INTRODUCTION

Haemophilia A (HA), inherited as an X-linked recessive trait, is the most common bleeding disorder caused by a deficiency or dysfunctional coagulation factor VIII (FVIII). Based upon the functional assay of factor VIII activity in the patient's plasma, haemophilia is classified as mild with > 5% activity, moderate 1-5% or severe <1% activity respectively. Individuals with haemophilia A (HA) are unable to produce FVIII due to various mutations at the FVIII gene. The most common genetic defect responsible for severe haemophilia A (45% of cases) is caused by inversion and translocation of intron 22. Others include missense mutations, nonsense, frame-shift splicing and insertional mutations.

Inhibitors are antibodies that neutralize the activity of a clotting factor. Inhibitor to factor VIII may arise as allo-antibodies in patients with HA who have been transfused with exogenous factor VIII. The formation of antibodies is a multi-factorial complex phenomenon involving both endogenous genetic as well as non-genetic risk factors. The most extensively studied genetic factor is the FVIII mutation, but there has been rising interest on the impact of polymorphisms in immune-regulatory genes. About 20 - 30% of severe HA patients and 5 - 15% of mild to moderate HA develop inhibitors whereas in HB inhibitor development is relatively uncommon occurring in 2 - 3%. It has been shown in studies that large deletions, stop codon mutations and inversions 22 were associated with greater chances to produce inhibitor as compared to small deletions and missense mutations (35% vs. 5%). Exposure to high purity products early in life and family history of inhibitors increase the risk of its development. In the present era development of virally attenuated, plasma derived coagulation factor products and recombinant FVIII and FIX concentrates, the complications from severe bleeding such as haemophilic arthropathy and danger of transmission of infections have almost been eliminated mainly in developed part of the world, leaving the development of inhibitory antibodies as the most serious and challenging complication of haemophilia therapy. The consequence of development of inhibitors in haemophilics is difficult-to-control haemostasis especially during acute bleeding episodes, and elective surgery thus increasing morbidity and mortality. Factor IX inhibitors pose specific health problems e.g. anaphylactoid reactions to FIX concentrates.

ORIGINAL ARTICLE

Frequency of Factor VIII (FVIII) Inhibitor in Haemophilia A

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ABSTRACT

Objective: To determine the frequency of factor VIII specific inhibitors in haemophilia A.
Study Design: Cross-sectional study.
Place and Duration of Study: National Institute of Blood Disease and Bone Marrow Transplantation, Karachi, from August 2007 to March 2009.
Methodology: Venous blood samples of diagnosed haemophilia A patients were collected in tubes containing 0.109 M (3.2%) trisodium citrate, centrifuged without delay at 1200 G for 15 minutes. Factor VIII inhibitors were screened by APTT based method using 50:50 patients' plasma mixed with normal plasma incubated together for 2 hours at 37°C. Quantitative assay was carried out to measure Bethesda units (BU). Samples were labelled as low titre inhibitor when less than 5 BU detected, while high titre inhibitor when more than 5 BU were detected.

Results: A total of 140 Haemophilia A patients were evaluated for allo-antibodies who received treatment with FVIII concentrates / FFP/ cryoprecipitate. Among them 21 patients (15%) were found to have positive screening test results for inhibitors. The mean age of patients with inhibitors was 11.9 ± 8.81 years. Thirteen were high responders (62%) while 8 were low responders (38%). The mean inhibitor level in low (titre) responders was 2.46 ± 1.31 BU while in high (titre) responders it was 29.15 ± 12.81 BU. According to severity of the disease 12/21 with severe haemophilia A (57.2%) developed inhibitors, whereas 8/21 with moderate (38%) and 1/21 with mild haemophilia A (4.7%) showed positive results for inhibitors.

Conclusion: Fifteen percent haemophilia A patients developed inhibitors in this cohort, majority with severe and moderate haemophilia A. Age and severity of disease were found to be main contributing factors in patients who developed inhibitors.

Key words: Inhibitors. Haemophilia A. Fresh frozen plasma. Cryoprecipitate.

