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Prevalence of Inhibitors to Factor VIII after 25 Exposures to Factor VIII Concentrates and/or Blood Products in Persons with Hemophilia A

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Abstract
Background and Objectives: *Hemophilias are the most common inherited coagulation factor deficiencies. Factor VIII deficiency accounts for 80% cases of Hemophilia. The treatment modalities available have drastically changed the lives of PWH in the last five decades. With the advent of factor replacement therapy, PWH experience the complications in the form of development of inhibitors to Factor VIII. The objective was to study the prevalence of these inhibitors using Bethesda assay.*
Methods: *This was a cross sectional study done among forty PWH at St. Johns Medical College Hospital. A follow up study was done in 47 percent of the low responders.*
Results and Conclusion: *Mean age of the PWH was 26.15 years.60 percent of the study population was diagnosed before 10 months of age and 90 percent were less than 40 years of age. Among the PWH studied 70 percent had severe hemophilia. The study included only the persons with congenital hemophilia and hence 100 percent were males. All PWH had mixed exposures to factor viii and blood products. The Bethesda assay was positive for 67.5 percent of the persons with hemophilia. Low responders were 42.5 percent and high responders were 25 percent. Among the low responders a repeat Bethesda was done on 47.05 percent PWH after a gap of one year to study the nature of inhibitors.37.5 percent of PWH in whom a repeat Bethesda was done turned negative and 62.5 percent remained positive*
There was a significant positive relation between age of the PWH at the time of study and age at the time of diagnosis with the level of factor VIII. PWH with older age at the time of study and at the time of diagnosis had better level of factor viii. We found that subjects in the study group were diagnosed at a younger age. Young age at the time of diagnosis was associated with worse pain and bleeding scores. PWH with a severe disease had worse bleeding and pain scores. PWH with multiple exposures to factor VIII and blood products had worse bleeding and pain scores.
There was a negative relation between level of inhibitor and age of diagnosis and level of factor VIII but it

was not statistically significant. A statistically significant correlation was found between the level of inhibitor and exposure to factor VIII but none of the PWH were exposed to factor VIII alone, the exposure was mixed including blood products. There was no statistically significant correlation between Bethesda score and total number of exposures. Most PWH had a bleeding and pain score of 2 which was not different in PWH with and without inhibitor. Similarly there was no significant difference in QOL in persons with or without inhibitors.

Keywords: *Hemophilia, PWH, Bethesda assay, Factor viii, FEIBA, ITC, QOL, Pain score, bleeding score*

Introduction

Hemophilia A is an X-linked recessive hemorrhagic disease due to mutations in the F8 gene. More than 500 different mutations have been identified in the F8 gene of patients with hemophilia A. One of the most common mutations results from an inversion of the intron 22 sequence, and it is present in 40% of cases of severe hemophilia A.

Classification

Hemophilia A is classified into three categories based on the level of factor VIII

1. Severe- When the Factor level is <1%
2. Moderate – When the Factor level is1-5 %
3. Mild – When the Factor level is 6-30 %

Prevalence

Hemophilia A(Factor VIII deficiency) affects 1 in 10,000 males worldwide, in all ethnic groups. Male subjects are clinically affected; women, who carry a single mutated gene, are generally asymptomatic. Family history of the disease is absent in 30% of affected individuals and in them, 80% of the mothers are carriers of the de novo mutated allele. Among the most serious problems in the treatment of patients with hemophilia A is the development of inhibitors (antibodies) against factor VIII[1]

International: Estimated prevalence of inhibitory antibodies is 5 to 7 % in Hemophilia A and 13%-20% in individuals with severe disease[2]

Preliminary data from Bangalore: Based on a screening test, estimated prevalence of inhibitor in Bangalore is 30%. This is taken from the data of our own pilot study. The confirmatory test is not easily available and our hospital is one of the few centers in India where Bethesda assay is done.

Clinical Features

In the severe and moderate forms, the disease is characterized by bleeding into joints, soft tissues and muscles either spontaneously or following minor trauma .Those with mild disease experience infrequent bleeding usually after trauma. Among those with residual Factor VIII activity more than 25% of normal, the disease is diagnosed only when bleeding occurs after major trauma or during routine pre -operative laboratory tests.

