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Original Research Article

Screening and quantitative estimation of factor VIII inhibitors by Nijmegen-Bethesda assay in hemophilia a patient of Southern Odisha, India

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ABSTRACT

Background: The current treatment of haemophilia is replacement of factor VIII or IX which is effective till development of inhibitor against factors. There has been no study on factor VIII inhibitors in Southern Odisha using Nijmegen–Bethesda assay. This study was planned with objectives to screen factor VIII inhibitors in hemophilia-A patients, to do quantitative estimation of it using Nijmegen-Bethesda assay and to explore factors associated with development of inhibitors.
Methods: This cross-sectional study was carried out from September 2016 to August 2018 in Department of pathology, MKCG medical college, Berhampur. Haemophilia-A patients coming to MKCG medical college and registered Haemophilia-A cases under Haemophilia society of Berhampur were included. Patients denying consent and having multiple clotting factors deficiencies were excluded. 1.8ml blood was collected. Mixing study was done to screen factor VIII inhibitors and then in positive cases inhibitors level measured by Nijmegen-Bethesda method. All data were analysed using SPSS (version 16.0).
Results: 70 cases of Hemophilia-A patients were studied. Majority (50%) were with severe hemophilia-A. 7 patients developed inhibitors where 3 were high and 4 were low responders. Inhibitor level ranged from 0.8 to 64 Nijmegen-Bethesda units. Patients with severe hemophilia A, more than 10 transfusions and who switched to receive recombinant FVIII from other blood products developed inhibitors which were significant.
Conclusions: Severity of hemophilia, increase frequency of transfusion and switching of blood products significantly increases chances of inhibitor development and hence intensive inhibitor screening is needed in these cases. Quantification of inhibitor is needed to monitor treatment and to manage bleeding episodes effectively.

Keywords: Bleeding disorder, Blood transfusion, Factor VIII inhibitors, Haemophilia, Nijmegen-Bethesda assay

INTRODUCTION

Haemophilia is the most common hereditary bleeding disorder which is caused by deficiency of Factor VIII (Hemophilia-A) and factor IX (haemophilia B).[1] Currently the mainstay of treatment of haemophilia is replacement of FVIII/FIX with the use of either plasma or recombinant FVIII/FIX concentrates. FVIII replacement is effective unless otherwise there is development of an alloantibody (inhibitor) against the factors which make them ineffective and necessitating use of most costly and less effective alternative bypassing hemostatic agents.[1] Both environmental and treatment related risk factors are associated with inhibitor

formation.[2] The high-risk group for inhibitor formation includes severity of factor deficiency, frequency of transfusion, types of blood product transfusion, family history of inhibitor and African descent. Formation of inhibitors of FVIII is the most common and challenging complication of haemophilia treatment in India. The overall prevalence of inhibitors is 8.2% in patients with inherited severe haemophilia-A in India.[3] Early detection of these inhibitory antibodies allows a proper intervention at an early stage resulting in decrease in morbidity and financial burden. These assays are performed in haemophilic patients during routine surveillance screening or when presence of inhibitors is suspected that is in case of abnormal bleeding episodes or poor response to FVIII treatment therapy.

As per 2011 census, India has 14718 patients with bleeding disorders with 11586 Hemophilia-A patients and 1687 Haemophilia B patients.[4] A disease registry for Haemophilia is available with the Haemophilia Federation of India (HFI).[5] HFI has 80 chapters with eastern zone having 16 chapters. One of them is in Berhampur in Odisha.[5] Inhibitors screening is frequently done in different tertiary care centres of India. There has been various studies on inhibitors of factor-VIII by many authors both inside and outside of India. There has been no study on inhibitors to factor VIII in Southern Odisha using Nijmegen–Bethesda assay. Therefore, this study was planned to know the inhibitor load in Hemophilia-A patients in southern Odisha. The present study is first of its kind in Southern Odisha to screen and do quantitative estimation of factor VIII inhibitors in Hemophilia-A patients using Nijmegen-Bethesda assay. The objectives of this study are to screen the factors VIII inhibitors by mixing study in hemophilia-A patients, to do quantitative estimation of factor VIII inhibitors in them using Nijmegen-Bethesda assay and hence to assess the burden of haemophilic patients with factor VIII inhibitors in the study place and to explore factors associated with development of inhibitors in the patients.

