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Effect of prolonged storage at 2°C–6°C for 120 h on the coagulation factors of thawed cryoprecipitate: Can we extend its shelf life post thaw beyond 4 h?

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Abstract:

BACKGROUND: Cryoprecipitate helps in replenishing important coagulation factors like fibrinogen, Factor VIII and von Willebrand factor without running the risk of volume overload. It is very useful in the treatment of trauma patients with active bleeding and works best when administered early. Extending the shelf life of thawed cryoprecipitate beyond 4 hours enables us to manage inventory better, reduces the burden of demand vs supply as well as minimizes wastage. It can also help in logistically supporting the transfusion services in making cryoprecipitate readily available in mass casualty scenarios (war, natural calamity) in remote locations by reducing the time required for thawing cryoprecipitate and the need for costly storage equipment. AIM: The aim of this study was to compare the levels of Factor VIII, Fibrinogen and von Willebrand factor on thawed cryoprecipitate after prolonged storage for 5 days at a temperature of 2-6°C.

METHODOLOGY: The above mentioned coagulation factors were analyzed in cryoprecipitate at the time of product thaw and again after 120 hours of 2 to 6°C storage using fully automated coagulation analyser (STA Compact Max). All parameters were expressed as Mean ± Standard deviation and were analyzed using paired t-test with level of significance, $P < 0.05$.

RESULTS: There was a significant decrease in the level of Factor VIII, whereas the levels of fibrinogen and von Willebrand Factor remained stable during the storage period. All the cryoprecipitate units retained factor activities above therapeutic range even after 5 days of storage at 2-6°C.

CONCLUSION: Although the levels of clotting factors are reduced during storage, they are still maintained above the therapeutic range. In scenarios where maintaining frozen cryoprecipitate inventory is a logistical challenge and emergency massive demands of cryoprecipitate are foreseen, the use of pre-thawed cryoprecipitate can be considered as a viable option.

Keywords:

Coagulation factors, prolonged storage, shelf life, thawed cryoprecipitate

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Introduction

Cryoprecipitate is the insoluble precipitate obtained by thawing and centrifuging fresh frozen plasma at 1°C–6°C. Cryoprecipitate is a rich source of fibrinogen, Factor VIII, Factor XIII, von Willebrand factor (vWF), and fibronectin.[1] When stored at a temperature below –18°C, it has a shelf life of 1 year. Post thaw, it has to be issued within 6 h if a closed system has been ensured and within 4 h if it is pooled in an open system, as per the American Association of Blood Banks and national guidelines.[1,2]

Cryoprecipitate is a critical component in the treatment of trauma cases requiring

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massive transfusion and consumptive or dilutional coagulopathies and in patients with uremia.[3-5] All these cases may need cryoprecipitate acutely on an emergency basis where any delay in issuing the product may put patient safety at stake. The time taken for thawing of cryoprecipitate often results in delay of issue of this vital product.

In a military setting, the forward deployment of troops is usually in remote locations with inhospitable conditions. Transporting cryoprecipitate in frozen state in supercooled containers with uninterrupted power supply while maintaining temperatures below −18°C becomes a huge logistical hurdle which may prevent timely supply of this life-saving product at these locations, especially in a wartime scenario.

In such cases, supply of thawed cryoprecipitate at 2°C–6°C in blood transport boxes is a viable solution if the shelf life of thawed cryoprecipitate is extended. This would also enable blood banks to maintain an inventory of prethawed cryoprecipitate, which will effectively address demands in emergency scenarios.

The national guidelines mandate each unit of cryoprecipitate to have at least 150 mg of fibrinogen, 80 IU of Factor VIII, and a minimum 40% activity of vWF at the time of administration to the patient.[2] This study was an endeavor to measure the changes in the vital clotting factors present in cryoprecipitate when it is stored for prolonged duration of 120 h at a temperature of 2°C–6°C post thaw and thereby exploring the possibility of extending the shelf life of this product.

Materials and Methods

Institutional ethical committee clearance for this study was taken in November 2016.

Sample size calculation

The sample size was calculated to test the following hypothesis for all variables of interest. Null hypothesis assumed no difference in the mean of hemostatic parameters in cryoprecipitate on day "0" (immediately post thaw) and day "5" (after prolonged storage at 2°C–6°C for 120 h). H0: $\mu d = 0$ against H1: $\mu d \neq 0$ where μd is the mean change in the respective variables after storage of cryoprecipitate for 5 days, with α being 5% and power of study being 80%. The minimum sample size worked out to be 15. However, thirty units of cryoprecipitate were studied to improve the strength of the study.

