ORIGINAL ARTICLE

Effects of Methylene Blue, Psoralen and Riboflavin Treatments on Fibrinogen and other Coagulation Factors Level of Fresh Frozen Plasma

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ABSTRACT

Introduction: Photochemical treatment is one of the pathogen inactivation method to treat plasma, part of a pro-active approach used for blood and blood component safety. Three photochemical treatments that have been used were methylene blue, riboflavin and psoralen treatment. This study was done on Fresh Frozen Plasma (FFP) to evaluate the treatment effects of psoralen, methylene blue and riboflavin on coagulation factors level. **Methods:** FFP was collected from apheresis plasma units and kept at 22°C to 24°C. A sum of 90 apheresis plasma units and segments were used, separated from each bag and a part used as controls, placed in a -30°C freezer for storage, thawed, and coagulation proteins function was evaluated before and after treatment, at immediate, 30 days and 270 days storage. **Results:** Significant differences in fibrinogen and coagulation factor levels between before and after treatment with methylene blue, psoralen and riboflavin. However, most of the mean values in treated plasma were within reference ranges. Methylene blue treated FFP showed the lowest changes in fibrinogen and other coagulation factors level whilst riboflavin treated FFP demonstrated the highest changes in coagulation proteins concentrations especially for fibrinogen, FV, FVIII, FIX and FXII. However, FXIII showed the best recovery for all three photochemical methods with reduction level of 3% to 8% compared to pre-treatment. Storage time comparison of immediate, 30 days and 270 days was inconclusive. **Conclusion:** The coagulation proteins in psoralen treated FFP and MB-FFP were adequately preserved, where MB-FFP showed better preservation than other two photochemical treatments.

Keywords: Pathogen inactivation, Methylene Blue, Psoralen, Riboflavin, Fresh Frozen Plasma

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INTRODUCTION

The overall quality and safety of blood and blood products supplied by the blood transfusion facilities improved tremendously for the past two decades. Various policies have been implemented to lessen the possibility of transfusion transmitted infection (TTI), such as restrictive donor criteria, meticulous examination and preparation of phlebotomy site, diversion of the first 35ml of blood into a pouch during donation, development and implementation of laboratory screening processes for infectious agents, lowering usage of allogeneic blood transfusion through autologous blood transfusion usage, modifying transfusion guidelines to conservatively use blood transfusion, or by means of introducing pharmacological agents and also direct modification of blood component.

While these implemented strategies have shown improvement on overall safety, there are still some risk of transmission of diseases still occurs especially in labile blood products for instance red blood cells, platelets and fresh frozen plasma (FFP). The presently employed methods to minimize the risk of TTI do not prevent bacterial contamination as the blood often contains trace quantities of endogenous bacteria, that may be from the gut, or pre-symptomatic infection, or may also be exogenous bacteria from the donor skin, trace level contaminations are nevertheless an issue when they are below detection levels of laboratory testing. Other contributing factor may be attributed to the incapability of the current level of test sensitivity in period of seroconversion (window period) of any infection, and in situation of non-seroconverting chronic (immunosilent)

carriers, and not to mention immunologically variants viruses and errors in laboratory test (1), and any newly emerging agents also continues to raise the potential of TTI.

In developing countries, transfusion transmitted infection is still a worrying issue instigating multitude of infectious cases predominantly human immunodeficiency virus (HIV), hepatitis C virus (HCV), hepatitis B virus (HBV), protozoa, tick-borne agents, and emerging viruses due to economic challenges for implementation of new and extensive laboratory testing. Traditional mechanical elimination such as washing and filtration methods were found to have inadequate success in diminishing the risks of cell-associated agents, for examples, cytomegalovirus (CMV virus), Parvovirus B19, Human T-lymphotropic virus (HTLV virus) and Human immunodeficiency virus (HIV). Although in developed countries, the laboratory testing for infectious agents has been properly implemented, the risk of protozoan and emerging infectious agents are still the main problems due to limited availability of the laboratory testing to detect presence of these pathogens (2).

