ORIGINAL RESEARCH PAPER
Volume - 11 | Issue - 02 | February - 2022 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

PREVALENCE OF FACTOR VIII INHIBITORS IN HAEMOPHILIA A PATIENTS

Haematology

Arumugam Pothipillai* Professor&Head (Department of Transfusion Medicine), The Tamil Nadu Dr.M.G.R. Medical University, Guindy, Chennai, Tamil Nadu, India. *Corresponding Author

Hamsavardhini Swathandran Associate Professor (Department of Transfusion Medicine), The Tamil Nadu Dr.M.G.R. Medical University, Guindy, Chennai, Tamil Nadu, India.

Deepak Jothy Junior Resident (Department of Transfusion Medicine), The Tamil Nadu Dr.M.G.R. Medical University, Guindy, Chennai, Tamil Nadu, India.

ABSTRACT

Introduction: Haemophilia A is an X - linked recessive bleeding disorder caused by dysfunctional or deficient production of coagulation Factor VIII. Development of antibodies against the exogenous Factor VIII is the major cause for refractoriness in the treatment of Haemophilia A. These antibodies are known as inhibitors.

Aim: Estimation of prevalence of Factor VIII inhibitors in Haemophilia A patients by inhibitor screening assay. Determination of Factor VIII activity by Factor VIII assay in these patients and Quantify Factor VIII inhibitors by Bethesda assay.

Methods: This study was carried out between January 2019 and December 2020. We studied 59 patients who were on "On-demand Plasma-derived factor VIII therapy" at the Haemophilia Treatment Centre- Royapetttah Government General Hospital, Chennai. Factor VIII level estimation, inhibitor screening assay and quantitative Bethesda assay were done at the Department of Transfusion Medicine, The Tamil Nadu Dr M.G.R Medical University.

Results: Out of 59 patients screened, 31, 26 and 2 were diagnosed as severe, moderate and mild Haemophilia A respectively. Five of them developed inhibitors, two were newly diagnosed and three were known cases. The prevalence was 8.5%. All patients with inhibitors had <1% residual Factor VIII activity. Three had positive family history. By Bethesda Assay, two had high and three showed low titre Factor VIII inhibitors.

Conclusion: The prevalence of Factor VIII inhibitor in our study is similar to other studies. We observed positive family history in majority of these patients. Since prophylactic factor VIII therapy delays inhibitor development, further study is recommended.

KEYWORDS

Haemophilia A, Factor VIII, Inhibitors, Bethesda Assay, Risk Factors, Plasma-derived factor VIII

1. INTRODUCTION

Haemophilia A is an X-linked recessive bleeding disorder caused by dysfunctional or deficient production of Coagulation Factor VIII. This Factor VIII gene is located in X chromosome. It occurs mostly in males, females are the carriers.[1] The incidence of Haemophilia A in the .world is approximately 1 in 5000 male live births.[2] The estimated frequency of Haemophilia A is approximately 1 in 10,000 births.[3]

The incidence of Haemophilia A in India in 2010 was 4 per 1, 00,000 male births and around 277 new patients are being registered each year in our country.[3] Haemophilia A prevalence is "the total number of reported or identified cases of Haemophilia A in the population at a given time divided by the total number of males in that population".[6] In India, the number of anticipated Patients with Haemophilia would be approximately 1,20,000. As per World Federation of Haemophilia (WFH) Global survey 2010 only 13,993 patients were documented which indicates the gross low-level of diagnosis, early deaths and lack of awareness to approach treatment facilities.[4] According to WFH Global survey 2020, 2, 41, 535 Haemophilia patients were identified and reported.[24]

