■ ORIGINAL ARTICLE

Study of the Impact of Leucocyte Reduction on the Coagulation Factors in Fresh Frozen Plasma

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ABSTRACT

Introduction: Plasma transfusion is required to arrest or prevent bleeding, for various congenital and acquired cases of coagulopathies such as inherited factor deficiencies, disseminated intravascular coagulation, liver disease, post major trauma, etc. Leukocyte reduced blood components are indicated to prevent the febrile nonhemolytic transfusion reactions, Human Leukocyte Antigen (HLA) alloimmunization, transmission of infections such as cytomegalovirus (CMV), and adverse transfusion reactions due to storage lesion. In the present study, the effect of plasma filtration on eight coagulation factors viz. fibrinogen, factors II, V, VII, VIII, IX, X and XI has been studied. **Materials and Methods:** The plasma separated from the whole blood donation from each of 25 donors was separated into two aliquots. Group-1 included unfiltered fresh frozen plasma; group-2 included fresh frozen plasma which was subjected to pre-storage leukocyte reduction by filtration. The levels of the coagulation factors Fibrinogen, II, V, VII, VIII, IX, X, XI were estimated in each sample in both the groups. **Results:** There was no statistically significant difference in the levels of any of the coagulation factors included in this study, between the unfiltered and the leukocyte reduced plasma. Group O positive individuals were found to have higher levels of all the coagulation factors. **Conclusion:** Filtration of plasma has no effect on the coagulation factors. This is the first study where the effect of plasma filtration on eight coagulation factors has been studied. Blood group O positive individuals were found to have higher levels of all the eight coagulation factors.

Keywords: Blood coagulation factor; Blood component transfusion; Filtration, Leukocyte reduction; Fresh frozen plasma.

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INTRODUCTION

Plasma is required for transfusion in various congenital and acquired cases of coagulopathies such as inherited factor deficiencies, disseminated intravascular coagulation, liver disease.[1,2] The major cause of death following major trauma, in 10-25% patients is acute traumatic coagulopathy.[3] Among the treatment options for various causes of acute bleeds, factor replacement is considered as the mainstay of treatment.[4]

Among the inherited bleeding disorders in Indian population, Hemophilia A is the commonest comprising 42.4% of cases, followed by platelet function defects comprising 39.2% of cases. Von Willebrand disease is relatively uncommon in Indians, comprising 8.5% of cases. The deficiencies of factors IX, X, XIII, V, VII, XI, XII, afibrinogenemia comprise 5.1%, 1.8%, 0.8%, 0.6%, 0.2%, 0.2%, 0.1%, 0.5% of cases respectively.[2]

Bioactive substances in Fresh frozen plasma (FFP) are considered to be responsible for the transfusion-related adverse events, especially in patients with sepsis and trauma.[5] Leukocyte reduced(LR) blood components are indicated to prevent the febrile nonhemolytic transfusion reactions, Human Leukocyte Antigen (HLA) alloimmunization, transmission of infections such as cytomegalovirus(CMV), and adverse transfusion reactions due to storage lesion.[6]

Transfusion of leucocyte reduced FFP reduces the allogenic immunogenicity induced by residual leucocytes.[7] The leukocytes in the plasma are cleared mainly by filtration.[7] Leukocyte reduction prevents the accumulation of cytokines released by the leukocytes into the storage bag.[6] The activities of coagulation factors were not found to be affected by whole blood filtration before component separation. However, the activation of the coagulation system by the filter material cannot be excluded.[8]

Previous studies have compared the levels of some of the coagulation factors/anticoagulants, between the unfiltered(UF) and leucocyte-reduced (LR) whole blood and plasma, wherein plasma filtration and its effect on the coagulation factors have been studied only by a few researchers. In the present study, the effect of plasma filtration on eight coagulation factors viz. fibrinogen, factors II, V, VII, VIII, IX, X and XI has been studied.

