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Original Article

Effect of gamma irradiation with 30 Gy on the coagulation system in leukoreduced fresh frozen plasma

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ABSTRACT

Background: Fresh Frozen Plasma (FFP) is a blood component prepared from whole blood or from apheresis donation. Donor leukocytes including lymphocytes are present in FFP in significant numbers inspite of freezing, responsible for Transfusion Associated Graft versus Host Disease (TA-GvHD).

Study design and methods: 75 units of FFP prepared at our centre were analysed. After thawing of FFP a small aliquot was made under sterile conditions and another after irradiating the product. In both the parts, variables of haemostasis were measured in parallel, using automated coagulation analyser. Activated partial thromboplastin time (APTT), prothrombin time (PT), International Normalized Ratio (INR), Thrombin time (TT), coagulation factors FI, FII, FV, FVIII, FIX, FX, FXI, FXII, vWF Ag, inhibitors of coagulation (protein C & S) and D-dimer were measured.

Results: Gamma irradiation of FFP with 30 Gy resulted in weak activation of coagulation system which was evident in the form of shortening of PT, APTT and TT. The activity of coagulation factors FIX, FX, FXI, and FXII were significantly raised after irradiation. No reduction in the activity of inhibitors of coagulation (protein C & S) or increase in D-dimers was observed following irradiation of FFP.

Conclusion: Gamma irradiation of FFP with 30 Gy resulted in a significant but very weak alteration of coagulation system in FFP.

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Introduction

Fresh Frozen Plasma (FFP) is the component of blood consisting of 90% water, 7% protein and colloids, and 2% to 3% nutrients, crystalloids, hormones, and vitamins. The protein fraction contains the soluble coagulation factors, fibrinogen, Factor XIII, von Willebrands factor and vitamin K dependent clotting factors F II, VII; IX & X. Clotting proteins are the

constituents for which transfusion of plasma is most often required. A unit of FFP contains approximately 200 units of each coagulation factor, or 7 percent of the coagulation factor activity of a 70 kg adult.¹

FFP is indicated for transfusion in liver disease, coagulopathies, before or after surgery to maintain haemostasis and other clinical conditions because of its coagulation factor constituents.

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Like any other component of blood, FFP is also associated with many adverse effects following transfusion, one among them being Transfusion Associated Graft versus Host Disease (TA-GvHD). TA-GvHD is caused by donor pool of T-Lymphocytes present in FFP. By and large FFP is considered as an acellular component containing no cells, but of late studies have proved that FFP contains significant number of WBCs in functioning state despite freezing below -18°C for a period of one year and thawing.^{2,3} WHO reported an average of 3.2×10^7 WBCs in FFP units with an average volume of 260 ml.⁴ Hence in immunologically incompetent patients FFP may be irradiated in order that the T-lymphocytes are destroyed. This in turn would prevent a catastrophic TA-GvHD.

In our study we evaluated the effect of gamma irradiation with 30 Gy on various coagulation assays in FFP i.e. PT, INR, APTT, TT, vWF Ag, Fibrinogen, FII, FV, FVIII, FIX, FX, FXI, FXII, vWF ristocetin factor, anticoagulants (protein C and protein S) and D-dimers.

Materials & methods

75 units of FFP prepared at our centre were analysed. After thawing of FFP a small aliquot was made under sterile conditions and one more after irradiating the product. In both the parts variables of haemostasis system were investigated in parallel. Haemostatic variables measured included Global coagulation tests (PT/INR, APTT, and TT), coagulation factors (FII, FV, FVIII, FIX, FX, FXI, FXII, vWF Ag), Inhibitors of coagulation (Protein C & Protein S), and markers of activation of haemostasis system (D-dimers).

