Original Research Article

Prevalence of inhibitors in hemophilia patients and quantitative estimation of FVIII Inhibitors in hemophilia patients of Odisha

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Abstract

Objective: To know prevalence of factor VIII and IX inhibitors in Haemophilia patients and Quantitative estimation of factor VIII inhibitors in Haemophilia A patients using Bethesda Assay. **Study Population**: Hemophilia Patients receiving blood products and recombinant factor transfusion. **Results:** Total 54 cases were screened for factor deficiency and inhibitors. 42 old cases were screened and 12 new cases of Hemophilia A were screened for development of inhibitors.The mean age of patients in the study population was 14.38+8.12 years with age ranging from 9 months to 68 years. Prevalence of Hemophilia A was 92.6%, prevalence of Hemophilia B was 5.5%. There were 46% severe hemophilia A cases and 44% moderate hemophilia A cases and 10% mild hemophilia A cases. 40.74% cases had development of target joints with knee joint which was most commonly effected. Prevalence of inhibitors in Hemophilia A was 8%.It was 13% in severe hemophilia cases. 25% i.e. 1 patient was high responder with inhibitor level of 64 BU, other 3 inhibitor positive patients were low responders with inhibitor levels of 3BU, 3.2 BU and 4.4 BU. **Conclusion:** Severe hemophilia patients need frequent factor transfusions and are at higher risk of inhibitor development. Patients with low inhibitor levels i.e.<10BU need high dose of recombinant factor VIII. Patients with high inhibitor levels >10 BU may require Recombinant factor VII with or without immune tolerance therapy. So inhibitor screening and Bethesda assay is needed at least once in every six months for prompt treatment.

Key Words: Hemophilia, Inhibitors, Recombinant factor, Bethesda.

Introduction

The World Federation of Hemophiliais known to be established in 1963[1]. The reported number of patients with haemophilia A in India is 11,586 and the estimated prevalence could be around 50,000 patients[2]. Bleeding disorders reportedly affect 1 in 1,000 men and women globally. Haemophilia A and B and von Willebrand disease, are the most prevalent types of bleeding disorders[2]. Hemophilia is a hereditary clotting disorder which is caused by deficiency of factor VIII (hemophilia A) or factor IX (hemophilia B). The most common congenital severe bleeding disorder is Hemophilia A[3]. Both Hemophilia A and B are X chromosome-linked bleeding disorders and are caused by mutations in the factor VIII (FVIII) and factor IX (FIX) genes[4]. The prevalence of hemophilia A is 1 in 5000 male live births, and that of hemophilia B is 1 in 30,000[4].

Currently the mainstay of treatment is the replacement of FVIII/FIX with the use of either plasma or recombinant FVIII/IX concentrates to achieve haemostasis. Using gene sequencing techniques, researchers cloned factor IX in 1982 and factor VIII in 1984. By 1992, recombinant factor VIII products for use in hemophilia A were available recombinant factor IX became available for people with hemophilia B in 1997[5]. FVIII replacement is effective uptill a patient develops an inhibitor which is an alloantibodyagainst the exogenous FVIII. In 1941 the first case of occurrence of inhibitor development after infusion of FVIII in a hemophilia A patient was described[6].

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FVIII inhibitors interfere with the infused factor concentrates and render them ineffective and thus more costly and less effective alternative haemostatic agents have to be used[3]. The formation of inhibitors of Factor VIII is currently the most common and challenging complication of haemophilia treatment. Both environmental and treatment related risk factors are seen to be associated with inhibitor formation[7].

Mixing studies are useful screening tests for inhibitors in patients with prolonged coagulation times.The screening method was modified from Kasper techniqueusing a commercial APTT reagent[8]. Patient plasma and an equal volume of pooled normal plasma were incubated both together and separately for 2 hours at 37 ◌̊C. At 2 hours, the separately-incubated plasma was mixed for immediate APTT testing[9]. The inhibitor assay is required to confirm when mixing studies tests does not correct.Inhibitor assays are performed in the blood of hemophilia A patients when the presence of inhibitors is suspected or during routine surveillance screening in the case of abnormal bleeding episodes or poor response to FVIII replacement therapy. When inhibitors are suspected, an activated partial thromboplastin time (APTT) mixing test with normal plasma is used for screening. A prolonged clotting time of the mixture may indicate the presence of inhibitors, but the presence of heparin and lupus anticoagulant has to be excluded[6]. It is generally accepted that inhibitor screening should occur before invasive procedures and at regular intervals during the initial 50 treatment days, as this is the highest risk period for inhibitor development[10]. As per the recommendation of The International Society on Thrombosis and Hemostasis Scientific and Standardization committee an inhibitor titre of 5 BU differentiates low- from high-responding inhibitors[11]. An antibody titre that is persistently below 5 BU despite repeat challenges with factor VIII is considered a low-responding inhibitor.

