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SCREEN AND FREQUENCY OF COMMON BCLI AND HINDIII FACTOR VIII GENE POLYMORPHISMS IN NORTH-EASTERN INDIAN CHILDREN WITH HEMOPHILIA-A

Paediatrics

Sushil Kumar Jaiswal — Doctoral fellow, Centre for Genetic Disorders, Institute of Science, Banaras Hindu University, Varanasi.

Sandeep H S — Department of Pediatrics, Institute of Medical Sciences, Banaras Hindu University, Varanasi.

Amit Kumar Rai* — Doctoral fellow, Centre for Genetic Disorders, Institute of Science, Banaras Hindu University, Varanasi. *Corresponding Author

ABSTRACT

Introduction- Hemophilia A, an X-linked recessive disorder due to deficiency of factor VIII coagulant activity, has prevalence of 1 male per 7000 of the male population in northern states of India. Intron 18 (BclI) and intron 19 (HindIII) polymorphism are considered very valuable in carrier detection by linkage analysis by previous reports from India. **Aim-** Objective of the study was to find out frequency of the two commonest intronic SNP's rs4898352 (A/T) in intron 18 (BclI) and rs4074307 (A/G) in intron 19 (HindIII) by restriction fragment length polymorphisms (RFLP) of Factor VIII gene in patients with hemophilia A of the most dense and populous states of India, Uttar Pradesh and Bihar. **Patient and Methods-** All cases were diagnosed by factor VIII assay. Intron 18 polymorphism in 59 male children of 56 families and intron 19 polymorphism was analyzed in 62 male children of 59 different families respectively of DNA extracted from blood leukocytes by genotyping using PCR-Restriction Fragment Length Polymorphism method. **Results-** Positive allele frequency for intron 18 it was 0.54 and intron 19 polymorphism was 0.34 whereas intron 19 negative allele had higher prevalence for affected sibling compared to positive allele. Number of individual affected with Hemophilia A in the pedigree and number of deaths due to Hemophilia related complications in the pedigree were noted. **Conclusion-** Frequency of two common polymorphisms in intron 18 and 19 were observed whereas heterozygocity rate was comparable to world ethnic groups of hemophilic children.

KEYWORDS

Hemophilia A, Factor VIII gene Polymorphisms, Restriction Fragment Length Polymorphism, Intron 18 polymorphism, Intron 19 polymorphism.

INTRODUCTION:

Hemophilia is an X-linked recessive disorder of coagulation due to deficiency of factor VIII (Hemophilia A) or factor IX (Hemophilia B) coagulant activity characterized by spontaneous or trauma related bleeding typically into the large joints and muscles resulting in progressive deterioration of function (1). There are an estimated 75,000 Hemophilia patients in India, of them only 16,000 have been diagnosed (2). 2500 new cases are added every year. With almost no government or insurance support, in India, Hemophilia places a considerable financial burden on families (3). Considering the complexity and cost of management, prevention of Hemophilia by carrier detection and pre-natal diagnosis is considered a priority, especially in developing countries like India (4). But, due to the size and complexity of the factor VIII gene, heterogenous nature of the mutations and limited government support available, direct gene analysis is difficult and is currently available only in two centers in India (1, 3, 4). Thus, carrier screening and prenatal diagnosis of Hemophilia often depends on haplotype analysis using Restriction Fragment Length Polymorphism (RFLP) to track the defective factor VIII gene within a family. Efficient gene tracking in family depends upon the informativity of individual polymorphisms and thus the heterozygosity rate of each marker within the ethnic group to which that family belongs (3).

Studies from different parts of India have shown different heterozygous frequencies of polymorphisms in restriction sites of the two most commonly used markers, two commonest intronic SNP's rs4898352 (A/T) in intron 18 (BclI) and rs4074307 (A/G) in intron 19 (HindIII) intron 18 (Bcl I) and intron 19 (Hind III), making a uniform selection of all places of India, difficult, for the purpose of carrier detection (5-12). The present study was, therefore, undertaken to find out frequency of two intragenic markers, intron 18 and intron 19 in hemophilic patient in the most populous state of India, Uttar Pradesh and Bihar (13).

