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Hemophilia and allied disorders care in India : A story of dismay and success

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INTRODUCTION

Hemophilia and allied conditions collectively referred to as "hemophiloid disorders" are a group of disorders due to inherited deficiency of blood coagulation factors leading to life long bleeding disorders. The factors most frequently found deficient in hemophilias are factors VIII (FVIII) and IX (FIX), whose genes are located on the X-chromosome and when mutated, cause the X-linked recessive traits called hemophilia A and B. The reported incidence of hemophilia A is 1 in 10,000 births and that of hemophilia B is 1 in 60,000. Deficiencies of other coagulation factors, which are transmitted as autosomal recessive traits and affect both sexes; are much rarer (1 in 500,000 or less). There is paucity of epidemiological data on the incidence of hemophilia in developing countries. However, as per WHO data 4.8 billion out of 6.0 billion people in the world live in the developing countries belonging to Asia, Africa and South America (1). There is tremendous social and economic diversity within this group leading into significant differences in the incidence, prevalence and management of inherited disorders (2).

Hemophilias occur in mild, moderate and severe forms (corresponding to plasma factor levels of 6-30%, 1-5% and less than 1% respectively). Hemophilia A and B are clinically indistinguishable and are characterized by delayed, prolonged and repeated bleeding episodes. Although patients with mild hemophilia usually bleed only after trauma or surgery, those with severe hemophilia bleed spontaneously or after trivial trauma particularly into joints and muscles, on average 20 to 30 times per year but sometimes more frequently. Hemorrhages within joints and muscles, unless treated adequately with deficient factors results in painful, progressive joint damage and muscle atrophy; resulting in severe disability and limitations of daily activities. These physical disabilities are compounded by associated psychological problems. Hence, *Comprehensive care of Hemophilias* are very essential for successful management of these patients.

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In this communication I will describe to you how we are able to establish the comprehensive hemophilia care in India: the problems, frustrations and ultimate success.

World Scenario in the end of 20th country : The modern management of hemophilia started in the 1970s, when the increased availability of plasma concentrates of coagulation factors and the widespread adoption of home replacement therapy led to the early control of bleeding and to the reduction of musculoskeletal damage typical of untreated or poorly treated patients. Furthermore prophylactic treatment was started in Sweden and other countries with very successful outcome of preventing the majority of bleeding episodes and also arthropathy. Therefore, on the whole during these years hemophilia care became one of the most gratifying examples of successful secondary prevention of a chronic disease. However, this optimistic perception changed dramatically in 1980s, when 60-70% of patients became infected with HIV virus. It was also found that they were also infected with hepatitis C virus (HCV) transmitted by factor concentrates manufactured from large pool of plasma.

The last 15 years witnessed the production of safer plasma concentrates of coagulation factors due to the efforts of the scientific communities. The availability of recombinant factors has been the result of progress in DNA technology. Analysis of patients DNA has permitted identification of the mutations in these disorders and have allowed secondary control of disease through carrier detection and antenatal diagnosis. New treatments have substantially improved the previously unfavorable prognosis of patients who develop alloantibodies (inhibitors) to F VIII and IX. Finally in the past few years the first experiments of somatic gene therapy started in persons with hemophilia which has promising results.

Indian Scenario:

Almost 100,000 patients with haemophilia exist in India, although there is no large epidemiological data available in our country. Inadequacy of health care facilities, lack of adequate knowledge of bleeding disorder among primary care physicians and poorly developed haematology services particularly with reference to good diagnostic facilities of bleeding disorders contribute to the fact that the vast majority of patients with hemophilia are undiagnosed Suffice to tell in short that it is an under diagnosed entity. The state expenditure on health is usually 1% of GDP. Considering the large population, the per capita expenditure on health is often $1 per annum. Diseases of greater public health importance i,e infectious diseases and malnutrition take major share from the health budget. The result is that "low-volume-high cost" disorders like hemophilia get little attention in the health planning. There are few laboratories that can provide accurate diagnostic services for bleeding disorders. The fact remains that the clinicians often have little interest in diagnosing diseases they cannot treat, leads to inadequate volume of work to sustain good laboratory services. It is also not enough to attract industry to market appropriate equipments, reagents and also the therapeutic materials i.e the factor

concentrates both recombinant and plasma derived. This again complicates in providing quality diagnostic services and management.