INTRODUCTION

Haemophilia A (HA), inherited as an X-linked recessive trait, is the most common bleeding disorder caused by a deficiency or dysfunctional coagulation factor VIII (FVIII). Based upon the functional assay of factor VIII activity in the patient's plasma, haemophilia is classified as mild with > 5% activity, moderate 1-5% or severe < 1% activity respectively. Individuals with haemophilia A (HA) are unable to produce FVIII due to various mutations at the FVIII gene. The most common genetic defect responsible for severe haemophilia A (45% of cases) is caused by inversion and translocation of intron 22. Others include missense mutations, nonsense, frame-shift splicing and insertional mutations.

Inhibitors are antibodies that neutralize the activity of a clotting factor. Inhibitor to factor VIII may arise as allo-antibodies in patients with HA who have been transfused with exogenous factor VIII. The formation of antibodies is a multi-factorial complex phenomenon involving both endogenous genetic as well as non-genetic risk factors. The most extensively studied genetic factor is the FVIII mutation, but there has been rising interest on the impact of polymorphisms in immune-regulatory genes. About 20 - 30% of severe HA patients and 5 - 15% of mild to moderate HA develop inhibitors whereas in HB inhibitor development is relatively uncommon occurring in 2 - 3%. It has been shown in studies that large deletions, stop codon mutations and inversions 22 were associated with greater chances to produce inhibitor as compared to small deletions and missense mutations (35% vs. 5%). Exposure to high purity products early in life and family history of inhibitors increase the risk of its development.

In the present era development of virally attenuated, plasma derived coagulation factor products and recombinant FVIII and FIX concentrates, the complications from severe bleeding such as haemophilic arthropathy and danger of transmission of infections have almost been eliminated mainly in developed part of the world, leaving the development of inhibitory antibodies as the most serious and challenging complication of haemophilia therapy. The consequence of development of inhibitors in haemophilics is difficult-to-control haemostasis especially during acute bleeding episodes, and elective surgery thus increasing morbidity and mortality. Factor IX inhibitors pose specific health problems e.g. anaphylactoid reactions to FIX concentrates.
Eighty percent of the haemophiliacs live in developing countries where only few people with haemophilia receive adequate care. In Pakistan, haemophilia is mostly a reflection of limited resources and lack of support to this disease requiring specialized care. Patient monitoring and management are best described as limited, insufficient and inadequate. Fresh frozen plasma (FFP) remains the most commonly used therapeutic modality in our patients due to non-availability of factor concentrates and high cost. With this background the study was designed to determine the frequency of factor VIII specific inhibitors and risk factors associated with inhibitor formation in haemophilia A patients treated with plasma derived products and factor concentrates.

METHODOLOGY

From August 2007 to March 2009, diagnosed haemophilia A patients from 3 main haemophilia centres of Karachi, were screened for inhibitors. Patients with other bleeding disorders were excluded. The study was approved by institutional ethics committee and was done in accordance with the declaration of Helsinki. Informed consent was obtained from all adult subjects, parents or legal guardians. This was a cross-sectional, multi-centre epidemiological study. A detailed history including onset of symptoms and signs, bleeding episodes, treatment details, type of factor concentrates, and duration was recorded on the study proforma. Venous blood samples were collected in tubes containing 0.109 M (3.2%) trisodium citrate in a ratio of 9 parts blood to 1 part anticoagulant, centrifuged without delay at 1200 G for 15 minutes. Factor VIII inhibitor was screened using APTT based method, 50:50 patient’s plasma mixed with normal pooled plasma incubated together for 2 hours at 37°C. Quantitative assay was carried out to measure Bethesda units (BU). Each Bethesda unit is defined as the amount of inhibitor which will neutralize 50% of one unit of added factor VIII in 02 hours at 37°C. FVIII inhibitors are time dependent and called low titre when less than 5 BU detected while high titre means more than 5 BU. An inhibitor activity graph was prepared. If a test sample has no inhibitor, the factor VIII activity in the test sample mixture should equal to the control, and the residual factor VIII activity should be 100%. If the residual FVIII: C activity in a sample was between 80% and 100%, it was considered that sample had no inhibitor. Statistical Package for Social Sciences (SPSS-13) was used to analyze data. Frequency and percentage were computed for categorical variables and mean and standard deviation were estimated for quantitative variables.

