ORIGINAL ARTICLE

Effect on Haemostatic Proteins in Plasma Prepared from Fresh and Overnight Stored Whole Blood

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ABSTRACT

Introduction: Fresh frozen plasma (FFP) is prepared within 8-10 hours after collection to ensure preservation of coagulation factors however, adherence to this time is a challenge. Extended processing time is an option to overcome it. This study was done to evaluate haemostatic proteins after extended time. **Methods:** Blood collected from a mobile donation centre was divided into three (3) groups before processed into plasma. Group 1 (n=42) was prepared within 8 hours post collection. Group 2 (n=42) was prepared after overnight and stored at room temperature. Group 3 (n=42) was prepared after overnight but stored at 2-6^{0}C. Plasma haemostatic proteins were measured in all groups and mean activity of each level was compared using One-way ANOVA. **Results:** There was no reduction in all the haemostatic proteins in plasma prepared from overnight storage (Groups 2 and 3) compared to Group 1 except for Factors VIII and V whilst PT was not significantly prolonged. aPTT was significantly prolonged in both Groups 2 and 3 compared to Group 1. There were 25.7% and 35.2% reduction of Factor VIII levels in Groups 2 and 3 respectively, however levels were above 60%. There is 8.7% reduction in Factor V level but the mean factor activity was above 90%. Comparing Groups 2 and 3, there was no significant difference in activity of all haemostatic proteins. **Conclusions:** Haemostatic proteins are preserved in plasma prepared from blood stored overnight. Prolongation of the APTT is reflected by reduction in Factor VIII activity but still within the normal reference range.

Keywords: Plasma, Preservation, Haemostatic proteins, Extended Processing Time, Factor VIII

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INTRODUCTION

Fresh Frozen Plasma (FFP) contains plasma haemostatic proteins including coagulation factors and natural anticoagulants. Since Factor VIII (FVIII) is the most labile coagulation factor in the FFP, the quality control (QC) parameter for FFP necessitates that coagulation (FVIII) activity level must be at least 70 IU per 100 mL (1). Several factors could affect the FVIII activity level; these include whole blood storage temperature before processing and the delay in plasma separation from whole blood collection, processing and separation before been frozen (2). Council of Europe suggests to process and separate plasma from whole blood preferably within six (6) hours but not exceeding 18 hours after collection provided the blood bag is refrigerated (1). Additionally, for those whole blood collected, the units are to be processed after 6 hours and less than 24 hours and the blood bag should immediately be cooled to 20-24^{0}C.

Current practice in National Blood Centre, Kuala Lumpur (NBCKL) necessitates whole blood should be processed within eight (8) hours of collection, as like other blood centres (3, 4). Nevertheless, the preparation of blood components from whole blood on a large scale especially in a big centres like NBCKL is undoubtedly challenging since most of the blood units are collected from many distant mobile donation sites. Thus, components processing within eight (8) hours after collection is remains a challenge. There are potential benefits to the transfusion service efficiency if plasma production period from whole blood is extended without causing deterioration of quality. Many studies have been conducted on this matter; however, results have been inconsistent in most of these studies.

The number of haemostatic proteins activity level involved was also different among studies. Some of them just assess labile coagulation FVIII alone and some others assess coagulation factors without natural anticoagulants all together. Therefore, our own institutional study is needed to adapt to the local requirements towards

improvement of transfusion service in Malaysia.

Thus, the objective of this study was to investigate the quality of plasma haemostatic proteins prepared from whole blood stored overnight at two different temperatures (room temperature and in the cold room). Two (2) different temperatures were compared because at 2-6ºC, platelets cannot be manufactured due to its deleterious effect, while labile FVIII is sensitive to temperature changes that could be affected if stored at room temperature. Both coagulation factors and natural anticoagulants activity level were evaluated in actual donation situations to the subsequent steps of components processing. This study was conducted in NBCKL.

