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RESEARCH ARTICLE

TO STUDY THE OCCURRENCE OF INHIBITORS IN CHILDREN AFFECTED WITH HEMOPHILIA 'A' AND HEMOPHILIA 'B' DISEASE

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ABSTRACT

Introduction: Use of factor VIII and factor IX concentrates has decreased the morbidity and mortality in patients with hemophilia. But with increasing use of factor concentrates, one major complication occurring is formation of inhibitors to Factor VIII and Factor IX which results in poor response to factor administration making control of hemorrhages more difficult. Different studies show enormous difference in inhibitor occurrence. The prevalence of inhibitors is estimated to be between 5 and 10% of all hemophilia A with 20% of severe hemophilia A patients and 3–5% of all hemophilia B patients

Aim of study: To study the occurrence of inhibitors in hemophilia A and hemophilia B affected children receiving factor replacement therapy.

Material and Methods: The study was conducted in department of pediatrics, S.M.S. medical College, jaipur, India from april 2011 to March 2013 and a total of 100 children with hemophilia were screened for inhibitors to factor VIII and IX with quantification done by Bethesda assay.

Results: Out of 100 hemophilia children screened 81(81.00%) had hemophilia A and 19 (19.00%) had hemophilia B. Among 81 Hemophilia A patients, 56 (69.14%) had severe, 13 (16.05%) had moderate and 12 (14.81) had mild hemophilia A disease. Among 19 hemophilia B patients, 15 (78.95%) and 4 (21.05%) had severe and moderate hemophilia B respectively. 4 patients, all belonging to severe hemophilia A, were found to be inhibitor positive. Thus 4.94% (4 out of 81) of total hemophilia A and 7.14% (4 out of 58) of severe hemophilia A patients were found inhibitor positive. None of the 19 hemophilia B patients showed inhibitors. Inhibitor titers ranged from 3.1 BU/mL to 128 BU/mL.

Conclusions: Percentage of patients showing inhibitor positivity is slightly lesser than previous studies. Inhibitor occerrance in still low in Indian patients than their western counterparts. However,with increasing use of factor concentrates in hemophiliac children in developing setups, inhibitor occurrence is expected to increase and screening at regular intervals advised , to detect inhibitors early.

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INTRODUCTION

Use of factor VIII and factor IX concentrates has undoubtedly, decreased the morbidity and mortality in patients with hemophilia. But with increasing use of factor concentrates in treatment of hemophilia, one major complication that has appeared and is posing a great challenge in hemophilia treatment is formation of inhibitors to Factor VIII and Factor IX. Inhibitors are polyclonal antibodies with majority belonging to IgG4 subclass (Anupam Sachdeva, 2012). These inhibitory antibodies bind to and neutralize the pro coagulant activity of factor, clinically resulting in an increased bleeding tendency not responding to factor concentrate infusion and thus making control of hemorrhages more difficult. Studies show enormous difference in the incidence of inhibitor development in patients with different types of hemophilia (Scharrer and Nentlzing, 1993; Ghosh *et al*., 2001). Incidence of inhibitors is much higher in patients with factor VIII (FVIII) deficiency than factor IX (FIX) deficiency. It is also well known that patients with severe hemophilia A tend to develop inhibitors more often than patients with mild or moderate hemophilia (Hoyer, 1995). The prevalence of inhibitors to FVIII is estimated to be between 5 and 10% of all cases and 20% of severe hemophilia A patients. Inhibitors to FIX are detected in only 3–5% of all hemophilia B patients (Longo 18th edition). In India, due to cost constrains, many hemophilia patients still

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receive blood products and receive very few factor concentrates. In India till now very few studies have been conducted to study inhibitor occurrence in hemophiliacs. We therefore attempted to get an insight into inhibitors detected through screening by this study.

MATERIALS AND METHODS

In this hospital based cross sectional descriptive study, based on hospital data, 100 hemophilia patients attending OPD (81hemophilia A and 19 hemophilia B) in 2012-2013 were screened for presence of inhibitors. 99 patients were upto18 years of age with one patient being 21 years. Blood samples for screening were collected by organizing three camps between April 2012 to September 2013. Blood samples taken from hemophilia patients were appropriately processed, stored and sent to National Institute of Immuno haematology (Indian Council of Medical Research) at K.E.M. Hospital, Mumbai, India where screening for the presence of inhibitors was done. Screening was done by preparing 1:1 mix of patients plasma (with prolonged APTT) and Normal Pooled Plasma (NPP) and incubating it for 1 and 2 hours along with simultaneous incubation of patients plasma and NPP separately for the same length of time at 37ºC. APTT was then performed on patients plasma, NPP taken as control, 1:1 mix Control: Patient incubated mix and on 1:1 Control : Patient immediate mix (50:50 mix from normal plasma and patients plasma incubated separately) at 1 hour and 2 hours and results of APTT expressed in seconds. Those mix samples which showed more than 10s prolongation of APTT after 1 hour and 2 hour incubation were further evaluated by Nijmegan modification of Bethesda Assay (Verbruggen *et al*., 1995) and results were expressed as Bethesda Units (BU)/mL. Family history was considered positive when any of the first or second degree relative of the patient had been diagnosed with hemophilia or had history of recurrent joint bleeds.

