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FV, FVIII and Fibrinogen Activity in Fresh Frozen Plasma, Frozen Plasma and Cryoprecipitate: Observational Cross-sectional Study

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Authors' contributions

This work was carried out in collaboration among all authors. Author IY designed the study. Author WH performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author SA managed the analyses of the study. Author SA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Objective: Studies proved that storing whole blood overnight at 4°C resulted in a decrease in the activity of coagulation factor FVIII, without significant loss of activity of coagulation factors FV or fibrinogen. This study is conducted to compare the activity of labile factors V and VIII as well as fibrinogen level in FFP with that of FP24 and to assess their levels in cryoprecipitate and cryosupernatant bags as well.

Materials and Methods: FFP bags separated from whole blood within 8 hours were compared to FP24 bags separated within 24 hours, cryoprecipitate and cryosupernatant after phlebotomy in terms of coagulation factors V and VIII activity and level of fibrinogen by standard methods using (automated coagulometer STA Compact, Stago, France).

Results: A statistically significant loss of factor VIII activity in FP24 compared to FFP was detected; while, the fall in activity of factor V and fibrinogen level was not statistically significant. A highly statistically significant difference was elicited regarding factor VIII, factor V and fibrinogen when

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comparing cryoprecipitate and cryosupernatant samples. On comparing FFP and cryoprecipitate regarding factor VIII, factor V and fibrinogen a highly statistically significant difference was elicited.
Conclusion: The retention in factor activities, in particular factor V, in FP, indicates the lack of relevant adverse changes when extending the hold period for plasma units. The reduction in factor VIII activity doesn't reduce the quality of FP.

Keywords: Factor V; factor VIII; fibrinogen; fresh frozen plasma; plasma frozen within 24 h; cryoprecipitate.

1. INTRODUCTION

Plasma is a crucial component of blood with albumin, coagulation factors and immunoglobulins being the most important components of plasma that can be transfused. There are many types of plasma such as fresh frozen plasma (FFP), plasma frozen within 24 h, single donor plasma, cryoprecipitate, cryoprecipitate – reduced plasma, pathogen inactivated plasma, and thawed plasma. FFP is human donor plasma frozen in a short period after the process of collection (often 8 h). Plasma frozen at later intervals (up to 24 h) after collection is referred to as frozen plasma (FP) [1].

Cryoprecipitate is a frozen blood product prepared from thawed FFP and contains fibrinogen, von Willebrand factor (VWF), FVIII, FXIII and fibronectin. It's used for treating patients with inherited or acquired hypo- or dysfibrinogenemias. It should no longer be the first choice in treating hemophilia A or von Willebrand disease given the widespread availability of recombinant or virally inactivated factors. Cryoprecipitate is prepared by thawing a unit of fresh-frozen plasma in a 1 to 6°C water bath and then the cryoprecipitated material is separated from the liquid plasma. The cryoprecipitate is then frozen and stored at temperatures not exceeding −18°C for up to 1 year [2].

A few studies have analyzed the stability of different coagulation factors when whole blood storage time is prolonged to 24 hours and compared this to FFP. The data available on the levels of coagulation factors (factor VIII, vWF, fibrinogen, and other proteins) in cryoprecipitate made from whole blood stored for 24 hours before component preparation is not enough [3]. The current regimen for the preparation of FFP within 8 hours of whole blood collection was implemented to maintain the activity of coagulation factors. When whole blood is stored at 4°C for short time intervals, factor VIII significantly decreases in the extracted plasma, while other coagulation factors keep unchanged [4]. The aim of this study is to analyze and compare the activity of coagulation factor V, VIII and fibrinogen level in fresh frozen plasma, frozen plasma and cryoprecipitate bags.

2. MATERIALS AND METHODS

2.1 Blood Collection and Processing

The study was conducted at the main blood bank and the coagulation laboratory of Ain Shams University Hospitals and extended over a period of 8 months. Only donors fulfilling the eligibility criteria were made to donate blood according to AABB standards [5]. Blood was collected by a clean, single venipuncture in either triple bag or quadruple plastic blood packs.