Establishment of Quality Laboratory: We established therefore good coagulation and hemostasis laboratory at I.I.H, Mumbai in the year 1993 which was recognized by WFH and ISTH in course of time. This is a tertiary centre and reference laboratory too. Regular quality control exercise is under taken here. Subsequently this centre was recognized as one of the International Training centre (IHTC) from the year 1999. The aim of the IHTC programme is to disseminate medical knowledge and experience in the diagnostic and management of haemophilia and other coagulation disorders in order to improve the quality of hemophilia care and services in developing countries. This overall activity is achieved by providing training to physicians, Surgeons and laboratory personnel's and also by holding regional workshops providing theoretical lectures and practical demonstrations on the care of haemophilia. These centres have been chosen by WFH for the excellence, for the appropriateness as role models within their region for the diversity of training which they can offer in various aspects of haemophilia care, for facilities they provide.

Carrier Detection and prenatal diagnosis in Hemophilia Families:

Since the treatment of hemophilia is very expensive in the developing country we decided to establish the "Prevention Programme" which is quite in-expensive compared to the huge cost of the management of PWH. It has a greater relevance in the developing country like India. These are achieved in the following ways:

A) Detection of hemophilia by pedigree analysis and phenotype assessment.

It is possible to detect hemophilia carriers by pedigree analysis and by performing some coagulation tests like factor assays both coagulant as well as antigenic.

However, both pedigree data and phenotype assessment are subjected to the limits of probabilistic evaluation, which in the best of the conditions carries no less than 3-20% of error rates. The lower values within the range have been obtained with rigorous testing procedures and sophisticated statistical analysis. Certainly this is an important parameter in many cases. The efficacy of the coagulation data in the classification of carriers and normal controls were assessed by us in our Indian population. (3) (Fig 1, 2)

In case of hemophilia A, the ratio of factor VIII : C and VWF was used as a discriminant and in case of hemophilia B, univariate analysis using factor IX: C was used as a discriminating parameter. In hemophilia A, with a ratio of 0.7 for F VIII: C and VWF Ag there was 92% agreement between the coagulation data and DNA analysis, where as in case of hemophilia B there was only 76% agreement between coagulation parameter and genetic analysis.

B) Carrier detection by DNA analysis:

After the cloning of factor VIII and IX genes, an accurate diagnosis of the carrier state and prenatal diagnosis of hemophilia A and B in the foetus is possible by DNA analysis.

There are two prevalent mutations in severe hemophilia A. About 36% of cases in our series were found to have inversion involving a gene within intron 22 of the F VIII gene, of which 2 further copies exist distal to the factor VIII locus on Xq 28. (4). Recently we also found inversion in intron 1 accounting for about 2% of cases. We have performed the carrier detection by linkage analysis with restriction fragment length polymorphism (RFLP). Usually polymorphic markers in and around factor VIII and IX genes are chosen. These markers are either biallelic or multiallelic and have successfully been used to track down the mutations through the hemophilic families. Following are the strategies for carrier detection in hemophilia A and hemophilia B families in our centre (Figs. 3&4). Our centre has, amongst the developing countries, experience of carrier detection in the largest number of cases so far and also prenatal diagnosis. The efficiency of three common intra and extragenic polymorphic sites of the factor VIII and IX genes has been examined by us in the Indian population (5). In the course of investigation we found a case of recombination between st 14 & the factor VIII gene. For our Indian population we found that for hemophilia A carrier detection Bcl 1, xba1 and taq 1 polymorphic sites for intron 18 and 22 and the extragenic locus st 14 respectively are most suitable amounting to 100% cumulative efficiency shown in Fig 5. For hemophilia B the polymorphic markers determination includes taq1, Dde1 and Hha1 for introns 4 & 1 and the 3' flanking region of the factor IX gene respectively. It indicated the low efficiency of the Taq1 restriction site (18%) in factor IX gene in our population as compared to 45% in caucasian.

