Review Article

Hemophilia and Its Treatment: Brief Review

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ABSTRACT

Hemophilia is a genetic bleeding disorder. Hemophilia A or B is treated by recombinant clotting factor VIII or factor IX and immunosuppressives to prevent formation of alloantibodies and inhibitors. Formation of inhibitors to these factors poses a challenge in treating hemophila. Plasma derived activated prothrombin complex concentrate and activated recombinant factor VII are used to treat patients with inhibitors. Treatment also varies with situations such as, pregnancy, surgery, and malignancy, as these trigger increased risk of bleeding. This review illustrates the various etiologies of hemophilia, methods of diagnosis, newer treatment options available for hemophilia, treatment in special circumstances and the future of hemophilia treatment. In the present times, long lasting and permanent cure for hemophilia is not available. Replacement by factors provides a temporary cure for hemophilia. Permanent cure may lie in the direction of gene therapy or stem cell therapy which is under development. Few successes are achieved by gene therapy in previous studies providing cure lasting for many months without need for recombinant factor replacement. Further modifications of therapy are required to achieve long lasting and permanent cure for hemophilia.

Keywords: Hemophilia, Bleeding disorders, Treatment of Hemophilia.

INTRODUCTION

Hemophilia derived from the Greek *haima* (Blood) and *philia* (Love), is a genetic bleeding disorder caused by the deficiency of clotting factor-VIII (Hemophilia-A) or factor-IX (Hemophilia-B) or factor-XI (Hemophilia-C). Hemophilia A (also known as classic hemophilia) and B (also known as Christmas disease) are X-linked recessive trait with defective *F8* and *F9* genes in long arm of X chromosome with incidence of one in 5,000 and one in 30,000 males respectively while Hemophilia C (also known as plasma thromboplastin antecedent (PTA) deficiency or Rosenthal syndrome) has autosomal recessive inheritance with defective gene in chromosome 4 and has incidence of one in 1,00,000 males and is more common in Jews of Ashkenazi.

Hemophilia can also be acquired due to the development of autoantibodies directed against the clotting factors. Acquired hemophilia is very rare with incidence of one in 1 million persons.[1-3]

In Hemophilia there is internal or external bleeding which may be spontaneous or with trivial trauma. It can lead to complications like chronic anaemia, haemarthrosis, intracranial hemorrhage and compartment syndrome. Early diagnosis and management is required to prevent the development of these complications.[4] The management varies in special situations such as, pregnancy, major surgeries, and malignancy. This review aims in presenting the current advances in diagnosis and treatment of hemophilia and the recent recommendations in the management of special conditions or procedures in hemophiliacs.

CAUSES OF HEMOPHILIA

When there is injury to the blood vessel, the platelets are activated at the site of injury which leads to activation of the clotting factors and formation of fibrin blood clot by the 'Intrinsic pathway' of coagulation. Factor VIII and factor IX are required to activate factor X which in turn activates prothrombin activator which converts prothrombin to thrombin. Thrombin helps in conversion of fibrinogen to fibrin which traps the platelets and forms clot. Factor XI is needed to activate factor IX.[5]

The factors VIII, IX or XI are genetically transferred to the offspring through X chromosome (F8 and F9) and chromosome 4 (F11). Any defect in these genes causes absence or reduced production of these factors. Sometimes antibodies labeled as 'inhibitors' to these factors may develop. Inhibitors may develop as a response to therapy with the clotting factors in hemophiliacs or idiopathically in normal subjects without any genetic defect. In these patients, even after therapy with clotting factor, activated partial thromboplastin time (aPTT) and prothrombin time (PT) are prolonged.[6] Various mechanisms are suggested in the genesis of genetic defect or inhibitor formation.

1. F8 gene mutation

Hemophilia A is caused by mutations in the *F8* gene. In hemophilia A, there are large DNA mutations like intron 22 and intron 1 inversion and many small mutations like missense mutations, nonsense mutations and frameshift mutations.[7] Factor 8 consists of 2332 amino acids and has domains A1, A2, A3, B, C1, and C2. Severity of the disease is correlated with the type of mutation in these domains.[8] According to Ryan *et al*, a point mutation causing amino acid substitution N1922S in A3 domain of F8 gene leads to

defective folding of A3 which ultimately stops the production of factor VIII.[9] There may also be Arg2150His substitution in C1 domain of factor 8 which results in reduced binding of factor 8 to Von Willebrand factor which leads to reduced stability of factor VIII.[10]

