

ORIGINAL ARTICLE

Gamma irradiation and red cell haemolysis: a study at the Universiti Kebangsaan Malaysia Medical Centre

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Abstract

Gamma-irradiation of blood components is regarded a safe procedure used for prevention of transfusion-associated graft-versus-host disease. However, reports showed that irradiation can cause erythrocyte haemolysis and damage to the RBC membrane. In Universiti Kebangsaan Malaysia Medical Centre (UKMMC), a number of suspected transfusion reactions (TR) featured unusual isolated episodes of red-coloured-urine or haemoglobinuria among paediatric patients without clinical features of acute haemolytic TR. Haemolysis of irradiated red cells was suspected as a cause. This study was conducted to evaluate haemolytic changes of RBC components following irradiation. A prospective, pre- and post- irradiation comparative study was conducted on 36 paired RBC-components in the blood-bank, UKMMC in the year 2013. Samples were tested for plasma-Hb, percent-haemolysis, plasma-potassium (K^+) and lactate dehydrogenase (LDH) level. Post-irradiation mean plasma-Hb and percent-haemolysis were significantly higher than pre-irradiation values at 0.09 ± 0.06 g/dl VS 0.10 ± 0.06 g/dl and $0.19 \pm 0.13\%$ VS $0.22 \pm 0.13\%$ respectively, while plasma- K^+ and LDH values did not show significant difference. However, the mean percent-haemolysis level was still within recommended acceptable levels for clinical use, supporting that irradiated RBC units were safe and of acceptable quality for transfusion. There was no conclusive reason for isolated haemoglobinuria following transfusion of irradiated red-cell products. Further research is suggested to investigate the other possible causes.

Keywords: irradiation, blood-unit, haemolysis, potassium, lactate-dehydrogenase

INTRODUCTION

Transfusion associated graft-versus-host disease (TA-GVHD) is an infrequent complication of blood transfusion with high mortality resulted from transfusion of immunologically competent lymphocytes to an immunocompromised recipient.¹ Gamma irradiation of blood components is the main procedure used for prevention of TA-GVHD. It has been shown that gamma irradiation of red cells with a dose of 25-30 Gy will suppress proliferation of residual donor lymphocytes and hence prevent TA-GVHD.² The primary target of irradiation is the DNA of lymphocytes. Ionizing radiation readily penetrates nucleated cells and damages nuclear DNA either directly or by generating ions and free radicals.³ Irradiation is generally documented to have no significant deleterious effect on

blood products which are then considered safe for transfusion. However, there have been some reports of erythrocyte haemolysis, damage to the RBC membrane and increased membrane permeability.^{1,4}

In Universiti Kebangsaan Malaysia Medical Centre (UKMMC), occasional cases of suspected transfusion reactions with unusual isolated episodes of red color urine or haemoglobinuria has been observed among paediatric patients.^{5,6} However, clinically, the vital signs of these patients were stable, without any other sign or symptom of haemolytic transfusion reaction (HTR). Investigations for transfusion reaction in these patients did not reveal serological evidence of HTR and these patients showed an increase of post-transfusion haemoglobin levels with clearing of haemoglobinuria over 12-24 hours. The reason

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for such post-transfusion red-colour-urine was not ascertained. One of the causes raised was post transfusion haemoglobinuria could be due to transfusion of lysed irradiated blood products. It is reasoned that since irradiation can cause excessive haemolysis of RBC resulting in increased plasma haemoglobin, the transfusion of such products can give rise to red-colour-urine or haemoglobinuria. Besides, it was postulate that red cells could also be damaged due to the (i) priming of the intravenous line or infusion with inappropriate intravenous fluids such as dextrose, before or during the transfusion; (ii) the small branula size used in paediatric patients or (iii) the quality or near expiry of blood products given to the patients as well as (iv) underlying medical causes related to the patients. As irradiation is one of the procedures performed in the blood bank, a study to evaluate the effect of gamma-irradiation on red cell components was deemed useful.

