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# Irradiating stored blood and storing irradiated blood: Is it different? - A study of serial changes in biochemical parameters of red blood cell units

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## Abstract:

**INTRODUCTION:** Gamma Irradiation of blood products accentuates biochemical changes in the blood stored at 4°C. This study tried to compare the changes in potassium, sodium, glucose, lactate, and lactate dehydrogenase (LDH) levels in packed red blood cell (PRBC) units irradiated at various time points and then stored versus those stored for a particular period then irradiated.

**METHODOLOGY:** One hundred and eighty units of RBCs were randomly assigned equally to be irradiated or not. Eighteen units each were irradiated by gamma irradiator using cobalt 60 (BI 2000) on day 1, 7, 14, 21, and 28 of their storage, respectively, in the irradiation group. All the units were assessed for their plasma levels of potassium, sodium, glucose, LDH, and lactate by clinical chemistry auto analyzer Beckman coulter AU680 weekly. The values were documented and analyzed by SPSS.

**RESULTS:** Baseline values on day 1 for studied biochemical parameters were comparable between irradiated and nonirradiated groups. Mean values of potassium, lactate and LDH were higher in irradiated than nonirradiated PRBC bags. In contrast, Sodium and Glucose mean values were lower than baseline values. Maximum cumulative mean values were noted in day-21 irradiated bags when the parameters were measured on day-28 for potassium and lactate levels. This was followed by day 14 irradiated bags, followed by day 7 irradiated bags.

**CONCLUSION:** The study indicates that irradiation of red cells later in their storage period had comparatively more detrimental changes in relation to potassium and lactate than irradiation in earlier days. Consideration of irradiation to be performed as close to the issue as possible to reduce a lesser number of days of storage postirradiation is to be explored.

## Keywords:

Blood storage, irradiation, serial biochemistry, storage lesions

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## Introduction

Gamma irradiation of blood products, performed to prevent Transfusion-associated Graft Versus Host disease in recipients, accentuates the various biochemical changes in the blood cells called "storage lesions." Potassium (K<sup>+</sup>) leaks out of the cell due

to the inactivation of the Na<sup>+</sup>-K<sup>+</sup> pump due to the reduction of adenosine triphosphates (ATPs). Red cells in stored blood metabolize glucose by anaerobic means, and lactate is produced as a result of it. The increase in K<sub>e</sub>, reduction in ATP, decreased pH, and increased lactate dehydrogenase (LDH) and plasma free hemoglobin have been greater in the irradiated than the non-irradiated units by Day 142,842.<sup>[1,2]</sup> Irradiation

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thus brings down the shelf life of packed red blood cells (PRBC) from 42 or 35 days to 28 days.<sup>[3,4]</sup>

Irradiated RBC are increasingly utilized for both children and adults immunosuppressed or on chemotherapeutic regimens. With this growing demand, there has been an increasing need for transfusion services to store previously irradiated blood until needed for transfusion.<sup>[5]</sup> Unfortunately, the facility to irradiate is not readily available at many centers. This necessitates the blood centers to irradiate and maintain the requisite stock well in advance of transfusion, whereas in centers wherein irradiation is readily available, it is performed just before transfusion. Therefore, we performed this study to compare these two practices with regard to levels of various biochemical parameters such as Na<sup>+</sup>, K<sup>+</sup>, glucose, lactate, and LDH in the blood products.

The objective of this study was to assess and compare the cumulative changes in levels of these biochemical parameters in packed red cells, which were irradiated and then stored, versus packed red cells, which were stored for a particular period and then irradiated. In addition, it intends to provide data reflecting on the effect of irradiation of RBCs should it be performed after a defined antecedent storage period, combined with the maximal recommended storage period after irradiation.

## Materials and Methods

### Design

This was a descriptive longitudinal study comparing two groups – irradiated and non-irradiated blood bags. Twenty blood bags consecutively from blood collected in citrate phosphate dextrose adenine and saline adenine glucose mannitol equally on the first working day every month were collected for nine consecutive months, starting July 2017. Component separation was done as usual into PRBCs, platelet concentrates, and fresh frozen plasma by the platelet-rich plasma method. Ten of these bags were randomly designated to be Irradiated or not. The irradiation on the bags was done on different storage days, namely Day 1, 7, 14, 21, and 28 after collection. Hence, we had 18 bags each, irradiated on these different days totaling 90 blood bags. Figure 1 summarizes the study design and procedure.

