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Activity of Labile Coagulation Factors, Factor X and Fibrinogen Level in Frozen Plasma versus Fresh Frozen Plasma

Transfusion Medicine Section

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ABSTRACT

Introduction: Fresh Frozen Plasma (FFP) is a blood component separated from whole blood and frozen below -30°C within 8 hours of donation for optimum preservation of coagulation factors. However, logistic and geographical reasons may hamper separation of plasma within 8 hours and the separation may have to be delayed to between 8 and 24 hours and then frozen below -30°C which is called as Frozen Plasma (FP). Plasma separated between 8 and 24 hours is a licensed blood component in the United States of America (USA) for therapeutic use similar to FFP. It is not licensed in India leading to frequent shortage of plasma.

Aim: To compare the activity of factors V, VIII and X and the level of fibrinogen between FFP and FP, so as to assess the therapeutic use of FP for formulating recommendation as licensed blood component.

Materials and Methods: A prospective observational study was conducted in the Department of Transfusion Medicine at Seth GS Medical College and KEM Hospital, Mumbai, Maharashtra, India. The duration of the study was 10 months, from January 2018 to October 2018. Fifty units each of FFP and FP matched for the camp location, age, gender and blood group were selected. There were 44 males and six females in each of FFP and FP groups. They were compared for the activity of labile coagulation factors (factors V and VIII) and stable factor X. The level of fibrinogen was also measured in both components. It was done within 30 days of preparation of plasma. The mean values of each of the four parameters for FFP and FP were calculated and compared for statistical significance (p) by using unpaired Student's t-test. Microsoft Excel 2016 was used for statistical analysis. The p-value <0.05 was considered statistically significant.

Results: The mean age of FFP and FP individuals (blood donors) was 31.2 and 31.3 years respectively, while the median age in years was 31 and 30.5, respectively. The activity/level of all the tested coagulation factors was lower in FP as compared to FFP. The difference was statistically significant for factor VIII (p-value <0.05). It was not significant for factor V, X and fibrinogen. The level/activity of coagulation factors in FP, though lower than that in FFP, fell within normal reference range in 90-95% of units.

Conclusion: FP may be used as a therapeutic alternative to FFP excluding patients of haemophilia A in whom factor VIII concentrate and cryoprecipitate are considered better therapeutic modalities. Results of similar multicentre studies will help in formulating recommendations regarding licensing.

Keywords: Blood components, Factor V, Factor VIII, Stable clotting factors

INTRODUCTION

The FFP is a blood component that is frozen below -30°C within a short specified period after collection (usually 8 hours) [1]. It contains plasma proteins and all the coagulation factors, including the labile factors V and VIII [2]. The use of FFP is indicated in patients with single coagulation factor deficiencies where the appropriate concentrate is not available (for example: factors V, XI), acute disseminated intravascular coagulation, plasma exchange for thrombotic thrombocytopenic purpura (TTP), and in some instances of liver disease, warfarin reversal, cardiopulmonary bypass, and massive transfusion [3]. When FFP is frozen within 8 hours of its preparation at below -30°C, levels of coagulation factors like V, VIII, II, IX, X, XI, and fibrinogen remain stable with good relevant activity [1,4]. Factors V and VIII are known labile factors, whereas, fibrinogen is a stable factor. Factor VIII level is also one of the quality control parameters for FFP as per the guidelines by Directorate General of Health Services (DGHS), India [5]. According to these guidelines, 75% of the units of FFP should have factor VIII levels greater than 0.7 IU/mL. To meet this requirement, plasma is usually separated from whole blood and frozen within 8 hours of collection [6,7].

These requirements for FFP preparation limit the number of units of whole blood that can be processed into FFP in large blood centres that collect and transport blood over a wide geographic area. In a few situations, it is not possible to separate the whole blood and/or freeze the separated plasma within 8 hours of collection due to logistic and administrative reasons [8-10]. These limitations result in not infrequent shortages of FFP [8]. Extension of the allowable processing time for plasma for transfusion i.e., FFP to 24 hours after collection of whole blood would alleviate this problem. This plasma that is frozen at later intervals (usually up to 24 hours) after collection has been referred to as plasma frozen within 24 hours after phlebotomy (PF24) [1]. There have been growing global interest and efforts invested in exploring the efficacy and benefits of using PF24 for transfusion purpose. PF24 is a licensed blood component in the USA [11,12]. Since, it is not yet licensed in India, it cannot be used for therapeutic purposes for indications similar to FFP.

