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Detection of Factor VIII Inhibitors in Hemophilia A Patients

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Abstract

Background: Factor VIII administration to hemophilia A patients results in an immune response (inhibitor formation) which significantly complicates the therapy. The present study was performed to determine the prevalence of inhibitor development in hemophilia A patients receiving recombinant factor VIII therapy.

Materials and Methods: This was an observational descriptive study. Clotting factor inhibitor screening was performed by activated partial thromboplastin time mixing studies using normal pool plasma collected from twenty healthy donors. Bethesda assay for quantitation of factor VIII inhibitors was performed on samples which were positive with screening tests.

Results: Out of 229 patients with hemophilia A enrolled in the hemophilia society of Pakistan, Lahore center, 50 patients were selected. The mean factor VIII level in these patients was 2.46 +3.14. Out of 50 patients, 29 (58%) had severe hemophilia A (factor VIII level <1%), 17 (34%) had moderate hemophilia A (factor VIII level 1-5%) and 4 (12%) had mild hemophilia A (factor VIII level >5-30%). In this study, 12 patients (24%) were positive for inhibitors. Most of them 9 (75%) were low responders (<5 Bethesda units) with a mean Bethesda units of 1.82+0.473, while 3 (25%) patients were high responders (>5 Bethesda units) with a mean BU of 11.33+5.85. Patients were divided into two groups on the basis of the number of factor VIII concentrate therapies of <50 (group 1) times and >50 times (group 2). Inhibitor positivity was high (34.5%) in group I, as compared to group II (9.5%). Bleeding episodes were also more common in inhibitors positive patients.

Conclusion: In this study, the inhibitor development in patients with hemophilia A receiving recombinant factor VIII concentrates therapy was 24% and the first fifty therapies were crucial for inhibitor development.

Keywords: Hemophilia A, inhibitors, Bethesda units**.**

Introduction

Hemophilia A is a sex linked hereditary disorder.[1,2] Patients present with bleeding manifestations like epistaxis, bleeding gum, hematoma, joint bleeding and hemarthrosis.[3] Bleeding episodes in these patients are difficult to control. Treatment consists of replacement of factor VIII (human source or recombinant) either to prevent or stop bleeding.[4] A major complication for the administration of deficient factor is the development of neutralizing antibodies against the factor VIII called inhibitors since factor VIII administration for an individual with normal immunocompetence results in an immune response.[5] Factor VIII inhibitors arise in up to 35% of patients with severe hemophilia A and a smaller proportion of patients with mild to moderate hemophilia [2,6]. Inhibitor formation greatly complicates therapy, because bleeding can no longer be effectively controlled or prevented by the factor VIII administration.[7,8] . Due to the higher incidence of hemophilia A in Pakistani population there is a need to evaluate these patients, and the determination of factor VIII inhibitors will provide guidance towards the proper management of our patients. The objective of the present study was to determine the prevalence of inhibitor development in hemophilia A patients receiving recombinant factor VIII therapy.

Material and Methods

This was an observational descriptive study, which was conducted in the department of Hematology, University of Health Sciences Lahore

from January 2010 to July 2010. A total of 50 patients with hemophilia A who were receiving recombinant factor VIII concentrate therapy were selected from 229 patients with hemophilia A, who were enrolled with the hemophilia society of Pakistan, Lahore center.

All the tests were performed using Sysmex CA-50 instrument. Activated partial thromboplastin time (APTT) was performed using commercially available kits (Global). The screening coagulation tests and one-stage APTT based factor assay were performed following well-established routine techniques [9]. Screening for inhibitors was done by immediate correction of prolonged APTT of patients with a 1:1 mix of patient's and control normal plasma (which was collected from 20 healthy donors), incubated for 2 hours along with simultaneous incubation of the patient's plasma (PP) and control normal plasma (CNP) separately for the same length of time at 37 °C. APTT was then performed on the mix and on the separate patient's plasma and normal control plasma (PP and CNP). Rosner's index was calculated using the following formula:

$$\text{Rosner's Index} = \frac{\text{1:1 Mix aPTT} - \text{CNP aPTT}}{\text{PPaPTT}} \times 100$$

Rosner's index value of <15 indicated the correction during the mixing study and the presence of clotting factor deficiency and Rosner's index value of >15 indicated failure to correct during the mixing study and the presence of clotting factor inhibitor.

