ORIGINAL RESEARCH PAPER

Pathology

STUDY OF INHIBITOR SCREEN IN HEMOPHILIA PATIENTS.

KEY WORDS: Hemophilia, inhibitors.

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ABSTRACT

Introduction: Hemophilia is X-linked disorder, due to deficiency of clotting factors namely Factor VIII, Factor IX or Factor XI causing Hemophilia A (HA), Hemophilia B (HB) & Hemophilia C (HC) respectively. Replacement therapy with factor concentrates is the standard of care. Development of inhibitors following replacement therapy occurs in 20% to 40% of patients with severe HA & 3-5% patients with severe HB. It increases mortality & morbidity. Patients with inhibitors do not respond to standard therapy, requiring use of expensive alternatives like FEIBA. This mandates need for screening for inhibitors. **Aim:** To evaluate the use of mixing-based inhibitor screening (MBIS) in the detection of inhibitors in Hemophilia patient. **Methods:** It is a Cross-sectional descriptive retrospective study conducted from Nov17 to June23 at a tertiary care hospital. MBIS by calculating difference between APTT values using PNP & patient's plasma were done for 86 patients. **Results:** Out of 86 patients, 1 patient had HB, 1 had APLA & rest 84 were cases of HA. Of 86 cases who underwent MBIS 26 cases (30.23%) showed presence of inhibitors. 49 cases were transfused more than 50 times; 21 cases less than 50 times throughout the course of their disease. Transfusion history couldn't be traced for 16 cases. Significant family history was noted in 41 cases, was absent in 17 cases and it could not be traced in 28 cases. Most common presentation was hemarthrosis in 42(48.83%) cases. **Conclusion:** Screening & testing for inhibitors is an essential aspect of any comprehensive Hemophilia program & allows timely & adequate treatment of inhibitors.

INTRODUCTION

Hemophilia is X-linked disorder, due to deficiency of clotting factors namely Factor VIII, Factor IX or Factor XI causing Hemophilia A (HA), Hemophilia B (HB) & Hemophilia C (HC) respectively. [1, 2,3] Hemophilia generally affects males on maternal side. [3]

Around 1/3rd of all cases are due to spontaneous mutation where there is no prior family history. [1,3]

Hemophilia A is more common than Hemophilia B, representing 80-85% of the total cases of Hemophilia, while Hemophilia C is a very rare condition. [1,2,3]

The incidence of Hemophilia A is 1 in 5000 to 10,000 male live births. [1,2,3,4]

Incidence of Hemophilia B is 1 in 25000 to 30000 live births. [1,3]

Hemophilia is characterized by a deficiency of coagulation factors that leads to a decrease in hemostasis, resulting in spontaneous bleeding. [1,4,5] Approximately 70–80% of bleeding episodes affect the joints causing hemarthrosis, mainly involving the large joints. [1,4]

Other bleeding manifestations include mucosal bleeds, recurrent epistaxis, prolonged bleeding due to trauma or surgeries and less frequently hematuria, gastrointestinal bleeding, bleeding in respiratory tract and CNS. [1,4]

There is variation in frequency and severity of bleeding episodes depending on the level of factor present in the plasma. [1,5]

- Mild deficiency (5–40% FVIII activity): Bleeding only after surgical procedures/trauma.
- Moderate deficiency (1 to 5% FVIII activity).
- Severe deficiency (<1% FVIII activity): Spontaneous bleeding episodes most commonly recurrent hemarthrosis, causing progressive damage of the joint. [1,6,7]

Replacement therapy with factor concentrates is the standard of care for treating acute bleeding episodes and preventing long term bleeding in patients with hemophilia. [3,8,9]

Development of neutralizing antibodies (inhibitors) against factors (VIII & IX) is the most severe and challenging complications of factor replacement therapy. [8,9,10]

It occurs in 20% to 40% of patients with severe Hemophilia A [1,7,9,10] and 1-5% patients with severe Hemophilia B. [10]

In patients with low-responding inhibitors (low-titre <5 Bethesda units [BU]), increased doses of FVIII or FIX may saturate the inhibitor and result in measurable hemostatic factor levels in the circulation (Immune tolerance induction). [7]

The presence of inhibitors makes the treatment and prevention of bleeds difficult, particularly for patients with high-responding inhibitors (high-titre >5 Bethesda units [BU]). It increases mortality, morbidity particularly of joint disease, pain and physical disability and decreases the quality of life. [9,11]

