Original Research

Assessment of prevalence of clotting factor inhibitors in patients with haemophilia

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ABSTRACT

Background: X-linked genetic changes in the coagulation factor production—which is crucial for preserving hemostasis—cause hemophilia, a bleeding disorder. The present study was conducted to assess prevalence of clotting factor inhibitors in patients with hemophilia.

Materials &Methods: 54 cases of hemophilia of both genderswas recruited. Based on the factor VIII bioassay and bleeding profile, phenotype analysis was carried out.In order to undertake mixing-based inhibitor screening, patient plasma and pooled normal plasma (PNP, which was obtained from 20 healthy donors) were mixed in a 1:1 ratio.

Results: All 54 patients were males. Type of haemophilia was A in 47, B in 5 and Hemophillia+ Von Willebrand disease in 2 patients. Factor VIII activity was mild in 7, moderate in 21 and severe in 26 cases. The difference was significant ($P< 0.05$). Mixing based inhibitor screening was positive in total 15 patients. Bethesda assay confirmed 10 cases with presence of inhibitor out of 15 cases.

Conclusion: Since follow-up investigations are more expensive and time-consuming than basic screening tests, mixing tests are a crucial first step in the evaluation of inhibitors in hemophilia cases.

Keywords: coagulation factor, Hemophillia, X-linked

Introduction

X-linked genetic changes in the coagulation factor production—which is crucial for preserving hemostasis—cause hemophilia, a bleeding disorder. Hemophilia A, the most prevalent kind, is caused by a factor VIII deficiency and affects 1 in 5000 to 10,000 male patients. Factor IX (FIX) deficiency is a component of hemophilia B, which affects roughly 1 in 34,500 men.[1] Despite being uncommon, both conditions can be fatal and costly to cure because the missing element must be continuously replaced. Plasmid-derived factors and recombinant factors are the two forms of factor concentrations that are linked to different inhibitor formation rates. The most dangerous side effect of hemophilia treatment is the creation of inhibitors, which has a significant financial impact.[2]

Lupus anticoagulants (LA) and other non-specific coagulation inhibitors, which often do not exhibit greater inhibition with time, can be distinguished from FVIII inhibitors using time dependency. FIX inhibitors don't depend on time.[3] Drawing blood several times after the replacement factor infusion might be very beneficial when attempting to evaluate the onset of an inhibitor. The formation of an inhibitor against that factor usually results in an acceleration of the fall-off rate of factor level displayed versus time.[4]

Although some patients develop transit inhibitors, which are typically low-titer inhibitors that never exceed 5BU/mL and eventually go away on their own, these inhibitors are typically categorized as "high-titer" inhibitors [activity of >5 Bethesda units (BUs)/mL] or "low-titer" inhibitors (<5 BU/mL) based on their plasma levels. After stopping FVIII treatment, many high-responder patients will show inhibitor titers that go away to low or undetectable levels.[5] Treatment-related factors (such as product type, age at first treatment/exposure, and treatment length and intensity) or patient-related factors (such as genetic, ethnic, or immunological characteristics) can be risk factors for inhibitor development.[6]The present study was conducted to assess prevalence of clotting factor inhibitors in patients with hemophilia.

Materials & Methods

The study was carried outon 54 cases of hemophilia of both genders.All gave their written consent to participate in the study.

Data such as name, age, gender etc. was recorded. Records were kept of the bleeding profile and FVIII transfusions in terms of IU/Kg/Year. APTT was carried out using an automatic coagulometer (stago)

as soon as the sample was collected.Based on the factor VIII bioassay and bleeding profile, phenotype analysis was carried out.In order to undertake mixing-based inhibitor screening, patient plasma and pooled normal plasma (PNP, which was obtained from 20 healthy donors) were mixed in a 1:1 ratio. After two hours of incubation at 37°C, APTT was done on the fresh (F) and incubated (I) mixes.Results thus obtained were subjected to statistical analysis. P value < 0.05 was considered significant.

Results

Table: I Distribution of patients

Total- 54		
Gender	**Male**	**Female**
Number	54	0

Table I shows that all 54 patients were males.

Table: II Assessment of parameters

Parameters	Variables	Number	P value
Type of hemophilia	Hemophilia A	47	0.01
	Hemophilia B	5	
	Hemophillia+ Von Willebrand disease	2	
Factor VIII activity	Mild	7	0.05
	Moderate	21	
	Severe	26	

Table II shows that type of haemophilia was A in 47, B in 5 and Hemophillia+ Von Willebrand disease in 2 patients. Factor VIII activity was mild in 7, moderate in 21 and severe in 26 cases. The difference was significant (P< 0.05).