All blood component preparation was performed as part of routine operation of the blood bank. Whole blood bag (450 mL) was centrifuged at 2,000 rpm (448×g) at 20°C for 11 minutes (Light spin) to prepare red cells and platelet-rich plasma (PRP) using Sigma 8KS centrifuge (Germany). PRP was subsequently centrifuged at 3,500 rpm (1372×g) at 20°C for 11 minutes (Heavy spin). Platelets in PRP were forced to the bottom of a satellite bag. The supernatant platelet-poor plasma (40-60 mL) was expelled into another satellite bag and stored at -18°C while the remaining bag contains platelet concentrate. Frozen plasma (FP24) was prepared by separating plasma from whole blood at later intervals within 24 hours after phlebotomy being stored at 4°C. Cryoprecipitate (20-40 mL) was prepared from freshly separated plasma by freezing at -70°C followed by thawing at 4 °C and heavy centrifugation. The plasma remaining after removing of cryoprecipitate is called cryo poor plasma (cryosupernatant).

2.2 Coagulation Studies

Samples were taken from 20 bags of each of FFP, FP 24, cryosupernatant and cryoprecipitate of randomly chosen blood groups. Plasma and

cryo samples were thawed in a 37°C water bath for 10 minutes immediately prior to performing the assay procedures. Factor V, Factor VIII and fibrinogen were measured using automated coagulometer STA Compact (Stago, France). Tests were run as per the manufacturer's instructions. FVIII was assessed using FVIII deficient reagent, STA-Immuno Def VIII (Stago, France). For FV assessment, STA FV deficient reagent (Stago, France) was used. STA-Liquid Fib (Stago, France) is the reagent used for the quantitative determination of fibrinogen level in plasma based on the Clauss method. For all assays, calibration was performed with STA-unicalibrator (Stago, France). The standards were automatically prepared by the analyzer by dilution according to the parameters supplied to the coagulometer for the assay. Positive and negative controls were run in order to ensure the accuracy and reproducibility of the results.

2.3 Statistical Methods

The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (SPSS 20) software package under Windows 8.1® operating system. Student t test was used to assess the statistical significance of the difference between two study group means. P value < 0.05 is considered significant.

3. RESULTS

A total of 20 units of FFP, FP24, cryosupernatant and cryoprecipitate were compared in terms of fibrinogen level which is a stable coagulation factor and activity of Factor V and VIII, which are labile factors. Levels of tested coagulation factors in all tested units are presented in Table (1).

Compared to FFP processed on Day 0, FP24 showed statistically significant losses of FVIII activity (42%), but the fall in FV activity (14%) or fibrinogen level (15%) was not statistically significant (Table 2 and Fig. 1).

On comparing FFP and cryoprecipitate regarding FV, FVIII and fibrinogen, a highly statistically significant difference was elicited (Table 3 and Fig. 2).

4. DISCUSSION

This observational cross-sectional study assessed and compared the level of fibrinogen, the stable coagulation factor, and activity of factor V and factor VIII, which are known to be labile factors in a total of 20 units of FFP compared with 20 units of FP 24, in addition to 20 cryoprecipitate and cryosupernatant units.

Table 1. Coagulation factor activities in FFP, FP24, cryoprecipitate and cryosupernatant units

Component Unit	Coagulation factor (Mean ± SD)		
	Factor VIII (%)*	Factor V (%)†	Fibrinogen (g/L)‡
FFP	81.1 ± 34.7	73.9 ± 14.9	2.5 ± 0.5
Frozen plasma (FP24)	47.1 ± 23.9	64.1 ± 18.2	2.1 ± 0.8
CRYO-PPT	147 ± 50.1	85.5 ± 11.3	2.9 ± 0.6
CRYO-supernatant	21.9 ± 16.5	8.6 ± 8.0	1.1 ± 0.3

**: Factor VIII reference interval: 60-150%; †: Factor V: 70-120%; ‡: fibrinogen: 2-4 g/L*

Table 2. Comparison of coagulation factors in FFP and FP24

Parameter (Mean ± SD)	FFP	FP24	P value	Significance
Factor VIII (%)*	81.1 ± 34.7	47.1 ± 23.9	0.001	S[ł]
Factor V (%) †	73.9 ± 14.9	64.1 ± 18.2	0.073	NS
Fibrinogen (g/L) ‡	2.5 ± 0.5	2.1 ± 0.8	0.079	NS

**: Factor VIII reference interval: 60-150%; †: Factor V: 70-120%; ‡: fibrinogen: 2-4 g/L*

Table 3. Comparison of coagulation factors in FFP and cryoprecipitate

Parameter (Mean ± SD)	Cryoprecipitate	FFP	P value	Significance
Factor VIII (%)*	88.6 ± 25.5	81.1 ± 34.7	0.44	NS
Factor V (%) †	85.5 ± 11.3	73.9 ± 14.9	0.009	S
Fibrinogen (g/L) ‡	2.9 ± 0.6	2.5 ± 0.5	0.006	S