### Sample size calculation

The sample size was calculated assuming an expected difference in the level of K<sup>+</sup> between the groups as 1 mEq/dL with a standard deviation of 2 mEq/dL at a power of 90% and an alpha error of 0.05. K<sup>+</sup> was chosen since the changes in the level of Na<sup>+</sup>, LDH would be relatively higher, and hence the one calculated would suffice to detect the significant changes in them.<sup>[6]</sup> The sample size was calculated as a minimum of 85 in each

group (170-total) by Open Source Calculator-SS Mean, Open epi version-3.

### Sample collection

On the day of sample collection, bag contents were mixed well; the segment was stripped off of the blood entirely and sealed with a clip at the start of the segment from the bag. The bag was made to stand undisturbed in the refrigerator overnight. The clip was removed slowly without disturbing the bag and allowed the supernatant plasma to fill up the segment of the bag. Then approximately 2–3 inches of the segment was sealed and taken for sampling procedure. First, the segment contents were taken into a test tube, and contents were centrifuged at 2000 rpm for 2 min to make sure red cells did not contaminate the sample and capped. Next, clear supernatant plasma was taken into a plain sampling tube and used for sample analysis. All samples were collected 1 h post-irradiation. None of the blood bags was leukoreduced.

### Study procedure

Irradiation of the units was performed using BI 2000 Cobalt 60; self-contained, turn-table gamma cell irradiator with 810 curie activity. The dose delivered to each bag was a minimum of 25 Gy requiring 4 min 30 s to 4 min 45 s of exposure, based on the decay rate.

Estimation of supernatant plasma potassium, sodium, glucose, lactate, and LDH was done by the Clinical Chemistry auto-analyzer, Beckman Coulter AU680, as per the departmental protocol and manufacturer's instructions. Data of serial biochemical values were noted at weekly intervals and documented in the pro forma for assessment at the end of the study.

### Statistical analysis

All the data were entered into Microsoft Excel, and statistical analysis was performed using SPSS for Windows version 20 (SPSS IBM Corp. Ltd. Armonk, NY). The distribution of data on categorical variables was expressed as percentages. The continuous data such as volume of PRBC, hematocrit in the bag, plasma Sodium, Potassium, Lactate, LDH, and glucose levels were expressed as mean with standard deviation. The association of continuous variables with irradiation was carried out by an independent Student's *t*-test. Generalized linear model was used to assess the changes in the levels of Na<sup>+</sup>, K<sup>+</sup>, glucose, lactate, LDH over time. A *post hoc* analysis was performed for these variables in the different groups irradiated and followed up at different time points. Statistical analysis was carried out at a 5% level of significance.

### Statement of ethics and consent

The Institutional Ethics Committee approved the study vide infra letter no. JIP/IEC/2016/1140 dated

