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A Review on Hemophilia it's diagnostic tests and the application of Extending Half Life Products

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ABSTRACT

Both haemophilia A as well as haemophilia B were X-linked congenital disorders which leads to bleeding owing to deficiency of coagulation factors in blood notably factor VIII (FVIII) and IX (FIX), respectively. Replacement treatment is chosen care option for those persons suffering from haemophilia. Prophylactic elimination of coagulation factor is the treatment of choice for patients with chronic condition; which have been proved to considerably reduce arthropathy, lowering number of bleeds, as well as boosting the patients' life quality. Normal recombinant factor prophylaxis needs with at minimum 2 (FIX) to 3 (FVIII) intravenous injections per week. Synthetic FVIII and FIX products with a longer half-life are being developed or have just been authorised. These products had documented average half-life increase of around 1.5 to 1.8 times than that compared to regular FVIII products as well as three to five times that of normal FIX products, thus, it possibly answers the demands of patients who are being treated by standard factor concentrations.

Keywords: Clotting factor, Excessive bleeding, Prophylaxis, Trauma

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INTRODUCTION

Mechanism of blood clotting

The process of blood clotting can be defined as a process of producing a clot in order to inhibit bleeding [1]. It is a complex process. The body depends on collaboration of 3
processes so as to inhibit bleeding:

First two processes are involved in primary hemostasis:

- Vasoconstriction: It is defined as body's first response to the injury caused in vascular wall. Whenever there is an injury, constriction of vessel walls occurs which leads to the decreased flow of blood towards the site of injury.
- Platelet plug: Just at site of damage, platelets collect. They act like a "plug" by acting in unison. In platelets, production of fibrin clot, termed as secondary hemostasis, is initiated [2].

Secondary hemostasis: There is no way for platelets to shield vessel wall from injury. When you have a blood clot, it should form at the wound site. The production of clot depends on different elements known as clotting factors. These elements are denoted from the roman numerals I till XIII. The clotting cascade occurs as these mechanisms interact with one another. When fibrinogen is cleaved into fibrin and a soluble solids, and fibrin, a – anti protein, this leads in a cascading effect Fibrin proteins produce a clot by linking together. Two separate yet interrelated mechanisms are used in the coagulation sequence the intrinsically and extrinsically routes [3].

Extrinsic pathway: External damage activates the extrinsic pathway, causing blood to leak from a vascular system. This pathway performs quicker function than that of intrinsic pathway. It functions with the help of factor VII.

Intrinsic pathway: The intrinsic cascade is triggered via damage within vascular system and may be triggered with the exposed collagen, platelets, hormones or endothelial cells. Since this route is gentler than that of extrinsic route, but this pathway is more significant. Factors XII, XI, IX, and VIII are involved [4].

Common pathway: A general pathway is carried out in which all the pathways combine and complete the clot formation pathway. Factors I, II, V, and X are all included in the general pathway. Factor IX (F-IX) and Factor IX (F-IX) , they are both X-linked hereditary bleeding disorders, which are associated with germline abnormalities in F-IX and F-VIII genes, respectively. Factor plasma concentration of 1% or less, 2–5%, and 6–40% may both engage in the internal method of coagulation factors, and afflicted people may have disease that is very mild, moderate, or severe, depending on the amount of factor present. One in every five thousand males is born having haemophilia A, whereas one in every thirty thousand males is born having haemophilia B. Hemorrhage in people with severe hereditary hemorrhagic telangiectasia is rather prevalent. Staphylococcus infections may lead to joint, nerve, or soft tissue haemorrhages for no apparent cause. Deadly hemorrhage outbreaks, like cerebral haemorrhages, may also occur [5]. Factor insufficiency seldom causes unintentional haemorrhage, but trauma or surgery-related haemorrhage is more likely to occur.

A variant of haemophilia called haemophilia the consequences from deficiency of the coagulation factor VIII; whereas another kind called haemophilia B is related to a deficiency of the clotting factors IX. They are often acquired via an X chromosome through one parent. Clotting antibodies may arise in early development because a novel mutation cannot be detected, or they may develop later in life due to haemophilia due to an increase in clotting antibodies. Other manifestations include low factor XI-deficient haemophilia C, and low factor V-deficient parahemophilia. Hemophilia may lead to tumours, autoimmune disorders, and pregnancy complications.

DISCUSSION

Genes Involved in Hemophilia Discussion

The F9 as well as F8 alleles both are present on X chromosome, positioned towards the end of a very long arm (Xq28 for F8 and Xq27 for F9).

The F8 gene is very massive (about 180 kb), with a very complicated structure (twenty six exons), while the gene F9 being much smaller (of about thirty four kb), and has a more straightforward structure (only eight exons). Hundreds of people who have the haemophilia mutations have been discovered. The overwhelming number of mutations revealed underscores the genetic complexity of haemophilia.

