Original Article

A study to determine the prevalence, clinical profile and incidence of formation of inhibitors in patients of hemophilia in North Eastern part of India

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Abstract

Introduction: Deficiency of factor VIII (Hemophilia A), factor IX (Hemophilia B) and Von Willebrand's factor are the most frequent coagulation defects. The incidence of inhibitors in patients of factor VIII deficiency is varies in different regions of India. **Aim:** To determine the prevalence, clinical profile and incidence of formation of inhibitors in patients of Hemophilia in north eastern part of India. **Methods:** Selected patients were under went for complete Blood Count (CBC), General Blood Picture (GBP), Prothrombin time (PT), Activated partial thromboplastin time (APTT), Thrombin time, Correction experiment to know the specific factor deficiency or inhibitors present by Normal Plasma, Normal aged serum, $Al(OH)_3$ adsorbed plasma. **Results:** 92 patients diagnosed as suffering with Hemophilia A or B were included in study. The age of patients ranged from 2.5 month to 53 years. Out of 92, seventy nine (85.87%) were Haemophilia A and thirteen were (14.13%) Hemophilia B patients. 3.50% (2/55) cases of treated Hemophilia A patient develop inhibitor. **Conclusion:** The prevalence of hemophilia and incidence of inhibitors in these patients is varies in different regions of India. This variation may be due to the type of product used as treatment, intensity of treatment or the genetic characteristics of the patients.

Keywords: Factor VIII, IX. Hemophilia, Inhibitors

Introduction

Deficiency of factor VIII (Hemophilia A), factor IX (Hemophilia B) and Von Willebrand's factor are the most frequent coagulation defects. Hereditary deficiencies of other coagulation factors are significantly less common.[1] In geographic or ethnic populations where consanguineous unions are common, recessively inherited bleeding disorders are more common. Hemophilia A occurs in 1 out of 10,000 male births, while hemophilia B, occurs in 1 out of 30,000 male births. The prevalence of haemophilia A varies with the reporting country, with a range of 5.4-14.5 cases per 100,000 male individuals.[2,3]

The most serious complication of replacement therapy in hemophilia is the development of inhibitors.[4] An inhibitor is a polyclonal high-affinity immunoglobulin G (IgG) that is directed against the FVIII protein. These antibodies can be either inhibitory or non inhibitory. Factor VIII have different types of domains. Antibody binding at these domains results in functional impairment of factor VIII. Development of these antibodies leads to an increase in the management cost, morbidity and mortality.

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The incidence of inhibitors in patients of factor VIII deficiency varies in different regions of India. This variation may be due to the type of product used as treatment, intensity of treatment or the genetic characteristics of the patients.[5,6] Antibodies to factor IX are rare in comparison to factor VIII, and seen only in 1-3% of hemophilia B patients. The data related to the inhibitors and prevalence of hemophilia are lacking from this part of country.

Objectives

1. To determine the prevalence and clinical profile of hereditary coagulation disorders, particularly Hemophilia A and B in North eastern part of India
2. To determine the incidence of formation of inhibitors in treated patient of Hemophilia.

Methods

The present study was conducted in the Division of Hematology, Department of Pathology, on patients with clinically suspected bleeding disorder, who were referred to us either from departments of our tertiary care hospital or from Hemophilia Society. For this purpose an informed consent was made and detailed performa containing the nature of bleeding episodes, age of onset, frequency of bleeding, family history, mode of inheritance and history of prior medications including blood transfusion was taken along with detailed physical examination. The following Investigations were done for hemostatic assessment: Complete Blood Count (CBC), General Blood Picture (GBP), tests for coagulation: Prothrombin time (PT), Activated partial thromboplastin time (APTT), Thrombin time, Correction experiment to know the specific factor deficiency or inhibitors present by Normal Plasma, Normal aged serum, $Al(OH)_3$ adsorbed plasma, Specific Factor Assay, Inhibitor Assay. All the tests were performed within 4 hours of blood collection. However, factor VIII assays was done after 1 or 2 days, for that plasma was stored at -20 to -30 degrees centigrade. The Bethesda assay is used to quantify the concentration of factor VIII inhibitor. Test plasma is mixed with a source of Factor VIII and is incubated for 2 hours at 37°C.

