ORIGINAL RESEARCH PAPER

Medical Science

HEMOPHILIA: DIAGNOSIS AND INNOVATIVE MANAGEMENT.

KEY WORDS: Hemophilia, Replacement Therapy, Gene Therapy, Pharmacokinetics.

Dr Farhin Saiyed BHMS, MBA Health management

Dr Wajid Saiyed B.D.Sc, MBA, MPH Australia

Dr Urvi Rangrej B.D.S, M.D.S Periodontology

ABSTRACT

Hemophilia is an acquired, X-linked, passive disorder caused by an insufficiency of utilitarian plasma clotting factor VIII (FVIII). In a critical number of cases, the disorder results from a novo mutation. It rarely manifests as an acquired autoimmune process. Research facility thinks about for suspected hemophilia include a total blood cell tally, coagulation ponders, and an FVIII assay. In patients with an established diagnosis of hemophilia, periodic research facility evaluations include screening for the presence of FVIII inhibitors and screening for transfusion-related or transmissible illnesses such as hepatitis and HIV contamination. Estimation of FVIII levels is critical for observing FVIII substitution treatment. Treatment of hemophilia includes prophylaxis, treatment of bleeding episodes, and induction of immune tolerance. Intended for the treatment and rehabilitation of the patients who are taking factor inhibitors and patients have hemophilic synovitis. Treatment for hemophiliacs should ideally be provided by a Hemophilia Day Care center. We discuss innovative management for the prevention of hemophilia through gene therapy, non-replacement therapy, EHL recombinant concentration, etc. we discuss the data from our studies and emerging results from other gene therapy trials in both hemophilia A and B. This analysis will provide evidence and information on the feasibility and data quality/completeness of the hemophilia database to assess the impact of innovative management on hemophilia outcomes in developing countries. We used a statistical method to analyze the data of adult and pediatric patients who come to hemophilia day care center for taking treatment. The data analysis was performed by the data collected monthly at the hemophilia day care center.

INTRODUCTION:

The meaning of hemophilia derives from the Greek words Haima meaning blood and Philia meaning Love [1]. Hemophilia is now and then called the "royal disease" since the hemophilia gene was acquired from Ruler Victoria, who afterward got to be Ruler of Britain in 1837 , to the ruling families of England, Germany, Russia and Spain in the 19th and 20th centuries [2]. In 1803, Philadelphia physician John Conrad Otto first published a paper stating that hemorrhagic bleeding disorders primarily affect men and occur in certain families. John Conrad Otto called this Disease; the males "bleeders" [3]. The term hemophilia was first documented in a paper by Johann Lukas Schönlein and his student Friedrich Hopp at the University Of Zurich, Switzerland [4]. Dr. Nasse portray about his first hereditary depiction of hemophilia in Nasse's Law. It states that hemophilia is transmitted only from an unaffected woman to her sons [5]. Hemophilia is an acquired bleeding disorder, ordinarily in which the blood does not clot appropriately. This will lead not as it were to spontaneous bleeding, but moreover to bleeding after injury or surgery. Platelets and proteins in your plasma work together to halt the bleeding by forming a clot over the injury [6].

Table 1:Type of hemophilia:

Types of Hemophilia	Missing Clotting Factor
A	8
B	9
C	11
Acquired	The auto-immune system attacks clotting factors

Cause Of Hemophilia:

Hemophilia is caused by a mutation or change, in one of the genes, that provides instructions for making the clotting factor proteins needed to form a blood clot. This change or mutation can prevent the clotting protein from working properly or to be missing altogether [8]. The genetic change that causes hemophilia is a recessive change in the X chromosome. Males have one copy of the genes in the X chromosome, and females have two copies [9]. Hemophilia A is caused by variants in the gene that encodes coagulation FVIII. Hemophilia B is caused by variants in the F9 gene that encodes coagulation FIX [10].

Classification Of Hemophilia:

Table 2: Hemophilia Severity, Factor Activity, and Hemorrhage Type

Classification	Factor Activity, %	Cause of Hemorrhage
Mild	6 – 40	Major trauma or surgery
Moderate	1 – 5	Mild-to-moderate trauma
Severe	< 1	Spontaneous

Table 3: Clinical classification.