Clinical signs and symptoms of Haemophilia A are easy bruising, spontaneous hemorrhage into the joints, muscles and soft tissues, excessive hemorrhage after trauma.[5] Classification of Haemophilia is based on plasma procoagulant levels of Anti Haemophilia Factor VIII with persons <1% factor characterized as severe, 1-5% as moderate, >5% and less than 30% as mild. In mild haemophilia, the patients bleed heavily only after trauma or surgery. Patients with Severe Haemophilia A have spontaneous hemorrhage or excessive bleeding after minor trauma, especially into joint muscles.[5]

Treatment of Haemophilia A patients depends on the administration of exogenous Factor VIII either in the form of FFP/ cryoprecipitate, Plasma-derived factor VIII or Recombinant Factor VIII.[25] The present-day standard of care for Haemophilia A patients is either "On-demand" or "prophylactic".

Development of antibodies against the exogenous Factor VIII is the major complication of the treatment of Haemophilia A. These antibodies are better-known as inhibitors, [8] and are of IgG immunoglobulins.

Inhibitors are categorized according to their levels in plasma as high titre inhibitors (≥5 BU/ml) and low titre inhibitors (<5 BU/ml). Few patients develop transient inhibitors in which low titre inhibitors that never exceed 5 BU/ml and disappear spontaneously with time.[9] One Bethesda unit is defined as the amount of inhibitor that will neutralize 50% of one unit of Factor VIII present in normal plasma after 2 hour incubation period at 37°C.[9]

Over several years, both treatments related and patient related risk factors have been identified. The development of inhibitors depends upon the severity of the disease, ethnicity, mutation involved in F8 gene, family history of Haemophilia A with inhibitors and treatment related risk factors like degree of Factor VIII exposure, early exposure to Factor VIII and type of Factor VIII.As per the severity of disease,an individual with severe Haemophilia A is having highest risk for inhibitor development .[10] The inhibitor formation varies between 20-30% in individuals with severe Haemophilia A and 3-13% among mild or moderate Haemophilia A.[9]

Iinhibitor positive in Haemophilia A is a challenging problem in the treatment of those patients to control bleeding manifestation, to maintain the haemostasis and overall quality of life. Screening procedure for Inhibitor assays are performed in Haemophilia A patients, when the presence of inhibitor is suspected with abnormal bleeding even after taking Factor VIII or showing poor response to Factor VIII replacement therapy. The development of Factor VIII inhibitors is the most concern aspect to detect and strategize treatment protocol.

With this background, our study was carried out to estimate Factor VIII level among Haemophilia A patients and to screen & quantify Factor VIII inhibitor by mixing study and Bethesda assay respectively.

2. PATIENTS AND METHODS

2.1 Patients

This Study included all Haemophilia A patients receiving "On-demand Plasma-derived Factor VIII Therapy". The diagnosis was based on clinical features and factor VIII assay at the time initiating factor VIII therapy. Both newly diagnosed and already established cases with Factor VIII inhibitors were included.

2.2 Ethics approval and consent to participate

The protocol was approved by the Research Council and Ethics

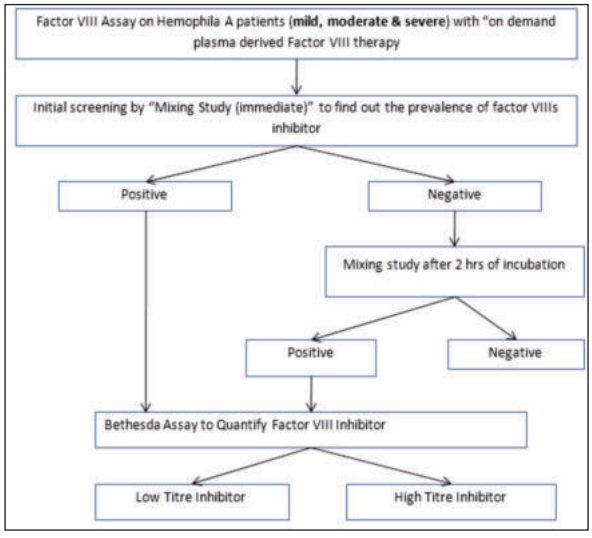
Committee of The Tamil Nadu Dr.M.G.R. Medical University. Written consent was obtained from all patients.