Blood processing and sample collection

A total of thirty units of nonpooled cryoprecipitate derived from single whole blood donation units, were randomly selected for this study. The whole blood units (450 ± 50 ml) were collected in top and top (Citrate Phosphate Dextrose Adenine bags, Polymed Medical Devices, Faridabad, India) CPDA-1 bags, each having 63 ml of the anticoagulant following the standard collection procedures. The component preparation and storage was done within 8 h of collection from the donor. Cryoprecipitate was prepared from fresh frozen plasma after thawing overnight at 4°C in a walk-in cold room. The cryoprecipitate thus formed was then stored in a blast freezer at a temperature below −30°C (−40°C ultralow freezer, NUAIRE, Plymouth, United States). Thirty units of cryoprecipitate were randomly chosen from this inventory and were used for the study. All the units chosen for the study were from nonleukodepleted products.

These units were then thawed at 37°C in a plasma thawer for 20 min while maintaining all the necessary precautions to avoid any contamination. These thawed cryoprecipitate units underwent quality control (QC) testing as per the national guidelines. Units which passed the QC tests were selected for evaluation in our study.

The "Day 0" samples (immediately post thaw) were collected from these freshly thawed cryoprecipitate units in a transfer bag using a sterile tube connecting device (TSCD II, Terumo BCT, Colorado, United States). Samples taken from the transfer bags were then used for laboratory analysis of various parameters. The cryoprecipitate bags were then kept at a temperature of 2°C–6°C in a blood storage cabinet (HHB III, Helmer Scientific, Bergen Blvd, Noblesville, IN 46060, United States). "Day 5" samples (after 120 h of storage at 2°C–6°C) were similarly collected from these stored units after prolonged storage of 120 h.

Laboratory analysis

Quantitative assays were performed on the "Day 0" and "Day 5" samples for three main parameters Factor VIII, fibrinogen, and vWF. All the parameters were tested on a fully automated coagulation analyzer (STA compact MAX, Daignostica STAGO, France). The quantitative determination of Factor VIII level was done in undiluted cryoprecipitate samples by clot-based method using STA Deficient VIII kit (Diagnostica Stago, Asnières sur Seine, France). Measurement of the levels of vWF: Ag (vWF antigen) was done in undiluted samples by immunoturbidimetric method using LIATEST vWF: Ag kit (Diagnostica Stago, Asnières sur Seine, France). Similarly, assessment of the fibrinogen levels was carried out using FIBRI-PREST kit 3 (Diagnostica Stago, Asnières sur Seine, France) by the clotting method of Clauss.

All the assays were standardized using a calibrator plasma STA-Unicalibrator (Diagnostica Stago, Asnières sur Seine, France).

Statistical analysis

The data were analyzed with SPSS 22 (IBM Corp. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp). Shapiro Wilk tests were used to establish normalcy of the collected data. Paired *t*-tests were then used to compare the means of the variables of day "0" and day "5" samples to check for the changes in the activities of the variables. $P < 0.05$ was considered statistically significant.

Results

All the values were expressed as mean (±standard deviation) [Table 1]. The levels of Factor VIII decreased from a mean of 159.26 (±27.30) IU/dL on day "0" to a mean of 120.16 (±17.13) IU/dL on day "5," which was a 24.55% drop in activity from the baseline and was found to be statistically significant ($P < 0.001$).

Similarly, there was a statistically significant ($P = 0.002$) drop in the levels of vWF (vWF: Ag) by 2.56% from a mean of 189.86 (±28.55) IU/dL on day "0" to a mean of 185.00 (±26.61) IU/dL on day "5."

Fibrinogen levels declined marginally by 0.48% from a mean of 461.26 (±104.30) mg/dL on day "0" to a mean of 459.03 (±103.19) mg/dL on day '"5;" however, this change did not achieve statistical significance ($P = 0.462$).

The levels of both fibrinogen and vWF remained relatively stable for the entire duration of storage post thaw. The changes in the various parameters are illustrated in Figures 1-4.