METHODS

This cross-sectional study was carried out from September 2016 to August 2018 in the Department of pathology, M.K.C.G medical college and hospital, Berhampur, Odisha which is a tertiary care referral centre in Southern Odisha. Inclusion criteria- Haemophilia-A patients attending department of pathology M.K.C.G. medical college, registered cases of Haemophilia A under Haemophilia society of Berhampur and patients who were diagnosed with hemophilia-A in clinical OPDs coming with complain of bleeding episodes were included in the study. Exclusion criteria- patient denying consent, patient having multiple clotting factors deficiencies and newly diagnosed cases of Haemophilia-A who had not yet received any blood products were excluded. Universal sampling method was adopted and all qualified patients between September 2016 to August 2018 were studied. Prior to the study ethical clearance was taken from ethical committee and well-informed consent was taken from all the patients. All the participants were subjected to clinical examination with proper detailed clinical history, family history and treatment history in a predesigned Proforma. Clinical history includes types of bleeding (superficial/ deep tissue bleeding and spontaneous/after trauma) and any excessive bleeding history during dental extraction or surgery. Family history includes complete family pedigree along with detailed family history affected members in the family, gender of the affected individuals, history of consanguinity among parents, history of bleeding during surgery in any of the family members were recorded. From each patient 1.8ml blood was collected in a collection tube containing 0.2ml of 0.109M of trisodium citrate anticoagulant (9:1 proportion) using a 21 gauge needle (23 or butterfly for infants).Collection tubes were made of plastic to avoid contact activation of intrinsic pathway as occurs in glass tubes. Care was taken for a clean venipuncture with minimal stasis because partially clotted blood or haemolysed blood may cause spurious factor activation resulting in false results. Blood and anticoagulant were mixed immediately by gentle inversion 5-6 times. Samples were processed at room temperature as quickly as possible within 1 hr of collection and testing was performed within 4 hrs. This was done to avoid in vitro loss of factor activity of labile factors (FVIII). Blood samples were drawn and collected in citrated tubes and plasma was collected after centrifugation. APTT (Activated thromboplastin time), Factor VIII assay were done. Mixing study was done to screen Factor VIII inhibitors and then the positive cases in screening tests were evaluated for inhibitors level by using Nijmegen-Bethesda method. Nijmegen-Bethesda assay is based on the principle of measurement of inactivation of FVIII during a fixed incubation time and the quantification of residual FVIII activity using a semi-log rhythmic plot. Equal volumes of the sample and a FVIII source (imidazole buffered normal pool plasma) are incubated during 2 hours at 37ºC with a control sample (normal plasma diluted 1:1with inhibitor-free FVIII-deficient plasma). At the end of the study, all data were entered and analysed using SPSS (version 16.0). Continuous variables were presented with mean with standard deviation and categorical variables as proportion and percentages. Chi square test was applied to explore the associations between variables, p value less than 0.05 was taken as statistically significant.

RESULTS

This cross-sectional study was carried out in a tertiary care hospital of southern Odisha among patients with Hemophilia A from September 2016 to August 2018. Total 70 cases of Hemophilia A patients were studied and their demographic and laboratory profiles were depicted in (Table 1). The mean age of participants was 13.1±8.2 years, which ranged from 2.5 to 42 years. Patients having factor VIII level less than 1% were categorized as severe hemophilia A, 1% to 5 % as moderate and 5% to 40% as

mild hemophilia A. According to that majority (50%) were with severe hemophilia-A followed by 42.94% as moderate and rest 7.1% as mild hemophilia-A. The aPTT level ranged from 55 to 106 seconds with mean as 89.2±11.7 seconds and factor VIII level ranged from 0.4 to 17.9 with mean as 2.28±3.0. Out of 70 patients, majority (64.3%) had history of receiving recombinant factor VIII. In the study 84.3% patients took blood transfusion for less than 10 times and only 2.9% took for more than 30 times. Most of the participants (64.3%) received last blood transfusion within 60 days (Table 1).

