Original article

Effects of gamma irradiation on red blood cells at different storage period: A comparative study

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Abstract

Objectives: The objective of this study was to explore the effect of gamma irradiation on the haematological and biochemical parameters of the allogeneic red blood cells units at different storage duration. ***Methods:*** A prospective comparative study was conducted at the Blood Bank unit, Department of Diagnostic Laboratory Services of Universiti Kebangsaan Malaysia Medical Centre (UKMMC) in year 2013. Total 36 allogeneic red blood cell units were selected by simple random sampling method and divided into two groups, 18 units in each group based on the storage period into i) up to 14 days and ii) >14 days to expiry. Pre and post irradiation samples were taken immediately before and after irradiation of the units to analyse for the haematological parameters such as plasma Hb level, percent red cell haemolysis, haemoglobin per unit blood (Hb/unit), haematocrit (HCT), red cell indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and biochemical parameters such as plasma sodium (Na^+), potassium (K^+) and LDH levels. Data was collected and statistical analysis was done. ***Results:*** Statistically significant changes were observed between red cell units of up to 14 days and > 14 days old and pre irradiated samples in terms of plasma haemoglobin level, percent red cell haemolysis and plasma Na^+, K^+ and LDH levels. However, pre and post irradiation samples in each group of 14 days and >14 days showed significant changes in the plasma Hb and % haemolysis, Hb/unit, HCT, MCV and MCH in 14 days group while only in plasma Hb, MCV and MCH in >14 days group without any effect on plasma Na^+, K^+ and LDH levels in both group. ***Conclusion*:** Comparison at two different red cells storage groups i.e. period of up to 14 days and >14 days, showed significant changes in haematological and biochemical parameters. In the pre irradiation group this reflects the gradual storage changes over time even without going through the irradiation process. On the other hand, the changes between pre and post–irradiation for both the groups of storage showed significant haematological changes indicating irradiation itself also causes significant changes. In view of all these findings, we recommend to irradiate the allogeneic red cell units within 14 days of collection to reduce the adverse effects resulted from irradiation on the cellular products.

Key words: gamma irradiation; red blood cells; storage period; percent red cell haemolysis

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Introduction

Gamma irradiation of the cellular blood components is the most widely used procedure for susceptible patients to prevent transfusion associated-graft versus host disease (TA-GVHD). This serious complication of blood transfusion develops as a result of engraftment of transfused donor lymphocytes in the susceptible host that subsequently multiply

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and mounts an immune response against the host[1]. Gamma irradiation suppresses the proliferation of donor lymphocyte and the recommended dose of irradiation to stop this proliferation is between 25 to 30 Gy[2]. However, there is evidence that irradiation of cellular products may cause some untoward effects particularly to the erythrocytes such as decreased post-transfusion viability of red cells, increased red cells haemolysis and leakage of intracellular potassium[3].

The effects of gamma irradiation on the quality of blood products have previously been studied, however, the researches vary in respect of methodology, irradiation dose, red cell additive media, storage period etc. The earlier research carried out by Moore and Ledford in 1985 showed increased plasma-free-haemoglobin (PFH) in stored red cells after irradiation with 40 Gy. However, the effects were minimal and irradiation does not seem to cause serious damage to red cells[4]. Subsequently, other researchers investigated and showed inconsistent results. Hillyer et al (1991)[5] showed the irradiation effect of 35 Gy on packed red cell units were significant increase in plasma potassium (K^+) as early as day-14, however the PFH and lactate dehydrogenase (LDH) were minimally significant on day-42. Davey et al, (1992) [6] demonstrated significant increase in PFH and plasma K^+ on irradiated (30Gy) red cell units by day-42 of storage. Mintz & Anderson (1993)[7] did not find any significant difference between the irradiated (30 Gy) and non-irradiated group, although the PFH and plasma K^+ were significantly higher in both groups after 35 days of storage. However, Agarwal et al (2005)[8] showed a significant increase in plasma Hb in the blood units immediately after irradiation with 25 Gy, at day-1 as well as in the stored irradiated blood. Reverberi et al (2007)[3] irradiate red cells with 25 Gy and showed significant differences in percent haemolysis between irradiated and control red cells, although the percent haemolysis was very low in the first weeks of storage and the values remained lower than 1% even after 5 weeks. Similarly, significant change occurred in filtered red cells following irradiation[9]. However, frozen red cells did not show any significant damage following irradiation as compared to non-irradiated controls[10, 11]. X -ray irradiation also showed an acceptable level of percent haemolysis and plasma K^+ compared to that of gamma radiation[12]. All these results showed contrasting and inconsistent results.

