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ORIGINAL ARTICLE

Coagulation Factor Activities Changes Over 5 Days in Thawed Fresh Frozen Plasma Stored at Different Initial Storage Temperatures

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Abstract Thawed plasma is fresh frozen plasma (FFP) that has been stored for 5 days at 1–6 °C. Duration of storage and different storage temperatures might affect the coagulation factor activity in thawed FFP. This study measured the changes of coagulation factor activities over 5 days in thawed FFP and stored at two different initial storage temperatures. Thirty-six units of FFP, which consisted of nine units each from blood groups A, B, AB, and O, were thawed at 37 °C. Each unit was divided into two separate groups (Group A and Group B) based on initial storage temperature. The first group was stored at 2–6 °C for 5 days (Group A). The second group was stored at 20–24 °C for initial 6 h followed by 2–6 °C for 5 days (Group B). Prothrombin time (PT), activated partial thromboplastin time (APTT), coagulation factor activities of fibrinogen, factor (F) II, FV, FVII, FVIII, FIX, FX, and von Willebrand factor antigen (vWF Ag) were assessed at baseline after thawing, at 6 h, and on days 1, 3, and 5 of storage for both groups. All coagulation factors mean activities in both storage groups decreased significantly over 5 days of storage. The mean FVIII activity at day 5 of storage was 36.9% in Group A and 39.8% in Group B. The other coagulation factors mean activities were > 50% on day 5 of storage in both groups. The coagulation factor activities of thawed FFP stored for 5 consecutive days were reduced in the two storage groups but most of the activities were still above 30%. This study suggests that thawed FFP stored for 5 days has the potential to ameliorate coagulation factor deficiencies in affected patients.

Keywords Fresh frozen plasma · Coagulation factors · Storage · Temperature

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Introduction

Fresh frozen plasma (FFP) contains almost normal plasma proteins, including procoagulants, inhibitory components of the coagulation system, acute phase proteins, immunoglobulin, and albumin [1]. It can be prepared either from whole blood or plasmapheresis within 8 h of donation, and it must be stored at − 18 °C or colder. The shelf life of FFP is 12 months, but it can be extended to 7 years if stored at − 65 °C [2]. FFP contains all stable and labile coagulation factors, such as factor (F) V and FVIII. Upon request for blood transfusion, FFP is thawed for 30 min at 37 °C [3].

Previously, after thawing, FFP was stored for 24 h at 1–6 °C if not transfused, and subsequently it was discarded, leading to wastage of valuable resources [4]. The

American Association of Blood Banks (AABB) introduced thawed plasma (TP), which is thawed FFP stored at 1–6 °C with an extended expiry date of up to 5 days [5]. In Australia, TP is known as Extended Life Plasma (ELP), and it has a shelf life of up to 5 days from the date of thawing and is stored at 2–6 °C [6]. ELP and TP are similar to FFP but have lower levels of coagulation factors V, VII, and VIII [4, 7, 8].

Currently, the National Blood Centre of Malaysia discards unused FFP after thawing [9]. This has led to wastage and increased in operational costs. With the implementation of massive transfusion protocol in most hospitals, the availability of thawed FFP in emergency departments provides a better outcome for patients with massive bleeding [10]. The aim of this study was to measure the changes in coagulation factor activities over 5 days in thawed FFP and to determine the effect of different initial storage temperatures on coagulation factor activities in this blood component.

Materials and Methods

Sample Collection and Processing

FFP was prepared from 36 whole blood donations collected in 450 ml bags containing citrate–phosphate-dextrose. Nine donors were randomly selected to represent each blood group (A, B, AB, and O). The donors were predominantly male; 28 males and eight females with mean age of 32 years old. The FFP was prepared within 8 h of collection. Consent to participate in this study was obtained from all eligible blood donors. After separation of plasma by two centrifugation steps (soft spin and hard spin) using a Thermo Scientific™ Heraeus™ Cryofuge™ 8500i, the FFP units underwent blast freezing (MBF 42, Dometic, Hosingen, Luxembourg). Volume of all FFP bags varied between 250 and 210 mL. The FFP bags were stored at − 30 °C before being thawed using a plasma thawer (CytoTherm™ DR, Trenton, NJ, USA) for 30 min at 37 °C. Only FFP were used in this study and the remaining components such as packed red blood cells and platelet concentrates were used for transfusion purpose.

