

DEPLETION OF FACTOR EIGHT (VIII) LEVEL IN FRESH FROZEN PLASMA AFTER ONE MONTH STORAGE

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ABSTRACT

Objective: To evaluate the efficacy and quality of fresh frozen plasma after one month storage.

Material and Method: It was a descriptive cross sectional study conducted in blood bank of Hayatabad Medical Complex Hospital Peshawar, Pakistan. In this study a total number of one hundred and thirty nine (Male n= 121, Female n= 18) healthy volunteer blood donors were enrolled. Whole blood (450 ml \pm 10%) was collected from all donors into triple packs blood bag (JMS, Germany). The blood components were prepared within 8 hours in a 2-step spin protocol. Whole blood was centrifuged at 40C with low spin (2500 rpm for 10 minutes) and platelets rich plasma was transferred into 2nd pack of triple bag followed by a high spin for 10 minutes at 3200 rpm. Platelets poor plasma (PPP) of 2nd pack was transferred into 3rd pack and fresh plasma was stored at -700C. Factor VIII assay was performed on freshly prepared FFPs and repeated after 30 days of storage on Stago STA compact Hemostasis analyzer according to manufacturer instructions. Data was analyzed by SPSS-20 and presented as mean \pm standard deviation.

Results: Total number of 139 healthy blood donors was enrolled in the present study. Majority of blood donors age was <20 years while only 1.4% blood donors were in the age of 60-70 years, with mean age of 31 years. Out of total donors 121 (87.1%) were male while 18 (12.9%) were female. In more than 95 % of day 1st FFP unites, level of factor VIII greater was 50%. On day 30th factor VIII level was decreased by 23% on average in 95 present of unites.

Conclusion: There is sufficient levels of relevant coagulation factor activity present in the plasma obtained from whole blood with in 8 hours of collection and stored at -18°C and would be an acceptable product for patients requiring FFP for bleeding disorders for one month.

Key Words: Volunteer, Blood Donors, Fresh Frozen, Plasma, Factor VIII

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INTRODUCTION

The aim of the various blood donor services is to provide safe and effective blood components which are beneficial for the recipients in terms of their requirements and at the same time safe from all hazards associated with transfusion of blood and blood products.¹ In order to make the process of blood collection, preparation of various products, their storage and transport of these products in accordance with

the guidelines given by Food and Drug Administration (FDA) all processes need to be monitored strictly for quality of all procedures, reagents, equipment, and the contents of the components.² At present fresh frozen plasma (FFP) is used commonly for bleeding disorders and is collected by plasmapheresis or separated from whole blood.³ According to guidelines FFP must be prepared and frozen within 6 or 8 hours of collection. Fresh frozen plasma must be frozen rapidly to a temperature required for the maintenance of normal activity of heat labile coagulation factors.⁴ Pakistan is a country with a high prevalence of inherited bleeding disorders and the fresh frozen plasma is the most commonly used product due to financial constraints.⁵ FFPs are mostly used in patients who are having coagulation factors deficiencies or actively bleeding.⁶ Examples may include massive trauma, routine surgical

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bleeding, liver disease, DIC, and when a specific disorder cannot be or has not yet been identified. They may also be used when a patient on warfarin must undergo surgery and there is not sufficient time for vitamin K to reverse the effect.⁷ Upon storage at -18°C or below, FFP maintain maximum levels of labile and non labile coagulation factors (about 1 IU per mL) and can be stored for almost 12 months. Even at -65°C FFP can be stored for up to 7 years.⁸

MATERIAL AND METHODS

The present study was conducted in pathology department of Hayatabad Medical complex Peshawar Pakistan. In this study the quality of FFP and factor VIII level that was stored for 30 days was evaluated. Whole blood samples were collected from normal volunteer donors according to standard procedures in the blood bank of HMC. Each Whole Blood sample ($450\text{ mL} \pm 10\%$) was collected into blood collection triple packs that contained a CPDA-1 anticoagulant. The blood components were processed within 4 to 6 hours in a 2-step centrifugation protocol. Whole blood was centrifuged with initial light spin ($2000 \times \text{rpm}$ for 5 minutes in a refrigerated centrifuge) followed by a heavy spin for 10 minute at 3200 rpm with temperature of 4°C . The plasma was removed, and the packed RBCs were resuspended in one of the triple blood bag. Plasma was stored at 18°C . The storage temperature was monitored by a digital thermometer with alarm system and log book was maintained.