MATERIALS AND METHODS

This was a cross-sectional study and conducted from January 2012 to May 2013. Ethics approval from the Ministry of Health Research and Ethics Committee (MREC) and Human Research Ethics Committee, Universiti Sains Malaysia (JEPeM) - FWA Reg. No.00007718; IRB Reg. No. 00004494) were obtained before this study was done. Whole blood was collected from donors who had donated their blood in a mobile collection center organized by NBCKL. Duration of blood collection was as in normal donation time, which was about 30 minutes from the point of registration to end of the process. Samples totalling 126 units (with volume 450ml ± 45ml) were collected using simple randomization in bags containing anticoagulant-preservative solutions (63mls CPDA and 100ml Optisol). These samples were then divided into three (3) groups before processed into plasma. Group 1 (n=42) was plasma prepared within eight hours post collection. Group 2 (n=42) was plasma prepared after overnight (hold 18-24 hours) and stored at room temperature. Group 3 (n=42) was plasma prepared after overnight (hold 18-24 hours) but stored at 2-6ºC.

Using Platelet Rich Plasma (PRP) method, the blood units were then separated into packed red cells, platelet concentrates and plasma based on current practice in NBCKL. Except for Group 1, platelet concentrates and plasma from Group 2 and Group 3 were not transfused to the patient as they were deviations from the current practice. About 6ml plasma sample was then taken using blood docking device system from each plasma bag just after plasma preparation into polypropylene tube and frozen to -30ºC or below for testing. Baseline samples were also taken and subsequently frozen within eight (8) hours after blood collection for Group 2 and Group 3. All samples were tested within one (1) month after collection, since there was heavy usage of the analyzer in routine service work and we had to wait for turns for the testing schedule.

After frozen samples were thawed at 37˚C water bath for 5-10 minutes, PT, APTT, fibrinogen, coagulation factors and natural anticoagulants were measured using IL ACL TOP 500 Analyzer (United States) according to manufacturer's recommendation. Baseline FVIII activity level was measured for Group 2 and Group 3 to compare changes after overnight storage for both groups at two different temperatures.

Computer software (IBM Statistical Package for the Social Sciences (SPSS) version 20.0) was used for data analysis where the Means and Standard Deviations (SD) were calculated. One-Way Analysis of Variance (ANOVA) was used to compare mean for three groups (Group 1, Group 2, and Group 3). A p-value less than 0.05 was considered significant.

RESULTS

A summary of plasma haemostatic proteins activity level is shown in Table I. Only Factor V and Factor VIII activity levels were found to be significantly reduced in plasma from Group 2 and Group 3 compared to plasma in Group 1. This is associated with significant prolongation of APTT.

Table I: Summary of plasma haemostatic proteins activity level

Factors	Group 1* n = 42	Group 2* n = 42	Group 3* n = 42	*p-value*
	Mean(SD)	Mean (SD)	Mean (SD)	
PT (Sec)	10.27 (0.809)	10.27 (0.818)	10.62 (0.830)	0.080
APTT (Sec)	33.86 (2.747)	35.53 (2.729)	35.94 (3.002)	0.002
Fib (mg/dL)	284.69 (47.052)	261.59 (49.334)	277.02 (48.137)	0.086
FII (IU/mL)	1.09 (0.112)	1.11 (0.106)	1.06 (0.080)	0.073
FV (IU/mL)	1.03 (0.196)	0.94 (0.185)	0.94 (0.143)	0.020
FVII (IU/mL)	1.11 (0.248)	1.10 (0.276)	1.04 (0.253)	0.393
FVIII (IU/mL)	1.05 (0.262)	0.78 (0.191)	0.68 (0.192)	<0.001
FIX (IU/mL)	1.12 (0.150)	1.05 (0.137)	1.06 (0.179)	0.072
FX (IU/mL)	1.05 (0.178)	1.01 (0.189)	1.01 (0.223)	0.606
FXI (IU/mL)	0.98 (0.189)	0.92 (0.193)	0.91 (0.172)	0.155
Antithrombin (IU/mL)	0.94 (0.115)	0.97(0.113)	0.93 (0.095)	0.185
Protein C (IU/mL)	1.01 (0.186)	0.98 (0.210)	0.98 (0.202)	0.814
Protein S (IU/mL)	0.85 (0.164)	0.85 (0.113)	0.80 (0.173)	0.186

