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Pathology

PREVALENCE OF INHIBITORS IN HEMOPHILLIA PATIENTS

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ABSTRACT **Background:** Hemophilia is a genetic disorder of coagulation due to deficiency of Factor VIII or Factor IX. Inherited as X-linked recessive pattern. Hemophilia A is due to mutations in the Factor VIII gene. Inversion of intron 22 sequence is the most common hemophilia A mutation. Hemophilia B is due to mutations in Factor IX gene. The peculiar thing about the transmission of this disease is that only the males suffer from it. The females are only the carriers. Unless and until the sufferer bleeds into a vital organ and dies, Hemophilia is not a killing disease. Coagulation profile shows only an isolated prolongation of activated Partial Thromboplastin Time. Persons with Hemophilia (PWH) have normal platelet counts and bleeding times. Specific determination of FVIII or FIX level is essential for diagnosis. Replacement of the missing Factor (FVIII or FIX Concentrates) is the treatment for bleeding hemophiliacs. The major complication of hemophilia is development of alloantibodies (inhibitors) to Factor VIII or Factor IX.

Materials & Method: This was an prospective/cross sectional study. 100 hemophillia patients were selected. Fully automated- The Stago STA compact coagulation analyser was used for the coagulation assays. Inhibitor assay was done using Mixing study.

Result & Conclusion: Out of 100 cases, 94% patients had Hemophillia A & 6% cases had Hemophillia B. Most patients were in age group of of first & second decade. All patients were Male. Among the 100 patients studied; 94% of patients having Hemophilia A & 6% of patients having Hemophilia B. This study reported the prevalence of inhibitors in hemophilia A was 17.02%, while in case of hemophilia B; it was 16.66%. Early identification of inhibitors through routine inhibitor screening at regular interval will prevent the adverse events with increase of success in inhibitor treatment. This would lower the cost and morbidity associated with inhibitor development in hemophiliac patients.

KEYWORDS : Hemophillia, factor VIII, factor IX, Inhibitors, Mixing study.

INTRODUCTION:

Hemophilia is an X-linked hereditary bleeding disorder, leading to deficiency of factor VIII or factor IX. Hemophilia A is due to mutations in the Factor VIII gene. Inversion of intron 22 sequence is the most common hemophilia A mutation. Hemophilia B is due to mutations in Factor IX gene. This deficiency results in recurrent bleeding into joints and muscles, leading to hemophillic arthropathy and contractures. The peculiar thing about the transmission of this disease is that only the males suffer from it. The females are only the carriers. Unless and until the sufferer bleeds into a vital organ and dies, Hemophilia is not a killing disease. Management of Hemophillia A or B is based on the lifelong replacement of coagulation proteins. Today, the most feared complication of this therapy is the development of inhibitors that neutralize the replaced clotting factors. Clinically, the presence of inhibitor is suspected if there is either inadequate response to administered clotting factor or increased frequency of bleeding episodes or allergic responses to administered clotting factor. Based on the level of Factor VIII or IX Hemophilia are classified into three categories; Mild when factor level is 6-30%, Moderate when factor level is 1-5% & severe when factor level is <1%. In the severe and moderate forms, the disease is characterized by spontaneous or trauma related bleeding into the large joints or muscles. In mild disease the patient experiences infrequent Bleeding especially after trauma. If the Factor VIII or IX level is >25% the disease is diagnosed only during routine preoperative screening tests or after major trauma. Coagulation profile shows only an isolated prolongation of activated Partial Thromboplastin Time. Persons with Hemophilia (PWH) have normal platelet counts and bleeding times. Specific determination of FVIII or FIX level is essential for diagnosis[1,2].

MATERIALS & METHODS:

Present study is a prospective study conducted at Department of Patholog00y, M. P. Shah Govt. Medical College, Jamnagar in a duration of 2 years. This study includes total 100 cases. We included diagnosed hemophillia patients who received factorVIII or Factor IX concentrate; whose bleeding can not be stopped by administration of factor treatment by the following physician & for whom there is clinical suspicion of inhibitor. A detailed history including onset of symptoms and signs, bleeding episodes, treatment details, type of factor concentrates and duration is recorded on study performa. Hemophilia patients using blood products and other blleding disorders were excluded. For each patient 4ml of blood sample was collected into 2 tubes containing 0.109M(3.2%) of trisodium citrate. All samples were centrifuged at minimum of 2000g for 15 min atleast to obtain platelet poor plasma(PPP). Pooled normal plasma(PNP) which was prepared from 20 healthy donors(Male=Female); Desorb-U, CK-PREST, Cacl2 and Fully automated The stago-STA compact coagulation analyser and STA reagents(DiagnosticaStago, Paris, France), was used for the coagulation assays. The screening of inhibitors was done by the APTT based method, 50:50 patient plasma mixed with pooled normal plasma and incubated together for 2 hours, at 37°C.The testing should preferably be completed within 2 hour of collection. Care must be taken not to disturb the buffy coat layer when removing the platelet poor plasma(PPP). If the problem is a simple factor deficiency, mixing the patient's plasma 1:1 with plasma that contains 100% of the normal factor level; results in a level >50% in a mixture & the APTT will be normal. Correction with mixing indicates factor deficiency; failure to correct indicates an inhibitor[3].

