Prevalence of Factor VIII Inhibitors among Hemophilia A Patients

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Abstract:
Background*:* Factor VIII (FVIII) replacement therapy to hemophilia A patients results in an immune response (inhibitor formation). Inhibitory antibody to exogenous FVIII is a major complication of hemophilia treatment.
Aim of the work: This study was conducted to evaluate the prevalence of FVIII inhibitors among a group of Egyptian patients with hemophilia A, after replacement therapy and related risk factors to develop such inhibitors.
Patients and Methods: From June 2016 to March 2017, 40 patients with hemophilia A who were attending the Pediatric Hematology Unit of Abu El-Rish Children's Hospital, Cairo University, were evaluated. For all patients, through history, physical examination, and laboratory investigations (CBC, PT, APTT, FVIII levels, and anti-FVIII antibodies detection by ELISA and Bethesda assay) were carried out. Data were collected and analyzed.
Results: The age range of the patients was 2.5-18 years. The inhibitor antibodies were detected in 10 patients (25%), and all were with severe hemophilia A. The mean ± SD level of inhibitor antibodies was 14 ± 19 BU/mL (range 2-64 BU/mL). Seven patients had inhibitor antibodies level ≥5 BU/mL (high responder) and three patients had inhibitor antibodies level <5 BU/mL (low responder).
Conclusion:

- Prevalence of FVIII inhibitors detected among our study population was 25%.
- Patients with severe hemophilia A had a substantially higher incidence of inhibitors development than mild or moderate disease.
- No significant difference in the risk of inhibitors development among patients treated by plasma-derived products (SD-cryoprecipitate) or those who were treated with recombinant products (rFVIII).

Recommendation: Regular screening of patients with severe hemophilia A every 3 months to detect FVIII inhibitors development.
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Key Words: Inhibitory antibody, FVIII, hemophilia A

1. Introduction

Development of inhibitory antibodies against FVIII is considered the most severe treatment-related complication in hemophilia A impacting on the efficacy of coagulation factor concentrates to treat bleeds, and creating a significant risk of life-threatening bleeds **(Mazurkiewicz-Pisarek et al., 2016).** The formation of antibodies is a multi-factorial complex phenomenon involving both endogenous genetic as well as non-genetic risk factors **(Borhany et al., 2012).** Patients with certain types of mutations are at higher risk for the development of inhibitors than those with others **(Hay et al., 2011)**. However, the role of non-genetic factors has been increasing, supported by evolving concepts of the immune response based on the "danger model" **(Gouw et al., 2007).**

However, mortality decreased significantly with the development of activated prothrombin complex concentrates**,** recombinant FVIIa and the introduction of immune tolerance protocols **(Kasper and Meirione, 2004).**

2. Patients and Methods

This cross-sectional descriptive study was done at the Pediatric Hematology Unit of Abu El-Rish Children's Hospitals, Cairo University, during the period from June 2016 to March 2017 on total of 40 patients with hemophilia A. Their ages ranged from 2.5 to 18 years with a mean ± SD age of 8.9 ± 4.6 years and all patients were males.

Patients with hemophilia A were selected by history, examination and laboratory evidence of the disease (prolonged APTT and Factor VIII level less than 40 % of normal).

According to detection of inhibitors, patients with hemophilia A were divided into two groups:

- **Group 1:** No inhibitors: anti-FVIII antibodies less than 1 Betheseda unit/mL (BU/mL).
- **Group 2:** With inhibitors: anti-FVIII antibodies equal or more than 1 BU/mL.

All participating individuals gave a written informed consent for applying in the study. Thorough history and physical examination including the age of the onset of bleeding, age of first exposure to FVIII, duration of bleeding with its frequencies per year and how to stop (spontaneous, local or systemic drug therapy), positive family history of similar bleeding condition and the nature of bleeding manifestations.

Blood samples were collected before receiving FVIII. The blood sample was then divided into 5 tubes with the following order: 2ml on EDTA tube for CBC and 4 samples of 1.8 mL on 3.2% tri-sodium citrate tube for measurement of PT, APTT, FVIII level, anti-FVIII antibodies by ELISA and Bethesda assay for quantification of the inhibitors if present. Plasma for sample analysis was obtained following centrifugation of whole blood at 3000 rpm for 5 minute to be assayed immediately or aliquot and stored at -20^{o} C to avoid loss of bioactivity and contamination.

- CBC was done using Sysmex KX-21 N automated cell counter, Symex Corporation, Japan.
- PT and APTT were done using Sysmex CA-1500 automated coagulation analyzer, Dade Behring Holdings Inc., USA.
- FVIII level measurement was done using STAGO STAR Max 58403 system, Diagnostics Stago Inc., USA.
- Anti-FVIII Antibodies were detected using FVIII antibody Screen 303283-F8S-IFUEN kit, immucor GTI diagnostics Inc., USA. It is qualitative solid phase ELISA antibodies reactive with rFVIII in human serum and plasma.
- Bethesda Assay was done using Factor VIII Inhibitor Reagent Kit REF 5152005, Austria. It was used for quantification of the inhibitors in BU, if present.

