Original Article

"Prevalence of Inhibitors in Hemophilia Patients and its Clinical Implications": A Study of 276 Patients in Western India

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Abstract

Introduction: Hemophilia is an X-linked congenital bleeding disorder caused by a deficiency of coagulation factor VIII (FVIII) in hemophilia A (HA) or factor IX (FIX) in hemophilia B (HB). Accurate diagnosis of hemophilia by factor assay to demonstrate deficiency of FVIII or FIX is essential for appropriate management. Inhibitor development results in partial or complete lack of the efficacy of replacement therapy, and it makes the management of patients more difficult with an increased risk of morbidity, serious bleeding, and disability, resulting in a substantial impact on patient's quality of life and health-care costs, compared to patients without inhibitors. **Aims and Objectives**: To assess the incidence of inhibitor development in HA and HB patients along with its consequences. **Materials and Methods:** The present study was carried out at a tertiary care teaching hospital in Western India. A total of 276 patients of hemophilia were included in the study. FVIII, FIX, and inhibitor screening were carried out in all patients sample as routine testing. Patients who were found positive in inhibitor screening were further evaluated for quantitative assay (Bethesda assay). **Results:** Out of total 276 patients, 243 patients of HA and 33 patients of HB were observed. The incidence of inhibitor development is 20.57% in HA and 6.06% in HB. The maximum number of patients and maximum number of inhibitors was between the age group of 11 and 30 years. There was more number of patients with severe disease as compared to mild and moderate forms. The concentration of inhibitor >5 BU was seen in 76% of HA patients and 100% of HB patients with inhibitor. Sixty-one patients came for follow-up. In three patients, inhibitor disappeared. The incidence of complications was more in patients who had developed inhibitor which increases the cost of treatment and increases the social suffering of the patients. **Conclusion:** Inhibitor development affect the severity and treatment of the disease significantly and there by increases the suffering and cost to the patient.

Keywords: *Factor IX, factor VIII, hemophilia, inhibitors*

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Introduction

Hemophilia is an X-linked congenital bleeding disorder caused by a deficiency of coagulation factor VIII (FVIII) in hemophilia A (HA) or factor IX (FIX) in hemophilia B (HB). The deficiency is the result of mutations of the respective clotting factor genes. Hemophilia has an estimated frequency of approximately one in 10,000 births. The estimated incidence of HA is one in every 5000–7000 live male births.[1] HA is more common than HB, representing 80%–85% of the total hemophilia population. Hemophilia generally affects males on the maternal side. However, both F8 and F9 genes are prone to new mutations, and as many as 1/3 of all cases are the result of spontaneous mutation where there is no prior family history. Accurate diagnosis of hemophilia by factor assay to demonstrate deficiency of

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FVIII or FIX is essential for appropriate management. Hemophilia should be suspected in patients presenting with a history of easy bruising in early childhood, "spontaneous" bleeding (bleeding for no apparent/known reason), particularly into the joints, muscles, and soft tissues, excessive bleeding following trauma or surgery. The classification of hemophilia was first described in 1958 by Biggs and Macfarlane[2] on the basis of the relation between bleeding and residual FVIII/FIX activity, which in 2001 was accepted by the Scientific and Standardization Committee of the International Society on Thrombosis and Hemostasis and is still valid today. Patients are categorized as having severe (FVIII: C/FIX: C <1 IU/mL), moderate (FVIII: C/FIX: C 1–5 IU/mL), and mild hemophilia (FVIII: C/FIX: C >5 IU/mL). The clinical phenotype of hemophilia is primarily dependent on the severity of deficiency. Approximately 60% are affected by the severe form, defined as factor levels of <1%. Another 15% are affected by the moderate form (factor levels of 1%–5%), and the remaining 25% are affected by the mild form (factor levels of 6%–30%).[3] Currently, the mainstay of treatment is the replacement of FVIII with the use of either plasma or recombinant FVIII concentrates to achieve hemostasis. FVIII replacement is effective unless a patient develops an alloantibody (inhibitor) against the exogenous FVIII. Inhibitor development results in partial or complete lack of the efficacy of replacement therapy[4] and it makes the management of patients more difficult with an increased risk of morbidity, serious bleeding, and disability, resulting in a substantial impact on patient's quality of life and health-care costs, compared to patients without inhibitors.[5,6] Inhibitors are classified as low responding (low titer) if the inhibitor level is always <5 BU/mL and high responding (high titer) if the historical peak titer is >5 BU/mL at least once due to the occurrence of anamnestic response after FVIII reexposure.[7] On the basis of inhibitor classification, the best approach to patients' treatment may vary substantially.[8] Patients with a low Bethesda titer (<5 U/ml), usually respond to high purity or recombinant human FVIII[9] Therapy with human FVIII is seldom successful in patients with high titer antibodies (>5 BU/ml), high-affinity antibodies, and infinite coagulation times.[10] As an alternative, the use of inhibitor – bypassing products may be useful. These include the prothrombin complex concentrate (PCCs), activated PCCs, and recombinent FVIIa.[11]

