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COAGULATION FACTORS LEVEL IN FRESH FROZEN PLASMA IN RWANDA

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ABSTRACT

Objectives: **To determine the level of coagulation factors and inherited inhibitors in Fresh Frozen Plasma (FFP) and to evaluate Prothrombin Time and activated partial thrombin time in fresh frozen plasma.**
Design: **Cross-sectional study.**
Setting: **Jomo Kenyatta University of Agriculture and Technology in Medical Laboratory Sciences.**
Subjects: **Eighteen blood bags collected from voluntary blood donors.**
Main outcome measures:**Coagulation factors and inhibitors levels, Prothrombin Time (PT) and Activated Partial thrombin Time (APTT)remained within the reference range requested by quality assurance regulations after three months of storage.**
Results: **APTT and PT show an increase from baseline to one month then remain constant up to three months, while, Fibrinogen, Factor II, Factor V, Factor VII, Factor X, Von Willbrand Factor, Protein C and Antithrombin decreased from baseline up to three months and then Factor VIII, Factor IX, Factor XI, Factor XII and Protein S, remained constant from baseline up to one month and decreased up to three months.**
Conclusion: **There is good retention of all coagulation factors and inhibitors in plasma produced from whole blood within eight hours of collection, stored at minus 18°C for three months.**

INTRODUCTION

Coagulation factors are plasma proteins that play a major role in blood coagulation process. These factors maintain haemostasis balance between clotting and anti-clotting (1).Fresh frozen plasma (FFP) is indicated for the treatment and prevention of bleeding disorders due to deficit in minor or major coagulation factors, defective management of massive bleedings, liver disease, disseminated intra-vascular coagulation or reverse anti-coagulant therapy (2). This study is therefore aimed at determining the levels of coagulation factors at minus 18°C temperatures.

Bleeding disorders due to deficiency of coagulation factors VIII and IX known as haemophilia A and B respectively, are the common and frequent clinical problem; then von Willebrand factor deficiency and the other factors rare deficiency come on the forth position as stated by World federation of haemophilia report on the annual global survey 2011. They are associated with increased morbidity, faster progression to other disease such as anaemia, decreased survival time, and increased mortality. Factor defect or deficit individuals who are severely anaemic experience due to excess bleeding in case of trauma or injury, functional impairment, decreased sense of wellbeing, and a poorer quality of life. For these reasons, it is imperative that clinicians in coagulopathy care are prepared to manage bleeding disorders related coagulation factors deficiency using fresh frozen plasma and factor concentrate where they are available and affordable. Understanding

the use and indication of fresh frozen plasma and knowing the level of each coagulation factor and the level require for plasma to be used for treatment purpose, helps to ensure that haemophiliac individuals and other patient suffering from bleeding disorders due to coagulation factors disorders receive appropriate treatment. In Rwanda there are many resource limited Referral, District hospitals and health centres laboratories, where factor assay are not done systematically on patients with bleeding disorders attending these centres and fresh frozen plasma is used without knowing the concentration of coagulation factors it contains. This could lead to the circulatory overload without any improvement; if many bags are used with low level of factors. Also the education about coagulopathy management and prevention given by health workers in most health facilities in Rwanda is not specific to the bleeding disorders related to coagulation factors deficiency and is not based on some frequent characteristics and aetiologic causes of coagulopathy, this can lead to inappropriate management.

Data from this study was aimed at guiding the management of patients with factor deficiency and monitoring. This study sheds light on level of fibrinogen, Factor II, Factor V, Factor VII, Factor VIII, Factor IX, Factor X, Factor XI, Factor XII, Factor XIII, Antithrombin or Factor III, von WillbrandFactor, Protein C, and Protein S in current used plasmas which was remains elusive. The need for levels of coagulation factors data in donated blood were important in order to provide information on quality of fresh frozen plasma prepared by National and Regional Centres for Blood Transfusion. It has also served as basis to renew policies and procedures regarding storage conditions and quality control prior to clinical use of fresh frozen plasma.Rwanda has adopted a guidelines which have not been assessed using our population while coagulation factors level can vary depending on several conditions including race, age, blood groups and other environment condition. The results of this study offer an ideal storage temperature for coagulation factors in fresh frozen plasma after preparation in order to improve storage conditions. Through this study substantial data on coagulation factors have been given, it constitutes preliminary data for other related studies.

MATERIALS AND METHODS

This descriptive study was conducted at Kigali, Butare and Ruhengeri Centres for Blood Transfusion and Kigali Health Institute at the Biomedical Laboratory Sciences, Haematology section. The sample population consisted of Fresh Plasma Bags from blood donors attending the centres for voluntary blood donation. Eighteen bags were sampled six from each centre enrolling in this study.