* Group 1 = processed within 8 hours, Group 2 = processed after overnight stored at room temperature, Group 3 = processed after overnight stored in the cold room

There was an 8.7% reduction in Factor V level in both Groups 2 and Group 3 compared to group 1. For Factor VIII level, there were 25.7% and 35.2% reduction in Group 2 and Group 3 respectively. Comparing Groups 2 and 3, there was no significant difference in activity of all plasma haemostatic proteins activity level. Comparison between three groups for Factor V and Factor VIII are shown in Figure 1 and Figure 2 respectively.

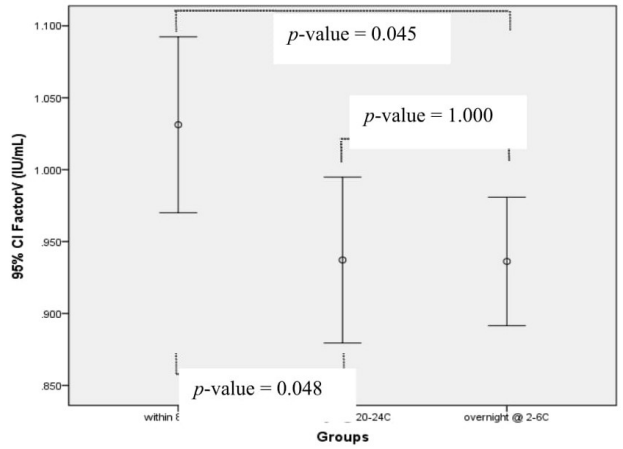

Figure 1: 95% confidence interval (CI) of the mean Factor V activity levels of the three sample groups

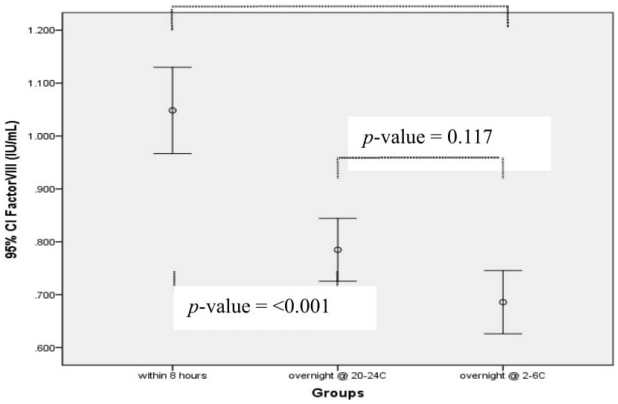

Figure 2: 95% confidence interval (CI) of the mean Factor VIII activity levels of the three sample groups

Although statistical analysis in between Group 2 and Group 3 for FVIII activity level was not significant, the level of FVIII activity level in the Group 2 had been always higher than in Group 3 throughout the processing duration (Figure 3).

