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The Effect of Pre-Storage Irradiation Blood on Quality of Red Blood Cells

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ABSTRACT

Background: Irradiation leads to increased storage lesions that may have harmful effects if transfused. Various storage lesions research has been carried out, and only very few articles are available on the impact of gamma irradiation on RBC storage lesions. Since there has been no study about finding the best time for irradiation, we decided to investigate the effect of irradiation on Red blood cells at different storage times after blood collection

Materials and Methods: A total of 40 units of red blood cells divided into two groups, irradiated and non-irradiated. Irradiated RBCs were divided into three groups and each group containing ten units. The remaining ten units were considered as non-irradiated controls. Sampling from these irradiated and non-irradiated blood units was performed weekly to evaluate biochemical parameters and free plasma hemoglobin/Hemolysis index levels.

Results: A significant increase in the mean values of plasma potassium, plasma Hb/Hemolysis index, and LDH, as well as a significant reduction in the mean value of 2,3 DPG and plasma sodium, were observed in both groups. Although the reduction of 2,3 DPG is extremely remarkable, it is compensated 24-48 hours after transfusion. Hence, the clinical result of 2,3-DPG-depleted RBC transfusion is known to be negligible. The irradiation group alteration was more notable than the non-irradiated one and the changes in the parameters were most significant in the group having been stored for a longer period after irradiation.

Conclusion: Our investigation on the impact of gamma irradiation on RBCs makes it possible to suggest a storage time up to 28 days after irradiation is permissible and the best time for irradiation after blood collection is up to 14 days. It is pointed out that the blood unit should be transfused as soon as possible after the irradiation.

Keywords: Red blood cells; Gamma irradiation; Storage lesions; Transfusion-associated graft-versus-host disease (TA-GVHD)

INTRODUCTION

Transfusion-associated graft-versus-host disease (TAGVHD) could be a debilitating, commonly fatal blood transfusion complication that arising from allogeneic donor white cells being grafted and clonally expanded. Gamma irradiation prevents TAGVHD but additionally affects and decreases the survival of red blood cells (RBCs). RBC concentrate is the portion suggested for patients with surgery, obstetric hemorrhage, anemia, or patients with an acute blood loss of more than 30 percent total blood

volume to increase the supply of oxygen to body tissue[1].

Pre-existing immunocompromised patient status, clinical difficulties, and usually fatal disease outcomes make it momentous to irradiate high-risk blood components[2].

Gamma Irradiation of blood elements is the preferred technique for preventing TA-GVHD in extremely immunocompromised recipients[3].

While gamma irradiation mainly targets lymphocyte nucleic acids that contaminate cellular blood components, it could also have impacts on non-lymphoid blood cells. Such results are minimal and probably medically insignificant. However, a notable modification in the properties of the elements is mentioned when RBCs are irradiated[1].

Gamma irradiation at a 25 Gy dose is efficient in prohibiting T-lymphocyte proliferation, but at the same time might contribute to red blood cell degradation[4, 5].

It has been shown that gamma irradiation and RBC damage made changes in intracellular hemoglobin, LDH, sodium, and potassium levels[4, 5].

Due to these findings, regulators have reduced the total storage period of irradiated RBCs. The U.S. Food and Drug Administration (FDA) has suggested that irradiated RBCs be stored until the end of their shelf-life, but not more than 28 days from the date of irradiation. The Council of Europe has advised that RBC irradiation not be conducted within 14 days of processing and that irradiated RBCs should not be processed within 14 days of irradiation[6].

Several kinds of researches with contrasting outcomes have been carried out and more research and studies are required in this field.

The main objectives of this study were to find the best time for irradiation after blood collection and evaluate the effect of changes during storage after blood collection.

To analyze the RBC lesions variations caused by various blood operations and storage time, we investigated the influence of irradiation on blood quality by measuring 2,3-diphosphoglycerate (2,3-DPG) levels, free hemoglobin (Hb)/ Hemolysis index, lactate dehydrogenase (LDH), potassium (K+) and sodium(Na+) concentration.

