

Haemophilia in the Indian Scenario

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ABSTRACT Deficiency of clotting factors VIII and IX leads to haemophilia A and B both inherited as sex-linked recessive pattern. There are significant variations of genetic make up in ethnic population of India as well as clinical variants of haemophilia A and B and may form crippling deformities due to bleeding at different sites if not treated early. In view of their rarity due to lack of awareness and non availability of high cost factor replacement, management of haemophilia is a problem in India. Moreover, the prevention of haemophilic birth is limited due to absence of facilities of detection of carriers of haemophilics and prenatal diagnosis. The use of factor VIII and factor IX prepared from human plasma for treatment leads to transmissible viral infection (HBV, HCV, HIV) so common in India. The absence of factors from aDNA technology limits the prophylaxis and curative treatment.

The inherited disorders of blood coagulation present providers of health and social care with formidable problems. The disorders are eminently treatable, even in their severest form. Untreated, they result in handicaps in early life, while proper treatment is expensive, inadequate treatment is even more so, both to the individual and to the community (Bhattacharya 1987). The commonest of the disorders is haemophilia A or clotting factor VIII deficiency. Haemophilia B or clotting factor IX deficiency is one fifth less common than haemophilia A. Both these disorders are inherited as sex linked recessive. Isolated deficiencies of other clotting factors are less common because they are usually inherited in autosomal recessive manner and this requires both parents to carry the abnormal gene. The disorder called von Willebrand disease, in which the factor VIII molecule is abnormal, is usually inherited as autosomal dominant fashion and is therefore the most common, but overall is the least severe of the inherited clotting disorders. No geographical, ethnic or racial variations in the incidence of either haemophilia A or haemophilia B are known. The incidence of haemophilia A is very low and it is easy to see how the disease can be overlooked in countries, like India, faced with major problems of malnutrition and infection. However ICMR Task Force (1990) estimates that 1330 children are born every year and 50000 patients of haemophilia A are there of whom less than 5% have access to medical facilities. The World Federation of Haemophilia (WFH) acknowledges the fact that the treatment is only presently available to around 20% of those with severe haemophilia in developed countries and the

number is insignificant in developing countries (Jones 1991). The task is therefore to do every thing possible to help persons with haemophilia in developing countries, arrange for the provision of effective therapy. Typically a severely affected person with haemophilia A bleeds 35 times a year on average necessitating supplement of factor 1000 unit per bleed. The bleeding frequency is likely to be higher in tissues previously damaged by uncontrolled haemorrhage. This gives an overall idea of cost of treating a haemophilic.

Discovery of the molecular structure of both factor VIII and factor IX has recently allowed the development of genetically engineered products, prepared using recombinant DNA technology. In the long term, Preparations presently prepared from human plasma may be superseded by recombinant clotting factor concentrates (aDNA).

Nearly one third of cases of haemophilia occur with no preceding family history, possibly from new genetic mutation. When recorded family history is available, efforts should be made to identify female carriers. Identification depends on family history, measurement of clotting profile and DNA analysis. Certain options are available of identifier of carriers of severe haemophilia. These include, preimplantal diagnosis of an embryo following in vitro fertilization, fetal diagnosis of chorionic villus sampling (CVS) using DNA technology, fetoscopy using clotting factor assay and amniocentesis in order to obtain confirmation of fetal status. One third of haemophilia carriers have factor VIII or factor IX levels below normal and are in danger of abnormal bleeding following injury, surgery or dental

extraction but not in pregnancy as factor VIII level rises normally (Choudhry et al 1996; Kashyap and Choudhury 2000).

GENETICS OF HAEMOPHILIA

Haemophilia A and B are X-linked recessive disorders that occur almost exclusively in males. About 30 percent of the mutations arise de novo. Factor VIII gene is very large, about 186kb, with about 9kb of exons. It contains 26 exons and 25 intervening sequences or introns. The size and complexity of the gene have made it difficult to pinpoint, on a routine basis, specific mutations that result in haemophilia. However, the factor VIII gene has now been cloned and sequenced and numerous specific mutations have been described (Williams 2001; Jayandharan et al. 2003).