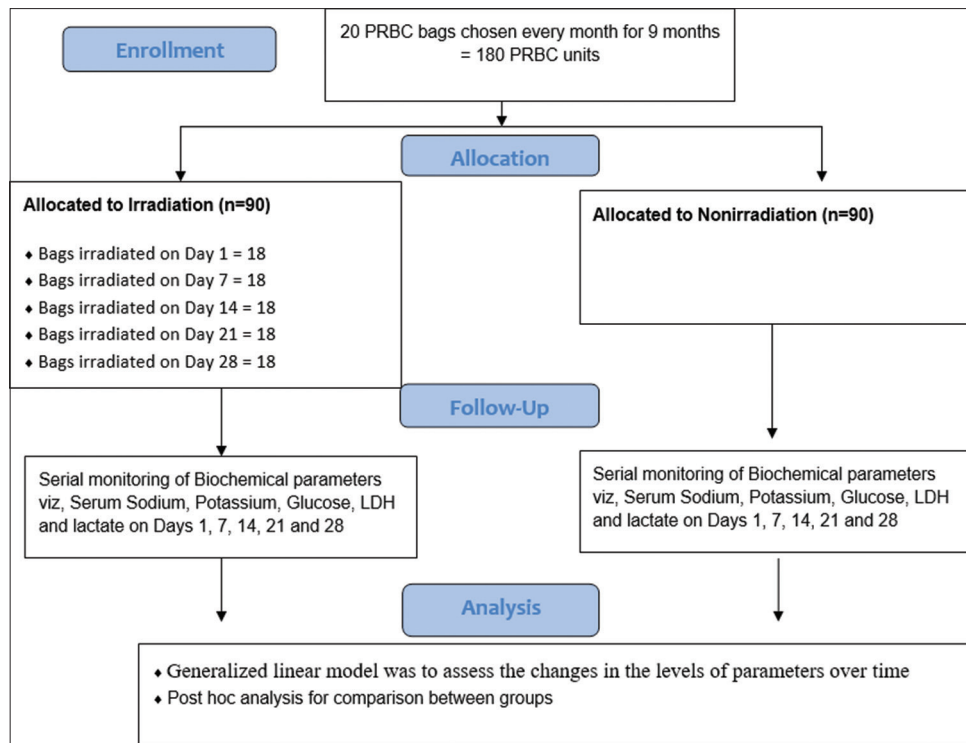


Figure 1: Flow diagram to show the study design and procedure

March 08, 2017 with a waiver of consent as there was no data collected from the study participants in any form.

## Results

A total of 180 PRBC bags, out of which 90 each were irradiated and non-irradiated, were included in the study. In the irradiated group, the irradiation was done on day-1, 7, 14, 21, 28 of storage for 18 bags each. The distribution of various blood groups in our study shows that O positive and B positive bags together contributed to more than two-third (one-third each) of the total bags studied. All the blood bags met the Quality Criteria as per departmental SOP, which is formulated based on the Director-General of Health Sciences (DGHS) criteria.

It was seen that there was no difference between irradiated and non-irradiated Day-1 baseline values for all the biochemical parameters. The mean values of biochemical parameters and their difference between non-irradiated and irradiated blood bags on Day-1 (baseline values) and Day-28 (last day of storage) are depicted in Table 1. The mean values were observed to be similar in Day-28 irradiated and non-irradiated groups for all the parameters. The tracings of mean values for the levels of potassium, sodium, glucose, lactate, and LDH are shown in Figures 2-6 respectively. Mean Potassium, Lactate, and LDH levels increased, and sodium, glucose values decreased from Day-1 to 28 in all the groups

### Potassium

The mean potassium levels in various groups (irradiated at different times) are shown in Table 2. There was no difference between mean values of plasma Potassium on Day-1 among various groups. The mean values are observed to be similar in Day-28 irradiated and non-irradiated groups. Maximum mean values are noted in Day-21 irradiated bags on Day-28. The non-irradiated group has the minimum mean potassium values of all. The cumulative potassium levels were almost linearly higher in the blood bags irradiated at a later date from the collection as shown in Figure 2 and were statistically significant in *post hoc* analysis

### Sodium

The mean sodium levels in various groups (irradiated at different times) are shown in Table 3. Mean plasma sodium values have reduced significantly from Day-1 to Day-28 in all the groups irrespective of irradiation status. However, the fall is more profound in irradiated groups than in non-irradiated groups ( $P < 0.05$ ). *Post hoc* analysis of sodium levels comparison showed that the cumulative fall in sodium was higher in Day 1, Day 7, and Day 14 irradiated groups than Day 21 and Day 28 irradiated blood bags, which was statistically significant.

### Glucose

The mean glucose levels in various groups (irradiated at different times) are shown in Table 4. Mean plasma Glucose values reduced significantly from Day-1

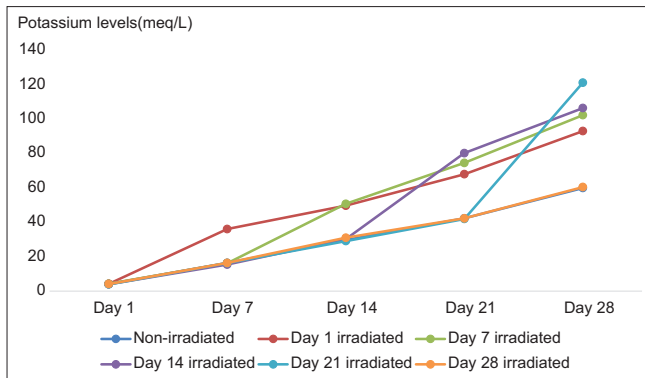


Figure 2: Changes in potassium levels over time in blood units irradiated at the various time period