2.3 Design of the study

Cross sectional study was conducted at the Haemophilia treatment centre, Government Royapettah General Hospital and the Department of Transfusion Medicine of The Tamil Nadu Dr MGR Medical University, Chennai from January 2019 to December 2020. The study design is depicted as flow chart in Figure 1.

2.4 Methods

The cases were classified into mild, moderate and severe from the available data based on initial Factor VIII assay done at the time of diagnosis. The known cases with Factor VIII inhibitor were also included for the purpose of calculating the prevalence during the study period.



Venous blood samples (3 ml) were withdrawn in tri-sodium-citrate tubes in the ratio of blood to citrate of 9:1 for performing coagulation studies. The sample was collected within one minute of tourniquet application without too much venous stasis and the sample was processed immediately. Platelet-poor plasma (PPP) was prepared by double centrifugation of a sample at 1700g per 10m at room temperature. The test was performed within four hours of collection. PPP was stored at -70 ° C for 6 months .[7]

Laboratory work of quantitative evaluation of factor VIII, detection of inhibitors by mixing study and then quantitative evaluation of inhibitors (Bethesda study) were done by using the hemostar -2 Channel coagulation analyzer (Tulip Diagnostics). The reagents used for the procedures were Factor VIII deficient plasma, APTT reagent and Calcium chloride (Tulip Diagnostics).

A control mixture is prepared by mixing Factor VIII deficient plasma and buffered-normal plasma pool and is also incubated. After 2 hours the Factor VIII activity of each mixture is measured. The Factor VIII of the test mixture is compared to that of the control and the percentage of residual Factor VIII is calculated.

The patients were on "On demand " therapy and hence 72 hours of wash out period was elapsed at the time of sample collection. One Bethesda unit (BU) is defined as that amount of inhibitor in the test plasma (patient) that results in 50% residual Factor VIII activity. Dilutions of patient plasma are also tested. Patient plasma producing a residual Factor VIII activity of 50% in an incubation mixture is considered to contain one *Bethesda unit per milliliter* (BU/mL). Relationship between the residual factor VIII activity in normal plasma and the inhibitor activity of the test plasma can be interpreted from the graph shown in the Figure 2. At 50% inhibition, the test plasma contains, by definition, 1 BU/mL. Note that the y-axis is a logarithmic scale

$$\text{Residual Factor VIII activity} = \frac{\text{Factor VIII activity (patient)}}{\text{Factor VIII activity (control)}} \times 100$$

Residual Factor VIII activity is then converted to BU/mL by using a standard chart as shown in Figure 2. The critical titre considered to decide the level of Factor VIII inhibitors was 5 BU/ml.

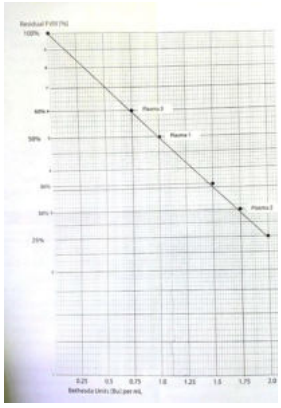


Fig 2

The Y axis is a logarithmic scale and the X axis is linear. The residual F VIII is plotted on the log Y axis and the BU titre on the linear X axis. Obtain the titre of the inhibitor from the graph and multiply it by the dilution to obtain the final titre.

2.5 Statistical analysis

The patients' data were collected using a case report form and entered in to a Microsoft Excel spreadsheet the dataset was completed, the data were analyzed using SPSS. Associated factors were analysed using Pearson's chi-squared test. Incidence and Prevalence were given in Percentages with 95% confidence interval.