Hence, the aims and objectives of the study are to analyze the prevalence of FVIII inhibitor in patients with HA and FIX inhibitor in HB, to evaluate the effect of severity of the disease in the development of inhibitor in patients with HA and HB, to study the titer of inhibitor and its effect on treatment, and to assess the incidence of development of the complication in HA and HB patient with inhibitor.

Aims and objectives

To assess the incidence of inhibitor development in HA and HB patients along with its consequences like development of complications and cost implications.

Materials and Methods

This study was conducted at a tertiary care teaching hospital in Western India during January 2015–December 2018. Ethics: The study has been cleared by the institutional ethics committee. The target population was patients with bleeding disorder who attended camps organized for hemophilia patients and patients referred to our hospital. Consent and detailed clinical history were obtained from the patients. A total of 310 samples were collected. Thirty-four patient samples were excluded from the study because 25 patients had von willebrand disease and nine had other (other than FVIII and FIX) factor deficiency. A total 276 of patients were included in the study.

Activated partial thromboplastin time (APTT), FVIII, FIX, and inhibitor screening were carried out in all patients sample to study the presence or absence of hemophilia, the severity of the disease, and presence or absence of inhibitor. Patients who were found positive in inhibitor screening were further evaluated for quantitative assay (Bethesda assay) from which we were able to differentiate the high responder patients from low-responder patients. Sixty-one patients came for follow-up. We studied the fate of inhibitor. We also studied the incidence of complications in hemophilia patients with or without inhibitor development.

All functional coagulation assays were performed on Fully automated elite pro coagulation analyzer (Manufacturer: Instrumentation Laboratory). The reagents used were commercially supplied by the same manufacturer company. The blood samples were collected in citrate tube and centrifuged at 2000 g for 20 min within 2 h of sample collection to prepare platelet poor plasma (PPP). Supernatant PPP was transferred in a 2 ml aliquot. These aliquots were stored at (−80°C) in deep freezer till further testing. For coagulation factor inhibitor test, minimum 2 aliquots of 2 ml PPP were stored.

Pooled normal plasma (PNP) is used as control plasma for inhibitor screening and Bethesda assay. Blood samples were taken in citrate tube from minimum 20 normal, healthy individuals (half of the control samples were obtained from males and another half from females) between 20 and 50 years, not taking medications which interfere with clotting factors and coagulation reaction to prepare PNP.

One-stage factor VIII assay based on activated partial thromboplastin time

It compares the ability of dilutions of standard and test plasmas to correct the APTT of plasma known to be totally deficient in FVIII but which contains all other factors required for normal clotting.