In our center, all red blood cell components are collected from the central blood collection centre; the 'National Blood Centre' and after collection, these components are stored in the inventory blood fridge. Irradiation is done at our center in a batch of 5-10 red blood cell units that are taken from the inventory, and then stored in the inventory blood fridge for future request before supply to the patients. Therefore, these irradiated blood units are usually not the freshly collected units; but stored units before irradiation. In view of the inconsistent results by other researchers on the effects of irradiation on red blood cells units of different storage period, the objective of this study was to evaluate the effects of irradiation on the haematological and biochemical parameters of red blood cells units.

Method

This research was designed to explore the haematological and biochemical changes in the donor red blood cell units immediately before and after the standard irradiation procedure. This was a prospective comparative study conducted on randomly selected donor red blood cell (RBC) components at the Blood Bank unit, Department of Diagnostic and Laboratory Services, of Universiti Kebangsaan Malaysia Medical Centre (UKMMC) in year 2013. In this present study, the storage period (based on duration from the time of donation) of these sampled blood bag units were divided into two categories which were up to 14 days and >14 days to expiry. Total 18 units were included in each group.

The main aim of the research was to evaluate the changes in the red blood cells units of different storage period following gamma irradiation. Paired samples were taken before and after irradiation of the units and were tested for the haematological parameters such as plasma haemoglobin (Hb) level, percent haemolysis, haemoglobin per unit blood (Hb/unit), haematocrit (HCT), red cell indices: mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and biochemical parameters such as plasma sodium (Na^+), plasma potassium (K^+) and plasma LDH levels. The pre-irradiated sample was taken just before the irradiation and was used as the baseline, whereas the post-irradiated sample

was the test sample to investigate the effects of the irradiation to see the differences from the baseline following the irradiation process. Paired samples of these donor blood units were used to eliminate the confounding factor caused by sample variability.

To measure the total red blood cell haemoglobin (RBC Hb), HCT, MCV, MCH and MCHC, full blood count (FBC) was performed using automated haematology analyzer, ('Beckman Coulter LH 750 analyzer'). Hb/unit was calculated using the formula Hb/unit = total RBC Hb X volume/100 where total RBC Hb was derived from FBC and blood volume was recorded from the blood bag label. Plasma Hb was measured by 'HemoCue Plasma/Low Hb System'. Percent haemolysis was calculated by using the formula: (100 - HCT) X plasma Hb (g/dl) / Total RBC Hb (g/dl) (Sowemimo- Coker 2002)[13] where HCT and total RBC Hb were derived from the FBC and plasma HB by HemoCue Plasma/Low Hb System. Na^+ and K^+ levels were determined by the Cobas Integra 800-ISE model. The quantitative determination LDH was also done by the COBAS INTEGRA systems.

Data was collected and analysed using statistical package for Social Sciences (SPSS) version 19. Data were presented as mean ± *Sd* values to describe the different variables. The comparison between the red cells of two different storage period of up to 14 days and >14 days in both pre and post irradiation sample was done using independent-samples't' test. Paired-samples t-test was used to compare between the red cells parameters at pre and post irradiation in each group of 14 days group and >14 days to evaluate any significant changes caused by irradiation process. The significance level was $p< 0.05$ and statistical power of 95%.

Ethical approval was received from the clinical trials ethics committee of Universiti Kebangsaan Malaysia Medical Centre (UKMMC) before data collection was initiated.

Results:

Table-1 and 2 showed comparison between two different storage periods of upto 14 days and >14 days in pre and post irradiated samples showed significant changes in the plasma Hb, percent haemolysis, plasma Na^+, K^+ and LDH levels. Other parameters such as HCT, Hb/unit and red cell indices did not show significant changes (table-1 and 2).

Table 1: Comparison between red cell components stored for 14 days and >14 days in the pre irradiated samples

Variables	14 days	>14 days	P value
	Mean (*SD*)	Mean (*SD*)	
Plasma Hb (g/dl)	0.06 (.03)	0.12 (.07)	0.005*
Haemolysis (%)	0.13(.06)	0.26 (.15)	0.001*
Hb / unit (g)	65.47 (8.90)	61.38(8.87)	0.176
HCT (%)	60.96 (7.04)	58.08 (6.88)	0.224
MCV (fl)	90.63 (6.51)	92.53 (4.19)	0.308
MCH (pg)	28.57 (2.32)	29.37 (1.42)	0.224
MCHC (g/dl)	31.5 (0.76)	31.77 (.60)	0.243
Na^+ (mmol/L)	133.64 (8.08)	125.74 (7.23)	0.004*
K^+ (mmol/L)	24.13 (3.52)	29.82 (0.76)	0.000*
LDH (U/L)	1144 (468.21)	1813.33 (472.23)	0.000*