Diagnosis

Typically, the global tests of coagulation show only an isolated prolongation of the APTT assay. Persons with haemophilia (PWH) have normal bleeding time and platelet count. The diagnosis is made after specific determination of Factor VIII clotting activity

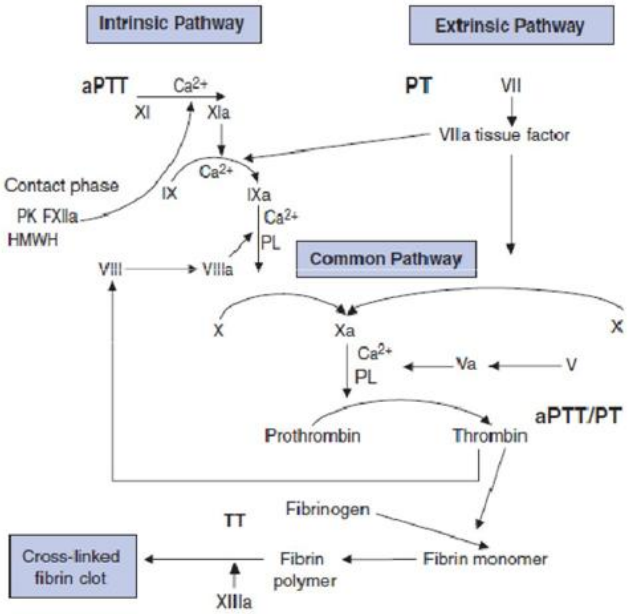

Figure 1: Coagulation cascade and laboratory assessment of clotting factor deficiency by activated partial prothrombintime (APTT), prothrombin time (PT), and thrombin time (TT)

Treatment

Persons with hemophilia (PWH), particularly those with severe disease, develop bleeding episodes that are treated with replacement of the missing factor (factor VIII)

1. **Factor VIII-** dosed in units. One unit is defined as amount of FVIII (100 ng/mL) or FIX (5 g/mL) in 1 mL of normal plasma. One unit of FVIII per kilogram of body weight increases the plasma FVIII level by 2%. One can calculate the dose needed to increase FVIII levels to 100% in a 70-kg severe hemophilia patient (<1%) using the simple formula below. Thus, 3500 units of FVIII will raise the circulating level to 100%.

FVIII dose (IU) = Target FVIII levels – FVIII baseline levels x body weight (kg) x 0.5 unit/kg. The FVIII half-life of 8–12 h requires injections twice a day to maintain therapeutic levels.

2. **Cryoprecipitate-** is enriched with FVIII protein (each bag contains 80 IU of FVIII) and was commonly used for the treatment of hemophilia A decades ago; it is still in use in some developing countries, but because of the risk of blood-borne diseases, this product should be avoided in hemophilia patients when factor concentrates are available.

3. **Fresh frozen plasma**

4. Nontransfusion Therapy

a.DDAVP (1-Amino-8-D-Arginine Vasopressin): DDAVP is a synthetic vasopressin analog that causes a transient rise in FVIII and von Willebrand factor (vWF), but not FIX, through a mechanism involving release from endothelial cells. Patients with moderate or mild hemophilia A should be tested to determine if they respond to DDAVP before a therapeutic application. DDAVP does not improve FVIII levels in severe hemophilia A patients, since there are no stores to release.

b. Antifibrinolytic Drugs: Bleeding in the gums, gastrointestinal tract, and during oral surgery requires the use of oral antifibrinolytic drugs such as -amino caproic acid (EACA) or tranexamic acid to achieve local hemostasis

Complications

One of the major complications in haemophilia (following therapy) is the development of an inhibitor; which usually occurs shortly after replacement therapy has been initiated. The inhibitors are antibodies (primarily IgG) directed against the specific deficient factor.

Inhibitors in Hemophilia A

The development of inhibitors is more common in patients with hemophilia A than in those with hemophilia B. Inhibitors in hemophilia A are more likely to develop in patients with severe disease. The pattern of response has been used to further subdivide patients with factor VIII. The risk of development of inhibitor is higher for PWH who receive multiple exposures to factor VIII, multiple plasma- derived products, continuous infusion as compared to intermittent treatment and on demand treatment as compared to prophylaxis. Factor VIII inhibitors have been reported in approximately 25-38% percent of patients with severe hemophilia A according to different studies.

High responders: Patients who develop titers above five Bethesda units at any time are considered high responders[4]. Such patients show an increase in antibody titer after each exposure; this response begins within 2 to 3 days, peaks at 7 to 21 days, and may persist for years in the absence of re-exposure.[5,6] Such high inhibitor levels render treatment with factor VIII preparations ineffective and usually require bypassing the deficient clotting factor.[7]

Low responders: Low responders have persistently low antibody titers (less than five Bethesda units) that do not increase after factor infusion and may disappear.[5,6] Such patients may continue to respond to treatment with factor VIII replacement therapy with minimal change in the factor VIII dose.