Table 1: Demographic and laboratory profile of study population (n= 70).

Variables	Value
Age (Mean±SD in years)	13.16±8.2
Severity of cases (Number %)	
Mild	5 (7.1%)
Moderate	30(42.9%)
Severe	35(50%)
Nature of bleeding (Number %)	
Spontaneous	50(71.4%)
After trauma	20(28.6%)
aPTT (Mean±SD in seconds)	89.2±11.7
Factor VIII level (Mean±SD)	2.28±3
Type of product transfused (Number %)	
Only recombinant factor VIII (r FVIII)	45(64.3%)
Switching of blood products (FFP/Cryoprecipitate/whole blood/r FVIII)	25(35.7%)
Frequency of transfusions	
0-10	59(84.3%)
11-20	6(8.6%)
21-30	3(4.3%)
>30	2(2.9%)
Exposer days (Days since last blood product transfused)	
0-60	45(64.3%)
61-120	12(17.1%)
121-180	0(0)
181-240	3(4.3%)
241-300	0 (0)
301-360	4(5.7%)
>360	6(8.6%)

Figure 1 showed the development of inhibitors in hemophilia-A patients to factor VIII. Out of 70 patients, only 10% (7 patients) developed inhibitors and rest 90% (63 patients) did not develop inhibitors. Among the seven who developed inhibitors, 3 were high responders (>5 Nijmegen-Bethesda units) and 4 were low responders (<5 Nijmegen-Bethesda units).

In the present study, total 7 cases developed inhibitors. Inhibitor level ranged from 0.8 Nijmegen-Bethesda units to 64 Nijmegen-Bethesda units. The average inhibitor level was 12.6 Nijmegen-Bethesda units. (Figure 2) showed the inhibitor level in patients developing inhibitors to factor VIII (Figure 2).

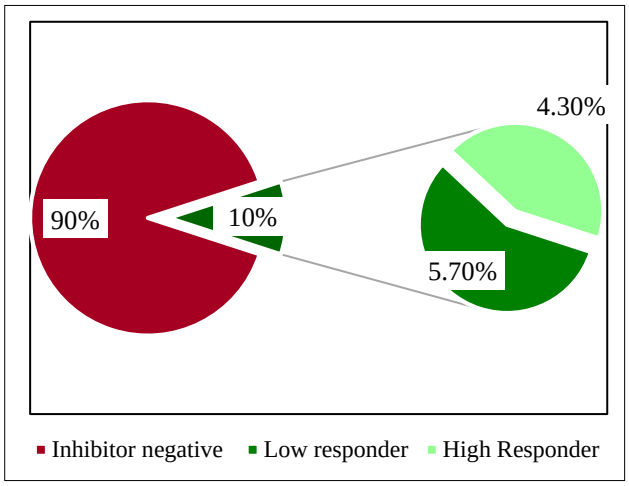

Figure 1: Development of inhibitors in study subjects (n=70).

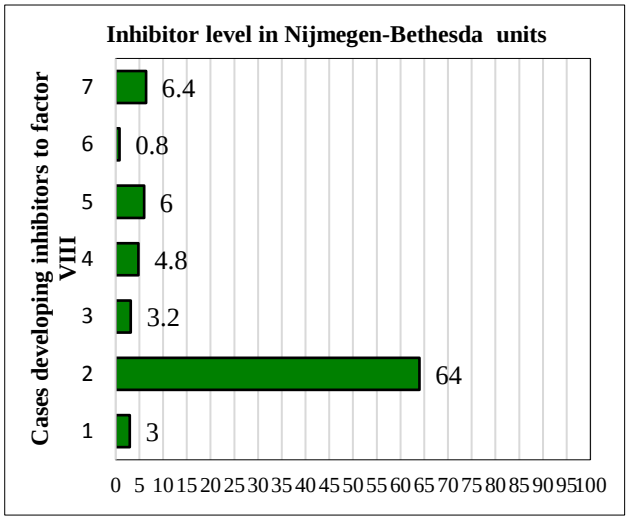

Figure 2: Inhibitor level in patients who developed inhibitor to factor VIII.