Since no similar research has been undertaken in our country, it is expected that this research will be useful as an analysis of irradiated products, which in effect will support the future management of blood products.

MATERIALS AND METHODS

The study was a random sample research design approved by the Iranian ethics committee of the High Institute of Research & Education in Transfusion Medicine. Clinical and laboratory data consisted of the date of blood collection, ABO blood group, gender, and age was performed. RBCs were prepared by using standard preparation methods and the blood was stored at 2 to 6℃.

A total of 40 units of WB in CPDA-1 anticoagulant solutions were collected from random, voluntary - healthy donors (Kerman Blood Centre, Tehran, Iran) and separated into red blood cells (RBCs). These RBCs are divided into two groups: irradiated and non-irradiated.

Irradiated RBCs were divided into three groups and each group containing ten units. Each blood unit was exposed to gamma irradiation of 25 Gy (Gammacell 3000 Elan; Best Theratronic, Ottawa, Canada).

In the first group, ten units were irradiated on Day +2, in the second group, ten units were irradiated on Day +9, and in the third group, ten units were irradiated on Day +14, after the date of blood collection. The remaining ten units were considered as non-irradiated controls.

In each group, sampling was performed weekly from 10 irradiated and 10 non-irradiated blood(control) units separately at the same time to evaluate biochemical parameters and Hemolysis index levels (first group on days 2, 9, 16, the second group on days 9, 16, 23, and third group on days 14, 21, 28, respectively).

The sodium (Na), potassium (K) concentrations were measured by direct ion-specific electrode method (Starlyte III, Alfa Wassermann, NJ, USA), lactate dehydrogenase was assayed using an LDH reagent kit Pars Azmoon company(Tehran, Iran).

The concentration of 2,3-DPG was measured using a 2,3-DPG ELISA kit (Zell Bio, Ulm, Germany), and free plasma hemoglobin concentration was determined based on spectrophotometrically

cyanmethemoglobin method by using Drabkin's Reagent kit (BaharAfshan, Tehran, Iran).
Plasma hemolysis levels were computed utilizing a validated formula as below:

Plasma Hb=(154.7*A_{415}-130.7*A_{450}-123.9*A_{700})
Hemolysis index (%) =Plasma Hb*100-HCT/ Total Hb

The results for irradiated and control units were compared at different storage periods. A Paired t-test of software SPSS 24.0 was used for statistical analysis and P=< 0.05 was considered to be significant and P>0.05 was considered non-significant different (N.S).

RESULTS

We assayed several parameters in the RBC units in both groups that were shown to change statistically. We have demonstrated that RBCs were modified by irradiation with 25 Gy.
During storage, the effect of gamma irradiation on RBC leads to increase LDH, hemolysis index, and potassium (K+).
All units of irradiated RBCs had potassium levels that were significantly higher than control. Immediately after irradiation, there was a slight and significant increase in the potassium concentration in the irradiated units compared to control. Changes in plasma potassium levels were evident by 24 hours and marked on day 7 after irradiation (Table 1).
On Day +14 after collection, there were no significant differences between irradiated RBCs and non-irradiated RBCs to the rate of hemolysis, LDH, and 2,3-DPG.
LDH concentrations increased with statistical significance for irradiated RBC on Days 14 and 28 (Table 1).
Hemolysis is one of the main RBC lesions. it is very notable about its medical consequences for transfused patients. As predicted, the hemolysis of RBCs during storage increased gradually. The mean rate of hemolysis in irradiated components was significantly higher than in non-irradiated ones.
On Days 28, statistically significant increases in RBC plasma free hemoglobin were indicated. Hemolysis was also increased in control units from a baseline level to the end of the shelf-life. In an irradiated unit, hemolysis was found to be significantly higher as compared to control at day 7 (Table 1).
The Na+ concentration of the non-irradiated group, served as the control and irradiation group was decreased during days 1–14, and it decreased to the lowest rate on day 14, But those of the irradiation group did not decrease significantly after day 14 (Table 1).
2,3-DPG plays an important role in RBC oxygen release. Along with the increasing storage time, the level of 2,3-DPG in each group decreased. It decreased rapidly ($p<0.001$) in the first three weeks of storage to almost undetectable levels. This level was not significantly different in the first week (Table 1).
Interestingly, after in vivo transfusion, levels of 2,3-DPG begin to recover within a few hours, however, may also take up to 72 hours to become virtually normal. For this purpose, it is established as a remarkable point to preserve the oxygen release role of stored RBC to prevent or reverse the 2,3-DPG decrease during storage.