When the titre is high or Immune tolerance induction (ITI) is not successful, bypass therapy must be used. Two products are available in the market, recombinant activated factor VII (abbreviated as rFVIIa) also known as NovoSeven; and activated prothrombin complex concentrate (aPCC) brand name FEIBA; Factor Eight Inhibitor Bypassing Activity for bypass therapy. [4,7,8,12]

The cumulative risk of inhibitor development ranged between 0% and 12.4% in patients treated with only one plasma-derived product compared with 20.3–33% in those treated with multiple plasma-derived concentrates and 36–38.7% in those treated with recombinant products. [15] The median number of exposure days (EDs) until inhibitors appear is typically about 10 days and they rarely develop after 100 Eds. [15,17]

Although these bypassing agents, are effective and safe in controlling bleeding in patients with inhibitors, their efficacy is not considered equal to that of coagulation factor replacement in patients without inhibitors. [12] Also, they are expensive resulting in inflated health care costs. [12]The annual cost of treating Hemophilia with inhibitors three times greater than that of treating Hemophilia without inhibitors. [3,13]

The increased complications, difficulty in controlling bleeding episodes, increased morbidity and mortality associated with inhibitor development, poor quality of life, the need to use expensive alternatives such as FEIBA and Novoseven, leads to overall social suffering of the patients with inhibitor development which mandates the need for screening for inhibitors.

Mixing based inhibitor assay is the screening test, while Bethesda assay is the gold standard for detection of inhibitors.

Aims and Objectives

- To evaluate the use of Mixing based inhibitor screening (MBIS) in the detection of inhibitors in Hemophilia patients.
- To find out the most common presenting complaint in patients with Hemophilia.
- To correlate type of Hemophilia, Number of transfusions with Inhibitor status.

MATERIALS AND METHODS

This Cross-sectional Retrospective Descriptive study was conducted at a tertiary care hospital during November 2017–June2023. The study has been cleared by the institutional ethics committee.

Of the 300 patients attending Hemophilia clinic, 86 patients underwent MBIS during this course. The details of the patients regarding the age, gender, family history, transfusion history, clinical profile and treatment received were obtained from Pune Hemophilia Registry Records and patient case papers. Prothrombin time (PT), Activation partial thromboplastin time (APTT) and mixing based inhibitor screening (MBIS) were carried out in all patients using fully automated STAGO machine.

The blood samples were collected in 3.2 % citrate tube. Pooled normal plasma (PNP) was used as control plasma for inhibitor screening. Pooled Normal Plasma was prepared by collecting blood samples in citrate tube from minimum 20 normal, healthy individuals (equal number of males & females) between 20 and 50 years, not taking medications which interfere with clotting factors and coagulation reaction.

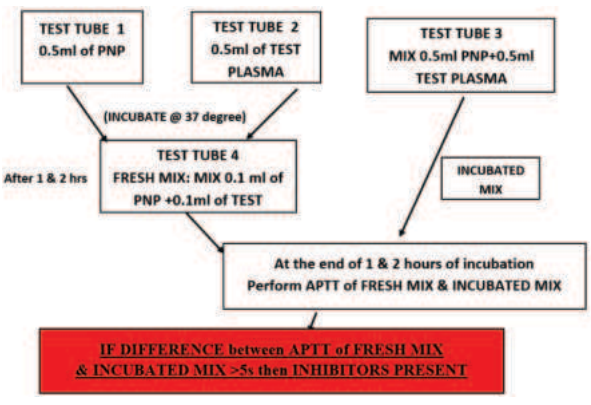


Image 1: Procedure Of Mixing Based Inhibitor Studies

Observations

A Total 86 cases were studied from Nov 2017 to June 2023. The highest number of cases studied were 25 in the year 2018.

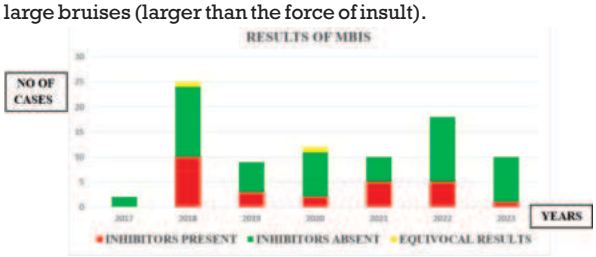
Out of 86 patients who underwent mixing based inhibitor screening 84 cases (97.76%) were of Hemophilia A, 1 case (1.17%) was of Hemophilia B and 1 case of APLA (Antiphospholipid antibody syndrome).