Incidence of inhibitors: Factor VIII inhibitors have been reported in approximately 25 percent of patients with severe hemophilia A.[8,9] They primarily occur early in treatment in young children and are much less common in patients with moderate and mild hemophilia A (3 to 13 percent).[8,9,10] These relationships were illustrated in a study in which 95 children who were not previously exposed to factor VIII were treated with recombinant human factor VIII; the median follow-up was 1.5 years.[8]

In one-half of the patients with inhibitors, the inhibitor titers were or became low or absent despite continued exposure. These inhibitors are termed "transient", and are more likely to occur in patients with a low inhibitor titer. The overall risk in patients with severe disease was estimated at 36 percent after 18 days of exposure, a value similar to 38 percent after 25 days of exposure in another recombinant factor VIII trial.[11]

Predisposing factors

Both host and product factors influence the likelihood of inhibitor formation.

Host related factors which influence the likelihood of inhibitor formation: The predilection for patients with severe disease is consistent with observations that inhibitors primarily occur in patients with large deletions and stop mutations, compared with small deletions or missense mutations.[12-14] There is a modest increase in antibody formation in patients with gene inversions.[15] Information taken from an international electronic database indicates the frequency of inhibitors as a function of mutation type[16,17]

Product-related factors which influence the likelihood of inhibitor formation: Product-related factors can lead to inhibitor formation with certain preparations.[18,19]

This possibility should be suspected when antibodies first form in multiple-infused patients who have changed to a new product. The suggestion that monoclonal antibody-purified or recombinant preparations might cause higher inhibitor rates than those seen with plasma-derived preparations has not been proven.[20-23]

Patient age at the time of initial replacement treatment, treatment intensity, and the early use of prophylaxis may influence inhibitor formation.

Clinical manifestations of factor VIII antibodies

The clinical manifestations of factor VIII antibodies in patients with hemophilia A depend in part upon the severity of the disease. In general, the inhibitor does not lead to a marked increase in the frequency of bleeding events except when a moderate or mild deficient patient is converted to a more severe state due to inhibitor development. However, patients with inhibitors have more difficulty in achieving hemostasis and tend to have more musculoskeletal complications. Inhibitors make the treatment of bleeding episodes more difficult. Thus, an inhibitor should be suspected when any bleeding episode is refractory to usual therapy, particularly in patients with severe hemophilia. On the other hand, bleeding episodes may be induced in patients with mild to moderate disease if the inhibitor causes a fall in factor VIII levels, converting the patient to a more severe phenotype.

Mechanism of action of factor VIII alloantibodies: The factor VIII allo antibodies that are formed after exposure to factor VIII concentrates are directed against specific epitopes on the factor VIII molecule. Factor VIII consists of a heavy chain with A1 and A2 domains, a connecting region with a B1 domain that is not required for clotting, and a light chain with A3, C1, and C2 domains.

Diagnosis of inhibitor: Factor VIII inhibitor activity generally is measured by the Bethesda assay, which establishes the diagnosis of a factor VIII inhibitor and well as quantifies the antibody titer. In Bethesda assay, serial dilutions of patient plasma are incubated with pooled normal plasma

at 37ºC for two hours; residual factor VIII activity then is measured using a clotting assay[24]. The reciprocal of the dilution of patient plasma that results in residual 50 percent factor VIII activity is the titer of the inhibitor in Bethesda units (BU). The higher the inhibitor titer, the greater the dilution required to demonstrate residual factor VIII activity.

The Nijmegen modification of the Bethesda assay appears to have improved specificity and reliability, especially with lower titer inhibitors, by enhancing the intrinsic buffering effect , following serial dilution in the assay.[25] Initial but limited information has been presented on an immunoassay for anti-factor VIII antibodies.[26]

Treatment of inhibitor: Comprehensive Hemophilia Treatment Centers provide expertise for these special subset of patients and should be consulted for planning treatment in a PWH with an inhibitor.[27]

The two components of therapy are treatment of active bleeding and inhibitor- ablation via immune tolerance induction.

Treatment of Active bleeding in High responders: In patients with usual bleeding episodes involving the joints and muscle who are high responders, inhibitor bypassing products are generally employed regardless of the present inhibitor titer. Bypassing products have included prothrombin complex concentrates (PCCs, or the original factor IX complex concentrates) and their activated counterparts (aPCCs: FEIBA®), and recombinant human factor VIIa (NovoSeven®).

Treatment of Active bleeding in Low responders: This is done by giving high purity factor viii concentrates in life-threatening hemorrhage or emergency surgery.

Inhibitor ablation via immune tolerance induction: Inhibitor eradication, also called immune tolerance induction (ITI), requires routine administration of the deficient factor to reset/tolerize the patient's immune system. A variety of ITI protocols exist utilizing a wide range of dosing regimens either with or without immunosuppressive therapy, or via the use of bypassing agents to suppress bleeding episodes. A closed international prospective ITI study in good-risk patients compared daily high dose FVIII (200 IU/kg/day) with a three times weekly lower dose FVIII (50 IU/kg/day) regimen with time to successful ITI as the primary endpoint.