Episodic (On Demand Therapy) – Therapy given in response to a bleeding episode. Dose of factor given is determined by the type of bleeding (Srivastava 2nd edition).

Prophylactic Therapy - Therapy given with factor in order to prevent bleeding.

Type: (Srivastava 2nd edition)

i. Continuous Prophylaxis: involves continuous factor treatment defined as the intent of treating for 52 weeks / year and receiving a minimum of an a priori defined frequency of infusions for at least 45 weeks (85%) of the year under consideration
ii. Intermittent (Periodic) Prophylaxis: treatment given to prevent bleeding for periods not exceeding 45 weeks in a year.

Classification of inhibitors as low titer and high titer
Low titer : defined as tier <5 BU/ml and High titer : defined as titer >5 BU/ml (Anupam Sachdeva *et al*., 2012)

Inhibitors were further classified as Low Responders and High Responders which depends upon how a person's immune response is stimulated on repeated exposure to factor VIII or IX. A low responder is one whose response to factor exposure is slower and weaker, and Bethesda titer persistently remain low (<5BU/mL) A high responder is one with high inhibitor titer (>5 BU/mL) or one with low titer but showing an an amnestic response in antibody titer to >5 BU/mL (Anupam Sachdeva *et al*., 2012; Carol K. Kasper, 2004)

RESULTS

The present study was a hospital based cross sectional descriptive study. This study is one of the very few studies being attempted to screen hemophilia affected patients for the presence of inhibitors. A total of 100 hemophilia A and B patients were screened for the presence of inhibitors to Factor VIII and Factor IX. Out of 100 hemophilia patients screened 81(81.00%) were hemophilia A patients and 19 (19.00%) had hemophilia B**.** All the hemophilia patients studied were males who had received factor concentrates or blood products at least once. Almost all the patients studied had received plasma derived Factor VIII and IX concentrates. All the patients screened had been receiving episodic (on demand) therapy. None was on prophylaxis. One patient in our study had previously been screened for presence of inhibitors but no records of inhibitor assay were available. As the patient had been suspected of being inhibitor positive, we included him in our study despite being 21 years of age. Mean age of hemophilia patients included was 10.08 ± 5.91 years with mean age of hemophilia A patients being 10.48 ± 6.04 years and that of hemophilia B patients being 8.34 ± 5.11years (Table 1). Among 81 Hemophilia A patients screened 56 (69.14%) were having severe hemophilia A, 13 (16.05%) had moderate and 12(14.81) had mild hemophilia A (Fig 1). The definition of severe hemophilia in the present study was kept as factor VIII or IX level <2% as most of the standard laboratories are now giving <2 % as cut off limit in their reports for the same. Also many previous studies have been conducted wih severe disease classified as factor VIII level <2%. Out of 19 hemophilia B patients studied 15 (78.95%) had severe hemophilia B and 4 (21.05%) had moderate hemophilia B. No patient belonged to mild hemophilia B category. During our study we found one patient having severe hemophilia A (FVIII level =1%) and mild hemophilia B (FIX level <17%). Also one patient was having combined deficiency of Factor V (FV level 4.5%) and Factor VIII (FVIII level 6%). For statistical purpose in our study they have been classified as severe and mild hemophilia A respectively.

Family history was considered positive when any of the first or second degree relative of the patient had been diagnosed with hemophilia or had history of recurrent joint bleeds. Among 100 patients of hemophilia included in our study, 59 were having positive family history, 40 patients showed negative family history and family history of one patient was not available. Thus 40 patients with negative family history might have been representing new spontaneous mutation cases. Out of 81 hemophilia A patients studied, 47 (58.02%) had a positive family history. 30 (53.57%) out of 56 severe, eight (61.54%) out of 13 moderate and nine (75%) out of 12 mild hemophilia A were having positive family history. Out of 19 hemophilia B patients studied 12 (63.16%) had positive family history. 10 (66.67%) out of 15 severe and two (50.00%) out of four moderate hemophilia B patients were having positive family history. However, no significant association was seen between positive family history and severity of disease. Majority of patients in had received factor infusions ≤ 20 times. 59 (72.84%) out of 81 hemophilia A patients and 18 (94.74%) out of 19 hemophilia B patients had received factor concentrates ≤ 20 times thus making it 77.00% of total.