2. Lack of f8 mRNA

In some hemophiliacs no defects were found in DNA. There is defective mRNA or lack of mRNA in some of them which prevents the relay of message.[11-13] In these patients, RT-PCR did not give any mRNA corresponding product which is specific for factor 8. This can be due to swift degradation of mRNA due to an unidentifiable mutation in f8 intron.[14]

3. F8 inhibitors

Inhibitor to factor VIII develops as a complication to therapy of hemophilia A, mostly in patients with deletion or nonsense mutation in *F8* gene. Alloantibodies develop against the replaced factor VIII and bind to A2 and C2 domain of F8 and inactivate F8 completely.[6] The incidence of inhibitor development is directly proportional to age of the individual. This may be due to decline of immune regulation function in old age.[15] According to Viel *et al,* mismatched factor VIII replacement by giving H1 and H2 types of factor VIII (present in white population) to black population in whom H3, H4 and H5 are present increases the incidence of developing alloantibodies to factor VIII in black population.[16] These patients don't respond or respond poorly to therapy.

In Acquired Hemophilia, autoantibodies mostly of IgG class are present against factor VIII in subjects who have normal factor VIII gene. It can be idiopathic or can accompany other conditions like autoimmune diseases, cancer and drug ingestion.[17] They bind to A2, A3 or C2 domain of factor VIII and inactivate factor VIII incompletely.[6] This results in reduced function of factor VIII and increased aPTT even after therapy.

4. Defect in tissue factor pathway

Tissue factor (TF) can initiate an alternate pathway of coagulation in the absence of factor VIII, IX or XI by forming TF-factor VII complex. This becomes TF-Factor VIIa complex which activates factor X to Xa. Absence of factors VIII, IX or XI is more significant when there is deficient TF concentration.[1,18]

DIAGNOSIS OF HEMOPHILIA

Diagnosis of hemophilia is made on the basis of clinical suspicion and laboratory tests. Mutation in a particular gene may be detected which helps in development of newer modalities of treatment like gene therapy.

a. Clinical diagnosis

Clinical conditions, such as hemarthrosis, intracranial bleeding, excessive bleeding in trivial trauma, prolonged bleeding after surgery and menorrhagia point strongly towards a diagnosis of hemophilia.[19] Bleeding can be spontaneous in severe cases or only as a response to trauma in mild cases and these are correlated with the laboratory tests.

b. Lab diagnosis

Hemophilia can be diagnosed by coagulation factor assays or activated partial thromboplastin time. Bleeding time, prothrombin time and thrombin time are normal in Hemophilia. Bleeding time is not affected as it shows only platelet function. Prothrombin time is not affected as it depends only on extrinsic pathway of coagulation and factors I, II, V, VII and X. Thrombin time is normal because it depends on fibrinogen.

Activated partial thromboplastin time

This measures the integrity of intrinsic pathway and common pathway.[20] In Hemophilia, there is prolongation of aPTT. As factors VIII, IX and XI are part of intrinsic pathway of coagulation along with other factors, aPTT is prolonged in all 3 types of Hemophilia.[21]

Coagulation factors F8/F9 assays

The type of hemophilia and degree of factor activity can be assessed by factor assay. The normal value of factor VIII is 50-150% and factor IX is 50-150%.[22] In hemophilia these values are reduced. Knowledge of the exact amount of activity of clotting factors help in grading the severity of the disease and its precise management.[19]

Thrombin generation assay

This measures the ability of blood to form thrombin. This is valuable in assessing the response to therapy in hemophiliacs with inhibitors as the conventional coagulation profiles are not useful in them.[23] It shows the overall assessment of hemostasis while aPTT shows only the time taken to form a clot.[24] Thrombin generation (TG) maximum peak and lag-phase time are used to find the severity of hemophilia.[25,26] Severity determined by conventional methods show discrepancies as many severe hemophiliacs may not have severe symptoms and bleeding.[27] This is because other pro-thrombotic factors play a role in bleeding in hemophiliacs which can be detected by TG assay.[28]

Thromboelastography

The integrity of coagulation can be assessed by thromboelastography. R-time and K-time shows the integrity of clotting factors.[29] R- time shows the time taken for onset of clotting. PT and aPTT show the integrity of coagulation till this point only. K time is the time taken from end of R until the clot reaches 20mm and it shows the speed of clot formation.[30] This helps in monitoring of patients on therapy.