Currently employed nucleic acid amplification testing (NAT) and leuco-depletion procedure have greatly improved the safety of blood, nevertheless with some limitations. Nucleic acid testing reduces the window period of infections but does not eliminate it totally (3). Therefore, technologies such as pathogen inactivation that broadly and non-specifically inactivate pathogens have been explored to further increase blood and blood component safety particularly for platelet and fresh frozen plasma (FFP). New promising techniques for pathogen inactivation in labile blood products could hopefully present some measure of safety alike in stable plasma-derived products (2). Several methods have been delved into for pathogen inactivation includes solvent/detergent, Cohn fractionation, heat treatment, and pasteurization.

Current method by using photochemical (photo-sensitizers) to target nucleic acid containing pathogens in non-cellular and non-nucleated cellular fractions of whole blood and apheresis plasma or platelet are still in development. The efficiency of this method against intracellular microorganism depends on their ability in penetrating the cell membranes. An effective photochemical treatment also must meet few criteria. It must be able to inactivate pathogens, must not change the product quality, must not introduce a toxicological risk to the recipient and finally the process must not give any impact to the safety, quality and efficiency of the treated blood products and its component, and lastly cost-efficient (4).

Three methods of photo-chemicals treatment are recognized to be useful for pathogen inactivation. Study have shown that methylene blue (MB), psoralen and riboflavin (Mirasol®) are an effective sensitizer of viral inactivation of blood products with deficient genomic nucleic acid for instance red cells, platelets and plasma proteins. Methylene blue possesses powerful attraction to negatively charged phospholipids and nucleic acid, while having negligible attraction to neutral phospholipids or albumin (5). Mirasol has negligible affinity to albumin or neutral phospholipids and reasonable attraction to nucleic acid. Whereas psoralen has strong attraction to negatively charged and neutral phospholipids, albumin and nucleic acid.

Generally, the effect of photochemical treatments for pathogens inactivation such as methylene blue, psoralen and riboflavin on coagulations factors level of fresh frozen plasma (FFP) was not well studied in Malaysia specifically. No similar study has been done in this centre. By comparing the effects of all three methods of photochemical treatments on fibrinogen and coagulation factors level, we can evaluate the appropriate methods for treatment of FFP.

MATERIALS AND METHODS

Sampling

The venue of this study is National Blood Centre (aka Pusat Darah Negara), Kuala Lumpur (NBCKL). NBCKL is the only centre that started to use methylene blue (Theraflex system) to treat FFP and psoralen (INTERCEPT system) to treat platelets in Malaysia. This study was conducted from January 2010 to October 2011. Donor age was between 20-40 years old with body weight more than 90 kg. A total of 30 apheresis donors at National Blood Centre (NBCKL) were involved in this study. Single unit of apheresis plasma was obtained from each donor. All apheresis plasma was kept in room temperature of 20°C to 24°C and processed within 8 hours post collection, and each plasma was divided into 3 bags for different photochemical treatments for methylene blue treatment (between 235ml- 315ml), psoralen (between 385 to 635 millilitres) and riboflavin (between 170 – 360 millilitres). 3-4 segments from each untreated bag were kept separately as control. Total number of treated bag was 90 bags and divided for 30 bags for each treatment. Each bag was barcode labelled, and post-treatments segments were taken. Both control and treated plasma were kept in a -30°C freezer.

Sample processing and assay parameters

Samples were removed from the freezer and immediately thawed at 37.0 °C as quickly as possible in water bath for 2-3 minutes (duration required for thaw varies depending on sample volume. Note: On average, a less than 1.0 ml sample would require 2-3 minutes to thaw, a more then 2.0 ml sample required 3-5 minutes to thaw). Plasma sample was then gently mixed using a transfer pipette. Within 2 hours of thawing, treated and untreated sample were loaded into necessary microtainers for protein analysis per standard

operating procedure. The proteins activity from treated segments was compared with control segments. The pre- and post-treatments levels were recorded for data analysis. Fibrinogen level was recorded as mg/dl unit meanwhile the level of coagulation factors was recorded as percentage (%). For storage duration comparison, a part of plasma was immediately analysed, a part was stored for 30 days and another part was stored for 270 days. The coagulation factors indices concentrations before and after methylene blue, psoralen and riboflavin treatment were analysed accordingly. All procedures were done following Good Manufacturing Practice (GMP). The measurement of fibrinogen and coagulation factor levels was done for the pre- and post- treated fresh frozen plasma by using ACL TOP 500 analyser.

