INTRODUCTION

Deficiency of clotting protein called factor VIII (FVIII) results in one of most common congenital bleeding disorders termed as Haemophilia A. On the basis of FVIII activity Haemophilia A may be severe (FVIII activity <01%), moderate (FVIII activity between 1-5%) or mild (factor VIII activity between 5-45%) respectively. Replacement therapy is very effective and forms the cornerstone in the treatment of haemophilia A unless patient develops an alloantibody (inhibitor) against exogenous FVIII. Although effective treatment has only become available in the recent decades, haemophilia was known to the ancient world.

The earliest written references to what appears to be haemophilia are encountered in Jewish texts of the second century AD. Rabbinical rulings exempted male boys from circumcision if two previous brothers had died of bleeding after the procedure. The first modern description of haemophilia is attributed to Dr John Conrad, who clearly appreciated the three cardinal features of haemophilia: an inherited tendency of males to bleed. However, the first use of the word haemophilia "appears in an account of the condition written in 1828 by Hopff (Über die haemophilieoder die erbliche Anlage zutödliche blutungen). Haemophilia is sometimes referred to as "the royal disease" because several members of royal families in Europe were affected by it. Queen Victoria had no ancestors with the condition but soon after the birth of her eighth child, Leopold in 1853 it become evident that he had haemophilia. Two of Queen Victoria's...
daughters were also carriers of haemophilia. The condition was transmitted through them to several Royal families. Perhaps the most famous affected individual was the son of Tsar Nicolas II of Russia, Tsarevich Alexis, who was born in 1904.

The rationale of the study was to find out the load of factor VIII inhibitors in factor VIII deficient patients so as to formulate a comprehensive and elaborative strategy for further investigations and treatment and will be implemented in all military health care setups.

PATIENTS AND METHODS

This cross-sectional study was conducted in the department of haematology, Armed Forces Institute of Pathology Rawalpindi and Combined Military Hospital Peshawar, over a period of six months, from 1st March 2016 to 31st August 2016. A total of 88 patients with haemophilia on replacement therapy were included in this study using non probability consecutive sampling. Sample size was calculated using WHO calculator taking 15% as the least proportion outcome variable, 5% margin of error and 95% confidence interval. Male patients between 2-70 years of age with known Haemophilia A on treatment for last 1 year with a factor VIII level activity between 0-45 units/dl were included in the study. Patients with known bleeding disorders other than Hemophilia A, liver disease, co morbid and using aspiring were excluded from the study. Patients with vitamin K deficiency were also excluded from the study. Informed consent was taken and purpose was explained to participants. aPTT based screening for immediate and delayed inhibitors was done by immediately and two hours after mixing the patients plasma with the normal plasma. Results were noted in a specially designed proforma by the researcher. Both host related and treatment related risk factors for inhibitor formation were checked. They were then followed during the treatment. Inhibitor presence and Strength of inhibitor were noted. Confounding variables were removed by strictly following the exclusion criteria. Care givers bias was removed by blinding the data. However, patients showing results (diagnostic of inhibitor) were communicated their results due to ethical reasons and included in study. Of 5 ml venous blood sample was collected in trisodium citrate bottle (9:1). The blood samples were centrifuged at 3500 rpm for 05 minutes (hard spin) to have platelet poor plasma. aPTT was performed on platelet poor plasma of test sample and readings of aPTT both in test and control plasma were taken. Mean of aPTT (test plasma and control plasma aPTT) was taken.

Test plasma aPTT + Control plasma aPTT

- 50µl of patient plasma and 50ul of control plasma was taken and aPTT was performed immediately. Inadequate correction of aPTT as compared to the mean value shows inhibitors. If there was now prolongation of aPTT on immediate test, three test tubes were incubated at 37°C for two hours, containing:
  - Test tube 1 100µl control plasma
  - Test tube 2 100µl patient plasma
  - Test tube 3 50µl control plasma + 50µl patient plasma

aPTT was performed after two hours on all the three test tube samples and a mean of test tube 1 and 2 reading was taken. Presence of inhibitor is confirmed if aPTT value is greater than mean value in test tube three. Factor assay was then performed to measure the Inhibitor strength. Data was stored in SPSS 16. Quantitative variables like age, was presented/measured as mean and standard deviation. Qualitative variables like gender, factor VIII inhibitor level was reported as frequency and percentages. Effect modifiers like age, gender, severity of haemophilia, any treatment and level of inhibitor was stratified and post stratification Chi square was applied. A p-value of <0.05 was considered significant.
RESULTS