A control mixture is prepared by mixing Factor VIII deficient plasma and buffered-normal plasma pool and is also incubated. After 2 hours the Factor VIII activity of each mixture is measured. The Factor VIII of the test mixture is compared to that of the control and the percentage of residual Factor VIII is calculated. One Bethesda unit (BU) is defined as that amount of inhibitor in the test plasma (patient) that results in 50% residual Factor VIII activity. Dilutions of patient plasma are also tested. A patient plasma producing a residual Factor VIII activity of 50% in an incubation mixture is considered to contain one Bethesda unit per mL. Relationship between the residual factor VIII activity in normal plasma and the inhibitor activity of the test plasma can be read off this plot. At 50% inhibition, the test plasma contains, by definition, 1 Bethesda inhibitor unit per ml. Note that the y-axis is a logarithmic scale [Figure 1].

Statistical analysis

Statistical analysis was done using SPSS 16. Student's *t* test, Mann Whitney U test and One Way Analysis of Variance was used to compare the significant difference in mean. For categorical variables, Chi square test and Fisher's – exact test were used. *P* value < 0.05 considered as statistically significant.

Results

92 patients diagnosed as suffering with Hemophilia A or B were included in study. The age of patients ranged from 2.5 months to 53 years. In our study, maximum number of patients were in age group 11-15 years. [Table 1] All patients were male. In our study, family history was present in 64.13% of patients, consanguinity was present in 5.43% of patients, 3.26% consanguineous patients were positive for family history. In our study, most common presenting clinical feature was Prolonged bleeding after cut (79.34%) [Table 2]. More than 3 joints were involved in 21.87% of haemophilia A patients. Arthropathy/deformity was present in 18.75% of patients. Bilateral knee joints were involved in 15.38% of Hemophilia B patients. Arthropathy/deformity was present in 7.69% of patients [Table 3]. The mean for PT was 14.06 ± 1.06 while mean for APPT was 80.33 ± 19.20 [Table 4]. Out of 92, 79 (85.87%) were Haemophilia A and 13 were (14.13%) Hemophilia B patients. The Mean Factor VIII value was 47.58 ± 29.33 (*P* value < 0.001).

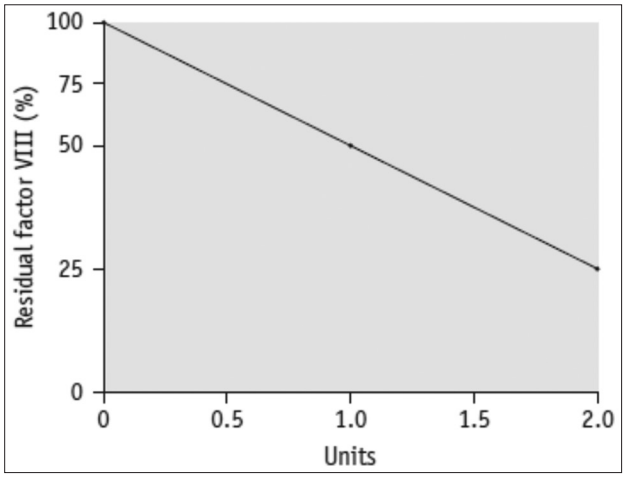

Figure 1: Measurement of factor VIII inhibitors

Table 1: Age distribution of patients (*n*=92)

Serial no.	Age group (year)	Male	
		No.	Percentage
1.	< 1	7	7.60
2.	1-5	17	18.48
3.	6-10	10	10.96
4.	11-15	23	25.00
5.	16-20	13	14.13
6.	21-30	18	19.56
7.	>31	4	4.35
	Total	92	100

Mean Age=14.23±10.69 years, Median age=14 years, Mode=15 years

Factor IX assay was done in 48 patients. Mean Factor IX value was 108.60 ± 23.93 (*P* value < 0.001). 63.298% patients of Haemophilia A were having <1% factor VIII concentration. In our study 61.54% cases of Haemophilia B were having <1% factor IX. [Table 5]. 36.70% patients were having transfusion ≥3 times, 37.97% were having <3 times. 3.50% (2/55) cases of treated Hemophilia A patient develop inhibitor. Table 6 showed the details of the patients with factor VIII inhibitors.

