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Quality Assessment of Antihemophilic Factor and Fibrinogen Levels in Fresh Frozen Plasma, Aden, Yemen, 2023

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Abstract

Introduction: Fresh Frozen Plasma (FFP) is mainly indicated for patients with active bleeding or for those preparing to undergo surgical procedures. However, the product should only be considered when factor concentrates are not available or when the benefit-to-risk ratio is maximum. This study aimed to assess whether the FFP meets the quality standards in regards to antihemophilic factor (FVIII) activity and fibrinogen level, with the acceptance limits of not less than 0.70 IU/ml for FVIII, and normal level for fibrinogen.

Methods: This cross-sectional study was conducted at the National Blood Transfusion and Research Center, Aden, Yemen from August to September 2022. A total of 206 FFP units were selected using stratified random sampling method, with 103 samples from O and 103 from non-O blood groups. FVIII and fibrinogen levels were measured in each sample at two points: baseline and post-thaw. The measurements were carried out using STart Max coagulation analyzer and reagents purchased from Diagnostica Stago. Statistical analysis was performed using SPSS version 26, with a significance level set at $P<0.05$.

Results: From baseline to post-thaw, a statistically significant reductions were observed in the average FVIII (20%) and fibrinogen (4.9%) levels. Of all tested FFP, 75.2% units had FVIII levels of ≥0.70 IU/ml.

Conclusion: The majority of FFP units met the international quality standards in regards to FVIII and fibrinogen levels, and were suitable for clinical use.

Keywords: Fresh Frozen Plasma, Factor VIII, Fibrinogen, Quality Assessment.

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تقييم جودة عاملي التخثر الثامن والأول في البلازما الطازجة، عدن، اليمن، 2023

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ملخص الدراسة

المقدمة: توصف البلازما الطازجة المجمدة للمرضى الذين يعانون من نزيف حاد أو لأولئك المقبلون على إجراء عمليات جراحية. ومع ذلك، ينبغي النظر في المنتج فقط عندما لا تتوفر عوامل التخثر المصنّعة أو عندما تكون نسبة فائدة إعطاء البلازما إلى مخاطرها في الحد الأقصى. هدفت هذه الدراسة إلى تقييم ما إذا كانت البلازما المجمدة الطازجة تفي بمعايير الجودة المحددة في المعايير الدولية فيما يتعلق بمحتواها من العامل المضاد للهيموفيليا (عامل التخثر الثامن) والفيبرينوجين (عامل التخثر الأول). وبحد مقبول ليس اقل من 0.70 وحدة دولية من عامل التخثر الثامن لكل مل لتر من البلازما، ومستوى طبيعي من الفيبرينوجين.

المنهجية: أجريت هذه الدراسة المقطعية في المركز الوطني لنقل الدم وأبحاثه (عدن، اليمن) في الفترة من أغسطس إلى سبتمبر 2022. استخدمت الدراسة أسلوب العينة العشوائية الطبقية لاختيار 206 عينة (103 عينة من زمرة الدم O و103 عينة من زُمر الدم الاخرى (A, B, AB). تم قياس تراكيز عاملي التخثر لكل عينة في نقطتين: قياسات أولية (ما قبل إنتاج البلازما المجمدة) وقياسات بعد انتاج وإذابة البلازما المجمدة. تم قياس جميع العينات باستخدام محلل تخثر وكواشف تمت شراؤها من شركة ستاجو. تم إجراء التحليل باستخدام البرنامج الإحصائي SPSS الإصدار 26، بمستوى دلالة إحصائية $P<0.05$.

النتائج: تم ملاحظة انخفاض ذو دلالة إحصائية في متوسطات العامل الثامن (20٪) والأول (4.9٪) في البلازما الطازجة المذابة مقارنة بالمتوسطات الأساسية. كما أظهرت النتائج أيضاً أن 75.2% من وحدات البلازما كانت تحتوي على تركيز 0.70 وحدة دولية أو أكثر من العامل الثامن/مل لتر من البلازما.