After thawing of FFP bags, 10 mL of baseline sample was taken. The bags were then divided equally into another four satellite bags and labelled based on storage temperature; Group A and Group B (A6 h, A1, A3, A5 and B6 h, B1, B3, B5). Bags in Group A were stored at 2–6 °C immediately after thawing, whereas bags in Group B were stored at room temperature (20–24 °C) for initial 6 h. Six hours after thawing, 10 mL samples were taken from bags labelled A6 h and B6 h. All the remaining bags from both groups were then stored at 2–6 °C for 5 days. At day 1, 3, and 5 of storage, 10 mL of sample was taken from bag labelled A1/B1, A3/B3, and A5/B5 respectively. All aliquots were placed into polypropylene test tubes and kept frozen at –70 °C before the coagulation factor activity tests were performed. Prothrombin time (PT), activated partial thromboplastin time (APTT), coagulation factor activities for factor II (FII), factor V (FV), factor VII (FVII), factor VIII (FVIII), factor IX (FIX), factor X (FX), fibrinogen and von Willebrand factor antigen (vWF Ag) were measured from each sample collected. Figure 1 showed the flowchart summary of the study design.

This study was approved by the Medical Research & Ethics Committee, Ministry of Health Malaysia and the Jawatankuasa Etika Penyelidikan Manusia of Universiti Sains Malaysia.

Laboratory Analysis

The frozen aliquots were thawed for 5–10 min in a 37 °C water bath prior to analysis. All coagulation tests were performed using an ACL TOP CTS 500 device (Instrumentation Laboratory, Bedford, MA, USA). Measurements of coagulation factors were conducted using one stage clotting factor assays. Fibrinogen was measured using the Clauss method, and vWF Ag was measured using the latex enhanced immunoassay. All reagents such as ACL PT

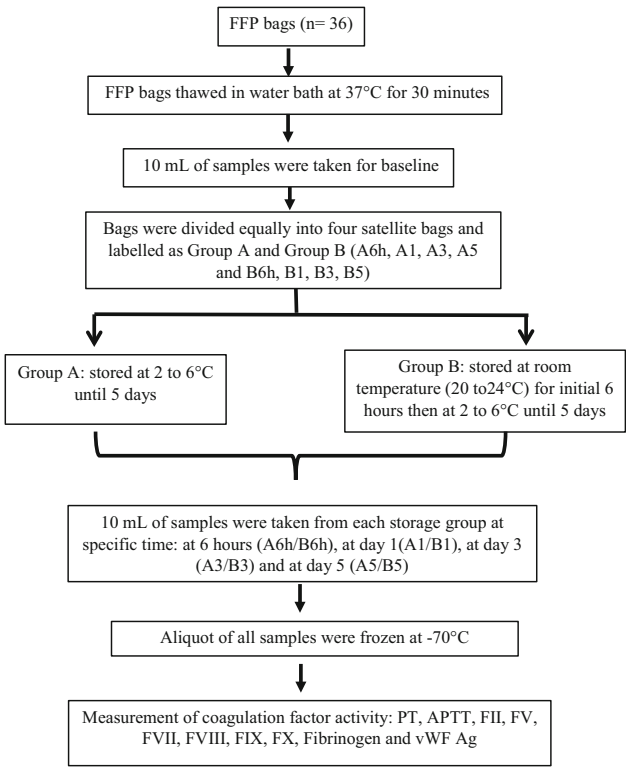

Fig. 1 Study flowchart

Recombiplastin, APTT Synthasil, vWF Ag, ACL Hemosil Fibrinogen Clauss and factors deficient plasma were obtained from Instrumentation Laboratories Company (United States). All the tests were performed according to the laboratory standard operating procedures with the manufacturer's recommendation.

Statistical Analysis

Data were analysed using SPSS version 22.0. Mean percentage activity ± standard deviation (SD) was expressed for all coagulation factors. Repeated measures analysis of variance (ANOVA) was used to compare coagulation factor activity level between and within groups over the duration of the test period. Differences between or within groups were considered significant at $p < 0.05$ with a confidence interval of 95%. For significant within-subject effects over time in repeated measures ANOVA, a paired *t* test with Bonferroni correction was performed. For significant within-subject effects over temperature in repeated measure ANOVA, a paired t-test was used.

Results

All coagulation factors showed significant reduction of activities over 5 days of storage in both test groups (Table 1). The baseline for all the coagulation factors activities were all more than 70%. By day 5, the activity of FVIII had decreased the most (36.9%) in storage group A, and the level was 39.8% in storage group B. The other blood coagulation activities, including FV, were > 50% in both storage groups at day 5. Significant differences in mean PT, APTT, FVIII, and vWF Ag were detected between the two storage groups at each time point.