Measurement of factor inhibitors was performed using the Bethesda assay which is based on the principle that if factor VIII is added to plasma containing an inhibitor and the mixture is incubated, factor VIII will be progressively neutralized. If the amount of factor VIII added and the duration of incubation are standardized, the strength of the inhibitor may be measured in units according to how much of the added factor VIII is neutralized. Serial dilutions of test plasma were prepared using Owren's buffer as diluent. CNP was added to all the dilutions. A dilution containing 1:1 mix Owren's buffer and CNP served as reference preparation, and was considered as 100% activity in the factor VIII assay at the end of 2 hours incubation at 37 °C. Factor assay was performed on all the incubation mixtures. Residual factor VIII activity in normal pool plasma was measured (after mixing with patient's plasma containing inhibitors). Residual factor VIII activity was calculated using the following formula:

$$\text{Residual activity} = \frac{\text{Factor VIII activity from patient sample}}{\text{PPaPTT}} \times 100$$

Calculation of Inhibitor level was done by plotting the results of residual factor VIII activity on the y-axis vs. inhibitor units on the x-axis using log/log graph paper. In the Bethesda method, the Bethesda unit is defined as the amount of inhibitor that will neutralize 50% of one unit of factor VIII in normal plasma after two hours of incubation at 37°C. Cut off for the detection of inhibitors was taken as 0.6 Bethesda units. Patients with < 5 Bethesda units were considered as low responders and patients with > 5 Bethesda units were taken as high responders [10].

Statistical Analysis

Data were analyzed using SPSS V16 (SPSS Inc, Chicago USA). Continuous variables were expressed as mean + SD, whereas categorical

Table1: Demographic and laboratory data of hemophilia A patients.

Parameter	Mean+ SD	Range
Age of Patients(years)	16.62 +11.15	2-54
Factor VIII Level (%)	1.93 + 2.80	0.4-16
Rosner Index	14.70 + 9.50	4.8-63
Bethesda Units (BU/ml)	4.20 + 4.99	1.25-18

variables were expressed in the form of proportion and percentages. Fisher's exact test was used to test the difference between proportions. P values < 0.05 were considered as statistically significant.

Results

Out of fifty patients, 29 patients (58%) had severe hemophilia A (factor VIII level <1%), 17 patients (34%) had moderate hemophilia A (factor VIII level 1-5%), and 4 patients (8%) had mild hemophilia A (factor VIII level >5-30%). The mean factor VIII level in this study was 1.93 + 2.80 (Table-1).

In this study, 12 patients (24%) were positive for inhibitors. Out of 29 severe hemophilia A patients, 9 patients (31%) were positive for inhibitors and out of 17 moderate hemophilia A patients 3 patients (18%) were positive for inhibitors. No patient with mild hemophilia A was positive for inhibitors. Data related to inhibitor positive patients are given in table 2.

Nine out of 12 positive patients were low responders (<5 Bethesda units), with mean Bethesda units and Rosner's index of 1.82+0.47 and 16.94+1.73 respectively, and only 3 patients (25%) were high responders (>5 Bethesda units), with mean Bethesda units and Rosner's index of 11.33+5.85 and 48.20+12.98 respectively.

All the patients included in this study were on recombinant factor VIII concentrate therapy. Number of therapies ranged from one to 260 (Figure 1). Patients were divided into two groups on the basis of the number of factor VIII concentrate therapies of <50 times and >50 times. Twenty nine patients received <50 times therapies and 10 patients (34.5%) out of them were positive for inhibitors, whereas out of 21 patients who received > 50 therapies, only 2 patients (9.5%) were positive for inhibitors. The prevalence of inhibitors was high in group who received <50 therapies with the difference being statistically significant (P value = 0.041).

Bleeding episodes (per month) were high among inhibitor positive patients 2.50+1.16 and low in inhibitor negative patients 1.58+0.91, and the difference was statistically significant (p value =0.007). Complications of hemophilia like arthropathy, epistaxis, easy bruising and CNS complications were present in 46 patients (92%) out of 50 patients and arthropathy was present in 45 patients (90%). The most affected joints were the knee and elbow with 14 patients (28%) and 8 patients (16%) respectively. While both the knee and elbow joints were simultaneously affected in 5 patients (10%). CNS complications developed in 2 patients (4%), one of them was found to be positive for inhibitors. Epistaxis was present in 3

Table2: Demographic and laboratory data of hemophilia A inhibitor positive patients.

No	Age (Years)	Inhibitor status	Rosner Index	BU*	F-VIII Level (%)	Joints Involved
1	16	Positive	15.5	2	0.8	Elbow
2	4	Positive	16	1.8	0.8	Elbow
3	2	Positive	16.8	1.25	1.7	Nil
4	10	Positive	16.2	1.5	0.6	Knee
5	15	Positive	19.07	1.6	0.8	Knee
6	20	Positive	16.4	2.5	0.9	Elbow
7	21	Positive	16.3	1.25	0.4	Toe
8	21	Positive	15.6	2	1.8	Knee, Elbow
9	50	Positive	20.6	2.5	1.2	Hip
10	7	Positive	38.7	9	0.7	Knee, Elbow
11	18	Positive	63	18	0.9	Knee
12	5	Positive	42.9	7	0.7	Nil

* Bethesda Units/ milliliter

patients (6%) and one was positive for inhibitors and a History of easy bruisability was present in 12 patients (24%).