Prenatal diagnosis: The strategy for prenatal diagnosis in our centre in the first trimester of pregnancy by CVS is shown in Fig 6. The prenatal diagnosis can be done either in the first trimester or second trimester of pregnancy in a carrier female. The first trimester diagnosis is based on RFLP method. The technique thus involves an index case of hemophilia in the family. In the second trimester the diagnosis is offered by determining the coagulation parameter like level of factor VIII:C activity and also VWF : Ag in the fetal blood sample obtained by cordocentesis under ultrasound guidance at 16 to 18 weeks of pregnancy. The difficulty arises when the index case in not available. Our experience has been published else where (5). The DNA diagnosis approximately costs $100 and is found to be cost effective. Chances of misdiagnosis is about 1-2%.

Genetic Counselling:

This is an important aspect of our procedure for carrier detection and antenatal diagnosis. It is mandatory that formal counselling should be done before any laboratory tests are even considered. Genetic counselling therefore remains an important aspect of hemophilia care helping obligate carriers / those with unknown status to make informed decision. Some of the important points that should be kept in mind while doing this procedure are:

1) The female who seeks antenatal diagnosis must have her carrier status confirmed by DNA analysis before CVS procedure.
2) The affected person in the family also should have a confirmed diagnosis and the factor level determined.

3) The coagulation factor level of the carrier female determined and if she is a symptomatic carrier care should be taken after the procedure if there is excessive bleeding.

4) All the relevant family members including the affected person should be available for investigations.

5) Improper blood sampling or inaccurate labeling may result in misdiagnosis. So, extreme caution is being applied to this procedure. Preferably the CVS sampling should be done in the same centre where the genetic tests are being offered and the person responsible should be present during the procedure.

6) The patients and their relatives should realize and give consent that occasionally the tests might end in inconclusive or inaccurate results based on the fact that not all females are informative for markers used or that there is recombination. However this occurs only in <0.5% of cases.

7) To eliminate maternal DNA contamination in CVS, one can have some different markers.

Now, with improved technology one can go for sequencing of the gene by automatic DNA sequencer to find out the mutation. It is also thought that impaired folding and /or altered conformation of the mutant factor VIII lead to both intra and extracellular instability, which in turn causes factor deficiency in plasma.

Development of Strategy for Economic use of clotting Plasma Products:

(a) Management during surgery: Attempt has been made by us to plan and manage with less amount of factor concentrate for patients with hemophilia who need an operation (6). A patient with an inherited bleeding disorder like hemophilia may need surgical intervention due to diverse common ailments just like his non-hemophilic counterpart. With the availability of factor concentrates in liberal amounts, the scenario of surgical management in patients with hemophilia in western countries has changed substantially except fro those with high inhibitor level. However, factor concentrates are costly and liberal use in major surgery for an adult patient may consume upto 50,000 to 80,000 I.U. of factor VIII in patients with severe hemophilia if carried out according to standards set by developed countries. These standards are arbitrary and were never established by double blind trials finding out the minimum. Under these circumstances there is a pressing need for planning the surgical operation in a patient with hemophilia in such a way that a limited amount of factor concentrates is used along with several other measures without risking excessive bleeding in the patient.

(b) Planning Operations: Any major or minor operative procedures must be well planned in a centre where experienced haematologists are available along with a good hemostasis laboratory, good blood bank services and surgeons having experience in managing such cases. Relevant imaging and other studies required to manage surgical procedure and postoperative period. The inhibitor

status determined preoperatively and if the patient has an inhibitor level of > 10BU/ml then the FEIBA or recombinant factor VII A may be made available.