الاستنتاج: معظم وحدات البلازما المجمدة استوفت معايير الجودة العالمية فيما يتعلق بمحتواها من عاملي التخثر الثامن والأول، وكانت مناسبة للاستخدام السريري.

الكلمات المفتاحية: البلازما الطازجة المجمدة، العامل المضاد للهيموفيليا، الفيبرينوجين، تقييم الجودة.

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Introduction

Blood transfusion has continuously advanced over the years. From the days of vein-to-vein transfusion, it has become possible to collect, separate, and personalize blood to address specific clinical conditions. This approach to transfusion therapy has been with the focus of ensuring the right product for the right condition at the right time and dose. However, the need for safe and effective blood products remains a top priority, especially after adverse events [1]. A case in point is the use of fresh frozen plasma (FFP). While it has proven benefits in reversing certain clotting disorders, administering low quality FFP can be more problems than benefits. It can potentially overload the circulation without improvement [2].

FFP is obtained from whole blood by centrifugation or by apheresis and frozen within six hours to a temperature of −18°C or lower to maintain the labile coagulation factors in a functional state [3]. It is mainly indicated for patients with active bleeding or for those preparing to undergo surgical procedures. However, the product should only be considered when factor concentrates are not available or when the benefit-to-risk ratio is maximum [4].

Quality monitoring of FFP is the function of labile coagulation factors, more precisely FVIII. This factor is the most sensitive marker of inappropriate processing of FFP. Stable coagulation factors, e.g., fibrinogen, remain relatively stable or only slightly decreased [5–8]. Immediately after thawing, FFP should have ≥0.70 IU FVIII/ml and ≥2.0 g fibrinogen/l [7, 9, 10].

Other guidelines such as the British Society for Hematology [5] and the New Zealand blood service require at least 75% of all tested FFP units containing ≥0.70 IU FVIII/ml [11]. Specific assays for coagulation factors should be used for quality monitoring of FFP. Screening test like activated partial thromboplastin time (APTT) and prothrombin time (PT) do not reflect the true levels of individual clotting factors [5,12,13]. APTT test is only sensitive to FVIII deficiency of 0.30 IU/ml or less, and neither APTT nor PT tests typically prolong until fibrinogen levels fall below 0.5 g/l [14,15].

Factor concentrates, either plasma-derived or recombinant, are preferred to FFP and considered safe and effective [14]. However, in resource-limited settings, like Yemen, access to these concentrates is not readily available or cost-effective, leaving FFP a more accessible, though less optimal, alternative [16]. In this context, it is important that the plasma used must be of the highest possible quality. The quality assessment of FFP is important in ensuring products efficacy and suitability for the clinical use. This study aimed to assess whether the FFP produced at the National Blood Transfusion and Research Center (NBTRC) meets the quality standards set by the international guidelines in regard to FVIII and fibrinogen levels. The acceptance limits were set as not less than 0.70 IU/ml for FVIII activity, and not less than 2.0 g/l for fibrinogen level.

Methods

This cross-sectional study was conducted at NBTRC from August to September 2022, with the aim of assessing the quality of FFP based on its FVIII and fibrinogen contents. In order to account for the lower FVIII level in blood group "O" individuals, the study utilized stratified random sampling method, dividing the FFP units into two categories, O and non-O blood groups. A total of 206 units were then equally selected from each category. Each unit was measured for FVIII and fibrinogen levels at two points, baseline (pre-freeze) and post-thaw (post-freeze). The measurements were carried out using the STart Max coagulation analyzer along with the reagents, calibrators and controls purchased from Stago company (Diagnostica Stago., Asnières sur Seine, France). All FFP units were donated by male donors. No woman attended the NBTRC to donate blood at the time of the study.

Preparation of baseline samples

The blood collection bags used at NBTRC were 450±45 ml primary bag to which small 30-50 ml ancillary bag is attached (Mitra Industries (P) Ltd., New Delhi, India). An initial amount of blood was first fed into the ancillary bag, from which 2.7 ml was transferred into 3.2% sodium citrate tube (BD Vacutainer®, Becton Dickinson, France). The tube was then quickly separated, aliquoted, and frozen for later baseline FVIII and fibrinogen measurements within two hours of blood collection.