Statistical analysis

Data were analyzed using SPSS© Statistics version 21 (IBM© Corp., Armonk, NY, USA). Normality of numerical data distribution was examined using the Shapiro-Wilk test. Non-normally distributed numerical variables were presented as median and interquartile range and intergroup differences were compared using the Mann-Whitney test. Nominal variables were compared using Fisher's exact test and ordinal variables using the chi-squared test for trend.

P-value <0.05 was considered statistically significant.

3. Results

Hemophilia A patients were diagnosed at a mean ±SD age of 7.27 ±7.57 months (range 0.25-30 months). They received FVIII therapy at a mean ±SD age of 10.66±12.21 months (range 0.5-60 months).

Family history of hemophilia was present in 22 patients (55%), 10 patients had a brother with hemophilia A and 12 patients had maternal uncle, cousin and/or brother.

32 out of 40 patients (80%) received FVIII on demand and 8 patients as a prophylaxis regimen.

The source of FVIII used in the replacement therapy, depended on the availability of factor concentrates or SD-cryoprecipitate at the hospital. The most common source was plasma derived factors. 12 patients (30%) received SD-cryoprecipitate, 3 patients (17.5%) received rFVIII, 21 patients (52.5%) received FVIII from both sources and 4 patients (10%) were shifted to rFVII due to poor response to FVIII (2 patients were receiving rFVIII and 2 patients were receiving FVIII from both sources).

The majority of patients had the severe form in comparison to moderate and mild form (27, 10 and 3 patients; 67.5%, 25% and 7.5% respectively).

The inhibitors were detected in 10 patients (25%). The mean ± SD level of the inhibitors was 14 ±19 BU (range 2-64 BU/mL). Seven patients had antibody level ≥5 BU/mL (high responder) and three patients had inhibitor antibody level <5 BU/mL (low responder).

Our results are presented in the following tables:

Table (1): Relation between FVIII Inhibitors and Demographic Characteristics of the Study Population

	No FVIII Inhibitors (N=30) (Group 1)		*FVIII Inhibitors (N=10) (Group 2)*				
Variable	**Median**	**IQR**	**Median**	**IQR**	**U**	**Z**	**P-value¶**
Age (year)	7.8	6.0 – 12.0	6.0	4.0 – 13.0	141.0	-0.282	0.778
Weight (kg)	24.3	18.0 – 35	17.8	15.0 – 40.0	133.0	-0.532	0.595
Age at diagnosis of hemophilia (month)	6.5	1.0 – 12.0	5.0	1.0 – 9.0	135.5	-0.456	0.648
Age at 1st exposure to FVIII (month)	8.0	2.0 – 120	9.0	6.0 – 12.0	131.5	-0.592	0.554

Data are median and interquartile range (IQR). IQR, interquartile range; U, U statistic; Z, Z statistic
¶Mann-Whitney U test.

– There was no statistically significant difference between the two groups regarding the demographic data (age, weight, age at diagnosis of hemophilia and age at first exposure to FVIII) as P > 0.05.

Table (2): Relation between FVIII Inhibitors and Coagulation Parameters

	No FVIII Inhibitors (N=30) (Group 1)		*FVIII Inhibitors (N=10) (Group 2)*				
Variable	**Median**	**IQR**	**Median**	**IQR**	**U**	**Z**	**P-value¶**
Platelet count (k/mm^3)	401	387 – 470	401	390 – 450	143.5	-.203	0.839
PT (s)	13	13 – 13	13	13 – 13	126.0	-.812	0.417
APTT (s)	73	45 – 90	109	48 – 140	86.0	-2.002	**0.045**

Data are median and interquartile range (IQR). IQR, interquartile range; U, U statistic; Z, Z statistic
¶Mann-Whitney U test.

– There was a statistically significant difference between the two groups (P= 0.045) as the activated partial thromboplastin time (APTT) was found to be more prolonged with patients with inhibitors than those without inhibitors.

Table (3): Relation between FVIII Inhibitors and Severity of Hemophilia A

	No FVIII Inhibitors (N=30) (Group 1)		*FVIII Inhibitors (N=10) (Group2)*				
Variable	**N**	**%**	**N**	**%**	**X^2**	**df**	**P-value**
Severity of hemophilia A					5.333	1	**0.021¶**
Severe (FVIII activity <1%)	17	56.7%	10	100.0%			
Moderate (FVIII activity 1-5%)	10	33.3%	0	0.0%			
Mild (FVIII activity >5%)	3	10.0%	0	0.0%			

Data are number (N) and percentage (%). X^2, chi-squared statistic; df, degree of freedom.
¶Chi-squared test for trend.

– There was a statistically significant difference between the two groups regarding the severity of hemophilia as patients with severe hemophilia A are more liable to develop FVIII inhibitors (P=0.021).