A convenient sampling method has been used to get sample of this study.To determine level of coagulation factor level in FFP, seventy two samples of plasma have been collected from eighteen fresh plasma bags; four samples per each bag well prepared before freezing regardless blood groups, sex and age. Whole blood units (450±50 mL) have been collected in JMC triple bag containing 63 mL CPDA-1 and centrifuged within eight hours of collection and then a semi-automated extractor have been used to separate the plasma and put it in accessory blood bags and in four plain tubes 5ml in each, through a connecting device. Immediately after detachment from the main bag, the FFP bags and plain tubes frozen at minus 18ºC.

After the agreement made between investigator and Kigali Health Institute collected samples taken to Kigali Health Institute, Biomedical Laboratory Sciences department, Haematology service, haemostasis section after collection, for Factor assay and APTT and PT tests. A cool box had been used in transportation of samples from National Centre for Blood Transfusion to Kigali Health Institute. After preparation before freezing, all samples were tested for HIV, Syphilis, hepatitis B and C, blood grouping and rhesus testing were done to ensure blood was safe to be given to another person.Only the first eighteen tubes were assayed for the level of coagulation factors and measurement of the APTT and PT before freezing or at baseline. The second assay was done on the second samples after one month, the sample was thawed by placement in a water bath at 30–37 °C for 30 minutes then prepared for assays of the levels of coagulation factors and measurement of the APTT and PT, the third was done after two months and the last session was assayed after three months of storage. This means, we did four rounds of analysis in each round we assayed eighteen tubes of 5mls each from eighteen bags, the whole bag was not brought to Kigali Health Institute for analysis and taken back to National Centre for Blood Transfusion for donation.

The coagulation factors have been assayed using a coagulation analyser named ACL 7000 a product of instrumentation Laboratory Company USA made in Italy, 2000 usingturbidmetric clot and chromogenic methods the same analyzer will be used to measure APTT and PT. This will provide reliable results by giving the exact amount of each coagulation factor in percentages, Prothrombin time and activated partial thrombin time in seconds. Two ways Anova was used and the P-value obtained from statistical tables was compared with the calculated value to get the effect of storage conditions (time and temperature) on the level of preserved fresh frozen plasma ($P<0.05$).

Ethical considerations: This study sought scientific approval from Rwanda Biomedical Centre division of Medical research committee, Ethical approval from

Rwanda National Ethics Committee, Research permit from Ministry of Education and Affiliation letter from National Centre for Blood Transfusion before excursion. Blood donors were informed about blood donation, blood product preparation, and the tests to be done on their samples, the risks and benefits of the research and need for their consent. The identity of the blood donors was treated with confidentiality. Results were sent to National Centre for Blood Transfusion management for them to know and to inform blood donors the level of their coagulation factors