Of the 84 cases of Hemophilia A inhibitors were present in 26 cases (30.95%), Inhibitors were absent in 56 cases (66.66%) and equivocal results were obtained in 2 cases (2.38%).

Inhibitor development was absent in the case of Hemophilia B and APLA syndrome.

The most common chief complaint was Hemarthrosis seen in 42 cases. A history Scalp hematoma (SDH) was seen in 9 cases of which 8 were children less than 12 years of age and only one case was of adult.

Other clinical features encountered were recurrent epistaxis, prolonged blood loss after dental extraction and trauma, large bruises (larger than the force of insult).

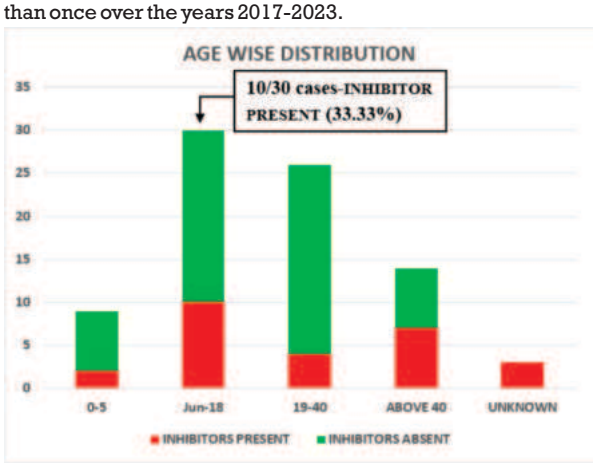


Graph 1: Results Of Mixing Based Inhibitor Screening

Of the total patients, 41 (48%) cases gave a history of a family member or distant relative affected with the same disease. 17 (20%) cases had no affected family member or any carrier females in the family, thus may be due to spontaneous mutations. While detailed family history could not be traced in case of 28 (32%) cases.

49 cases (57%) received Factor VIII transfusion more than 50 times; 21 cases (24%) received transfusion less than 50 times throughout the course of their disease. While transfusion history could not be traced in 16 cases (19%).

Repeat Cases: For 15 cases, mixing studies were done more than once over the years 2017-2023.



Graph 2 : Age Wise Distribution Of Cases & Development Of Inhibitors.

Out of these, for 12 cases mixing studies were done two times, of which same results were obtained in both studies for 10 cases. For 6 cases inhibitors were Absent both the times, and for 4 cases inhibitors were Present in both studies.

For The Rest 2 Cases (32 & 7 Year Old Male) Studies Conducted On A Earlier Date Showed Presence Of Inhibitors. After Receiving Proper Treatment When Studies Were Repeated At A Later Date, Conversion Of Status From Presence To Absence Of Inhibitors Was Seen.

For 3 cases mixing studies were done three times over the years, of which for one case inhibitors were absent in all three studies.

For The Rest 2 Cases (7 & 40 Year Old Male) Studies Conducted On A Earlier Date Showed Presence Of Inhibitors.

After Receiving Proper Treatment When Studies Were Repeated Later, Conversion Of Status From Presence To Absence Of Inhibitors Was Seen.

DISCUSSION

Hemophilia A is the second most common inherited bleeding disorder.[3]

Hemophilia A is more common than Hemophilia B, representing 80-85% of the total cases of Hemophilia, while Hemophilia C is a very rare condition.[1,2,3]

In our study, out of 86 patients, 84 cases (97.76%) were of Hemophilia A, 1 case (1.17%) was of Hemophilia B and 1 case of APLA (Antiphospholipid antibody syndrome).

In Patricia Pinto Et Al study conducted in 2014, out of 1505 cases studied, 1285 cases (85%) were of Hemophilia A and 160 cases (10.63%) of Hemophilia B.[10]

In Shah Et Al study conducted in 2019, out of 276 patients, 243cases (88%) were of Hemophilia A and 33 cases (11%) were of Hemophilia B.[3]

The most common presenting symptom is recurrent hemarthrosis, which accounts for 70–80% of bleeding episodes in patients with Hemophilia.[1,4] The most common chief complaint in our study too was hemarthrosis, which was seen in 42 cases.

Development of neutralizing antibodies (inhibitors) against factors is the most severe and challenging complications of factor replacement therapy.[8,9,10] It occurs in 20% to 40% of patients with severe Hemophilia A [1,7, 9,10] and 1-5% patients with severe Hemophilia B.[10]

In our study inhibitor development was seen in 26 (30.95%) out if 86 cases. All the cases were of Hemophilia A. This is comparable with overall percentages of inhibitor development.