*= significant

Table 2: Comparison between red cell components stored for 14 days and >14 days in the post irradiated samples

Variables	14 days	>14 days	P value
	Mean (*SD*)	Mean (*SD*)	
Plasma Hb (g/dl)	0.07 (.03)	0.13 (.07)	0.007*
Haemolysis (%)	0.18 (.08)	0.28 (.15)	0.021*
Hb / unit (g)	59.91(6.15)	61.76 (9.44)	0.613
HCT (%)	57.31 (2.98)	57.91 (4.69)	0.651
MCV (fl)	89.98(6.83)	92.18(3.95)	0.247
MCH (pg)	28.27 (2.64)	29.15 (1.60)	0.238
MCHC (g/dl)	31.53 (.62)	31.55 (.65)	0.938
Na^+ (mmol/L)	132.96 (6.34)	125.71 (7.62)	0.004*
K^+ (mmol/L)	24.09 (3.20)	29.82 (0.78)	0.000*
LDH (U/L)	1161.44 (497.13)	1856 (426.28)	0.000*

*= significant

Table-3 showed the comparison between the pre and post irradiated red cell parameters in each group of 14 days and >14 days. It showed significant changes in the plasma Hb, percent lysis, Hb/unit, HCT, MCV and MCH in 14 days group while only in plasma Hb, MCV and MCH in >14 days group. However, changes in Na+, K+, and LDH were not significant in both groups.

Table 3: Comparison between pre and post irradiated red cell components stored for 14 days and >14 days

Up to 14 days	Variables	Pre	Post	Sig diff
	Plasma Hb	0.06 (03)	0.07 (.03)	0.001*
	% lysis	0.13 (.06)	0.18(.08)	0.001*
	Hb / unit	65.47 (8.9)	59.91 (6.15)	0.005*
	HCT	60.96 (7.04)	57.31(2.98)	0.051*
	MCV	90.63 (6.51)	89.98 (6.83)	0.001*
	MCH	28.57 (2.32)	28.27(2.64)	0.038*
	MCHC	31.50 (0.76)	31.53 (0.62)	0.768
	Na^+	133.64 (8.08)	132.96 (6.34)	0.599
	K^+	24.13 (3.52)	24.09 (3.20)	0.947
	LDH^+	1144.00 (468.21)	1161.44 (497.13)	0.435
>14 days to expiry	Plasma Hb	0.12(07)	0.13(.07)	0.002*
	% lysis	0.26 (.15)	0.28(.15)	0.410
	Hb/unit	61.38 (8.87)	61.76 (9.44)	0.797
	HCT	58.08 (6.88)	57.91 (4.69)	0.901
	MCV	92.53 (4.19)	92.18(3.95)	0.057*
	MCH	29.37(1.42)	29.15 (1.60)	0.009*
	MCHC	31.77 (0.60)	31.55 (0.65)	0.110
	Na^+	125.74 (7.23)	125.71 (7.62)	0.970
	K^+	29.82 (0.76)	29.82 (.78)	0.331
	LDH	1813.33 (472.23)	1856.00 (426.28)	0.357

*= significant

Discussion

This study gives us an insight about the effects of irradiation on stored red blood cells. The American Association of Blood Banks (AABB) recommends a dose of 25 Gy to the central area of the component with no portion receiving <15 Gy for an effective irradiation procedure[14]. In US, the irradiated red cells products can be stored up to 28 days after irradiation or up to original outdate whichever one comes first and there is no restriction on the age of the blood product for irradiation[15]. UK guideline recommends a minimum dose required is 25 Gy with no part should receive more than 50 Gy. They recommended red cells of up to 14-day after collection can be irradiated and stored for at least a further 14 day without significant loss of viability[2, 7]. This strict guideline concerns about the quality of the red cell products because it is evidenced that gamma irradiation can cause reduced red cell recovery after transfusion and extracellular potassium level. . Studies showed that red cells units irradiated within 24 hours after collection and subsequently stored for 28 days had a reduced 24hour recovery compared to the non irradiated red cells; but the level was still above the minimum acceptable level of 75%, while units stored for 42 days had unsatisfactory viability[16, 6, 17] . Loss of viability was the same whether the cells were irradiated on day-1 or day-14[16, 7, 17]. In addition to that irradiation also results in accelerated leakage of potassium into the plasma causing an increase in the level of extracellular potassium[2]. The mechanism of radiation induced bio-molecular damage involve the generation of free radicals followed by their attack on protein, lipid and carbohydrates[18].