Deletions, point mutations, rearrangements/ inversions and insertions are found in the F9 and F8 have all been linked to haemophilia A and B in people who exhibitthese mutations [8]. While these mutations are somewhat prevalent in HA and HB, the frequency varies. in particular, the gross genetic flaws contribute to around 7% of HB cases, but HA has half of its severe instances resulting from rearrangements in the genes, wherein inversion of the 22nd intron is the most common.

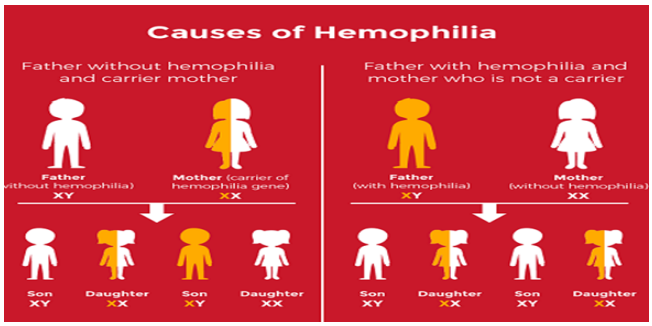

Figure 1: Demonstration of causes of haemophilia [9].

Difference between both the haemophillas

- Studie have shown that patients suffering from haemophilia B have a lower incidence of admissions into hospitals at all stages of seriousness, implying that these patients suffers from a milder phenotype of bleeding than patients suffering from hemophilia A.
- Other data acquired from the 4,343 male people experiencing from haemophilia among their age categories of 2-19 years included their bleeding incidence, age, insurance status, family history, orthopaedic operations, prophylaxis usage, age of the patient, and the very first haemophilia treatment centre (HTC) visit. Patients with HB experienced much fewer instances of bleeding, regardless of an age or a severity. Others having Hemophilia A had more joint mobility limitation than with Hemophilia B.
- In Italy, a single-center, case-control trial was conducted to assess the function of genotype and endogenous thrombin potential (ETP) as one of the cause of patients' clinical manifestations with extreme hemophilia. The investigators compared patients with an unusually the mild bleeding tendency to those having normal bleeding tendency. In research, the probability of developing a mild type [11] of disease were 5 times greater in Hemophilia B patients than in serious Hemophilia A patients.
- By contrasting the utilization of prophylaxis, investigators found that thirty two percentage of the patients having moderate Haemoglobin and 69 percent of patients with extreme HA received prophylaxis. However, it is uncertain if this discrepancy is due to an actual or perceived variation in clinical physiology or merely represents the conventional therapeutic approach to HB patients.

The study in pathophysiology

In 2002, scientists introduced the first real intravenous paracetamol under the trade name Perfalgan. To establish a stable composition of paracetamol IV, the consistency issue with in paracetamol composition needs to be rectified. Paracetamol is changed during the breakdown process to 4-aminophenol, which is rapidly transformed to hepatotoxic chemical N-acetyl-p-benzoquinoneimine (NAPQI). To avoid hydrolysis and thus transformation to 4-aminophenol, paracetamol should be produced at a pH of around 5-6. On the

other side, material oxidation processes must be resisted. This is done by inflating nitrogen into to the IV liquids to reduce the available oxygen, as well as by utilising enclosed oxygen-impermeable glassware vials packed with a ready-to-use solutions which doesn't need reconstitution with an extra ampoule.

Diagnosis of Hemophilia:

Screening procedures and clotting factor tests are used in the diagnosis. Screening checks are the blood tests to determine whether or not there is a clotting happening in blood correctly. It also checks, also known as factor assays, are used to determine the occurrence of a bleeding condition. This blood test determines the form and severity of hemophilia. Screening checks are blood tests to determine whether [13] or not the blood is clotting correctly. Screening measures are classified into the following categories:

Complete Blood Count (CBC): This common test for haemoglobin (the red pigment contained in red blood cells that carries oxygen) measures the quantity of haemoglobin, the quantity and red blood cells' shape, the variety of several kinds of platelets and WBCs, and the number of various kinds of red blood cells. CBC levels are stable in persons with haemophilia. Hemoglobin as well as red blood cell count may also be inadequate, if someone has haemophilia and bleeds for a lengthy length of time.

Activated Partial Thromboplastin Time (APTT) test: The quantity of time required by blood to clot is determined during this test. Factor XII (12), Factor XI (11), Factor IX (9), and Factor VIII (8), have a higher likelihood of clotting (12). A deficiency in one of these clotting pathways slows clotting down to a slower rate than usual. Patients suffering with haemophilia A or B have a prolonged clotting time, as the outcomes of this test show.