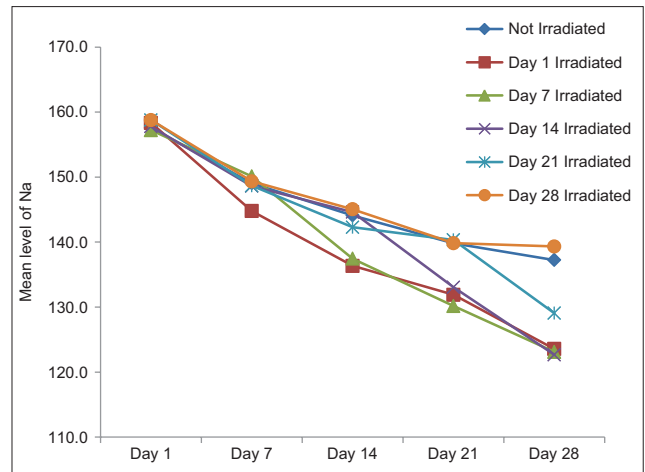


Figure 3: Changes in sodium levels over time in blood units irradiated at the various time period

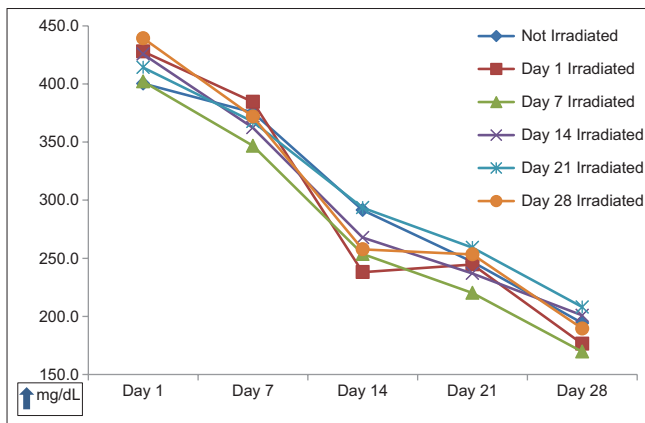


Figure 4: Changes in glucose levels over time in blood units irradiated at the various time period

to Day-28 in all the groups irrespective of irradiation status. The fall is more profound in irradiated groups than non-irradiated groups ( $P < 0.05$ ). The cumulative fall in glucose levels was comparatively more in Day 7 and Day 14 irradiated than Day 21 and Day 28 group; however, this was not statistically significant. Irradiation after Day 14 did not make much difference to the glucose levels.

### Lactate

The mean levels of lactate in various groups (irradiated at different times) are shown in Table 5. It is seen that level of plasma lactate was maximum in Day-21 irradiated bags of all groups. This was followed by Day 14 irradiated, followed by Day 7 irradiated bags, followed by Day 1 irradiated bags. Minimum increment is noted in the nonirradiated group and Day-28 irradiated group of all. The longer the blood bag was stored before irradiation, the larger was the cumulative lactate content in the blood bag, which was statistically significant with a  $P < 0.001$ .

**LDH:** The comparison of LDH levels for blood bags irradiated at different days is given in Table 6. It shows that the levels of LDH between the non-irradiated and irradiated groups are significantly different ( $P < 0.001$ ) at day-28. The values were noticed to increase over time

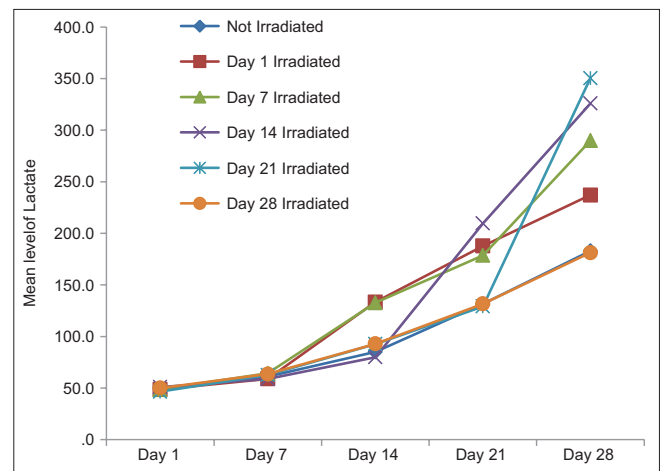


Figure 5: Changes in lactate levels over time in blood units irradiated at the various time period

and with irradiation. The mean levels of LDH in various groups are shown in Figure 6. It is seen that level of plasma LDH is maximum in Day-1 irradiated bags of all groups. Minimum increment is noted in the non-irradiated group and Day-28 irradiated group of all. The *post hoc* analysis for comparison of LDH levels shows that the levels of LDH between the non-irradiated and day 1 irradiated groups are significantly different ( $P < 0.001$ ) at day 7. In a similar manner Day, 7 irradiated group had mean LDH levels higher than subsequently irradiated units at later dates; Day 14 irradiated had higher levels than the ones irradiated on Day 21 or Day 28 irradiated bags and so on. The increase in mean values was maximum in the week following irradiation, respectively, in all the groups.