Efficient utilization of blood components with a good knowledge about their quality and maintenance help us improve the management of the blood bank inventory and efficient intervention with strategic transfusion therapy prevents the death in case of major trauma.[1,3]

Abbreviations Used

FFP: Fresh frozen plasma
UF: Unfiltered
LR: Leukocyte reduced/Leukocyte reduction
CI: Confidence interval

Objectives

The objectives of this study are to compare the levels of factors I, II, V, VII, VIII, IX, X, XI between unfiltered FFP and LR FFP, and between different blood groups. This helps us to find out the effect of filtration on the factors and thus its impact on the management of patients with coagulopathies.

MATERIALS AND METHODS

The study was commenced after getting approval from the Institutional Human Ethics Committee. Whole blood collected from 25 healthy donors who consented for participation in the study and fulfilled the eligibility criteria were separated into components. The donors who participated in this study belonged to blood groups A positive, B positive and O positive. The plasma from each donor was separated into two aliquots, so that there are two groups each of 25 plasma aliquots. Group-1 included unfiltered FFP, that is, plasma freshly separated within 6 hours and frozen immediately after separation; group-2 included FFP which was subjected to pre-storage leukocyte reduction by filtration with Terumo Penpol Imugard III polyurethane filter within 6 to 8 hours after blood collection.

The aliquots were frozen at a temperature between -30 and -40°c until analysis. Analysis of coagulation factors was carried out within 24 hours after blood collection. Before analysis, they were thawed and the levels of the coagulation factors Fibrinogen, II, V, VII, VIII, IX, X, XI were estimated in each sample, with the fully automated coagulation analyzer ACL Elite/Elite Pro (Instrumentation Laboratory Co.) in both the groups. The biological reference range for plasma Fibrinogen, Factors II, V, VII, VIII, IX, X, XI are 180-360 mg/dl, 79 to 131%, 62 to 139%, 50 to 129%, 50 to 150%, 65 to 150%, 77 to 131% and 65 to 150% respectively (8–16).

Statistical analysis was done using GraphPad Prism software. Descriptive statistics including mean, standard deviation, range, confidence interval was performed. Comparison between the unfiltered and LR samples was done with multiple t test and Wilcoxon signed rank test. The results were considered to be statistically significant if

Table 1: Comparison of the levels of coagulation factors between unfiltered and leukocyte reduced plasma.

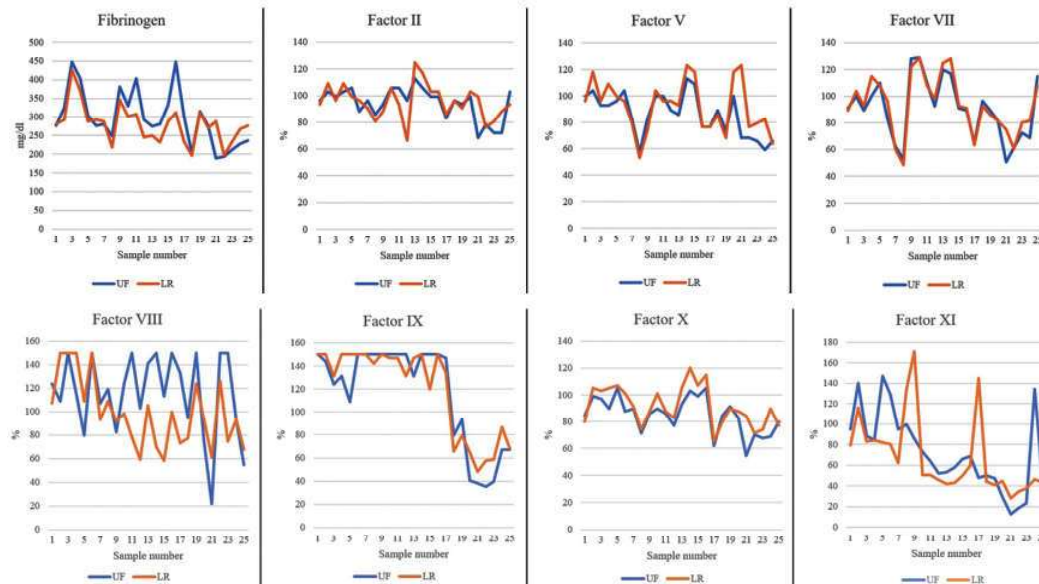| Coagulation factor	Unfiltered			Range		Leukocyte reduced			Range		P value
	Mean	SD	95% C.I.	Min	Max	Mean	SD	95% C.I.	Min	Max	
Fibrinogen	298.2	73.56	30.36	189	447	280.6	51.98	21.45	196	427	0.33
Factor II	94.54	11.93	4.93	68.2	113	95.62	12.8	5.28	66.4	125	0.76
Factor V	86	16.1	6.66	57.1	113	92.07	19.29	7.96	53.3	123	0.24
Factor VII	90.62	22.63	9.33	50.6	129	93.49	21.86	9.02	48.5	129	0.65
Factor VIII	116	34.4	14.19	21.8	150	98.7	29.5	12.18	58.3	150	0.07
Factor IX	115.8	43.69	18.03	35.2	150	119	38.4	15.86	48.2	150	0.78
Factor X	84.9	13.46	5.56	54.4	105	91.6	14.5	6.01	65.3	120	0.10
Factor XI	72.34	37.43	15.45	12.6	147	67.9	37.4	15.44	27.9	171	0.68