Factor VIII inhibitor screening

Procedure

0.5 ml of PNP in test tube no. 1 and 0.5 ml of test plasma in test tube no. 2 were placed. In the third tube, mixer of 0.5 ml of PNP and 0.5 ml of test plasma was placed. All the tubes were placed in a water bath (37°C). At the end of 1st h, 0.1 ml of PNP and 0.1 ml of test plasma were mixed. At the end of 1st hr, take 0.1 ml of PNP from tube No. 1 and 0.1ml of test plasma from test tube No.2 and mixed it in separate test tube to perform APTT (FRESH MIX). Performed an APTT from the sample taken from tube no. 3 (INCUBATED MIX). The same procedure was done after 2nd h. The difference between APTT value of fresh mix and incubated mix more than 5 s, indicates the presence of inhibitors and <5 s indicates the absence of inhibitors. In the sample positive for inhibitor, we did inhibitor assay.

FVIII inhibitor assay (Bethesda assay)

Procedure

Labeled 10 plastic test tubes from 1:2 on 1st test tube to 1:1024 on 10th test tube. Took 200 µl imidazol buffer (ph 7.4) in all test tube. 200 µl of test plasma was added in test tube labeled as 1:2 and then did serial dilution till test tube 1:1024. Then labeled 12 glass tubes. In tube 1: 150 µl CONTROL (PNP) +150 µl of BUFFER (imidazole), in Tube 2: 150 µl of TEST PLASMA + 150 µl of CONTROL (PNP) and in tube 3–12: 150 µl of respective diluted test plasma (from 1:2 to 1:1024). 150 µl of control plasma (PNP) was added in all test tube labeled from 3 to 12. (all the tubes were plugged with nonabsorbent cotton). All the tubes were incubated at 37°C in the water bath for 2 h. FVIII assay was performed on each incubation mixture. The FVIII activity of the control and the patient incubation mixtures were determined from the FVIII assay value. The residual FVIII activity was determined using the FVIII activity of the control and the dilution of the patient plasma having an FVIII activity that yields a residual FVIII activity approximately 50%.

$$\text{Residual FVIII activity (\%)} = \frac{\text{FVIII activity (patient)} \times 100}{\text{FVIII activity (PNP + Buffer)}}$$

Definition of inhibitor unit (Bethesda unit)

It is defined as the amount of antibody that will inactivate 50% of added normal plasma FVIII activity after 2 h incubation at 37°C.

For FIX inhibitor, only 15 min incubation at 37°C water bath was done. If the difference of APTT between fresh mix and incubated mix was more than 5 s, the screening for inhibitor was considered positive and that patient's sample was further evaluated for quantitative assay. The inhibitor quantitative assay used was the Bethesda assay, and the results were expressed as Bethesda units (BU/ml).

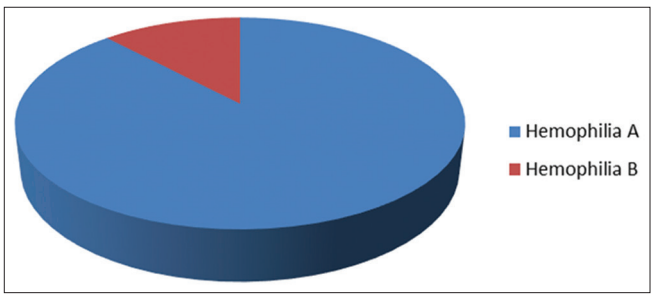
Results

Out of total 276 patients of hemophilia, 243 patients were of HA and 33 patients of HB. The incidence of inhibitor development in HA was 20.57% (50 patients developed inhibitor among 243 patients), but it was 6.06% in HB (only two patients developed inhibitor in 33 patients). In both HA and HB, the maximum number of patients were between the age group of 11 and 30 years. Maximum numbers of inhibitors were also developed in that age group. Out of 183 patients of HA with severe disease, 48 developed inhibitors, whereas in HB out of 17 patients with severe disease only two developed inhibitors. The concentration of inhibitor >5BU was seen in 76% of HA patients with inhibitor and 100% of HB patients with inhibitor. Inhibitors disappeared in three patients. The incidence of complications was more in patients who had developed inhibitor.