Emergency Surgery:

If a known hemophiliac and the factor level is known then the patient should receive 100% factor correction before undergoing surgery. A problem arises when a patient with mild or moderate hemophilia who does not know about his disease comes for emergency surgery or even for elective surgery. The diagnosis can only be made after proper investigations. One of the most important things is to know about bleeding diathesis of the patient by collecting his past history about how he has tackled the hemostatic stress like previous operation, child birth, dental extractions, injuries etc and also the detail family history.

Our emphasis was on reducing the factor concentrate use and we are successful in doing so by following the steps described below:

(a) Use of sealant :

Cryoprecipitate which contains fibrinogen and tranexamic acid was taken in one syringe and thrombin in another syringe connected with plastic "Y" connector and then spread together on the wound surface. Immediately the reaction takes place, fibrin glue is generated and hemostasis obtained. This home made product is cheap and was able to stop bleeding from the surgical wounds where a large raw area of oozing remains following surgery. We have used this technique for circumcision with gratifying results and the patient needed only single dose of factor concentrate just before operation.

(b) Use of DDAVP:

DDAVP is known to increase factor VIII levels in patients with hemophilia A 3 to 5 times. So, milo & moderate hemophilia cases can be given the drug just 30 to 60 minutes before operation. It is usually administrated at a dose of 0.3mg/kg in 50ml of normal saline as an intravenous infusion. The medicine is contraindicated in cases of hypertension and coronary artery disease . But the problem is tachyphylaxis development and not all patients do responds to it. So, one has to find out the response in each case. The patients can be given initially daily for 3-4 days & then on alternate days for another 4-5 days keeping an eye on factor VIII level.

(c) Use of Drugs inhibiting Fibrinolysis:

We have found that concomitant use of fibrinolysis inhibitor drugs like EACA and tranexamic acid cut down the factor concentrate requirements. Infect in an in vitro study, we have been able to show that EACA may specifically improve the factor VIII economy in the patients with inhibitors. Tranexamic acid is a better inhibitor of fibronolysis than EACA (Epsilon Amino Caproic Acid). We use EACA more liberally except in urological surgeries and hematuria in patients with hemophilia. Antifibrinolytic drugs can be used locally over the operation field at a dose of 100mg/ml of EACA or 10mg/ml of Tranexamic acid. We have used EACA successfully for orthopedic, procedures like open reduction and plating in cases of fractured femur.

In our patients needing orthopedic surgical procedures we have been able to wean these patients of factor concentrates by 12 days and subsequently used 10IU/ Kg twice or thrice weekly during the beginning of active physiotherapy for first 2 wks. Hence it may be said that antifibrinolytic agents should be used in severe cases of hemophilia more liberally.

d) Problems with patients having an inhibitor:

It is a difficult decision to operate on patients with hemophilia A who have developed inhibitors. Of course the magnitude of the problem of this kind is low, we have successfully used FEIBA and EACA in high doses along with cryoprecipitate in some of the cases.

e) Economizing on the use of Factor concentrates in the post operative period:

Continuous use of factor concentrates has been shown to be one of the effective ways of maintaining constant level of haemostatic factors leading to substantial saving of factor concentrates. However, it is a must to have > 80% factor VIII level and > 60% factor IX level during the operation but subsequently keeping trough level at 30% with other measures will ensure adequate hemostasis. Adequate level of clotting factors can also be maintained by frequently giving relatively lower doses of the factor. The use of FFP and cryoprecipitate also cut down the factor concentrate amount.