3. RESULTS

In our study on 59 patients, those who were diagnosed as Haemophilia A. and on "On-demand Plasma-derived factor VIII therapy", 21 (35.5%) were less than 20 years of age, 19(32.2%), were between 21 and 30 years, 11(18.6%) were above 30 and below 40 years of age, the remaining patients were above 40 years of age. 31(52.5%) patients had less than 1% residual factor VIII activity and were classified as severe haemophiliacs, 26 (44.1%) were moderate haemophiliacs with residual factor VIII activity between 1% and 5% activity. 28(47.5%) patients belong to B Blood Group and 21(35.7%) belong to O Group, 5(8.5%) each belong to AB and A Blood Group.

Five (8.5%) of the 59 patients [3 known cases & 2 Newly diagnosed cases] showed the presence of Factor VIII inhibitor by Inhibitor Screening Assay[Mixing Study] presented in Table 1. Two had high titre Inhibitor with more than 5 BU/mL and three cases had low titre Inhibitor, the data is provided in Tables 2&3. The demographic details and laboratory parameters of all 5 cases with Factor VIII inhibitors are summarily given in Table 4.

All 5 cases with positive inhibitor status belong to severe haemophilia with factor VIII activity <1%.

Three of 5 patients with inhibitors had positive family history of Factor VIII inhibitor development. In all 5 cases with inhibitors, the age at which Factor VIII exposure was more than 6 months. Number of exposure days was more than 50 in all 5 cases with inhibitors. 2 of the 5 patients had high titre Factor VIII inhibitors.

Table 1 Inhibitor Status (prevalence)

	Frequency	%
Positive	5[a]	8.5
Negative	54	91.5
Total	59	100.0

[a][3+2] Known cases: 3 & Newly Diagnosed: 2

Table 2 Inhibitor [based On The Bethesda Units Titre] Distribution

	Frequency	%
Negative	54	91.5
High titre	2	3.4
Low titre	3	5.1
Total	59	100.0

Table 3 Inhibitor Titre In Bu/ml Among 5 Patients With Inhibitor

S.No	Age (yrs)	Residual Factor VIII activity (%)	INHIBITOR TITRE BU/ml	High/Low Type Inhibitor
1.	17	0.64	3.4	Low
2.	19	0.71	9.6	High
3.	31	0.51	1.3	Low
4.	37	0.78	4.86	Low
5.	39	0.59	10	High

Table 4 Summarized Details Of Haemophilia A Cases With Factor Viii Inhibitors

S. NO	Age/ Sex	Classification	Mode of Rx	Response To Rx	TTI	G&T	aPTT	FVIII level	Mixing Study	Inhibitor Screening				Inhibitor Assay
							(Secs)		at 2hrs Incuba -tion (Secs)	1 hr Fresh Mix (Secs)	1 hr Incubated Mix (Secs)	2 hrs Fresh Mix (Secs)	2 hrs Incubated Mix (Secs)	BU/ml
1	17/M	†	‡	No	NEG	O+	109	0.64%	75	59.6	62.3	69.2	78.4	3.4
2	19/M	†	‡	No	NEG	B-	104.2	0.71%	98.4	61.1	64.5	86.2	106.8	9.6
3	31/M	†	‡	No	NEG	B+	119.1	0.51%	73.1	44	51.4	52.4	69.4	1.3
4	37/M	†	‡	No	NEG	B+	102.1	0.78%	98.4	59.6	64.5	86.2	106.8	4.86
5	39/M	†	‡	No	NEG	O+	114.1	0.59%	84.5	45.9	64.3	71.3	93.8	10

†Severe
‡On Demand Therapy

4. DISCUSSION:

In our study, among 59 patients on "On-demand Plasma-derived factor VIII therapy", 5 (8.5%) developed inhibitors to Factor VIII. The prevalence of Factor VIII inhibitor from similar studies as mentioned in the Table 5 ranges from 3.9% to 19 %. The prevalence of Factor VIII inhibitors among Indian Population varies from 5.1% to13%. [14,15,16] Studies done in countries other than India, had shown prevalence between 3.9% and 19%, the patients in these studies were both on "Prophylactic" and "On-demand" therapy. [17,18,19]