A number of studies have been performed to investigate the effect of extended storage of blood prior to plasma separation, especially on coagulation factors [6,8,10,13-19]. Only one out of these studies is from India, which was conducted in a Northern Indian state [13]. Considering this background, the authors undertook this prospective observational study with the overall goal to determine whether separation of plasma after 8 hours of blood collection has any effect on the level of coagulation factors. To achieve this goal, the activity of labile coagulation factors (V and VIII), factor X and level of fibrinogen in FFP were compared with those in the plasma units that were separated and frozen after 8 hours of collection but before 24 hours of blood collection (FP).

MATERIALS AND METHODS

A prospective observational study was conducted in the Department of Transfusion Medicine at Seth GS Medical College and KEM Hospital, Mumbai, Maharashtra, India. The duration of the study was 10 months, from January 2018 to October 2018. The blood centre collects on an average 2000 blood units per month indoor, as well as, from outdoor blood donation camps. Ethical approval was granted by the Institutional Ethics Committee as per approval letter no. IEC/116/2016 and continuation permission letter IEC (I)/OUT/1907 dated 28/8/2017.

Sample size calculation: The study was performed on plasma samples obtained from the segments of plasma units. It was performed on 50 units each of FFP and plasma separated after 8 hours (FP). The sample size calculation was based on the level of significance (p-value=0.05), the desired margin of error and standard deviation [20].

The following formula was used:

$n=(\text{for } Z\sigma d/E)^2$ where Z=confidence level=1.96 for 95%. σd=standard deviation=71.2% for factor VIII level in FFP as obtained from one year quality control data of factor VIII on FFP at tertiary blood centre. The authors considered standard deviation of factor VIII in calculation as it is a heat labile coagulation factor and a quality control parameter for FFP. E=desired margin of error=20% as considered for the present study.

$N=(1.96\times71.2/20)^2=48.68\approx50$ units each of FFP and FP

Inclusion criteria: Samples from plasma units separated between 8 and 24 hours and those separated within 8 hours of collection matched for age, gender and ABO blood group were included in the study.

Exclusion criteria: Study samples which showed visible lipemia and/or which were reactive for any of the Transfusion-Transmissible Infections (TTIs) (i.e., HIV, hepatitis C, hepatitis B, malaria, syphilis) were excluded from the study.

Study Procedure

Plasma separation: The blood units that were collected in double bags 350 mL capacity (Polymedicure Ltd., India) containing Citrate Phosphate Dextrose Adenine (CPDA) anticoagulant- preservative-solution. Double blood bags were used for preparation of red cell concentrate and plasma only. Clean and continuous flow of blood was ensured during blood collection since intermittent and slow flow affects the activity of coagulation factors [11]. They were maintained at a temperature of 2 to 6°C during transportation and processing. The whole blood units that were received in the blood bank from the campsite within 6 hours of collection were separated into FFP to ensure that, the separation and freezing were completed within 8 hours limit. Blood units received from the same campsite after 8 hours were stored between temperatures 2 to 6 °C overnight (Walk-in Chiller, BlueStar Limited, India) and then separated within 24 hours of collection (FP). In both the above cases, separation of plasma units was performed on cold centrifuge machine (Thermo Scientific Cryofuge 6000 i centrifuge, Thermo Fisher Scientific, Germany) at 4000 rpm at 4°C for 7 minutes as per the standard operating procedure. The tubings of the plasma bags were carefully stripped and refilled so that, the composition of plasma in the whole plasma bag including the attached tubing is uniform and the plasma sample in the tubing is representative of the whole plasma unit. Segments of approximately 3 cm length were made using tube sealer. They were not detached from the plasma bag but were labelled with the original plasma unit number. The separated plasma units along with the attached segments were frozen immediately at -80°C using ultra low freezer (-80°C) (Cryo Scientific Systems Pvt., Ltd., Chennai, India).