Table 2 shows the association of inhibitors development to factor VIII with various factors. There was a significant association between inhibitors development and severity of cases (p value= 0.007) and frequency of blood product transfusion (p value= 0.000). In the present study, patients only with severe hemophilia-A developed inhibitors to factor VIII. Patients who had received less than 10 blood product transfusions did not developed any inhibitors, but patients with more than 10 transfusions developed inhibitors to factor VIII. The association between inhibitor development and switching of blood products received was significant (p value= 0.038). Out of 25 patients who switched to receive recombinant FVIII from either FFP or cryoprecipitate or whole blood, 20% of them developed inhibitors. Whereas out of 45 patients who received only recombinant FVIII, only 4.4% developed inhibitors to factor VIII (Table 2). Majority of

patients who developed inhibitors to factor VIII received last transfusion within 60 days. A maximum of 90 days were there between last transfusion and development of inhibitors against factor VIII. However, the association between inhibitor development and days since last blood product transfused was not significant (p value= 0.729) (Table 2).

Table 2: Association of inhibitors development with different factors in patients.

Factors	Total cases	Inhibitor developed No. (%)	Inhibitors not developed No. (%)	χ2 value	p value
Severity of cases					
Severe	35	7(20%)	28(80%)		
Moderate	30	0(0)	30(100%)	7.34	0.007*
Mild	5	0(0)	5(100%)		
Switching of products					
Only r FVIII	45	2(4.4%)	43(95.6%)		
Switching of blood products (FFP/Cryoprecipitate/whole blood/r FVIII)	25	5(20%)	20(80%)	4.3	0.038*
Frequency of transfusion					
0-10	59	0(0)	59(100%)		
11-20	6	4(66.7%)	2(33.3%)	42.2	0.000*
21-30	3	2(66.7%)	1(33.3%)		
>30	2	1(50%)	1(50%)		
Exposer days (Days since last blood product transfused)					
0-60	45	6(13.3%)	39(86.7%)		
61-120	12	1(8.3%)	11(91.7%)		
121-180	0	0(0)	0(0)		
181-240	3	0(0)	3(100%)	2.037	0.729
241-300	0	0(0)	0(0)		
301-360	4	0 (0)	4 (100%)		
>360	6	0(0)	6(100%)		

DISCUSSION

The development of inhibitors to factor VIII is one of the most serious complications in hemophilia therapy and 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 patients' death.[6] 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 and 70 cases of Hemophilia-A were screened during the period of September 2016 to August 2018. These cases were further screened to assess the development of inhibitors to factor VIII. The inhibitors level was further quantitated using Bethesda Assay and Nijmegen-Bethesda assay in cases those came positive in screening for inhibitors.

In this study demographic data showed mean age of participants was 13.1±8.2 years which ranged from 2.5 to 42 years. Mohsin et al, in his study found the mean age was 16.62+11.15 years.[6] Dubey et al, in their study reported age of patients with Hemophilia-A ranged from 1 year to 53 years with median age being 16 years.[7]

Depending upon the factor level patients were divided into mild moderate and severe cases. In the present study out of 70 patients, majority (50%) were with severe hemophilia-A followed by 42.94% as moderate hemophilia-A and rest 7.1% as mild hemophilia-A. Similar findings were also found by Mohsin et al, Sharifian et al, and Eshghi et al, Mohsin et al, reported that in his study 58% cases had severe Hemophilia-A, 34% cases had moderate hemophilia-A and 12% cases had mild Hemophilia-A.[6,8,9] Sharifian et al. found 49.6% cases had severe Hemophilia-A, 21.6% cases had moderate Hemophilia-A and 28.8% cases had mild hemophilia-A.[8] Eshghi et al. also found 47% cases had severe hemophilia.[9] But Dubey et al. in their study found that majority (46.9%) had moderate hemophilia-And only 12.3% had severe hemophilia.[7]