Fig. 1: Comparison of the levels of coagulation factors between unfiltered and leukocyte reduced FFP.
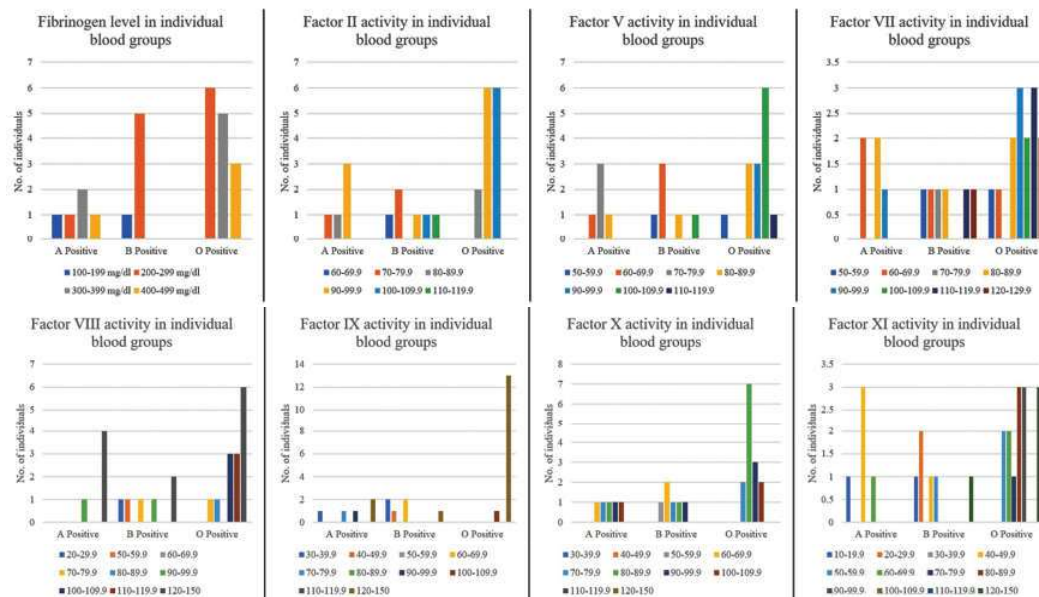

Fig. 2: Coagulation factors activities in UF-FFP in individual blood groups.

the 'p' value is <0.05. Quality control was ensured with routine quality assurance methods such as periodic calibration of the equipment, processing of commercial QC samples, participation in EQAS, quality control checking of blood bags.