RESULTS

The level of coagulation factors and inhibitors were determined and analysed over time with a p-value < .005 (p < .005) findings reported with a p-value < .00325 (p < .00325) to avoid type one error; comparing baseline results and up to three months of storage based on both age, sex, weight and blood group of blood donors, we found significant decrease of fibrinogen (-10%), FII (-8%), FV (-15%), FVII (-13%), FX (-15%), FXIII (-5%), PC (-7%), and ATIII (-5%), show a decrease from baseline up to three months, whereas FVIII (-8%), F IX (-4%), FXI (-6%), FXII (-3%), FPS (-3%), and VWF-Ag (-7%) have been constant without significant change (+/-0%) from baseline to one month then changed also significantly over time and decreased up to three months. Prothrombin Time and Activated Partial Thrombin Time increased (0.5-1.5 second) from baseline up to one month of storage and from one month up to three months of storage had been stable.
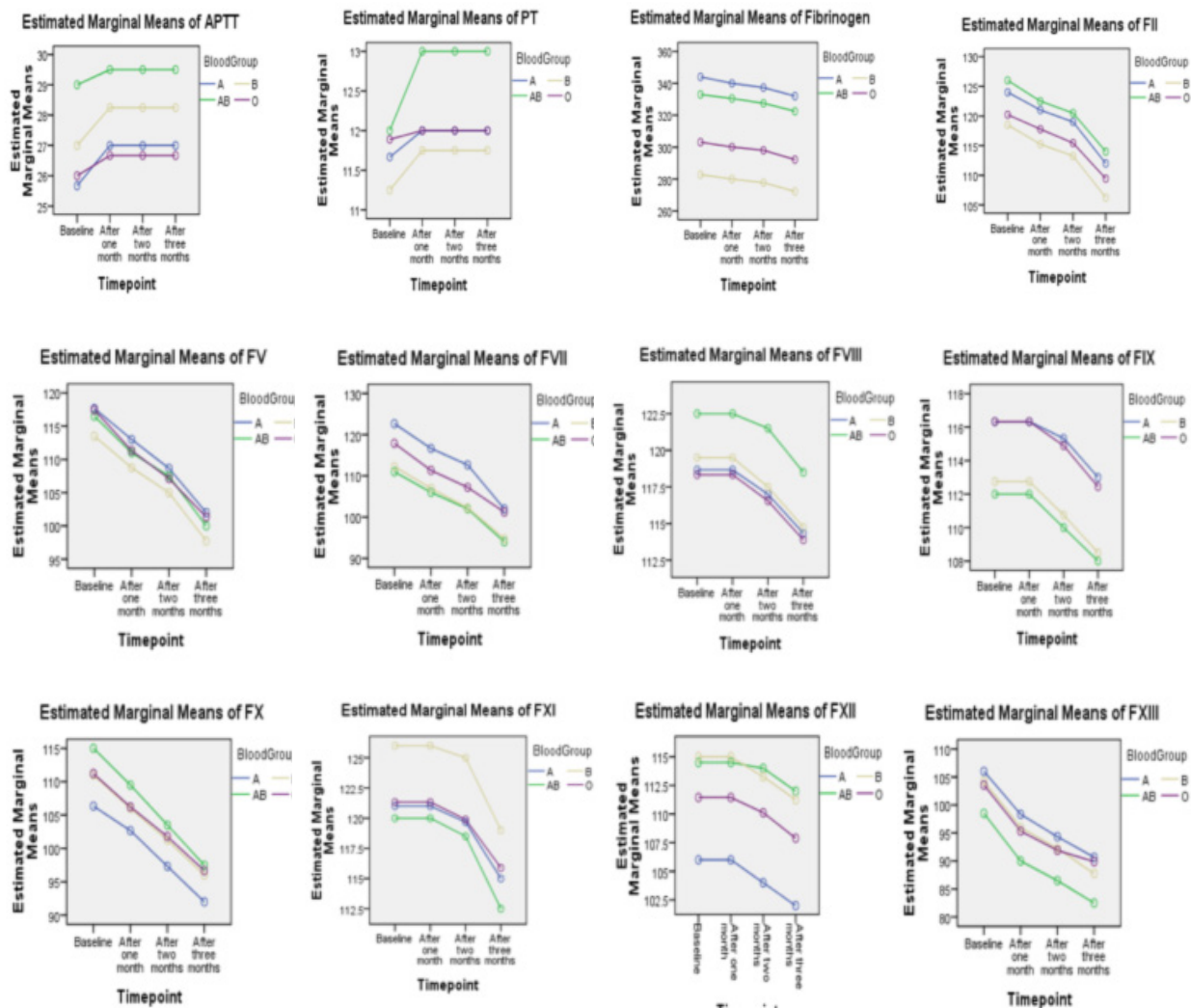
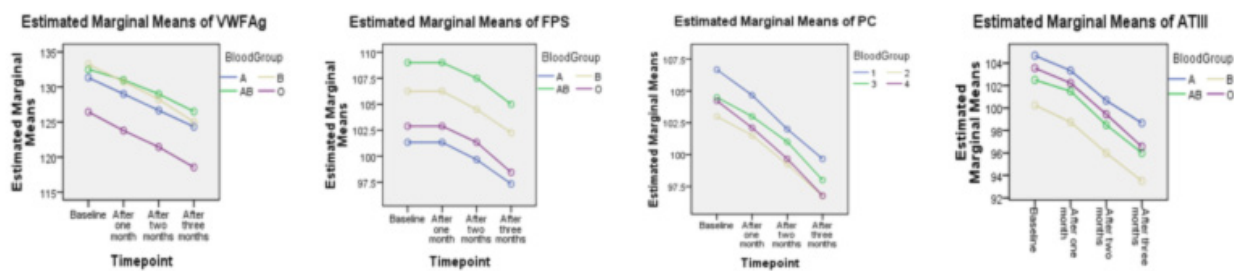**Plot of coagulation factors levels in fresh frozen based on time point and sex**
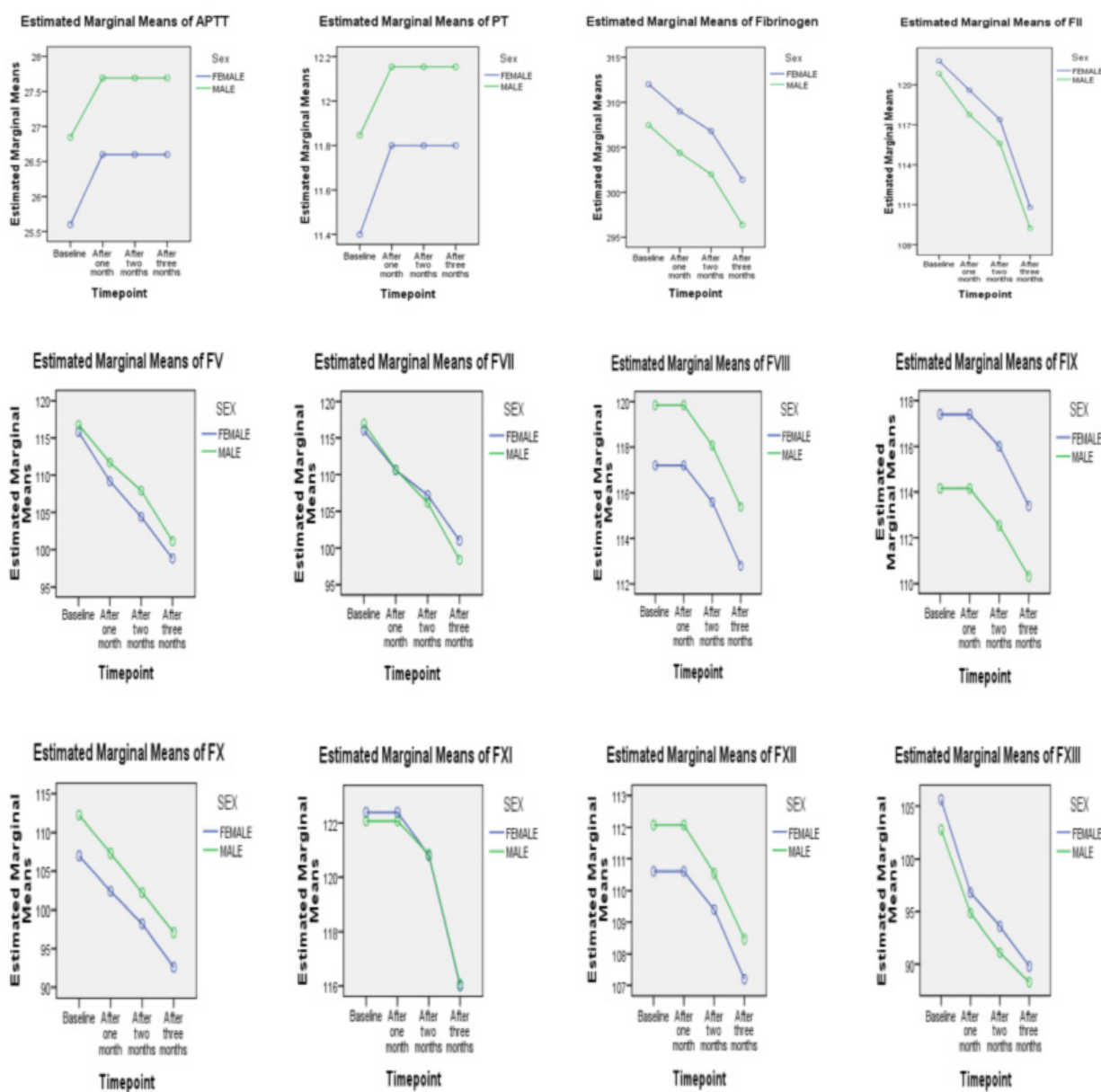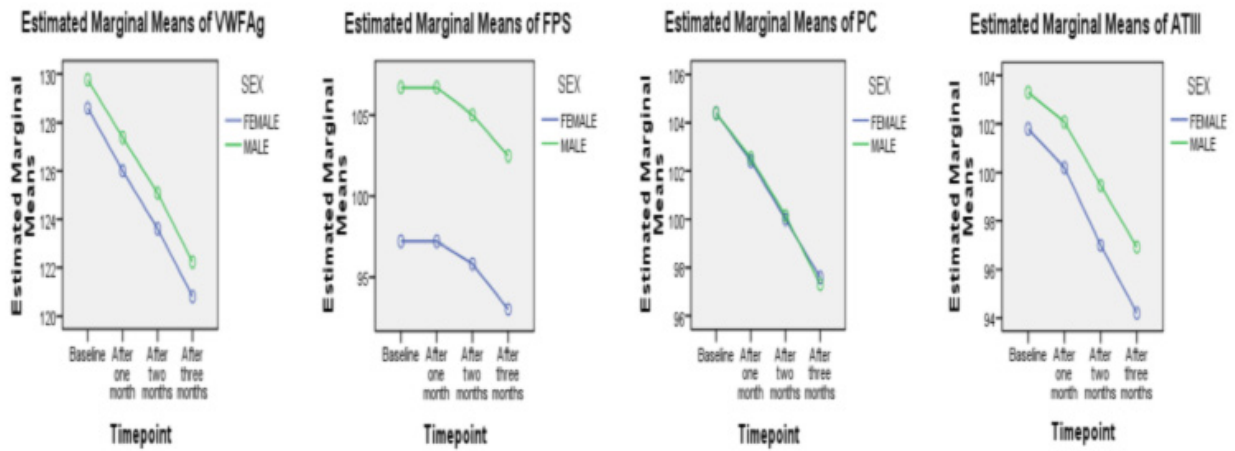