Figures 2, and 3 show the significant interaction ($p < 0.05$) of FVIII and vWF Ag for the two storage groups over the course of 5 days. Figure 4 shows the percent decrease of coagulation factors activities from baseline until day 5 of storage for each group. FVIII decreased the most, followed by vWF Ag. PT, APTT, and vWF Ag levels were significantly influenced by time and temperature interaction.

Discussion

Implementation of TP in clinical setting has been proven to reduce wastage [4, 11] Furthermore, in cases of massive trauma, there was a reduction in time to transfuse the first unit of plasma, a decreased in blood product usage for the first 24 h of admission and also a reduction by 60% in 30-day mortality when TP is available for immediate transfusion in emergency department [12]. Moreover, a study by Holcomb et al. had shown that severely injured patients who received immediate prehospital transfusion with TP had an improved early outcome, decreased use of blood products over 24 h and a reduction in the first 6-hour mortality [13].

This study described the effect on coagulation factors in thawed FFP that was stored for 5 days. The coagulation factors activities were measured immediately after thawing (at baseline), at 6 h post thawing, and at day 1, 3, and 5 of storage. This study also assessed the effect of different initial 6 h of storage temperature (2–6 °C versus 20–24 °C) on coagulation factors activities.

The baseline for all the coagulation factors activities were above 70% with FVIII level of 71.6%. This result was in contrast with the other study which reported that baseline for most of the coagulation factors activities were more than 100%. It might be due to the difference in the current study methodology which used repeated thawing and freezing prior to sample measurement as compared to immediate measurement of coagulation factors activities after sampling [14].

In this study, all coagulation factors activities in the two different storage groups showed significant reduction over 5 days. Wang et al. [8] reported the same finding. Previous study by Feng et al. [15] reported that PT, APTT, FVIII, FX and fibrinogen measurement between two storage temperature (4 and 25 °C) were about the same for the initial 6 h of storage. By 8–24 h of storage, the coagulation activities in 25 °C storage group were lower than in 4 °C.

Most of the coagulation factor activities remained at > 50% after storage for 5 days; the exception was FVIII, for which its mean levels of activity were 36.9 and 39.8% at day 5 for the Group A and Group B respectively. However, other studies reported that activities of most coagulation factors, including FVIII, were > 50% at day 5 of storage [4, 14, 16, 17]. The different results might be because the baseline FVIII activity in this study was already low (71.6%) as compared to baseline of 86–126% in those studies.

Activity of FVIII, which is a heat labile factor, decreased the most over 5 days, followed by vWF Ag, FVII, and FV. Milam et al. reported that FVIII activity decreased rapidly during first 4 h after thawing, with a loss of 26%, but that the reduction was less during the next 20 h of storage [18]. Other studies reported FVIII activity reductions ranging from 14 to 47% from baseline until day 5 of storage, which was comparable with results of the current study [7, 19, 20].

FV appeared to be relatively stable during the first 24 h after thawing as compared to FVIII in this study. Milam et al. also found that FV was relatively stable for 24 h regardless of whether it was stored at 25 or 4 °C [18].

Table 1 Coagulation factors activities in thawed fresh frozen plasma stored for 5 days