MATERIALS AND METHODS

This was a prospective cross-sectional study performed on donor red blood cell (RBC) units at the Blood Bank, Department of Diagnostic Laboratory Services, Universiti Kebangsaan Malaysia Medical Centre (UKMMC) in the year 2013. The study population consisted of donor blood bag units from the inventory in the blood bank at different storage periods. This study used a random sampling method. 36 donor packed cell units in additive solution (SAGM) were investigated for changes of red blood cells in pre- and post-irradiated samples taken from the stored blood components. The parameters investigated were the haematological values of plasma haemoglobin (Hb) and percent haemolysis, and biochemical values of plasma potassium (K⁺) and lactate dehydrogenase (LDH). Samples were taken just before and after irradiation of the red cell units and analyses were done within 1 hour of sampling.

Plasma Hb was estimated by the HemoCue Plasma/Low Hb analyzer and percent haemolysis was calculated using the following formula: $(100 - \text{HCT}) \times \text{plasma haemoglobin (g/dl)} / \text{Total haemoglobin (g/dl)}$.⁷ Here, the total Hb content and haematocrit was measured by automated haematology analyzer, the Beckman Coulter LH 750 analyzer. The quantitative measurement of plasma electrolyte K⁺ was performed using the automated biochemistry analyzer 'Cobas Integra 800-ISE model'. The blood LDH level was also measured by Cobas Integra System.

Data analysis was conducted using the Statistical Package for Social Sciences (SPSS) version 19. Descriptive analysis was performed to find out the mean $\pm$ SD values to describe the different variables. Paired student t-test was used to compare the mean of the pre- and post-irradiated red cells to find out if there was a significant difference between the two groups. In this study the statistical power determined was 0.95 and the p value of significance was set to 0.05.

RESULTS

Table 1 shows the haematological values of plasma Hb and percent haemolysis in the samples. For pre-irradiated samples, plasma Hb and percent haemolysis were 0.09 ± 0.06 g/dl and $0.19 \pm 0.13\%$ respectively, while for post-irradiation samples, the values were 0.10 ± 0.06 g/dl and $0.22 \pm 0.13\%$ respectively. This difference was statistically significant (p value = <0.05).

Table 2 shows the biochemical values of plasma K⁺ and LDH in pre and post-irradiated samples. The levels of plasma K⁺ and LDH in pre-irradiated samples were 26.97 ± 3.83 mmol/L and 1478.66 ± 574.45 U/L respectively, while in post irradiation samples, the values of plasma K⁺ and LDH were 26.95 ± 3.70 mmol/L and 1508.72 ± 576.50 U/L respectively. There were no significant changes observed between pre- and post-irradiated plasma K⁺ and LDH levels.

DISCUSSION

Plasma haemoglobin (Hb) is the free Hb in the plasma which gradually increases during the storage of RBCs⁸ and irradiation of RBCs has been shown to have some significant effect.⁹ The supernatant plasma Hb is measured as percent haemolysis which is described as a percentage of free Hb in relation to the total. Currently, the accepted threshold level of haemolysis at the end of the storage period of red cell products is 1% in the United States and 0.8% in the European Union countries.^{10,11} In Malaysia, according to the National Blood Centre guidelines, the accepted threshold is up to 0.8%.¹² This present study showed that mean red cell haemolysis was significantly increased in post-irradiated samples, however, the actual percent haemolysis demonstrated were still beneath the accepted threshold level of 0.8% – 1.0% as recommended by the US, UK as well as Malaysian national guidelines. Therefore the observed increase in

TABLE 1: Comparison of plasma Hb and percent haemolysis between the pre- and post-irradiated samples

Variables	Mean	SD	Minimum value	Maximum value	P value
Pre plasma Hb (g/dl)	0.09	0.06	0.02	0.26	0.000*
Post plasma hb (g/dl)	0.10	0.06	0.02	0.32	
Pre haemolysis (%)	0.19	0.13	0.02	0.50	0.005*
Post haemolysis (%)	0.22	0.13	0.05	0.64	

*= statistically significant

plasma Hb or percent haemolysis did not translate into clinical significance as an adverse effect of gamma-irradiation.