Inhibitor to factor concentrate and management of such cases:

Hemophilia Patients with inhibitors pose a formidable challenge for patient management. This is particularly problematic in developing countries where porcine factor VIII, FEIBA, factor VIIa or immunoadsorption column are generally unavailable. We investigated both in vivo and in vitro, the effect of EACA on the inhibitory activity of the inhibitor to factor VIII. It was found that the in vitro EACA substantially inhibited the activity of the inhibitor and had no effect on other immunological reaction like red cell agglutination. The same was confirmed by antigen binding ELISA system also (7). Factor VIII inhibitors are IgG alloantibodies that arise during replacement therapy in 25 to 50% of patients with severe hemophilia A. The hydrolysis of factor VIII by anti-factor VIII antibodies has been found as a mechanism of inactivation of factor VIII. Of course not all antifactor VIII antibodies are found to be proteolytic or catalytic antibodies (8,9). It has an implication in the treatment of cases of hemophilia A having inhibitors.

We also have developed monoclonal antibody to factor VIII:C which we utilize for estimation of inhibitor to factor VIII by ELISA technique. Patent has been applied for the same.

Management of Chronic synovitis and Hemophilia Arthropathy:

Prevention of chronic synovitis is the key to management of hemophilic arthropathy. Hemophilic arthropathy is often seen in India due to inadequate management of the early bleed owing to the nonavailability of factor concentrate. About 30% of the total six hundred hemophiliac patients treated at our centre present with various grades of synovitis. Apart from the

factor infusion on demand, immobilization of the patient during an acute episode of bleeding forms an important aspect of management in chronic synovitis. The immobilization time may vary from couple of days to a few weeks, depending on individual patient response. Walking is not allowed till the patient is free of pain on weight bearing. Along with immobilization in appropriate splints, compression with elastocrepe bandage is applied.

Subsequent to immobilization, graded mobilization using appropriate exercise regimen is mandatory. Mobilization is generally started following infusion of factor concentrate like cryoppt. Mobilization and appropriate exercise regime is carried out by physiotherapists having experience in handling hemophilia cases.

Synoviorthesis:

It is a method of choice prescribed in the treatment of chronic synovitis, who present with repeated bleeds to the same joint. Two widely used methods are chemical synoviorthesis, radioactive synoviorthesis. We have carried out chemical synoviorthesis using rimpicin intraarticularly in some of our cases with gratifying results. (10).

Rehabilitation intervention is equally important to address the complications in chronic arthropathy which is usually done by: (a) management of the acute bleed in chronic synovitis. (b) Improving range of movements of joints. (c) Muscle strengthening. The details of these have been described elsewhere (10). Regular physiotherapy exercises are taught to the hemophiliacs by the physiotherapist. This procedure strengthens the muscles and reduces instability in the joints. Ultimately bleeds are found to be less.

Education:

Realizing the fact that where resources are scarce education remains the corner stone of hemophilia care, we prepared some Educative materials in Hindi, Gujarati & Marathi language for the patients and their family members.

Von Willebrand Disease:

Incidence, carrier detection and molecular basis; Although VWD is considerably more frequently encountered in clinical practice than hemophilia A, the common VWD variants are generally quite mild clinically. Type 3 von willebrand disease is severe and is inherited as autosomal recessive manner and has got very low or undetected level of VWF. Paucity of data regarding incidence, spectrum of clinical manifestations prompted us to undertake this study at our centre. In includes screening of 217 patients for VWD with a bleeding tendency. Out of these, 36 patients were diagnosed as VWD. On investigating the family members of these patients, 10 additional cases of VWD were diagnosed. The laboratory investigations include the screening coagulation test like PT, APTT, TT the factor VIII:C and VWD:Ag levels and VWF:Rco and multimeric pattern. The results are tabulated in tables 1-5.

For carrier detection polymorphisms in intron 40 of the VWF gene was studied. 300 normal controls were also screened for the various alleles in the VWF 1 and VWF2

VNTR polymorphic markers of the intron 40. Apart from the 8 alleles in VWF 1 and 6 alleles in VWF2 markers of VWF intron 40, we have found new alleles VNTR 9 consisting of 111bp in VWF 1 and VNTR 7 and VNTR 8 made up of 178 base pairs and 182 base pairs respectively in the VWF 2 markers. Details of the study are given in table 6. This data obtained subsequently was used in carrier detection in 2 severe type 3 VWD families.

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