A high-responsive inhibitor is applied if the assay has been greater than 5 BU at any time.

The Bethesda assay, measures the plasma levels of FVIII inhibitors, and is the accepted standard method for detecting FVIII inhibitors in hemophilia A patients[12]. The overall prevalence of inhibitors was 8.2 % in patients with inherited severe haemophilia A in India[13]. Still for Indian haemophilia patients blood product transfusions are commonly used at many places and factor transfusions are usually given on an 'on-demand' basis because of the prohibitive costs involved. In case of inhibitor development, the cost of management of bleeding episodes also increases significantly with the need for expensive bypassing agents in most cases[13].

As per 2011 census India has 14,718 patients with bleeding disorders with 11,586 hemophilia A patients and 1687 hemophilia B patients. Odisha has 377 registered hemophilia cases[2]. In terms of proportionate burden of patients, data indicated that India harbours 5 and 9 per cent of the global number of patients with bleeding disorders and with haemophilia A respectively[2].

In USA and Brazil the highest prevalence is reported which is over 4 per 1,00,000 population[2]. While the number of prevalent cases in India is 0.9 per 1,00,000 population, and this may be due to underdiagnoses, mortality and incomplete case recording which could be a reason for lower case reporting in India[2]. In India, the data on records of hemophilia is registered and maintained by Hemophilia Federation of India (HFI)[14]. Patients with inhibitors can be treated with rFVIIa but the disadvantage is that it has a very short half-life (2.3-2.7 hours); therefore, repeat dosing is generally indicated ever two to three hours. The recommended dose of rFVIIa is 90 mcg/kg every 2-3 hours[15].

Inhibitor screening is frequently done in different tertiary care centres of India and data is reported. There has been no study on inhibitors in Odisha till date though it has high prevalence of hemophilia. So the study was done to know the inhibitor load in the patients treated for hemophilia A and hemophilia B.

Materials and Method

Data Collection: All existing cases of Hemophilia of Odisha attending Department of pathology, M.K.C.G Medical College Berhampur were taken into the study. Patients coming to emergency department for an acute joint bleed or trauma induced bleed or spontaneous bleed into muscles, or bleeding gums or cephalohematoma were first tested for any bleeding disorder and those diagnosed as Hemophilia were taken into the study. After obtaining a well informed consent, all patients were subjected to clinical examination, detailed clinical history, family history and treatment history. Blood samples were drawn on routines basis and collected in citrated tubes and then centrifuged and then plasma was collected and mixing studywas done to screen for inhibitors and then the cases with positive results in screening tests were quantitated for inhibitor levels using Bethesda study.

At the end of the study, the data were analysed using Microsoft excel and SPSS (VER20) and ethical clearance was taken. Verbal consent was taken from every patient before conducting the test. APTT estimation was done in Automated Coagulometer Sysmex CA600 for the testing. The machine is based on the principle of optical scatter.

Method of Mixing study

Three plastic tubes were prepared – A,B and C. 0.5 ml of normal plasma into tube A, 0.5ml test plasma into tube B and 0.2 ml each of normal and test plasma into tube C were taken. A,B and C were incubated for 60 minutes at 37 C.A 50:50 mix from tubes A and B were made in tube D, which serves as an immediate mix. A APTT was performed on A,B,D and C.Then if incubated mix D – Fresh mix C > 10 seconds, these cases were taken as positive for screening. Then Bethesda Assay is done to quantitate inhibitors.

