

Evaluation of transfusion-related complications along with estimation of inhibitors in patients with hemophilia: A pilot study from a single center

Anju Dubey, Anupam Verma, Priti Elhence, Prashant Agarwal

Department of
Transfusion Medicine,
Sanjay Gandhi
Postgraduate Institute
of Medical Sciences,
Lucknow, India

Abstract:

Background: Apart from inhibitor development in patients with hemophilia (PWH) the old problems of blood borne viral infections and red cell alloimmunization still persist in PWH from developing countries. This study was planned to detect the presence of inhibitors in our PWH and to determine the presence of transfusion transmitted infections (TTI) markers and clinically significant red cell alloantibodies in these patients. **Materials and Methods:** One hundred fourteen PWH were screened for various laboratory tests. Screening for inhibitors was done by mixing study. Blood grouping, TTI testing and red cell alloantibody detection were done as per the departmental standard operating procedures. **Results:** Out of 114 patients evaluated 98(86%) had hemophilia A and remaining 16(14%) had hemophilia B. Five (5.1%) patients of hemophilia A were positive on inhibitor screening. On Bethesda assay, one patient was high responder (14.4 BU/ml) and rest 4 were low responders (<5 BU/ml). Overall, 19 PWH were positive for TTI markers and two had clinically significant red cell alloantibody (anti-E and anti-Jk^b). **Conclusion:** This is probably first comprehensive study from our state on laboratory testing in PWH. The specialty of Transfusion Medicine can be a core part of hemophilia care. The overall prevalence of inhibitors in our hemophilia A patients was 5.1%, which is less as compared to majority of published studies.

Key words:

Hemophilia, inhibitor, transfusion-related complications

Introduction

Throughout life, hemophiliacs are challenged with complications of both the disease and the treatment. The latter includes development of inhibitors due to exogenous replacement factors, transfusion transmitted infections (TTI), and red cell alloimmunization due to blood products transfused. The development of inhibitors to factor VIII/IX is one of the most serious complications in hemophilia therapy and is an important challenge in hemophilia care. 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.^[1]

The present study was conducted with the aim of estimating the burden of transfusion-related complications in patients with hemophilia (PWH) at our hospital, which caters to the most populous state of India. We also wanted to know the prevalence of inhibitor in our PWH, as there is limited data in this context from the developing countries.

Material and Methods

This study was conducted by Department of Transfusion Medicine at Sanjay Gandhi Postgraduate

Institute of Medical Sciences, Lucknow, Uttar Pradesh (India), which is a tertiary care referral hospital. A total of 114 PWH were screened in a hemophilia camp visit for various laboratory tests. Citrated and ethylenediamine tetraacetic acid (EDTA) samples were collected from the patients and their clinical details were recorded. Activated partial thromboplastin time (APTT), factor assay (VIII and IX), and inhibitor screening (mixing study) were done on citrated plasma using semi-automated coagulation analyzer (STart4, Diagnostica Stago, Japan). Screening for inhibitors was done by mixing study. Briefly, 1:1 mix of patient's plasma (PP) and normal pooled plasma (NPP) was incubated for 2 hours along with simultaneous incubation of PP and NPP separately for the same length of time at 37°C. APTT was performed on the mix and then separately on PP and NPP. Any of the mix samples showing non-correction of prolonged APTT was evaluated by classical Bethesda assay in duplicate and the results were expressed as Bethesda units (BU).^[2]

Blood grouping, TTI testing by ELISA (Biomerieux, France), and red cell alloantibody detection (Diamed gel cards, Switzerland) were done using EDTA sample as per the departmental standard operating procedures.

Results

Out of 114 patients screened, 98 (86%) had

Access this article online

Website: www.ajts.org

DOI: 10.4103/0973-6247.106714

Quick Response Code:



Correspondence to:

Dr. Anupam Verma,
Department of Transfusion
Medicine, Sanjay Gandhi
Postgraduate Institute
of Medical Sciences,
Lucknow – 226 014, India.
E-mail:
aver2211@gmail.com

hemophilia A and the remaining 16 (14%) had hemophilia B. The age range of patients with hemophilia A was 1-53 years (median age, 16.0 years) and that of hemophilia B was 3-37 years (median age, 13.5 years).

