Prevalence of factor VIII inhibitors among Afghan patients with hemophilia A: a first report

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Prevalence of inhibitors in Afghan hemophilia patients has not been reported previously. Our aim was to determine the prevalence of factor VIII inhibitors among hemophilia A patients from the Kabul province of Afghanistan to identify and characterize the pattern of inhibitor formation. Clinical information and blood samples were collected from three hemophilia centers in Kabul, Afghanistan. Plasma samples were obtained from 62 patients with severe (80.5%) and 15 patients with moderate hemophilia A (19.5%) in this cross-sectional study design. All the patients were receiving on-demand treatment. The Nijmegen modification of the Bethesda assay was used to detect inhibitors. Multiplex PCR, inverse-PCR, Multiplex ligation-dependent probe amplification and direct sequencing were performed for genotyping. Inhibitor activity was detected in one out of 15 (6.7%) patients with moderate hemophilia and in six out of 62 (9.7%) with severe disease. Apart from the intron 22 inversion, five different mutations including one missense, two large and two small deletions were detected. This is the first report showing that the prevalence of inhibitors in Afghan hemophilia A patients is much lower than in other populations. ***Blood Coagul Fibrinolysis*** **29:697–700**

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Introduction

Hemophilia A is the most common severe lifelong bleeding disorder, affecting approximately one in 5000 men, caused by quantitative or qualitative deficiency of factor VIII (FVIII). The severity of the disorder is inversely correlated with the residual plasma FVIII activity (FVIII:C). FVIII:C below 1% being categorized as severe, 1–5% as moderate and 5–40% as mild [1]. To date, the main effective therapy for hemophilia A is replacement treatment with plasma-derived FVIII (pdFVIII) or recombinant FVIII, which are associated with varied rates of inhibitor formation. The development of inhibitor against infused FVIII in patients with hemophilia A remains the most significant complication of this disorder [1]. Around one-third of previously untreated patients (PUPs) with severe hemophilia A develop inhibitors, usually within the first 10–15 days of treatment with factor concentrates [2,3]. The risk of inhibitor development in PUPs, who have been exposed to FVIII concentrates for more than 50–150 days declines, becoming almost negligible. However, the risk never disappears, persisting throughout life, and shows a slight increase in the elderly [4]. There are several different risk factors attributed to inhibitor development. They can be patient-related (e.g., genetic, ethnic, or immunological factors) or treatment-related factors (e.g., type of product used, age at the first treatment/exposure, and treatment duration and intensity) [5–7]. It has been estimated that the overall prevalence of inhibitors in unselected hemophiliacs is 5–7%, while the incidence of inhibitor development is about 25–35% in severe hemophilacs and 3–13% in mild/moderate ones [8] Various types of mutations in the *F8* gene are responsible for the bleeding. The most common mutation is the intron 22 inversion, accounting for 45–50% of the severe cases [9], which was about 47% in our previous study [10]. Molecular studies on patients with inhibitors show that mutations which interfere with FVIII transcription and biosynthesis (large deletions, nonsense mutations, intron 1 and 22 inversion mutations) have a significantly higher risk of inhibitor development than missense mutations or small deletions [11]. Afghanistan is a country in the Central Asia with nearly 32 million inhabitants. External invasions and internal ongoing wars have crippled the country in the past 30 years. Although there are very limited facilities for the diagnosis and treatment of patients with inherited bleeding disorders, in 2011, the hemophilia laboratory was inaugurated in Esteqlal Hospital in Kabul, the capital city, and in July 2012 the Afghanistan Hemophilia Patient Association (AHPA) was established and became a member of the World Federation of Hemophilia (WFH) [12,13]. Based on a report by the 2014 Annual Global Survey published by the WFH, only 273 patients with hemophilia A have been identified so far, which accounts for nearly 4% of the total expected prevalence for Afghan population. Most

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patients have severe hemophilia and their main problem is lack of proper access to FVIII concentrates. Recently the WFH and some other charity organizations provided them with some FVIII concentrates [14], so at the present pdFVIII (Kedrion Biopharma, Castelvecchio Pascoli, Italy) and ALPHANATE [antihemophilic factor/von Willebrand factor complex (human)], cryoprecipitate or fresh frozen plasma (FFP) are available for on-demand replacement therapy. The aim of this study was to assess the prevalence of inhibitors in Afghan patients with hemophilia A and to identify the relationship between the *F8* genotype and inhibitor development.