These plots show estimated marginal means of APTT and PT expressed in seconds while in coagulation factors and inhibitors are expressed in percentages. Based on sex and time point as Independent variables, we found that APTT and PT increased from baseline up to one month of storage and became constant up to three months, instead fibrinogen, FII, FV, FVII, FX, FXIII, VWFAg, PC and ATIII show a decrease from baseline up to three months, while FVIII, FIX, FXI, FXII and FPS have been constant from baseline to one month then decrease up to three months.
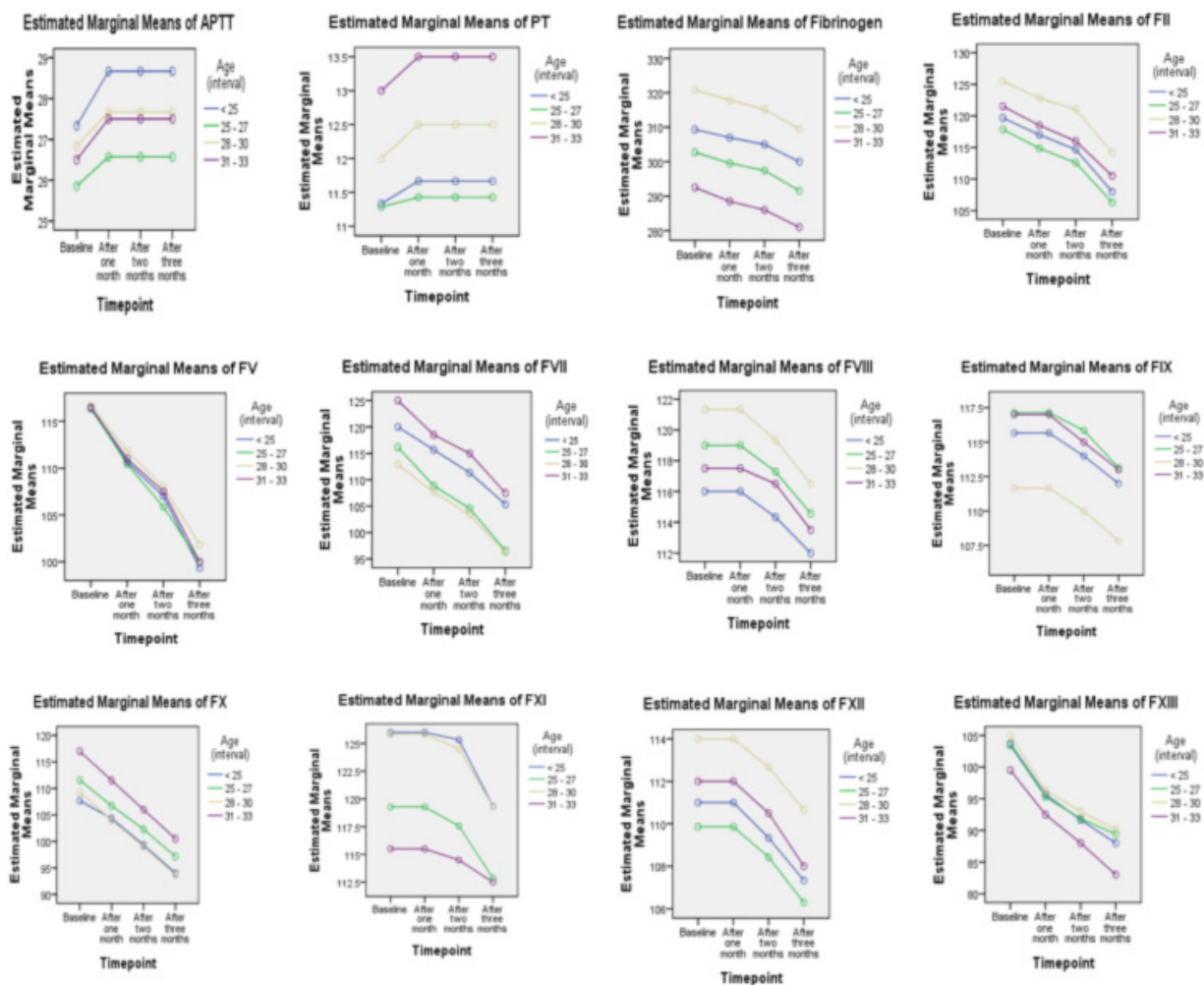
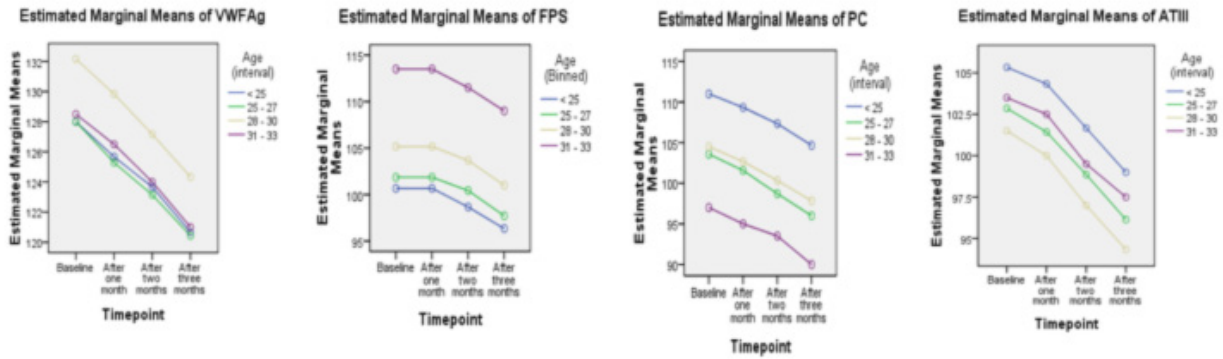**Plot of coagulation factors levels in fresh frozen based on time point and blood group**