*łS: Significant; NS: Non-significant; HS: Highly significant; *: Factor VIII reference interval: 60-150%; †: Factor V: 70-120%; ‡: fibrinogen: 2-4 g/L*

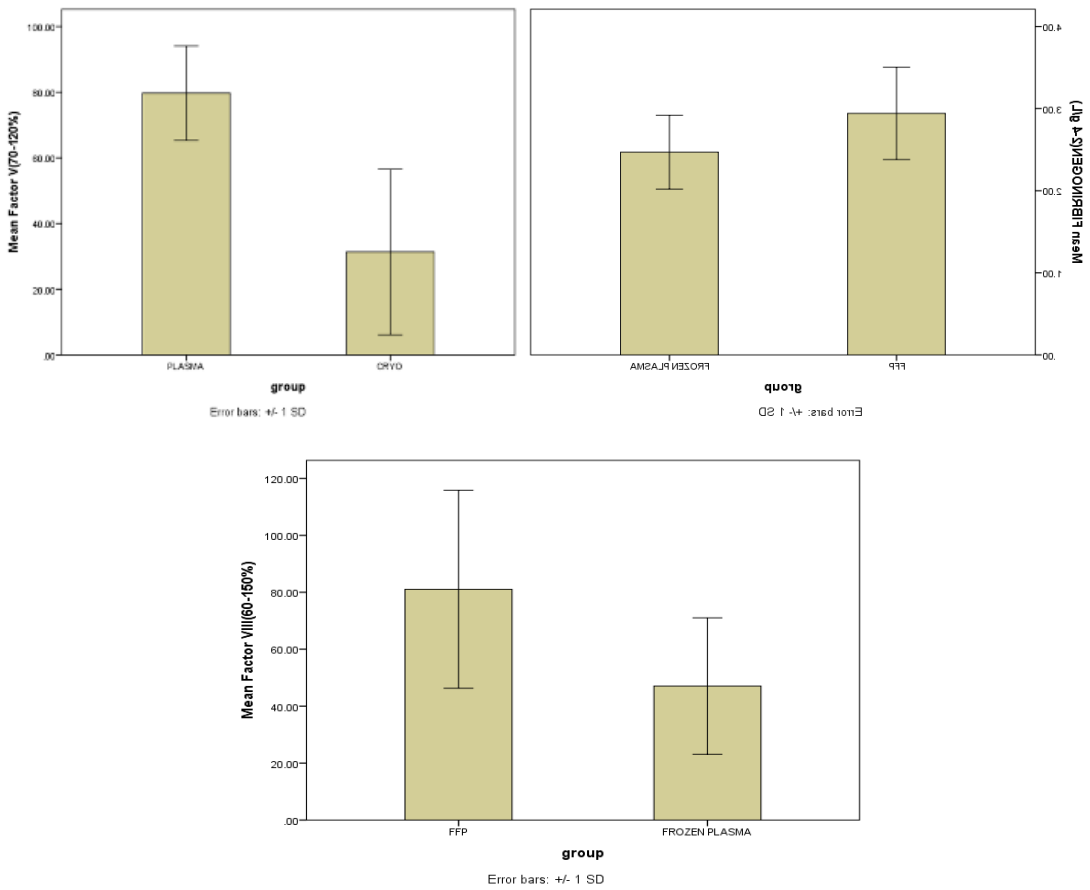

Fig. 1. Coagulation factor levels in FFP and FP24

Studies proved that storing whole blood overnight at 4°C resulted in a decrease in the activity of coagulation factor FVIII, without significant loss of activity of coagulation factors FV or fibrinogen [6]. The use of plasma frozen within 24 hours of phlebotomy is now preferred by many blood centers, as that not solely provides operational flexibility and efficiency, however can indirectly enhance component safety, and would maximize the potential to use plasma from male donors to avoid the hazards of HLA antibodies, and the potential occurrence of transfusion-related acute lung injury (TRALI) on the grounds that antibodies against human leukocyte antigens (HLA) in the plasma of female donors are the principle cause of TRALI. Typically, the reactions causing this injury are mediated by donor anti-HLA or neutrophil specific antibodies, which bind to recipient neutrophils leading to non-cardiogenic pulmonary edema, hypoxia, and sometimes death. The prevalence of anti-HLA antibodies is highest in donors who have previously been pregnant. Holding blood overnight could contribute to a reduction in the risk of TRALI by increasing the number of “male-only” donations available for FFP production [7].

