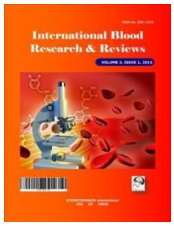

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Storage of Blood with or without Irradiation: Effect on Lipid Profile

Kiran Dahiya[1*], Monica Verma[1], Deepika Dalal[1], Veena Singh Ghalaut[1], P. K. Sehgal[2], Rakesh Dhankhar[3], Rajesh Kumar[1] and Sunita Kumari[1]

[1]Department of Biochemistry, Pt. BD Sharma PGIMS, Rohtak, Haryana, India.
[2]Department of Transfusion Medicine, Pt. BD Sharma PGIMS, Rohtak, Haryana, India.
[3]Department of Radiotherapy, Pt. BD Sharma PGIMS, Rohtak, Haryana, India.

Authors' contributions

This work was carried out in collaboration between all authors. Authors KD, MV, DD and PKS designed the study. Authors KD, MV and RD wrote the protocol and wrote the first draft of the manuscript. Authors RD, RK, DD, SK and VSG managed the literature searches, analyses involved in the study. Authors MV and DD managed the experimental process. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To observe the effect of storage on lipid profile in pre-irradiated blood and non-irradiated blood before transfusion.
Study Design: Prospective study.
Place and Duration of Study: Department of Biochemistry and Department of Transfusion Medicine, Pt. BD Sharma PGIMS, Rohtak, Haryana, India from April 2015 to July 2015.
Methodology: Blood for transfusion (450 ml) was drawn from 60 healthy volunteer donors into CPDA-1 anticoagulant (63 ml) and stored at 2-4℃ wit h 30 randomly selected blood bags subjected to 25 Grey gamma radiation before storage. Blood sample from each bag was analyzed at 0, 3, 7, 14 and 21 days interval for lipid profile and was compared statistically between two groups.
Results: A statistically significant decrease in levels of triglycerides (TG) and very low density cholesterol (VLDL-C) while an increase in low density cholesterol (LDL-C) was observed in pre-irradiated samples as compared to non-irradiated ones in 14th day and 21st day samples. The difference in other parameters and at lesser storage durations was not found to be

**Corresponding author: E-mail: kirandahiya_2002@yahoo.com;*

statistically significant.
Conclusion: The blood to be transfused should be fresh, non-irradiated or stored upto a duration of two weeks after radiation especially if recipients are at increased cardiac risk. The findings need to be supported with further studies with larger sample groups.

Keywords: Lipid profile; stored blood; irradiation; blood transfusion; duration of storage.

1. INTRODUCTION

Blood transfusion is necessary for treatment of anemia seen in various pathological conditions or hemorrhage caused by trauma or surgery [1]. When blood is stored in the blood bank, there occur alterations in the biochemical and physical properties of red blood cells (RBCs) because of storage conditions. These are referred to as 'storage lesions' [2].

The safety and efficacy of blood or its individual components, to be transfused, is of prime importance as degeneration in blood and cellular components occur as soon as it is withdrawn from the donor's body. There is a constant debate in the scientific community for the safe time frame between donation and transfusion [3].

Blood to be transfused needs to be stored for future use. Citrate, phosphate, dextrose, adenine (CPDA-1) is the most commonly used additive. It preserves the blood and also prevents coagulation. It has been claimed to provide a shelf-life of the stored blood at 2-4℃ for 35 days. It minimizes the biochemical changes but different storage lesions like decrease in pH, increased hemoglobin affinity to red blood cells (RBCs), leakage of ions like sodium and potassium from RBCs do take place [4]. Reports are available in literature regarding these changes but hardly any report is there to comment on the status of lipid profile of the blood stored in blood bank for transfusion.

Irradiation of blood before storage has also become a standard practice to prevent graft versus host disease (GVHD) in the recipients especially immunodeficient ones [5]. Irradiated RBCs as well as extracellular compartment, on storage, are documented to show some in-vitro biochemical changes [6-8] but reports are not available on any effect on the lipid profile of such blood.