Table 1. Level of parameters in irradiated and control red blood cells

Day	R	C	P
Potassium (mmol/L)			
Group 1			
2	6.71± 1.28	4.17 ± 0.34	0.05
9	15.94±3.95	11.60±1.84	0.001
16	22.01± 4.44	15.48±2.28	0.001
Group 2			
9	16.50±2.06	11.60±1.84	0.001
16	20.30±3.76	15.48±2.28	0.001
23	26.30±5.36	20.16±2.92	0.001
Group 3			
14	20.85±2.03	14.73±2.11	0.001
21	22.62±2.82	19.02±2.53	0.001
28	33.25±2.21	23.94±3.50	0.001
LDH (mmol/L)			
Group 1			
2	233.10± 38.02	224.10± 45.26	N.S
9	362.00±58.61	304.00±43.33	N.S
16	614.50± 120.53	529.80±78.72	N.S
Group 2			
9	508.70±163.69	304.00±43.33	N.S
16	705.60±181.91	529.80±78.72	N.S
23	953.20±264.23	817.00±148.09	N.S
Group 3			
14	577.50±198.70	514.00±116.65	N.S
21	820.20±216.11	749.20±134.04	0.03
28	999.30±185.80	920.80±125.80	N.S
Hemolysis index (%)			
Group 1			
2	0.02± 0.01	0.03 ± 0.17	N.S
9	0.05 ±0.02	0.05± 0.03	N.S
16	0.08± 0.01	0.09 ±0.04	N.S
Group 2			
9	0.06±0.04	0.05±0.03	N.S
16	0.08±0.04	0.09±0.04	N.S
23	0.16±0.06	0.13±0.05	N.S
Group 3			
14	0.12±0.08	0.07±0.02	N.S
21	0.15±0.08	0.12±0.04	N.S
28	0.23±0.08	0.19±0.07	N.S
Sodium (mmol/L)			
Group 1			
2	149.70 ± 1.15	148.50± 4.64	N.S
9	136.80±2.52	145.60±2.31	0.05
16	129.30±2.83	142.00± 2.53	0.05
Group 2			
9	143.40±1.17	145.60±2.31	0.05
16	133.50±2.67	142.00±2.53	0.05
23	123.50±3.13	136.50±2.95	0.04
Group 3			
14	133.90±2.18	143.00±2.58	0.05
21	125.10±3.03	137.40±4.83	0.04
28	120.60±2.50	135.60±3.59	0.04
2,3 DPG (µmol/ml)			
Group 1			
2	2.26±0.83	2.06±0.66	N.S
9	1.86±0.28	1.97±0.61	N.S
16	1.56±0.25	1.27±0.63	0.02
Group 2			
9	2.15±0.99	1.97±0.61	0.03
16	1.89±0.69	1.27±0.63	0.04
23	1.32±0.58	1.11±0.52	0.01
Group 3			
14	1.76±0.69	1.44±0.62	0.03
21	1.46±0.35	1.16±0.65	0.03
28	1.18±0.38	1.01±0.73	0.01