Table 5 Prevalence Of Factor Viii Inhibitor From Similar Studies

Study	Year	Place	Number of Participants	Inhibitor Prevalence
Our Study	2020	Chennai	59	8.5%
Dubey et al.,[15]	2013	Lucknow	118	5.1%
Mathews et al.,[16]	2009	CMC, Vellore	200	13%
Ghosh et al.,[14]	2001	Mumbai	352	8.2%
Wang et al.,[17]	2010	China	1435	3.9%
Rosineide A et al[19]	2016	Brazil	184	19%
Wight et al.,[18]	2003	UK	1770	5-7%

In a study by, Sultan et al., Aledort,. L et al Franchini.M et al[20,21,22] it was found that the there is no significant difference in development of Factor VIII inhibitors between Plasma-derived and Recombinant Factor VIII therapy among haemophilia patients. Iorio and colleagues et al.., [23] in their study revealed Factor. VIII inhibitor development in 14% and 27% of patients who were on plasma-derived and recombinant factor VIII therapy respectively.

In our study, 3[60%] of the 5 patients had family history of inhibitor development. The study by Gill et al.[13] revealed that the family history of inhibitor development is in 50% of haemophiliac patients with inhibitors.

The Factor VIII therapy had been initiated only after 6 months of age in all 5 patients who developed inhibitors, in our study. The study by Lorenzo et al., [12] observed that age of onset of therapy before 6 months as one of the risk factors for development of Factor VIII inhibitors. However, 2 of the 54 patients in our study who had been started with Factor VIII therapy before 6 months of age did not develop inhibitors. The study by Gouw et al.., [11] found that there was no apparent correlation between age of onset of therapy and inhibitor development.

All 5 patients with inhibitors were 'severe haemophiliacs' with less than 1% Factor VIII activity at the time of diagnosis. This is in concordance with study by Rosineide A et al., [19] and Wang et al., [17]

The patients in our study had developed inhibitors after 50 exposure days to Factor VIII therapy. However, the number of exposure days was less than 50 in a study by Gouw et al.[11]

In our study 2 of 5 patients with inhibitors developed high titre Factor VIII inhibitors with ≥ 5 Bethesda units. These 2 patients had family history of Factor VIII inhibitor development. 1 of the 2 newly diagnosed patients with Factor VIII inhibitor clinically presented with shortened therapy free interval and joint bleeds-hemarthrosis unresponsive to the treatment. Since 1 of the 3 already diagnosed patients with inhibitor was refractory to Factor VIII therapy, he had been started with Recombinant activated Factor VII therapy.[14]

The number of participants and Inhibitors prevalence in the above studies are cited in the Table 5.

CONCLUSION

Our study has reiterated the importance of factor VIII inhibitor screening among Haemophilia A patients not responding to "On-demand Plasma-derived factor VIII therapy". The prevalence is almost same as that of other similar studies. The positive family history of inhibitor development suggests that it could be one of the predictive factors for resistance to the Factor VIII therapy. It is also absolutely essential to estimate Factor VIII inhibitor by Bethesda Assay to design further treatment protocol. Since it was established in other studies that the prophylactic factor VIII therapy delays inhibitor development, a study may be conducted on Haemophilia A patients with this treatment protocol.

Acknowledgements

This study was conducted from The Tamil Nadu Dr.M.G.R. Medical University Grant. Our sincere gratitude to the Vice-Chancellor**,** the Registrar**,** Dean of Students**,** Members of Research Council and Ethics committee of The Tamil Nadu Dr. M.G.R. Medical University. We thank **Dr.S.Malathy M.D,** Associate Professor, Department of Medicine & Haemophilia Care Clinic at Government Royapettah Hospital for having provided clinical inputs of the patients.

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