	Baseline	6 h		Day 1		Day 3		Day 5	
		Group A	Group B	Group A	Group B	Group A	Group B	Group A	Group B
PT (seconds)	11.3 ± 0.83	11.4 ± 0.93	11.3 ± 0.95	11.9 ± 0.95*	12.0 ± 0.96*	12.8 ± 0.99*	13.0 ± 1.08*	13.3 ± 1.01*	13.5 ± 1.10*
APTT (seconds)	38.5 ± 3.26	39.6 ± 3.86*	38.6 ± 3.21*	41.4 ± 4.58*	40.2 ± 4.07*	42.9 ± 4.29*	42.0 ± 4.85*	43.7 ± 4.78*	43.0 ± 4.59*
FII (%)	106.2 ± 14.86	103.8 ± 20.98	103.3 ± 21.54	100.9 ± 11.67**	102.0 ± 10.69**	96.9 ± 15.05**	96.5 ± 12.54**	94.9 ± 10.67**	92.3 ± 10.91**
FV (%)	85.7 ± 20.80	83.8 ± 19.20	84.2 ± 20.08	80.3 ± 18.49**	78.0 ± 19.05**	73.4 ± 16.20**	71.4 ± 15.60**	66.3 ± 15.72**	66.1 ± 15.52**
FVII (%)	104.8 ± 23.22	100.5 ± 23.54**	102.7 ± 25.99	90.9 ± 21.79**	91.1 ± 23.83**	76.6 ± 18.94**	75.0 ± 18.81**	70.6 ± 15.7**	70.2 ± 18.51**
FVIII (%)	71.6 ± 22.40	61.9 ± 24.29*	65.9 ± 20.43*	51.2 ± 24.24**	53.8 ± 21.66**	40.9 ± 19.07*	45.3 ± 23.20*	36.9 ± 17.75**	39.8 ± 18.04**
FIX (%)	96.2 ± 13.22	93.4 ± 13.06**	95.1 ± 13.62	90.9 ± 19.48	93.0 ± 12.48**	92.7 ± 14.36	91.1 ± 13.84**	91.5 ± 12.48**	90.6 ± 13.21**
FX (%)	101.0 ± 18.02	99.3 ± 6.6	99.6 ± 18.03	99.7 ± 16.01	96.8 ± 23.08	98.3 ± 16.43	96.1 ± 17.33	96.3 ± 17.09	96.1 ± 17.29
vWF Ag (%)	110.8 ± 39.01	98.7 ± 43.31*	104.8 ± 39.16*	85.3 ± 44.17*	93.2 ± 46.09*	75.6 ± 41.87*	83.0 ± 45.94*	67.0 ± 35.52*	77.4 ± 44.65*
Fibrinogen (mg/dL)	238.4 ± 55.02	225.8 ± 55.50**	227.2 ± 54.62**	224.8 ± 56.17**	221.5 ± 54.22**	220.5 ± 53.22**	219.8 ± 53.88**	219.3 ± 53.03**	220.3 ± 55.27**

Group A = FFP bags stored at 2–6 °C immediately after thawing until Day 5, Group B = FFP bags stored at room temperature (20–24 °C) for initial 6 h after thawing followed by storage at 2–6 °C until Day 5

Data presented as mean ± SD. Reference range: PT, 9–12 s; APTT, 25–39 s; FII, FV, FVII, FVIII, FIX, FX, vWF Ag 50–150%; fibrinogen, 150–450 mg/dL

* $p < 0.05$ between two storage categories at each time point

** $p < 0.0125$ when compared to baseline for each storage condition using paired t-test with Bonferroni correction

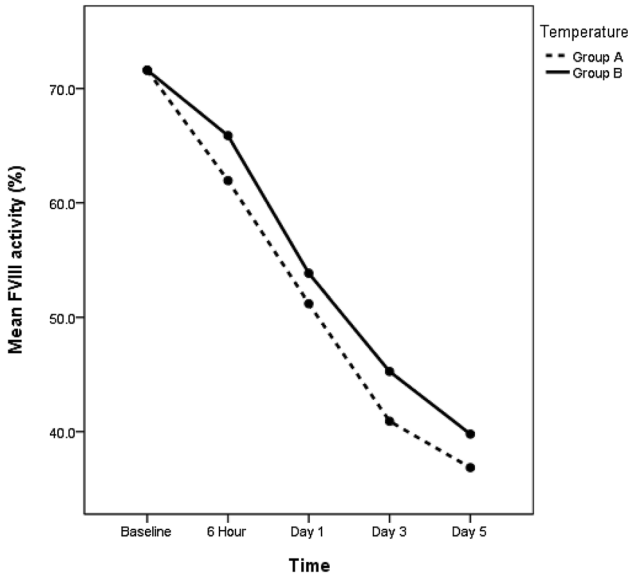

Fig. 2 FVIII activity changes over 5 days of storage

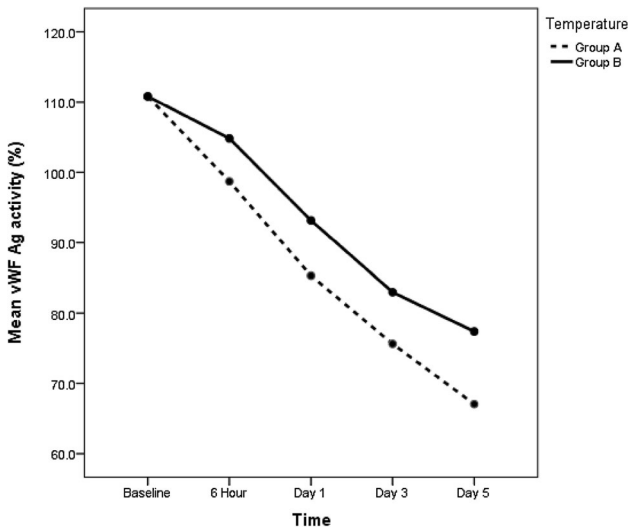

Fig. 3 vWF Ag changes over 5 days of storage

Other studies reported a significant reduction in FV activity of 9 to 21% over 5 days of storage [7, 19]. Although FV and FVIII are homologous coagulation cofactors, their B domain differs, as FV has 25 potential *N*-linked glycosylation sites but FVIII has 19. This may explain why FV appeared to be more heat stable than FVIII [21].

vWF Ag level underwent a gradual significant reduction over 5 days of storage. This could be due to variation of the vWF Ag level among the different blood groups. Franchini et al. previously reported that group O individuals have lower plasma VWF levels than non-O individuals [22].