Sample selection: The sample selection was done after two days of collection to ensure that, the mandatory ABO blood grouping and TTI testing of the blood units were completed. At first, the data of the FP units were accessed and the donor unit numbers were recorded in a separate register. The results of TTI testing and ABO grouping of the blood units, as well as, the demographic data of the corresponding blood donors (age and gender) were recorded. All the details were obtained from the mandatory documentation in the blood bank. Before the samples were selected, all the plasma units (FFP and FP) were inspected to ensure that, they are not visibly lipemic. Then FP units that tested non-reactive for TTI were selected and recorded. The records of FFP units prepared from the same blood donation camp were inspected. The numbers of TTI nonreactive FFP units that matched the FP units with respect to ABO blood group, age and gender were noted down. In case, there were more than one matching units, the selection was done by using chit method. The matching for ABO blood group, age and gender was done because these factors are known to influence the level/activity of coagulation factors [21]. The O blood group individuals have lower levels of factor VIII in their plasma as compared to other ABO blood groups (non O blood groups) [22]. Hence, they were matched as O blood group and other non O ABO blood groups.

The data of the FP units were reviewed first followed by matching with FFP data because, the latter were more in number (out of total separated plasma, FFP and FP units were 90% and 10% respectively). Hence, it was easier to find ABO and age matched FFP units corresponding to the FP units. Thus, 50 units each of FP and FFP were selected over a period of one month from eight camp locations. Their numbers were noted in tabular format in an Excel sheet. Case record forms were prepared for each unit separately, mentioning FFP/FP, unit number, age, gender, ABO blood group, date of collection, date and time of separation, date of testing, results of coagulation tests (factors V, VIII, X and fibrinogen).

Testing of plasma samples: The quality control requirements states that, 4 units or 1% of all blood component units (whichever is more) should be tested [6]. As per this, coagulation factor testing as a part of quality control of FFP is performed within one month of component preparation. To maintain uniformity across all the batches tested, the authors chose 21st day after separation of FP to perform the required coagulation tests. FFP samples that were obtained from the camp location and were matched for age, gender and ABO blood group were also tested simultaneously, in the same batch as FP to avoid any batch related bias. Since, FFP units were separated and frozen one day earlier than FP, it corresponded to 22nd day of separation of FFP while it was 21st day of separation for FP. The attached tubing segments of FFP and FP were cut and thawed in plasma bath at 37°C for 30 minutes to ensure that, all the plasma was liquefied. The liquefied plasma was drained into plastic tubes labelled with the corresponding unit numbers. The coagulation tests were performed on ECLL412-Four Channel Semiautomated Coagulation Analyser (Transasia Biomedicals Ltd.,) as per the manufacturer's instructions and the instrument operator manual. The Erba (Transasia) factor deficient plasma was used for factors V, VIII and X quantitation. Erba thrombin reagent was used for fibrinogen determination.

Factors V, VIII, X: The quantitative measurement of factors V, VIII and X was done by one stage method [23]. A dilution of test plasma was mixed with factor deficient plasma and the clot time of the mixture was determined. The degree of clot time correction with the patient plasma was compared to the correction with a reference material, allowing the percentage activity of the test plasma to be determined.

Fibrinogen: Fibrinogen concentration was determined based on Clauss method [24]. The clotting time of dilute plasma was measured after addition of thrombin. The clot time is proportional to fibrinogen concentration (reference range: 200-400 mg/dL) [25].

STATISTICAL ANALYSIS

The mean values of each of the four parameters (factors V, factor VIII, factor X and fibrinogen) were calculated for both the groups (FFP and FP). They were compared for statistical significance (p) by using unpaired Student's t-test. MS Excel 2016 was used for statistical analysis. The (p-value <0.05) was considered statistically significant.