In this study, the aPTT level ranged from 55 to 106 seconds with mean as 89.2±11.7 seconds. The factor VIII level present in all Hemophilia-A patients ranged from 0.4 to 17.9 with mean as 2.28±3.0. Dubey et al. in their study reported that factor VIII levels were in the range of 0.5-76.1%, median being 5.65%, the median APTT was 89.8 seconds and range of APTT was 43-120 seconds.[7] Mohsin et al. in his study found mean factor VIII level in this study was 1.93±2.80%.[6] Out of 70 patients, majority (64.3%) had history of receiving recombinant factor VIII. In the study 84.3% patients took blood transfusion for less than 10 times and only 2.9% took for more than 30 times. Most of the participants (64.3%) received last blood transfusion within 60 days.

In the present study, only severe hemophilia-A patients developed inhibitors to factor VIII. Out of total 35 patients with severe hemophilia A, 7(19.4%) had developed inhibitor and among those 7, 4 were low responder and 3 were high responder. However, no one with moderate and mild hemophilia-A had developed inhibitors to factor VIII. In this study, the overall percentage of patients who developed inhibitors in Hemophilia-A patients was 10%. In the studies done in Lucknow by Dubey et al. the prevalence of inhibitors was 5.1% among the hemophilia-A patients.[7] Similarly Kraiem et al, Yee et al, Company et al, and Kavakali et al, reported the overall percentage of patients who developed inhibitors to FVIII in hemophilia-A patients were 5%, 6.3%, 19.2% and 22.6% respectively.[10-13] Mohsin et al, in Pakistan found that 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 patients developing inhibitors 75% were low responders and 25% patients were high responders.[6] Sharifian et al, did inhibitor study in Iran showed that the overall prevalence of inhibitor was 14.4%, and inhibitor prevalence in severe hemophia A patients was 22.8%. Inhibitor prevalence in moderate and mild hemophilia was 9.4%, 3.5% respectively.[8] Pinto et al studied the inhibitor incidence in different parts of India and reported 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 %.[14]

In this study author found a positive association between inhibitor development and severity of hemophilia (p=0.007). This data is in accordance with the study of Sharifian et al, Iorio et al, also observed that inhibitor development increases with hemophilia-A severity.[8,15] There was a significant association between inhibitor development and frequency of transfusion. Patients with more than 10 transfusions developed inhibitors to factor VIII. Similarly significant association was found between inhibitor developments and switching of blood products received. Iorio et al, found a positive correlation between inhibitor development and switching of blood products and observed that inhibitor incidence was lower in cohorts using single concentrates as compared to multiple concentrates. Sharifian et al, observed that high percentage of inhibitors was seen in groups with more exposure to different blood products.[8] Lee et al, in his study found that hemophiliacs receiving single blood product had a lower inhibitor incidence than those with repeated switching between blood products.[16] Rosendaal et al, also had found same observation in his study.[17]

CONCLUSION

From the present study, it can be concluded that severe hemophilia patients are at higher risk of inhibitor development. Increase in the frequency of blood products transfusion and switching of blood products significantly increases the chances of inhibitor development. As inhibitor development complicates the therapy and patient may need higher doses of transfusion factors, intensive inhibitor screening is needed in severe hemophilic, patients with more numbers of transfusions, after any product change and in initial exposer days. In other cases, inhibitors screening is only needed annually. Quantification of inhibitor is needed to monitor the treatment and to manage bleeding episodes effectively. Prediction and early detection of inhibitor development leads to prevention of inhibitors formation thus helps in better management of the patients.

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