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Prevalence of FVIII Inhibitors Among Children with Hemophilia A: Experience at the Jordanian Royal Medical Services

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ABSTRACT

Introduction: Replacement therapy is constantly required by Hemophilia A (HA) patients lacking coagulation factor VIII (FVIII). The most serious complication of this treatment is the development of neutralizing antibodies (inhibitors). **Aim:** The aim of this study is to determine the frequency of FVIII inhibitors among children treated for HA at the Jordanian Royal Medical Services. **Methods:** A total of 165 diagnosed HA patients receiving on-demand treatment, were tested for FVIII inhibitors between 2003 and 2018. The age range was 6 months to 16 years. Coagulation and inhibitor screening assays were performed, followed by Bethesda assay for inhibitor-positive samples to quantify FVIII inhibitor titers. **Results:** Out of the 165 patients, 111 had severe hemophilia with FVIII level < 1%, 26 had moderate hemophilia with FVIII levels of 1–5% and 28 had mild hemophilia with FVIII levels of > 5%. Twenty patients had FVIII inhibitors, of whom 18 had high titers, 2 had low titers. The mean inhibitor level in low (titer) responders was 2.40 ± 0.85 BU, as opposed to 116.25 ±169.25 BU in high (titer) responders. In terms of disease severity, 18 of the 20 patients with FVIII inhibitors had severe HA, whereas two had moderate HA. No inhibitors were encountered in the mild HA group. **Conclusion:** Inhibitors only developed in moderate and severe cases of HA. The severity of the disease and age were the main contributing factors. The association between family history of inhibitors and the incidence of inhibitor formation warrants genetic evaluations to look for relevant mutations.

Keywords: Hemophilia A, FVIII inhibitors.

1. INTRODUCTION

Hemophilia A (HA) is a hemorrhagic diathesis that is ascribed to a specific deficiency or dysfunction of factor VIII (FVIII), which is a vital factor in the intrinsic pathway of coagulation (1). HA together with hemophilia B and Von Willebrand disease makes up more than 95% of all hereditary coagulation factor deficiencies (2). It is inherited in an X-linked recessive manner or occurs as a result of some de novo gene mutations in the FVIII gene located on the X-chromosome, which is seen in one-third of the cases. A variety of FVIII genetic defects may result in defective production, and the most common is intron 22 inversion, which is seen in about 45% of severe HA patients (3).

HA affects 1 in 5000–10000 males and presents with different patterns of bleeding, characteristically hemarthroses. The presenting symptoms depend on the severity of the disease, which is classified into three categories according to the degree of FVIII activity: severe, moderate, and mild (<1%, 1–5%, and 5–35%, respectively) (4). For decades, the mainstay of treatment has been FVIII replacement by repeated infusions of pathogen-free concentrate, either prophylactically or on-demand. Different kinds of plasma-derived and recombinant human FVIII (rFVIII) preparations are on the market as a consequence of technological advances and molecular cloning. Unfortunately, a lack of resources in many countries limits replacement to cryoprecipitate and fresh frozen plasma (5).

One of the current challenges in treatment is the financial burden. On one hand, prophylactic transfusions enable HA children to lead normal lives in terms of span and quality. On the other hand, the yearly treatment cost for an HA patient ranges between $ 50,000 and $ 300,000 in the United States, depending on the severity and treatment options (6, 7).

The development of inhibitors against the infused FVIII is currently the most challenging complication (8). Inhibitors are polyclonal IgG alloantibodies directed against the infused exogenous FVIII protein, thus inhibiting its function in activating factor X and impeding control over hemorrhages (9). Such cases exhibit resistance to treatment, require higher doses of factor replacement, and are more susceptible to arthropathy, bleeding episodes, and a worse quality of life in general (10). This also poses an enormous economic burden as it increases treatment costs by 2 to 10 times (11).

2. AIM

The aim of our study is to determine the prevalence of FVIII inhibitors among children treated for HA at the Jordan Royal Medical Services.

3. METHODS

Data were collected retrospectively for 165 children with HA who were diagnosed and received on-demand treatment at the Jordanian Royal Medical Services during the time period of 2003 to 2018. A 4.5-mL volume of venous blood was collected from each patient in a 3.2% trisodium citrate tube and immediately sent to the coagulation department at the Princess Iman Research and Laboratory Sciences Center. The sample was then centrifuged without delay at 3000 RPM for 10 minutes at a temperature of 4°C to obtain platelet-poor plasma. Coagulation tests were run, including PT, APTT, FVIII level, and FVIII inhibitor screening tests using an STA Compact® analyzer.