In our centre, the donor blood units are subjected to gamma irradiation of 25 Gy and stored up to 28 days after irradiation or up to original outdate whichever one comes first. If the age of the blood product is more than 28 days, then after irradiation they stored for further 5 days or up to original outdate whichever one comes first. So the red blood cells at different period of storage are subjected to irradiation. Therefore, the current research is designed to identify the effects of gamma-irradiation on the quality of the red blood cells at different storage period at our center.

Red cell haemolysis is an important marker to assess the changes of the red cells following irradiation. In the present study, comparison at two different red cells storage groups i.e. period of up to 14 days and >14 days, showed significant changes in red cell haemolysis. In pre irradiated samples, this reflects the gradual storage changes over time even without going through the irradiation process. On the other hand, the changes between pre and post–irradiation in each group of up to 14 days storage and >14 days storage showed significant changes in the plasma haemoglobin indicates irradiation itself also causes some changes (table-3). This study showed similarity with previous study where free haemoglobin levels were increased in stored irradiated red cell components (Weiskopf et al, 2005)[19] but remain within acceptable limits.

Regarding the biochemical values of Na^+, K^+ and LDH, comparison at different storage period of 14 days and >14 days showed significant changes in Na^+, K^+ and LDH which reflects gradual storage changes in both pre and post irradiated samples. The changes in the pre irradiated samples reflects gradual storage change (Table-1 and 2). However, there were no significant changes between pre and post –irradiation in each group of up to 14 days storage and > 14 days storage (table-3). Potassium efflux form RBC and concomitant influx of Na^+ occurs

during the storage of RBC and gamma irradiation enhances the phenomena[7]. This present study complies with the previous studies on the storage changes of Na^+ and K^+. Previous study have shown that the Na^+ level gradually decreased in the stored blood from 169 mmol/l at day 0 to 159 mmol/l at day-14 and 153 mmol/l at day-35[20] which is higher than our present study. Our study results showed the K^+ level of 24.13mmol/L and 24.09mmol/L in the 14 days stored blood group which has similarity with that of the seven day old blood units where the K^+ concentration was 23 mmol/L as mentioned by Vengelen-Tyler (1999)[21]. Significant changes at different storage period could be explained by the fact that during storage, red cells showed gradual storage changes. The storage lesion involves the influxe of Na+ ions and effluxe of K^+ ions since the Na-K pump is inactive at 4°C. There is massive entry of Na+ ion into the cell while K+ ions exit from the cell. However, this is a reversible process and it takes 24 hours to restore the physiological gradients for sodium, and up to 4 days for potassium[22]. The increased plasma potassium imposes a risk to the neonates and children undergoing cardiac surgery, or exchange transfusion and any patient receiving massive transfusion such as trauma surgery and liver transplantation as hyperkalaemia induces cardiac arrhythmia leading to cardiac arrest[23].

Elevated serum LDH levels have been observed due to haemolysis of red cells. Yong & Rudmann(1995)[20] showed in their experiment that during storage, LDH levels gradually increases from 296U/L at the beginning to 1222U/L at day-14 and 1816 U/L at day-35 in red blood cells. Our study has shown similar higher results during storage. However, the irradiation procedure did not significantly changes the biochemical markers in both the groups.

Conclusion

Gamma irradiation of RBCs from donor units resulted significant storage changes at different time period of 14 days and >14 days as evident from the parameters of plasma Hb level, % red cell haemolysis, plasma Na^+, K^+ and LDH levels. These are important parameters for assessing the quality of the red cells. However, radiation effect on the red cells in each group of up to 14days and >14 days showed significant changes in the plasma Hb, MCV and MCH without any effect on plasma Na^+, K^+ and LDH levels. In view of the statistically significant changes in red cells stored up to 14 days and >14 days, it is recommended to irradiate the red cells units that are within 14 days, thus to minimise the adverse effects added on by the irradiation process especially the high K^+ effect which is harmful for the neonate. We recommend to irradiate the donor red cell unit within 14 days of collection to increase the effectiveness of red cell usage as well as patient safety. Further studies are suggested to evaluate the changes in the stored irradiated red cells at different time period. So that more precise result can be obtained in view of the red cell changes with irradiation and subsequent storage duration.

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