RESULTS

The results of the analysis are given in Table 1 and Figure 1. In the unfiltered plasma, the level of Fibrinogen ranged from 189 mg/dl to 447 mg/dl and the mean value was 298.2 mg/dl (95% CI = 30.36); the activities of Factor II ranged from 68.2% to 113% with a mean of 94.54% (95% CI = 4.93); Factor V ranged from 57.1% to 113% with a mean of 86% (95% CI = 6.66); Factor VII ranged from 50.6% to 129% with a mean of 90.62% (95% CI = 9.33); Factor VIII ranged from 21.8% to 150% with a mean of 116% (95% CI = 14.19); Factor IX ranged from 35.2% to 150% with a mean of 115.8% (95% CI = 18.03); Factor X ranged from 54.4% to 105% with a mean of 84.9% (95% CI = 5.56); Factor XI ranged from 12.6% to 147% with a mean of 72.34% (95% CI = 15.45).

In the leucocyte reduced plasma, the level of Fibrinogen ranged from 196 mg/dl to 427 mg/dl and the mean value was 280.6 (95% CI = 21.45) mg/dl; the activities of Factor II ranged from 66.4% to 125% with a mean of 95.62% (95% CI = 5.28); Factor V ranged from 53.3% to 123% with a mean of 92.07% (95% CI = 7.96); Factor VII ranged from 48.5% to 129% with a mean of 93.49% (95% CI = 9.02); Factor VIII ranged from 58.3% to 150% with a mean of 98.7% (95% CI = 12.18); Factor IX ranged from 48.2% to 150% with a mean of 119% (95% CI = 15.86); Factor X ranged from 65.3% to 120% with a mean of 91.6% (95% CI = 6.01); Factor XI ranged from 27.9% to 171% with a mean of 67.9% (95% CI = 15.44).

There was statistically significant difference in the levels of coagulation factors Fibrinogen, FV, FVIII, FX between the unfiltered and leukocyte reduced plasma. The difference in the levels of FII, FVII, FIX, FXI were not significant.

The individuals included in the study belonged to blood groups A positive, B positive and O positive. When the individual blood groups were compared for the activities/levels of coagulation factors, in blood group 'A positive', most of the individuals had Fibrinogen levels in the range of 300 – 399 mg/dl; Factor II activity in the range of 90 – 99.9%; Factor V in the range of 70 – 79.9%; Factor VII in the range of either 60 – 69.9% or 80 – 89.9%; Factor VIII in the range of 120 – 150%; Factor IX in the range of 120 – 150%; Factor XI in the range of 40 – 49.9%. All ranges of Factor X activity were equally distributed among the A positive individuals.

In blood group 'B positive', most of the individuals had Fibrinogen levels in the range of 200 – 299 mg/dl; Factor II activity in the range of 70 – 79.9%; Factor V in the range of 60 – 69.9%; Factor VIII in the range of 120 – 150%; Factor IX in the range of either 30 – 39.9% or 60 – 69.9%; Factor X in the range of 60 – 69.9%; Factor XI in the range of 20–29.9%. All ranges of Factor VII activity were equally distributed among the B positive individuals.

In blood group 'O positive', most of the individuals had Fibrinogen levels in the range of 200–299 mg/dl; Factor II activity in the range of 90 – 109.9%; Factor V in the range of 100 – 109.9%; Factor VII in the range of either 90 – 99.9% or 110 – 119.9%; Factor VIII in the range of 120 – 150%; Factor IX in the range of 120 – 150%; Factor X in the range of 80 – 89.9%; Factor XI in the range of either 80 – 99.9% or 120 – 150%. (Figure 2).

DISCUSSION

In July 1998, UK Transfusion services implemented universal leucodepletion, that is, leucodepletion of all blood units, to prevent the risk of transmission of variant Creutzfeldt-Jakob disease via blood transfusion. Though filtration of red cells has already been in practice in the UK, this new guideline initiated the process of filtration of FFP.[9]