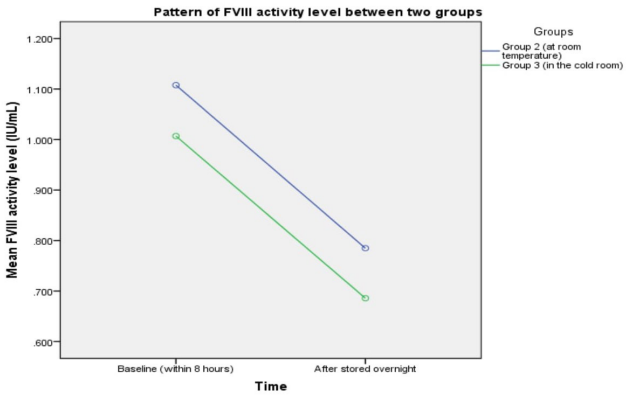

Figure 3: Pattern of FVIII activity level between Group 2 and Group 3

Out of 42 units of plasma in each three groups, percentage of plasma units contained at least 0.70 IU/ml FVIII activity level were 90.5%, 61.9% and 40.5% in Group 1, Group 2 and Group 3, respectively. The percentage of plasma units tested and contained at least 0.5 IU/mL FVIII activity level were 100%, 95.2% and 83.3% for Group 1, Group 2 and Group 3 respectively.

DISCUSSION

In the current study, the influence of two factors on plasma haemostatic proteins activity level were assessed. First, the effect of time in which delayed in plasma preparation from whole blood stored overnight instead of standard practice in National Blood Centre, Kuala Lumpur (NBCKL) that is within eight (8) hours after blood collection (Group 1). Second, the effect of temperature in which whole blood stored either at room temperature at 20-24°C (Group 2) or in the cold room at 2-6°C (Group 3 plasma) prior to plasma preparation.

In this study, except for Factor V (FV) and Factor VIII (FVIII), all other coagulation factors (Factor II, Factor VII, Factor IX, Factor X, Factor XI) and natural anticoagulants (antithrombin, Protein C, Protein S) were minimally affected by delay in plasma production and were not influenced by two different storage temperatures. As there was only a minimal effect on coagulation factors in the tissue factor pathway, prothrombin time (PT) was also not significantly affected.

On the other hand, there were significantly lower level of FV and FVIII activities with prolonged mean activated partial thromboplastin time (APTT) value in plasma prepared after stored overnight (Group 2 and Group 3) compared to plasma prepared within eight (8) hours after collection (Group 1). There was no significant difference between plasma from Group 2 and Group 3. These findings indicate that FV, FVIII and APTT are affected by delayed in plasma production and not by two (2) different whole blood storage temperatures both at room temperature and in the cold room.

Although statistical analysis showed significant reduction of FV activity level, it was only marginally significant. Mean FV activity levels in all groups were still within normal limits and the lowest level was still high with 0.9 IU/mL in both Group 2 and Group 3. Compared to the plasma produced in Group 1, the plasma produced in the Group 2 and Group 3 had significantly lower FVIII activity level with p-values of <0.001. Mean FVIII activity level in Group 2 plasma and Group 3 plasma were 35.2% and 25.7% lower than in plasma processed within eight (8) hours after collection, respectively. Even after comparing to their own baseline sample, which was taken within eight (8) hours after collection, plasma in the Group 2 and Group 3 showed reductions of 30.3% and 32.7% FVIII activity level respectively.

However, whole blood storage temperatures both at room temperature (20-24ºC) and in the cold room (2-6ºC) did not play a significant role on FVIII activity level in current study. There was no significant difference in the mean FVIII activity level in Group 2 plasma when compared to the Group 3 plasma. Furthermore, there was no time and temperature interaction effect on FVIII activity level as displayed clearly in the Figure 3. This means that the effect of delayed in plasma preparation from whole blood on FVIII activity level was independent of that of whole blood storage temperatures.

Although statistical comparison in between plasma produced from whole blood in Group 2 and Group 3 was not significantly different, the mean FVIII activity level was higher in the Group 2 than Group 3. Unfortunately, this study cannot conclude that the higher level of mean FVIII activity in the Group 2 plasma was due to different whole blood storage temperatures effect. This is because starting from baseline sample; mean FVIII activity level was already higher in the Group 2 plasma than in the Group 3 plasma as showed in the Figure 3. Possible reason is due to natural donor variability as the donors were different for all three groups (5, 6, 7). This effect was seen too for the mean anti thrombin activity level.

