Original Research Article

The Prevalence of clotting factor inhibitors in patients with hemophilia

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Abstract

Background: Hemophilia A and B are X-linked diseases that predominantly affect male patients. Patients can develop coagulation factor inhibitors, which exponentially increases the treatment cost. **Objective:** This study aimed to determine the prevalence of factor VIII inhibitors. **Materials and methods:** This was an observational descriptive study. Clotting factor inhibitor screening was performed by activated partial thromboplastin time mixing studies using normal pooled plasma. Bethesda assay for quantitation of factor VIII inhibitors was performed on samples which were positive with screening tests.**Results:** Study was performed in total of 62 patients with Hemophillia. Out of 62 patients, Hemophilia A and Hemophilia B was observed in 92% cases and 7% cases respectively and 1 case was with Hemophillia and von willebrand disease(1%). Out of 62 patients, 39(63%) had severe hemophilia A, 18(29%) had moderate hemophilia A, and 5(8%) had mild hemophilia A. Mixing based inhibitor screening was positive in total 14 number of patients. Bethesda assay confirmed 10(16%) cases with presence of inhibitor. 4(40%) out of 10 patients were low responders (<5 BU), with mean BU of 2.88, and 6(60%) patients were high responders (>5 BU), with mean BU of 39.2. Diagnostics of mixing based inhibitor screening showed sensitivity and specificity of 75% & 60% at difference of ≥5 seconds and 60% & 100% for difference of ≥10 seconds.**Conclusion:** Mixing tests are an important first step in the investigation of inhibitors in cases with hemophilia as the follow up investigations are more costly and time consuming than the basic screening tests.

Key words: Hemophilia, Inhibitors, Bethesda Units.

Introduction

Hemophilia is a bleeding disorder that is caused by X-linked genetic alterations in the production of coagulation factors, which are important for maintaining hemostasis. The most common type is hemophilia A, which involves factor VIII (FVIII) deficiency and affects male patients at a prevalence of 1: 5000 to 10,000. Hemophilia B involves factor IX (FIX) deficiency, and its prevalence is approximately 1: 34,500 male patients. [1]

Although both disorders are rare, they can be life threatening and expensive to treat, as they require constant replacement of the deficient factor. There are 2 types of factor concentrates (plasma-derived factors and recombinant factors), which are associated with varying rates of inhibitor formation. The development of inhibitors is the most serious complication of hemophilia treatment, and creates an enormous economic burden. [3]

Inhibitors in haemophilia are usually polyclonal [4,5] and predominately of IgG class, although other immunoglobulin classes are also seen. [6,7,8] The reaction of FVIII inhibitors is time and temperature-dependent; they react slowly to inactivate FVIII and are most effective at 37°C. [9,10] Time dependence is used to distinguish FVIII inhibitors from lupus anticoagulants (LA) and other non-specific inhibitors of coagulation, which usually do not show increased inhibition over time. [11]. FIX inhibitors are not time-dependent. [12] It can be very helpful when trying to assess the emergence of an inhibitor to draw blood at multiple times following the infusion of the replacement factor. An acceleration in the fall-off rate of factor level plotted against time will typically occur with the development of an inhibitor against that factor. [2]

Standard practice for detection of inhibitor is Mixing based inhibitor screening followed by quantification of inhibitor titers by Bethesda assay. [13]

These inhibitors are usually classified according to their plasma levels as “high-titer” inhibitors [activity of >5 Bethesda units (BUs)/mL] or “low-titer” inhibitors (<5 BU/mL), although some patients develop transit inhibitors (usually low-titer inhibitors that never exceed 5BU/mL and disappear spontaneously over time). [14-17] Many high-responder patients will exhibit inhibitor titers that resolve to low or undetectable levels after abstinence from FVIII treatment. The risk factors for inhibitor development can be patientrelated factors (e.g., genetic, ethnic, or immune factors) or treatment-related factors (e.g., type of product used, age at the first treatment/exposure, and treatment duration and intensity). [18-23] The presence of inhibitors has major effects on bleeding control, arthropathy status, and quality of life. Unfortunately, severe hemophilia cases become more resistant to the replacement therapy and require high doses of factor replacement to control their bleeding symptoms [15].

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Table1: Classification of Hemophilia A and B [2]

	CLASSIFICATION		
	Severe	**Moderate**	**Mild**
Percentage of patients	50–70	10	30–40
Factor VIII or factor IX activity, %	<1	1–5	6–30
Pattern of bleeding episodes	≈2–4 per month approximately	≈4–6 per year approximately	Uncommon
Cause of bleeding	Spontaneous	Minor trauma	Major trauma Surgery

The reported prevalence of factor inhibitors are ≤30% among patients with hemophilia A and ≤ 5% among patients with hemophilia B. [15,18,20-23]

Material and Methods

Our study group comprised of a series of CWH (cases with hemophilia) from the population of Baroda. A total of 62 diagnosed CWH were included in the study after prior consent as per the protocol. Samples were collected at hemophilia camp and study was conducted in department of pathology, in a tertiary care centre.