RESULTS

A total of 140 haemophilia A male patients were evaluated for allo-antibodies who received treatment with FVIII concentrates / recombinant / virally inactivated products / FFP / cryoprecipitate. Of these, a total of 21 patients (15.0%) were found to have positive inhibitors. The mean factor VIII level was 1.2 ± 0.54% in the inhibitor group while in the non-inhibitor group 6.7 ± 3.25%. Thirty patients (62%) were high responders while 8 were low responders (38%). The mean inhibitor level in low responders was 2.46 ± 1.31 BU while in high responders it was 29.15 ± 22.81 BU. Out of 21 haemophilia A patients who developed inhibitors majority were of severe type (57.2%), and moderate type (38%). The age of patients with inhibitors ranged from 6 months to 28 years with mean of 11.9 ± 8.81 years. Those without inhibitors were from 1 to 58 years with mean 17.8 ± 13.61 years. No positive family history was observed in any patient with inhibitors. Treatment details showed that (n=126, 90%) patients received treatment with FFP and its products, plasma derived factor concentrates were used by (n=49, 35%) and recombinant factor concentrates by only (n=14, 10%) patients. Patients switched from one source of FVIII product to the other depended on their availability. Similarly, sub-optimal treatment was also a problem contributing to physical disability and crippling arthropathy in these patients. Bleeding episodes per month was 3 - 24 in the inhibitor group as compared to 1 - 10 per month in patients without inhibitors. Patients' characteristics are given in the Table I.

Table I: Patients characteristics [n = 140].

<table>
<thead>
<tr>
<th>Variables</th>
<th>Inhibitor no = 21/140</th>
<th>No inhibitor no = 119/140</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>6 months - 28 years</td>
<td>1 month - 58 years</td>
</tr>
<tr>
<td>Mean</td>
<td>11.9 years ± 8.81 SD</td>
<td>17.8 years ± 13.61 SD</td>
</tr>
<tr>
<td>Age of 1st exposure (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>1 month - 22 years</td>
<td>2 months - 25 years</td>
</tr>
<tr>
<td>Mean</td>
<td>2.5 years ± 1.1 SD</td>
<td>3.97 years ± 2.82 SD</td>
</tr>
<tr>
<td>Severity of haemophilia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe (&lt; 1%)</td>
<td>12 (57.2%)</td>
<td>60 (50.4%)</td>
</tr>
<tr>
<td>Moderate (1-5.0%)</td>
<td>08 (38%)</td>
<td>54 (45.3%)</td>
</tr>
<tr>
<td>Mild (&gt; 5.0%)</td>
<td>01 (4.7%)</td>
<td>05 (4.2%)</td>
</tr>
<tr>
<td>Inhibitor level (BU).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>1.1 - 192 BU</td>
<td>–</td>
</tr>
<tr>
<td>Low responder n=8</td>
<td>2.4 BU ± 1.31 SD</td>
<td>–</td>
</tr>
<tr>
<td>High responder n=13</td>
<td>29.15 BU ± 22.81</td>
<td>–</td>
</tr>
</tbody>
</table>

DISCUSSION

Haemophilia is an X-linked inherited bleeding disorder with high-cost treatment, and technology-intensive therapy is not a high priority for the governments of developing countries. A previous study on the spectrum of congenital bleeding disorders and other local studies have shown that HA is the most common bleeding disorder in Pakistani population. Patients with haemophilia and other bleeding disorders are treated with factor replacement therapy based mainly on fresh frozen plasma and its components. At best, most
Inhibitors (allo-antibodies) can be found when a person with haemophilia has an immune response to the clotting factor concentrates. Allo-antibodies development is 20 - 30% in haemophilia A patients, more common in severe type as compared to mild to moderate types. This study found inhibitor development mostly in severe type as compared to moderate and mild types. Several factors are known to influence the risk of inhibitor development like type and severity of HA, nature of gene defect, ethnicity, intensive factor exposure at the time of surgery, and prophylactic or on-demand treatment regimen. The source of FVIII employed for replacement therapy may also have an effect on inhibitor development. The influence of FVIII source on the risk of inhibitor development originated from two ground-breaking studies and a systematic review by Wight and Paisley, highlighted that in HA patients treated exclusively with recombinant FVIII (rFVIII) the cumulative incidence of inhibitors was more than 2-fold higher than in those treated exclusively with plasma-derived FVIII (pdFVIII). Retrospective cohort studies by Gouw et al. and Chalmers et al. has also shown similar findings. A cohort study by Gouw et al. found no significant difference in the risk of developing inhibitors between patients receiving FVIII from these two different sources. In this study, patients were mainly treated with plasma and its products; plasma-derived factor concentrates was used by 35% and recombinant type by only 10%. Patients switched from one source of FVIII product to the other depended on their availability. A recent systematic review by Iorio et al. highlight that, currently, it was not possible to either prove or disprove the hypothesis that there is a higher risk of inhibitor development associated with the use of rFVII product when compared with pdFVIII concentrates. Thus necessitat evalution of this issue in a randomized controlled clinical trial before adjusting clinical practices.