Methylene blue treatments

Plasma bag was connected to the Plasma flex Plas 4 filter set using sterile docking device. The plasma bag was hanged on the stainless-steel stand and the white part of the Plasma flex set was set to break to allow plasma to pass methylene blue (MB) dry pill (85µg) presenting estimated concentration of 1 µmol /L methylene blue per 235 to 315 ml of plasma into the treatment (illumination) bag. The mixed plasma bag was illuminated under UV light at 180 J/cm2 (590nm) for 20 minutes at room temperature in (Maco-tronic, MacoPharma) device. Then, the plasma was circulated by gravity through Blueflex filter to remove MB and its photoproducts. The treated plasma in the storage bag was stored in a -30 oC freezer.

Psoralen (INTERCEPT) treatment

Amotosalen was added via gravity flow to sterile docked plasma to a final concentration of 150 µM. The amotosalen containing plasma was illuminated at 3J cm2 UVA light (320-400nm) in an INTERCEPT illuminator, then by gravity, it circulated through the compound adsorption device (CAD) for 20 minutes. Plasma was uniformly disseminated into the storage containers and stored in a -30°C freezer.

Riboflavin (Mirasol) treatment

Plasma was sterile docked and intermixed with 35ml of riboflavin at 500 µM in an illumination bag. The bag was then positioned in an illuminator and subjected to UV light (6.24J/ml). The plasma was then transferred to a barcoded bag and it was stored in a -30°C freezer. Diluted samples/plasma were corrected by applying dilution correcting factor. The Mirasol dilution correction factor: Final Illumination Volume = (Plasma Volume + Riboflavin volume) / Plasma Volume.

Statistical analysis

Statistical Package for the Social Sciences (SPSS) version 18.0 was used for data analysis. For each parameters, arithmetic mean and standard deviations were computed. The mean ± SD for methylene blue + UV light, psoralen + UV light and riboflavin + UV light plasma treatments were described in tables. Standard paired t-test was used for determining significance value. All mean values for each unit were compared with the reference range to evaluate significant differences in percentage of retention proteins level between treated and untreated FFP.

RESULTS

Methylene blue treated plasma

Coagulation factor levels were quantified via standard functional assays for control plasma and plasma treated with methylene blue (MB-FFP). 30 units of MB-FFP were prepared. Mean ± SD was determined and compared to untreated FFP and accepted reference range. Fibrinogen was measured in mg/dl, other coagulation factors as percentage (%) (IU/dl) and seconds for PT/APTT (Table I). There was no significant reduction in fibrinogen level in a pre- as compared to post- methylene blue treatment

Table I: Methylene blue treated plasma

Factors	N	Pre-treatment		Post-treatment		% Change Mean ± SD Lower		p value Upper	95% Confidence Interval	
		Methylene Blue								
		Mean ± SD		Mean ± SD						
Fib	30	291.37	57.42	290.37	57.42	-	-	-	-	-
FII	30	116.85	12.41	112.01	12.61	4.84	5.73	0.00	2.70	6.98
FV	30	129.55	26.32	115.06	25.27	14.49	11.60	0.00	10.16	18.82
FVII	30	111.30	23.71	107.67	23.78	3.63	8.00	0.02	0.64	6.62
FVIII	30	86.36	24.16	72.52	19.48	13.83	10.15	0.00	10.04	17.62
FIX	30	120.79	27.29	111.11	26.96	9.68	10.69	0.00	5.69	13.67
FX	30	116.47	23.86	103.74	17.96	12.73	11.44	0.00	8.45	17.00
FXI	30	99.89	23.28	77.88	18.31	22.01	7.21	0.00	19.32	24.70
FXII	30	77.34	28.70	68.10	26.61	9.24	8.23	0.00	6.16	12.31
FXIII	30	76.89	20.89	67.65	16.82	9.24	10.22	0.00	5.43	13.06
PT	30	10.77	0.85	11.28	0.88	-0.52	0.21	0.00	-0.60	-0.44
APPT	30	32.15	3.15	34.01	2.68	-1.86	1.61	0.00	-2.46	-1.25