Materials and methods

Patients

From 29 January 2017 to 30 January 2018, 77 patients with hemophilia A (between 1 and 35 years, with an average age of 13) from 61 unrelated families were invited to participate in this study, after obtaining informed consent. They included 62 patients (80.5%) with severe and 15 (19.5%) with moderate hemophilia A. Citrated blood samples were collected at three centers, the Esteqlal and Indira Gandhi Hospitals and the center run by the AHPA. The plasma samples were sent to the reference laboratory of the Iranian Comprehensive Hemophilia Care Center (ICHCC), in Tehran, Iran, for inhibitor screening and the whole blood samples for genetic analysis to Dr Zeinali's Medical Genetics Lab., Kawsar Human Genetics Research Center in Tehran. All patients were investigated to determine their age at diagnosis, type of factor replacement, bleeding history and joint or organ with recurrent bleeding. The majority of them have had many spontaneous hemorrhages in joints and muscles and arthropathy.

Methods

Plasma samples collected outside Kabul city were frozen at −20 °C, shipped to the central laboratory in Kabul on dry ice and then transferred to the ICHCC in Tehran, Iran. FVIII inhibitors were measured using the modified Nijmegen–Bethesda method [15]. To determine mutations in patients with inhibitors, patients DNA was obtained by the standard salting-out method on whole blood [16] and patients were screened for the inversion mutation of intron 1 using multiplex PCR [17] and for the inversion of intron 22 by an inverse-PCR, as previously described with some modifications [10]. Also, possible existence of large deletions were confirmed by the Multiplex ligation-dependent probe amplification, according to the manufacturer's recommendation (MRC-Holland Amsterdam, Netherland) and direct sequencing techniques.

Results

Inhibitor development

Of 77 patients with hemophilia A, 62 (80.5%) used both FFP and plasmatic FVIII concentrates, and 15 (19.5%) used only plasmatic FVIII concentrate. Table 1 shows the prevalence of inhibitors in different age groups. Prevalence was 9.1% (7/77), of whom 5/77 had high titer inhibitors (>5 BU/ml) (Table 2). In patients with severe and moderate hemophilia A, prevalence were 9.7% (6/62) and 6.7% (1/15), respectively. In patients with low titers inhibitors, two of 77 (2.6%) had inhibitor titer less than 1 BU/ml.

Table 1 The frequency of inhibitors in different age groups

Age group (year)	Inhibitor negative no. (%)	Inhibitor positive no. (%)	Overall no. (%)
0–10	30 (85.7)	5 (14.3)	35 (100)
11–25	31 (96.9)	1 (3.1)	32 (100)
25–35	9 (90)	1 (10)	10 (100)
Overall	70 (90.9)	7 (9.1)	77 (100)

Distribution of *F8* gene mutations in inhibitor patient

Apart from the intron 22 inversion mutation in hemophilia patients (2/7; 28.6%), five different mutations were found, including two large deletions, two small deletions and one missense mutation. Among the detected mutations, two (28.5%) were novel and had not been described in the HAMSTeRS mutation registry, including one large deletion and one small deletion (Table 3).