In the coagulation profile of hemophilia A patients [Table 1], range of APTT was 43-120 seconds (normal control = 32 seconds; median, 89.8 seconds). Factor VIII levels were in the range of 0.5-76.1% (median, 5.65%). Based on factor level, these patients were categorized as follows: mild, 28 (28.5%); moderate, 46 (46.9%); and severe, 12 (12.3%). The remaining 12 (12.3%) patients had Factor VIII level >30%. Five patients (5.1%) were positive on inhibitor screening using the mixing study. Bethesda assay was performed to quantify the inhibitors in these five hemophilia A patients [Table 2].

In the coagulation profile of hemophilia B patients [Table 1], range of APTT was 46.4-111.7 seconds (normal control= 32 seconds; median, 70.4 seconds). Factor IX levels were in the range of 0.8-64.6% (median, 4.9%). Based on factor levels, these patients were categorized as follows: mild, 5 (31.2%); moderate, 7 (43.8%); and severe, 1 (6.2%), while 3 (18.8%) patients had Factor IX level >30%. However, no patient was positive on inhibitor screening using the mixing study.

The blood group distribution did not vary much between two groups, with blood group B being the most common. On TTI testing, two patients were seroreactive for HIV, two for hepatitis B surface antigen (HBsAg), and 15 for Hepatitis C virus (HCV) [Table 3]. Red cell alloantibodies (anti-Jk^b and anti-E) were detected in two patients.

Discussion

The study was conducted to estimate the coagulation parameters, prevalence of inhibitors, red cell alloimmunization, and TTI markers in hemophilia patients who were registered in the camp at our center. It is imperative to collect data about patients, their present clinical condition, and coagulation parameters with assessment of inhibitors besides any transfusion complications for better planning and structuring of hemophilia services. Most patients presented with hemarthroses, with knee joint followed by elbow joint being the most commonly involved sites. On analyzing the coagulation factor levels, majority of the hemophilia patients were categorized as moderate, followed by mild and severe, which is in contrast to other studies where the majority of patients had severe disease.^[3,4] This difference could be attributed to the fact that baseline factor levels in the patients were not compared; besides, the majority of our patients were either taking or had taken treatment with factor concentrates. The ratio of hemophilia A:B at our center was found to be 6.1:1, whereas a ratio of 4.2:1 has been reported from another study in India.^[5]

Data are limited on prevalence of inhibitors in PWH in India. The prevalence of inhibitors in our study was 5.1% among the hemophilia A patients. Most of these patients were previously being managed with cryoprecipitate units and only recently they were supported with plasma-derived factor concentrates supplied free of cost from the state government. However, this on-demand factor replacement therapy had been erratic with some patients receiving wet plasma products in between, due to unavailability of factor concentrates for some time. The inhibitor prevalence in our study is lower than that reported from other studies in India, which

is 8.2-13%.^[6] Similarly, in a study among Chinese hemophilia A patients treated only with plasma-derived FVIII, cryoprecipitate, or fresh frozen plasma (FFP), the overall prevalence of inhibitors was 3.9%.^[7] However, the prevalence in patients of severe hemophilia A reported from developed countries is as high as 30%.^[8]

The cause for a comparatively low prevalence of inhibitors in our patients, which holds true for developing countries like India, may be due to delayed initiation of factor replacement therapy and scarce/erratic availability of purified factor concentrates. This is in accordance with the findings of the CANAL study, in which the intensity of treatment was associated with a higher risk for inhibitors when compared to most of the other risk factors examined.^[9] In our study, only one patient was found to have a high titer of inhibitors (14.4 BU/ml) and the rest four had a low titer of inhibitors (<5 BU/ml). The former was treated with recombinant factor VIIa and tranexamic acid and latter, with increasing dosage of factor VIII concentrates in order to yield satisfactory results.