The use of markers for restriction fragment length polymorphism (RFLP) is simpler than direct sequencing of the coding region of the factor VIII gene, but the use of technique requires that the pedigree analysis includes at least one haemophilia male whose mother is heterozygous for the one or more RFLP markers. Haemophilia B is clinically indistinguishable from haemophilia A. It is a sex-linked recessive disorder characterized by decrease of factor IX clotting activity. The factor IX gene is on the long arm of the X chromosome and is about 33kb in length, much smaller than the gene of factor VIII. Because it is less complex, the factor IX gene has been studied in greater detail. Many gene variations of haemophilia B have been described including point mutation, frameshifts, deletions and other abnormalities. However, haemophilia B inheritance is same as to that of haemophilia A (Williams 2001).

Factor VIII Gene Polymorphism in the Asian Indian Population

An extensive study was undertaken to explore the heterozygous frequency of polymorphism markers within and flanking the factor VIII gene in Indians and identify those most informative for carrier screening and prenatal diagnosis. Factor VIII gene polymorphism analysis at intragenic and extragenic sites was carried out by the polymerase chain reaction (PCR) method and Southern blot procedure. Sixty-three Asian Indian haemo-

philiacs and their families were screened. A control group of 150 women from nonhaemophilic families were screened for two markers, HindIII and BclI. Among intragenic markers studied, the HindIII restriction fragment length polymorphism (RFLP) showed the highest heterozygous frequency (0.52) followed by the intron 13 (0.47), intron 22 (0.44) and short tandem repeat (STRs). Among extragenic markers, TaqI had the highest heterozygous frequency (0.75) followed by BglII (0.54). The intron 22 inversion mutation was observed in eight (40%) of 20 severe cases. In the population studied the most diagnostic polymorphism were the intragenic markers, intron 22(70%) STR followed by the intron 13(52%) STR and HindIII (52%) RFLP, and the TaqI (50%) extragenic marker. Application of HindIII, BclI and the intron 2 dinucleotide repeat combined were diagnostic in 87.2% of haemophilia A families studied (Chowdhury et al. 2000).

Elsewhere (Shetty et al. 1998) intron 1 and 22 inversions were looked for in 80 severe haemophilia A patients using PCR and multiplex Long-Distance Sub cycling-PCR, respectively. Intron 1 inversion was in 3(3.75%) and intron 22 inversion was seen in 35 (43.75%) patients. Of severe haemophilics, 47.5% had either of these inversions. It is thus suggested that screening for inversions may be the first step in genetic testing of Indian haemophilics.

Blood samples suitable for DNA analysis are needed in order to advise patients requesting carrier detection or prenatal diagnosis. The absence of specimen from a relative with haemophilia can make the diagnosis of carrier impossible.

Detection of Haemophilia A Traits in Carriers

Haemophilia A which has an incidence of 1 in 10000 male births (28), imposes a severe strain on the affected individual and his family as well as on the blood transfusion services of the country. It is therefore essential to detect the potential carriers of haemophilia A, so that they can be given precise genetic counseling.

Blood samples were collected from normal women and obligate carriers of haemophilia A in the reproductive age group. The obligate carriers were the mothers of known haemophilics who are being treated at the center. Blood (4.5 ml) was divided into aliquots, one of which was taken for prothrombin time (PT), activated thromboplastin

time (APTT) and factor VIII coagulant activity (FVIII C) measurements while the other was snap frozen and stored at -70°C for later use in factor VIII related antigen (FVIII R: Ag) determination. The APTT was significantly higher in carriers. The levels of FVIII C and R: Ag were significantly lower in carriers than in normal individuals. The ratio of FVIII C to FVIII R: Ag in carriers was 0.55 while that in normal was 1.0. By applying the linear discriminating the result of the study indicates that the ratio of FVIII C to FVIII R: Ag can be of use for the routine detection of obligate carriers of haemophilia A (De et al. 1989).

Carrier Detection of Haemophilia Families with DNA Polymorphism Analysis

Linear discriminants that include data on factor VIII: C and von Willebrand factor antigen levels are well established tools in estimating the probability of carriership in haemophilia A families. A comparison between the conventional coagulation data, i.e. the ratio of factor VIII: C and von Willebrand factor antigen, and the DNA analysis techniques was made. The lowest misclassification rate, i.e. among the carriers, was seen when a cut-off value of 0.7 was chosen. In the case of normals all were outside this cut-off value. Thus, it was considered as a workable reference value for classifying the carriers in haemophilia A families. The optimal service for haemophilia A carrier diagnosis must include above coagulation test, probabilities as DNA marker studies. However, it is recommended that the smaller laboratories in developing countries can benefit immensely by only establishing factor VIII: C and von Willebrand factor antigen estimation (Shetty et al. 1998a, b).