Discussion

- In the present study, a total of 92 patients with abnormal bleeding manifestations were investigated. Amongst these, 85.87% (79/92) was suffering from Hemophilia A, whereas 14.13% (13/79) from Hemophilia B. The age at presentation of disease ranged from 2.5 months to 53 years, out of which 61.96% (57/92) patients belong to paediatric age group (0-18 years) and 38.04% (35/92) patients belongs to adult age group (19-53 years). Maximum number of patients 25% (23/92) were of the age group 11-15 years, followed by the age group 21-30 years 19.56% (18/92) >1-5 years 18.48% (17/92)>16-20 years (14.13%)>6-10 years (10.96%) > less than 1 year, and the minimum number of patients belongs to more than 31 years' age group. Mean age was 11.25 ± 8.84, median age of the patients was 10 years and the most frequent age i.e. mode was 15 years. In a study done by Gupta *et al.* the age at presentation ranged from 2-47 years with a median age of 32.2 years.[7] Sajid *et al.* reported the age at presentation ranged from 3 to 57 years with a median age of 17 years. Mostly patients with mild deficiency presented and diagnosed in adult group.[8] Munira *et al.* reported mean age of 15.8 years.[9] All patients in the present study were male. As Haemophilia A and B are X-linked disorders, it most commonly affect males, female act as carrier. In the present study, most common presenting clinical feature in was prolonged bleeding after cut (79.34%) >Ecchymosis/bruise (45.65%) >Haemarthrosis (41.30%) >Haematoma (30.43%) >Petechiae (13.04%) >Epistaxis (8.69%) and Gum bleeding (8.69%) >Bleeding after tooth extraction (4.34%) >Bleeding after tonsillectomy (3.26%) >Post circumcision bleeding (3.26%) >Umbilical bleeding (3.26%) >Haematuria (2.17%) and least common Haematemesis (1.08%). Ahmed *et al.* reported most common presenting feature in hemophilia as hemarthrosis (82%).[10] Munira Borhany *et al.* reported Haemarthrosis in 72.85%, Haematoma (51.4%), post circumcision bleeding (37.14%), Bleeding after trauma (28.51%) followed by haematuria, bruise, gum bleeding.[9] In the present study, 40.50% have experienced joint swelling at least once in life involving one, two or multiple joints, most commonly knee joint. Sajid *et al.* also reported knee joint as most common joint involvement (48%) and in 36% more than one joint was involved.[8] 63.29% (50/79) cases of hemophilia A were having <1% factor VIIIc designated as severe hemophilia A, 22.78% (18/79) were having 1-5% factor VIIIc designated

Table 2: Frequency of bleeding symptoms in patients (*n*=92)

Clinical Symptoms	No. of Patient	Percentage (%)
Prolonged bleeding on cut/trauma	73	79.34
Ecchymosis/bruise	42	45.65
Haemarthrosis	38	41.30
Haematoma	28	30.43
Petechiae	12	13.04
Epistaxis	8	8.69
Gum bleeding	8	8.69
Bleeding after tooth extraction	4	4.34
Bleeding after tonsillectomy	3	3.26
Post-circumcission bleeding	3	3.26
Umbilical bleeding	3	3.26
Haematuria	2	2.17
Haematemesis	1	1.08

Table 3: Hemarthroses in Haemophilia A and Haemophilia B

Features Haemophilia A (*n*=32)	Number	Percentage
Joint swelling	27	84.37
Unilateral knee joint	13	14.62
Bilateral knee joint	14	43.75
>B/L knee+ other joint	7	21.87
Pain during walking/working	14	43.75
Decreased range of motion	18	56.25
Arthropathy/deformity	6	18.75
Features Haemophilia B (*n*=13)	**Number**	**Percentage**
Joint swelling	4	30.77
Unilateral knee joint	2	15.38
Bilateral knee joint	2	15.38
>B/L knee+ other joint	0	0
Pain during walking/working	2	15.38
Decreased range of motion	2	15.38
Arthropathy/deformity	1	7.69

Table 4: PT, APTT (*n*=92)

PT (seconds)			
Test		Control	
Range	Mean±SD	Range	Mean±SD
12-18	14.06±1.06	12-14	13.29±0.54
APPT (seconds)			
Test		Control	
Range	Mean±SD	Range	Mean±SD
42-93	80.33±19.20	24.4-34.5	28.35±2.02

Table 5: Factor VIIIc and Factor IX concentration in Haemophilia A and Haemophilia B patients

Factor VIII concentration (%)	Number	Percentage (%)
<1	50	63.29
1-5	18	22.78
5-50	21	26.58
Factor IX concentration	**Number**	**Percentage (%)**
<1	8	61.54
1-5	3	23.08
5-50	2	15.38

Table 6: Patient with factor VIII inhibitor details (*n*=2)