The screening test involved mixing studies. Four tubes were prepared for each patient. The first contained normal plasma, the second contained the patient's plasma, and the third had a 50:50 mixture of normal and patient plasma. The three tubes were incubated for 2 hours at 37°C and then placed on ice. A fourth tube was prepared with a 50:50 mixture of normal and patient plasma without incubation. APTT was then measured for all tubes. A positive screening test for time-dependent inhibitors was inferred from normal APTT in tubes 1 and 4 and prolonged APTT in tubes 2 and 3.

The next step was to confirm the result and quantitate the inhibitors present using the Bethesda assay. This assay involved incubating a 50:50 mixture of patient plasma (serially buffer-diluted) with normal pool plasma for two hours at 37°C. The residual FVIII activity was then compared to that of a control mixture containing equal amounts of buffer and normal pool plasma and incubated under the same conditions. The test results are reported in Bethesda units (BU) per mL.

A Bethesda unit represents the amount of inhibitor that neutralizes 50% of one unit of factor activity after 2 hours at 37°C. Depending on the inhibitor level, each sample was labeled as "high titer" when the level was >5 BU /mL (high responder) or "low titer" when it was ≤5 BU/mL (low responder). Data were analyzed using the software IBM SPSS®, and a P-value of <0.05 was considered significant. This study was approved by the ethics committee at the Jordan Royal Medical Services.

4. RESULTS

A total of 165 male patients who were treated on-demand for HA were enrolled in the study. The age of the patients ranged from 6 month to 16 years with a mean of 5.1 years. All patients were classified according to their FVIII activity level into three groups. The majority of patients (111 patients; 67.3%) had severe hemophilia with FVIII level < 1%, while 26 (15.7%) had moderate hemophilia with FVIII level of 1–5%, and 28 (17%) had mild hemophilia with FVIII levels > 5. Out of all 165 subjects, 20 (12.1%) were positive for FVIII inhibitors.

The age of patients with inhibitors ranged from 6 months to 15 years with a mean of 5 ± 4.9 years. Eighteen were high responders, while 2 were low responders. The mean inhibitor level among low responders was 2.40 ± 0.85 BU, as opposed to 116.25 ±169.25 BU among high responders (Table 1). An association was found between age at onset of treatment and inhibitor titer level ($P < 0.05$), particularity among patients < 5 years old. The patients' characteristics are detailed in Table 2.

Range	1.8 – 563 BU
Low responders n=2	2.40 BU ± 0.85 SD
High responders n=18	116.25 BU ± 169.65 SD

Table 1. Classification of FVIII inhibitor titer level (BU).

Patient	Age (years)	Inhibitor titer (BU)
1	0.5	51.2
2	0.5	6.11
3	0.5	19
4	0.5	454
5	1	358
6	1.5	1.8
7	2	230
8	2	20.4
9	2	563
10	3	39
11	3	104
12	3	3
13	4	11.36
14	7	8
15	9	5.6
16	10	18.4
17	11	51
18	12	64
19	14	18.56
20	15	71

Table 2. Characteristics of patients with inhibitors

Severity of hemophilia A	Number of Patients	Number of patients with inhibitors	Percentage
Severe	111	18	16.2%
Moderate	26	2	7.6%
Mild	28	0	0%
Over all	165	20	12.1%

Table 3. The prevalence of inhibitor in relation to hemophilia A severity.

According to the severity of the disease, 18 out of the 111 severe HA patients (16.2%) and two out of the 26 moderate HA patients (7.6%) developed inhibitors. No inhibitors were encountered in the mild HA group (Table 3). The presence of inhibitors in severe and moderate cases but not mild cases suggests an influence of disease severity on inhibitor formation ($P < 0.05$).

Six out of the 20 (30%) patients with FVIII inhibitors had a positive family history. Furthermore, each of the three different families had two siblings with severe disease and were positive for FVIII inhibitors. An association was found between the development of inhibitors and the family history of inhibitor formation ($P < 0.05$).

5. DISCUSSION

In the management of HA, we aim to prevent major bleeding and its complications, as well as to give patients a chance at living normal lives. Current medical practice at our institute includes plasma-derived FVIII concentrates, which constitute 80% of all FVIII used, and rFVIII concentrates, which comprise the remaining 20%. The prophylactic treatment has also been introduced recently. The majority of our patients, however, still receive on-demand treatment. Inhibitor testing was performed in three situations: after on-demand transfusions, in the assessment of treatment-resistant cases, and before surgeries.