Discussion

One of the main uses of cryoprecipitate was for the treatment of hemophilia A. Replenishing the thermolabile Factor VIII levels, therefore, used to be the major beneficial effect of transfusing cryoprecipitate units and hence, the decline in its activity was the limiting factor in prolonged storage of cryoprecipitate beyond 6 h.[6] The advent of recombinant clotting factors along with other effective modalities of treatment (e.g., desmopressin) has limited the role of cryoprecipitate for F VIII replenishment in the modern clinical practice.[7,8]

Table 1: Changes in various parameters in cryoprecipitate after prolonged storage at 2°C–6°C post thaw

Parameter studied	Mean±SD		*P*
	Day "0"	Day "5"	
Factor VIII (IU/dL)	159.26±27.30	120.16±17.13	<0.001
Fibrinogen (mg/dL)	461.26±104.30	459.03±103.19	0.462
vWF (IU/dL)	189.86±28.55	185.00±26.61	0.002

SD=Standard deviation, vWF=Von Willebrand factor

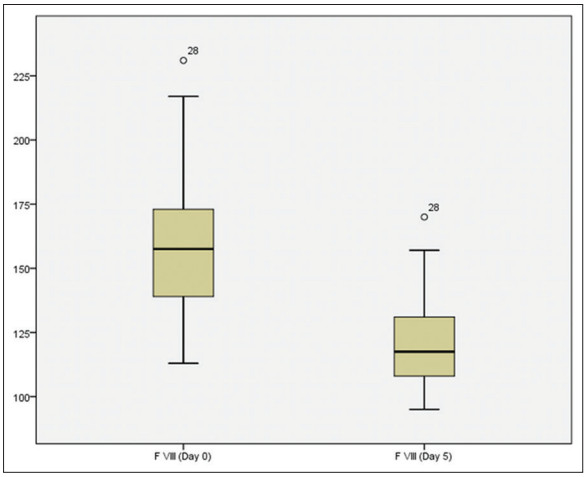

Figure 1: Changes in Factor VIII levels (IU/dL)

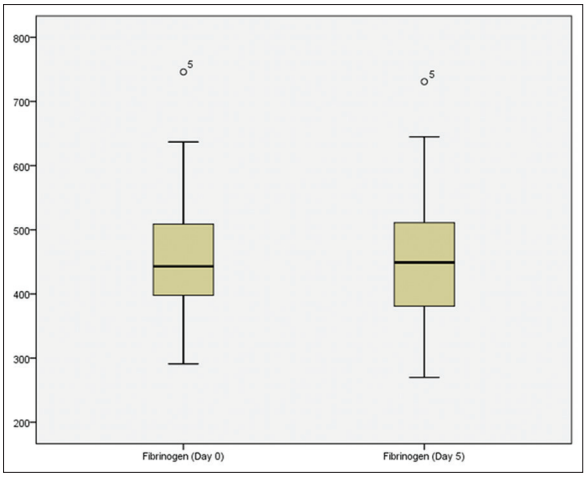

Figure 2: Changes in fibrinogen levels (mg/dL)

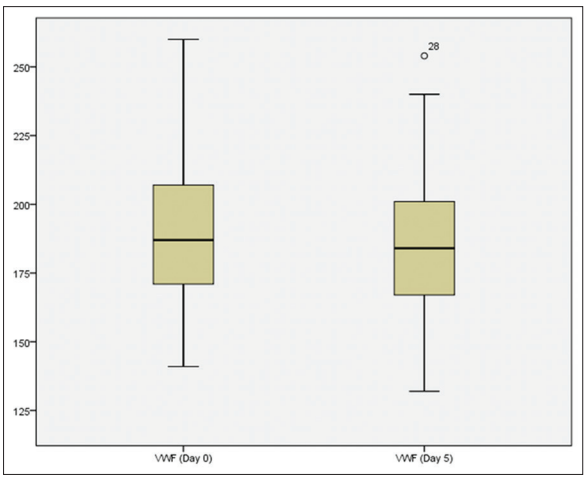

Figure 3: Changes in von Willebrand factor levels (IU/dL)