CLASSIFICATION	SEVERE	MODERATE	MILD
FACTOR VIII OR IX ACTIVITY	< 1%	1% to 5%	6% to 30%
FREQUENCY OF CASES	50% TO 70%	10%	30% to 40%
CAUSES OF BLEEDING	Spontaneous	Minor trauma, rarely spontaneous	Major trauma, Surgery
FREQUENCY OF BLEEDING	2/4 times per Month	4/6 times per Year	Uncommon
PATTERN OF BLEEDING	Joint, Soft tissue bleeding after circumcision, neonatal ICH	Joint, Soft tissues, ±bleeding after circumcision, ±neonatal ICH	Joint, Soft tissues, ±bleeding after circumcision

Symptoms Of Hemophilia

Bleeding into joints. [May cause joint swelling, pain and tightness; It affects on the knees, elbows, and ankles]. Bleeding into the skin (causing bruising), and post-vaccination bleeding. Head bleeding in infants after Dystocia, Hematuria [blood in urine] or blood in stool [Melena/ Hematochezia] [17]. Hematomas, [bleeding into the muscle or soft tissues], Bleeding of the mouth and gums, bleeding after circumcision (surgery performed on male babies to remove the hood of skin, called the foreskin, covering the head of the penis), Frequent and hard-to-stop nose bleeds. [18]

Diagnosis:

Diagnosis of hemophilia is based on clinical suspicion and laboratory tests.

1. Clinical Diagnosis

Clinical manifestations such as arthritis, intracranial hemorrhage, excessive bleeding from minor trauma, persistent bleeding after surgery, and menorrhagia are highly suggestive of 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.

2. Lab Diagnosis

Hemophilia can be diagnosed by blood clotting factor testing or activated partial Thromboplastin time. Bleeding time, Prothrombin time, and thrombin time are normal in hemophilia. Bleeding time is not affected as only platelet function is displayed. Prothrombin time is also unaffected, as it depends only on the extrinsic coagulation pathway and factors I, II, V, VII, and X. Thrombin time is ordinary because it depends on fibrinogen. These are the Following Testes done in Laboratory [20].

Activated Partial Thromboplastin Time:

This measures the integrity of the intrinsic pathway and common pathway. In Hemophilia, there is a prolongation of APTT [21]. As factors VIII, IX, and XI are part of the intrinsic pathway of coagulation along with other factors, APTT is prolonged in all 3 types of Hemophilia [22].

Coagulation Factors F8/F9 Assays:

The type of hemophilia and level of factor activity can be assessed by factor testing. Normal levels of factors VIII and IX are 50-150% [23]. These values are reduced in hemophilia. Knowing the exact degree of clotting factor activity helps assess the severity of the disease and provide accurate treatment.

Thrombin Generation Assay:

This measures the capacity of blood to make thrombin. This is valuable in assessing the response to therapy in hemophiliacs with inhibitors as the conventional coagulation profiles are not useful for them [24]. It shows the overall evaluation of hemostasis whereas APTT shows only the time taken to make a clot. Thrombin generation maximum peak and lag-phase time are used to find the severity of hemophilia.

Thromboelastography:

Coagulation integrity can be assessed using Thrombo elastography. R-time and K-time show the integrity of clotting factors. R-Time shows the time taken for the onset of clotting. K time is the time taken from the end of R [25].

Thrombodynamics Test

Also called "spatial clot proliferation assay". There are clotting and elongation stages, which are activated by intrinsic or extrinsic pathways. Thin, fragile clots form, causing bleeding. These friable clots are formed because of a weak positive feedback mechanism involving thrombin [26].

Genetic Diagnosis:

Genetic testing helps in the confirmation of diagnosis and identification of carriers. This increases the index of suspicion and aids in early prenatal diagnosis of the fetus, which is important for treatment at birth. This is essential in genetic counseling and in the meticulous care of pregnant mothers and, in some cases, children with hemophilia [27].

Carrier detection:

This can be basic in hereditary counseling and vigilant care of pregnant mothers and conceivably a hemophilic children. Hemophilia A carrier women have a wide variation in levels of FVIII and rarely may show mild bleeding tendencies. This can be because of X-inactivation in women amid embryonic life [28]. Hemophilia carrier women are at risk for post-partum hemorrhage and hemophiliac child is at risk of intracranial hemorrhage.

Prenatal diagnosis:

It is done by surveying chorionic villous examining at 11-14 weeks of development or amniocentesis after 15 weeks of gestation or cordocentesis after 20 weeks of gestation [29]. This is advantageous in fetuses with a strong family history of moderate to severe hemophilia. In the early 1980s hemophilia was prenatally diagnosed mainly by immunoradiometry, factor VIII: CAg and factor VIII: Ag assays.

