Research Article

Seroprevalence of HBsAg, Anti-HCV and Anti-HIV in Hemophilia 'A' and 'B' Patients

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Abstract: The aim of the study was to find out seroprevalence of HBsAg, anti-HCV and anti-HIV in hemophilia "A" AND "B" patients. To find out the association of hepatitis B, hepatitis C and HIV infection with various factors like age, sex, family history, number of transfusions of blood products and factor concentrates. It was a hospital based cross sectional descriptive study. Hemophilia affected children were selected and evaluated in detail with regards to the age of onset of symptoms, mode of presentation, family history of bleeding/ hemophilia, severity of bleeding episodes and their relation to trauma , number of bleeding episodes with respect to time, most common site of bleeding, most common joint involved, therapies taken for bleeding episodes including blood products and / or factor concentrates, number of times therapy being taken, response to therapy, age of using blood product / factor for the first time, prophylactic or episodic (on demand) therapy. This study revealed that infection rates were higher with higher number of blood products transfusion. Hemophiliacs who received only factor concentrates were less prone for infection with above studied viral infection as compared to the ones who received blood products. Hence, we recommend the use of factor concentrates over blood products in hemophilia patients. Also, routine screening, for transfusion related infections like HCV is advisable for better outcome.

Keywords: Hemophilia, Blood transfusion, Hepatitis, HIV, Clotting Factors.

INTRODUCTION

Hemophilia 'A' and Hemophilia 'B' are X-linked recessive disorders of coagulation occurring due to mutations in F8 gene (Hemophilia A) or F9 gene (Hemophilia B) leading to deficiency of coagulation factors VIII & factor IX respectively [1]. Combined incidence for both Hemophilia A and Hemophilia B is estimated to be approximately 1: 5,000 live male births [2].

Hemophilia disease has been classified, depending upon factor level in blood as severe <1%, moderate 1-5% and mild 6-30% disease [3, 4]. Clinical manifestations include unusual bleeding like easy bruising, spontaneous bleedings in joints, soft tissue and excessive bleeding. Clinical manifestations of hemophilia A and B are indistinguishable [5, 6].

Treatment history dates back to 1937 when Patek and Taylor, two doctors at Harward corrected the clotting factor deficiency by adding a substance from plasma in blood that was called anti hemophilic globulin [7]. In 1950's and 1960's patients with hemophilia were treated with Fresh Frozen Plasma (FFP). In 1960s Cryoprecipitate was discovered which was found to be rich in factor VIII. In 1968, factor concentrates containing factor VIII and factor IX made from plasma became available. In early 1990's genetically engineered recombinant clotting factor concentrates came into market. Now a days hemophilia patients are treated with replacement therapy consisting of recombinant factor VIII and factor IX concentrates and in some developing countries cryoprecipitate and fresh frozen plasma are also being used. Children in our setup are receiving treatment with factor concentrates and fresh frozen plasma and sometimes with cryoprecipitate [8, 9].

Replacement of haemostatic concentrations of the deficient factor is the mainstay of treatment for bleeding episodes, according to the type and severity of bleeds and until complete resolution of symptoms. Patients with haemophilia treated with multiple blood transfusions and unheated clotting factor concentrates, including factors I, VIII, and IX have a high risk of acquiring hepatitis C, hepatitis B, HIV and other viral

infections. Subsequent to widespread blood-borne virus transmission in the late 1970s and early 1980s caused by the use of pooled plasma in the manufacture of factor concentrates, the need for improved safety of treatment became crucial for the haemophilia community. As a result, viral inactivation techniques for the production of plasma-derived factor concentrates were implemented. Most important advance was recombinant gene technology and protein purification techniques that enabled the development of highly purified recombinant FVIII (rFVIII) and FIX (rFIX) products [8, 10, 11].

Patients with haemophilia face a great risk of acquiring several viral infections by virtue of their need for plasma derived blood products and multiple coagulation factor concentrate transfusions. Among these viral infections, hepatitis B and C (HBV, HCV) and human immunodeficiency virus (HIV) infections are particularly important for several reasons, including difficult management and poor outcome. As a result, transfusion-transmitted diseases, including HIV infection and hepatitis C or B, may be the major long-term cause of morbidity and mortality. Therefore, controlling these infections is one of the most important goals of haemophilia management. Therefore, the current study was undertaken with the aim to test all haemophiliacs for antibodies to HCV, HIV and HBsAg detection, and to find out the seroprevalence of these infections.