Patients and Methods:

Patients in the present study partcipated from two states of India, Uttar Pradesh and Bihar. Clinical details were recorded from history, detailed clinical examination and relevant laboratory studies. These clinical details could be recorded in 61 children. In few cases, some of the clinical details could not be documented. Clinical details recorded from history include age at the time of examination, age of onset of first bleeding episode, history of joint, muscle, gum bleeds, echymotic patches, intra-cranial hemorrhage, hematuria and gastro-intestinal bleeds and history of spontaneous bleeds. Frequency of bleeding was divided into 3 groups, those with bleeding once or more in a month, those with bleeding once or more in a year and those with bleeding once in few years. Minor echymotic patches were excluded while considering frequency of bleeding. Detailed pedigree analysis was done and number of affected siblings and number of affected people in the entire pedigree, number of deaths due to complications of Hemophilia were noted. Detailed clinical examination was done to assess number of joint involvement and those with age 4 or more years were assessed with Functional Independence Score In Hemophilia (FISH), a performance based assessment tool (15, 16). Factor level and Activated Partial Thromboplastin Time (APTT) with respect to controls were noted.

After informed written consent, around 5 ml peripheral blood was drawn in heparinized syringe under strict aseptic precautions from 62 children with Hemophilia A of 59 different families diagnosed by factor VIII assay. DNA isolation was done following standardized protocol according to standard guidelines (14). Quality and quantity of DNA was assessed. Genotyping for two intragenic markers, intron 18 (Bcl I) in 59 children and for intron 19 (Hind III) in 62 children, of factor VIII gene were done by PCR-RFLP method.

RESULTS:

Frequency of the two commonest intronic SNP's rs4898352 (A/T) in intron 18 (BclI) and rs4074307 (A/G) in intron 19 (HindIII) by restriction fragment length polymorphisms (RFLP) of Factor VIII gene in patients with hemophilia A of the most dense and populous states of India, Uttar Pradesh and Bihar (**Figure 1 and 2**).

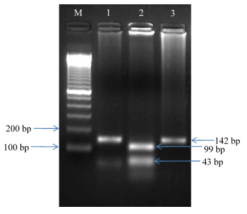

Figure 1: Detection of Intron 18 (BclI) polymorphism: Male 142 bp DNA fragment from intron 18 was digested with restriction enzyme BclI and analysed by electrophoresis using 3% agarose gel. Lane M: 100bp DNA molecular weight marker. Lanes 1 show undigested PCR amplicon of 142bp. Lane 2 shows +ve allele with two fragments of 99 and 43bp. Lane 3 shows –ve allele with undigested fragment of 142 bp.

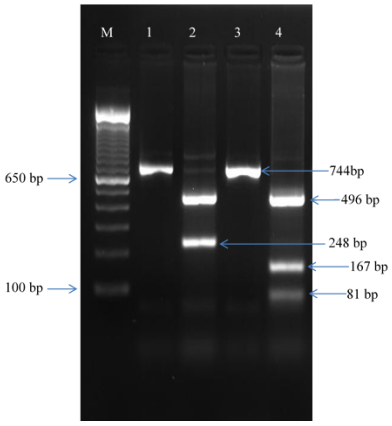

Figure 2: Detection of Intron 19 (HindIII) polymorphism: Male744 bp DNA fragment from intron 19 was digested with restriction enzyme Hind III and analysed by electrophoresis using 3% agarose gel. Lane M: 100bp DNA molecular weight marker. Lanes 1& 3 show undigested PCR amplicon of 744 bp. Lane 2 shows –ve allele with two fragments of 496 and 248 bp. Lane 4 shows + allele with three fragments of 496, 167 and 81 bp.

Positive allele frequency for intron 18 and intron 19 marker was 0.54 and 0.34 respectively. (**Table 1**).

Number of individuals affected with Hemophilia in the pedigree and number of deaths due to Hemophilia related complications in pedigree also showed an increased prevalence for intron 19 negative allele but that was not statistically significant.

DISCUSSION:

Manifestation of different clinical features had high heterogeneity among patients. Several studies have been done to look for informativeness of the intron 18 and 19 makers in context to carrier detection, present study and comparing with allele frequency distribution of particular marker in other population triggers to screen other markers in context to clinical manifestation in patients. In present study negative allele of intron 19 was found to be significantly associated with having an affected sibling compared to positive allele. Number of individuals affected with Hemophilia in the pedigree and number of deaths due to Hemophilia related complications in pedigree also showed an increased prevalence for intron 19 negative allele but was not statistically significant. Intron 18 alleles didn't show any trend for association of alleles with clinical features.