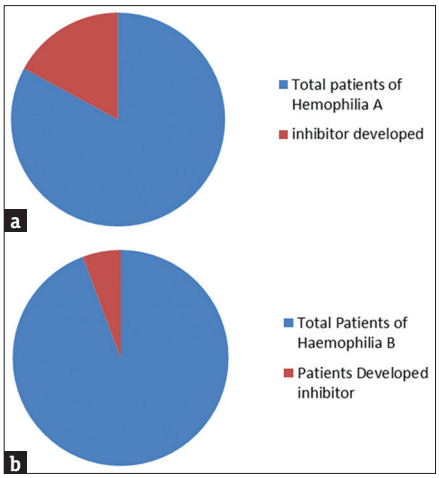
Discussion

The main purpose of this study was to evaluate the prevalence of development of FVIII and FIX inhibitor in patients with hemophilia and what its clinical implication is. HA is the second-most common of the inherited bleeding disorders. Estimates of its incidence range from 1 in 20,000 to as high as 1 in 10,000 persons.[1] HA is four to eight times more common than HB.[2] In our study, the number of HA patients were 243 and number of HB patients were 33. The ratio is 1:7.33 [Pie Chart 1].

Previous studies show a broad range of inhibitor prevalence (10%–30%) for HA and (2%–5%) for HB.[12,13] In our study, we have observed that the



Pie Chart 1: Pie chart title – Number of patients with hemophilia A and B



Pie Chart 2: Pie chart title-percentage of inhibitor in hemophilia A and B. (a) Inhibitor developed in 20.57% patients of hemophilia A. (b) Inhibitor developed in 6.06% patients of hemophilia B

occurrence of inhibitor against FVIII is 20.57% (n = 50) among 243 patients with HA [Pie Chart 2a] and 6.06% (n = 2) among 33 patients with HB [Pie Chart 2b]. In persons with HB, alloimmunization is relatively low but has life-threatening implications such as anaphylaxis or severe allergic reactions on infusion of any type of FIX-containing product. Hence, it is very important to detect alloantibody against FIX and be prepared for the treatment of shock when such kind of patients comes.[14]

All age groups of hemophilia were included in our study. The maximum number of patients was in the age group of 11–20 years (32.09%) and 21–30 years (21.39%) of HA and 36.36% and 24.24% of HB, respectively [Tables 1 and 2]. The lower prevalence observed in the youngest age group (1–10 years) compared with 11–30-year old most likely reflect delay in diagnosis of milder cases.[15] In our study, the prevalence of disease in older age group 41–50 (6.99%) and age group >50 years (7.81%) in HA and 9.09% and 0% in HB is lower than 11–30 age group [Tables 1 and 2]. It may be because of the lack of effective treatment in the peripheral rural areas which resulted in excess mortality among the oldest generation of cases, particularly those with severe disease resulted in low prevalence in older age groups.[15] In addition, middle and older generations of cases were likely reduced by the epidemic of AIDS and hepatitis introduced into the population through the use of plasma-derived factor concentrates.[16,17]

Soucie *et al*. study showed that total 2156 patients had HA. Of those with available F VIII measurements, 1140 (43%) had severe (F VIII <1%), 684 (26%) had moderate (F VIII 1%–5%), and 848 (31%) had mild (F VIII 6%–30%) disease.[3] In our study,

Table 1: Age-group wise distribution of patients with hemophilia A with inhibitors

Age (years)	Hemophilia A (n=243), total (%)	Inhibitor positive (n=50), total (%)
<1	2 (0.8)	1 (2)
1-10	47 (19.34)	10 (20)
11-20	78 (32.09)	16 (32)
21-30	52 (21.39)	8 (16)
31-40	28 (11.52)	6 (12)
41-50	17 (6.99)	4 (8)
>50	19 (7.81)	5 (10)
Total	243	50

Table 2: Age-group wise distribution of patient with hemophilia B with inhibitors