These plots show estimated marginal means of APTT and PT expressed in seconds while in coagulation factors and inhibitors are expressed in percentages. Based on blood group and time point these: APTT and PT show an increase from baseline to one month then remain constant up to three months, but: fibrinogen, FII, FV, FVII, FX, VWFAg, PC and ATIII have decreased from baseline up to three months and then FVIII, FIX, FXI, FXII and FPS remain constant from baseline up to one month and decreased up to three months.
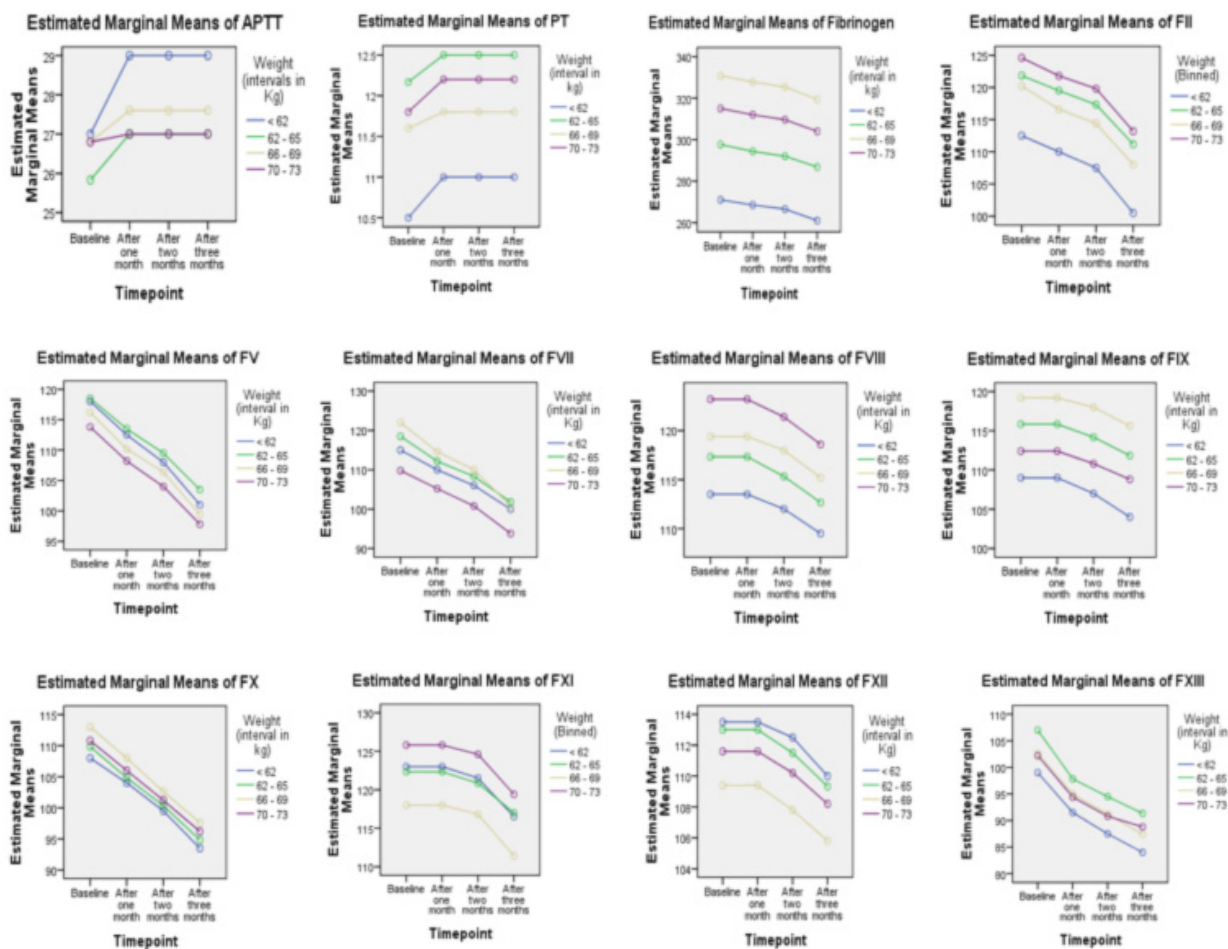
Plot of coagulation factors levels in fresh frozen based on time point and age
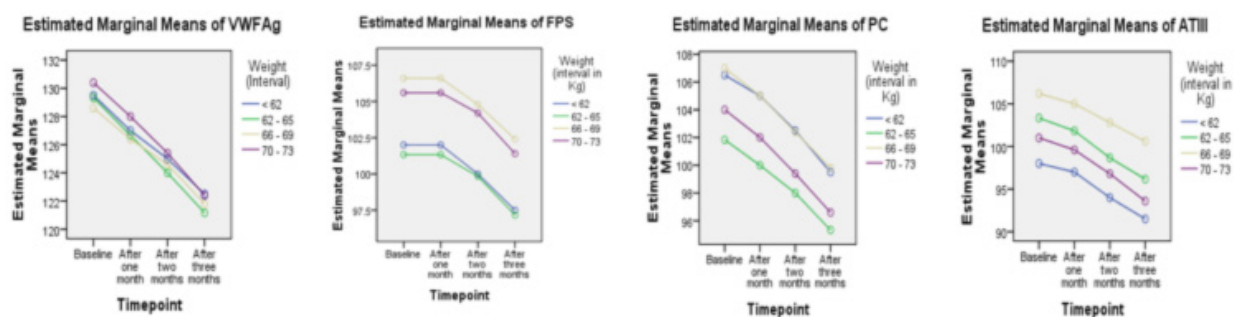
These plots show estimated marginal means of APTT and PT expressed in seconds while in coagulation factors and inhibitors are expressed in percentages. Based on Age and time point: APTT and PT show an increase from baseline to one month then remain constant up to three months, while; fibrinogen, FII, FV, FVII, FX, VWFAg, PC and ATIII have decreased from baseline up to three months and then FVIII, FIX, FXI, FXII and FPS remain constant from baseline up to one month and decreased up to three months.

