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Quantification and Frequency of Inhibitors in Congenital Coagulation Disorder Patients; An Experience at Tertiary Care Hospital

***Neelam Mazhar*[1]**
***Shazia Yaseen*[2]**
***Sarah Rafi*[3]**
***Saima Farhan*[4]**

Department of Haematology and Transfusion medicine, CH&ICH Lahore.

Address for Correspondence
Dr. Neelam Mazhar
Department of Haematology and Transfusion medicine, CH&ICH Lahore
neelam_mazhar@hotmail.com

Abstract

Objective: To quantify and calculate the frequency of inhibitors in patients with congenital coagulation disorders using the Bethesda assay.
Methodology: A cross-sectional study was conducted in the Department of Hematology at Children's Hospital Lahore, Pakistan, from September 7, 2016, to March 7, 2017. 350 Pediatric patients of 1-15 yrs of age of both genders having congenital coagulation disorders were selected in the study. Patients having Hepatitis B, C, chronic liver disease evidence, HIV or malignancies were excluded from the study. The Chi-square test was applied, and a P-value of ≤0.05 was considered statistically significant.
Results: A total of 350 children were enrolled in the study, comprising 277 (79.1%) males and 73 (20.9%) females. The age of the children ranged from 1 to 15 years. Among them, 127 (36.3%) were diagnosed with Hemophilia A, 73 (20.9%) with Hemophilia B, 38 (10.9%) with rare bleeding disorders, and 112 (32%) with Von Willebrand disease. Sixteen (5.77%) patients developed inhibitors after receiving treatment, all of whom were males aged 1 to 4 years. The quantification of inhibitors in these patients was performed using the Bethesda Assay.
Conclusion: Coagulation factor inhibitors may develop against any coagulation factor, with FVIII (Haemophilia-A) being the most common target. The evaluation of coagulation factor inhibitors involves the Bethesda assay to measure inhibitor titers, which aids in treatment decision-making.
Keywords: Congenital bleeding disorders, hemophilia A, Nijmegen-Bethesda Assay.

Introduction

An inhibitor is a polyclonal high affinity antibody, immunoglobin IgG which acts against the procoagulant activity of the concerned clothing factor and decreases its efficacy. Inhibitors are mostly subtyped as high or low responders. Congenital coagulation disorders are found in all racial groups and have worldwide distribution. In developing countries like Pakistan, there is limited data regarding their prevalence.[1]

Most commonly, factor inhibitors develop in patients of Hemophilia A, who are undergoing treatment with factor replacement therapy. The immune process of development of FVIII inhibitor is quite complex. These antibodies are generated from a complex cellular cascade from antigen presenting cells to T lymphocytes to B lymphocytes. [2]

Development of inhibitors in patients can complicate the clinical course of Hemophilia, making the treatment challenging and affecting the prognosis of disease.[3] Presence of inhibitors leads to increased risk of bleeding in the patients that is resistant to treatment. Chronic joint disease and restriction of physical ability can impair the quality of life in patients suffering from inhibitors and hence poorly controlled bleeding. [4]

The possibility of presence of inhibitors should always be kept in mind when a patient presents with bleeding symptoms without any prior history of bleeding diasthesis.[5]

The inhibitors are usually quantified by using the technique of Bethesda assay. Many specific inhibitors are time dependent and will not be detected unless a test is repeated after 2 hour incubation according to a study on FVII inhibitors published in Blood Journal in 2013. The cumulative incidence of development of inhibitors in patients of non-severe hemophilia was 5.3%.[6]

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Methodology

A cross-sectional study was conducted in the Department of Hematology and Transfusion Medicine at Children's Hospital & Institute of Child Health, Lahore from January 2017 to June 2017. Permission was obtained from the Institutional Review Board of the hospital, and written consent was acquired from the parents of the participants. The study included 350 pediatric patients aged 1-15 years, of both genders, diagnosed with congenital coagulation disorders. Patients with Hepatitis B, Hepatitis C, chronic liver disease, HIV, or malignancies were excluded from the study.

Blood samples were collected in Na-citrate (blue top) vials from the study population for Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT), inhibitor screening, and quantification by the Bethesda assay.