Inhibitor detection is affected by the continuously evolving management system. Other limitations include a lack of treatment guidelines and limited awareness among caregivers about inhibitors, especially in remote villages, which leads to the delay or even failure of patient referral to specialized centers. Given the retrospective nature of our study, it was difficult to collect comprehensive data regarding treatment type and intensity, as well as to determine the drug history and genetic defects. On a national scale, no protocols for screening, registration, or reporting are currently in place.

Approximately 12% of all HA patients developed inhibitors in the present study (16.2% in severe HA). An association was found between the severity of disease and the incidence of inhibitors ($P<0.05$). Awidi et al. identified five novel FVIII gene mutations and reported similar rates in Jordan (about 10% overall and 14% in severe HA) (12). Few studies have been published on the prevalence of HA inhibitors among Arabs with HA. Studies from Saudi Arabia, Egypt, and Tunisia reported prevalence rates of 29.3%, 18.2%, and 5%, respectively (13, 14, 15). In other countries, rates of 14–28% rates were reported in Iran (16), 6–21% in various geographical locations in India (17), 25–30% in Japan (18), and 9% in Afghan patients (19). Furthermore, a very low rate of 3.9% was reported by Wang et al. in a Chinese population (20). Differences in prevalence rates could be attributed to treatment protocols, ethnicity, and study design.

Studies in the United States noted varying rates depending on ethnicity, family history of inhibitors, and treatment. There were higher rates among Hispanic, Black, Asian, and Indian groups and those with positive family history. Patients on prophylactic treatment had lower rates (21, 22).

Many factors influence the development of inhibitors, with some involving patient characteristics (e.g., genetic factors, immune response, and ethnicity), while others are treatment related (e.g., duration and intensity, age at the first exposure, and product types) (13). The type of product plays a major role. Sande et al. found that the risk was twice as high when using rFVIII compared to plasma-derived FVIII (23). Peyvandi et al. also found that the incidence of inhibitors with rFVIII was 87% higher than that with plasma-derived FVIII (24). Only 20% of our study subjects received rFVIII, which contributed to our lower rate of inhibitors.

Treatment duration, intensity, and younger age at first exposure also increase the risk. The age range of the 20 HA patients with inhibitors in our study was 6 months to 15 years, and 13 (65%) of them were less than 5 years old. This is explained by their need for repeated exposures starting at an earlier age. We found an association between the age at the onset of treatment and the inhibitor titer level ($P<0.05$), particularly among patients < 5 years old. The literature indicates a higher incidence of inhibitors among patients who start replacement therapy before the age of 6 months (25, 26). Moreover, a study in the United Kingdom found approximately half of new inhibitor cases presented before the age of 5 years in severe HA (27). High inhibitor titers occurred in 18 out of the 20 patients who developed FVIII inhibitors, and only 2 had low titers. This finding calls for further exploration of immune tolerance induction treatment in the high inhibitor titer group since most of these patients have uncontrollable bleedings and do not respond to replacement therapy, in contrast to the low-inhibitor titer group (28, 29). FVIII genotype is an important determinant of inhibitor development in patients with severe HA. It is well established that the nature of FVIII mutation (e.g., interon 22 inversions, large gene deletions, and stop codons) is a predisposing factor (3, 30, 31, 32).

Notably, 6 of the 20 inhibitor-positive patients (30%) in our study belonged to three families where severe HA occurred in two siblings each. There was an association between the development of inhibitors and the family history of inhibitor formation among our patients ($P<0.05$). Unfortunately, the genotype study for these patients was not available at our center. However, Awidi et al. found that 13% of Jordanian HA cases who carry null mutations and 6% who had loss-of-function mutations developed inhibitors against FVIII (12). An association between positive family history of inhibitors and the incidence of inhibitor formation has been well established in previous studies (33, 34). Genetic evaluations should be considered to look for high-risk gene mutations that predispose to the development of inhibitors in severe HA patients, which would facilitate the early identification and better management of patients with inhibitors.

6. CONCLUSION

Inhibitor development poses heavy economic and health burdens and it is not uncommon in Jordanian

HA patients, particularly in severe cases. Prospective studies using centralized inhibitor titer measurement as well as standardized and comprehensive data collection are needed to evaluate modifiable and non-modifiable (e.g., genetic) factors that contribute to inhibitor development. Furthermore, prospective protocols and new strategies need to be implemented to reduce the risk, identify positive cases, and conduct proper screening for families. The adoption of a national screening and counseling program could also facilitate early identification and better management.

Abbreviations: HA = hemophilia A; FVIII = factor VIII; BU = Bethesda units; rFVIII = recombinant human FVIII

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