Discussion

Development of antibodies against factor VIII protein is one of the most serious adverse effects that 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 [11].

In this study the overall prevalence of factor VIII inhibitors was 24%, which was similar to the findings of Wang et al. (32%) [12] and Sharifian et al. (22.2%)[13]. Lusher et al. reported that the overall prevalence of factor VIII inhibitors was 24.8%, and high titer inhibitors were found in 18% of patients[14]. Similar percentages have been reported in other international studies like 28% [15, 16] and 19% [17]. Bray et al. reported that the prevalence of inhibitors was 38.4% and the high titer inhibitors were present in 11.3% [18]. Patients receiving recombinant factor VIII concentrates have might be at a greater risk for inhibitor development as compared to patients receiving plasma-derived products. All the patients included in our study were receiving recombinant factor VIII therapy. However, studies conducted on previously untreated patients (PUP) had not shown any significant difference between the recombinant and the plasma-derived products [8].

In the present study all of the high responders (>5Bethesda units/ml) belonged to the group of severe hemophilia A patients (factor VIII activity <1%). The prevalence of the high responders (25%) was low in this study as compared to the prevalence reported by Knobe et al. (58%) [19]. The low frequency of high responders among our patients may be due to fairly infrequent factor VIII concentrate support since they were not on prophylactic therapy. They only visited hemophilia care center in case of occurrence of a bleeding episode. It is interesting to find that lower titer (<2 Bethesda units/ml) was found more frequently (58%) in the present study compared to other studies. This should be considered in the follow-ups of hemophiliacs to verify the natural history of low titer inhibitors and studies should be performed to rule out the presence of transient inhibitors.

It is reported in studies done by Hay et al. and Bray et al. that if the inhibitor does not develop before 50 therapy exposure days, then the probability of development is very low [2, 18]. In our

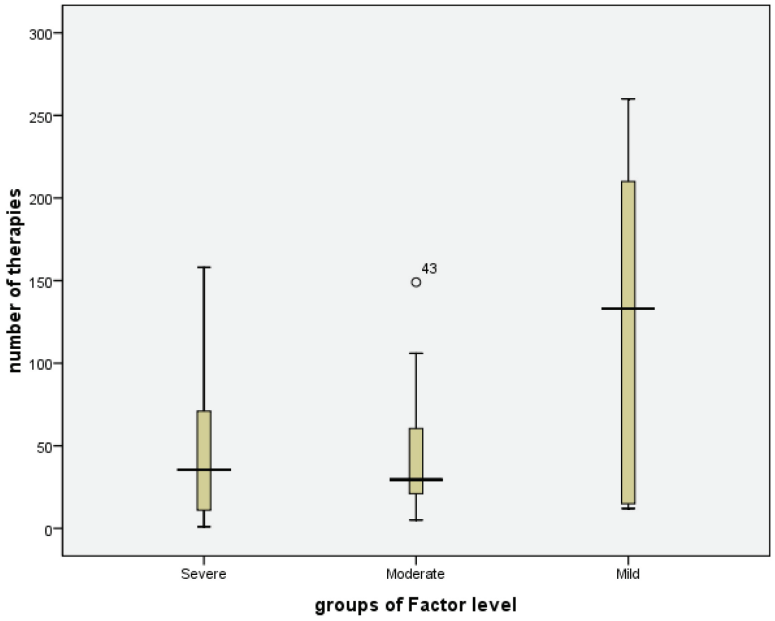

Figure1: The relation between the factor level and the number of recombinant factor VIII therapies in hemophilia A patients.

study the positivity of inhibitors was significantly high in <50 therapy group. This data suggests that the first 50 therapies are high risk therapies for inhibitor development. Production of antibodies occurs due to lack of factor VIII in these patients as recombinant factor VIII is considered as foreign by their immune system [20]. The risk of inhibitor formation also increases significantly in patients with a positive family history of inhibitors.

In this study bleeding episodes in inhibitor positive patients were significantly higher (2.50+1.17) as compared to inhibitor negative patients (1.58+0.91). This implies that inhibitor positive patients cannot benefit fully by substitution therapy. Inhibitor testing was rarely done previously. Many hemophiliacs experience very serious bleeding episodes in procedures such as circumcisions, dental extractions and other surgical interventions. Incorrect therapies causes unnecessary financial costs due to prescription of mega doses of factor concentrates over long periods which often results in no response in patients with inhibitors.

Conclusion

The results of our study show that inhibitors develop frequently in patients with hemophilia A receiving recombinant factor VIII concentrates therapy. Our results also indicate that the first fifty therapies are crucial for inhibitor development.

It may be recommended that hemophilia A patients should always be screened for factor VIII inhibitors on regular intervals. Once inhibitors development occurs it is advisable that these patients should be treated with specific products such as recombinant factor VIIa or activated prothrombin complex concentrates (APCCs) for bleeding episodes or during their surgical procedures.

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