(from 291.37 mg/dl to 290.37 mg/dl). There were significant reduction in protein levels for post treatment for coagulation factors of Factor II until Factor XIII with p value <0.05, however the levels were still within reference range (range of 50-150%). MB-FFP showed reduction of Factor II, VII, IX, XII and XIII level around 3% to 9% as compared to untreated FFP. Meanwhile for Factor V, VIII and X, MB-FFP showed 12% to 14 % reduction of proteins level and 22% reduction of Factor XI. PT and APTT for MB-FFP were within normal range but increase 0.51 sec and 1.86 sec respectively if compared to pre-treated plasma.

Psoralen (INTERCEPT) treated fresh frozen plasma

Coagulation factor levels were quantified via standard functional assays for control plasma and psoralen treated fresh frozen plasma (psoralen-FFP). 30 units of psoralen-FFP were prepared. Mean ± SD was determined and compared to untreated FFP and accepted reference range. Fibrinogen was measured in mg/dl, others as percentage (%) (IU/dl) and seconds for PT/APTT (Table II). Fibrinogen and other coagulation factors concentration reduced when comparing pre- and post-psoralen treatment ($p < 0.05$). However, fibrinogen and most of coagulation factors level were within the reference range. Psoralen-FFP showed slight reduction of Factor V and XIII which about 8% to 9% compared to untreated FFP. Meanwhile the reductions were 11% and 12% for Factor II, VII, XI and XII. More reduction of proteins level was seen in Factor IX and X which was 18% to 19% and FVIII showed level reduction by 22%. The mean values for both PT and APTT for psoralen-FFP were within reference range. When compared to untreated FFP, PT and APTT showed time prolongation of 0.37 sec and 1.72 sec.

Riboflavin (Mirasol) treated fresh frozen plasma

Coagulation factor levels were quantified via standard functional assays for control plasma and riboflavin treated FFP (riboflavin-FFP) were prepared. Mean ± SD was determined and compared to untreated FFP and accepted reference range. Fibrinogen was measured in mg/dl, others as percentage (%) (IU/dl) and seconds for PT/APTT (Table III). All final Riboflavin treated proteins measurements were multiplied with mirasol dilution correction factor. Riboflavin-FFP showed significant reduction of fibrinogen and all coagulation factors level with p value < 0.05, however, except Fibrinogen, Factor VIII, XI and XII, all other coagulation factors values were within reference range (Table III). The mean values for fibrinogen level was within reference range although the difference was highly significant (130.46 ± 47.75%) if compared to pre-treated FFP. Slight reduction was seen in Factor XIII which was 4%. Factor II, VII and XII showed reduction by 22% to 25% whereas Factor X showed 33% reduction. Factor VIII and IX showed significant reduction of proteins level by 38 %. Factor XI was the most affected protein which the level reduced about 40% compared with untreated fresh frozen plasma. The mean values for both PT and APTT for riboflavin treated FFP surpassed the upper threshold of normal reference value (11.5 sec) by 0.66 sec and 1.84 sec (36.0 sec). When compared to untreated FFP, PT and APTT showed time prolongation of 1.39 sec and 5.69 sec.

Storage time comparison of coagulation factor concentration between photochemical treatments of FFP For short term storage, fibrinogen and all coagulation factor levels showed significant reduction whether treated with methylene blue, psoralen or riboflavin (Table IV). All protein levels showed significant in comparison