### Discussion

All the bags met the DGHS and AABB criteria of volume and minimum hematocrit values.<sup>[7,8]</sup> Irrespective of the day of irradiation, the increase in potassium in

**Table 1: Mean differences between day 1 and day 28 in levels of various biochemical parameters between irradiated and nonirradiated blood**

Variables	Nonirradiated			Irradiated			t-test score, P
	Day 1	Day 28	Mean difference±SD	Day 1	Day 28	Mean difference±SD	
Potassium	4.05	59.99	55.9±8.9	4.1	101.85	92.5±25.6	12.81, <0.001
Sodium	157.58	137.23	-20.4±8.6	158.17	127.5	-30.6±9.6	7.53, <0.001
Glucose	400.25	194.29	-205.9±111.9	421.76	188.8	-232.9±94.9	1.741, <0.001
Lactate	49.8	182.93	133.1±42.9	48.9	276.9	227.9±87.4	9.233, <0.001
LDH	232.65	943.16	710.5±338.4	229.6	2063.65	1924.9±885.01	12.159, <0.001

\*Negative sign indicates a reduction in average values. SD=Standard deviation, LDH=Lactate dehydrogenase

**Table 2: Comparison of serial mean plasma potassium levels on different days of storage in irradiated and nonirradiated groups**

Group	Mean±SD					Statistical significance (P)
	Day 1	Day 7	Day 14	Day 21	Day 28	
Not irradiated	4.06±0.79	16.37±4.50	29.65±6.43	42.30±7.32	59.99±9.01	<0.001
Day 1 irradiated	4.18±0.88	36.08±10.76	49.68±6.15	67.94±6.24	93.00±16.54	<0.001
Day 7 irradiated	4.28±0.53	16.16±4.10	50.69±6.92	74.52±14.62	102.23±17.57	<0.001
Day 14 irradiated	4.03±0.69	15.43±4.48	30.20±5.31	80.21±11.40	106.36±16.96	<0.001
Day 21 irradiated	3.92±0.95	16.29±4.47	29.07±5.48	41.93±6.42	121.08±17.02	<0.001
Day 28 irradiated	4.12±0.77	16.37±3.48	31.01±6.16	42.30±8.15	60.44±10.78	<0.001
Statistical significance (P)	0.800	<0.001	<0.001	<0.001	<0.001	<0.001

SD=Standard deviation

**Table 3: Comparison of serial mean plasma sodium levels on different days of storage in irradiated and nonirradiated groups**

Group	Mean±SD					Statistical significance (P)
	Day 1	Day 7	Day 14	Day 21	Day 28	
Not irradiated	157.59±6.79	149.04±7.23	144.09±7.66	139.82±5.57	137.23±5.90	<0.001
Day 1 irradiated	158.33±5.02	144.78±4.31	136.33±5.32	131.89±2.35	123.56±6.10	<0.001
Day 7 irradiated	157.22±6.70	150.11±6.96	137.44±4.06	130.17±4.12	123.11±4.57	<0.001
Day 14 irradiated	157.78±6.79	148.61±7.32	144.61±6.47	133.06±5.51	122.67±4.26	<0.001
Day 21 irradiated	158.78±6.17	148.67±6.43	142.28±8.14	140.33±5.36	129.06±6.05	<0.001
Day 28 irradiated	158.78±7.19	149.33±5.21	145.06±7.17	139.83±6.01	139.33±6.79	<0.001
Statistical significance (P)	0.593	0.204	<0.001	<0.001	<0.001	<0.001

SD=Standard deviation

**Table 4: Comparison of serial mean plasma glucose levels on different days of storage in irradiated and nonirradiated groups**