In Patricia Pinto Et Al study, overall, only 6% cases of Hemophilia A demonstrated inhibitor development, but in Chennai the rate of development of inhibitors was 20.99%. In the same study, 1% cases of Hemophilia B developed inhibitors.[10]

In Shah Et Al study 20.57% cases of Hemophilia A and 6.06% cases of Hemophilia B developed inhibitors over the course of their treatment.[3]

In Peter W Collins Et Al study 2014, wherein all cases of Hemophilia A were included, 26% cases developed inhibitors.[14]

The average age of inhibitor development was 6-18 years in the present study, was comparable with other studies as it was 19 years in Patricia Pinto Et Al study and 11-20 years in Shah Et Al study.[3,10]

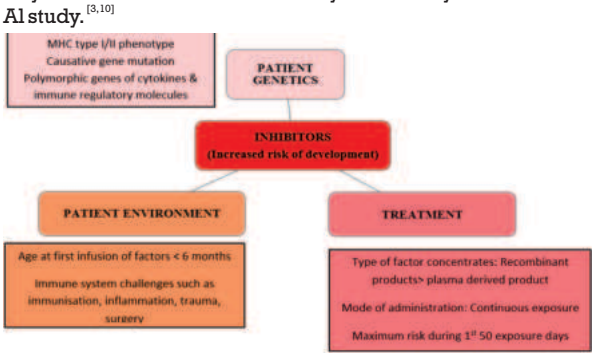


Image 2: Summary Of Factors That Influence The Risk Of Inhibitor Development In Patients With Hemophilia[15]

Familial factors and genetic factors have been implicated in increasing risk of development of inhibitors in Hemophilia patient.[15] Hemophilia generally affects males on maternal side. Inhibitors are more prevalent in siblings (50%) than in extended hemophiliac relatives (9%). Both Factor VIII and factor IX genes are prone to new mutations, and as many as 1/3rd of all cases are due to spontaneous mutation where there is no prior family history.[1,3]

The risk of inhibitor development seems to be twice as high in patients with nonsense mutations.[15] In our study of the total patients, 41cases gave a history of a family member or distant relative affected with the same disease. 17 cases had no affected family member or any carrier females in the family, which may be due to spontaneous mutations.

A higher incidence of inhibitors in patients starting replacement therapy before the age of 6 months has been described.[15] In a Spanish study, the cumulative incidence of inhibitors at 3 years of age in patients with Hemophilia A treated with factor concentrates prior to the age of 6 months, between 6 and 12 months of age or after 1 year of age was 41%, 29% and 12%, respectively.[15,18]

Our study could not record the age of starting the replacement therapy in patients in order to avoid recall bias. (as 77/86 patients were more than 5 years of age)

The median number of exposure days (EDs) until inhibitors appear is typically about 10 days and they rarely develop after 100 Eds.[15,17] Our study could not record the number of exposure days but we have recorded the median number of exposures. 49 cases (57%) received Factor VIII transfusion more than 50 times; 21 cases (24%) received transfusion less than 50 times throughout the course of their disease.

The cumulative risk of inhibitor development ranged between 0% and 12.4% in patients treated with only one plasma-derived product compared with 20.3–33% in those treated with multiple plasma-derived concentrates and 36–38.7% in those treated with recombinant products.[15] This could not be assessed in our study as most patients had received more than one type of factor concentrate over the years of treatment. Few patients also showed a switch in treatment from plasma derived factors to recombinant factors over years.

This study has few limitations

- Bethesda Assay was not performed.
- This study could not analyze the association between number of blood transfusions and development of inhibitors.
- This study could not assess the incidence of development of inhibitors depending upon the severity of Hemophilia.

CONCLUSION

The results of this study are corresponding to the overall incidence of Hemophilia & incidence of development of inhibitors.

Our study emphasizes that screening & testing for inhibitors is an essential aspect of any comprehensive Hemophilia program & allows timely & adequate treatment & management of inhibitors.

MBIS at a cut-off of 5s can be considered as an effective screening test especially in low- resource situations as the reagents are readily available, comparatively cheaper and tests are less time consuming, easy to perform and interpret. They have a sensitivity, specificity, PPV & NPV of 90%, 95%, 94.5%, 90.5% respectively.[16]

It also highlights that adequate and effective treatment for inhibitors (FEIBA, Novoseven), a conversion of the inhibitor

status from present to absent can be achieved on repeating testing post therapy and thus the development of bleeding complications can be prevented.

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