Table II: Psoralen (INTERCEPT) treated fresh frozen plasma

Factors	N	Pre-treatment		Post-treatment		% Change Mean ± SD Lower		p value Upper	95% Confidence Interval	
		Intercept								
		Mean ± SD		Mean ± SD						
Fib	30	291.37	57.42	225.53	52.67	65.84	15.08	0.00	60.21	71.47
FII	30	116.85	12.41	105.16	11.60	11.69	7.06	0.00	9.05	14.33
FV	30	129.55	26.32	120.73	26.38	8.82	16.12	0.01	2.80	14.84
FVII	30	111.30	23.71	99.89	22.41	11.41	12.69	0.00	6.67	16.15
FVIII	30	86.36	24.16	64.32	18.71	22.03	10.70	0.00	18.04	26.03
FIX	30	120.79	27.29	102.49	24.40	18.30	10.52	0.00	14.37	22.22
FX	30	116.47	23.86	97.79	18.10	18.68	11.55	0.00	14.36	22.99
FXI	30	99.89	23.28	87.84	29.62	12.05	15.83	0.00	6.14	17.96
FXII	30	77.34	28.70	65.40	27.07	11.94	9.15	0.00	8.53	15.36
FXIII	30	76.89	20.89	68.71	17.98	8.18	7.11	0.00	5.53	10.84
PT	30	10.77	0.85	11.16	0.98	-0.39	0.46	0.00	-0.57	-0.22
APPT	30	32.15	3.15	33.87	3.06	-1.72	1.84	0.00	-2.40	-1.03

Table III: Riboflavin (Mirasol) treated fresh frozen plasma

Factors	N	Pre-treatment		Post-treatment		% Change Mean ± SD Lower		p value Upper	95% Confidence Interval	
		Mirasol								
		Mean ± SD		Mean ± SD						
Fib	30	291.37	57.42	160.92	55.37	130.46	47.75	0.00	112.63	148.28
FII	30	116.85	12.41	92.19	14.35	24.66	13.75	0.00	19.53	29.80
FV	30	129.55	26.32	87.29	30.45	42.27	16.87	0.00	35.97	48.57
FVII	30	111.30	23.71	87.31	18.64	23.99	14.76	0.00	18.48	29.50
FVIII	30	86.36	24.16	47.45	15.91	38.90	19.26	0.00	31.71	46.09
FIX	30	120.79	27.29	82.68	24.85	38.11	23.90	0.00	29.18	47.04
FX	30	116.47	23.86	83.08	19.32	33.39	14.19	0.00	28.09	38.69
FXI	30	99.89	23.28	59.19	19.43	40.70	16.40	0.00	34.58	46.82
FXII	30	77.34	28.70	54.61	15.94	22.73	25.17	0.00	13.33	32.13
FXIII	30	76.89	20.89	73.10	19.16	3.79	7.34	0.01	1.05	6.53
PT	30	10.77	0.85	12.16	1.36	-1.39	0.91	0.00	-1.73	-1.05
APPT	30	32.15	3.15	37.84	3.50	-5.68	2.23	0.00	-6.52	-4.85

Table IV: Comparison of coagulation factors indices concentrations of fresh frozen plasma

Factors	N	Pre-Treatment		Post -Treatment					
		Mean ± SD		Methylene Blue % Change Mean ± SD		Intercept % Change Mean ± SD		Mirasol % Change Mean ± SD	
Fib	30	291.37	57.42	-	-	65.84	15.08	130.46	47.75
FII	30	116.85	12.41	4.84	5.73	11.69	7.06	24.66	13.75
FV	30	129.55	26.32	14.49	11.60	8.82	16.12	42.27	16.87
FVII	30	111.30	23.71	3.63	8.00	11.41	12.69	23.99	14.76
FVIII	30	86.36	24.16	13.83	10.15	22.03	10.70	38.90	19.26
FIX	30	120.79	27.29	9.68	10.69	18.30	10.52	38.11	23.90
FX	30	116.47	23.86	12.73	11.44	18.68	11.55	33.39	14.19
FXI	30	99.89	23.28	22.01	7.21	12.05	15.83	40.70	16.40
FXII	30	77.34	28.70	9.24	8.23	11.94	9.15	22.73	25.17
FXIII	30	76.89	20.89	9.24	10.22	8.18	7.11	3.79	7.34
PT	30	10.77	0.85	-0.52	0.21	-0.39	0.46	-1.39	0.91
APPT	30	32.15	3.15	-1.86	1.61	-1.72	1.84	-5.68	2.23