A detailed history pertaining to bleeding profile and FVIII transfusions in terms of IU/Kg/Year were recorded. APTT performed immediately after collection using automated coagulometer (stago). Phenotype analysis was done based on bleeding profile and factor VIII bioassay.

Mixing based inhibitor screening was performed by mixing patient's plasma and pooled normal plasma (PNP- which was collected from 20 healthy donors) in 1:1 ratio and performing APTT on fresh (F) and incubated (I) mix after 2 hours incubation at 37·c . MBIS was carried out on all 62 samples.

Cut-offs for positive MIBS are not clearly defined and are based on personal experiences and communication with other labs performing these assays. When difference between two APTTs was ≥5 seconds, were considered as positive for mixing based inhibitor study. Divided in two groups, Difference of 5-10 seconds and >10 seconds. Inhibitor assay was carried out in all screening positive cases.. Additionally in one patient because of clinical history relevant to inhibitor development, in difference of <5 seconds inhibitor assay was performed.

Interpretation of the result

In factor deficiency: PNP adds sufficient clotting factors to overcome the deficiency and correct the clotting time.

If an inhibitor is present, it typically inhibits the clotting factors in patient plasma and PNP so the clotting time remains prolonged. Mixing study results aid in selection of further coagulation testing, such as assays for specific factor deficiencies or inhibitors. [24]

Bethesda assay was used as gold standard to quantify inhibitors. Screening positive cases were sent to another tertiary care center where Bethesda system study is well established and results were acquired.

Results

Mixing based inhibitor study was performed in total of 62 patients with Hemophillia.

Table 2: Patients characteristics and disease manifestations

Parameters	**Results**		
Type of hemophilia	Hemophilia A- 57 cases(92%) Hemophillia B- 4 cases (7%) Hemophillia+ von willebrand disease-1 case(~1%)		
Sex	Male (100%) Female (0%)		
Factor VIII activity	<1%(severe)- 39 cases(63%) 1-5%(moderate)- 18 cases(29%) >5%(mild)-5 cases(8%)		
Age group wise distribution and prevalence of inhibitors	**Age group**	**Number of pt**	**Inhibitor positive**
	0-10	15	2
	11-20	20	4
	21-30	12	2
	31-40	10	1
	41-50	3	1
	51-60	0	0
	61-70	1	0
	71-80	1	0

Out of 62 patients, Hemophilia A was observed in 57 cases(92%), Hemophilia B was observed in 4 cases(7%) and 1 case was with Hemophillia and von willebrand disease.

No case with hemophilia detected in female.

Out of 62 patients, 39 patients (63%) had severe hemophilia A(factor VIII level <1%),18 patients (29%) had moderate hemophilia A (factor VIII level 1-5%), and 5 patients (8%) had mild hemophilia A (factor level >5-30%).

In this study, 10 patients (16%) were positive for inhibitors. All patients were presented with severe hemophilia (factor VIII- <1%).

Most common age group for development of inhibitor is 11-20 yrs.

Table 3: Laboratory data of hemophilia A inhibitor positive patients

SR NO	**Positive MBIS samples**	**AGE(Years)**	**Inhibitor status**	**Past history of inhibitor positive**	**Previous factor 8 level**	**Present factor 8 level**	**BU/ml**	**Factor consumed last year**
1	H3	22	Positive	Yes	3%	Severe<1%	1.5	3
2	H4	9	Positive	Yes	Moderate	Severe<1%	23.2	6
3	H14	21	Positive	No	4%	Severe<1%	2.4	13

4	H23	12	Positive	Yes	Severe<1%	Severe<1%	76.8	1
5	H30	45	Positive	Yes	Severe<1%	Severe<1%	3.2	0
6	H35	16	Positive	Yes	Severe<1%	Severe<1%	19.2	2
7	H41	4	Positive	No	Severe<1%	Severe<1%	18.4	32
8	H55	13	Positive	Yes	Severe<1%	Severe<1%	8.0	16
9	H58	12	Positive	Yes	Severe<1%	Severe<1%	89.6	4
10	H61	34	Positive	-	Severe<1%	Severe<1%	4.4	-

Titres for Bethesda assay ranged from 1.5 to 89.6.

4 (40%) out of 10 patients were low responders (<5 Bethesda units), with mean Bethesda units of 2.88, and 6 (60%)patients were high responders (>5 Bethesda units), with mean Bethesda units of 39.2.