We did not find inhibitors in any of the 14 hemophilia B patients. In another study from India, out of 35 hemophilia B patients, only one patient developed an inhibitor.^[10] Inhibitor prevalence of 1.5-3% has been reported for hemophilia B patients in western countries by DiMichele.^[11]

The TTI seropositivity of hemophilia patients in the present study was 1.75% for HIV, 1.75% for HBsAg, and 13.15% for HCV. This prevalence is much lower than that reported from a study done in western India in which the prevalence of HIV, HBsAg, and HCV has been reported to be 3.8%, 6%, and 23.9%, respectively.^[12] In another study from a neighboring country where a total of 173 multitransfused male hemophiliacs showed a prevalence of

Table 1: Profile of hemophilia patients (n = 114)

Parameter	Hemophilia A (n = 98)	Hemophilia B (n = 16)
Age (yrs) Median Range	16 (±9.77) 1-53	13.5 (±9.38) 3-37
APTT (sec) Median Range	89.8 (±21.4) 43-120	70.45 (±20.7) 46.4-111.7
FVIII/IX level (%) Median Range	5.65 (± 15.1) 0.5-76.1	4.90 (±17.77) 0.8-64.6
Severity		
Mild	40 (40.8%)	08 (50%)
Moderate	46 (46.9%)	07 (43.75%)
Severe	12 (12.3%)	01 (6.25%)

Table 2: Characteristics of patients positive on inhibitor screening (n = 5)

Patient No.	Age (yrs)	APTT (sec Control = 32)	FVIII (%)	Inhibitor titer (BU/ml)
1	20	102.8	3	2.2
2	20	116.3	0.5	14.4
3	07	117.8	0.8	2
4	36	97.4	6.6	1.2
5	24	74.3	1.2	1.4

Table 3: TTI reactivity in hemophilia patients (n = 114)

Infectious marker	No. (% of total patients)
Anti-HIV antibodies+ P24 antigen	02 (1.75)
HBsAg	02 (1.75)
Anti-HCV antibodies	15 (13.15)

51.4% for HCV, 1.73% for hepatitis B virus (HBV), and nil for HIV.^[13] This study also showed that HCV infection was more frequently identified than HBV and HIV infections in PWH. However, there may be selection bias in reporting the prevalence of TTI, especially HIV infections; besides, the variation in TTI seropositivity may be due to different policies, namely, stringent policy on blood usage, comparatively greater use of purified factor concentrates, universal hepatitis B vaccination, and better methodologies for TTI screening of blood products at various centers. An interesting observation was that all TTI-seropositive patients were from hemophilia A group, whereas there were no seropositive cases in hemophilia B. This differential pattern of seropositivity may be due to a relatively larger number of patients, more number of bleeding episodes resulting in greater use of cryoprecipitate units in hemophilia A patients. An adult dose of cryoprecipitate units used in hemophilia A is derived from multiple blood donors (up to 20) as compared to 2-4 FFP units transfused in hemophilia B patients with exposure to 2-4 blood donors only. Of late due to government support, all hemophiliacs are now being treated with plasma-derived factor concentrates, which should now further decrease the seroprevalence at our center.

Another transfusion-related challenge in care of hemophilia patients, especially those whose are multitransfused due to recurrent bleeding episodes, is alloimmunization to red cell antigens. In our study, we found two patients of hemophilia A who had developed antibodies to Jk^b and E antigens. These patients were successfully transfused with crossmatch compatible corresponding antigen-negative red cell units to alleviate the risk of any hemolytic transfusion reaction. Red cell antibodies of variable clinical significance against minor red cell antigens are frequent findings in any multitransfused patients; however, to the best of our knowledge, there are no published studies on alloimmunization in PWH from the Indian subcontinent.

The main limitation of our study is small cohort of patients with snapshot evaluation of PWH, which has not included the systematic screening of all patients registered at our center; hence, real magnitude of the problem is still unclear in this region. It is hoped now that with regular factor replacement therapy and periodic screening of patients for inhibitor formation and transfusion-related complications a paradigm shift towards a better management care awaits for PWH at our center.