The incidence of congenital bleeding disorders may vary depending on the ethnic origin.[2] While Von Willebrand disease is the most common inherited bleeding disorder in the industrialized world, Hemophilia A is found to be the most common and qualitative platelet defect is the second most common inherited bleeding disorder in India.[2] In the study by Gupta M et al, platelet function defects were more prevalent among females,

whereas the coagulation defects were rare.[2] The incidence of VWD is lower in India, as compared to the west, because of the fact that only symptomatic patients presented to the outpatient department.[2] Factor X deficiency is a very rare hereditary bleeding disorder and it is found to be more common in communities accepting consanguineous marriages.[10]

Leukocyte reduction prevents the release of cytokines by the leukocytes into the storage bag.[6] The concentrations of bioactive substances such as Histamine, Myeloperoxidase, Eosinophil cationic protein were found to be higher in unfiltered FFP thawed after storage, compared to FFP samples which are filtered before storage, and FFP samples which are unfiltered and tested before freezing and storage.[5] LR filters have a variable effect on the activities of coagulation factors which may be attributed to the possibility of adherence of the coagulation factors to the surface of the filter which is made of either polyester or polyurethane.[11,12]

Management of coagulopathy in patients post major trauma is difficult and FFP transfusion should be started during the primary survey phase of resuscitation, instead of considering as a product for volume replacement, during massive transfusion.[3]

In our study, none of the coagulation factors showed any significant difference between the unfiltered and leukocyte reduced plasma. Various studies have analyzed the effect of filtration over the coagulation factors. In the study by Alhumaidan et al, PT, APTT, activities of factors V, VII, VIII, X, XI, fibrinogen, antithrombin III, protein C and free protein S were compared between filtered and unfiltered plasma.[11]

The paired plasma aliquots were stored at -18°c until assessment. Then the aliquots were thawed and the coagulation assays were performed. They were stored at 1 to 6°c until further analysis on days 5 and 7. Factors VII, VIII, IX showed decrease whereas factors V, X, fibrinogen showed no difference between the filtered and unfiltered plasma.[11] Shooshtari et al studied sixty units of plasma separated from whole blood for the activities of coagulation factors V, VII, VIII, IX, XI, Fibrinogen, Antithrombin, Antitrypsin. The filtration had been done between 4 and 20 hours of blood donation.

Except for the negligible change in the activity of factor VII, there was no significant difference in the activities of the coagulation factors and inhibitors involved in this study, between the filtered and unfiltered plasma.[12] Williamson et al studied the effect of whole blood filtration on the coagulation factors in plasma separated from the whole blood. There was decrease in factor VIII, increase in factor V, and no changes in factors IX, X, fibrinogen during 12 months of storage, but without statistical significance.[13] In the study by Cardigan et al, the coagulation factors in filtered FFP were evaluated, employing either whole blood or plasma filters. Significant reduction in factors V, VIII, IX, XI, XII was observed after filtration.[9]

In the study by Heiden M et al, there were no significant differences between the coagulation factor activities of unfiltered FFP and FFP obtained from whole blood filtration.[14] In the study by Solheim et al, pre-storage leukocyte filtration had been done with whole blood filter and the levels of coagulation factors were found to be improved with it.[15] In the study by Chabanel et al, the levels of coagulation factors had been maintained within the normal reference range in the plasmas stored at -30°c for 6 months.[16]

In our study, the filtration of plasma was done within 6 to 8 hours after blood collection, immediately after separation into components, that is, before storage. Separation by centrifugation was done within 6 hours after blood collection. In the study by Cardigan et al, whole blood or plasma were filtered within 8 hours of blood collection.[9] Neutrophils get activated and elastase is released if whole blood is filtered after storage at room temperature.[12] Hence pre-storage leukocyte reduction is preferable.

Factor VIII is affected by ABO blood group.[17] Blood from group A individual contains higher amounts of factor VIII activity and antigen than that from a group O individual.[18] But in our study, higher levels of all the coagulation factors were found in group O positive individuals.

CONCLUSION

Filtration of plasma does not have any effect on the coagulation factors. Hence leucocyte reduction can be done in the plasma for patients

who need it. This is the first study where the effect of plasma filtration on eight coagulation factors has been studied. Blood group O positive individuals were found to have higher levels of all the eight coagulation factors.

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Conflicts of Interest: None

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