A total of 88 known patients of haemophilia A, on treatment for the last one year were included in this study during the study period of six months. Patients’ ages ranged between 2-30 years with a mean age of 6.68 ± 4.90 years with 83 (94.3%) patients under the age of 15 years (table-I). All patients were male. Eighteen (20.4%) patients had mild, 43 (48.9%) had moderate and 27 (30.7%) had severe haemophilia (fig-1). Out of 88 patients, 81 (92%) had been under treatment with replacement therapy for <10 years and 7 patients (8%) had been under treatment for >10 years with a mean treatment period of 4.69 ± 3.72 years. FVIII inhibitors were found in only 4 (4.5%) patients. The mean FVIII inhibitor level was 3.47 ± 1.2 BU. The patients were stratified according to age (table-II), severity of haemophilia (fig-2) and duration of treatment (fig-3).

DISCUSSION

The studies have been limited by the enrollment of small, heterogeneous study populations and the use of several factor VIII products, and comparisons among studies have been difficult because of different study designs. 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. The influence of FVIII source on the risk of inhibitor development originated from two groundbreaking studies and a systematic review by Wight and Paisley, high-lighted that in HA patients treated exclusively with recombinant FVIII (rFVIII) the cumulative incidence of inhibitors was more than 2-fold higher than in those patients treated exclusively with plasma-derived FVIII (pdFVIII). Retrospective cohort studies by Goudemand et al11 and Chalmers et al has also shown similar findings12. A cohort study by Gouw et al13 found no significant difference in the risk of developing inhibitors between patients receiving FVIII from these two different sources13. Studies have shown that large deletions, stop codon mutations and inversion patients had mild, 43 (48.9%) had moderate and 27 (30.7%) had severe haemophilia (fig-1). Out of 88 patients, 81 (92%) had been under treatment with replacement therapy for <10 years and 7 patients (8%) had been under treatment for >10 years with a mean treatment period of 4.69 ± 3.72 years. FVIII inhibitors were found in only 4 (4.5%) patients. The mean FVIII inhibitor level was 3.47 ± 1.2 BU. The patients were stratified according to age (table-II), severity of haemophilia (fig-2) and duration of treatment (fig-3).

Table-I: Distribution of haemophiliac patients on replacement therapy by age (n=88).

<table>
<thead>
<tr>
<th>Age (Year)</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-15</td>
<td>83 (94.3)</td>
</tr>
<tr>
<td>16-30</td>
<td>05 (05.7)</td>
</tr>
<tr>
<td>Total</td>
<td>88 (100)</td>
</tr>
</tbody>
</table>

Table-II: Stratification of haemophiliac patients on replacement therapy with inhibitors according to age (n=88).

<table>
<thead>
<tr>
<th>Age (Year)</th>
<th>Factor VIII inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present</td>
</tr>
<tr>
<td>2-15 (n=83)</td>
<td>4 (4.8%)</td>
</tr>
<tr>
<td>16-30 (n=5)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Total (n=88)</td>
<td>4 (4.5%)</td>
</tr>
</tbody>
</table>

A p-value=0.615
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. 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. Twenty Two
cidence 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

![Figure-2](image1.png)
A p-value = 0.001

**Figure-2: Stratification of haemophiliac patients on replacement therapy according to severity of haemophilia and inhibitor.**

![Figure-3](image2.png)
A p-value=0.547.

**Figure-3: Stratification of haemophiliac on replacement therapy according to duration of treatment.**

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 after adjusting for genetic factors. Clinically significant FVIII inhibitors usually present as a lack of response to replacement therapy. In the current study, 4.5% factor VIII inhibitors found in known haemophils. These results are comparable with the study carried out by Borhany et al.
CONCLUSION

Development of FVIII inhibitors was not found to be a major problem in our haemophilia A patients and only 4.5% haemophilia A patients developed inhibitors, while on replacement therapy. Inhibitors were more frequently seen in patients with severe haemophilia. Inhibitors were not encountered in patients on FVIII replacement therapy for more than 10 years duration.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

REFERENCES