Mixing based inhibitor screening was positive in total 14 number of patients.

Bethesda assay confirmed 10 cases with presence of inhibitor out of 14 cases.

Inhibitor positive cases (10 cases) divided in to two groups, APTT difference (difference of patients's APTT and incubated mix APTT) of ≥5-10 seconds and >10 seconds.

Table 4: 2x2 table showing BA as gold standard vs MBIS at difference of 5-10 seconds

		Bethesda Assay		**TOTAL**
		Positive	**Negative**	
MBIS(I-F) >5 sec	Positive	3	4	7
	Negative	1	6	7
TOTAL		4	10	14

Sensitivity-75%, Specificity-60%

Table 5: 2x2 table showing BA as gold standard vs MBIS at difference of >10 seconds

		Bethesda Assay		**TOTAL**
		Positive	**Negative**	
MBIS(I-F) >10 sec	Positive	6	0	6
	Negative	4	4	8
TOTAL		10	4	14

Sensitivity-60%, Specificity-100%

Difference of 5-10 seconds is more reliable and sensitive for mixing based inhibitor screening.

Discussion

Development of antibodies against factor VIII protein is one of the most serious adverse effects that can occur after administration of blood products in hemophilia A patients. The patients with inhibitors do not respond to treatment with factor VIII concentrate during bleeding episodes, which results in continuous bleeding and may sometimes lead to patient's death. [25]

In this study the overall prevalence of factor VIII inhibitors was 16%, which was similar to the findings of Wang et al. (32%) [26] and Sharifian et al. (22.2%) [27]. Lusher et al. reported that the overall prevalence of factor VIII inhibitors was 24.8% [28]. Similar percentages have been reported in other international studies like 28% [29,30] and 19% [31]. Bray et al. reported that the prevalence of inhibitors was 38.4%. Patients receiving recombinant factor VIII concentrates might be at a greater risk for inhibitor development as compared to patients receiving plasma-derived products. All the patients included in our study were receiving recombinant factor VIII therapy.[32]

In the present study all of the high responders (>5Bethesda units/ml) belonged to the group of severe hemophilia A patients (factor VIII activity <1%). The prevalence of the high responders (60%) was comparable to the prevalence reported by Knobe et al.(58%). [33]

In an Egyptian study, inhibitors were detected in 18.2% of the patients with hemophilia A and in 9.1% of the patients with hemophilia B, and although mild-to-moderate hemophilia was more common than severe hemophilia, inhibitors were more common in patients with severe hemophilia, prevalence of inhibitor is comparable to our study. [34] In a Tunisian study, the prevalence of FVIII and FIX inhibitors was much lower (5%). [35] Pakistan is another Eastern Mediterranean country, although 1 study found inhibitors in only 15% of 140 patients with hemophilia A; these patients exhibited various degrees of severity and different replacement treatments (FVIII concentrate, fresh frozen plasma, or cryoprecipitate). [36] Nevertheless, these discrepancies may be related to differences in the study populations, management trends, and testing strategies.

A study of 102 Iranian patients with hemophilia A (44 severe cases, 28 intermediate cases, and 30 mild cases) found that only 20 patients (19.6%) had inhibitors (11 severe cases, 5 intermediate cases, and 4 mild cases) [37], these findings are similar to our present findings. A large Indian study of 1285 patients with hemophilia A found that only 6.07% of the patients had inhibitors, although there were remarkable regional variations (the highest prevalence was 20.99%). [38] We also found a higher prevalence of inhibitors among patients who were receiving recombinant factors. [39-41]

Namrata et al. in their study they showed in difference of 5-10 seconds(APTT difference in mixing based inhibitor study) , sensitivity and specificity were 100% and 68.18% respectively, which is 75% and 60% respectively in present study and in difference of >10 seconds, sensitivity and specificity were 79.31% and 100% respectively, which is 60% and 100% respectively in present study. Sensitivity is more in 5-10 seconds difference result which is comparable to Namrata et al. [13]

Conclusion

Studies to detect the emergence of an inhibitor may be undertaken periodically, and most particularly when doses of replacement factor therapy thought sufficient to control a bleeding challenge prove unsuccessful. It may be recommended that hemophilia A patients should always be screened for factor VIII inhibitors on regular intervals.

The economic and health burdens of hemophilia are significant, and inhibitor development can further exaggerate these burdens.

Mixing tests are an important first step in the investigation of inhibitors in cases with hemophilia as the follow up investigations are more costly and time consuming than the basic screening tests.

Once inhibitors development occurs it is advisable that these patients should be treated with specific products such as recombinant factor VIIIa or activated prothrombin complex concentrates(APCCs) for bleeding episodes or during their surgical procedures.

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