Preparation of post-thaw samples

The unit of blood, which was stored for about one hour at 20-24°C, was centrifuged (Jouan, KR 4i Thermo Fisher Scientific, France) to finally yield platelets-poor plasma (PPP). Prior to freezing, a segment of PPP tubing long enough to contain at least 0.3 ml of plasma was heat-sealed and detached (Composeal, Fresenius Kabi, Germany). The segment was then quickly aliquoted and frozen for the next day post-thaw FVIII and fibrinogen measurements. All baseline and post-thaw aliquots were frozen at or below -40°C (Frimed plasma freezer, Italy); and thawed at 37°C for two minutes (QuickThaw® System, Helmer Scientific Inc. Noblesville, USA).

Factor VIII and fibrinogen measurements

FVIII and fibrinogen assays were based on one-stage and Clauss methods respectively. The assay procedures involve sample dilution with Owen-Koller solution, followed by incubation and addition of either deficient VIII reagent and cephascreen activator (for FVIII assay) or thrombin reagent (for fibrinogen assay) to initiate clot formation. The clotting time is then measured and compared against the unicalibrator curve. For FVIII, the activity is expressed as a percentage relative to normal plasma, with linearity range of 1.5% to 150%. The normal plasma range of FVIII in the adult population is usually 60-150% [17]. The fibrinogen level is expressed as g/l, with linearity range of 1.0 to 8.0 g/l. The normal plasma fibrinogen level in the adult population is usually in the range of 2-4 g/l [18].

Statistical analysis

FVIII and fibrinogen levels were expressed as mean ± SD. Paired t test was used to compare mean baseline and post-thaw FVIII and fibrinogen levels while one-sample t test was used to compare mean post-thaw FVIII with that established by the guidelines. A simple descriptive statistic was used to calculate the number and percentage of units that met the acceptable limits of FVIII in FFP. Statistical analysis was accomplished using SPSS version 26, with a *P*-value less than 0.05 as significant.

Ethical consideration

The study was carried out in accordance with strict ethical principles after taking due approval from the Research and Ethics Committee of the Faculty of Medicine and Health Sciences, University of Aden; and permission from NBTRC**.**

Results

The participants had median age (range) (years), weight (kg) and Hgb level (g/dl) of 27 (18-50), 68 (50-127) and 14.8 (13.4-17.5) respectively. Results obtained for FVIII and fibrinogen levels are shown in Figures 1-3. A statistically significant decrease (*P*=0.001) was noted in the average FVIII (20%) and fibrinogen (4.9%) from baseline to post-thaw (Figure1).

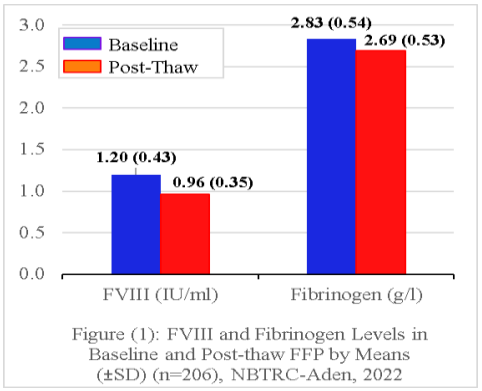

Figure (1): FVIII and Fibrinogen Levels in Baseline and Post-thaw FFP by Means (±SD) (n=206), NBTRC-Aden, 2022

The analysis of post-thaw FFP compliance with guidelines based on average FVIII and fibrinogen levels is shown in Figure 2.