Discussion

Over 3 decades of civil war in Afghanistan, the medical infrastructures have been destroyed and it has also hampered proper medical progress, particularly for patients with lifelong or chronic disorders. Prior to 2012, none of Afghan hemophilia patients had any access to FVIII and FIX concentrates or the necessary facilities to assess the coagulation factor levels. During these years, blood transfusion and FFPs were used for treatment. In 2011, the hemophilia laboratory was inaugurated in Esteqlal Hospital in Kabul, Afghanistan and later on, in July 2012, the AHPA was established, and became a member of the WFH in the same year [12,13]. In 2012, the WFH and some charities organizations provided to our patients with FVIII and FIX concentrates. However, after 6 years, patients still have inadequate access to medication and diagnostic facilities. Sixty two hemophilic patients (80.5%) older than 7 years of age have been treated with FFP and FVIII concentrates, and 15 additional patients (19.5%) aged less than 7 years had only used FVIII concentrates. Frequency of FVIII inhibitors have been reported to vary from 5 to 50% in different populations [18–22]. Our study shows that 9.1% of patients with

Table 2 The prevalence of inhibitor in relationship to hemophilia A severity

Severity of hemophilia	Number of patients	Inhibitor positive	Percentage
Severe	62	6	9.7
Moderate	15	1	6.7
Overall	77	7	9.1

Table 3 Different characteristics of patients with inhibitors

Patients no.	Age (years)	Ethnicity	Severity of hemophilia (%)	Inhibitors titers (BU/ml)	Mutations	Mutations type
1	3	Pashtun	0.9	4.3	c.6274–6_6279deletionTGGTAGGTGGAT	**Small deletion**
2	6	Pashtun	0.8	5.8	c.6274–6_6279deletionTGGTAGGTGGAT	**Small deletion**
3	3	Pashtun	0.4	25	Intron 22 inversion	**Inversion**
4	11	Hazara	1.5	3	c.5177G>C	**Missense**
5	33	Tajik	0.7	2529	Deletion of exon 1–25	**Large deletion**
6	1	Tajik	0.9	22	Intron 22 inversion	**Inversion**
7	4	Tajik	0.8	162	Deletion of exon 1–25	**Large deletion**

hemophilia A (7/77) had inhibitors against FVIII, so the inhibitor prevalence in Afghan patients is much lower than in other populations [23]. Most of our patients who had developed inhibitors were below the age of 12 (six of seven) and only one was 33 years old. In one case the level of inhibitor was extremely high 2529 BU and in others were 162, 25, 22, 5.8, 4.3 and 3 BU. It is noteworthy that four of seven patients were from two families, two were brothers and two others were uncle and nephew. We determined mutations in inhibitor patients for *F8* gene, in the first step. The samples were then tested for inversion mutations in intron 22 and intron 1. Two patients with inhibitor level of 22 and 25 BU had the intron 22 inversion. Two additional patients had a point mutation in exon 14 or splice site mutation in exon 22. The remaining case had a large gene deletion covering exon 1–25th. As mentioned above, two patients from one family with this large gene deletion also had abnormally high inhibitor level. The first patient, aged 33, had inhibitor level of 2529 BU, and the second patient, a niece of the same person aged 4, had a 162 BU inhibitor level.

This is the first study to investigate the frequency of inhibitors in the sample of hemophilia A patients from the capital city of Afghanistan. In various studies conducted in neighboring countries (such as Iran, Pakistan, India, Uzbekistan and China), the incidence of inhibitors in hemophilia A varies from 4 to 14% [24–29]. Our findings are closer to the two studies conducted in India by Jayandharan *et al.* [26] and Ghosh *et al.* [27]. Other studies done in other countries have reported inhibitor prevalence between 9 and 40% [30–35].

A national screening for carriers, symptomatic and asymptomatic patients will facilitate early identification of at risks patients, to help them to take proper family planning actions (including prenatal diagnosis), also provide the patients with better treatment or counseling and managing those with inhibitors. International collaborations and supports are seriously needed to fulfill the urgent need for prevention and treatment programs by a war-torn country such as Afghanistan.

Conclusion

The prevalence of inhibitors in Afghan hemophilia A patients is much lower than in other populations. The most probable reason would be inadequacy or improper treatment.

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Conflicts of interest

There are no conflicts of interest.

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