This study was planned to observe the effect of storage on pre-irradiated blood sample and to compare with non-irradiated blood on the lipid profile.

2. MATERIALS AND METHODS

The present study was conducted in collaboration of departments of Biochemistry and Blood Transfusion. Blood (450 ml) was drawn from 60 healthy volunteer donors into CPDA-1 anticoagulant (63 ml) with adequate safety measures to avoid contamination and infection. Blood donors were screened as per regulations of Drugs and Cosmetics Rules [9]. All subjects were serologically examined for hepatitis B virus, hepatitis C virus and HIV before blood donation. Thirty of these blood bags, group I, (randomly selected) were carefully stored in a quarantine shelf in the blood bank at 2-4℃ while remaining thirty bags (group II) were exposed to gamma radiation of 25 Gy and then stored in the similar manner.

A blood sample measuring to 50 ml was taken for study purpose from each blood bag and stored in plain bags. Rest of the blood was used for transfusion purpose. Effect of storage was analyzed at 0, 3, 7, 14 and 21 days interval by withdrawing 8 ml blood each time from all bags in both the groups. Plasma triglycerides (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) were analyzed by standard kit methods using automated clinical chemistry analyzer (Randox suzuka) according to the manufacturer's specifications and using proprietary reagents. The levels of very low density lipoprotein cholesterol (VLDL-C) were calculated using Friedewald's formula (VLDL-C= TG/5). The levels of low density lipoprotein cholesterol (LDL-C) were also calculated using the formula [LDL-C= TC- (HDL-C+ VLDL-C)] [10-12]. The results were analyzed by applying suitable statistical methods.

3. RESULTS AND DISCUSSION

The plasma levels of TG, TC, HDL-C, LDL-C and VLDL-C in both non-irradiated and pre-irradiated samples at specified duration of storage is shown in Table 1. A significant decrease in levels of TG and VLDL-C while an increase in LDL-C was observed in 14^{th} day and 21^{st} day samples as compared to those in day 0 and previous samples (P<0.05). The TG and VLDL-C levels

were found to be decreased significantly in pre-irradiated samples as compared to non-irradiated ones at a duration of 14 day (*P* value 0.045 and 0.042 respectively) and 21 day (*P* value 0.014 and 0.041 respectively). A significant rise in levels of LDL-C was found in pre-irradiated samples as compared to those in non-irradiated samples on 14th day (*P* = 0.020) and 21st day (*P*= 0.018). The difference in other parameters was not found to be significant as compared to day 0 and previous sample in the corresponding categories. In addition, the difference at corresponding duration of storage was also not found to be significant in non-irradiated and pre-irradiated categories for other parameters.

3.1 Discussion

In the present study, the levels of TG and VLDL-C were found to be decreased on storage from day 0 (baseline) to day 21 but difference became significant only on 14th day ($p<0.05$) followed by a significant decrease again on 21st day ($p<0.05$) in both the categories i.e. non-irradiated and pre-irradiated ones. The levels were further found to decrease in pre-irradiated samples as compared to non-irradiated ones significantly on 14th and 21st day samples ($p<0.05$).

The changes suggest that triglycerides are getting degraded in the stored blood sample. Exact mechanism could not be found in the literature but changes in configuration of erythrocytic membranes and acidic environment might be the cause. Acidic conditions have been reported to lead to hydrolysis of triglycerides [13,14]. The ester linkage with palmitoyl moiety is most susceptible to acid hydrolysis [15]. The pH of blood on storage has been reported to decrease due to formation of lactic acid under anaerobic conditions [8,16].

Exposure to gamma radiation before storage increases propensity of damage to plasma membranes of erythrocytes along with increased generation of free radicals [17-20]. Free radicals might act on fatty acid component of triglycerides, oxidizing it and leading to its dissociation from TG molecule. These are only speculations and concrete mechanism is yet to be found in literature.