Feature	Case 1	Case 2
Age	19 years	40 years
Sex	Male	Male
Age at onset of bleeding	9 month	1 year
Site of bleeding	Joints and muscles	Joints
Nature of bleeding	Spontaneous	Spontaneous
Frequency of bleeding (per year)	10-15	15-18
Prolonged bleeding on cut/trauma	Present	Present
Malena	Present	Present
Ecchymosis	Present	Absent
Mode of control	Factor VIII infusion	Factor VIII infusion
Family history	Present	Present
Any systemic disorder association	Absent	Absent
Platelet	Adequate	Adequate
PT	14.9 (C=14)	13.2 (C=13)
APTT	96.9 (C=32)	82.0 (C=29)
Correction with normal plasma	Not corrected	Not corrected
Factor VIII assay	Less than 1%	Less than 1%
Inhibitor screening	positive	Positive
Inhibitor assay	8 BU	4 BU

as moderate hemophilia A and 13.92% (11/79) were having > 5% factor VIIIc designated as mild hemophilia A. In a study by Ahmed *et al.* 77.8% cases of severe hemophilia A. 14.4% of moderate hemophilia A, and 7.75% cases of mild hemophilia A were reported.[10] Sajid *et al.* reported 37.2% (79) mild, 41% (87) moderate, 21.6% (46) severe Haemophilia A out of 212 patients of Haemophilia A.[8] These data are comparable with clinical presentation of the patients. Patients having very less concentration of factor VIIIc presented to clinics due to very severe symptoms, while patients with mild to moderate factor VIIIc deficiency did not came to clinics because symptoms were less server and managed locally. In present study, 61.54% cases of hemophilia B having <1% factor IX designated as severe hemophilia B, 23.08% cases of hemophilia B were having 1-5% of factor IX designated as moderate hemophilia B, and 15.38% cases were having 5-50% of factor IX concentration designated as mild hemophilia B. In the study of Ahmed *et al.*, hemophilia B patients have 69.6% severe, 19.2% moderate and 11.2% mild disease.[10] Haemophila can be referred to as a disorder that causes joint damage leading to limitation in conducting daily activities and changes in social functioning. In the developed countries haemophiliacs have a quality of life very similar to that seen in general population due to provision of safety factor concentrates and multidisciplinary comprehensive care approach, but in countries like ours, haemophiliacs are not treated with safe products and appropriate qualities of the products because of cost related issues, so lack of adequate treatment can result in pain, arthropathy and disability. Although hemarthrosis was the leading cause of presenting feature of the haemophilia in children in the present setting, bruises and hematoma either spontaneous or traumatic were the main features at very onset of presentation of these children. So, the presence of these features in an otherwise normal child should be considered for evaluation of hemophilia. Out of 79 hemophilia A patients, 59 (74.68%) have received treatment more than once in form of whole blood, fresh frozen plasma, cryoprecipitate or recombinant factor VIII. These patients have been transfused with either whole blood (10.2%), fresh frozen plasma (33.9%), recombinant FVIII (32.2%), or combination of recombinant FVIII + fresh frozen plasma + whole blood (23.8%). Amongst these, 50.8% (30/59) patients received transfusion less than 3 times and 49.2% (29/59) received transfusion more than 3 times. Therefore, only these patients were subjected for antibody detection against factor VIII by the mixing studies. In the present study, we found factor VIII inhibitor in only 2 out of 59 (3.39%) treated patients. One of the patients had inhibitor titre of 8BU, and the other had 4BU. The details of these patients have shown in Table 6. Wight and Paisley, reported an overall inhibitor prevalence of 5-7% and when limited to patients with severe disease is much higher at 12-13%.[11] Lusher *et al.* reported incidence of new factor inhibitor in patients with severe factor VIII deficiency is approximately 30%. About 60% of those inhibitors are of high titer (>5BU) and remaining (40%) are of low titre (<5BU).[12] Most factor VIII allo antibodies are directed against epitopes in the A2 and A3-C1 domain of factor VIII, or auto antibodies, those that suddenly appear in person with normal factor VIII gene and previously normal plasma level of plasma factor VIII, causing acquired hemophilia.[13-15] Factor affecting development of inhibitor classified as non-modifiable and modifiable. Non-modifiable risk factors include high risk hemophilia genotype, co-stimulatory genotype-immuno genotype interaction, ethnicity and positive family history. Modifiable risk factors include environmental influence that is implicated in increasing the risk of inhibitor formation. Environment factors include age at start of prophylaxis, type of replacement therapy product and intensity of treatment.[10,16] In India, for hemophilia, inhibitor screening and other genetic test are not available therefore the identification and management of these patients is difficult. Care of haemophilia is complex, often requiring health management beyond the prevention and treatment of bleeding. Medical experts like family physician or physiotherapists can help in managing the recovery from bleeding or can apply aggressive hemostatic techniques or can assist patients capable of self administered factor therapy.[17]

Conclusion

The prevalence of hemophilia and incidence of inhibitors in these patients is varies in different regions of India. This variation may be due to the type of product used as treatment, intensity of treatment or the genetic characteristics of the patients.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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