Age (years)	Hemophilia B (n=33), total (%)	Inhibitor positive (n=2), total (%)
<1	0 (0)	0 (0)
1-10	5 (15.15)	1 (50)
11-20	12 (36.36)	1 (50)
21-30	8 (24.24)	0 (0)
31-40	5 (15.15)	0 (0)
41-50	3 (9.09)	0 (0)
>50	0 (0)	0 (0)
Total	33	2

Table 3: Relation between the severity of disease and development of inhibitors

Patients	Total number of patients	Inhibitor positive	Percentage positive
Hemophilia-A	33	0	0
Mild			
Moderate	27	2	7.40
Severe	183	48	26.22
Hemophilia-B	05	0	0
Mild			
Moderate	11	0	0
Severe	17	2	11.76

183 (75.30%) patients had severe HA, 27 (11.11%) had moderate HA, and 33 (13.58%) had mild HA [Table 3]. The proportion of severe disease was quite high. Maybe because, mild and moderate disease patients are less symptomatic, and hence, they may not have approached the hospital or camp for their treatment and so not included in the study. Severe HB was present in 17 patients (51.51%), moderate HB in 11 patients (33.33%), and mild HB in 5 patients (15.15%) in our study [Table 3].

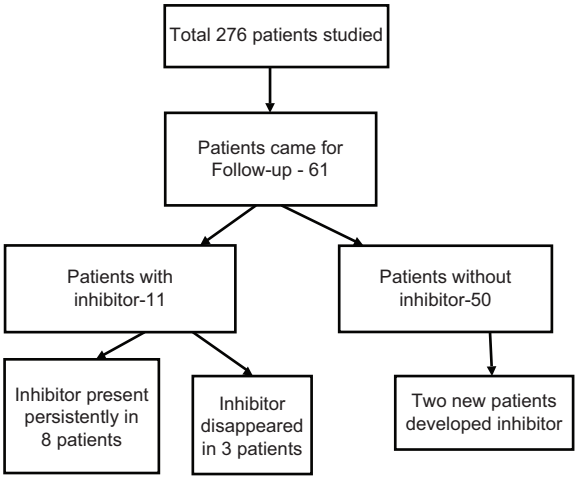
On an average, various studies by Garagiola *et al*., Lillicrap *et al*. suggest that cumulative incidence of inhibitors in severe hemophiliacs is 25%–30%[18,19] regardless of FVIII concentrate origin and purity.

Muto *et al*. and Uchida *et al*. suggested that the incidence of inhibitor is more in sever and moderate hemophilia.[20-22] In our study, the prevalence of inhibitor development in severe HA (FVIII level <1%) was 26.22% (48 patient in 183 severe hemophiliac) and 7.40% (2 patients in 27 moderate hemophiliacs) ([Table 3]. Brettler showed that in person with HB alloantibody occurs in only 1%–3% of severely affected person.[14] While in our study, it was 18.18% (2 patients in 11 patients) which is different from this study [Table 3]. In severe hemophiliacs the prevalence of inhibitor is more because they require more amount of factors more frequently for treatment. Hence because of repeated exposure, they develop more antibodies.

The most widely used tool for quantifying inhibitors is the Bethesda assay. Using this tool, inhibitor titers are measured in BU/ml. One BU is defined as the amount of plasma inhibitor per milliliter that results in 50% residual FVIII activity. Patients with titers of <5 BU are considered to have low titer inhibitors, whereas patients with titers of >5 BU are considered to have high titer inhibitors. Generally speaking, high titer inhibitors are increasingly difficult to treat.[23] There is a high prevalence of high titer of inhibitor.[24] It is nearly impossible to achieve in patients with high alloantibody titers (>5 BU);[25] thus, these patients may require bypassing agents, either as prophylaxis or on-demand therapy.

In our study, in HA 76% of inhibitor positive patients have >5 BU/ml. Moreover, 24% of inhibitor positive patients have <5 BU/ml. In HB, all the inhibitor positive patients had >5 BU/ml [Table 4].