Table showing characteristics and distribution of patients with hemophilia including inhibitor positive patients

PATIENTS	Number of patients	Mean Age (in years)	Positive Family History	Number of Inhibitor Positive	Percent age of positive patients	Range of inhibitor (BU/mL)	Patients with high titer (>5BU/mL)
HEMOPHILIA A	81	10.48 ± 6.04	47	4	4.94 %	3.1 – 128	3
Severe	56		30	4	7.14%	3.1 – 128	3
Moderate	13		8	-	-	-	-
Mild	12		9	-	-	-	-
HEMOPHILIA B	19	8.34 ± 5.11	12	-	-	-	-
Severe	15		10	-	-	-	-
Moderate	4		2	-	-	-	-
Mild	0		-	-	-	-	-
TOTAL	100	10.08 ± 5.91	59	4	4%	-	3

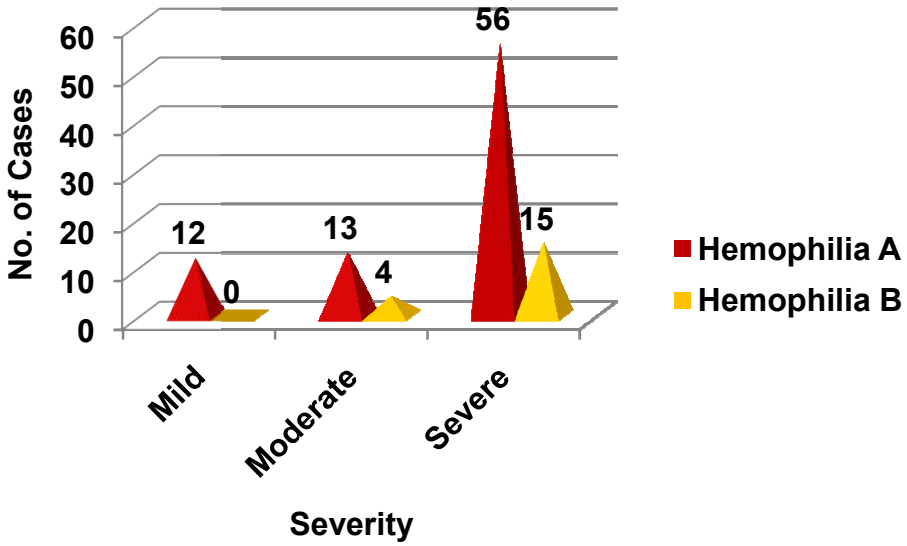

Figure 1. Distribution of patients according to severity & type of hemophilia

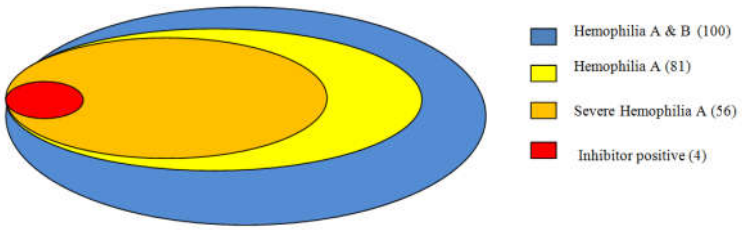

Figure 2. Distribution of hemophilia patients showing hemophilia and inhibitor positive patients

87 out of 100 had received blood products with 71 (87.65%) out of 81 hemophilia A patients and 16 (84.21) out of 19 hemophilia B patients had received blood products mainly in form of Fresh Frozen Plasma.

Inhibitor occurance

Out of total 100 patients screened four (4.00%) were found to be inhibitor positive. All the inhibitor positive patients belonged to hemophilia A group. Thus out of 81 hemophilia a patients screened we found four (4.94%) as inhibitor positive. None of the 19 hemophilia B patients studied showed presence of inhibitors. Furthermore it was observed that all four inhibitor positive patients belonged to severe hemophilia A group. Thus out of 56 severe hemophilia A patients screened 4 (7.14%) were inhibitor positive (Fig 2). Although the sample size taken in our study was smaller than some of the above mentioned studies, we obtained a percentage of inhibitors positive hemophilia patients lower than observed with majority of the other studies conducted.

