Original Research Article

Modified method for partial purification of human blood clotting factor VIII from fresh frozen plasma

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Abstract

Human blood is an irreplaceable commodity. Its essentiality has its own limitations in Transfusion Medicine like its proteinaceous structure, the compatibility and others. Introduction of individual coagulation factors like Blood clotting Factor VIII to the haemophilic patient provides the better way of management of the hemophilic patients. There is dire need to make available Clotting Factor VIII from freeze dried anti-haemophilic cryoprecipitate. The cryoprecipitate of fresh plasma with Factor VIII of coagulation has longer shelf life (2 years) and can be introduced to the patient with less interference of immunological complications. The present study established the partial purification procedure for cryoprecipitate - rich in Coagulation Factor VIII without significant loss of activity. The gradual treatment of change in temperature followed by lyophilisation produced enriched fraction. Thus the Coagulation Factor VIII was made available for infusion to the required haemophilic patient readily.

Key Words: Cryoprecipitate, Stages of lyophilisation, Blood Safety and Quality regulations (BSQR), Clotting Factor VIII

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INTRODUCTION

The Patients with the coagulation factor deficiencies are at increased risk of hemorrhagic complications. The replacement whole blood or its fractions in treatment of hemophilic patients is always a difficult part of the treatment. The diagnostic skills and knowledge of the disorder processes improved the need of whole blood and its components for specific deficiencies which can be diagnosed by improved techniques. Moreover, these components needed to be relatively pure and concentrated so that they could be administered in optimal amounts with minimum side effects.[1,2,3] The fresh plasma which is essentially separated aseptically from donor's blood, contains proteins, immunoglobulin and various other coagulative factors such as Fibrinogen, Factor VIII, factor V, Factor XIII etc.[1] This plasma transfusion is beneficial, if transfused to the patient within 24 hours of collection of blood as the activity of coagulation factor starts to deplete after that. Cryoprecipitate, which contains factor VIII and other factors, is used for the correction of inherited and acquired coagulopathies. Measurement of factor VIII concentration in blood plasma is commonly determined by the clotting and other tests[4,5,6], useful in transfusion medicine. The factor VIII mainly used in treatment of Hemophilia A, von Willebrand's disease, hypofibrinogenemia, deficiency of the Coagulation Factor XIII, Disseminated intravascularcoagulation (DIC),acute leukemia, dilutionalhy pofibrinogenemia, fibrinolysis[2-4] Various research groups utilized the techniques viz. apheresis and leucofiltration to achieve partial purification of blood clotting factor VIII.[5,7] No standard techniques are available to achieve the enrichment of the required fraction along with retention the activity. The efforts were also done with whole blood and leucodepleted plasma using whole blood filters and apheresis machine[7,8,9] They could achieve about 79-40%% activity. There is a need to increase the efficiency

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of the process and obtain higher activity. The study has been directed to get the enriched fractions with maximum activity.

MATERIALS AND METHODS

The human blood of voluntary blood donors (Age 25-45 years) was collected aseptically in units of special triple bags.. In order to maintain the blood viscosity and to prevent it from coagulation, the anticoagulants like SAGM (saline-adenine-glucose-mannitol), CPD-A (citrate-phosphate-dextrose adenine), Warfarin were added[7,9,10]. The written informed consent from healthy volunteers was taken at the time of donation of blood. The volunteers had no history of malaria, dengue coagulopathy or anticoagulant/antiplatelet medication in the last 14 days before blood donation. Prior to the blood collection, registering physical and personal details of blood donor was done. The clinically healthy subjects (25 years to 45 years) tested negative for HBsAg, HCV, HIV and VDRL were selected for the study. A total of 300 samples were selected for Cryoprecipitation.(BSQR). Thus freeze-dried human antihaemophillic cryoprecipitate was prepared from fresh human plasma and the amount of Factor VIII was estimated. The percentage recovery of Factor VIII was calculated before and after lyophilisation. Percentage recovery was obtained by mathematical calculation and for product approval it has to be greater than or equal to 60 %.

Collection of Cryobags: Fresh frozen plasma(FFP), was separated from whole blood within 6 hours of donation and snap frozen at or below -30^{0}C. FFP contains all coagulation factor plus complements/coagulation factors

Preparation of Cryo-vials: Under laminar air flow plasma was pooled from all bags. Equal amount of Glycine buffer was added to the pooled plasma. With the help of glass rod it was stirred for proper homogenation. This plasma was then transferred in one 1000ml glass bottle under laminar air flow. Ten aliquots of10 ml were prepared and these vials were stored at or below -60^{0}C in refrigerator overnight. These were then transferred to the chamber of laboratory Lyophiliser, before temperature of vials reached to its eutectic point

Lyophilisation and activity check: During lyophilisation, temperature of vials was increased slowly from -45^{o}C-20^{o}C at specific time intervals using LYOVAC GT2 Laboratory Lyophiliser. Sysmax CA-50. The blood coagulation analyser was employed for assessment of the activity using Von Clauss Method[5,6].