Prenatal Diagnosis in Haemophilia A

The heterogeneous nature of the mutation, the size, and the complexity of the factor VIII gene makes direct mutation analysis of haemophilia A families in India an option that is not very feasible and practical. Thus, carrier screening and prenatal diagnosis of haemophilia A often depends on haplotype analysis using restricted fragment length polymorphisms (RFLP) and short tandem repeat (STR) markers to track the defective factor VIII gene within a family. Study was designed to assess the utility of using polymerase chain reaction (PCR) based five

polymorphic markers, four intragenic Hind III, Bc II, intron 13, intron 22 STRs and one extragenic marker St14 in prenatal diagnosis. Linkage analysis with the combined use of these five PCR based polymorphic markers, gives good informativeness of 87.8% in the Indian population. Of the 41 CVS tested, 21 were found to be male fetus and of these 13 were found likely to be affected with haemophilia A. Only in 12.2% of the families were none of the markers was informative (Chowdhury et al. 2003).

Molecular Characterization of Haemophilia A and B

Forty-seven haemophilia A patients from 43 pedigrees and 34 haemophilia B patients from 31 pedigrees were screened for the presence of mutations by Southern blotting using factor VIII and cDNA and genomic DNA probes. Three deletions and two restriction site variants were detected among 47 haemophilia A patients and one deletion and two restriction site variants were detected in 34 haemophilia B patients. Overall, the frequency of mutations in haemophilia A was 10.6%; the frequency of deletion was 6.4% and that of point mutations was 4.2%. in haemophilia B the frequency of mutations detected was 9%; deletions 9% and point mutations 6%. The present report, the first from India, shows that like other earlier published reports from Europe and the United States, mutations in haemophilia are heterogeneous, and that RFLP using Southern blotting did not detect most of the mutations in disorder and is an insensitive and inefficient procedure (Shetty et al. 1998).

Heterogeneous mutations in factor IX gene cause haemophilia B and a large number of mutations have been characterized. However, reports on gene defects among Indian haemophilia B patients are rare despite a high estimate of such patients in the country. One report of identification of 22 independent mutations including five novel mutations in 24 unrelated patient showed novel gene defects include two point mutations, two deletion and one insertion of a LINE 1 element. Majority of the mutation (14 of 24) occurred on the same haplotype background, but do not suggest any founder effect. Direct identification of mutations can be utilized to perform the carrier detection and prenatal diagnosis, especially in families with isolated patients. (Mukherjee et al. 2004)

Gene polymorphism and carrier analysis for haemophilia A & B was utilized in the three common intra and extragenic polymorphic sites of the factor VIII and IX genes. The approach for haemophilia A carrier detection included tests for BclI, XbaI, and TaqI polymorphic sites for introns 18 and 22 and the extragenic locus St 14, respectively, whereas for haemophilia tests include detection of TaqI, DdeI, and HhaI polymorphic site for introns 4 and 1, and the 3' flanking region of the factor IX gene respectively. In haemophilia A, the cumulative efficiency of these three polymorphism has been found to be 100%. A low effectiveness of TaqI restriction site in carrier analysis of haemophilia B families has been observed. (Shetty et al. 1997)

Management of Haemophilia in Developing Countries

The problems with management of haemophilia in developing countries are poor awareness, inadequate diagnostic facilities and scarce factor concentrates for therapy (Shrivastava et al. 1998). The priorities for services for haemophilia include training care providers, setting up care centers, initiating a registry, educating effected people and their families about the condition, providing low-cost factor concentrates, improving social awareness and developing a comprehensive care team. A coagulation laboratory capable of reliably performing clotting times with correction studies using normal pooled, FVIII and FIX deficient patient plasma and factor assay is most essential for diagnosis. More advanced centralized laboratories are also needed. Molecular biology techniques for mutation detection and gene tracking should be established for accurate carrier detection and antenatal diagnosis. In India, there is no support from the government. Services, Import of factor concentrates are organized by the Haemophilia Federation of India with support from other institutions. Haemophilia is managed with minimal replacement therapy. Developing world providing haemophilia services should define standards for care and set achievable goals. Haemophilia societies act as strong media in educating affected families. In developing countries FVIII replacement is not easily available for mass treatment. In Malaysia the system is fully supported by the government and moderate level of factor concentrate are available on