Prothrombin Time (PT) test: The time it would take for blood to clot is also measured during this procedure. It mainly tests the clotting capabilities of the following five factors: Factor VII, Factor V, Factor IX, Factor I, and Factor II. In the event that any of these contributing factors is deficient, the blood clotting slows down. Because this therapy has only been studied on individuals with haemophilia A and B, it is unknown whether people with haemophilia A and B would have normal results.

Fibrinogen test: Additionally, this process aids physicians in identifying an ability of patient to form a blood clot. This testing is performed in combination with the other coagulation assays or when a patient's PT or APTT external icon result is abnormal, or both [17]. Additionally, coagulation factor I is referred to it as fibrinogen.

Clotting factor tests: Clotting factor tests, also known as factor assays, are utilized to determine the reason of a bleeding condition. This blood test determines the form and severity of haemophilia. It is important to consider the form and severity in order to develop the right recovery strategy [18].

Results obtained from above mentioned tests are then matched with the threshold level of factor VIII or IX in blood as shown in Table 1.

Table 1: Levels of factor VIII or IX in blood [19].

Condition of an Individual	Amount of Factor VIII or IX present in blood
Severe hemophilia	<1%
Mild hemophilia	>5% & <50%
Moderate hemophilia	1% - 5%
Normal Individual	50% - 100%

Treatment

The clinical severity of the disease determines treatment for patients with haemophilia. Mild to moderate haemophilia A patient normally does need temporary care to resolve their clotting deficiency before surgery, after trauma, and before dental extractions, as well as conservative treatment involving local anaesthesia. Since these patients still have some of the circulating factor VIII, prescribing desmopressin – which is a synthetic version of hormone vasopressin - can cause retained factor VIII to be released from endothelial linings of blood vessels. Hormone desmopressin is an inadequately found in patients suffering with haemophilia B, who need factor IX replacement therapy. Individuals suffering from severe haemophilia A and haemophilia B face the bulk of health issues[20]. The most frequent symptom is accidental internal bleeding, which normally involves significant weight-bearing joints like the feet, thighs, and elbows. Repeated bleeding into joints corrodes the synovium which may cause disabling and irreversible injury, resulting in discomfort and impairment.

Bleeding can occur anywhere, either naturally or as a result of a traumatic incident. For those with serious haemophilia, the only possible therapy is to restore the deficient clotting component.

These formulations need to be regenerated with an intravenous drip. The amount of factor VIII or IX delivered is influenced by weight, location, and length of bleeding. Factor concentrates are normally administered three days a week to maintain a level above 0.03 to 0.04 IU/dl for the factors VIII or IX in the majority of children and young adults with serious haemophilia. Now that the treatment has been completed, the child or young person is mildly affected by haemophilia rather than being seriously affected and has a reduced risk of developing debilitating joint haemorrhage.

Prophylaxis is a kind of treatment where children who use it are less likely to have arthritis or otherwise injure their joints, and are exposed to a comfortable background as their peers. Additional facts are that the clinical course of haemophilia remains the same regardless of treatment, and family life gets simpler to manage with steady treatment.

Extending half-life requirements: Factor VIII and Factor IX are massive, complex proteins with comparatively short half-lives, necessitating repeated dosing to sustain therapeutic levels. EHL coagulation factors are modified structurally to have longer half-lives, such as chemical changes or fusion of the factor protein to another molecule with a longer half-life. In principle, a medication with a longer half-life could result in greater commitment to care and better prophylactic results by allowing for fewer doses. The best approach for extending the half-life of a coagulation factor does not alter its biological activity or protection. The concept of a scientifically significant half-life extension is typically based on certain practical requirements such as dosing plan and planned therapeutic use (e.g., on-demand vs prophylaxis). The lower clearance rate of EHL factors has the ability to minimize treatment pressure by providing less injections while retaining or enhancing effectiveness without increasing total factor intake[23]. This provides for more versatility in tailoring prophylaxis to the needs of the patient, resulting in stronger adherence and, as a result, an increased quality of treatment in haemophilia.

Prolonged half-life factor VIII as well as factor IX products: Some of new rFIX as well as rFVIII formulations with an enhanced plasma half-lives are now eligible for being used for a purpose of clinical use. To maximize the half-life of rFIX, and rFVIII and different technologies have been used. Both rFVIII and rFIX undergo PEGylation, including like albumin (rFIX) or the fragment of igg G1 f-crystalllizable (Fc) element (both rFVIII and rFIX). Because of these differences in the structure and processing techniques required by such diverse molecules, it is necessary to identify unique product safety and effectiveness. Because of the dissimilarities present in structure and processing methods provided by such diverse molecules, it is necessary to identify unique product protection and effectiveness. As compared to the widely stated half-life for regular FIX concentrates (18–30 h), half-life of FIX is extended via EHL–rFIX preparations by 3-5 times[24], leading in preservation of greater plasma FIX trough concentrations. Which further enable individual to acquire effective prophylaxis once with weekly or fornight in the scenerio of adults. The extension of half-life is more modest for the products of EHL-rFVIII products. Some of the examples of recombinant drugs being approved by FDA for hemophilia are mentioned in the table (Table 2) below.