Objectives

Primary

- To study the prevalence of antibody to factor VIII after 25 exposures to factor VIII and/or blood and blood products in persons with Hemophilia A using Bethesda assay and to quantify the antibody(inhibitor)

Secondary

- To assess the Quality of life in PWH with and without inhibitor

Methodology

It was a Descriptive, Cross- sectional Study.

Sample Size and Source of Data:

PWH diagnosed on the basis of clinical features and factor VIII assay, after 25 exposures either in the form of factor VIII or blood and blood products, were the subjects of this study.

Number of Factor VIII concentrate/ transfusions:

It was taken as the number of times a PWH is exposed to infusion of factor VIII or blood products. If the infusion is given multiple times on the same day, it is taken as a separate exposure.

Duration of study: One year.

Inclusion Criteria

1. PWH between the age of 10 and 70 years, who have had 25 or more exposures to factor VIII and/or blood and blood products.

Exclusion Criteria

1. PWH who have had less than 25 exposures.

2. Persons with Acquired Hemophilia.

Methodology

All PWH who were willing to participate in the study were evaluated as per the proforma. The initial factor level of the patient was recorded to categorize the patient as mild, moderate & severe hemophilia from the medical records of the patient. History regarding the number of exposures PWH has had to the factor VIII or blood & blood products was taken. If the PWH has had more than 25 exposures to Factor VIII or blood & blood- products, consent to participate in the study was taken. The above mentioned PWH underwent Bethesda assay to assess the inhibitor level .Pain score and bleeding score as set by the World Federation of Hemophilia were used to assess the severity of the disease in PWH. Quality of life(QOL) was assessed using QOL questionnaire. It is a validated disease specific instrument approved by World Federation of Hemophilia known as HEAMO-QOL-A.

Table 1: Pain Score

SYMPTOMS	SCORE
No pain No functional deficit No analgesic use (except with acute hemarthrosis)	0
Mild pain Does not interfere with occupation or with activities of daily living. (ADL) May require occasional non-narcotic analgesic	1
Moderate pain Partial or occasional interference with occupation or ADL Use of non-narcotic analgesic. May require occasional narcotic medications	2
Severe pain Interference with occupation or ADL Requires frequent use of non-narcotic and narcotic medication	3

Table 2: Bleeding Score

None	0
No major or 1-3 minor+	1
1-2 major or 4-6 minor+	2
3 or more major or 7 or more minor+	3

Minor

A. Mild pain
B. Minimal swelling
C. Minimal restriction on motion
D. Resolves within 24 hours of treatment.

Major:

A. Pain
B. Effusion
C. Limitation of motion
D. Failure to respond within 24 hours

+ This is measured by the number of minor and major hemarthroses per year

Statistical Methods

Statistical analyses were performed with SPSS. Normal data were summarized using mean and standard deviation (SD). Pearson correlation coefficient with single tailed analyses was done to correlate various clinical variables. QOL was analyzed using specific instrument.

Results

The mean age of the population studied was 26.15+/- 13.45. The mean age at presentation was 26 years and 90 percent of the study population was younger than 40 years. 60 percent of the study population was diagnosed before 10 months of age. As hemophilia is X linked, all patients studied were males. Based on factor levels, 70 percent had severe, 20 percent moderate and 10 percent mild hemophilia.

Table 3: Number of exposures to factor VIII

Number of exposures to factor VIII	Number of patients	%
<50	21	52.5
50-75	9	22.5
75-100	9	22.5
>100	1	2.5
Total	40	100.0

Mean ± SD: 54.82±31.93

The mean exposure to factor viii was 54.82. 52.5 percent had less than 50 exposures to factor viii.

Table 4: Number of exposures to cryoprecipitate

Number of exposures to cryoprecipitate	Number of patients	%
Nil	4	10.0
1-10	18	45.0
10-20	11	27.5
>20	7	17.5
Total	40	100.0

Mean ± SD: 20.95±47.10

The mean exposure to cryoprecipitate was 20.95. 82.5 percent had less than 20 exposures to cryoprecipitate

Table 5: Number of exposures to FFP

Number of exposures to FFP	Number of patients	%
Nil	10	25.0
1-10	24	60.0
10-20	4	10.0
>20	2	5.0
Total	40	100.0

Mean ± SD: 6.95±10.02

The mean exposure to FFP was 6.95. 95 percent had less than 20 exposures to FFP

Table 6: Number of exposures to Whole blood

Number of exposures to Whole blood	Number of patients	%
Nil	17	42.5
1-10	15	37.5
10-20	4	10.0
>20	4	10.0
Total	40	100.0

Mean ± SD: 8.92±19.79

The mean exposure to whole blood was 8.92.