MATERIALS AND METHODS
The study was conducted in the department of Pediatric Medicine, Sir Padampat Mother and Child Health Institute, S.M.S. Medical College, Jaipur over a period of one year.

Study Design: It was a hospital based cross sectional descriptive study.

Subjects: Children affected with severe, moderate and mild hemophilia A and hemophilia B disease already diagnosed with factor assay attending the OPD, Emergency or getting admitted in wards of SPMCHI, S.M.S. Medical College and who had received replacement therapy in form of factor concentrates or blood products at least once.

Sample Size: 100 hemophilia affected children.

Laboratory Tests Done: Detection of HBsAg, anti HCV and anti HIV.

Statistical Analysis: Categorical variables were assessed using the chi- square test or Fisher exact test and continuous variables using student t-test.

Inclusion Criteria
- Children between 18 months -18 years.
- Children affected with severe, moderate and mild hemophilia A and hemophilia B disease who had received replacement therapy in the form of factor concentrates or blood products at least once.

Exclusion Criteria
- Clinically relevant coagulation disorders other than hemophilia "A" or "B".
- Those who have never received any blood products/factor concentrates.

METHODOLOGY
Hemophilia affected children were evaluated in detail with regards to the age of onset of symptoms, mode of presentation, family history of bleeding/ hemophilia, severity of bleeding episodes and their relation to trauma, number of bleeding episodes with respect to time, most common site of bleeding, most common joint involved, therapies taken for bleeding episodes including blood products and / or factor concentrates, number of times therapy being taken, response to therapy, age of using blood product / factor for the first time, prophylactic or episodic (on demand) therapy.

Records were evaluated for age at diagnosis, factor VIII or factor IX levels at the time of diagnosis, treatment received.

The patients were classified into Hemophilia A and Hemophilia B depending upon Factor VIII and Factor IX levels. Further classification into Severe, Moderate and Mild Hemophilia A and B was done on the basis of factor VIII or Factor IX levels at the time of diagnosis.

Blood samples for screening were collected randomly from April 2012 to March 2013 from hemophilia patients and sent to concerned laboratory at SMS medical college. Blood samples were collected in plain vial, labeled accurately and were transferred immediately to laboratory where samples were tested for HBsAg, anti HCV and anti HIV.

RESULTS
Out of total 100 patients screened 80(80%) patients were having hemophilia A and 20 (20%) patients were having hemophilia B.

Hemophilia patients were classified, depending upon their factor level, into having Severe, Moderate and Mild disease with factor levels <2 %, 2-5 % and 5-30% respectively. The distribution of patients is as shown in table 1.

Table 1: Distribution of patients according to severity & type of hemophilia

Severity	Type of Hemophilia		Total
	A	**B**	
Mild	12 (12.00%)	02 (02.00%)	14 (14.00%)
Moderate	25 (25.00%)	13(13.00%)	38 (38.00%)
Severe	43 (43.00%)	05 (05.00%)	71 (71.00%)
Total	80 (80.00%)	20 (20.00%)	100 (100.00%)

Mean Age of hemophilia A patients was 08.98±5.91 years. Mean Age of hemophilia B patients was 06.60±5.11 years. The Hemophilia A & B patients were almost equally distributed among different age groups.

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. Out of 100 patients studied family history of hemophilia A or B was found to positive in 56 (56%) patients.

We tried to explore the association between severity and family history. Among 80 hemophilia A patients, a total of 43 (53.75%) were found to have a positive family history. Out of these 43 patients 23 were suffering from severe hemophilia A.

Among 20 hemophilia B patients, a total of 13 (65.00%) were found to have a positive family history. Out of these 15 patients 5 patients were suffering from severe hemophilia B.

The use of factor concentrates was also taken into consideration in patients. Seventy six (76) patients out of 80 hemophilia A patients (95.00%) and 18 patients out of 20 hemophilia B patients (90.00%) had received factor concentrates previously.

The number of times factor concentrates received by the patients before undergoing serological testing was taken into consideration based on history given and records available and is shown in table 2.

Table 2: Distribution of patients according to no. of times factor infused & type of hemophilia

No of times Factor Infused	Hemophilia Type		Total
	A	**B**	
0-20	62 (62.00%)	19 (19.00%)	81 (81.00%)
>20	18 (18.00%)	1 (1.00%)	19 (19.00%)
Total	80 (80.00%)	20 (20.00%)	100 (100.00%)

The use of blood products by the patients is as follows. Fifty seven (57) patients out of 80 hemophilia A patients (71.25%) and 15 patients out of 20 hemophilia B patients (75.00%) had received blood products mainly in form of Fresh Frozen Plasma.