Many patients have only transient inhibitors, and perhaps, only 5%–10% of hemophiliacs have persistent inhibitors. It is recognized, particularly in patients with mild hemophilia, that 50%–75% of inhibitors are subclinical, occur relatively early (median of 10 days) after product exposure, are of low titer, are transient, and often resolve even with continuing product exposure.[26]



Flow Chart 1: Fate of inhibitor

In our study, we observed that from 276 total patients, 61 patients came for follow-up, among them, 11 patients had antibody present previously. In the follow-up, we found that in three patients antibodies disappeared [Flow Chart 1].

With the development of inhibitor, there is an increase chance of development of complications in hemophilia patients. The development of inhibitors inactivating clotting factor concentrates has represented one of the most challenging complications of hemophilia treatment because it does compromise the effectiveness of replacement therapy, increasing the risk of limb-and life-threatening bleeding, severe arthropathy, physical disability, and mortality.[27,28]

In our study, we have noticed six types of complications. They are hemarthroses, hematomas, hematuria, neurologic complications, mucous membrane hemorrhage, and dental and surgical bleeding. Among these complications, hemarthroses is most common complication seen in 84.61% of patients with inhibitor and 70.53% of patients without inhibitor. Dental and surgical bleeding is the second-most common complication having more prevalence in patients with inhibitor (80.76% in patient with inhibitor, 65.62% in

Table 4: Inhibitor positive patients with level of BU/ml

Type of hemophilia	Bethesda assay (BU/ml)	Number of patients (%)
Hemophilia A	<5 BU/ml	12 (24)
	>5 BU/ml	38 (76)
Hemophilia B	<5 BU/ml	0 (0)
	>5 BU/ml	2 (100)

Table 5: Incidence of complications in patients with inhibitor and without inhibitor in hemophilia A (total patients=243, inhibitor=50) + hemophilia B (total=33, inhibitor=2)

Complications	Number of patients (%)	
	With inhibitor total 52 patients	Without inhibitor total 224 patients
Hemarthroses	44 (84.61)	158 (70.53)
Hematomas	32 (61.53)	104 (46.42)
Hematuria	11 (21.15)	13 (5.80)
Neurologic complications	2 (3.84)	3 (1.33)
Mucous membrane hemorrhage	26 (50.0)	103 (45.98)
Dental and surgical bleeding	42 (80.76)	147 (65.62)

patient without inhibitor [Table 5]. Gringeri *et al*. studied that from the fifty-two hemophilia patients with inhibitors enrolled, 81% had at least one bleeding event: 72% had hemarthrosis, and 44% had hematomas.[29] Same way in all other complications also the incidence was more in patients with inhibitor than in patients without inhibitor.

Inhibitor development is not only the most problematic complication facing the world of hemophilia treatment today but it is also the most expensive.[30] The annual cost of treating hemophilia with inhibitors is more than three times greater than that of treating hemophilia without inhibitors.[31]

Hence when inhibitor develops in hemophilia patients, it reduces the efficiency of FVIII and FIX and increase the requirement of factors. It increases the incidence of complications in the hemophilia patient, thereby increasing the cost of treatment and the social suffering of the patients.

Conclusion

This study aimed to study the prevalence of the development of inhibitor in hemophilia patients and the effect of the inhibitor on various aspects of treatment as well as the fate of patients. After taking disease history from individual patients, the tests for the FVIII and FIX assay, FVIII and FIX inhibitor screening and FVIII and FIX inhibitor assay were performed. From evaluation of the results and the history, we come to the following conclusions:

- Development of inhibitor of FVIII and FIX is significantly affecting the treatment of hemophilia patients
- Severity of the disease has a positive correlation with inhibitor development
- Fate of inhibitor. It may disappear after some time or it may persist
- The risk of development of complication is more in patients who have developed inhibitors.

Cost of the treatment is more in the patients who have developed inhibitors.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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