Method of Bethesda assay for FVIII: C inhibitor

Eppendorf micro tubes were taken and 200 microliters of OVB is added in all tubes. Then 200 microliter of plasma was added in first tube and serial dilutions were done. In siliconised glass tubes were taken up to 8 (depending on the dilution required) in the first tube in which 100 microliter NPP was taken with 100 microlitre of imidazole/OVB buffer, this acts as the Buffer bank. Then 100 microliters of NPP were added in each tube, and 100 microliters of each respective dilution from step 1.If the residual factor VIII: C activity is between 80% and 100%, the plasma sample does not contain an inhibitor. If the residual activity is less than 60%, the plasma unequivocally contains an inhibitor. Values between 60% and 80% are borderline, and repeated testing on additional samples is needed before the diagnosis can be established. Statistical analysis was performed using the SPSS 21.0 software. Chi-Square tests were used to compare frequencies. The significant level was set at $p < 0.05$. Continuous variables were expressed as mean + SD, whereas categorical variables were expressed in the form of proportion and percentages. [16]

Discussion

To our knowledge, this is the first report on inhibitor development in haemophiliacs of Odisha. The most serious complication faced by hemophilia patients on treatment is the complication of development of inhibitors to factor VIII/IX that occur due to administration of blood products or recombinant factors that is administered to Hemophilia A/Hemophilia B patients. The patients with inhibitors do not respond to the treatment with the deficient factor concentrate during bleeding episodes which results in continuous bleeding and may sometimes lead to patient's death[16]. Treatment of Hemophilia patients with inhibitors is an important challenge in hemophilia care. The present study was carried out in Department of Pathology M.K.C.G Medical College, Berhampur. 54 cases of Hemophilia were screened during the period of October 2014- September 2016. Among them there were 13 new cases which were diagnosed during this 2 year period. These cases were further screened for development of inhibitors. The cases which came positive in screening were further quantitated using Bethesda Assay. In the present study prevalence of Hemophilia A was 92.6% and Hemophilia B was 5.5%. Hemophilia patients are divided into mild moderate and severe cases. In the present study of the 50 hemophilia A cases 23 i.e. 46% cases had severe Hemophilia A (factor VIII level <1%) and 22 i.e. 44% cases had moderate Hemophilia A (factor VIII level 1-5%) and 5 i.e. 10% cases had mild Hemophilia A.(Table 1)

Table I: Frequency of involvement of Joints in Hemophilia Cases (n= 22)

Joints Involved	Number of Cases	Percentage(%)
Knee Joint	10	45.45
Ankle Joint	2	9.09
Elbow Joint	2	9.09
Knee and Elbow Joint	4	18.18
Knee and Ankle Joint	2	9.09
Elbow and ankle Joint	2	9.09

In our study demographic and laboratory data shows that the mean age of patients in the study population was 14.38+8.12 years with age ranging from 9 months to 68 years.(Table 2)

Table II: Prevalence of inhibitors in Hemophilia A patients

Severity of hemophilia A	Total Number of Cases	Inhibitor positive	High responders	Low Responders	Percentage prevalence
Severe	23	3	1	2	13.04%
Moderate	22	1	0	1	4.5%
Mild	5	0	0	0	0%

The mean factor VIII level was 2.57% and the range was varying from 0.4-17.5%.The range of APTT in our study was 55 seconds to 105 seconds and mean APTT was 87.96 + 10.52 sec with control being 30seconds.The average number of transfusions taken for treatment was 12.72 with a range of 1 to 59 transfusions. Most patients presented with hemarthroses; 40.74% cases had development of target joints with knee joint which was most commonly effected i.e. 45.45% (10) of cases followed by involvement of elbow joint with knee joint seen in 18.18% (4) cases[10] reported that knee joint followed by elbow joint being the most commonly involved site for hemarthrosis in his study. Our study had similar findings(Table I).

The patients were treated with blood products and factors, 61.1% of the cases were treated with recombinant factors only and 39.9% (21) cases were treated with blood products like FFP, Cryoprecipitate with recombinant factor. With transfusion of blood products risk of infections transmitted by blood increases, in our study, shows 1.8% patients had Transfusion Transmitted Seropositivity, with 4.76% patients were positive for Hepatitis C and none for HIV and HBsAg.

In our study(Table II), 13.04% i.e. 3 of 23 severe Hemophilia A cases developed inhibitors. 33% of them i.e. 1 of the 3 cases were high responders having inhibitor level >5 BU. In moderate Hemophilia A 1 patient developed inhibitor which was a low responder with value <5BU. None of the Mild Hemophilia A cases developed inhibitors. The overall prevalence of inhibitors in Hemophilia A patients in our study was 8%. And out of 29 severe hemophilia A patients 31% were positive for inhibitors and out of 17 moderate hemophilia A patients 18% were positive for inhibitors. No patient with mild hemophilia A was positive for inhibitors. Among them 75% patients were low responders (<5 Bethesda units) and only 25% patients were high responders (>5 Bethesda units). None of the Hemophilia B patients in our study developed inhibitors. The demography and laboratory data of the hemophilia A patients who developed inhibitors is mentioned in table III.