Group	Mean±SD					Statistical significance (P)
	Day 1	Day 7	Day 14	Day 21	Day 28	
Not irradiated	400.26±92.72	375.54±82.35	291.48±79.80	246.88±72.84	194.30±62.56	<0.001
Day 1 irradiated	427.89±70.19	384.56±101.22	238.11±53.57	244.72±79.19	176.50±51.55	<0.001
Day 7 irradiated	402.11±79.20	346.72±69.06	253.67±65.80	220.22±55.23	169.72±41.15	<0.001
Day 14 irradiated	425.61±72.15	362.28±68.35	267.78±87.63	236.94±40.12	200.83±56.20	<0.001
Day 21 irradiated	414.11±79.84	367.56±67.01	293.50±89.24	259.22±71.11	208.00±60.13	<0.001
Day 28 irradiated	439.33±93.90	371.89±67.79	257.67±71.16	253.39±67.26	189.56±63.75	<0.001
Statistical significance (P)	0.452	0.736	0.046	0.595	0.342	<0.001

SD=Standard deviation

the irradiated group was more in comparison to the nonirradiated group. The difference of means of plasma potassium in nonirradiated bags was 55.9 mmol/L, and in irradiated bags, it was 92.5. This difference was also statistically significant, with a  $P < 0.001$ . The difference of plasma potassium mean was comparable to a study conducted by Jeter *et al.*<sup>[5]</sup> The efflux of potassium can

explain this change out of the cell during the storage period. These changes are enhanced by irradiation, which is the reason for increased mean potassium in irradiated groups. It is to be noted that at 37°C, increased permeability caused by radiation is counterbalanced by the activity of the Na<sup>+</sup>-K<sup>+</sup> pump. However, since these blood units are stored at around 4°C–6°C, and

**Table 5: Comparison of serial mean plasma lactate levels on different days of storage in irradiated and nonirradiated groups**

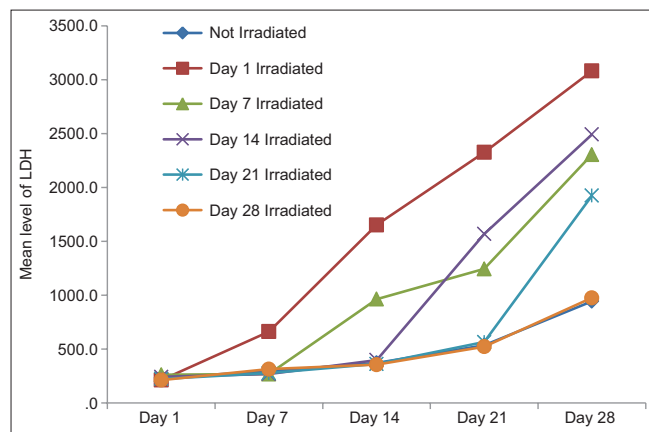
Group	Mean±SD					Statistical significance (P)
	Day 1	Day 7	Day14	Day 21	Day 28	
Not irradiated	49.81±13.29	61.11±13.84	85.03±12.49	131.58±23.15	182.96±41.05	<0.001
Day 1 irradiated	48.61±15.08	59.00±10.15	133.33±16.49	187.78±29.78	237.11±46.89	<0.001
Day 7 irradiated	48.83±10.58	64.33±10.49	132.67±12.64	178.67±24.02	289.89±64.28	<0.001
Day 14 irradiated	51.00±15.68	58.94±13.93	79.94±11.55	209.61±38.04	325.83±65.55	<0.001
Day 21 irradiated	46.56±11.80	62.78±12.66	92.72±9.55	129.17±20.94	350.39±83.80	<0.001
Day 28 irradiated	50.06±10.23	63.67±11.66	92.94±12.30	131.67±14.86	181.28±46.98	<0.001
Statistical significance (P)	0.929	0.699	<0.001	<0.001	<0.001	

SD=Standard deviation

**Table 6: Comparison of serial mean plasma lactate dehydrogenase levels on different days of storage in irradiated and nonirradiated groups**

Group	Mean±SD					P
	Day 1	Day 7	Day 14	Day 21	Day 28	
Not irradiated	232.66±55.11	294.97±73.86	371.77±142.02	534.51±202.84	943.17±332.91	<0.001
Day 1 irradiated	211.61±50.90	661.44±161.38	1651.06±420.75	2325.17±248.14	3080.50±239.91	<0.001
Day 7 irradiated	261.17±47.33	268.00±68.68	963.39±287.15	1243.83±333.03	2304.83±650.17	<0.001
Day 14 irradiated	241.67±60.78	268.22±68.82	397.17±203.12	1567.56±224.19	2490.50±457.06	<0.001
Day 21 irradiated	222.33±60.58	275.89±78.56	361.06±127.61	564.22±173.85	1923.17±691.16	<0.001
Day 28 irradiated	211.78±51.11	313.72±77.02	355.11±111.66	521.44±155.90	974.39±550.49	<0.001
Statistical significance (P)	0.054	<0.001	<0.001	<0.001	<0.001	