Amniocentesis:

This is often done between 34 to 36th weeks of gestation. Beneath ultrasound direction, through the maternal abdominal wall, a needle is embedded into the amniotic sac and amniotic liquid containing amniocytes (Fetal cells) is obtained. Coordinate mutation detection or linkage examination is utilized to discover affected fetuses [30].

Cordocentesis:

It is also called Percutaneous Umbilical Blood Sampling [PUBS]. This is done when the results of other tests are uncertain. Umbilical cord blood is taken using an ultrasound-guided needle and factors VIII and IX in fetal blood are measured [31].

Pre-implantation genetic diagnosis:

In this, in-vitro fertilization is done and affected embryos are identified and only healthy embryos are returned to the uterus. It is done by linkage analysis to detect F8 intron 22 inversions in blastomeres obtained by biopsy of the embryo [32].

Chorionic villus sampling:

It is the most common method of prenatal diagnosis. It happens in between the 10 to 13 weeks of gestation. Sampling of chorionic villi may be obtained transcervically or transperitoneally under direct ultrasound guidance and subjected to indirect or direct mutational or linkage analysis to diagnose affected fetuses [33].

Non Invasive tests-digital PCR:

Invasive tests always carry a risk of fetal loss. To prevent that, noninvasive tests are done by analyzing fetal DNA circulating in maternal plasma [34]

Cell-free fetal DNA present in maternal plasma has amplified Y chromosomes (Y-PCR) which can be tested. Digital PCR known as the relative mutation dosage approach can be used for the detection of hemophilia [35].

Genetic diagnosis in adults:

In adults with clinical signs and symptoms of a bleeding disorder, genetic testing is done to confirm the 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 [36].

Treatment Protocol:

I] Prophylaxis -

The administration of clotting factors concentrates in anticipation of or to prevent bleeding

- **Continuous:** Routine replacement of FVIII/IX via infusion of factor concentrates, FVIII 3 days per week (or) FIX 2 days per week for 45 to 52 weeks per year.
- **Intermittent:** Prophylaxis is given for < 45 weeks per year

II] On Demand - Replacement of deficient factor concentrate is infused when a bleed occurs. [37]

Treatment Modalities Available:

A] Factor concentration:

- **FVIII Concentrate:** Treatment of choice for hemophilia A
- **FIX concentrate:** Treatment of choice for hemophilia B

Two classes of FIX concentrate are available

I] Pure FIX concentrate:

Pure FIX concentrates are preferred for treatment because Prothrombin complex concentrates carry the risk of thrombosis or disseminated intravascular coagulation (DIC).

II] Prothrombin complex concentrate (PCCs): It contains factors II, VII, IX, and X. [38]

B] Cryoprecipitate:

Cryoprecipitate is utilized as it were when FVIII concentrates are not accessible. It is still in use in developing countries. It carries the risk of transmitting blood-borne diseases.

C] Fresh frozen plasma (FFP):

It contains all coagulation variables. Cryoprecipitate is preferable to FFP for the treatment of hemophilia A. FFP and cryo-poor plasma can be used for the treatment of hemophilia B, as both products contain FIX.

D] Non-transfusion therapy in hemophilia:

Apart from conventional coagulation factor concentrates other agents used in hemophilia are;

- Desmopressin
- Tranexamic acid
- Epsilon aminocaproic acid. [39]

Innovative Management Of Hemophilia

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

Gene therapy:

Gene therapy may provide a complete lifelong cure for hemophilia. Gene therapy offers the potential for a cure for patients with hemophilia by establishing continuous endogenous expression of factor VIII or factor IX (FIX) following transfer of a functional gene to replace the hemophilic patient's own defective gene [40]. Early efforts of Gene Therapy focused on retroviral vectors, which proved to have many problems including the following: efficient insertion of vector occurs only in actively dividing cells; the inserted gene tends to be silenced, leading to rapid fall off in circulating factor levels; poor expression levels; the development of antibodies to foreign protein; concerns about insertional mutagenesis with vectors related to tumor-inducing viruses; and difficulty in obtaining sufficiently high titers of virus [41]. Great strides have been made in the development of gene therapy for hemophilia in the last decade. First, adeno-associated virus (AAV)-derived vector-mediated transfer of a normal FVIII or FIX gene is most advanced in clinical development. Hemophilia gene therapy products for AAV- mediated gene transfer will soon be approved and will create revolutionary challenges for patients, providers, industry and health systems [42]. Recently, efforts have focused on the r- adeno-associated virus (rAAV) as a preferred delivery method, although three trials currently underway will utilize lenti-viral gene delivery combined with auto-logous stem cell transplantation for patients with HA (NCT04418414) and one trial for HB (NCT3961243). Onetrial for patients with HB is underway using a gene-editing approach. The following Tables summarize the prior and current, ongoing trials using a rAAV to deliver the F8 or F9 Trans gene for people with HA or HB respectively [43].