FVII activity decreased by 33% over 5 days of storage, which was similar to results of another study [7]. For the other coagulation factors activities such as fibrinogen, FII, FIX and FX, they exhibited statistically significant changes over 5 days of storage with FX activity decreased the least from baseline. However, previous studies demonstrated no significant changes in activities of FII, FIX, and FX over 5 days of storage. The different results might be due to small sample sizes in those studies, which may have made it impossible to detect statistically significant changes [4, 8, 23].

In the present study, only PT, APTT, FVIII, and vWF Ag showed significant differences between the two storage groups at each time point. The percentage difference between the two storage groups was $< 3\%$ for PT and APTT, but it was markedly different for FVIII and vWF Ag. By day 5 of storage, fibrinogen, FVIII, and vWF Ag showed more reduction in Group A (initially stored at 2–6 °C) as compared to Group B (initially stored at room temperature for 6 h). In contrast, Lamboo et al. reported no significant changes in PT, APTT, FV, and fibrinogen during storage at room temperature for up to 6 h [24]. This might possibly be due to cold activation of FVIII and vWF Ag during the initial 6 h of storage at temperature 2–6 °C (Group A) as compared to storage at room temperature (Group B) [25].

In this study, the methodology used involved repeated thawing and freezing of samples. A study by Dzik et al. [26] reported that coagulation factors from plasma samples that underwent repeated freezing and thawing were always within the normal range but had a small statistically significant reduction in PT, APTT, FV, and FVIII activities when comparing twice frozen FFP to units frozen only once. Moreover, Ben-Tal et al. [27] showed that mean level of FII, FVII, FIX, FX, and fibrinogen did not vary greatly if repeated thawing and freezing were performed. As such, it was expected that some coagulation factors may have deteriorated during repeated freezing and thawing cycles in this current study.

In conclusion, this study showed that all coagulation factors activities in thawed FFP stored for 5 consecutive days were reduced in both storage groups. However, activities of the coagulation factors present in the samples were still sufficient to treat bleeding patients, as the activities were $> 30\%$ [28]. This study showed that FFP can be stored at room temperature for an initial period of up to 6 h after thawing without significant loss of most coagulation factors activities. Given the potential cost-saving benefit of reducing the amount of discarded post thawed FFP and the immediate availability of plasma for early resuscitation, thawed FPP should be considered as part of the massive transfusion protocol for the treatment of

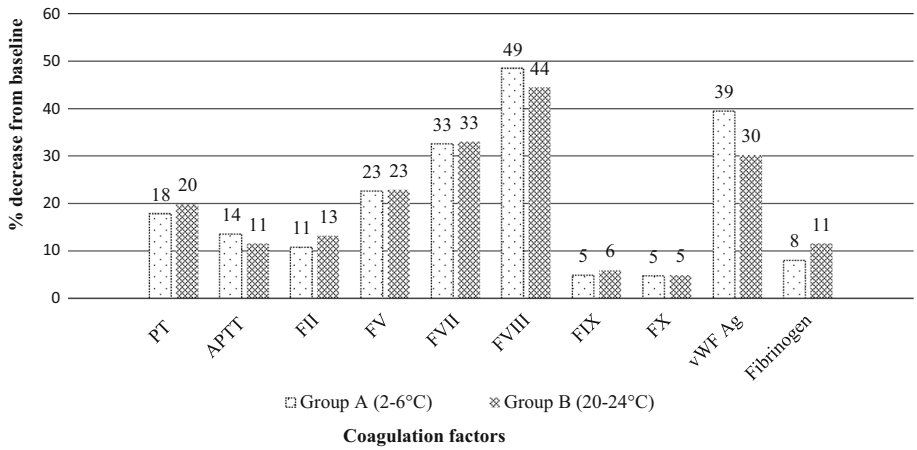

Fig. 4 Percentage decrease in coagulation factors activities over 5 days for each storage category

substantial bleeding and be made available especially in emergency department.

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Compliance with Ethical Standards

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.

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