For the biochemical values of K^+ and LDH, the overall comparison between pre- and post-irradiated samples did not show any significant difference. It has been reported that in stored red blood cells, intracellular K^+ leaks due to inhibition of the membrane ATP pump and gamma irradiation potentiates the K^+ leak further by inhibiting the Na-K pump.¹³ Potassium level is an important indicator of blood safety as high K^+ can lead to serious adverse effects particularly in neonates. Slow RBC transfusion has little effect on serum K^+ concentration in the patient. However, massive blood transfusion may cause complications by increasing the plasma K^+ level especially when the patient's blood volume is very low and the K^+ concentration is very high in the transfused unit. Increased plasma K^+ poses a risk to neonates and children undergoing cardiac surgery or exchange transfusion, and to patients receiving massive transfusion such as during trauma, surgery and liver transplantation, as hyperkalaemia can induce cardiac arrhythmias leading to cardiac arrest.¹³ The K^+ concentration of 7-day-old blood units (packed RBC stored in AS-1) was reported to be around 23mmol/L whereas that of the expired blood (42-days-old) stored in AS-1 was approximately 50 mmol/L.¹⁴ A higher K^+ concentration of 70 mmol/L has also been reported in a 28-day-old irradiated packed cells stored in SAGM.^{15,16} In another

report by Nakaqawa *et al*¹⁷ the K^+ concentration was reported as 50meq/L (50mmol/L) one week after irradiation. Compared to these reports, our findings of 26.97mmol/L and 26.95mmol/L in pre- and post-irradiated blood can be considered below the threshold level.

Serum LDH levels were also elevated in our study but no significant difference was observed between the two groups. This finding was similar to that of Weinmann *et al*¹⁸ where no significant differences were detected in post-irradiated red cells after irradiation at different doses starting from 10-150 Gy at 2 hours, 24 hours, 48 hours and 72 hours. Baumler *et al*² and Moreira *et al*¹⁹ also did not find any significant difference in lactate concentration between non-irradiated and irradiated RBCs.

Regarding the suspected transfusion reaction featuring isolated red-colour-urine or haemoglobinuria without other evidence of acute haemolysis seen in a few of our paediatric patients, it is unlikely that the suspected transfusion reactions were secondary to irradiation of the red cell products as the amount of red cell haemolysis was not clinically significant.

CONCLUSION

This study showed that gamma-irradiation significantly increases the plasma Hb and percent haemolysis in post-irradiated red cell units although the quantitative results were beneath

TABLE 2: Comparison of plasma K^+ and LDH levels between pre- and post-irradiated samples

Variables	Mean	SD	Minimum value	Maximum value	P value
Pre K^+ (mmol/L)	26.97	3.83	19.54	30	0.936
Post K^+ (mmol/L)	26.95	3.70	18.04	30	
Pre LDH (U/L)	1478.66	574.45	367	2810	0.233
Post LDH (U/L)	1508.72	576.50	335	2830	

the recommended threshold levels for clinical significance. There was no significant changes in plasma K⁺ and LDH levels between the pre- and post-irradiated samples. These results suggest that irradiated RBC units are clinically safe and of acceptable quality for transfusion. This study was not able to conclude that irradiation was the cause of isolated episodes of red-colour-urine following transfusion. Further research is required to investigate the other possible causes which may lead to post-transfusion haemoglobinuria in paediatric patients such as the venous line and infusion set priming method, size of branula used for transfusion, maintenance of the cold chain of the blood bag from supply to transfusion as well as medical causes that contribute to transfusion associated red-coloured-urine or haemoglobinuria.

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