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A CROSS-SECTIONAL STUDY OF PREVALENCE OF FACTOR VIII (FVIII) INHIBITORS IN HEMOPHILIA-A PATIENTS.

Hematology

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ABSTRACT

Background: The treatment of Hemophilia A includes Factor VIII replacement therapy which poses a major complication of developing Factor VIII inhibitors. This makes bleeding difficult to control and prevent, resulting in increased morbidity, mortality, and cost of care. An insight into the prevalence of Factor VIII inhibitors and its relations if any, with associated conditions would help to switch these factors to alternate therapies available. The purpose of this study was to determine the frequency of Factor VIII inhibitors among patients with Hemophilia A. **Methods:** This descriptive analytical study included 70 patients with Hemophilia A. All the blood samples were received in Advance Hematology Lab, SMS Medical College, from May 2021 to March 2022. Coagulation tests including Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), Factor VIII levels and Factor VIII inhibitor levels using Bethesda assay were done. **Results:** 14.3% (10) patients had severe hemophilia A with FVIII levels <1%, 44 (62.9%) had moderate hemophilia A with FVIII levels 1-5% while 16 (22.9%) had mild form with FVIII levels >5%. 13 patients (18.6%) were found positive for FVIII inhibitors among which 11 had high titre (>5 Bethesda Unit/mL) and 2 had low titre of FVIII inhibitors (<5 Bethesda Unit/mL). Furthermore, 40% of patients with severe hemophilia A and 20% with moderate disease developed inhibitors whereas none were detected in mild cases. **Conclusion:** This study reveals that development of inhibitors is more common in severe hemophilia A. Early detection of FVIII inhibitors in patients with hemophilia A can facilitate better management and prevent perilous complications.

KEYWORDS

Hemophilia A, Factor VIII, Inhibitors, Bethesda Assay.

INTRODUCTION:

Hemophilia A is a recessive X-linked coagulation disorder resulting from a qualitative and/or quantitative defect of Factor VIII caused by the mutation in F8 gene [1, 2]. It affects approximately 1 of every 5,000 to 10,000 live-born males[3]. The treatment of haemophilia A includes two types of factor concentrates- plasma derived Factor VIII and recombinant Factor VIII which may be administered prophylactically or during episodes of bleeding [2,4].

One of the major complications affecting the replacement therapy is the formation of alloantibodies or inhibitors against the transfused products [2]. Inhibitors are neutralizing immunoglobulin G (IgG) alloantibodies that are targeted against either specific clotting factors or phospholipids i.e., a lupus anticoagulant [5]. Inhibitors block the activity of Factor VIII, thus decreasing or even eliminating the effectiveness of factor replacement [6]. These are usually classified according to their plasma levels as high-titer (activity of >5 Bethesda units/mL) or low-titer (<5 Bethesda Units/mL).

Development of inhibitors makes bleeding difficult to control and prevent, thus resulting in increased morbidity and mortality, increased cost of care, and decreased quality of life [6]. Therefore, detection and estimation of levels of inhibitor against factor VIII can be used to avert major bleeding episodes and its complications.

The objective of this study was to determine the frequency of Factor VIII inhibitors in Hemophilia A patients, classify them in low and high titer groups and to study the association between severity of hemophilia A and development of inhibitors.

MATERIALS AND METHOD:

This was a cross-sectional study conducted between May 2021, and April 2022. Detailed Informed consent was attained from the patients or their attendants before the beginning of study. Ethics clearance was duly obtained from Institutional Ethics Committee.

All consecutive eligible patients fulfilling following inclusion and exclusion criteria were included:

Inclusion Criteria:

All diagnosed patients of hemophilia A were included in this study.

Exclusion Criteria:

Patients with known history of inhibitors were excluded from this study.

A total of 70 patients with Hemophilia A were included in the study. Demographic and detailed clinical history was obtained from all the patients including duration of disease, age at onset of bleeding, site of bleeding, nature (spontaneous or intermittent) and frequency of bleeding, history of blood transfusion and treatment, family history and history of inhibitors.

Procedure:

Venous blood sample was taken in trisodium citrate vial for coagulation tests including PT, APTT and Factor VIII activity level using the Automated Coagulation Analyzer STA Compact. Factor VIII inhibitors were measured using the Bethesda Assay [7].

RESULTS:

Our study involved 70 male patients of hemophilia A. The age of the patients ranged from 2.5 years to 49 years with a mean of 17.36 ± 11.07 years. The mean duration of Haemophilia A in these patients was found to be 11.69 ± 8.94 years with mean age at onset of disease being 6.69 ± 8.94 years and mean age at onset of treatment 7.50 ± 9.30 years.

The most common sites of bleeding were knee joint hemarthroses (20%), bleeding from teeth & gums (17.1%), and hematomas in elbow, ankle and hip joints (14.4%). These were followed by traumatic bleeding (14.3%), hematomas over scalp & cheek (10.0%), skin ecchymoses (8.6%), gastro-intestinal bleeding (2.9%), and bleeding from other sites (epistaxis, ears, urethra). The nature of bleeding was either spontaneous or intermittent. The average frequency of bleeding in these patients was 2.41 ± 1.22 times per month.