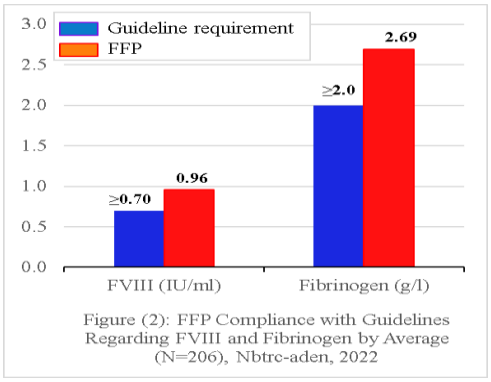

Figure (2): FFP Compliance with Guidelines Regarding FVIII and Fibrinogen by Average (N=206), Nbtrc-aden, 2022

Of post-thawed FFP units, 75.24% (155/206) had FVIII activity of at least 0.70 IU/ml (Figure 3).

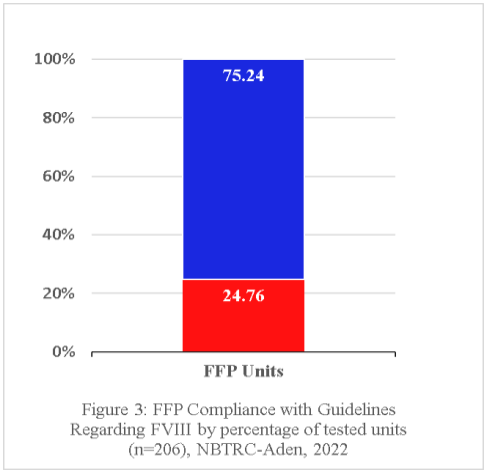

Figure 3: FFP Compliance with Guidelines Regarding FVIII by percentage of tested units (n=206), NBTRC-Aden, 2022

Discussion

Despite the decrease in the average post-thaw FVIII and fibrinogen levels compared to baseline, the majority of FFP units were in line with quality standards set by the international guidelines.

The processing of FFP from blood donation to thawing involves several steps, which can reduce the activity of one or more coagulation factors. Guidelines on the quality of blood and blood products have studied these steps and established a minimum acceptable limit of ≥0.70 FVIII/ml, and normal fibrinogen level in FFP, among the guidelines, the United Kingdom Blood Services [9], the Australian Red Cross [10], and World Health Organization [7]. In the current study, the average post-thaw FVIII met the average limits outlined by the guidelines, and so did fibrinogen with overall average fell within the reference range of 2.0 – 4.0 g/l.

The findings of the present study were compared with previous relevant studies that evaluated the quality of FFP by assessing FVIII with or without fibrinogen levels (Table 1). Sultan *et al*. [19] and Loganathan *et al*. [20] reported lower average FVIII and fibrinogen. Agus *et al* [21] and Dekhili *et.al.* [22] reported nearly similar average FVIII. Bala *et al*. [23] observed lower average FVIII but higher average fibrinogen to ours. Notably, nearly most studies met international guidelines requiring average FVIII ≥0.70 IU/ml and fibrinogen ≥2.0 g/l.

Table 1: Comparison of FVIII and Fibrinogen in FFP with Existing Studies by Average and Percentage of Units Meeting FVIII Requirement

Study	FVIII (IU/ml)	SD	% units compliance [a]	Fib. (g/l)	SD
Present Study (2023)	0.96	0.35	75.2	2.69	0.53
Bala et al. (2019)	0.80	0.086	97.5	3.04	0.53
Sultan *et al.* (2019)	0.84	0.15	95.0	2.47	0.50
Agus *et al.* (2012)	1.0	-	75.0	-	-
Loganathan *et.al*. (2019)	0.68	0.20	< 75.0	2.55	-
Dekhili *et.al.* (2018)	0.99	0.52	70.0	-	-

[a] % of tested FFP units that had FVIII of 0.70 or more. SD: Standard Deviation
Fib.: Fibrinogen

Moving beyond a simple average, the British Society of Hematology guideline [5] and the New Zealand blood service [11] require a minimum of 0.70 FVIII/ml in at least 75% of all tested FFP units. The findings in the present study satisfied this quality standard. Even though the remaining 24.76% of the units contained FVIII activity below 0.70 IU/ml, they were still expected to retain their efficacy in treating general coagulation disorders and active bleeding. FVIII activity in 100% of the units was greater than the minimum threshold of 0.3 IU/ml necessary for sufficient hemostasis [24]. Regarding fibrinogen, on the

other hand, the guidelines set no percentage of units that must contain ≥2.0 g/l fibrinogen.