The most significantly affected factor was factor VIII, which showed a statistically significant loss of 42% when FFP was compared with FP. This comes in concordance with the results of Dogra et al. [6], who found a similar loss of 18.4% in factor VIII activity. Similar results were also reported by Alakech et al. [8] who found a loss of 29% of factor VIII activity, Sheffield et al. [9] who also detected a loss of 30-35% of factor VIII activity and Alhumaidan et al. [3] who found a loss of 25% of factor VIII activity. A significant loss of factor VIII activity of 23% and 25% was also reported by Cardigan et al. and Agus et al. [10,7] respectively.

The non-significant loss of Factor V activity of 14%, together with the fall of 15% in fibrinogen level in FP when compared to FFP in our study comes along with the findings of Dogra and colleagues, Alhumaidan et al. and Sheffield et al. [6,3,9] who also reported loss of factor V and fibrinogen level activity which was unlikely to be

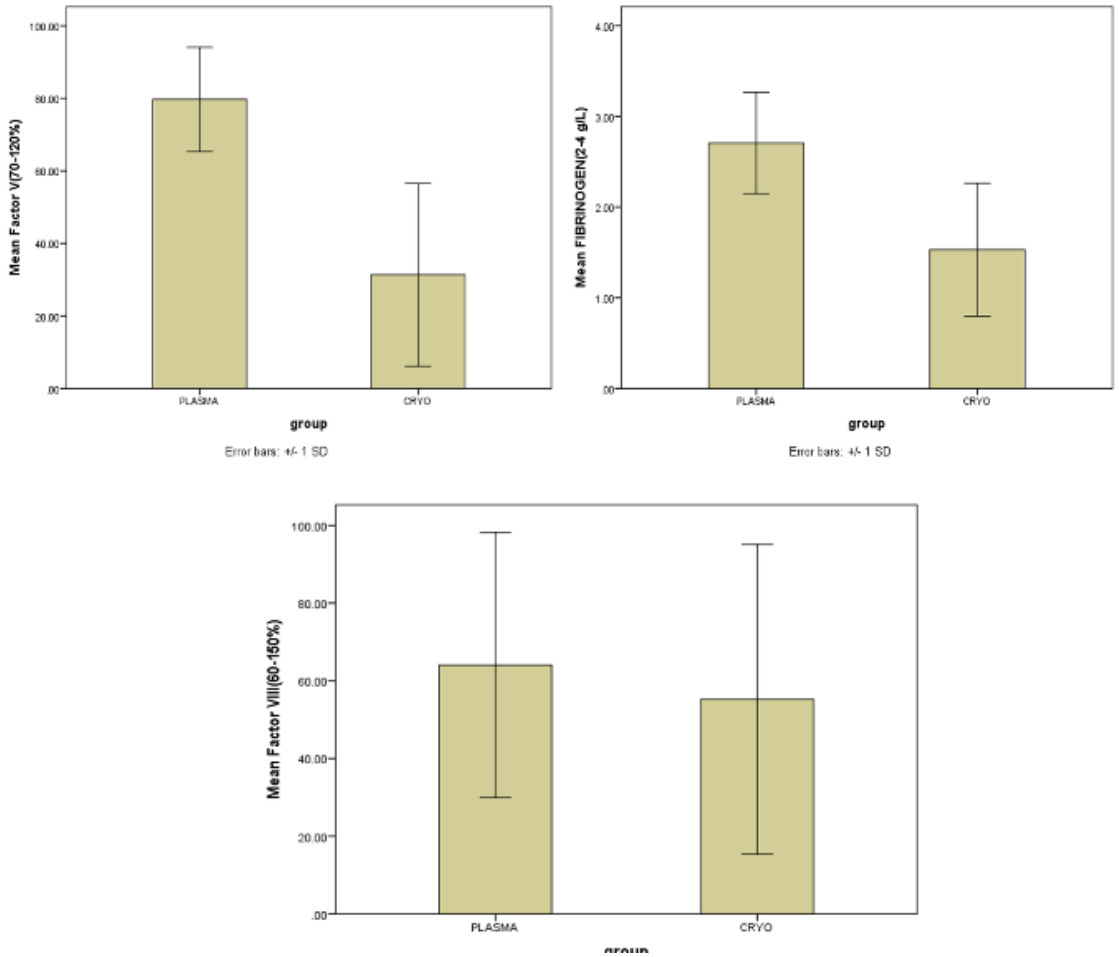

Fig. 2. Coagulation factors levels in FFP and cryoprecipitate

of clinical significance. However, Cardigan et al. [10] discovered a loss of 15% of FV activity together with 12% loss of fibrinogen level which was significant. The differences in testing methods between this study and other mentioned studies might have accounted for the significant loss of fibrinogen.