The permission from the local ethics committee was obtained for the studies.

RESULTS

Table 1: The samples of fresh whole blood (10 aliquots)were given following treatment within 6 hours of procurement The Sequence of Lyophilisation Process

Sr. No.	Duration	Temperature	Observations
1	Day 1 Zero hour:overnight freezing removed	-45^{o}c	Completely freezed vials kept to lyophilize
2	After 2 hours	-35^{o}c	Complete yellow Cake formation at base observed.
3	After 3 hours	-30^{o}c	Cake formation started from side walls of vials by turning from yellow to white.
4	After 4 hours	-25^{o}c	Cake appears in the middle of the vial as it slightly moves upwards by pressure.
5	After 5 hours	-20^{o}c	Cake at base with yellow centre moiety.
6	After 6 hours	-10^{o}c	White side walls appear with small yellow centre moiety and keep it for overnight**.**
7	Day – 2 :	0^{o}c	Complete dry cryoprecipitate formed.
8	After 25 hour	10^{o}c	Cryoprecipitate maintained.
9	After 30 hours	20^{o}c	Cryoprecipitate maintained.

Activity check: The Cryo-samples were analyzed for the activity of Coagulation factor VIII before and after lyophilisation (Table 1 and Table 2). Sysmex CA-50 blood coagulation analyser was used for analysis. The method used was von Clauss[5] with modifications, this produced a visible clot, which is detected by a light absorption method. The elapsed time from thrombin addition to the generation of a visible clot is inversely proportional to the coagulation Factor VIII concentration. A standard of known concentration is used to generate a standard curve, from which concentrations of Factor VIII can be deduced.[8,9,10]

Table 2: Percentage Recovery after Lyophilisation

	Before Lyophilisation mgs	After Lyophilisation mgs
Weight	119.8 + 0.416	91.2+ 0.047*
% Recovery	100.0%	76.13%*
Shelf life	24 hours	24 months

***p<.001**

Thus the method could provide enriched fraction with 76.13% activity. Earlier reports quoted about 70%-30% % activity[11,13,14].

DISCUSSION

Blood which is an essential commodity, is having short shelf-life and in short supply By using blood component therapy, various blood components such as Fibrinogen, Factor VIII, von Willebr and factor and other coagulative factors can be qualitatively fractionated and utilized for supplementation effectively.[7,9] This approach increases not only shelf-life of the products but also efficiency and utility of the blood. Few guidelines are available in the literature for this[10,13,14]. The present study was undertaken to optimize and analyse the procedure of the extraction of Factor VIII production from human fresh plasma or Cryoprecipitate. In this study. various analytical approaches involved in the Lyophilisation technique were studied[4,5,7]. The preparation of Cryoprecipitate was the first practical method towards preparation of the more concentrated form of anti-haemophiliac factor and its commercialization. It was prepared by controlled thawing of frozen plasma to precipitate high molecular weight proteins, including Factor VIII, fibrinogen and von Will brand factor.[13,15] The present study optimized the procedure of purification and procuring the fraction rich in Coagulation Factor VIII from freeze dried lyophilised Cryoprecipitate. The percentage recovery of the Clotting Factor VIII from FFP was about 76 %. With switching of shelf life of the fraction from 24 hours to two years was remarkable, which has long implications.[14,16]

CONCLUSION

Factor VIII was enriched successfully from freeze dried anti haemophilic Cryoprecipitate by following stepwise warming protocol. The major advantage of this protocol was that, it ensures immunological reactions-free option for the haemophilics. It is easy to handle and so it reduces also storage cost. By following this protocol The minimal loss in activity of Factor VIII at different stages of Cryoprecipitation was occurred during the new process of lopholization. This produced Cryoprecipitate containing Factor VIII with shelf life of 365 days only. During transfusion this cryoprecipitate would have to undergo pretransfusion test for its activity and then would be transfused to the patient by adding appropriate volume of saline to it. A typical adult dose is two to five-donor pools (equivalent to 10 single donor units) containing 3–6 g factor VIII in a volume of 200 to 500 ml. One such treatment administered to an adult would typically raise the plasma factor VIII level by about 1 g/l. Further study in this direction will provide newer and better options of treatment of the hemophilic patients

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