Graph: I Assessment of parameters

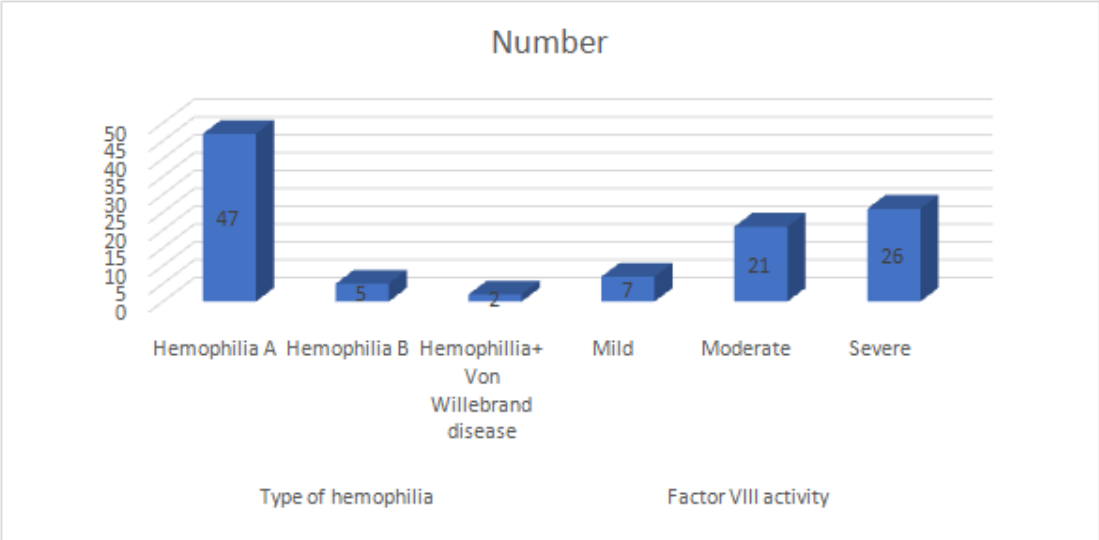

Table: III BA as gold standard vs MBIS at difference of 5-10 seconds

		Bethesda Assay		Total
		Positive	Negative	
MBIS(I-F) >5 sec	**Positive**	4	4	8
	Negative	1	6	7
Total		5	10	15

Table III shows that mixing based inhibitor screening was positive in total 15 patients.

Table: IV 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	5	9
Total		10	5	15

Table IV shows that Bethesda assay confirmed 10 cases with presence of inhibitor out of 15 cases.

Discussion

Treatment-related factors (such as product type, age at first treatment/exposure, and treatment length and intensity) or patient-related factors (such as genetic, ethnic, or immunological characteristics) can be risk factors for inhibitor development.[7] Inhibitors have a significant impact on quality of life, arthritis condition, and bleeding control.[8] Unfortunately, severe instances of hemophilia require substantial dosages of factor replacement to treat their bleeding symptoms because they become more resistant to the replacement medication.[9,10]The present study was conducted to assess prevalence of clotting factor inhibitors in patients with hemophilia.

We found that all 54 patients were males. We observed that type of haemophilia was A in 47, B in 5 and Hemophillia+ Von Willebrand disease in 2 patients. Factor VIII activity was mild in 7, moderate in 21 and severe in 26 cases. Sodha et al[11]determined the prevalence of factor VIII inhibitors. 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. 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 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.

We found that mixing based inhibitor screening was positive in total 15 patients. Bethesda assay confirmed 10 cases with presence of inhibitor out of 15 cases.Sharifan et al[12]determined the prevalence of inhibitors in Iran hemophilia A patients exposed to blood products, 1280 hemophilia A patients (age range 9 months-84 years). All patients received several blood products such as fresh frozen plasma (FFP), cryoprecipitate, and factor VIII. 635 of 1280 patients (49.6%), 277 patients (21.6%) and 368 patients (28.8%) had severe, moderate and mild disease, respectively. 184 of 1280 patients (14.4%) developed inhibitor. The prevalence of inhibitor for severe, moderate and mild in hemophilia A patients was 22.8%, 9.4%, and 3.5% respectively. 41 patients (22.2%) and 143 patients (77.8%) were high responder and low responder respectively. Among 184 patients with inhibitor, 67 patients (36.4%) had blood group O and for B, A, AB blood groups, number of patients with inhibitor was 55 (29.9%), 50 (27.2%), 12(6.5%) respectively and 153 patients (83.1%) had Rh blood group.

Mansouri et al[13] in their study a total of 102 patients with hemophilia A were selected. Results showed that of 102 participants, three had used fresh frozen plasma (FFP) during the last six months; 10 used cryoprecipitate, 73 used factor VIII concentrate, four used both FFP and factor VIII concentrate and 12 patients had used both factor VIII concentrate and cryoprecipitate. Amongst them, 20 (20%) had factor VIII inhibitor: One patient who had used FFP; among 10 patients who used cryoprecipitate, three; from 73 patients who used factor VIII concentrate, 14; one who used both FFP and factor VIII; and one patient who used both factor VIII and cryoprecipitate had factor VIII inhibitor. It seems that preparation of coagulation factor concentrates is one of the most problematic aspects of taking care of patients with hemophilia in developing countries. In our group, only 73 (72%) patients had used factor VIII concentrate and three (3%) used FFP and 10 (10%) had used cryoprecipitate. This shows that either these patients do not have enough access to coagulation factor concentrates, or the distribution of coagulation factors was not appropriate.

The shortcoming of the study is small sample size.

Conclusion

Authors found that since follow-up investigations are more expensive and time-consuming than basic screening tests, mixing tests are a crucial first step in the evaluation of inhibitors in hemophilia cases.

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