Table (4): Relation between FVIII Inhibitors and Regimen and Forms of FVIII Replacement

	No FVIII Inhibitors (N=30) (Group 1)		*FVIII Inhibitors (N=10) (Group 2)*		
Variable	**N**	**%**	**N**	**%**	**P-value¶**
Regimen of FVIII replacement					0.192
On demand	23	76.7%	9	90.0%	
Continuous prophylaxis	6	20.0%	0	0.0%	
Intermittent prophylaxis	1	3.3%	1	10.0%	
Forms of FVIII replacement					**0.080**
SD-cryo	*11*	*36.7%*	*1*	*10.0%*	
rFVIII	*2*	*6.7%*	*3*	*30.0%*	
SD-cryo&rFVIII	*17*	*56.7%*	*6*	*60.0%*	

Data are number (N) and percentage (%).
¶Fisher's exact test

- There was no statistically significant difference between the two groups regarding the regimen and the forms of FVIII replacement as P value > 0.05.

Table (5): Relation between FVIII Inhibitors and Frequency of Bleeds and Hospital Admissions during the Previous Year (2015-2016)

	No FVIII Inhibitors (N=30) (group 1)		*FVIII Inhibitors (N=10) (Group 2)*				
Variable	**Median**	**IQR**	**Median**	**IQR**	**U**	**Z**	**P-value¶**
Frequency of bleeds during the previous year	24	10 – 30	27	12 – 50	98.5	-1.625	0.104
Frequency of hospital admissions during the previous year	3	0 – 5	5	4 – 10	86.0	-2.022	**0.043**

Data are median and interquartile range (IQR). IQR, interquartile range; U, U statistic; Z, Z statistic
¶Mann-Whitney U test.

- There was a statistically significant difference between the two groups regarding frequency of hospital admissions during the previous year as patients with more frequent hospital admissions were more likely to develop FVIII inhibitors(P=0.043).
- On the other hand, patients with FVIII inhibitors were more likely to develop severe bleeds and frequent hospital admissions.

4. Discussion

Factor VIII replacement therapy to hemophilia A patients results in an immune response (inhibitors formation).

Our study showed the prevalence of FVIII inhibitors among patients with hemophilia A was 25 % which is close to **Mohsin et al., (2012)** results.

In addition, **Klukowska et al., (2011)** and **Nesheli et al., (2013)** reported lower results, (5.1% and 17.3% respectively). **Klukowska et al., (2011)** reported that the product used in the study was exclusively Octanate®, which is a plasma-derived, human, von Willebrand factor-stabilized FVIII product. Also some antibodies were transient and disappeared over time. However, **Nesheli et al, (2013)** reported a wide age-range of the patients (4-60) years.

On the other hand, **Owaidah et al., (2017)** found the prevalence was higher (29.3%). That difference may be due to in our study, patients received infrequent FVIII support; and most of the patients received SD-cryoprecipitate. This infrequency of infusion could allow their immune system to adequately control the tendency to develop the inhibitors. In addition, most of our patients received pdFVIII as SD-cryoprecipitate which had much less potential to induce FVIII inhibitors than highly purified factor concentrates **(Goudemand et al., 2006) (Chalmers et al., 2007).**

The present study did reveal the significance of hemophilia severity on the development of inhibitors. This result is in agreement with all previously published papers. The explanation of this result is that the majority of included cases either inhibitor positive or negative had severe hemophilia A (27 out of 40 patients with severe hemophilia A (67%) and 10 out the 27 patients developed inhibitors). Patients with severe hemophilia are much more likely to develop inhibitors than those with moderate or mild hemophilia, possibly because the severe phenotype with presumably absent fetal exposure to FVIII, is a major predisposing cause of allo-antibody formation following exogenous clotting factor exposure. In addition, studies of hemophilic patients with all degrees of severity usually demonstrate lower inhibitor prevalence than studies that include only severe cases of hemophilia.

The current work found no significant difference in the risk of developing inhibitors among patients who were treated with plasma-derived products (SD-cryoprecipitate), those who were treated with recombinant products (rFVIII) or both (P>0.05). This is in agreement with the results reported by **Gouw et al., (2007).** On the otherhand, two retrospective cohort studies by **Goudemand et al., (2006)** and **Chalmers et al., (2007)** found higher prevalence of inhibitors among patients who received rFVIII than patients who received pdFVIII. Therefore, the influence of the type of FVIII concentrate remains controversial and this question might be addressed by new studies.

Conclusion

- Prevalence of FVIII inhibitors detected among our study population was 25%.

- Patients with severe hemophilia A had a substantially higher incidence of inhibitors development than mild or moderate disease.
- No significant difference in the risk of inhibitors development among patients treated by plasma-derived products (SD-cryoprecipitate) or those who were treated with recombinant products (rFVIII).

Recommendation:

- Regular screening for patients with severe hemophilia A every 3 months to detect inhibitors development.
- Studies that reveal the interactions between genetic and environmental risk factors are required to develop patient-specific risk algorithms to reduce the likelihood of inhibitors development.

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