Distribution of patients with hemophilia and having HBsAg in their blood are as shown in table 3, anti-HCV is shown in table 4 and anti-HIV in table 5.

Table 3: Distribution of patients according to type of hemophilia and presence of HBsAg

Type of Hemophilia	HBsAg		Total
	Positive	**Negative**	
A	0 (0.00%)	80 (80.00%)	80 (80.00%)
B	1 (1.00%)	19 (19.00%)	20 (20.00%)
Total	1 (1.00%)	99 (96.00%)	100 (100.00%)

$\chi^2 = .57$ d.f.=1   p=.450   NS

Table 4: Distribution of patients according to type of hemophilia and presence of anti HCV

Type of Hemophilia	Anti HCV		Total
	Positive	**Negative**	
A	2 (2.00%)	78 (78.00%)	80 (80.00%)
B	0 (0.00%)	20 (20.00%)	20 (20.00%)
Total	2 (2.00%)	98 (98.00%)	100 (100.00%)

$\chi^2 = .03$ d.f.=1   p=.862   NS

Table 5: Distribution of patients according to type of hemophilia and presence of anti HIV

Type of Hemophilia	Anti HIV		Total
	Positive	Negative	
A	0 (0.00%)	80 (80.00%)	80 (80.00%)
B	0 (0.00%)	20 (20.00%)	20 (20.00%)
Total	0 (0.00%)	100 (100.00%)	100 (100.00%)

Association between number of blood products infused and presence of viral infection is shown in table 6.

Out of 95 patients with <10 times blood products infused 2 (2.10%) were found to be tested positive for presence of viral infection. Out of 5 patients with >10 times blood products infused 1 (20.00%) were found to be tested positive for presence of viral infection. Risk of viral infections was higher in those patients who had received more numbers of blood transfusions (20%).

Association between no. of factor concentrates infused & presence of viral infection is shown in table 7.

Out of 81 patients with <20 times factor concentrates infused 3 (3.70%) were found to be tested positive for presence of viral infection. Out of 19 patients with >20 times factor concentrates infused, none were found to be tested positive for presence of viral infection.

Table 6: Distribution of patients according to no. of blood products infused & presence of viral infection

No. of times Blood products infused	HBsAg/anti HCV		Total
	Positive	Negative	
<10	2 (2.00%)	93 (93.00%)	95 (80.00%)
>10	1 (1.00%)	4 (4.00%)	5 (5.00%)
Total	3 (3.00%)	95 (95.00%)	100 (100.00%)

$\chi^2 = .89$ d.f.=1   p=.345   NS

Table 7: Distribution of patients according to no. of factor concentrates infused & presence of viral infection

No of times Factor concentrates infused	HBsAg/anti HCV		Total
	Positive	Negative	
<20	3 (3.00%)	78 (78.00%)	81 (81.00%)
>20	0 (0.00%)	19 (19.00%)	19 (19.00%)
Total	3 (3.00%)	97 (97.00%)	100 (100.00%)

$\chi^2 = .01$ d.f.=1   p=.920   NS

DISCUSSION

The present study was a hospital based observational study conducted in SPMCHI, Jaipur over a period of one year. This study is one of the very few studies attempting to screen hemophilia affected patients for the presence of viral infections. A total of 100 hemophilia A and B patients were screened for the presence of HBsAg, anti HCV and anti HIV antibodies.

Out of 100 hemophilia patients screened 80(80.00%) were hemophilia A patients and 20 (20.00%) 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.

Mean age of hemophilia A patients was 08.98 ± 6.04 years and that of hemophilia B patients was 6.60 ± 5.11years. The Hemophilia A & B patients were almost equally distributed among all the age groups.

Among 80 Hemophilia A patients screened in our study 43 (43.00%) were having severe hemophilia A, 25 (25.00%) had moderate hemophilia A and 12(12.00%) had mild hemophilia A. Among 20 hemophilia B patients, those with severe, moderate and mild hemophilia B disease were found to be 05, 13 and 02 respectively.

The percentage of patients with severe, moderate and mild hemophilia A as well as hemophilia B varied greatly in different studies conducted previously as mentioned below.

Valizadeh N *et al*. [12] studied 35 hemophilia patients for the seroprevalence of hepatitis C, hepatitis B and HIV viruses, out of which 5 (14.7%), 3 (8.8%), 26 (76.5%) had mild, moderate and severe hemophilia A respectively. Only 1 had severe hemophilia.