Family history for Haemophilia A was positive in 22 (31.42%) patients, out of which in 7 cases first degree relatives were affected while in 15 cases second degree relatives had history of Haemophilia A. 9 patients i.e., 12.8% had known history for FVIII inhibitors. Out of 70 patients, 38 (54.3%) were receiving prophylactic FVIII therapy while the rest (32 patients i.e., 45.7%) received on-demand treatment

for Haemophilia A. Among 9 patients with known history of inhibitors, 8 patients were being treated with Tablet Feiba and NovoSeven, while one patient was on prophylactic treatment with recombinant FVIII concentrates. The average duration of FVIII infusion among all the patients was 10.03 ± 6.74 years.

At the time of presentation of patients, mean PT was found to be 12.60 ± 0.86 seconds while mean APTT was 66.54 ± 14.2 seconds. The average FVIII activity levels in all the patients with haemophilia A was 3.58 ± 5.44%. All patients were classified into three groups depending on their FVIII activity levels into mild, moderate, and severe Haemophilia A. 10 (14.28%) patients had severe haemophilia A with FVIII activity less than 1%, 44 (62.86%) patients had moderate haemophilia A with FVIII activity levels between 1% to 5% and 16 (22.86%) patients had mild haemophilia A with FVIII activity levels > 5% *(Table 1)*.

Table 1- Severity of Haemophilia A (mild, moderate, and severe) according to FVIII activity (in percentage) and prevalence of FVIII inhibitors in relation to Haemophilia A severity.

Severity Of Hemophilia A	Fviii Activity (%)	Number Of Patients With Hemophilia A	Percentage Of Patients With Hemophilia A (%)	Number Of Patients With Inhibitors	Percentage Of Patients With Inhibitors (%)
Severe	<1	10	14.3	4	40
Moderate	1-5	44	62.9	9	20
Mild	> 5	16	22.9	0	0

Of all 70 subjects, 13 (18.6 %) patients were found to have Factor VIII inhibitors. The age of patients with FVIII inhibitors ranged from 3 to 48 years with a mean age of 23.54 ± 14.3 years *(Chart 1)*. The inhibitor range was found to be 2.4 BU - 486.4 BU with a mean of 77.77 ± 142.73 BU. All 13 patients with inhibitors were classified into low and high titre groups depending on the inhibitor levels. "High titre" was labelled when the Inhibitor level was <5 BU (high responders) and "low titre" when it was >5 BU (low responders). The mean inhibitor level among low responders (N= 2 i.e., 15.4%) was 3.0 ± 0.85 BU as compared to 91.37 ± 152.07 BU among high responders (N= 11 i.e., 84.6%) *(Table 2)*.

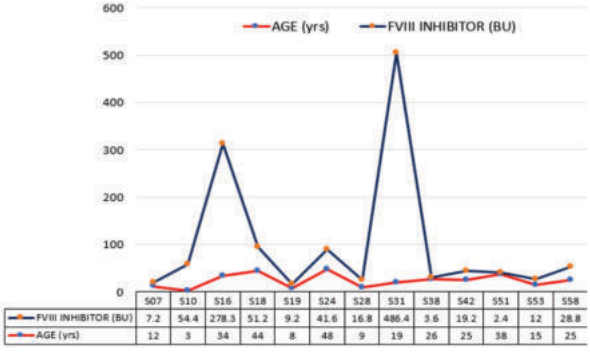

Chart 1- Characteristics of patients with inhibitors and there FVIII inhibitor titre levels.

Table 2- Classification of FVIII inhibitor titre levels (BU).

TITRE GROUP	NUMBER OF PATIENTS	INHIBITOR RANGE (BU)
LOW RESPONDERS (< 5 BU)	2	3.0 ± 0.85
HIGH RESPONDERS (> 5 BU)	11	91.37 ± 152.07
TOTAL	13	2.4 to 486.4

According to the severity of Haemophilia A, 4 out of 10 cases (40%) with severe Haemophilia A developed FVIII inhibitors as compared to 20% (9 out of 44) patients with moderate Haemophilia A. None amongst the mild group developed inhibitors *(Table 1)*. A statistically significant association was found between severity of Haemophilia A and development of inhibitors using the ANOVA test ($p < 0.001$).

DISCUSSION:

Development of factor VIII inhibitors against the replacement therapy in patients of hemophilia A is one of the major challenges in preventing bleeding episodes. Inhibitors are targeted against either specific clotting factors or phospholipids i.e., a lupus anticoagulant [8]. The incidence of FVIII Inhibitors differs in different geographical regions varying from 6.2 % in the French to 30% in the Japanese population [9]. In India the overall prevalence of inhibitors is reported to be 8.2 % in patients with severe hemophilia A [9].