All FFP units were prepared at our centre from a donation of 350 ml or 450 ml whole blood donations using CPD (citrate phosphate dextrose) as anticoagulant. All FFP units were prepared within 6 h of blood donation. All units were stored below -18°C until thawing. FFP were thawed at 37°C for 30 min before issue. A small aliquot of 5 ml was made under sterile conditions after thawing and was used as pre irradiation sample for measuring haemostatic variables. The remaining plasma in original FFP bag was subjected to Gamma

irradiation. Gamma irradiation was done using Gamma cell 3000 Elan (Canada) having Cesium 137 as source (Fig. 1).

A small aliquot was made immediately after irradiation with careful mixing and was used as post irradiation sample for measuring haemostatic variables. From both irradiated and non irradiated aliquots, samples were transferred to labelled test tubes for further haemostatic analysis. Haemostatic variables were measured using automated coagulation analyser (STA Compact, Diagnostica Stago France) (Fig. 2). All coagulation parameters were measured on the same day of collection of samples. Measurements of both irradiated and non irradiated part of FFP were always performed in the same run.

Results

Range of different global coagulation tests measured in 75 units of FFP along with their *p* value is shown in Table 1. PT, APTT and TT were shortened following irradiation. The reduction in time was significant for all 3 tests measured in terms of *p* value (*p* value < 0.05). But in all samples PT, APTT and TT remained within normal range. Following irradiation FIX, FX, FXI and FXII levels were slightly and significantly elevated (*p* value < 0.05), but post irradiation values were within normal range. There was no significant difference in vWF Ag, fibrinogen, FII, FV, FVIII and vWF ristocetin cofactor activity levels following irradiation (Table 2). These changes suggest that there is weak activation of coagulation system. There was no significant change in these anticoagulant levels (protein C and protein S) following irradiation. Since there was no change in D-dimers level following irradiation, this signifies there occurred no substantial fibrin production as a consequence of activation of coagulation system (Table 3).

Discussion

In our study we evaluated effect of gamma irradiation on quality of FFP i.e. on global coagulation tests (PT/INR, APTT,

Fig. 1 – Showing gamma cell irradiator (Gammacell Elan, Canada).

Fig. 2 – Showing automated coagulation analyser (STA Compact, Diagnostica Stago France).

TT), coagulation factors (Fibrinogen, FII, FV, FVIII, FIX, FX, FXI, FXII and vWF Ag, vWF ristocetin cofactor), anticoagulants (Protein C and Protein S) and on fibrinolytic system (D-dimers).

Besides the costs and the time delay it causes in transfusion, no counterargument has risen against Gamma irradiation of FFP. It is generally assumed that gamma irradiation with 30 Gy has no effect on the quality of FFP. Although there are several reports describing the effect of gamma irradiation with a dose between 20 and 35 Gy on the quality of RBCs and PLT concentrates, knowledge regarding effect of gamma irradiation on the quality of FFP is limited. In previous studies, where gamma irradiation in extremely high doses between 2500 and 100,000 Gy was applied to FFPs in the frozen state to achieve virus inactivation, a loss of activity of FVIII, FIX, and of vWF antigen between 1.1 and 60 percent had been observed after irradiation.^{5,6}

The combined analysis of all 75 units of FFPs prepared at our centre showed that gamma irradiation of leukoreduced FFPs with 30 Gy resulted in a highly significant but weak activation of the coagulation system with no effect on fibrinolytic system and natural anticoagulants present in FFP. Leukocyte-reduced FFP (LR-FFP) prepared using a filter can

achieve counts of $<2.0 \times 10^5$; up to five units can be processed per filter.

How γ irradiation might have caused the observed effects in FFP is not clearly understood. One possibility is that reactive free radicals, which are known to be generated in water during gamma irradiation, might exert the effect by direct modification of coagulation factors or by oxidation of phospholipids stemming from residual platelets in FFP. Albumin which is present in large amounts in plasma is known to be an excellent radical scavenger.⁶

Irradiated phospholipid membranes exhibit significantly enhanced bilayer rigidity due to free radical mediated reaction of lipoxy radicals. Membrane oxidative damage is generally

Table 1 – Shows global coagulation tests in 75 samples before and after irradiation with 30 Gy.