90 percent had less than 20 exposures to whole blood

Table 7: Bethesda Assay of patients studied

Bethesda Assay	Number of patients	%
Negative (<0.6)	13	32.5
Low responders (0.7-5)	17	42.5
High responders (>5.0)	10	25.0
Total	40	100.0

Mean ± SD: 6.95±17.26

The Bethesda assay was positive for 67.5 percent of the persons with hemophilia. Low responders were 42.5 percent and high responders were 25 percent

Table 8: Repeat Bethesda assay

Bethesda Assay	Number of patients	
Test turned negative <0.6	3	37.5
Persistent low responders	5	62.5
Total Repeat test done	8	100.0

37.5 percent of PWH in whom a repeat Bethesda was done turned negative and 62.5 percent remained positive

Table 9: Pain score of patients studied

Pain score	Number of patients	%
Nil	2	5.0
1	11	27.5
2	21	52.5
3	6	15.0
Total	40	100.0

52.5 percent of the PWH had a pain score of 2.

Table 10: Bleeding score of patients studied

Bleeding score	Number of patients	%
Nil	2	5.0
1	10	25.0
2	18	45.0
3	10	25.0
Total	40	100.0

45 percent of the PWH had a bleeding score of 2.

Table 11: Evaluation of Quality of life score

Items	None of the time (n=40)	A little of the time (n=40)	Some of the time (n=40)	A good bit of the time (n=40)	Most of the time (n=40)	All of the time (n=40)	Mean	SD
1.Loss of joint mobility affects how I walk	4(10%)	6(15%)	12(30%)	9(22.5%)	5(12.5%)	4(10%)	2.43	1.43
2.It is hard for me to climb the stairs	10(25%)	8(20%)	3(7.5%)	10(25%)	3(7.5%)	6(15%)	2.15	1.77
3.It is easy for me to perform daily activities	6(15%)	9(22.5%)	5(12.5%)	4(10%)	11(27.5%)	5(12.5%)	2.5	1.72
4.I am unable to leave the house because of my hemophilia.	11(27.5%)	8(20%)	7(17.5%)	8(20%)	5(12.5%)	1(2.5%)	1.78	1.47
5.I have to adjust my activities because of my pain	4(10%)	8(20%)	12(30%)	7(17.5%)	6(15%)	3(7.5%)	2.3	1.41
6.I am able to complete household task	6(15%)	9(22.5%)	8(20%)	6(15%)	5(12.5%)	6(15%)	2.33	1.67
7.It is easy for me to lift heavy objects	8(20%)	16(40%)	5(12.5%)	6(15%)	2(5%)	3(7.5%)	1.68	1.47
8.I depend on others to carry out activities around the home	17(42.5%)	4(10%)	10(25%)	4(10%)	1(2.5%)	4(10%)	1.5	1.64
9.I am able to participate in sports	18(45%)	3(7.5%)	6(15%)	5(12.5%)	2(5%)	6(15%)	1.7	1.88
10.I have difficulty travelling because of my hemophilia	15(37.5%)	7(17.5%)	7(17.5%)	4(10%)	2(5%)	5(12.5%)	1.65	1.74
11.I am afraid of being far from a health care center with emergency care facility	11(27.5%)	1(2.5%)	11(27.5%)	3(7.5%)	9(22.5%)	5(12.5%)	2.33	1.78
12.I am hopeful about the future	-	9(22.5%)	11(27.5%)	5(12.5%)	5(12.5%)	10(25%)	2.9	1.53
13.I worry about accidents	5(12.5%)	4(10%)	13(32.5%)	5(12.5%)	7(17.5%)	6(15%)	2.58	1.58
14.I am afraid of being hit or bumped	4(10%)	4(10%)	8(20%)	11(27.5%)	9(22.5%)	4(10%)	2.73	1.45
15.I feel less confident than others	15(37.5%)	7(17.5%)	7(17.5%)	5(12.5%)	3(7.5%)	3(7.5%)	1.58	1.63
16.I enjoy life	8(20%)	7(17.5%)	6(15%)	3(7.5%)	8(20%)	8(20%)	2.5	1.86
17.I feel much older than my years	20(50%)	2(5%)	7(17.5%)	3(7.5%)	6(15%)	2(5%)	1.48	1.72
18.I am afraid of internal bleeding	7(17.5%)	3(7.5%)	9(22.5%)	8(20%)	9(22.5%)	4(10%)	2.53	1.60
19.I am in control of my life	4(10%)	5(12.5%)	11(27.5%)	5(12.5%)	9(22.5%)	6(15%)	2.7	1.57
20.I feel like I am taking a risk when I do things	7(17.5%)	5(12.5%)	11(27.5%)	4(10%)	9(22.5%)	4(10%)	2.38	1.62
21.I feel frustrated because I can't do what I	9(22.5%)	3(7.5%)	9(22.5%)	7(17.5%)	6(15%)	6(15%)	2.4	1.73