Studies from different parts of India have shown different allele frequencies for factor VIII gene markers of intron 18 and intron 19 in different ethnic groups of the world. This is understandable, considering the strong ethnic diversity within India (**Table 1and 2**) (3, 5-12).

Table 1: Allele frequency for intron 18 and intron 19 marker of factor VIII gene:

Polymorphic marker	Restriction enzyme	Number of patients	Alleles	Allele frequency
Intron 18	Bcl I	59	-ve allele (142 bp)	0.46
			+ve allele (99, 43 bp)	0.54
Intron 19	Hind III	62	-ve allele (496, 248 bp)	0.66
			+ve allele (496, 167, 81 bp)	0.34

Table 2: Allele frequency distribution in different studies from India for factor VIII gene intron 18 and intron 19 markers:

Allele frequency in different study from India				
Study	**Year**	**Number of cases**	**Intron 18 positive allele frequency**	**Intron 19 positive allele frequency**
Shetty S *et al.*	1997	37	0.41	---
Choudhury MR *et al.*	2000	63	0.43	0.35
Srinivasan A *et al.*	2002	112	0.68	0.33
Prabhavati P *et al.*	2002	43	0.67	---
Jayandharan G *et al.*	2004	50	---	0.29
Raza ST *et al.*	2009	100	0.57	0.38
Present study	**2018**	**59 and 62 respectively**	**0.54**	**0.34**

Table 3: Heterozygosity rates in different ethnic groups across the world for factor VIII gene intron 18 and intron 19 markers:

Ethnic group	Heterozygosity rate for intron 18 marker	Heterozygosity rate for intron 19 marker
Caucasian	0.39	0.38
Japanese	0.42	---
Chinese	0.33	0.37
Malays	0.33	0.35
American Black	0.31	0.34

Positive allele frequency in intron 19 showed almost same trend as reported in other studies from India (**Table 3**). We observed different positive allele frequency (0.54) for intron 18 marker compared to that reported by Shetty S et al. 1997 (0.41) in 37 unrelated families of west India who showed maximum heterozygosity (47%) compared to other population studies. Choudhury MR et al. 2000 reported 0.43 positive allele frequency for intron 18. This study was carried out in 63 hemophilia A patients and 102 of their female relatives of Northern India where highest heterozygosity of 52% was seen for intron 19 (Hind III) and positive allele frequency was 0.35 which matched with 0.34 in our population. Hind III, Bcl I and the intron 22 dinucleotide repeat combined were diagnostic in 87.2% of hemophilia A families in this population. A previous study from our population by Srinivasan A et al. 2002 reported 0.68 positive allele frequency for intron 18 (Bcl I) whereas in our study it came out to be 0.54 representing highly polymorphic marker but positive allele frequency for intron 19 is almost same in both studies 0.35 (Srinivasan A et al. 2002) and 0.34 (our). Srinivasan A et al. 2002 diagnosed about 64% of the families from the target population by intron 18 (Bcl I) marker alone and intron 19 (Hind III) marker was effective in 50% families. Prabhavati P et al. 2002 reported 100% cummulative efficacy of BclI and Xba I polymorphic site in intron 18 and 22 for carrier detection in 15 families of South India. Positive allele frequency for intron 18 (Bcl I) was 0.67 whereas in our study it was 0.54. A study on 100 patients and their relatives from North India by Raza ST et al. 2009 reported positive allele frequency 0.57 and 0.38 for intron 18 and intron 19 respectively which had almost same trend reported by us i.e. 0.54 and 0.34 respectively.

Some of the previous studies have calculated heterozygosity rates in different severity of cases but none of them have correlated with clinical details (3). Limitations of our study are smaller sample size and absence of complete mutational analysis of cases studied for RFLP. Studies with smaller sample size may not pick up subtler clinical differences in different heterozygous groups which may be useful in deciding appropriate management and follow-up plans for individual cases. This is evident in our study, has to be confirmed with further studies with larger sample size. This study gives an indication to screen more number markers in different population of hemophilia either individually or in different combinations.

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Conflict of interest: None

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