between pre- and post-treatment with p value of <0.05 except fibrinogen level in fresh frozen plasma treated with methylene blue which showed no significant reduction. The disparity was significant for fibrinogen treated with riboflavin with mean changes of 130.46± 47.75%. Although there were significant reductions of the proteins level, most of the means values were within reference range except riboflavin showed the highest changes compared to methylene blue and intercept treatment especially for Factor VIII, V, IX and XI. Among the three pathogen reduction techniques, methylene blue showed the lowest changes compared to psoralen and riboflavin except in coagulation Factor XI and Prothrombin time (PT) with changes of 22.01 ± 7.21% and -0.52 ± 0.21% respectively. There were significant differences of coagulation protein levels between before and after treatment using pathogen inactivation techniques that stored for 30 days (Table V). However, Factor II and VII treated with methylene blue and Factor V treated with psoralen revealed no significant differences between pre- and post-treatment. Fibrinogen treated with riboflavin showed a significant biggest change 98.39 ±164.39% and Prothrombin treated with methylene blue showed significant smallest changes of – 0.64 ± 0.20%. There were significant differences of coagulation protein levels between before and after treatment using pathogen inactivation techniques after 270 days of freeze storage (Table VI). However, only Factor XI in psoralen-FFP and Factor XIII in riboflavin treated FFP showed no significant differences. Overall, those treated with methylene blue showed some significant low changes as compared to those treated

Table V: Comparison of coagulation factors indices concentrations of fresh frozen plasma after 30 days

Factors	N	Pre-Treatment Mean ± SD		Post -Treatment (30 days)					
				Methylene Blue % Change Mean ± SD		Intercept % Change Mean ± SD		Mirasol % Change Mean ± SD	
Fib	13	324.55	63.64	-	-	74.78	8.72	98.39	164.35
FII	13	116.04	3.99	-0.15	3.64	6.32	4.15	8.72	23.84
FV	13	143.66	28.34	13.28	12.51	6.62	22.46	30.44	53.70
FVII	13	116.39	29.78	4.46	10.69	13.55	17.02	18.26	40.77
FVIII	13	92.74	23.61	14.20	7.02	22.86	11.34	28.14	55.93
FIX	13	137.23	21.95	9.91	13.34	16.27	14.01	23.11	63.42
FX	13	125.24	29.01	14.09	15.32	21.95	15.77	28.23	48.64
FXI	13	115.64	21.04	26.08	6.69	18.11	8.31	37.70	63.12
FXII	13	82.18	20.66	11.21	11.84	11.77	10.95	12.14	34.28
FXIII	13	79.28	22.90	15.20	12.78	10.63	9.27	0.15	11.20
PT	13	10.38	0.93	-0.64	0.20	-0.52	0.40	-2.07	-0.90
APPT	13	30.12	2.98	-2.35	1.71	-2.02	1.53	-7.10	-4.10

Table VI: Comparison of coagulation factors indices concentrations of fresh frozen plasma after 270 days

Factors	N	Pre-Treatment Mean ± SD		Post -Treatment (270 days)					
				Methylene Blue % Change Mean ± SD		Intercept % Change Mean ± SD		Mirasol % Change Mean ± SD	
Fib	17	266.00	36.87	-	-	59.00	15.51	129.75	43.56
FII	17	117.47	16.31	8.66	3.73	15.79	6.01	31.07	11.17
FV	17	118.76	19.18	15.41	11.17	10.50	9.26	42.42	15.43
FVII	17	107.41	17.79	2.99	5.41	9.77	8.25	19.77	9.53
FVIII	17	81.48	24.10	13.55	12.23	21.40	10.50	36.51	16.17
FIX	17	108.22	24.51	9.50	8.57	19.85	6.88	34.17	12.78
FX	17	109.76	17.03	11.68	7.66	16.17	6.31	29.53	10.71
FXI	17	87.85	17.18	18.90	6.07	7.42	18.70	33.28	4.49
FXII	17	73.64	33.75	7.73	3.45	12.08	7.86	22.36	29.94
FXIII	17	75.06	19.73	4.69	3.98	6.31	4.29	2.35	5.48
PT	17	11.06	0.68	-0.42	0.17	-0.30	0.49	-1.32	0.90
APPT	17	33.71	2.33	-1.48	1.47	-1.48	2.06	-5.75	2.09

with psoralen or riboflavin. Fibrinogen level in riboflavin treated fresh frozen plasma showed the highest changes with 129.75% ± 43.56% meanwhile Prothrombin time in MB-FFP showed the lowest changes, - 0.42 ± 0.17%.