Studies have shown that large deletions, stop codon mutations and inversion 22 are associated with greater chances to produce inhibitor as compared to small deletions and missense mutations (35% vs. 5%) and a positive family history of inhibitor formation. FVIII and FIX mutation analysis should be done in all patients with haemophilia A and B, especially in newly diagnosed patients. In Pakistan, molecular diagnostics of haemophilia has been started and need studies to explore the relationship between the generation of inhibitors and particular FVIII deficiency genotypes in the local population. These antibodies are exclusively IgG isotype. These inhibitors neutralize the procoagulant activity of factor by blocking functional epitopes as well as they hydrolyse the antigen for which they are specific.

The presence of inhibitors has significant clinical implications as the response to treatment becomes uncertain, morbidity is increased and life expectancy reduced. The development of neutralizing allo-antibodies ('inhibitors') while receiving the deficient clotting factor is relatively common during the patient's initial treatment. Among treated patients with haemophilia who do not develop inhibitors early on, the later incidence is considerably lower. Inhibitors develop in patients with severe haemophilia A after a median of 9-12 treatment days but may arise at any time in the patient's life. Inhibitor development is less common in patients who have received more than 150 exposure days of factor concentrate replacement. In this study haemophilia A patients with inhibitor age at first exposure ranged from 1 month - 22 years and the mean was 2.5 years showing inhibitor occurrence earlier in their life due to exposure to FVIII products mostly during the first 20 days of exposure. Literature review shows a higher incidence of inhibitors in patients starting replacement therapy before the age of 6 months. In a Spanish study, the cumulative incidence of inhibitors at 3 years of age in patients with haemophilia A treated with factor concentrates prior to the age of 6 months, between 6 and 12 months of age or after 1 year of age was 41%, 29% and 12%, respectively. A similar trend was observed in The Netherlands study as well. But in another case-control study, no association was seen between inhibitors and treatment initiated before 11 months of age after adjusting for genetic factors.

Clinically significant FVIII inhibitors usually present as a lack of response to replacement therapy. In the study, a total 21 haemophilia A patients (15%) were identified with inhibitors among them while 8 patients had low inhibitor titre BU and 13 patients had high BU. No positive family history was observed in any patient with inhibitors. Among the low titre group only one patient received treatment because he had acute haemarthrosis not responding to usual treatment with plasma and plasma derived FVIII concentrates. He was then treated with high dose plasma derived FVIII 50 IU per kg per day. He responded to treatment and his inhibitor disappeared after 3 months. Similarly, among the high titre group only 3 patients were treated through immune tolerance induction (ITI). Other patients did not have life threatening bleeding and, therefore, were followed-up in the clinic. To justify ITI strategies in adults, careful cost-utility considerations are required. However, ITI may represent a suitable approach in patients with frequent bleeding, which is not satisfactorily controlled by bypassing treatment and/or when orthopaedic surgery is
CONCLUSION

Though development of inhibitors has not yet become major issue in our population and only 15% haemophilia A patients developed inhibitors in this cohort. Mainstay of their treatment was plasma and its products. Age and severity of the disease were found to be a frequent factors in patients who developed inhibitors.

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REFERENCES