want to do.								
22.Because of my hemophilia, I have	8(20%)	2(5%)	7(17.5%)	9(22.5%)	6(15%)	8(20%)	2.68	1.76
difficulty planning for the future								
23.I worry about finding or losing a job.	8(20%)	7(17.5%)	12(30%)	7(17.5%)	0(0%)	6(15%)	2.05	1.6
24.I worry about missing work or school because of my hemophilia	9(22.5%)	4(10%)	7(17.5%)	7(17.5%)	6(15%)	7(17.5%)	2.45	1.79
25.I experience restrictions at work or school	10(25%)	3(7.5%)	11(27.5%)	5(12.5%)	7(17.5%)	4(10%)	2.2	1.68
26.I feel like a burden to my family.	18(45%)	3(7.5%)	10(25%)	3(7.5%)	4(10%)	2(5%)	1.45	1.6
27.I worry about having children.	21(52.5%)	2(5%)	12(30%)	5(12.5%)	-	-	1.03	1.16
28.Hemophilia interferes with my relationships with my friends.	12(30%)	6(15%)	13(32.5%)	3(7.5%)	4(10%)	2(5%)	1.68	1.49
29.I worry about not being able to provide for my family.	7(17.5%)	10(25%)	10(25%)	4(10%)	5(12.5%)	4(10%)	2.05	1.58
30.I am afraid to go to crowded places like concerts or bars for fear of being bumped or injured.	9(22.5%)	2(5%)	1(2.5%)	10(25%)	13(32.5%)	5(12.5%)	2.78	1.76
31.I feel different from others because for my hemophilia	13(32.5%)	4(10%)	9(22.5%)	4(10%)	5(12.5%)	5(12.5%)	1.98	1.79
32.I feel I have same opportunities to succeed in life as others.	2(5%)	9(22.5%)	9(22.5%)	3(7.5%)	7(17.5%)	10(25%)	2.85	1.67
33.Others treat me differently.	20(50%)	7(17.5%)	2(5%)	2(5%)	4(10%)	5(12.5%)	1.45	1.88
34.I feel I can carry out a normal life like the rest of the society	8(20%)	8(20%)	11(27.5%)	-	9(22.5%)	4(10%)	2.15	1.68
35.Hemophilia interferes with my ability to have an intimate relationship with another person	9(22.5%)	6(15%)	8(20%)	9(22.5%)	3(7.5%)	5(12.5%)	2.15	1.65
36.I am afraid of having a bleed in public	7(17.5%)	1(2.5%)	2(5%)	13(32.5%)	13(32.5%)	4(10%)	2.9	1.58
37.My hemophilia treatment interferes with my daily activities.	9(22.5%)	9(22.5%)	8(20%)	7(17.5%)	4(10%)	3(7.5%)	1.93	1.55
38.My infusions for hemophilia are stressful	7(17.5%)	5(12.5%)	8(20%)	10(25%)	8(20%)	2(5%)	2.33	1.50
39.I worry about my safety of my treatment	9(22.5%)	7(17.5%)	11(27.5%)	4(10%)	5(12.5%)	4(10%)	2.03	1.62

40.I worry about being treated by health care providers who do not know how to treat	9(22.5%)	4(10%)	9(22.5%)	6(15%)	7(17.5%)	5(12.5%)	2.33	1.71
41.I worry about the availability of hemophilia products.	9(22.5%)	2(5%)	7(17.5%)	5(12.5%)	10(25%)	7(17.5%)	2.65	1.81

Table 12: Correlation with different variables

		Age	Pain score	Bleeding Score	Factor level	Beth-esda Assay	Total ED	Age Diagnosis Months
Age	Pearson	1	.075	-055	.369**	-.014	.213	.626**
	Correlation Sig. (1-tailed)		.322	.368	.010	.466	.093	.000
	N	40	40	40	40	40	40	40
Pain Score	Pearson	.075	1	.560**	-327*	-.077	.293*	-336*
	Correlation Sig. (1-tailed)	.322		.000	.020	.319	.033	.017
	N	40	40	40	40	40	40	40
Bleeding Score	Pearson	-.055	.560**	1	-463**	-257	0286*	-307*
	Correlation Sig. (1-tailed)	.368	.000		.001	.055	.037	.027
	N	40	40	40	40	40	40	40
Factor level	Pearson	.369**	-327**	-463**	1	-133	-.165	0801**
	Correlation Sig. (1-tailed)	.010	.020	.001		.207	.154	.000
	N	40	40	40	40	40	40	40
Bethesda y Assay	Pearson	-.014	-077	-257	-.133	1	-.080	-.009
	Correlation Sig. (1-tailed)	.466	.319	.055	.207		.311	.478
	N	40	40	40	40	40	40	40
Total E.D.	Pearson	.213	.293*	.286*	-.165	-.080	1	-111
	Correlation Sig. (1-tailed)	.093	.033	.037	.154	.311		.247