The other finding noted in this study is that the percentage of plasma units with at least 0.70 IU/mL FVIII activity level for Group 1, Group 2 and Group 3 were 90.5%, 61.9% and 40.5% respectively. Meanwhile, the percentage of plasma units tested and contained at least 0.5 IU/mL FVIII activity level were 100%, 95.2% and 83.3% for Group 1, Group 2 and Group 3 respectively. The level of 0.5 IU/mL is the minimum level for Standard Haematology Reference Range (8). Therefore, the bigger number of plasma units can be obtained if we could use the minimum level of 0.5 IU/mL of the FVIII activity level to be present in the plasma for QC monitoring. Council of Europe has specified that average FVIII activity level after freezing and thawing is not less than 70 IU /100 mL (0.7 IU/mL) with no specification for percentage of units tested (1).

The United Kingdom (UK) guideline specified that plasma units must meet FVIII activity level of more than 0.70 IU/mL for at least more than 75% of the plasma units tested (9). Thus, only plasma produced within eight hours after blood collection meets both Council of Europe and UK specifications for plasma quality requirement in term of FVIII activity level. However, plasma produced from whole blood stored overnight could still benefit for plasma transfusion, even though they did not fulfil the current QC requirements according to European and UK guidelines for several reasons.

Firstly, Council of Europe Guideline has specified that pathogen inactivated plasma must contained an average of at least 50-70 IU/100 mL FVIII activity level for their quality monitoring (1). In current study, plasma produced after overnight stored both at room temperature and in the cold room contained even higher FVIII activity level than those minimum specified QC monitoring level for pathogen-inactivated plasma. If pathogen-inactivated plasma is effectively used for clinical transfusion, plasma in current study supposed to have a potential place in clinical transfusion as well.

Secondly, all coagulation factors including FVIII can exert their functional coagulation activity at a minimum level of 30% activity (10). At the same time, FVIII is an acute phase protein and usually not reduced to a very low level in person under stress (11, 12, 13). Therefore, except for haemophiliac patients, the majority of patients requiring plasma transfusion in which they are under a stress condition, would have high FVIII level (14). Even if the FVIII activity level dropped in acute condition, the level will be replenished soon after (15).

Thirdly, the mean FVIII together with other coagulation factors and natural anticoagulants activity levels in current study were still within a normal reference range according to Standard Haematology Reference Ranges (9). Furthermore, FVIII activity level reduction does not necessarily reflect the final clot formation. As reported by a recent study, even though their FVIII activity level was reduced to about 23%, their thrombin generation to form a clot as a final product was normal (16). Therefore, the residual FVIII and other coagulation factors activity level in plasma from our current study, perhaps, have the potential to generate normal final clot formation as well.

In addition, the need for higher level of FVIII activity in the plasma for clinical transfusion is less relevant as plasma is indicated to treat multiple coagulation factors deficiency rather than for FVIII replacement for which FVIII concentrate is safer and more effective (17).

Furthermore, plasma produced after overnight stored of whole blood is issued out for clinical use separately from FFP depending on the patient's clinical condition. This is effectively practiced in certain country such as in United States (11).

Quality control (QC) monitoring for plasma produced after stored overnight needs revision separately from those for FFP according to institutional requirements and needs. Canada is one example that defines QC for this type of plasma separately from FFP (18, 19). This plasma must contain at least 0.52 IU/mL FVIII activity levels in a minimum 75% of plasma units tested. At the same time, the level of FVIII is influenced by ABO blood group, where the level is known to be lowest in individuals with blood group O (7). Thus, to set QC level of FVIII at a minimum level of 0.7 IU/mL is sometimes difficult especially in a community with high prevalence of blood group O donors.

CONCLUSION

In conclusion, there was relatively good retention of plasma haemostatic proteins activity level in the plasma produced from whole blood stored overnight regardless of storage at either room temperature or in the cold room except for FVIII.

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