R: irradiated red cells, **C:** control, *P for each parameter at different storage time (Independent sample T- test). P=< 0.05 was considered significant and P>0.05 was considered non-significant different (N.S), ****Group 1**= Day 2,7,16 after blood collection, **Group 2**= Day 9, 16, 23 after blood collection, **Group 3**= Day 14, 21, 28 after blood collection

DISCUSSION

The impact of RBC storage lesions is imperfect and the clinical aspects of the safety and efficacy of stored RBC are still being assessed[7]. In this study, we realized that irradiation may lead to substantial damage in RBC and heightening the RBC lesions. Irradiation resulted in an extremely considerable difference in all measured variables relative to the non-irradiated group. Although gamma irradiation exacerbates storage lesions, the outcome of this study confirms that it is the accepted method to prevent TAGVHD[8].

As Reverberi et al.[9] pointed out, probably it is the best practice to irradiate the blood just before transfusion(9).we found that irradiation, in prestorage may cause significant damage in RBC and intensify the RBC storage lesions. Compared with the non-irradiated group, irradiation resulted in a significant difference in all measured factors. Due to oxidative damage caused by storage lesions, hemoglobin, lactate dehydrogenase (LDH) ,and potassium cations are released into the supernatant[10].

This study has found significant differences between irradiated and non- irradiated RBC. There has been an enhancement in potassium, LDH , and hemolysis levels, which has also been observed by previous studies [4,11-15]. Irradiation leads to distinctive leakage of potassium ions and LDH from Day + 21 to the end of the examined storage period. On Day +28, the difference in potassium levels in the supernatants between irradiated and nonirradiated units was higher than on other Days. RBC lesion induces a gradual increase in hemolysis. Besides biochemical parameters, it was observed that RBC lysis occurred late during storage.

There have also been significant differences between the levels of plasma haemolysis in irradiated and non-irradiated groups. The hemolysis rate on Day +28 was only slightly enhanced in irradiated units.

Therefore, Hemolysis is regarded as an important marker for the performance evaluation of stored RBCs as other researchers have also stated[14, 16].

our attention was drawn to some serious modifications. While on day 28, the potassium concentration of the irradiation group was over 40 mmol/L and the sodium concentration was below 85 mmol/L. The High concentration of potassium might lead to acidosis and potassium toxicity.

RBC oxygen affinity is known to increase during storage and 2,3 DPG controls the ability to release oxygen from RBC by increasing the affinity of Hb oxygen.

2,3 DPG plays a remarkable impress in RBC oxygen release. A reduction in the 2,3-DPG levels specifically enhances the RBC affinity for oxygen, decreases RBC's viability after infusion, extends the duration of RBC movement via capillaries, promotes inflammation ,and directly modifies the clinical infusion, However, the 2,3 DPG level will be compensated 24-48 hours after transfusion[17].

The parameters tested yielded similar outcomes to earlier researches in developed countries. After long time of storage, the 2,3-DPG concentration levels were very low in both groups, indicating serious damage to the RBCs.

Although some storage lesions may occur within days or weeks, evaluated potassium and LDH levels may be identified within hours of storage[18]. All these changes affect blood rheology and may lead to transfusion difficulties[19].

In conclusion, our result highlighted the importance of RBC damage during storage. Also, pre-storage irradiation might result in serious harm during storage, which will be affecting the efficacy of RBC transfusion directly.

Although the storage of irradiated blood affects their quality, in many cases, despite changing parameters such as sodium, hemolysis index, LDH, product specifications eventually fall within the permissible quality control range of the product.

On the other hand, considering the significant change of parameters such as 2,3DPG and potassium in the second and third groups of irradiated samples, it was found that the best irradiation time which has the least adverse effect on product quality is the first group (until day 14). Reducing the adverse transfusion reactions and also maintaining the RBC function to ensure successful transfusion is one of the key issues that require to be further studied.

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CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

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