RESULTS

The mean age of FFP and FP individuals (blood donors) was 31.2 and 31.3 years, respectively while the median age in years was 31 and 30.5, respectively. There were 44 males and six females in each of FFP and FP groups. There were 22 O blood group donors while 28 were non O group donors. The mean values of factors V and VIII (labile coagulation factors) and factor X and fibrinogen (stable coagulation factors) were compared between FFP and FP [Table/Fig-1]. The coagulation factor activity/level in FFP was taken as baseline as FFP is a licensed blood component, prepared regularly as blood component preparation activity and is not subjected to delay in preparation. The levels of all the studied coagulation factors were lesser in FP units as compared to those in FFP units as observed by percentage decrease in their mean values. However, unpaired Student's t-test showed that, the difference was not statistically significant for factor V (p-value=0.133), factor X (p-value=0.228) and fibrinogen (p-value=0.415). Conversely, factor VIII level was significantly lower (p-value=0.00065) in FP units as compared to FFP units.

Parameters	FFP	FP	Percentage decrease	p-value
Factor V mean activity (%)	86.46	80.59	6.78	0.133
Factor X mean activity (%)	90.66	87.01	4.02	0.228
Factor VIII mean activity (%)	80.99	62.00	23.45	0.00065
Mean fibrinogen level (mg/dL)	310.28	296.91	4.30	0.415

[Table/Fig-1]: Student's t-test to compare the activity of factors V, X and VIII, and level of fibrinogen in FFP and FP.

DISCUSSION

The study compared 50 samples of FFP and FP for the activity of heat-labile coagulation factors and heat-stable factor X, as well as, level of fibrinogen. The objective was to assess the effect of storage of whole blood beyond 8 hours at 2-6°C on the coagulation factors. The present study, showed 23.45% lower activity of factor VIII in FP plasma as compared to FFP. This was statistically significant (p-value <0.05). The levels of factor V, factor X and fibrinogen were also lower by 6.78, 4.02 and 4.3%, respectively in FP as compared to FFP in the present study. However, the difference was not statistically significant. Kakaiya RM et al., compared two preparations of plasma separated and frozen within six hours (FFP I) and within 18-20 hours (FFP II) from CPDA-1 whole blood units [17]. Factor VIII activity was significantly lesser in FFP II (p<0.01). Factor V activity in both the products was equivalent. Cardigan compared FP separated from leucodepleted whole blood after storage at 4°C overnight (18-24 hours) with FFP separated within 8 hours from leucodepleted whole blood [6]. Factor VIII level in the former was 23% lower than that in the latter. They also found fibrinogen level to be 12% lower in the former which was statistically significant. Agus N et al., separated and froze plasma after 24 hours storage of whole blood at 4°C. This plasma showed 25% reduction in factor VIII levels as compared to FFP [18]. In a similar study by Dogra, 24 hour-separated plasma units showed 18.4% lower factor VIII activity when compared to that in FFP. However, fall in the activity of factor V was 6.52% which was not statistically significant [13]. The level of fibrinogen fell by 1.81% which too was not significant. In a study by Alhumaidan H et al., plasma units were prepared from Platelet-rich Plasma (PRP) after 8 hours and 24 hours room temperature hold. Additional plasma units were separated from whole blood donations kept at room temperature for 24 hours. Plasma units separated after 24 hours showed factor VIII and factor V levels 20% and 6% lesser than those in FFP. However, the fall in factor V level was not statistically significant [14]. A study by Afifi OAH et al., showed reduced factor VIII and fibrinogen in plasma separated within 24 hours (PF24) as compared to FFP with a conclusion that PF24 can be used for same indications as FFP except indications requiring FVIII and/or fibrinogen replacement [26]. In a study by Yazer MH et al., coagulation factor levels in plasma frozen within 24 hours of phlebotomy (FP24) were estimated over 5 days of storage at 1°C to 6°C. The results showed comparable factor assay levels in thawed plasma prepared from FFP and FP24 [27]. Dumont LJ et al., showed reduced factor VIII level in plasma that was separated after 20 to 24 hours of room temperature holding as compared to FFP [28].