Table III: Demography and laboratory data of Hemophilia A cases positive for inhibitor.

Sl no.	Age (yr)	Factor VIII level	Transfusion history	Last transfusion	Total number of transfusions	Inhibitor level
1	14	4.2%	BP+r FVIII	15d	56	3BU
2	22	0.8%	BP+r FVIII	3m	20	4.4BU
3	2	0.6%	r FVIII	10d	18	64BU
4	20	0.8%	BP+r FVIII	15d	50	3.2BU

Pinto et al[13] studied the inhibitor (Table IV) in different parts of India and reported that the highest incidence of FVIII Inhibitors was seen in South India (13.04 %).

Table 4: Inhibitor development in different cities of India[13]

Indian Cities	No. of cases of Hemophilia A	Inhibitor development HA	>5BU	<5BU
Chennai	81	17(20.99%)	7(41.2%)	10(58.82%)
Hyderabad	15	2(13.33%)	2(100%)	00
Jammu	101	10(9.9%)	5(50%)	5(50%)
Guwahati	47	47(8.51%)	1(25%)	3(75%)
Kolkata	117	4(3.42%)	1(25%)	3(75%)
Mumbai	246	13(5.28%)	9(69.2%)	4(30.77%)
Jaipur	100	4(4%)	3(75%)	1(25%)
Lucknow	135	6(4.44%)	2(33.3%)	4(66.66%)
Our study	50	4(8%)	1(25%)	3(75%)

The highest incidence of 20.99 % was observed in Chennai, followed by Hyderabad (13.33 %), Jammu (9.90 %) and Guwahati (8.51 %), respectively. The other regions showed an inhibitor incidence <8 %. Then inhibitor incidence in our study was 8% which is in par with the study done in Indian population

In our study we found a positive association between inhibitor development and severity of hemophilia (p=0.024)

Summary

In the present study Total 54 cases were screened for factor deficiency and inhibitors. 42 old cases were screened and 12 new cases of Hemophilia A were screened for development of inhibitors.The mean age of patients in the study population was 14.38+8.12 years with age ranging from 9 months to 68 years. Prevalence of Hemophilia A was 92.6%, prevalence of Hemophilia B was 5.5%. Prevalence of transfusion Transmitted Seropositivity was 1.8%. There were 46% severe hemophilia A cases and 44% moderate hemophilia A cases and 10% mild hemophilia A cases. 40.74% cases had development of target joints with knee joint which was most commonly effected. Prevalence of inhibitors in Hemophilia A in study population of Odisha was 8% and 13% in severe hemophilia cases.One Hemophilia B new case and 2 old cases of hemophilia B were negative for inhibitor screening test.Among the 4 inhibitor positive Hemophilia A patients 3 cases that developed were having severe hemophilia, and 1 had moderate hemophilia. By statistical analysis, it was seen that it has a positive correlation between number of blood product and factor transfusions to the development of inhibitors and it was statistically significant (P value= 0.004).There is increase in inhibitor development on switching of blood products and recombinant factors. The association in our study was not statistically significant (p=0.29) which may be attributed to the smaller size of study population. 25% i.e. 1 patient was high responder with inhibitor level of 64 BU, other 3 inhibitor positive patients were low responders with inhibitor levels of 3BU, 3.2 BU and 4.4 BU. By analysing severity of Hemophilia and inhibitor development we found there is positive association (odd ratio=0.826) between severity of Hemophilia and inhibitor development which is significant (p-value= 0.024).

Conclusion

Severe hemophilia patients need frequent factor transfusions and are at higher risk of inhibitor development. Inhibitor screening is done by mixing study and then quantification is done by Bethesda assay. Inhibitor development complicates the therapy and the patient may need higher doses of factor or immune tolerance therapy. Inhibitor level is expressed in Bethesda units. Patients with low inhibitor levels i.e.<10BU need high dose of recombinant factor VIII. Patients with high inhibitor levels >10 BU may require Recombinant factor VII with or without immune tolerance therapy. So inhibitor screening and Bethesda assay is needed at least once in every six months for prompt treatment of patients.

Conflict of Interest

Nil.

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