SD=Standard deviation

**Figure 6:** Changes in lactate dehydrogenase levels over time in blood units irradiated at the various time period

these pumps are almost inactive due to lack of ATP, the changes persist. The sodium usually counterbalances the efflux of potassium, and hence their levels in the plasma supernatant fall.<sup>[5,9]</sup>

When we observe Day-1 values of plasma mean potassium, there is no significant difference seen in all the six groups. It is baseline values for all the bags, and the readings are taken within few hours after component preparation, including the irradiated group, wherein sampling is done within 2–3 h after irradiation. This means that there are no immediate effects of irradiation on red cell storage lesions.

The biochemical changes in nonirradiated and 28-day irradiated red cells units followed a similar trend, and the changes were observed to be the least when compared with other irradiated red cell groups, and they had the least number of lesions among all the groups. However, day-1, 7, 14, 21 irradiated groups had storage lesions in increasing order. Maximum lesions were found in Day-21 irradiated group. This infers that the later the irradiation of PRBCs in the storage period, the more the storage lesions were noted. However, many studies have found that changes in biochemical parameters do not directly cause any adverse event in a recipient and can be safely transfused up to 28 days' postirradiation.<sup>[1,2]</sup>

A recent study by Eshghifar *et al.* showed similar results to our study. They investigated the impact of gamma irradiation on RBCs and suggested a storage time that the best time for irradiation after blood collection is up to 14 days. They also pointed out that the blood unit should be transfused as soon as possible after the irradiation.<sup>[10]</sup> Results in our study showed the changes in various biochemical parameters during different days of the storage period. It is seen that plasma potassium, lactate, and LDH levels gradually increased during the storage period, whereas sodium and glucose levels decreased significantly over time. It was noted that irradiated bags had more lesions than nonirradiated bags. It was also inferred that irradiation of red cells at an earlier storage period had comparatively fewer lesions than irradiation later from the collection. Red cells in stored blood

metabolize glucose by anaerobic means, and lactate is produced as a result of it. Glucose is used by red cells for their metabolism leading to a fall in their levels during storage.<sup>[11]</sup> LDH levels fall due to a fall in pH as they are released due to oxidative stress injury.<sup>[12]</sup> The homeostatic mechanism missing in the storage bag may increase enzyme formation with no scope for clearance.<sup>[13]</sup>

The proposition that the aged cells are more amenable to damage from ionizing radiation comes from the theory of blood cell antioxidant enzyme activities decreasing with age.<sup>[14]</sup> Ionizing radiation has shown to worsen the already unbalanced oxidant-antioxidant status in aging cells. Membrane damage response efficiency of cells is also shown to decline with age.<sup>[15]</sup>

Although these changes may not be of clinical significance in adults, hyperkalemia can be a worrisome complication in neonates, especially when large volumes are transfused as in exchange transfusion.<sup>[16]</sup> This would also affect the transfusion practice in neonatal intensive care units where a "mother bag" group O negative is irradiated and then used for aliquots for either one patient or multiple patients

The strength of our study was that the parameters were analyzed at weekly intervals irrespective of irradiation status, in contrast with most of the studies where they have analyzed parameters at 2–3 different points and not continuous. The limitation of the study was that the other parameters such as ATP, pH, supernatant hemoglobin, and 2, 3 DPG were not assessed in our study. Donor variables that affect biochemical results could not be negated in the study.

Studies can be undertaken to check *in vivo* survival and any adverse events in recipients to correlate laboratory values and *in vivo* effects of these changes.

## Conclusion

The study indicates that irradiation of red cells after the storage period had comparatively more detrimental metabolic changes than irradiation in earlier days in relation to potassium and lactate. Performing irradiation as close to the issue as possible may be considered, thereby reducing the storage post-irradiation to as many lesser days as possible. It may be advisable to choose the relatively fresh units for irradiation when they are not being transfused immediately.

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## Conflicts of interest

There are no conflicts of interest.

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