphate @100mg/mL of pooled plasma. Barium sulphate is added to the test tube containing pooled normal plasma placed in a water bath at 37 ℃ . After 15 minutes, the mixture is centrifuged at a slow spin @1000 rpm/min for 3 minutes. The supernatant is collected and a prothrombin time is done. If the prothrombin time is between 60 to 80 seconds, the process is complete. However, if PT is less than 60 seconds, we add more Barium sulphate and centrifuge again and do a repeat PT. If the PT is more than 80 sec, we add more pooled normal plasma and centrifuge it , followed by a PT. Upon obtaining a desired PT of 60 to 80 seconds, the adsorbed plasma is stored in labeled cuvets at -30 ℃ . Aged serum can be prepared by incubating normal serum for 24 hours at 37 ℃ . Barium sulphate adsorbs clotting factors II, VII, IX and X. Hence adsorbed plasma is deficient in clotting factors II, VII, IX and X. Aged serum is deficient in Factor V and Factor VIII. We performed a baseline PT and APTT of the test samples. Then we perform Mixing studies by mixing equal volumes of test sample and pooled normal plasma; test sample and adsorbed plasma; test sample and aged serum and repeat the test. We look for any correction of time of the prolonged PT or APTT. A correction of time indicates deficiency of Factor, no correction indicates presence of inhibitor (heparin, LA, Factor inhibitor, FDP etc) as shown in Table 1 and Table 2.

Results:

Total number of cases in the study was 36. There were 31 male patients and 5 female patients. Majority of the cases were in the age group 0-09 years. We performed PT and APTT tests for all the cases. Then we performed the mixing study to look for any correction in time.

14 cases showed correction with adsorbed plasma and pooled normal plasma; and were reported as Haemophilia A. 2 cases showed correction with aged serum and pooled normal plasma; and were reported as Haemophilia B. 3 cases did not show any correction of time with adsorbed plasma, aged serum and pooled normal plasma. Those three cases were reported as having presence of inhibitor. The remaining 17 cases had normal values. Factor assay was advised for the cases showing abnormal results.

Discussion

The rationale in mixing studies is that, since normal plasma contains all the factors, any prolongation in time due to deficiency of Factor in the test plasma, will be corrected when they are mixed correcting the prolonged time. If the prolongation in time is due to an inhibitor the normal plasma will also be inhibited and there will be no correction of the prolonged time.

There are several methods available to interpret whether mixing studies has shown correction or no correction of time. One such method is the Index of circulation anticoagulant [ICA], also known as the "Rosner Index".[4,5,6]

The ICA is identified by the formula:

ICA={[1:1 mixCT-NPPCT]/patient CT} x 100

Where CT= clotting time of the test under investigation, and NPP=normal pool plasma. In general the cut-off value will range from 10 to15%.[4,5,6,7,8.]

Another commonly used method is the "percent correction method' also known as the Chang method.[9]

The percent correction is identified by the formula:

% correction={ patient CT- 1:1 mix CT]/ [patient CT – NPPCT]} x 100

Where CT is the clotting time of the test under investigation, and NPP is the normal pool plasma. In general the cut-off value will range from 65 to 80%.[4,5,9]

Studies by Losos M et al,[3] Favaloro E,[10] Favaloro EJ,[11] Mohammad E et al,[12] Choi SH et al,[13] Kershaw G,[4] have highlighted the importance of mixing studies to help distinguish the etiology of coagulopathy.

Conclusion

Conclusion: Mixing studies aids laboratories in the investigation of abnormal coagulation tests. They help in differentiating whether the abnormal prolongation is due to deficiency of factors or due to presence of inhibitors. The reagents for mixing studies can be prepared in house and are easily available. The present study highlights the importance of mixing studies as a cost effective diagnostic tool in screening Haemophilia cases in a resource limited hospital setting.

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Conflict of interest: none

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Table 1: Normal PT and prolonged APTT seen in deficiency of factor VIII, IX, XI, XII

Defect in test plasma	APTT	Aged serum	Adsorbed Plasma	Normal plasma
VIII	abnormal	No correction	Correction	correction
IX	Abnormal	correction	No correction	correction
XI/XII	Abnormal	correction	Correction	correction
Inhibitor	abnormal	No correction	No correction	No correction

Table 2: Prolonged PT and APTT seen in deficiency of factor II, V, VII, X

Defect in test plasma	PT	APTT	Aged serum	Adsorbed plasma	Normal plasma
II	abnormal	abnormal	No correction	No correction	Correction
V	abnormal	abnormal	No correction	Correction	Correction
VII	abnormal	normal	Correction	No correction	Correction
X	abnormal	abnormal	Correction	No correction	Correction

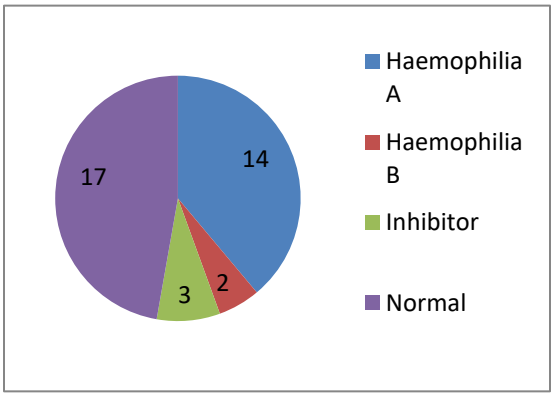

Fig 1: No of cases

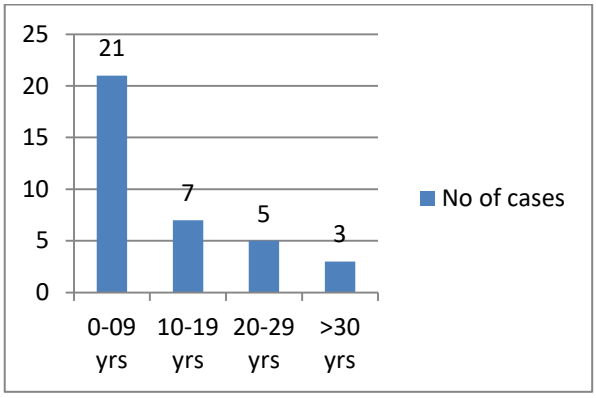

Fig 2: Age group in years