demand. Haemophilia care in South Africa is provided through major public hospitals intermediate purity factor concentrate are locally produced at low cost. Treatment of severe haemophilia and related disorder is dependent on availability of preparations containing factor VIII. Factor VIII is present in fresh frozen plasma, lyophilised fresh plasma, cryoprecipitated factor VIII concentrate either human or porcine factor VIII concentrates (ãDNA). Factor IX is present in fresh frozen plasma cryoprecipitate removed plasma or lyophilized, factor IX concentrate (human) or ãDNA. In haemophilia A other suitable drugs may be used (Sundar et al. 1993).

Replacement of Factor VIII in Haemophilia A

Management of haemophilia is almost exclusively based on administration of commercial Factor VIII concentrates prepared from plasma obtained from thousands of donors. Treatment with commercial Factor VIII concentrate presents two major problems *viz.*, (a) it is very expensive as often it has to be imported; (b) there is a high risk of contamination with hepatitis B virus, hepatitis C virus as well as HIV, from the large number of donors.

Production of good quality cryoprecipitate from plasma was achieved, as assessed by quantitating Factor VIII coagulant activity (F VIII C) and Factor related antigen (F VII R: Ag). The method used resulted in good recovery of FVIII in the cryoprecipitate containing FVIII C: Fibrinogen 0.82 ± 0.015 IU/mg. The method therefore appears suitable for indigenous preparation of F VIII in standard blood banks as a replacement therapy, which is not expensive (De et al. 1989, 1991). High purity cryoprecipitate may be used in surgery (Ghosh et al. 1998).

Viral transmission in Multitransfused Haemophiliacs

Seropositivity of HBV in multitransfused patient of haemophilia A, haemophilia B, from Eastern India, was found to be high. HIV seropositivity was detected in patients of haemophilia A (4.4%) who received plasma components. Seropositivity both HbsAg and HIV was found in one patient of haemophilia. The universal voluntary blood donation programme, screening of blood for HBV and HIV by sensitivity tests, early immunisation and periodic

monitoring of HBV and HIV status are prerequisites for the management of haemophilia (De et al. 1990).

Elsewhere in a study of multitransfused haemophiles in Central India 15 (12.1%) were confirmed to be positive for HIV infection. All except one had received both foreign and Indian cryoprecipitate. However, one haemophilic seroconverted within 4 months of receiving a cryoprecipitate manufactured in India (Singh et al. 1991).

In a more recent study of transfusion dependent non-A-non-B (HCV) in haemophiles from Eastern India shows a high prevalence of such viral disease (Bhattacharya et al. 1992). An enzyme immunoassay (EIA) recently introduced for the detection of antibodies to HCV (anti-HCV) was employed for monitoring NANBH. The frequency distribution of NANBH on the basis of serum alanine aminotransferase (ALT) showed wide variations and the assay of anti-HCV was considered a suitable parameter for identification of NANBH. An assay of anti-HCV could determine relative prevalence of NANBV in high risk recipients of blood and blood products, who had concomitant hepatitis B virus (HBV) infection. The frequency distribution of NANBV *vis-a-vis* HBV in transfusion dependent haemophiles was seen in 20 haemophiles (A=18, B=1, C=1) who were tested for antibodies to hepatitis C virus (anti-HCV) and markers for hepatitis B virus (HBV). The seropositivity for anti-HCV in haemophiles was 25 percent. The subjects who were seropositive to anti-HCV had additional exposure to HBV. Anti-HCV positivity was not related to the age of the subject nor the number of blood components transfused. Screening of blood donors for anti-HCV, apart from HBV, may minimize the hazards of post transfusion hepatitis in high risk recipients like transfusion dependent haemophiles (Raypaladhi et al. 1990; Bhattacharya et al. 1992; Sengupta et al. 1992).

A comprehensive study of transfusion related human immunodeficiency virus (HIV) hepatitis B (HBV) and hepatitis C (HCV) from Western India suggests a reduction in blood product related HIV transmission in severe and moderately affected haemophiles but more stringent policy for blood product usage, universal hepatitis C screening, hepatitis B vaccination and continuous awareness programmes for medical staff, general public and patients is needed to reduce

the incidence of these diseases in haemophiles (Ghosh et al. 2000).