	N	40	40	40	40	40	40	40
Age at Diagnosis Months	Pearson	.626**	-336*	-.307*	.801**	-.009	-.111	1
	Correlation Sig. (1-tailed)	.000	.027	.027	.000	.478	.247	
	N	40	40	40	40	40	40	40

** Correlation is significant at the 0.01 level (1-tailed) * Correlation is significant at the 0.01 level (1-tailed)

There was a significant positive relation between age of the PWH at the time of study and age at the time of diagnosis with the level of factor viii.

PWH with older age at the time of study and at the time of diagnosis had better level factor viii.

Lower levels of factor viii were associated with earlier diagnosis.

Young age at the time of diagnosis was also associated with worse pain and bleeding scores.

Bleeding and pain scores were worse in PWH with a severe disease.

PWH with more total exposures to factor viii and blood products had worse bleeding and pain scores.

The more severe the bleeding score, more was the pain score.

Table 13: Correlation of clinical variables with Bethesda Assay using ANOVA

Clinical variables	Bethesda Assay			P value	F value
	Negative (n=13)	Low responders (n=17)	High responders (n=10)		
Age in years	29.15±16.00	24.82±12.13	24.5±12.89	0.631	0.466
Age at the time of diagnosis (months)	53.38±136.22	10.47±11.82	28.10±45.11	0.365	1.032
Level of Factor VIII	3.42±6.41	1.99±2.05	0.85±0.16	0.294	1.267
Number of exposures to factor VIII	37.31±23.06	65.71±30.28	59.10±37.33	0.044*	3.405
Number of exposures to cryoprecipitate	16.77±13.36	30.94±70.85	9.40±9.43	0.492	0.724
Number of exposures to FFP	4.08±4.41	8.82±12.44	7.50±10.73	0.440	0.840
Number of exposures to Whole blood	4.00±7.20	13.18±27.10	8.10±15.92	0.459	0.795
Total exposure	62.15±28.03	118.65±105.05	84.10±60.06	0.143	2.054

Table 14: Correlation of Quality of life score with Bethesda Assay

Quality of Life score	Bethesda Assay			P value
	Negative (n=13)	Low responders (n=17)	High responders (n=10)	
day-to-day activities	21.15±5.35	23.47±8.65	21.90±5.93	0.661
mood and feelings	26.69±9.18	25.18±6.76	28.20±8.12	0.631
work or school life	27.54±12.69	30.94±8.64	28.20±9.86	0.641
Hemophilia treatment	10.00±6.24	11.65±5.13	12.20±4.87	0.589
Total score	85.38±27.56	91.24±21.39	90.50±18.43	0.771

There was no significant difference of QOL in persons with or without inhibitor

Discussion

In this study on 40 patients with hemophilia A, mean age of the PWH was [26].15 years. 60 percent of the study population were diagnosed before 10 months of age and 90 percent were less than 40 years of age. Among the PWH studied, 70 percent had severe hemophilia. The study included only the persons with congenital hemophilia and hence 100 percent were males. All PWH had mixed exposures to factor viii and blood products. The Bethesda assay was positive for 67.5 percent of the persons with hemophilia. This was much in excess of the observations by Lusher JM8 and Y. Sultan28 who reported incidences of 20% and 6.2% respectively.

In our study of 40 patients the prevalence of inhibitor was 67.5 percent. High responders constituted 25 percent and low responders 42.5 percent. This was greater than the incidence noted by Lusher JM8 where it was 31.25%. This high prevalence can be explained by the fact that we looked at the point prevalence in which case PWH who had transient inhibitors were also termed positive.

To study the nature of inhibitor and the reason for such a high prevalence, we repeated the Bethesda assay after a gap of one year in 47 percent of PWH who had low inhibitor. Out of the 8 PWH with low inhibitor on repeat assay 3 became negative. This was comparable to the findings of Lusher JM8 who recorded that inhibitor levels became negative in 44.4% of the patients with low inhibitor levels.