Thrombodynamics test

It is also called spatial clot growth assay.[31] It has clotting phase and elongation phase which can be activated by intrinsic or extrinsic pathway. In hemophilia, clotting

phase (initiation phase) when activated by intrinsic pathway is delayed whereas activation by extrinsic pathway is normal. Elongation phase (which indicates clot thickening) and rate of clot growth are poorer. Thin friable clots are formed which results in bleeding. These friable clots are formed because of weak positive feedback mechanism involving thrombin.[32]

c. Genetic diagnosis

Genetic testing helps in confirmation of diagnosis and identification of carriers. This can increase the index of suspicion and aid in early prenatal diagnosis of fetuses which is crucial in management during delivery.[33]

Carrier detection

This is imperative in genetic counseling and in vigilant care of pregnant mother and possibly hemophilic child. Hemophilia A carrier women have wide variation in levels of FVIII and rarely may show mild bleeding tendencies.[19] This is because of X-inactivation in women during embryonic life.[34] Hemophilia carrier women are at risk for post partum hemorrhage and hemophiliac child is at risk of intracranial hemorrhage. Inverse PCR (I-PCR) or inverse shifting PCR (IS-PCR) can be used to detect intron 1 and intron 22 inversions.[35,36]

Prenatal diagnosis

Prenatal diagnosis of fetuses with hemophilia is crucial during management of labour. It is done by assessing chorionic villous sampling at 11-14 weeks of gestation or amniocentesis after 15 weeks of gestation or cordocentesis after 20 weeks of gestation.[37] This is beneficial in fetuses with a strong family history of moderate to severe hemophilia. In early 1980s hemophilia was prenatally diagnosed mainly by immunoradiometry, factor VIII:CAg and factor VIIIR:Ag assays.[38]

1. Amniocentesis

This is done between 15 to 18th week of gestation. Under ultrasound guidance, through maternal abdominal wall, a needle is inserted into the amniotic sac and amniotic fluid containing amniocytes (Foetal cells) is obtained. Direct mutation detection or linkage analysis is used to find affected fetuses.[39]

2. Cordocentesis

It is also called percutaneous umbilical blood sampling. This is done if the results of other tests are uncertain. Umbilical cord blood is taken using ultrasound guided needle and factors VIII and IX in fetal blood are measured.[40]

3. Preimplantation genetic diagnosis

In this, in-vitro fertilization is done and affected male embryos are identified and only healthy embryos are returned to uterus. It is done by linkage analysis to detect F8 intron 22 inversion in blastomeres obtained by biopsy of embryo.[41] Cell sample is lysed and PCR is done followed by direct genotyping and mutation analysis.

4. Chorionic villus sampling

It is the most common method of prenatal diagnosis. It is done between 11 and 14th week of gestation.[40] Chorionic villus is taken through transcervical or transabdominal route under direct ultrasound guidance and indirect or direct mutation analysis or linkage analysis is done to diagnose affected foetus.[39]

5. Non invasive tests-digital PCR

Invasive tests always carry a risk of foetal loss. To prevent that, non invasive tests are done by analyzing foetal DNA circulating in maternal plasma.[42,43] Cell free foetal DNA present in maternal plasma has amplified Y chromosomes (Y-PCR) which can be tested.[37] Digital PCR known as relative mutation dosage approach can be used for the detection of hemophilia.[44,37]

Genetic diagnosis in adults

In adults with clinical signs and symptoms of bleeding disorder, genetic testing is done to confirm genetic or other etiology of hemophilia and its appropriate management. It can be done through many methods like direct mutation detection, targeted mutation analysis or inverse PCR.[19,45]

MANAGEMENT OF HEMOPHILIA

Initially in early 1960's concentrates of antihemophilic globulin[46] and glycene precipitated factor VIII were used in the treatment of hemophilia.[47] Then in 1970's and 80's plasma derived products and cryoprecipitate were used to treat hemophilia.[48] But this increased the incidence of HIV and Hepatitis A and B in hemophiliacs and increased the development of antibodies to factors VIII and IX.[49-51] By the beginning of 1990, recombinant- DNA derived antihemophilic factors were used to treat hemophilia.[52,53] Following that desmopressin and immunosuppressive therapy were used.[54,55] Activated prothrombin complex concentrate was used to treat patients with inhibitors.[56] In patients with liver cirrhosis or hepatitis due to transfusion, liver transplantation was done which provided good results and cured factor VIII and factor IX deficiencies.[57-59]

ADVANCES IN MANAGEMENT

In recent times, many advances are made in the treatment of hemophilia. They include modification of time old therapy or breakthrough of new drugs.