Parameter	Before irradiation	After irradiation	p value
PT (sec)	11.8–17	11.8–17.1	0.0002
INR	1–1.33	1–1.42	0.002
APTT (sec)	31–48.4	30.8–48.1	0.0000001
TT (sec)	15.8–20	15.8–19.8	0.000004

Table 2 – Coagulation factors in 75 samples of FFP before and after gamma irradiation with 30 Gy (range and p value).

Coagulation factors	Before irradiation	After irradiation	p value
VWF%	58–240	65–238	NS
Fibrinogen (mg/dl)	103–372	141–380	NS
FII%	52–91	55–97	NS
FV%	40–78	40–78	NS
FVIII%	63–128	63–140	NS
FIX%	52–140	51–131	0.00002
FX%	58–124	59–124	0.009
FXI%	74–132	71–133	0.0000000003
FXII%	79–131	82–130	0.0000000005
VWF ristocetin cofactor activity (%)	58–114	56–112	NS
NS = not significant.			

Table 3 – Inhibitors of coagulation and D-dimers in 75 units of FFP before and after gamma irradiation with 30 Gy.

Parameter	Before irradiation	After irradiation	p value
PC%	73–110	71–110	NS
PS%	60–112	61–112	NS
D-DI (ng/ml)	0.2–0.4	0.18–0.38	NS
NS = not significant.			

mediated by the degradation of phospholipids. It is observed that membrane lipids are easily peroxidized by Reactive Oxygen Species (ROS) produced by ionizing radiation, causing structural and functional impairment. Oxidative damage leads to an alteration in both lipid bilayer fluidity and permeability properties. In the context of cellular response to radiation, the contributions of radiation oxidative damage to membranes as well as to DNA damage via ROS appears rather complex and these pathways of initiators of cytotoxicity seem to be intimately linked in the development of radiation-induced deleterious cellular effects. A biphasic dose response relationship has also been described between gamma irradiation and changes in the permeability of unilamellar dipalmitoyl phosphatidylcholine liposomes. This result was interpreted in terms of radiation-induced chemical oxidative damage,⁷ in this study; the highest permeability of liposomes was seen at approximately 500 Gy. Further studies are needed to clarify the mechanisms which are responsible for the effects we observed.

The interpretation of this study is gamma irradiation of FFP with 30 Gy resulted in weak activation of coagulation system. Activation of coagulation system is evident in the form of shortening of PT, APTT and TT following irradiation. Even though PT, APTT and TT were shorter after irradiation, the changes are very small and the results were within normal range in all 75 samples. Hence shortening of coagulation system parameters was statistically significant but not clinically relevant. Activities of coagulation factors FIX, FX, FXI, and FXII were significantly raised after irradiation. The changes in these variables were small and the values after irradiation were still within the normal range. The absence of an increase of the fibrin degradation product D-dimer indicates that no substantial fibrin production occurred as a consequence of the activation of the coagulation system. The irradiation of FFP resulted in no reduction of the activity of inhibitors of coagulation (protein C and protein S). Hence overall gamma irradiation of FFP with 30 Gy showed very weak, statistically significant and clinically not significant activation of coagulation system, with no effect on fibrinolytic and anticoagulant system.

Plasminogen anti plasmin (PAP) complex an improved marker for the activity of fibrinolytic system, F VII activity ratio, F XIIIa, and fragment 1& 2 – markers for activation of the coagulation system were not analysed and hence could be considered as limitations of this study.

Intellectual contribution of authors

Study concept: Brig RS Sarkar.

Drafting & Manuscript revision: Prathip Kumar, Col J Philip, Brig RS Sarkar, Amardeep Pathak.

Statistical analysis: Prathip Kumar, Amardeep Pathak.

Study supervision: Brig RS Sarkar, Col J Philip.

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Conflicts of interest

All authors have none to declare.

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