Plot of coagulation factors levels in fresh frozen based on time point and weight

These plots show estimated marginal means of APTT and PT expressed in seconds while in coagulation factors and inhibitors are expressed in percentages. Based on Weight and time point these: factors APTT and PT show an increase from baseline to one month then remain constant up to three months, whereas; fibrinogen, FII, FV, FVII, FX, VWFAg, PC and ATIII have decreased from baseline up to three months and then FVIII, FIX, FXI, FXII and FPS remain constant from baseline up to one month and decreased up to three months.

DISCUSSION

Fresh frozen plasma (FFP) is indicated for the treatment and prevention of bleeding disorders due to deficit in minor or major coagulation factors, defective management of massive bleedings, liver disease, disseminated intra-vascular coagulation or reverse anticoagulant therapy (2).

According to United States of America and European guidelines for production of blood and blood product define Fresh Frozen Plasma (FFP) as the liquid portion of human blood that has been centrifuged, separated with blood cells, solid at minus 18 degrees C or colder within eight hours of blood donation, and store up to three months contains all coagulation factors in normal concentration 0.5 to 2IU / ml or (50-200%) to be used in blood transfusions (3).This study provides the first comprehensive analysis of the level of coagulation factors in fresh frozen in Rwanda.

The reported results in this study on PT and APTT increase of 0.5-1.5 seconds from baseline up to three months is however prolonged than the time reported by Von Heymann et al., 2006 (4) which show an increase of 0.1-0.5 seconds, in their study titled "Coagulation parameters of thawed fresh-frozen plasma during storage at 1-6°C for five days" in German and Thompson et al in Thailand in a study of coagulation factor activities in apheresed thawed fresh-frozen plasma at 1-6°C for five days with an increase of 0.1-0.5 seconds.