Carmo RA *et al.* [13] studied 469 live hemophiliac patients, 209 (44.6%) were anti-HCV-ELISA-3.0 positive . serological, virological, clinical and epidemiological assessments were completed for 162 (77.5%). Anti-HCV-ELISA-3.0 serologic status was confirmed with the anti-HCV-RIBA-3.0 antibody in 155 (95.7%) of the 162 hemophiliacs studied.

Mansour-Ghanaei F *et al.* [14] investigated 101 patients to determine the seroprevalence of hepatitis B and C viruses out of which 37 (36.63%) were having severe hemophilia) and 27 (26.7%) had moderate with rest 37 (36.63%) had mild hemophilia.

In accordance with Haldane hypothesis which predicts that one-third of all patients with an X-linked lethal disorder should represent new mutations. Spontaneous mutations should account for about 33% of all hemophilia cases.

Among 100 patients of hemophilia included in our study, 60 were having positive family history, 40 patients showed no family. Thus 40 patients with negative family history might have been representing new spontaneous mutation cases.

Out of 80 hemophilia A patients studied, 56 (56.00%) had a positive family history. 23 (28.75%) out of 43 severe hemophilia A patients, 14 (17.50%) out of 25 moderate hemophilia A and 6 (7.50%) out of 12 mild hemophilia A were having positive family history.

Out of 20 hemophilia B patients studied 13 (65.00%) had positive family history. 5 (25.00%) out of 5 severe hemophilia B patients, 7 (35.00%) out of 13 moderate hemophilia B patients and 1(5.00%) out of 2 mild hemophilia B patients were having positive family history.

On studying association of family history with severity of disease, we found it to be statistically not significant for both hemophilia A and hemophilia B.

Infection Occurance

In our study, out of total 100 patients screened 3 (3.00%) were found to be positive for studied viral infection.

Out of total 100 patients screened, 1 patient was found to be HBsAg positive (1%) and the patient belonged to hemophilia B. Thus out of 20 hemophilia B patients screened 1 were positive (5.00%) and 19 were negative. None of the 80 hemophilia A patients studied showed the presence of HBsAg.

Out of total 100 patients screened 2 (2%) were found to be anti HCV positive. Both patients belonged to hemophilia A. Thus out of 80 hemophilia B patients screened, 2 (2.50%) were positive and 78 (97.50%)

were negative. None of the 20 hemophilia B patients studied showed the presence of anti HCV.

None of the Hemophilia patients in our study showed the presence of anti HIV.

Ghosh.K *et al.* [15] investigated 400 patients (323 severe and 77 moderate hemophilics) and found 15 out of the 400 patients were positive for HIV (3.8%), 24/400 were HBsAg positive (6%) and 45/188 (23.9%) were positive for HCV (28 for both non-structural and core antigen, 13 for core only and 4 for non-structural antigen only).

Batool Sharifi-Mood *et al.* [16] in his study found seroprevalence of HCV to be 29.6%, and HBsAg was positive in 4.9%. Four cases had HCV and HBV co-infection.

Valizadeh N *et al.* [12] study in 35 hemophilia A patients showed a 8.57% prevalence of HCV infection. Abdelwahab MS *et al.* [15] studied 100 hemophilics and showed a prevalence of 40% for HCV antibodies.

Mansour-Ghanaei F *et al.* [17] surveyed 101 hemophilia patients and found 29 patients (28.7%) with elevated alanine aminotransferase (ALT), 27 (26.7%) and 72 (71.3%) were positive for HBsAg and HCV-Ab respectively.

Barbosa AP *et al.* [18] studied to investigate the hepatitis C virus (HCV) infection prevalence in hemophilics and found it to be 63.3% (CI 95%: 53.0-72.7).

Although the sample size taken in our study was smaller than some of the above mentioned studies, we obtained a percentage of infection positive hemophilia patients lower than observed with majority of the above mentioned studies.

As compared to these, our prevalence was considerably low, which partly reflects the impact of mandatory serological testing for these viral markers in the blood banks of India.

CONCLUSION

This study revealed that infection rates were higher with higher number of blood products transfusion. Hemophiliacs who received only factor concentrates were less prone for infection with above studied viral infection as compared to the ones who received blood products. Hence, we recommend the use of factor concentrates over blood products in hemophilia patients. Also, routine screening, for transfusion related infections like HCV is advisable for better outcome.

Limitations

It is quite possible that we might have missed some infected hemophilia patients during infectious window period who are undergoing seroconversion and repeat testing was not done.

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