Compared to existing literature (Table 1), the review indicated variability in the percentage of units meeting the guideline requirement. Bala *et al*. and Sultan *et al*. reported higher percentage (97.5% and 95% respectively), while Agus *et al*. [21] reported nearly similar percentage (75%). Non-compliance rates were reported by Loganathan *et al*. [20]. (<75%) and Dekhili [22] (70%).

Factors influencing the quality of FFP are numerous. However, the primary ones are related to the methods of plasma collection, the delay of plasma processing, and the speed and temperature at which plasma is frozen. A difficulty venipuncture by the first attempt, an unusually slow blood flow, or insufficient mixing of blood with anticoagulant may cause activation of the coagulation system. In addition, A collection time exceeded 15 minutes from the start of phlebotomy, or a failure to strip (mix) the residual blood in the bag tubing with its content immediately after blood donation may also activate the coagulation system. In any case, the activity of FVIII and fibrinogen would be reduced, and the collected blood is inappropriate for FFP preparation [25].

A delay of either whole blood or plasma for over eight hours before freezing causes a major decrease in FVIII. Plasma's freezing speed should be fast enough to reach a core temperature of -18°C or lower within 60 minutes. A slow freezing causes plasma salts to be concentrated around and exposed FVIII to high salt concentrations, which leads to its inactivation. At a high freezing rate, only small uniformly clusters of solutes trapped in the ice. To achieve the highest yield of FVIII, plasma should be frozen at -18°C or lower [6].

The results of this research have significant implications for the field of transfusion medicine and for NBTRC. Interpreted in the context of the quality standards, the levels of FVIII and fibrinogen in FFP stored at or below -40°C for 24 hours were acceptable and suitable for clinical use. Furthermore, adherence to guidelines could motivate the NBTRC to boost its performance.

While this study provides valuable insights into the quality of FFP, it is not without limitations. The study only assessed FVIII and fibrinogen levels as a measure of FFP quality. In addition, the study did not assess the levels of clotting factors at different temperatures, or after 24 hours storage period.

Conclusion

The majority of the tested FFP units met the quality standards, and were suitable for clinical use in settings where FVIII and fibrinogen concentrates are not available. Future studies extending to measure other quality parameters such as FV may add validation.

References

1. Puri V, Dhal I, Singh K, Sharma G, Rai P, Sharma S. Critical audit of fresh frozen plasma transfusion practices in obstetric and gynecology departments in a tertiary care hospital-Where and what needs to be

improved? Global Journal of Transfusion Medicine. 2019; 4(2):175.