Procedure:

1. Run APTT of Pooled normal plasma(PNP)-(Sample A)
2. Run APTT of patient's plasma-(Sample B)
3. Take 500µl of PNP + 500µl of patient's plasma
4. Immediately run APTT of this freshly mixed sample-(Sample C)
(a)Keep this mixed sample for incubation at 37°C for 2 hours.
(b)Keep both PNP and patient's plasma separately for incubation at 37°C for 2 hours.
5. After 2 hours of incubation run APTT of mixed sample-(Sample C)
6. Run APTT of incubated PNP-(sample A) and patient's plasma(sample B) after mixing them. [500µl+500µl each] (for comparison purpose only).

INTERPRETATION OF THE INHIBITOR SCREEN BASED ON THE ACTIVATED PARTIAL THROMBOPLASTIN TIME(APTT)

SAMPLE No.	CONTENT	APTT		
Sample A	Pooled normal plasma(PNP)	Normal	Normal	Normal
Sample B	Patient's plasma	Long	Long	Long
Sample C	50:50 mixture, patient:PNP;no incubation(freshly mixed)	Normal	Long	Normal

Sample C	50:50 mixture, patient:PNP; 2 hr of incubation	Normal	Long	Long
INTERPRETATION:		**Deficiency**	**Immediately acting inhibitor**	**Time-dependent inhibitor**

OBSERVATION & RESULT:

100 patients with Hemophillia were enrolled among which 94(94%) were carrying Hemophillia A and 6(6%) were carrying Hemophillia B. Out of 94 patients of Hemophilia A; 10.64% were mild, 21.28% were moderate & 68.08% cases were of severe hemophilia A. Prevalence of inhibitor development among Mild, Moderate & Severe Hemophilia A was; 10%, 25% & 17.18% respectively. Overall prevalence among Hemophillia A was 17.02%. Out of 6 cases of Hemophilia B; 33.33% cases are moderate & 66.66% cases are of severe Hemophilia B. Prevalence of inhibitor development among Mild, Moderate & Severe Hemophilia B was; 0%, 0% & 25%. Overall prevalence among Hemophillia B was 16.66%. So, Among the 94 patients out of 100 of Hemophilia A; prevalence of inhibitor development among them was 17.02% & 6 patients out of 100 of Hemophilia B; Prevalence of inhibitor development among them was 16.66%. So, Overall Prevalence is 17%. Most patients were of first & second decade with age group of 10-19 years.

Table 1: Type Of Hemophilia Among The Participants

Total number of patients	Hemophilia A	Hemophilia B
100	94 (94%)	6 (6%)

Table 2: Classification Of Hemophilia Patients

HEMOPHILIA A			HEMOPHILIA B			TOTAL
MILD	MODERATE	SEVERE	MILD	MODERATE	SEVERE	
10(10.64%)	20(21.28%)	64(68.08%)	0(0%)	2(33.33%)	4(66.66%)	
Total: 94 (Hemophilia A)			Total: 6 (Hemophilia B)			100

Table 3: Hemophilia A: Distribution According To Severity & Positive Cases For Inhibitors

	Total patients of hemophilia Number	Positive cases for inhibitors Number %
Mild	10(10.64%)	1(10%)
Moderate	20(21.28%)	4(25%)
Severe	64(68.08%)	11(17.18%)
Total:	94	16 (17.02%)

Table 4: Hemophilia B: Distribution According To Severity & Positive Cases For Inhibitors

	Total patients of hemophilia Number	Positive cases for inhibitors Number %
Mild	0(0%)	0 (0%)
Moderate	2(33.33%)	0 (0%)
Severe	4(66.66%)	1 (25%)
Total:	6	1 (16.66%)

Table 5: Prevalence Of Inhibitor Development Among Hemophilia Patients

Type of Hemophilia	No. of patients(Out of 100)	Inhibitor development among hemophilia patients
Hemophilia A	94	16(17.02%)
Hemophilia B	6	1(16.66%)
Total:	100	17(17%)

Table 6: Age Distribution Of Hemophilia Patients

Age	No. of patients
<10	24(24%)
10-19	38(38%)
20-29	18(18%)
30-39	12(12%)
40-49	5(5%)
>50	3(3%)
Total:	100

DISCUSSION:

We acknowledge that our study had limited statistical power because of the small number of patients and that the results require confirmation. We could have enrolled many patients living with hemophillia in Saurashtra region but some of them live so far from the centre. The prevalence of inhibitor in our study was 17.07% in Hemophillia A and 16.66% in Hemophillia B. So, overall prevalence was 17%. Bethesda Nijmegen test was not performed. Study among the factors associated to presence of inhibitors, only the severity of hemophilia was statistically associated. The frequency of inhibitors in severe hemophilia A was 17.18% and in severe hemophilia B it was 25%; this is correlated with world federation of hemophilia literature[4]. In our study, Total 100 patients with Hemophilia was enrolled; 94(94%) out of 100 was of Hemophilia A & 6(6%) out of 100 was of Hemophilia B. This is in concordance with study by Patrica Pinto et. al[5] who enrolled total 92 patients; of that 79(85.87%) of Hemophillia A & 13(14.13%) of Hemophillia B and Munira et. al[6] who enrolled total 173 patients; of that 140(81%) of Hemophillia A & 33(19%) of Hemophillia B. In our study, maximum number of patients are in age group of 10-19 years with mean of 18.998 & standard deviation of 14.1377 who develops inhibitors. This is in concordance with study by Patrica Pinto et. al[5], in which the mean age of patients was 19.31 years and by Munira et. al[6] in which the mean age of patients was 15.8 years. In our study, prevalence of inhibitor development among Hemophilia A patients was 17.02% and among Hemophilia B patients was 16.66%. This was correlate with previous study of Patricia et. al[5] and close to the 10-50% usually described in various literature[5,7,8&9]. In our study, 10% cases was of mild, 25% cases was of moderate & 17.18% cases was of severe hemophilia A. This was correlated with study by Sharifian et. al[10] in which 3.5% mild, 9.4%moderate and 22.8% severe Hemophillia A; a study by Ahmed et. al[11] showed 7.75% mild, 14.4% moderate and 77.8% severe Hemophillia A. A study by Sajid et. al[12] showed 37.2% mild, 41% moderate and 21.6% severe Hemophillia A. In our study, 25% cases was of severe hemophilia B. this was correlated with a study by Ahmed et. al[11] showed 69.6% severe hemophillia B cases.

CONCLUSION:

This study reporting the prevalence of inhibitors of Hemophillia A was 17.07%, while in case of Hemophillia B, it was 16.66%. So, overall prevalence in our study was 17%. Early identification of inhibitors through routine inhibitor screening at regular interval will prevent the adverse events with increase of success in inhibitor treatment. This would lower the cost and morbidity associated with inhibitor development in hemophilic patients. Moderate and Severe cases of hemophilia A or B must be screened in priority for inhibitors at regular interval.

REFERENCES:

1. Hemophilia Federation in India; https://hemophilia.in
2. Anthony S. fauci, MD; Dennis L. Kasper, MD; Dan L. Longo, MD; Eugene Braunwald, MD; Stephen L. Hauser, MD; J. Larry Jameson, MD,Phd; Joseph Loscalzo, MD, Phd , HARRISON's Principle of Internal Medicine 17th edition; Part 6: oncology & Hematology; Section-3 Disorders of hemostasis; 109:726-727
3. Barbara J. Bain, Imelda Bates, Michael A. Laffan; Dacie and Lewis, Practical Hematology. 12th edition Elsevier Churchill Livingstone. 2017 p. 376,389, 390
4. World federation of Hemophilia literature; https://www.wfh.org/en/page.aspx?pid=653.
5. Patrica pinto, Tejashree Shelar et al: the epidemiology of factor VIII inhibitors in Indian hemophilia A patients. Indian journal of hematology and blood transfusion December 2014; 30:356-363
6. Borhany M, Shamsi T, Naz A, Khan A, Parveen K, Ansari S, et al. Congenital bleeding disorders in Karachi, Pakistan. Clin Appl Thromb Hemost. 2011;17:E131–7
7. Goudemand J. Les anticorps anti-facteur VIII chez 1'hemophile. Hematologie 2001; 7:170-83
8. Carol K. Kasper: Diagnosis and management of inhibitors to factors VIII and IX An introductory discussion for physicians, Treatment of hemophilia September 2004(34), world federation of hemophilia.p. 1
9. DiMichele DM, Inhibitors in hemophilia: A primer. Haemophilia 2008; 7:1-413
10. Sharifian R, Hoseini M, safai R, Tugeh gh, Ehsani AH, Lak M et al. Prevalence of inhibitors in a population of 1280 hemophilia A patients in Irn. Acta Medica Iranica 2003; 41(1):66-8
11. Ahmad F, Kannan M, Ranjan R, Bajaj J, Choudhary P, Saxena R. Inherited platelet function disorders versus other inherited bleeding disorders: An Indian overview. Thromb Res. 2008;121:835–41
12. Sajid R, Khalid S, Mazari N, Azhar W, Khurshid M. Clinical audit of inherited bleeding disorders in a developing country. Indian J Pathol Microbiol. 2010;53:50–3.