Statistical analysis was performed using SPSS22 software. Quantitative variables such as age were expressed as mean and standard deviation, while qualitative variables such as gender and inhibitor were presented as frequency and percentage. The data were also depicted in tables. The Chi-square test was applied, and a P-value of ≤0.05 was considered statistically significant.

Results

A total of 350 children were enrolled in the study out of which 277 (79.1%) were males and 73 (20.9%) were female. Regarding the age distribution 153 (43.7%) children were in the age group of 1-5 years, 165 (47.1%) were from 5-8 years and the rest 32 (9.1%) were in the age group 8-15 years. All of the study population were diagnosed cases of congenital coagulation disorders out of which Hemophilia turned out to be the commonest diagnosed in 127 (36.3%) children. Evidence of Hemophilia B was found in 73 (20.9%) children. Frequency of rare bleeding disorders was 38 (10.9%) and that of Von Willebrand disease was 112 (32.0%). (Table I)

We also calculated frequency of diagnosis according to age distribution of patients. Hemophilia A, being the commonest coagulation disorder, 65 (42.5%) patients fell in the age group of 1-5 years, 58 (35.2%) in age group of 5-8 years and only 4 (12.5%) were 8-15 years of age. All patients of hemophilia A and B were male, whereas 51 (45.9%) patients of VWD were male and 60 (54.1%) were female. (Table II)

Table I: Demographic and Diagnostic Characteristics of Pediatric Patients with Congenital Coagulation Disorders.

Age in Children	N	%
1-5 years	153	43.7
5-8 years	165	47.1
8-15 years	32	9.1
Total	350	100.0
Distribution of Gender		
Male	277	79.1
Female	73	20.9
Total	350	100.0
Diagnosis		
Hemophilia A	127	36.3
Hemophilia B	73	20.9
Rare Bleeding Disorder	38	10.9
Von Willebrand Disease	112	32.0
Total	350	100.0

When the inhibitor screening of these patients was done, positive screen was found in 16 (5.77%) patients, remaining 261(94.22%) were absent. Regarding the

Table III: Stratification for inhibitors with respect to age.

		Inhibitors		Total	
		yes	no		
Age in children	1-4 years	16	137	153	P-value 0.001
		10.5%	89.5%	100.0%	
	5-8 years	0	165	165	
		0.0%	100.0%	100.0%	
	Above 8 years	0	32	32	
		0.0%	100.0%	100.0%	
Total		16	334	350	
		4.6%	95.4%	100.0%	

Table II: Diagnosis Frequency by Age and Gender

Diagnosis	1-4 years	5-8 years	Above 8 years	Gender distribution	
				Male	Female
Hemophilia A	65(42.5%)	58(35.2%)	4(12.5%)	127(100%)	0
Hemophilia B	34(22.2%)	36(21.8%)	3(9.4%)	63(100%)	0
Rare Bleeding Disorder	17(11.1%)	18(10.9%)	3(9.4%)	36(73.5%)	13(26.5%)
Von Willebrand Disease	37(24.2%)	53(32.1%)	22(68.8%)	51(45.9%)	60(54.1%)
Total	153(100%)	165(100%)	32(100%)	277(100%)	73(100%)

positive inhibitor screen, the inhibitions were found only in male gender in age group of 1-5 years. No inhibitors were formed in 5-15 years of age or in female gender (Table III & IV).

Next, the quantification of inhibitors was done by using Bethesda Assay by taking plasma dilutions in which values were 50% of the residual Factor VIII and Factor IX activity (Table V & VI).

Discussion

Inhibitors are proteins or immunoglobulin in nature that bind to function epitopes present on the molecule of coagulation factors, so inhibiting their function. The inhibitors can be allogenic or autologous.[7] Inhibitor quantification assays are used worldwide to calculate the concentration of inhibitors in plasma, two kinds of assays are commonly used, Bethesda assay and Nijmegen assay. These assays follow a similar principal which includes the measurement of clothing factor activity that is decreased in a 50:50 mix of normal plasma and factor deficient inhibitors containing plasma.[7, 8] False positive cases of Bethesda quantification can also be seen, the main causes can be lupus anticoagulants, EDTA plasma, anticoagulant therapy or laboratory errors/analytical issues. [9] The male to female distribution of our study population (350 cases) was 4:1 with dominance of male gender which is comparable to 3:1 male to female ratio in a study conducted in 2018.[10] Regarding the distribution of diagnosis in pediatric age group, other studies have also reported Haemophila A as the most common congenital coagulation disorder in the pediatric age group.[11] Another study has reported Haemophilia A as the second common coagulation disorder after rare bleeding disorder which our study shows Von Willebrand disease as second commonest after Hemophilia A in children. [10, 11]