a. Immunosuppressives

Steroids alone or steroids with cyclophosphamide or rituximab or cyclosporine are used as first line therapy. People who did not have complete remission with first line are given second line therapy with steroids, cytotoxics and rituximab. According to Collins *et al*, first line therapy with combination of steroids and cyclophosphamide gives a stable remission.[60]

b. Gene therapy

Gene therapy may provide complete lifelong cure for hemophilia. Gene therapy for hemophilia B using adeno-associated viral vector shows promising results in some studies. It provides remission temporarily and further studies are required to achieve permanent cure.[61,62] There are some limiting factors to its use like humoral and cellular immune response, toxicity and safety issues which must be rectified in future.[63] There is development of antibodies to recombinant proteins in hemophilia A. This can be prevented by giving adenoviral vector expressing factor VIII to neonates as their immune system is immature.[64]

c. Therapy under development

Many new treatment options are tried in the recent times. Bone marrow transplantation and hematopoietic stem cell transplantation are being tried in mice.[65,66] In vitro studies are done using solulin to increase clot stability in whole blood.[67] Further studies are required to achieve long lasting and permanent cure for hemophilia.

MANAGEMENT IN SPECIAL SITUATIONS

Management of hemophilia is challenging in special situations like pregnancy, newborn, and surgeries. They require special attention and a need to watch for and prevent undue hemorrhage and complications.

a. Management in pregnancy

Management in carriers of hemophilia during pregnancy requires prenatal diagnosis of hemophilia by chorionic villous sampling or amniocentesis or cordocentesis and careful watch for post partum hemorrhage (PPH).[37] According to study by Kadir *et al* 48% of hemophilia carriers had primary PPH. Pregnancy in carriers should be managed by multidisciplinary approach in a tertiary care center.[68] In case of PPH, fibrinogen supplementation in the form of fresh frozen plasma, cryoprecipitate or fibrinogen concentrate or antifibrinolytic agents like tranexamic acid or recombinant activated factor VII can be given.[69]

In neonates born of carriers, there is increased risk of intracranial or extracranial hemorrhage during delivery.[70] This can be prevented by prenatal diagnosis of affected foetus and carrier and taking proper precautions. The best possible mode of delivery is under debate. According to James *et al*, the optimal mode of delivery is planned caesarian delivery before labor and this will reduce 85% risk of intracranial hemorrhage.[71] According to Ljung *et al*, planned vaginal delivery without use of instruments like vacuum or forceps is the optimal mode of delivery in hemophilia carriers.[72] Mode of delivery should be decided based on maternal and fetal factors and individualized.

b. Management in neonate

If hemophilia is suspected in neonate, it should be diagnosed and confirmed by analysis of cord blood. Recombinant factor VIII or factor IX concentrates or fresh frozen plasma are given to affected children. If intracranial bleed is suspected, factor should be given immediately before confirming the diagnosis by cranial ultrasound or CT or MRI scan.[73]

c. Management in acute hemarthrosis

In acute hemarthrosis, there is bleeding into the joint which results in rapid joint swelling, pain and reduced movements. Radiological confirmation is not indicated routinely. Therapy with recombinant factors VIII or IX is the first line of treatment. It is followed by resting the joint, applying ice, compression bandage, leg elevation and physiotherapy.[74,75] In profuse hemarthrosis, arthrocentesis is done after complete correction of factor deficit.[74] In case of repeated hemarthrosis, synovectomy or angiographic embolization is done.[76]

d. Management in minor/major surgical procedures

Hemorrhage is a common complication during surgery and postoperatively increasing the mortality and morbidity. During surgical procedures in hemophiliacs, activated recombinant factor VII (rFVIIa) is used to achieve hemostasis.[77,78] In major cardiac surgeries, rFVIIa is used customarily during surgery and postoperatively.[79] Desmopressin is used prophylactically in open heart surgery patients with hemophilia.[80]

e. Management in malignancy

Cancer in hemophiliacs should be treated in same way as non hemophiliacs. But the risk of bleeding is higher due to therapy induced thrombocytopenia. Replacement therapy should be given as continuous prophylaxis during chemotherapy and radiotherapy when accompanied by thrombocytopenia. If cancer surgery is done, low molecular weight heparin should be given postoperatively to prevent thrombosis.[81]

f. Management in patients with inhibitors

Development of inhibitors is a complication of treatment of hemophilia with replacement therapy. It is because of the formation of alloantibodies to factor VIII. This reduces the efficacy of therapy by recombinant factors VIII or IX.[82] Patients with inhibitors need a bypassing agent to control bleeding. Plasma derived activated prothrombin complex concentrate and activated recombinant factor VII are used as bypassing agent to aid in hemostasis.[83]

CONCLUSION

In conclusion, early diagnosis of hemophilia and early management helps in improving the quality of living of the patient and prevents early development of complications. Replacement of factors provides effective control of hemophilia for short term. Newer research based options are required to provide long lasting and complete cure for hemophilia which may be possible by gene therapy in the near future.

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