Inhibitor titers and range

In our study inhibitor titers in positive patients ranged from 3.1 BU/mL to 128 BU/mL. Out of four inhibitor positive patients, three (75.00%) patients showed high inhibitor titers (>5 BU/mL) and one patient had low inhibitor titers (<5BU/mL). The mean inhibitor level was 10.0 BU/mL (excluding one patient with high inhibitor level of 128 BU/mL).

Some other observations in inhibitor positive patients:

i. *Family History* - Out of four inhibitor positive patients, two had positive family history and 1 had negative family history. Family history of one patient was unknown.

ii. *Use of blood products* – all four inhibitor positive patients had received blood products before screening.
iii. *Number of times factor infused* – the four inhibitor positive patients with inhibitor levels (in BU/mL) 3.1, 10.8, 16 and 128 had received factor concentrates 12, 20, 60 and 70 times respectively.

In our study we observed that inhibitor levels in positive patients were higher in those patients who had received more numbers of factor infusions.

DISCUSSION

In our study inhibitors were present in 4.94% of total hemophilia A and 7.14% of severe hemophilia A patients. The results of our study show inhibitors in slightly lower percentage than shown by other studies conducted previously. Sultan (1992) studied 3,435 hemophiliacs and showed a prevalence of 6.2% for the overall population. Prevalence of inhibitors was found to be 7% in the population of hemophilia A patients and 12.8% in the population of severely affected ones. The prevalence of inhibitors in hemophilia B patients was 2% and in severely affected hemophilia B patients, it was 4%. Ghosh *et al* (2001) investigated 407 patients (352 Hemophilia A and 55 Hemophilia B) and their study showed an overall inhibitor prevalence of 8.2 %. In their study 24 (8.21%) out of 292 severe and two (5.55%) out of 36 moderate hemophilia 'A' patients showed the presence of inhibitors. Out of 35 hemophilia B patients studied, only one patient developed an inhibitor. Oren *et al*. (1999) study in 58 hemophilia A patients aged 1-18 years (mean 9.5 ± 4.7 years) showed a 10% prevalence of inhibitors at the end of the study. Our study showed lower percentage of inhibitors than this study. Sharifian *et al*. (2003) surveyed 1280 hemophilia A patients and found 184 patients (14.4%) developing inhibitors. 145 patients of 635 with severe hemophilia A (22.8%), 26 patients of 277 with moderate hemophilia A (9.4%) and 13 patients of 368 with mild hemophilia A (3.5%) developed inhibitor. Wight and Paisley (2003) in their systematic review of epidemiology of inhibitors in hemophilia done from 50 papers also showed the overall prevalence of inhibitors in unselected hemophiliac populations to be 5-7%. They showed substantially greater prevalence, between 12% and 13% amongst severe hemophiliacs (FVIII:C < 2%). Farzad Company *et al* in 2011 studied 104 patients with hemophilia A and found 20 patients (19.2%) had factor VIII inhibitors Jacob katz (1996) showed that factor IX inhibitors were much less common (1.5%) in patients with hemophilia B than in patients with hemophilia A.

The values observed in our study were lower than many previous studies including mostly western studies probably due to the following reasons

- Most of patients in our set up receive blood products having lesser potential to induce inhibitors than factor concentrates as affordability is still an important issue in our setup where majority of haemophilia children belong to low socioeconomic status. More and more efforts are now being made to make factor available to affected individuals either free of cost or at subsidized rates, as factor concentrates infusion therapy is the recommended therapy which results in decreased disability and morbidity and improved quality of life. Moreover, there is no risk of transmission of blood borne infections with factor replacement therapy.
- Also, the factor concentrate available to most of patients are plasma derived which have lower potential than recombinant factor concentrates for induction of inhibitors (Escuriola-Ettingshausen *et al*., 2006; Wolfhart Kreuz *et al*., 2008).
- Ethnicity also affects the prevalence of inhibitors with higher incidence occurring in African- American and Latin-American patients, our patients did not belonged to any of these groups (Kleigman, 19th Edition; Aledort *et al*., 1998).

Also, it is quite possible that we might have missed some transient inhibitors which are well known to occur in haemophilia patients which might not be present at the time of screening. But now with more and more use of factor concentrates within hemophilia affected patients, inhibitor occurrence is expected to increase and screening at regular intervals should be done so as to detect inhibitors early and make sufficient arrangements to cope up with this important complication using therapies so as to improve management and quality of life of inhibitor positive patients.

Conclusion

Percentage of patients showing inhibitor positivity is slightly lesser than previous studies. With increasing use of factor concentrates in hemophiliac children in Indian set up, inhibitor occurrence is expected to increase and screening at regular intervals should be done, to detect inhibitors early and make arrangements to manage such patients.

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