The most common site of bleeding in our study was knee joint hemarthroses, bleeding from teeth & gums followed by large joint hematomas. These were followed by traumatic bleeding, hematomas over scalp & cheek, skin ecchymoses, gastro-intestinal bleeding, and bleeding from other sites (epistaxis, ears, urethra). Sanya Arshad et al. similarly found that bleeding in joints, gums & ecchymoses were the most common manifestations [10]. Aneel A. Ashrani et al. also concluded that the most common joint involved was knee joint followed by ankle, elbow, and hip joints [11]. E. C. Rodriguez-Merchan, Sandra A. Morais and Teja Thorat too had similar observations [12,13,3].

In this study, family history for Haemophilia A was positive in 22 (31.42%) patients, out of which in 7 cases first degree relatives were affected while in 15 cases second degree relatives had history of Haemophilia A. This finding is similar to A. Shirahata et al. who found positive family history in 29% patients [14]. This is a little less than that of Neha Singh et al. who found a positive family history in 50% of the patients with Haemophilia A and Sanya Arshad et al. who found it in 65% cases [15,10].

Upon classification of all the patients into three groups depending on their FVIII activity levels, we found that majority of patients (62.86%) had moderate haemophilia A followed by mild (22.86%) and severe (14.28%) haemophilia A. Nabil Abdelrazik et al. also observed that in 45 cases of Egyptian haemophilia A patients, mild and moderate Haemophilia cases (57.7% and 28.8%, respectively) were more frequent than severe cases (13.3%) in their study [16].

Among 70 patients, we found that the prevalence of FVIII inhibitors to be 18.6% (13 patients). This is similar to the findings of Nabil Abdelrazik et al. who studied prevalence of FVIII inhibitors in 45 Haemophilia A patients in Mansoura, Egypt and found the prevalence to be 18.2% [16]. Peyman Eshghi et al. reviewed 3957 Haemophilia A patients in Iran where inhibitor development was reported to be 14-28% [17]. This prevalence is much greater than that of J. Michael Soucie et al. who found the prevalence to be 2.4% in 950 patients of Haemophilia A in United States [18]. Patricia Pinto et al. studied prevalence of inhibitors in various states of India in 1285 patients of Haemophilia A [9]. The overall prevalence was 6.07% but it varied highly between states. The highest incidence of FVIII Inhibitors was seen in South India (20.99 % in Chennai), followed by Hyderabad (13.33 %), Jammu (9.90 %) and Guwahati (8.51 %), respectively. The other regions in their study showed an inhibitor incidence <8 % including Rajasthan. Other Indian studies like Sachin David et al., Neha Singh et al., Ghosh K. et al., Sanya Arshad et al., too found the prevalence much less than our study [19,15,20,10].

Majority of patients with inhibitors in our study were high responders (84.6%) as compared to low responders (15.4%). AK Taresh et al. similarly found high titres in 82% patients with inhibitors as compared to low titres in 18% patients [21].

According to the severity of haemophilia A, 40% cases in our study with severe Haemophilia A developed FVIII inhibitors as compared to 20% patients with moderate Haemophilia A. This finding is in accordance with Clevia Rosset et al. who found that inhibitor development in severe type of haemophilia in their study was 42% [22]. A. Shirahata et al. observed that most inhibitors developed in severe Haemophilia A patients (90.2%) as compared to moderate Haemophilia A (9.8%) [14]. Sayed H. Mousavi et al. and Tarek Owaidah et al. also concluded that most patients who developed inhibitors had severe haemophilia [23,24].

A statistically significant association was found between severity of Haemophilia A and development of inhibitors using the ANOVA test ($p < 0.001$) in our study. This indicates that the risk of development of inhibitors increases with the severity of the disease. Hence, it needs to be detected at the earliest so that replacement therapy can be started at the earliest, as prophylaxis is associated with a decreased risk of further inhibitor development as compared with on-demand treatment. The limited cross-sectional study design is a major limitation of the present study and needs a more detailed data.

CONCLUSION:

Inhibitor development continues to be one of the most arduous tasks in controlling and preventing bleeding episodes in Hemophilia A patients. It not only hinders the treatment, but affects the overall quality of life of patients. Timely detection and estimation of inhibitors can thus play a pivotal role in preventing major bleeding complications due to the same. A lot of studies have been conducted pertaining to this issue, but very few covered the profile of western India. We studied the prevalence of FVIII inhibitors in patients with hemophilia A in Jaipur, Rajasthan and found that it is a little higher than some of the previous studies done in this region. Therefore, we conclude that it is imperative to detect and document prevalence of FVIII inhibitors in patients with Hemophilia A.

Statements & Declarations:

Conflict of Interest:

The authors have no competing interests to declare that are relevant to the content of this article.

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Ethics Approval:

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Informed Consent:

Written Informed consent was obtained from all individual participants or their legal guardians included in the study.

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