Rare Inherited Coagulation Disorder in India

A comprehensive study of twenty four cases with rare coagulation disorders were diagnosed over a 4 year period. These included 8 patients with factor X deficiency, 7 with factor XIII deficiency, 4 each with fibrinogen and factor VII deficiency and 1 with factor V deficiency. All the patients had presented with bleeding manifestations. Two patients with factor X deficiency showed interesting clinical presentation, one recurrent deep vein thrombosis and another had a pseudotumor of the thigh (Kashyap et al. 1996).

Congenital abnormalities of fibrinogen are rare disorders and all cases reported in the literature indicate that the incidence of afibrinogenemia is much higher than hypofibrinogenemia. Of total 20 cases reported from other parts of India only one was congenital hypofibrinogenemia. In contrast, eight patients with congenital hypofibrinogenemia among a total of nine unrelated North India patients with a fibrinogen abnormality were found. This disproportionately high incidence of hypofibrinogenemia suggests the existence of a distinct genetic defect in the North Indian population (Pati et al. 1995). A combined factor V and factor VIII deficiency has been reported (Shetty et al. 2000). Clinically significant inheritors in haemophilia A patients from India tend to persist (Shetty et al. 1999).

Acquired Haemophilia

Acquired haemophilia often presents a perplexing clinical course. A report of haematological features in 10 patients with acquired haemophilia showed that three had FVIII inhibitors following pregnancy while in six the cause for development of inhibitors could not be determined. One patient had acquired von Willebrand's disease. Lupus anticoagulant coexisted with factor VIII inhibitors in the three patients. All patients presented with sudden onset of bleeding without any past or family history of a bleeding disorder. Factor VIII inhibitor levels ranged from 8 to 512 Bethesda units in the nine patients. Immunosuppressive therapy was given 8 patients, consisting of corticosteroids with endoxan or cyclosporin. Seven patients had clinical and laboratory responses and one

patient did not respond. One patient had seven postpartum bleeding with acute shock which was controlled with FEIBA. Diagnosis and management of idiopathic acquired haemophilia, thus, continues to be a major challenge, and among them postpartum haemophilia has good prognosis (Saxena et al. 2000).

Prevention of Haemophilia

Modern techniques, including DNA technology have extended the choices open to couples planning their families. When there is a family history of haemophilia it is not possible to identify accurately most females who carry the haemophilia gene. Women who know they are carriers may have options for prenatal diagnosis to obtain information of fetal status. Very rarely haemophilia occurs because the parent is a mosaic in which two or more genetically different cell lines develop from a single zygote- for instance fertilization of an ovum from a nest of genetically distinct cells in the maternal ovary may result in a haemophilic son of a mother who herself test a normal on DNA analysis (Jones 1991).

Haemophilia Services in India

With a population of 853 million there should be 51,204 patients with haemophilia A in India assuming a prevalence of 6/1000,000 population. With the current birth rate of 32/1000, 1,300 new patients with haemophilia A will be born each year. Hospital base data suggests that haemophilics in India suffer from preventable morbidity because doctors do not know enough about the disease and its management, because laboratory diagnostic facilities are inadequate and because there is not enough therapeutic material even if it is available the patients do not have the resources to purchase it. The current status of haemophilia in India and measures to improve haemophilia services with the health care infrastructure available in the country is meagre (Shrivastava et al. 1998).

CONCLUSION

Haemophilics Comprise a group of patients whose diagnosis and management are complex and costly. The rarity of the disorder, its life long number, variable severity and the fact that they are apparently well unless bleed add to the

complexity. The lack of prompt and appropriate treatment in developing country like India lead to prolonged immobilization, wastage of expensive factor replacement and burden to health care system. The rise of transfusion related viral disease in haemophilics over weights the risk of death or crippling disorder as result of bleeding. Blood donors from areas of low prevalence of HIV and HCV provide safe product until factors from aDNA is widely available.

Infrastructure needs to be built up for preventive measures of haemophilia like early diagnosis, carrier detection and prenatal diagnosis in major centres in order to minimize birth of haemophilic child. Haemophilia Federation of India (HFI) in collaboration with world Federation of Haemophilia (WFH) has established a network for supplementation of factor for replacement in Haemophilia.

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