Stem Cell Therapy:

ES cells can secrete blood clotting factor VIII, they have the potential to treat hemophilia A patients. These stem cells provide the perfect solution to this lack of clotting factors. This was shown in a study of mice transplanted with embryonic stem cells (ESCs) [46]. New insights and attractions have been absorbed to novel therapeutic options including cell therapy and BMT to overcoming current problem using manipulation of hematopoietic stem cells. Hematopoietic stem cells are easy and capable of being manipulated ex vivo, making them an attractive target for hemophilia therapy. BM transplantation cured hemophilia A through reconstitution of mononuclear cells and MSC [47]. Stem cell therapy is evolving as a new medical application concept in pharmacology. For all practical purposes, human embryonic stem cells are used in 13% of treatments, whereas fetal stem cells are used in 2%, umbilical cord stem cells in 10%, and adult stem cells in 75% of cases [48]. We found one study of mouse, based on this study, all authors conclude that cell therapy using genetically engineered endothelial progenitor and stem cells can generate functional coagulation factor VIII (FVIII) and provide stable long-term therapy for hemophilia A. [49].

The Anti-Coagulant Pathway:

Fitusiran is an Anti-thrombin RNA impedances particle (ALN-AT3; Fitusiran, (Alnylam/Sanofi) which diminishes Anti-thrombin (AT) delivery person RNA expression within the liver. Fitusiran, an RNA-interference drug that "silences" part of the action of the generesponsible for hemophilia, is now in Phase III trials. It is projected to be a once- monthly injection that both inhibits Anti-thrombin (which prevents clotting) and promotes the production of thrombin (which encourages it). It is intended for use not only in hemophilia A, but also in hemophilia B (due to low levels of factor IX) [50].

Prophylactic Hemophilia Replacement Therapy:

Prophylaxis in hemophilia consists of regular administration of therapeutic products aimed at maintaining hemostasis to prevent bleeding, especially for joint hemorrhages, which would lead to Arthropathy and disability [52]. The use of prophylactic therapy is always preferred over temporary therapy. Hemophilia patients who started prophylaxis early (that is, primary or secondary prophylaxis) had the best long-term outcomes. Hemophilia replacement therapy can help stop ongoing bleeding or can be used prophylactically (as a preventive measure) to stop or lessen the severity of a bleeding event before it occurs [53].

Prophylaxis for hemophilia A and B

Regular prophylaxis begins at an early age and is given in inappropriate doses. Until long- acting drugs and treatments become available, this is considered the standard of care for hemophilia. [54].

CONCLUSIONS:

From the analysis of the information displayed in this survey, it can be effortlessly concluded that the treatment of hemophilia has presently reached a high degree of quality; without a doubt, it is likely the foremost efficacious and safe treatment accessible for a monogenic disorder. Programs for the future will include the production of more factor concentrate (both plasma-derived and recombinant) to satisfy the needs of developing countries and the development of molecules with a longer half-life (such as pegylated factor concentrates) and less immunogenicity. In the coming years, the results of the prospective randomized trials currently underway will help us to clarify the role of secondary prophylaxis in joint status and quality of life in people with hemophilia and to identify the best immune tolerance regimen for patients with inhibitors.

The awareness regarding prophylaxis and on-demand is less in the community. To overcome the dark side of these factors antenatal pre-diagnosis during pregnancy should be increased in a practice. If early detection can be done in suspected cases then information, education, and communication can be done vigorously in hemophilic patients. A cure of hemophilia through gene transfer is being

attempted, but relatively, it is far from being implemented on a large scale. Through the accessibility of new-therapeutic apparatuses such as variables VIII and IX with longer half-lives, more powerful bypassing agents, and components extricated from the drain of transgenic creatures. The World federation of Hemophilia passes on coagulation figure concentrates and emicizumab in low- and low–middle-income countries through a helpful help program that has improved the life of a restricted number of PWH within the creating world.

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