DISCUSSION

In this study, overall distribution of the blood groups was 10% type A, 40% type B, 23.3% type AB and 26.7% type O. The results revealed that there were significant differences in fibrinogen and coagulation factor levels between before and after methylene blue, psoralen and riboflavin treatment ($p< 0.05$). However, most of the mean values changes were within reference range of internationally accepted values (6), except for F VIII, FXI, FXII, PT and APTT level in riboflavin-FFP. Activity levels of certain coagulation proteins especially Factor VIII and vWF were affected mainly in blood group O compared to blood group A and B (7). Meanwhile fibrinogen, Factor II, VII, X, XI and XII were not seen to be influenced by blood group, although blood group A plasma shown to have 20% more FVIII than blood group O (8).

The degree of discrepancy of coagulation protein levels were different for different treatments. Among the three pathogen inactivation techniques, methylene blue showed the lowest changes in coagulation protein levels compared to intercept and riboflavin treatment. Fibrinogen level in methylene blue treated plasma showed no difference before and after MB treatment. Meanwhile, there are significant reductions of fibrinogen level in riboflavin-FFP which the result showed mean changes of (130.46 ±47.75%). For psoralen-FFP, the

mean changes for fibrinogen was (65.84 ± 15.08%). In MB-FFP, the plasma indices showing highest reduction were Factor XI (reduced by 22.01%), PT and APTT which showed time prolongation of 0.51 sec and 1.86 sec respectively when compared to pre-treatment samples. For FII, FV, FVII, FVIII, FIX, FX, FXII and FXIII reduction, MB-FFP range (5%-15%) compared to pre-treatment. For fibrinogen and FVIII level, the losses of activities of 0% and 13.83%. These findings are comparable to Rainer et. al., 2010 with loss of activities for fibrinogen and FVIII at 14.4% and 15.4% respectively and 4% loss of FII level (9). Politis et. al., 2007 reported an 18% loss of fibrinogen and F VIII level after MB treatment (10).

Contradictorily, it was different in psoralen-FFP, which the plasma indices that show the highest reduction was Factor VIII (reduced by 22.03%), meanwhile for riboflavin-FFP, both Factor VIII and FV showed reduction within the range of 40%-42%. When compared to methylene blue treated FFP, psoralen treated fresh frozen plasma showed quite a significant reduction in fibrinogen and Factor VIII level, with 65.84 ± 15.08% and FVIII with 22%, conferring 8% lower than MB FFP. However, both fibrinogen and FVIII level were still within the reference range. For other factors in psoralen treated FFP especially FV, FXI and FXIII showed better recovery with mean values changes of 8.82%, 12.05% and 8.19% compared to pre-treatment samples.

Fibrinogen and FVIII were more sensitive to psoralen treatment with both level (showed 26% mean reduction) (11), and for other factors, FII, FV, FVII, FIX, FX, FXII and FXIII, the mean levels were as good as with pre-treated plasma with range 3% to 19% reductions (11). Meanwhile for psoralen-FFP, the reduction level for FII and FIX were 11% and 18% which was comparable to Singh et. al., 2006 with 28-29% reduction of fibrinogen and Factor VIII activity, whilst loss of 2-22% for FII, FV, FVII, F IX, FX, FXI, FXIII (12).

For riboflavin treated FFP, there was significant reduction in fibrinogen level and mean values for FVIII, FXI and FXII which was lower than the lower limit of reference range, significantly difference with pre-treatment FFP and both MB-FFP and psoralen treated FFP. Other factors were within normal range although lower than pre-treatment FFP. For FII, FVII and FXII, the reduction levels were 20% to 24% compared to pre-treatment. Meanwhile, FXIII showed the best recovery with reduction level of 4%. These finding was not comparable to Smith and Rock 2008, in which fibrinogen in riboflavin-FFP was 33% lower from baseline and FV, FVIII, FIX and FXI level reduced by 22% to 27% compared with pre-treatment, although FXIII remained normal in post- treatment FFP (13).