Recent study was conducted in 2020 published in International Journal of Pediatric Research in which total

Table IV: Stratification for inhibitors with respect to gender.

		Inhibitors		Total	
		yes	no		
Gender	Male	16	261	277	P-value 0.036
		5.8%	94.2%	100.0%	
	Female	0	73	73	
		0.0%	100.0%	100.0%	
Total		16	334	350	
		4.6%	95.4%	100.0%	

Table V: Calculation of Bethesda Units (BU) in Plasma Samples of patients with inhibitors

Patients	Plasma Dilution(in which values are near to 50% 0f residual factor viii activity)	% Residual VIII	Calculation BU× Dilution	Inhibitor inBU
1	1 in 10	60	0.70 ×1	=0.07
2	1in 5	33	1.60 ×5	=0.80
3	1in 10	55	0.85 ×10	=8.5
4	1 in 15	68	0.55 ×15	=8.3
5	1in 5	40	1.30 ×5	=6.5
6	1 in 10	55	0.85 ×10	=8.5
7	1 in 15	61	0.70 ×15	=10.5
8	1 in 20	65	0.60 ×20	=12
9	1 in 15	64	0.70 × 15	=10.25
10	1 in 5	33	1.35 × 5	= 6.75
11	1 in 20	54	0.60× 20	=12
12	1 in 25	68	0.70 × 25	=17.5

Table VI: Quantification of Inhibitors Using Bethesda Assay

Patients	Plasma Dilution (in which values are near to 50% of residual factor IX activity)	% Residual IX	Calculation BU× Dilution	Inhibitor in BU
1	1 in 5	45	1.65 × 5	=8.25
2	1 in 10	60	0.77× 10	=7.7
3	1 in 15	61	0.69 × 15	=10.35
4	1 in 5	55	1.60× 5	=8.00

93 patients were enrolled, this study showed highest disease prevalence in age group of 11-18 yrs whereas, in or study the highest number of cases were diagnosed in 1-5 years age group.[12]

Inhibitor formulation was observed in the diagnosed patients who presented with bleeding symptoms during treatment. Our study showed inhibitors in 16 (5.77%) patients out of total 350 number which is comparable to another study conducted in India which showed inhibitor frequency of 1.07%. Despite the advances in treatment of the congenital coagulation disorders, the formation of the

inhibitors have not been possible to avoid throughout. Therefore, it is essential to have the inhibitor testing assays facility in every center that provides diagnosis and treatment for these patients. [14, 15]

In the past, clot-based assays were used which are now been replaced by chromogenic testing which is more specific and this will decrease the required number of inhibitors tests and hence will decrease burden on laboratories. Inhibitors are usually quantified by Bethesda Assay or Nijmegan modification as done in our study. [17] We found out 16 patients with inhibitors out of 350 total study population and we quantified inhibitors on all of our 16 patients by Bethesda Assay. 12 out of 16 (75%) patients had FVIII inhibitors while 4 patients (25%) had FIX inhibitors. The individual liter of inhibitors was calculated and measured by Bethesda Assay. The internationally accepted cut off value for Bethesda based inhibitor Assay is 0.6 BU/ml and our study population showed values higher than this in all 16 patients. [18, 19]

Conclusion

Coagulation factor inhibitors can develop against any clotting factor as they are neutralizing antibodies that reduce the function of the clotting factors. The most common inhibitors are formed against Factor VIII in patients of Hemophilia A. These inhibitors are commonly seen in male gender in age group of 1-5 years. The assays used to quantify. These inhibitors are used worldwide, most common being Bethesda assay which aids in the early detection and prompt treatment of these patients.

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