Plasma for transfusion is regularly utilized for correction of coagulation factor deficiencies, for which a specific concentrate is not available, when there is active bleeding or in nonbleeding patients before invasive procedures or surgery whenever there are abnormal coagulation screening tests [10]. The most widely recognized causes of acquired coagulopathies are vitamin K antagonist coagulopathy, disseminated intravascular coagulation (DIC), liver disease and dilutional coagulopathy [11]. Level of factor VIII in liver disease is not decreased and may be even increased, as many chronic liver diseases are associated with chronic inflammation, so factor VIII replacement is not considered. On the other hand, DIC and dilutional coagulopathy can be associated with transiently diminished factor VIII levels. However, factor VIII is considered an acute phase protein that whenever diminished will bounce back quickly [3]. Therefore, the observed decrease in the level of coagulation factors is unlikely to be of importance clinically and FP can be used for the same indications as FFP [6].

The non-significant decrease in factor activities, in particular factor V, in FP, indicates the lack of relevant adverse changes when extending the hold period for plasma units. The reduction in factor VIII activity doesn't reduce the quality of FP because this component should not be utilized for treating hemophilia A. Rather, factor VIII concentrates, recombinant or plasma derived, DDAVP and rarely cryoprecipitate are the products of choice. This leads to the conclusion that the major rationale for the preparation and storage of FFP is, therefore, its use as a source of factor V.

While comparing FFP and cryoprecipitate, mean fibrinogen and FVIII level per cryo unit in our study was lower than observed in other studies. The possible reasons for these differences could be that the previous studies had smaller sample size (n = 10 on an average), preselection of donors (based on blood group) for the study, and analysis of stored samples in a batch after thawing.

Subramaniyan and colleagues [12] stated that cryoprecipitate has approximately 40–70% of the factor VIII activity and 30–50% of fibrinogen of the starting plasma. Where factor VIII (IU/bag) and fibrinogen (mg/bag) levels in FFP were higher than cryoprecipitate prepared using two different techniques. Factor VIII was analyzed using the single-stage clot-based assay, and fibrinogen was measured by the Clauss method. One explanation to this discrepancy in factor VIII and fibrinogen results is the possible effect posted by the difference in coagulation factors assay methods (Chromogenic assay versus single stage clotting assay for aPTT).

Another possible reason for these differences in factor VIII and fibrinogen levels between our study and other studies could be the different variables that may affect the techniques for the preparation of cryoprecipitate. As factor VIII is a labile coagulation factor, all steps of CRYO production should be optimized to prevent a reduction in factor VIII activity. Factors such as the time and temperature between donation and the start of the freezing process may affect FVIII activity. Besides, donor variation in factor VIII level, the blood group of the donor and the volume of the final product of CRYO may affect factor VIII level as well.

Omidkhoda et al. [13] measured FVIII activity in cryoprecipitate in duplicate using both a chromogenic assay and a one-stage clotting assay on the same coagulometer. The difference between the results was statistically significant. Accordingly, they recommended that a chromogenic assay is used to measure FVIII activity in manufactured concentrates and a clot-based assay for plasma samples. A number of studies have shown the importance of using the chromogenic assay for measuring FVIII activity in concentrates. Chandler et al. [14] stated that the chromogenic FVIII activity assay was the optimal method, showing good precision, the best overall correlation with other assays. In another study by Barrowcliffe and coworkers [15], they reported that most manufacturers of concentrates use the chromogenic method, which is more precise and is the reference method of the European Pharmacopoeia and the International Society on Thrombosis and Haemostasis (ISTH).

5. CONCLUSION

The retention in factor activities, in particular factor V, in FP, indicates the lack of relevant adverse changes when extending the hold period for plasma units. The reduction in factor VIII activity doesn't reduce the quality of FP.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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