Table 1. Comparison of plasma lipid profile on specified days in non-irradiated and pre-irradiated samples

Parameter (mg%) / Day		Triglycerides (mg%)	Cholesterol (mg%)	HDL-C (mg%)	LDL-C (mg%)	VLDL-C (mg%)
Day 0	NI	170.7±59.6	140.8±25.96	31.6±6.69	74.8±20.3	34.03±11.86
	PI	158.8±56.32	142.25±22.22	32.4±6.79	78.10±19.04	31.75±11.33
	P Value	0.483	0.839	0.798	0.567	0.501
Day 3	NI	156.9±57.4	140.7±25.66	29.9±5.86	78.4±17.91	31.5±11.49
	PI	156.3±58.24	146.9±221.5	29.75±5.97	79.95±18.63	31.2±11.66
	P Value	0.971	0.222	0.930	0.769	0.941
Day 7	NI	154.2±58.7	141.8±23	29.6±6.59	82.7±17.4	31.4±11.7
	PI	146.7±57.7	147.7±17.37	28.6±7.57	83.9±23.99	29.3±11.7
	P Value	0.338	0.338	0.622	0.892	0.945
Day 14	NI	152.3±54.07*#	137.3±23.2	37.2±28.8	78.6±14.6	30.5±10.6*#
	PI	129.8±36.7*#	141.8±13.6	27.2±7.38	88.8±14.9*#	25.8±7.14*#
	P Value	0.045	0.436	0.133	0.020	0.042
Day 21	NI	147±16.2*	135±14.7	35.4±21.4	79.2±15.0	29.5±12.2 *
	PI	122.0±17.2*#	145±12.3	25.3±20.8	94.2±15.4*#	22.5±9.21*#
	P Value	0.014	0.501	0.121	0.018	0.041

Abbreviation: NI non-irradiated, PI pre-irradiated

** P value <0.05 as compared to day 1 sample in respective category*

 # P value <0.05 as compared to previous sample in respective category

The levels of LDL-C were found to be increased in pre-irradiated samples as compared to non-irradiated ones at specified durations but the levels were found to be significant only in 14 day (P= 0.020) and 21st day samples (P= 0.018). The levels of total cholesterol are also seen increased and HDL-C levels decreased in all samples subjected to radiation as compared to non-irradiated samples though the difference is not statistically significant. The exposure to gamma radiation is associated with an increase in free radicals while a decrease in antioxidants producing a state of increased oxidative stress which leads to a variety of changes in the subcellular compartment of blood cells [21]. These changes may be responsible for increased release of cholesterol from cells into plasma and increased concentration of oxidized LDL. But why there is increased concentration of cholesterol carried in LDL and low in VLDL is difficult to be explained.

CPDA solution was developed in 1968 and shown to permit whole-blood storage for 5 weeks [22]. The citrate prevents coagulation by binding or chelating to calcium, phosphate acts as a buffer hence, maintains the pH of the blood. Dextrose serves as substrate for the blood cells, while adenine maintains high adenosine tri phosphate (ATP) level in the RBCs. Most blood collection bags (adult) contain 63 mL CPDA anticoagulant which is sufficient to anticoagulate and ensure the viability of blood cells in 450 mL ± 10% blood for up to 28-35 days when the blood is stored at 2-8℃ [20]. Citrate also inhibits glycolysis but may add to the acidic environment. Though the stored whole blood is not a good sample to analyze lipid profile for which serum samples should be frozen for longer periods [23], this study was undertaken to observe the effect of irradiation on the stored blood which was to be administered to a needy patient.

4. CONCLUSION

Thus, to conclude, the exposure of blood for transfusion to irradiation before storage has lower content of triglycerides and VLDL-C while higher concentration of LDL-C which may not be suitable if patient's cardiac health is already compromised or at risk. Therefore, such patients may be transfused fresh blood or blood stored for less than two weeks and preferably non-irradiated one. Though further studies with larger sample size are needed to support these findings.

CONSENT

All authors declare that written informed consent was obtained from the patients for publication of this article.

ETHICAL APPROVAL

All authors hereby declare that the ethical issues in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki were duly taken care of.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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