Table 2: Recombinant Approved for Hemophilia by FDA [25].

Approved Recombinant Drug	Year of Approval
Hemlibra	2017
Rebinyn	2017
Jivi	2018
Esperoct	2019
Wilate	2020
Sevenfact	

Techniques for maximizing half-life of recombinant clotting factors include: Among the methods for prolonging the half-life of recombinant coagulation factors are:

- Covalently attaching the coagulation factor to polyethylene glycol (PEG; PEGylation) to minimize contact with clearance receptors; i
- Fusing coagulation factor with the fractured crystallizable (Fc) part of the immunoglobulin G1 (IgG1) molecule to redirect the molecule away from lysosomal degradation and thereby postpone its clearance or
- Combining both the coagulation factor and the recombinant albumin to save endocytosed proteins from the intracellular degradation pathway; and
- Single-chain technology for augmenting the intracellular degradation pathway.
- Single-chain technology for increasing molecule stability.

PE Gylation: PEGylation, which includes the covalent attachment of PEG to FVIII or FIX, may alter the pharmacokinetic and pharmacodynamic properties of coagulation factors. PEGylation increases the circulating half-life of PEGylated factor concentrates. This decreases the binding ability of PEGylated proteins to their clearance receptors [26]and, as a result, their degradation.

Fusion protein technology (Fc fusion and albumin fusion): fusion protein technology entails genetic protein fusion which has been an exceptionally longer half-life, like albumin as well as immunoglobulins (Fc fusion). Albumin and IgG are the proteins that occurs naturally and have long half-lives (i.e. greater than 20 days) that accounts for approximately 80% of the total proteins in plasma, making them valuable instruments for fusion protein technologies. Albumin and IgG have longer half-lives as a result of neonatal Fc receptor (FcRn)-mediated recycling, a naturally occurring process

of recycling which diverts the proteins away from lysosomal degradation, resulting in delayed clearance and longer functional plasma half-lives. Human FVIII is a heterodimeric structure made up of a light chain (A3-C1-C2 domains) as well as a heavy chain (A1-A2-B domains) and linked by noncovalent bonds, making it relatively unstable and readily dissociable from inactivated FVIII chains [27].

Considerations when introducing expanded half-life goods to patients: A factor product has to be carefully evaluated before to being presented to patients. Clinical examination is critical in that, similar to the case with conventional key component replacement therapy, patients using EHL goods would profit from plasma factor levels being assessed; these individuals will also need to have medical procedures performed to detect bleeds and other joint-injury related issues that otherwise might go unnoticed.

Prolonged durations at low factor levels until an effective regime is created may result in breakthrough bleeding. Doctors should ensure that their patients' requirements are addressed prior to transitioning to guarantee that their patients' demands are met.

Lowering the amount or frequency of EHL drug use in individuals who have not had any negative effects might be an option. You can create the very same stable factor concentration by administering low dosages of an EHL component, and this will not increase factor intake.

CONCLUSION

The status of hemophilia has been changed drastically from the state of being neglected tand sometimes fatal inherent hemorrhagic disease to a state of well-known molecular entities, within the last three decades. There still studies going on to make the treatment of hemophilia as the most efficacious and safe among the other abundant monogenic disorders like thalassemia, cystic fibrosis, muscle dystrophy. Patients are encouraged to diverge from on-demand treatment towards the prophylaxis due to EHL factor products. Therefore, as a result of this there is a reduction in burden for frequent injections, decrease for central venous catheter need for children, encourages adherence, improves results and assists to give more active lifestyles. EHL-rFIX products EHL–rFIX drugs does have ability for substantially decrease the infusion prevalence as well as boost through concentrations in the patients suffering from haemophilia B, resulting in an improvement in efficacy.

No data have surfaced about longer half-life FVIII inhibitor-forming products increasing the chance of forming an FVIII inhibitor. Histologically confirmed individuals who are FVIII-tolerant and have a lower risk of developing this consequence are at least as bad to be benefited as patients who have not previously been treated. Despite being safe and immunogenic, no clinical evidence has yet been found that demonstrates the immunity of EHL products in individuals at high risk for inhibitor development, such as formerly uncontrolled or poorly treated neonates, and individuals having a family background of inhibitors. Even with its problems, the introduction of new EHL factor products might bring about a big improvement in the care of haemophilia. If they could find a way to improve the overall care environment, they would be able to dictate the eventual outcome.

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