The level of coagulation factors and inhibitors were determined and analysed over time with a p-value < .005 (p < .005) findings reported with a p-value < .00325 (p < .00325) to avoid type one error; comparing baseline results and up to three months of storage based on both age, sex, weight and blood group of blood donors, we found significant decrease of fibrinogen (-10%), FII (-8%), FV (-15%), FVII (-13%), FX (-15%), FXIII (-5%), PC (-7%), and ATIII (-5%), show a decrease from baseline up to three months, whereas FVIII (-8%), F IX (-4%), FXI (-6%), FXII (-3%), FPS (-3%), and VWF-Ag (-7%) have been constant without significant change (+/-0%) from baseline to one month then changed also significantly over time and decreased up to three months. All clotting factors and inhibitors remained within the reference range requested by quality assurance regulations. This is compared to the results of a study done in German on Activity of clotting factors in FFP by Von Hermanny *et al*, immediately after thawing there was a significant decrease of fibrinogen (-9%), FII (-7%), FV (-14%), FVII (-12%), FX (-11%), FXIII (-20%), PC (-7%), and ATIII (-4%), while FVIII (+8%), F IX (+1%), FXI (+11%), FXII (-1%), FPS (-1%), and VWF-Ag (-6%) remained stable. Over 6 days after thawing fibrinogen, ATIII (+2%) and VWF-Ag (+2%) remained stable whereas FXII (+2%), FXIII (+6%), and PC (+3%) , FII (-8%), FV (-16%), FVII (-31%), FVIII (-47%), F IX (-12%), FX (-10%), FXI (-25%), and FPS (+/-0%).(5). The level of coagulation factors stated in my study is a bit different from the results of this study done in German, the variation in coagulation factors might have been caused by variation in temperature and number of days or time FFP has been stored. Mine were frozen at minus 18oC while Von Hermanny removed from freezer and kept them at 1-6°C for six days. The decrease of coagulation factor level is normal for FFP kept for long time at high temperature; the more temperature is low the more you preserve enough level of coagulation factors. (AABB,2002), I did not found an increased level, more study are needed to provide information on the cause of increase in same coagulation factors level during storage as stated by von Hermanny.

The current study's results are a bit different compared to the results of another research done by Von Heymann *et al*., 2006 in German on Thawing procedures and the time course of clotting factor activity in fresh frozen plasma, they have confirmed

a significant decrease of FVII (130% and 100%, p < 0.001) and the relative stability of FVIII (150% and +/- 1%, p < 0.001), F IX (145% and +/- 1%, p < 0.001), FXI (130% and +/- 0%, p < 0.001), FXII (128% and +/- 1%), VWF-Ag (130% and +/- 1%, p < 0.001), and FPS (115% and +/- 0%, p < 0.001). Solely the decrease of fibrinogen (299 to 290 mg/dL, p < 0.001) and FV (130% and 116%, p < 0.001) which was not in accordance with their previous results, this showed an insignificant increase of both factors reported in their previous study (4).

The reported decrease of coagulation factors level in this study is however lower than the decrease reported by Mark H. et all in America on Coagulation factor levels in plasma frozen within 24 hours of phlebotomy over 5 days of storage at 1 to 6°C, including FV, FVII and FVIII activity, which decreased by 40%, 20% and 50% respectively on Day 5, the activity of PS was at the low end of the normal range and it declined to slightly below its normal level by Day 5. vWF activity remained within its normal range by Day 5.(6).The facts that the findings of this study are different from ours temperature and frozen time after phlebotomy, may have contributed to the observed variation.

CONCLUSION

Our results demonstrate that there is good retention of all coagulation factors and inhibitors in plasma produced from whole blood within eight hours of collection, stored at minus 18 ºC for three months and that such plasma would be an optional useful product for most patients with minor to major coagulation factor deficits to prevent blood loss. Moreover, this plasma will be used in treatment of disseminated intra-vascular coagulation, liver disease patients and reversal of therapy with vitamin K antagonists(8). Especially in emergency situations, transfusion of this plasma will deliver clotting factors without aggravating existing delusional coagulopathy induced by crystalloid or colloid volume replacement solutions (9).

Recommendation:It is recommended that to measure at least labile factors before providing fresh frozen plasma preserved for more than three months. Prothrombin time and activated partial thrombin time might be included in pre-transfusion test to evaluate the activity of intrinsic and extrinsic coagulation factors in fresh frozen plasma before use. Whereas the findings of this study seem to suggest that Fresh Frozen Plasma might be store at minus 18ºC for three months, for better management of bleeding disorders that need transfusion of Fresh Frozen Plasma.

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