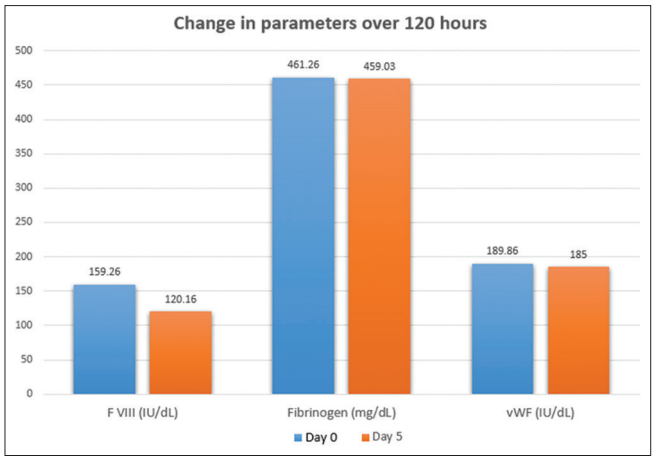

Figure 4: Graphical overview of the observed changes. (All graphs and tables represented above are original based on the data collected at the Department of Immunohematology and Blood Transfusion, AFMC, Pune)

At present, the use of cryoprecipitate is mainly intended to replenish fibrinogen in patients with acute bleeding. It has been seen in various studies that in severe trauma cases, early administration of cryoprecipitate may actually decrease transfusion requirements in addition to effectively controlling the bleed.[9,10] This can be facilitated by the provision of an inventory of prethawed cryoprecipitate in the blood banks. Therefore, fibrinogen levels, if maintained in thawed cryoprecipitate after prolonged storage, could be the primary therapeutic reason in favor of increasing its postthaw shelf life.

The present guidelines mandate the use of cryoprecipitate within 6 h of thawing (4 h in case of pooled cryoprecipitate).[2] This narrow postthaw shelf life makes it impossible to maintain an inventory of thawed cryoprecipitate, to avoid unnecessary wastage. This leads to a critical delay in the administration of cryoprecipitate in the setting of emergency requirement in patients. In addition, alternatives such as lyophilized fibrinogen concentrates are not cost-effective and do not replenish other factors such as vWF, Factor VIII, and Factor XIII.

In our study, we found that the levels of fibrinogen and vWF were maintained at almost the same levels even after prolonged storage of 120 h in a thawed state. The levels of vWF dropped by 2.56%, and the fibrinogen levels showed a minimal decrease by only 0.48%. These findings show that the most important component of cryoprecipitate, i.e., fibrinogen, remains extremely stable even after prolonged storage of 120 h postthaw at 2°C–6°C. A previous study by Lokhandwala *et al*. also exhibits similar findings with no significant decline in the activities of vWF and fibrinogen.[11] Fibrinogen stability in thawed pooled cryoprecipitate has also been shown to be maintained for 72 h postthaw in the study by Green *et al*.[12] For vWF, although the reduction in activity was significant in our study, the levels of the factor were well above the therapeutic range.

Factor VIII levels showed a decline in activity by 24.55% after which the mean Factor VIII levels on day "5" was 120.16 IU/dL, which is still above the therapeutic range for the product as per the national QC guidelines. FVIII decreased by 10% in the study by Lokhandwala *et al*., which is less than that seen in our study. Similarly, there was a lesser decline in FVIII levels in the study by Green *et al*. Both these studies have kept the postthaw cryoprecipitate at room temperature. A greater decline in FVIII activity has been demonstrated on prolonged storage at 1°C–6°C as compared to those samples which were stored at room temperature in a previous study by Spivey *et al*.[13] However, these studies were conducted in countries with a much lower ambient temperature (the USA and the UK) as compared to ours (India). This potentially poses a greater risk of microbial contamination on prolonged storage at room temperatures. Therefore, storage at 1°C–6°C may be beneficial in our scenario as it reduces the risk of microbial growth in the stored units while the FVIII levels were still maintained above the therapeutic range. Our study had the advantage of a greater sample size (n = 30) as compared to the previous studies (n = 20 for Lokhandwala *et al*., n = 16 for Green *et al*).

Therefore, we can see that although there is a significant drop in the levels of Factor VIII, the other parameters remain relatively stable in this product after 5 days. Even the levels of Factor VIII were above the therapeutic range (>80 IU) in our study.

Therefore, the study provides evidence in support of exploring the possibilities of extending the shelf life of cryoprecipitate. This would enable us to manage inventory better, reduce wastage, as well as have a ready stock of prethawed cryoprecipitate which can be issued at a very short notice in case of emergencies like in a wartime scenario/natural calamities/transport to remote locations in thawed state.

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Conflicts of interest

There are no conflicts of interest.

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