2. Denise M. Harmening., Brown MR. Red Blood Cell and Platelet Preservation: Historical Perspectives and Current Trends. In: Denise M. Harmening, editor. Modern Blood Banking and Transfusion Practices. 7th ed. F. A. Davis Company; 2019. p. 1–23.
3. Jason Acker, Anna Razatos. Whole Blood and Apheresis Collection of Blood Components Intended for Transfusion. In: Claudia S. Cohn, Meghan Delaney, Susan T. Johnson, Louis M. Katz, editors. AABB Technical Manual. 20th ed. Maryland: AABB; 2020. p. 141–65.
4. Association for the Advancement of Blood & Biotherapies (AABB), American Red Cross, America's Blood Centers, Armed Services Blood Program. Circular of information for the use of human blood and blood components. AABB; 2021. p. 64.
5. Green L, Bolton-Maggs P, Beattie C, Cardigan R, Kallis Y, Stanworth SJ, *et al*. British Society of Haematology Guidelines on the Spectrum of Fresh Frozen Plasma and Cryoprecipitate Products: their handling and use in various patient groups in the absence of major bleeding. British Journal of Haematology. 2018;181(1):54–67.
6. European Directorate for the Quality of Medicines & HealthCare of the Council of Europe (EDQM). Guide to the preparation, use and quality assurance of blood components. 21st ed. Vol. 0, Transfusion Medicine. Strasbourg: EDQM; 2023. 265–317 p.
7. World Health Organization. Collection, Testing and Storage of Blood Products. In: Educational Modules on Clinical Use of Blood. 1st ed. Geneva, Switzerland: WHO; 2021. p. 183.
8. Nadine Shehata, Yunchuan Delores Mo. Hemotherapy Decisions and Their Outcomes. In: Cohn CS, Delaney M, Johnson ST, Katz LM, editors. AABB Technical Manual. 20th ed. Maryland: AABB; 2020. p. 553–73.
9. Norfolk D, editor. Handbook of Transfusion Medicine. 5th ed. Norwich, UK: TSO; 2013. 170 p.
10. Australian Red Cross. Component Information : An Extension of Blood Component Labels. Melbourne: Australian: Australian Red Cross Lifeblood; 2023. 52 p.
11. Kirwan S. Blood Component Monograph - Plasma Fresh Frozen. 2017. p. 1001–3.
12. James C. Fredenburgh, Jeffrey I. Weitz. Overview of Hemostasis and Thrombosis. In: Ronald Hoffman, Edward Benz, Leslie Silberstein, Helen Heslop, Jeffrey Weitz, Mohamed Salama SA, editor. Hematology: Basic Principles and Practice. 8th ed. Philadelphia: Elsevier Inc.; 2023. p. 1906–17.
13. Acker JP, Marks DC, Sheffield WP. Quality assessment of established and emerging blood components for transfusion. Journal of Blood Transfusion. 2016;2016:28.
14. Srivastava A, Santagostino E, Dougall A, Kitchen S, Sutherland M, Pipe SW, *et al*. World Federation of Hemophilia (WFH) guidelines for the management of hemophilia, 3rd ed. Haemophilia. 2020;26(S6):1–158.
15. Hellstern P. Composition of plasma. In: Toby L. Simon, Eric A. Gehrie, Jeffrey McCullough, John D. Roback, Edward L. Snyder, editors. Rossi's Principles of Transfusion Medicine. 6th ed. Incorporated: John Wiley & Sons Ltd; 2022. p. 200–8.
16. Tahiraj A. Haemophilia care in Arabic countries compared to western European countries (Yemen and Germany as examples). Ludwig

Maximillians University in Munich; 2012.

17. Diagnostica Stago S.A.S. Immuno-depleted Plasma for Factor VIII Assay. Asnières-sur-Seine, France: Diagnostica Stago; 2018. 2 p.
18. Diagnostica Stago S.A.S. Sta-Liquid Fib. Asnières-sur-Seine, France: Diagnostica Stago; 2019. 1 p.
19. Sultan S, Zaheer HA, Waheed U, Baig MA, Rehan A, Irfan SM. Internal quality control of blood products: An experience from a tertiary care hospital blood bank from Southern Pakistan. Journal of Laboratory Physicians. 2018; 10(01):64–7.
20. Loganathan R, Kulkarni RG, Kar R, Abhishekh B, Basu D. Assessment and association of coagulation factors (FVIII and fibrinogen) with the mode of collection and storage of fresh frozen plasma. Global Journal of Transfusion Medicine. 2019; 4(2):204–7.
21. 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 Transfusion. 2012; 10(2):191.
22. Dekhili DR, Nasri F. Controle de qualite des plasmas frais congeles issus d'un don de sang total au centre de transfusion sanguine du chu TLEMCEN. 2018.
23. Bala G, Gupta A, Suri V, Chhabra S, 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 Society for Contemporary Medical Research. 2019;6(8):28.
24. Draman R, Yousuf R, Abdul Aziz S, Ding CH, Zainol S, Leong CF. A Preliminary Study on Coagulation Parameters and Sterility of Thawed Refrozen Fresh Frozen Plasma. Indian Journal of Hematology and Blood Transfusion. 2020;36:112–6.
25. Association for the Advancement of Blood & Biotherapies (AABB). Technical manual: Methods and appendices. 20th ed. AABB; 2020. 196 p.