Two factors that probably cause significant reduction of coagulations proteins in riboflavin-FFP were dilution effect and colour of the riboflavin solution. The dilution effect can be contributed by 35 millilitres of riboflavin solutions which was added as part of riboflavin (mirasol process). Although dilution correction factor has been applied to all final protein measurements based on plasma volume, however, the volume can be inaccurately determined due to underestimate of volume loss during transferring process which can lead to more dilutional effects of samples. The other factors which can interfere with the coagulation protein measurements, was the riboflavin colour interference. Yellowish colour of riboflavin may be interfered with the light passing through the medium onto a photo detector to determine the clot point during coagulation proteins measurement. Further study should be done to evaluate this colour interference.

Freeze-thawing methods may play a role in reduction of coagulation proteins levels. Freeze- thawing of plasma showed to have minimal effect on the coagulation factor activity (14). A study shows a slight loss of FXII (2%) and a small increment of FVII (1%) and FIX (4%) after thawing (15).

Duration of storage was another factor that can give an effect to coagulation protein levels in fresh frozen plasma. In this study, the sample was further divided based on duration of storage before testing, 13 units FFP from each treatment were tested after 30 days of storage meanwhile another 17 units FFP were tested after 270 days of storage. The test analysis of samples thawed after 30 days revealed that there were significant differences between before and after photochemical treatment except for FII and FVII treated with methylene blue and Factor V treated with psoralen showed no significant differences between pre- and post-treatment. However, most of the mean values for the factors were within reference range except for FVIII and FXII in riboflavin-FFP. Meanwhile, fibrinogen treated with riboflavin showed a significant change 98.39 ± 164.39% and Prothrombin treated with methylene blue showed significant smallest changes of -0.64 ± 0.20%. For samples thawed after 270 days of storage, the results showed significant differences between before and after treatment except for Factor XI in psoralen treated FFP and Factor XIII in riboflavin treated FFP which showed no significant differences. Overall, after 270 days of storage, MB-FFP showed a slightly significant changes compared to those treated with psoralen or riboflavin. Fibrinogen level in riboflavin treated FFP showed the highest mean changes with 129.75% ± 43.56% meanwhile Prothrombin time in MB-FFP showed the lowest changes, -0.42 ±0.17%. However, in this study, comparisons of results between samples thawed after 30 days and 270 days of storage could not be made due to imbalance number of samples available for the test. In other study, no significant differences were seen for FII, FXII and FXIII, compared to both MB-FFP and psoralen-FFP measured immediately post-treatment and after 30 days of frozen storage, and retention level for FV, FVII, FVIII, FIX and FX in

psoralen treated FFP were either equivalent to or higher than MB-FFP (16). A study showed most coagulation proteins level in riboflavin treated FFP measured after 36 weeks (252 days) of freeze storage retained 70% to 100% levels compared to controls, apart from FVII and FV which showed (67 ± 9%) and (64 ± 4%) reduction respectively (17).

Finally, there were variable factors that could contribute to variability of the results between the present study with other researchers including method of collection, time of processing, method of measurement of the coagulation proteins levels, and operator skill. Although, all three photochemical methods were relatively easy to be done, further studies are essential to evaluate cost-effectiveness of the execution of photochemical treatments of fresh frozen plasma. Further studies also should be done to evaluate the side effects of photochemical treatment on treated patients.

We reported that to the best of our knowledge, there was no other study investigating similar effect of fresh frozen plasma preservation method in Malaysia at the time of the study.

CONCLUSION

In this study, there was sufficient preservation of coagulation proteins in psoralen treated FFP and MB-FFP were adequately preserved, where MB-FFP showed better preservation than other two photochemical treatments. These data provide a rationale for further investigation of the significance of usage of these methods to better preservation of fresh frozen plasma in other blood centre in Malaysia.

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