Though, separation and freezing within 8 hours is the standard prescribed protocol for preparation of blood components in India, logistic and geographical reasons may cause difficulty in implementing the standard practice on day to day basis. Non separation of plasma within 8 hours due to long distance blood donation campsite with additional hindrance from traffic conditions would frequently lead to FFP being in short supply [10,16]. The purpose of the present study was to assess the clinical utility of a plasma component that was separated and frozen after 8 hours of whole blood donation. The two types of blood components (plasma separated within 8 hours of blood collection and between 8 to 24 hours of collection) showed statistically insignificant difference in the level of fibrinogen and activity of factors X and V. Though, the factor VIII activity was significantly lower in FP, in 90-95% of the tested FP, the activity fell within normal range of 50% to 150% [29]. Thus, 24 hours plasma should prove clinically beneficial in most patients with coagulation factor deficiencies, except those with haemophilia A. However, factor VIII concentrates and cryoprecipitate are better therapeutic modalities for haemophilia A [30-32].

Limitation(s)

The study was limited to single blood centre.

CONCLUSION(S)

Plasma that is separated from whole blood after 8 hours of collection retains the level/activity of coagulation factors. The outcome of similar studies in future from different blood centres in India, will help in formulating recommendations on therapeutic use of plasma that is separated between 8 and 24 hours of collection.

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REFERENCES

[1] Stanworth SJ, Tinmouth AT. Plasma transfusion and use of albumin. In: Simon TL, editor. Rossi's Principles of Transfusion Medicine. 4th ed. Oxford, West Sussex, New Jersey: Blackwell Publishing; 2009. Pp. 287-97.
[2] Apfelroth S. Standard terminology for plasma products. Transfusion. 2003;43(7):983.
[3] O'Shaughnessy DF, Atterbury C, Bolton Maggs P, Murphy M, Thomas D, Yates S, et al. Guidelines for the use of fresh-frozen plasma, cryoprecipitate and cryosupernatant. Br J Haematol. 2004;126:11-28.
[4] Anderson KC, Hillyer CD, Ness PM, Crookes RL, Silberstein LE, Roback JD. FFP and related products. 2 nd ed. Blood Banking and Transfusion Medicine. 2007. Pp. 259-60.
[5] Quality assurance in blood transfusion. In: Saran RK, editor. Technical Manual. 2 nd ed. New Delhi: Directorate General of Health Services; 2003. Pp. 354.
[6] Cardigan R, Lawrie AS, Mackie IJ, Williamson LM. The quality of fresh frozen plasma produced from whole blood stored at 4 °C overnight. Transfusion. 2005;45:1342-48.
[7] Bala G, Gupta A, Suri V, Chhabra S, Shaffy, Gupta R. Quality control of fresh frozen plasma using factor VIII and fibrinogen levels as measure: one year study in a tertiary care hospital. International Journal of Contemporary Medical Research. 2019;6(8):H1-H3.
[8] O'Neill EM, Rowley J, Hansson-Wicher M, McCarter S, Ragno G, Valeri CR. Effect of 24 hour whole blood storage on plasma clotting factors. Transfusion. 1999;39:488-89.