In conclusion, we have tried to provide the synopsis of laboratory care for PWH at our center; however, there is an urgent need to develop laboratory infrastructure in government-supported hospitals where facilities for laboratory tests for TTI viral markers, identification of alloantibodies, and testing for inhibitors in PWH are not available. In India, management options vary widely given the socioeconomic diversity among PWH and kind of financial support state governments are providing. The central/state governments should continue to support these patients and help in establishing the centers of excellence for holistic management

of hemophilia patients across India. The specialty of transfusion medicine can be a core part of hemophilia care by providing the laboratory services in the form of hemostasis and serology testing along with factor concentrates and blood component support.

References

1. Darby SC, Keeling DM, Spooner RJ, Wan Kan S, Giangrande PL, Collins PW, *et al.* The incidence of factor VIII and factor IX inhibitors in the hemophilia population of the UK and their effect on subsequent mortality, 1977-99. *J Thromb Haemost* 2004;2:1047-54.
2. Kasper CK, Aledort L, Aronson D, Counts R, Edson JR, van Eys J, *et al.* Proceedings: a more uniform measurement of factor VIII inhibitors. *Thromb Diath Haemorrh* 1975;34:612.
3. Sharifian R, Hoseini M, Safai R, Tugeh GH, Ehsani AH, Lak M, *et al.* Prevalence of inhibitors in a population of 1280 Hemophilia A patients in Iran. *Acta Medica Iranica* 2003;41:66-8.
4. Bhopale GM, Nanda RK. Blood coagulation factor VIII: An overview. *J. Bioscience* 2003;28:783-9.
5. Kar A, Potnis-Lele M. Descriptive epidemiology of haemophilia in Maharashtra, India. *Haemophilia* 2001;7:561-7.
6. Mathews V, Nair SC, David S, Viswabandya A, Srivastava A. Management of hemophilia in patients with inhibitors: The perspective from developing countries. *Semin Thromb Hemost* 2009;35:820-6.
7. Wang XF, Zhao YQ, Yang RC, Wu JS, Sun J, Zhang XS, *et al.* The prevalence of factor VIII inhibitors and genetic aspects of factor VIII inhibitor development in Chinese patients with haemophilia A. *Haemophilia* 2010;16:632-9.
8. White GC 2nd, Rosendaal F, Aledort LM, Lusher JM, Rothschild C, Ingerslev J; Factor VIII and Factor IX Subcommittee. Definitions in hemophilia. Recommendation of the scientific subcommittee on factor VIII and factor IX of the scientific and standardization committee of the International Society on Thrombosis and Haemostasis. *Thromb Haemost* 2001;85:560.
9. Gouw SC, van der Bom JG, Marijke van den Berg H. Treatment-related risk factors of inhibitor development in previously untreated patients with hemophilia A: the CANAL cohort study. *Blood* 2007;109:4648-54.
10. Ghosh K, Shetty S, Kulkarni B, Nair S, Pawar A, Khare A, *et al.* Development of inhibitors in patients with haemophilia from India. *Haemophilia* 2001;7:273-8.
11. DiMichele D. Inhibitor development in haemophilia B: An orphan disease in need of attention. *Br J Haematol* 2007;138:305-15.
12. Ghosh K, Joshi SH, Shetty S, Pawar A, Chipkar S, Pujari V, *et al.* Transfusion transmitted diseases in haemophiliacs from western India. *Indian J Med Res* 2000;112:61-4.
13. Borhany M, Shamsi T, Boota S, Ali H, Tahir N, Naz A, *et al.* Transfusion transmitted infections in patients with hemophilia of Karachi, Pakistan. *Clin Appl Thromb Hemost* 2011;17:651-5.

Cite this article as: Dubey A, Verma A, Elhence P, Agarwal P. Evaluation of transfusion-related complications along with estimation of inhibitors in patients with hemophilia: A pilot study from a single center. *Asian J Transfus Sci* 2013;7:8-10.

Source of Support: Nil, **Conflict of Interest:** None declared.