Five milliliters aliquots of FFP was stored in freezer at 18°C and thawed in a water bath at 37°C for the analysis of coagulation factor. Factor VIII assay was performed on stored samples on Stago STA Compact Haemostasis Analyzer according to manufacturer's instructions. Data are presented as mean \pm standard deviation.

RESULTS

The FFP units which were tested for coagulation factors level showed average activities of more than 80%. The level of Factor VIII (F VIII) was used as quality marker in accordance with guidelines given by AABB and FDA. The rationale to calculate F VIII levels as quality control for FFP is based on the fact that it is the most common labile clotting factors present in plasma. Thus F VIII level in the finally collected product is measured to assess if the storage condition and temperature are properly maintained. As the factor concentrates available are very expensive and some factor concentrates are not available like factor V and factor XI therefore it is necessary that the activities of these factors should be sufficient in the final product to provide enough factor

level to patients suffering from these factor deficiencies. Our study showed that for patients with bleeding disorders the activity FVIII is sufficient even after a storage temporarily at above -18°C .

In more 95 percent of Day 1 FFP units factor FVIII level was found to be greater than 100% as compared to that of FFP units stored for 30 days whose FVIII level was reduced by 23 percent in about 92 percent of units.

DISCUSSION

Fresh frozen plasma may be produced from whole blood or apheresis collections.⁹ According to FDA, FFP must be frozen inside 8 hours of collection provided CPD or CPDA-1 was used as anticoagulant while within 6 hours if the preservative was ACD.¹⁰ FFP can be stored at -18°C or lower for 12 months or for 7 years if stored at -65°C . FFP is required to contain the maximum levels of both stable and labile clotting factors, about 1 international unit (IU) per mL. FFP has slightly reduced levels of labile factors, factor VIII and FV. Therefore to assess the quality of FFP Factor VIII levels are measured. The level of FVIII should be $\geq 80 \text{ IU/L}$ (0.8 IU/ml) or 80%. According to BCHS guidelines the standard FFP after being thawed should have minimum of 70 IU/ml of coagulation FVIII in at least 75% of the units.¹¹

Fresh frozen plasma is still used an essential blood product to provide coagulation factors in acquired and hereditary bleeding disorders in Pakistan due to financial constraints or non-availability of required products.¹² Therefore, it is important to know the effect of temperature and time from collection to freezing on FVIII activity in order to make best use of the product. In this study we prepared FFP from whole blood collected from healthy blood donors and kept units of FFP at -18°C for one month and evaluated the effect of temperature variations and storage periods on the FVIII activity in FFP.¹³

The mean activity of factor VIII in the healthy blood donors in this study was 1.0 iu/ml with standard deviation ± 0.498 this correlates with the study conducted by Grazzini et al in which mean factor VIII levels were 0.99 iu/ml in whole-blood plasma produced by the 10 Italian regions.¹⁴ The study conducted in Nigeria demonstrated the mean factor VIII was 1.90 iu/ml .² In certain areas of Nigeria the normal factor VIII activity level was found to be about $0.65\text{--}5.55 \text{ iu/ml}$ with a mean value of 1.90 iu/ml .¹⁵

The study conducted by R. Cardigan showed the FVIII level in more than 87 percent of Day 1 FFP units were greater than 0.50 IU per mL (50%).¹⁶ on the other hand in our study the level was greater than 0.10 IU per mL (100%).

The study conducted by Lamboo et al show that FVIII level was decreased to a level of 45% after a storage period of 2 weeks while in our study it was reduced to 23 % after 30 days of storage which correlates with this study.¹⁷

In a study did by Moog Rainer it was observed that if FFPs are stored temporarily above a temperature

of -25°C , there will be no down grading in the quality of clotting factors.¹⁸ similarly Our data suggest that for bleeding patients the activity is sufficient even after a temporary storage above -18°C . On day 1 in 95% of the FFP units, levels of factor FVIII was found to be greater than 0.1 IU per mL compared with the level of FVIII after being stored for 30 days, in whom level was reduced by 23 percent with final levels above the lower limit of the normal range in more than 92 percent of units.¹⁹

CONCLUSION

Significant activity of the most common coagulation factor is detected in the plasma obtained from whole blood within 8 hours of donation and this activity could be retained if stored at -18°C degrees C for one month, thus this would be an acceptable product for most patients requiring FFP for various bleeding disorders. Compared with the usual reference ranges, the differences were not significant enough.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

- Zahoor S:** Main Idea, Data analysis.
Khan K: Data Collection, Overall supervision.
Zahoor M: Data analysis.
Zahoor S: Bibliography.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.