[9] Cardigan R, Van der Meer PF, Pergande C, Cookson P, Baumann-Baretti B, Cancelas JA, et al. Coagulation factor content of plasma produced from whole blood stored for 24 hours at ambient temperature: Results from an international multicenter BEST Collaborative study. Transfusion. 2011;51(Suppl 1):50S-57S.
[10] Smith JF, Ness PM, Moroff G, Luban NL. Retention of coagulation factors in plasma frozen after extended holding at 1-6°C. Vox Sang. 2000;78:28-30.
[11] Kakaiya R, Aronson CA, Julleis J. Whole blood collection and component processing at blood collection centers. In: Roback JD, Grossman BJ, Harris T, Hillyer CD, eds. Technical Manual. 17th ed. Bethesda, MD: American Association of Blood Banks; 2011:187-226.
[12] Horstman EE, Christopher A. Plasma products for transfusion: an overview. Ann Blood. 2022;7:4.
[13] Dogra M, Sidhu M, Vasudev R, Dogra A. Comparative analysis of activity of coagulation Factors V and VIII and level of fibrinogen in fresh frozen plasma and frozen plasma. Asian J Transfus Sci. 2015;9:06-08.
[14] Alhumaidan H, Cheves T, Holme S, Sweeney J. Stability of coagulation factors in plasma prepared after a 24-hour room temperature hold. Transfusion. 2010;50:1934-42.
[15] Nilsson L, Hedner U, Nilsson IM, Robertson B. Shelf-life of bank blood and stored plasma with special reference to coagulation factors. Transfusion. 1983;23:377-81.
[16] Kakaiya RM, Morse EE, Panek S. Labile coagulation factors in thawed fresh frozen plasma prepared by two methods. Vox Sang. 1984;46:44-46.
[17] Sohmer PR, Bolin RB, Scott RL, Smith DJ. Effect of delayed refrigeration on plasma factors in whole blood collected in CPDA-2. Transfusion. 1982;22:488-90.
[18] Agus N, Yilmaz N, Colak A, Liv F. Levels of factor VIII and factor IX in fresh-frozen plasma produced from whole blood stored at 4°C overnight in Turkey. Blood Transfus. 2012;10:191-93.
[19] Naghadeh HT, Roudkenar MH. A study of the quantity of some stable and labile coagulation factors in fresh-frozen plasma produced from whole blood stored for 24 hours in Iran. Blood Transfus. 2009;7(1):39-42.
[20] Suresh K, Chandrashekara S. Sample size estimation and power analysis for clinical research studies. J Hum Reprod Sci. 2012;5(1):07-13.
[21] Wang Z, Dou M, Du X, Ma L, Sun P, Cao H, et al. Influences of ABO blood group, age and gender on plasma coagulation factor VIII, fibrinogen, von Willebrand factor and ADAMTS13 levels in a Chinese population. Peer J. 2017;5:e3156.
[22] O'Donnell J, Laffan MA. The relationship between ABO histo-blood group, factor VIII and von Willebrand factor. Transfus Med. 2001;11(4):343-51.
[23] Penner JA. The University of Michigan Medical School Blood Coagulation Laboratory Manual, 14th Ed., University Publications, Ann Arbor, 1979; 72-78.
[24] Clauss A. Gerinnungsphysiologische Schnellmethodezur Bestimmung des Fibrinogens. Acta Haematol. 1957;17:237-46.
[25] Hantgan RR, Francis CW, Scheraga HA, Marder VJ. Fibrinogen structure and physiology. In: Colman RW, Hirsh J, Marder VJ, Saizman EW, editors. Hemostasis and Thrombosis-Basic Principles and Clinical Practice. Philadelphia: J.B. Lippincott Company; 1987. Pp. 269-88.
[26] Afifi OAH, Abdelsalam EMN, Makhlouf AAEAM, Ibrahim MAM. Evaluation of coagulation factors activity in different types of plasma preparations. Indian J Hematol Blood Transfus. 2019;35(3):551-56.
[27] Yazer MH, Cortese-Hassett A, Triulzi DJ. Coagulation factor levels in plasma frozen within 24 hours of phlebotomy over 5 days of storage at 1 to 6 degrees C. Transfusion. 2008;48:2525-30.
[28] Dumont LJ, Cancelas JA, Maes LA, Rugg N, Whitley P, Herschel L, et al. The bioequivalence of frozen plasma prepared from whole blood held overnight at room temperature compared to fresh-frozen plasma prepared within eight hours of collection. Transfusion. 2015;55(3):476-84.
[29] Baig MA, Swamy KB. Comparative analysis of chromogenic vs clot based one stage APTT assay for determination of factor VIII level. Indian J Pathol Microbiol. 2021;64:123-27.
[30] Nascimento B, Goodnough LT, Levy JH. Cryoprecipitate therapy. Br J Anaesth. 2014;113:922-34.
[31] Samuelson Bannow B, Recht M, Négrier C, Hermans C, Berntorp E, Eichler H, et al. Factor VIII: Long-established role in haemophilia A and emerging evidence beyond haemostasis. Blood Rev. 2019;35:43-50.
[32] Nester T, AuBuchan JP. Hemotherapy decisions and their outcomes. In: Roback JD, Grossman BJ, Harris T, HillyerCD, eds. Technical Manual. 17th ed. Bethesda, MD: American Association of Blood Banks; 2011: 594.

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