Most studies done earlier looked at exposures only to recombinant factor VIII and the development of inhibitors. In our study we had 100 percent of the study population being exposed to multiple plasma derived products along with recombinant factor VIII. Exposure to multiple plasma- derived products is a known therapy -related risk factor for the development of inhibitor as established by Wight J1. And this could also add to prevalence being high.

Since the study was cross sectional, the time interval between the last exposure to blood products or factor VIII was not considered and this could add to the probability of high prevalence, because the inhibitors primarily occur early in treatment in young children and are much less common in patients with moderate and mild hemophilia A (3 to 13 percent).[29,30,31]

70 percent of the study population was with severe hemophilia. Presumably, the virtually complete lack of circulating factor VIII in patients with severe disease prevents fetal induction of tolerance and predisposes them to antibody formation after exposure to normal exogenous

factor VIII. The concept that self factor VIII, even if altered, contributes to tolerance to factor VIII is supported by the lower frequency and often transient nature of inhibitors in patients with less severe hemophilia A. [29,30,31]

There was a significant positive relation between age of the PWH at the time of study and age at the time of diagnosis with the level of factor VIII. PWH with older age at the time of study and at the time of diagnosis had better level factor VIII. Lower the levels of factor viii earlier were they diagnosed. This could be explained by the fact that severe disease manifests in the form of hemarthrosis and bleeding episodes once the child starts crawling and in our study 60 percent of them were diagnosed before the age of 10 months. We found that younger subjects in the study group had lesser age at the time of diagnosis. Young age at the time of diagnosis was also associated with worse pain and bleeding scores. PWH with a severe disease had worse bleeding and pain scores. PWH with more total exposures to factor VIII and blood products had worse bleeding and pain scores. The more severe the bleeding score was, the more was the pain score. This was similar to the findings of Y Sultan28 who noted that earlier the presentation, lower the factor levels and worse the incidence of pain and hemarthrosis.

A statistically significant correlation was found between the level of inhibitor and exposure to factor viii but none of the PWH was only exposed to the factor VIII, the exposure was mixed. There was no statistically significant correlation seen with total number of exposure to factor VIII and blood products.

Most PWH had a bleeding and pain score of 2 which was not different in person with and without inhibitor. Similarly there was no significant difference of QOL in persons with or without inhibitor which was also reported by Alessandro Gringeri[29] where approximately two thirds of patients reported "some/moderate problems" in the physical sphere, specifically for mobility, and pain/discomfort. Approximately half of them had some problems in performing usual activities, whereas only one third reported "some/moderate problems" in self-care and anxiety/depression. No more than 2 (4%) patients reported "extreme problems" in one or more dimensions.

Conclusion

An observational clinical study with 40 patients exposure to factor VIII, was undertaken to study the prevalence of Inhibitors to factor VIII after 25 exposures to factor viii and or blood products using Bethesda assay.

Mean age of the PWH was 26.15 years and 90 percent were less than 40 years of age. Thus the patients included in the study were young adults. Among the PWH studied, 70 percent had severe hemophilia. The study included only the persons with congenital hemophilia and hence 100 percent were males.

All PWH had mixed exposures to factor VIII and blood products. The Bethesda assay was positive for 67.5 percent of the persons .Low responders were 42.5 percent and high responders were 25 percent. Among the low responders, a repeat Bethesda was done on 47.05 percent PWH after a gap of one year .Out of the 8 PWH with low inhibitor, on repeat assay, 3 became negative.37.5 percent of PWH in whom a repeat Bethesda was done turned negative and 62.5 percent remained positive

There was a significant positive relation between age of the PWH at the time of study and age at the time of diagnosis with the level of factor VIII. PWH with older age at the time of study and at the time of diagnosis had better level of factor VIII. Lower the levels of factor VIII,earlier was the diagnosis.

Young age at the time of diagnosis was also associated with worse pain and bleeding scores. Bleeding and pain scores were worse in PWH with a severe disease. PWH with more total exposures to factor viii and blood products had worse bleeding and pain scores. The more severe the bleeding score , higher the pain score

A statistically significant correlation was found

between the level of inhibitor and exposure to factor VIII but none of the PWH was exposed to only factor viii; the exposure was mixed. There was no statistically significant correlation seen with total number of exposure to factor VIII and blood products. There was a negative relation between level of inhibitor and age of diagnosis and level of factor viii but it was not statistically significant. Most PWH had a bleeding and pain score of 2 which was not different in persons with and without inhibitor.

There was no significant difference of QOL in persons with or without inhibitor

As it stands now, in a developing country like India we do not routinely evaluate all PWH for inhibitors. However the Bethesda assay is done whenever there is a clinical suspicion as in the PWH does not respond to usual doses of factor VIII and preoperatively to avoid complications.

Further a larger study over a longer period of time may help evaluate the profile of inhibitor development in Indian scenario.

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