

# In-Vitro Red Blood Cells Integrity and Morphology Changes after Passing Through Volumetric Peristaltic Infusion Pump

Esfahani H <sup>\*1</sup>, Dehghan A <sup>1</sup>, Hosseini H <sup>1</sup>, Esfahani S <sup>2</sup>

1. Pediatric Hematology/Oncology department, Besat hospital, Hamedan University of medical science, Hamedan, Iran.

2. Hamedan University of medical science, Hamedan, Iran.

**\*Corresponding Author: Esfahani H, Email: hesfehiani@yahoo.com**

Submitted: 10-05-2013 , Accepted: 23-01-2014

## Abstract

**Background:** It is not clear whether the use of volumetric peristaltic infusion pumps, affect the quality of the erythrocytes during packed cell transfusion or not. In the present study in vitro quality of red blood cells was assessed after using this type of infusion pumps in a prospective study.

**Materials and Methods:** In this study 60 units of packed red cells were examined before and after passing through volumetric peristaltic infusion pumps. The shelf life of each bag was up to 7 days, and the transfusion rate was set to 40 milliliter/hour. Pre- and Post- infusion pump samples were analyzed for erythrocyte morphology and hemoglobin, potassium, lactate dehydrogenase and bilirubin levels. Samples obtained before the blood going through the infusion pumps were considered as the reference.

**Results:** Infusion pump did not significantly increased the free hemoglobin, potassium, total bilirubin and lactate dehydrogenase increment, or fragmentation of the red blood cells, compared to the reference.

**Conclusion:** Due to insignificant increase in free hemoglobin caused by erythrocyte destruction after passing through the volumetric peristaltic pumps in this in-vitro study, we suggest that these pumps can be safely used for transfusion of fresh packed cells. However clinical in-vivo studies on children and neonates are strongly recommended to ensure the safety and effectiveness of this new method of transfusion.

**Keywords:** Infusion, blood, transfusion, hemolysis, erythrocytes.

## Introduction

Generally, anemia is defined as decreased hemoglobin concentration or red cell mass according to patients' age <sup>1</sup>. Patients' treatment may differ according to their final diagnosis, but blood transfusion as a supportive care is lifesaving in severe cases. In these patients, acute or delayed transfusion reactions are preventable, if proper measures are in place. One of the most important acute complications is improper volume or rate of transfusion estimation, which may lead to volume overload and acute heart failure <sup>2</sup>. With the increasing use of blood transfusions and the progress made to prevent its complications, the infusion methods are also progressing to achieve more precise control of the blood volume and the transfusion rate, especially in light-weighted newborns and infants. Non-secure transfusion methods and improper volume estimation, may

damage erythrocytes and harm patients due to mechanical effects, or cause volume overload. Physical stress caused by transfusion practices has been evaluated in several studies since the 1960s, which has led to the use of new technologies and significant improvements in fluids infusion safety. Among these, are needles with different diameters, vascular access catheters, filters and various fittings, which make blood transfusions safe and secure.

Infusion pumps are one of the most useful instruments to control infusion rate and are approved for fluid infusion in children. These devices act with three mechanisms: gravity, as well as applying positive or negative pressure as the driving force. Rapid infusers such as syringe pumps use positive pressure, and volumetric ones such as peristaltic pumps, use negative pressure. These devices are useful in controlling the infusion

volume, but may harm the blood cells and cause complications, such as mechanical cell damage<sup>3</sup>. Hemoglobin and potassium efflux hurt recipients by causing electrolyte imbalance and kidney damage. Pump effects on RBCs are influenced by the type of infusion devices, packed cell age, infusion rate and in-line filters directly, but this is not true for the type and diameter of needles and the length of injection hoses<sup>4</sup>. Micro infusion sets (micro-set) with a burette volume of 100 milliliter, and an air bubble used in transfusion hose, are the two most commercially used methods in low volume transfusion. These methods may cause infection or inaccurate volume estimation of infusates. Increasing use of infusion pumps for liquid injections with air and pressure sensors, improves infusion accuracy, but there is not sufficient proof for the safety of their use in packed cells transfusion. Erythrocyte damages have been reported by some investigators during the blood passage through infusion pumps<sup>5-9</sup>. In the present study, blood from packed cell bags was compared before and after passing through volumetric peristaltic infusion pumps, to study the integrity of red blood cells.

## Materials and Methods:

This experimental laboratory study was performed on 60 Packed Red Blood Cells with less than 7 days of shelf age, prepared and ready to be transfused in pediatric hematology department. Blood bags were examined at the start of this study to assess their appearance, the color of blood, any leak or other apparent damage, or possible physical or chemical injuries to ensure damaged bags had not been used. Two milliliters of blood for biochemical assays (Potassium, Lactate

dehydrogenase, Bilirubin), and one milliliter for hematologic evaluation (Hemoglobin) was taken before and after the blood passing through the infusion pumps in coagulated and EDTA tubes respectively, and peripheral blood smears were prepared simultaneously. A volumetric peristaltic infusion pump (JMS, model: OT-601, Japan), approved for liquid infusion in neonates and children from Medical Device Agency set up to 40 milliliter/hour was used<sup>10</sup>. Prepared samples were sent to laboratory and examined by a single pathologist. Free plasma hemoglobin was evaluated by spectrophotometer (Pars Azmoon, Iran), and the hematocrit using micro-hematocrit testing. Because of the low plasma in packed cells, they were diluted with normal saline and were examined using ISE method (Easy-light instrument, Medica, USA) to assess potassium concentrations and by enzymatic method (Pishtaz teb kit, Iran; Mindray B-380 instrument, China) to assess lactate dehydrogenase (LDH) and bilirubin concentrations, after centrifugation. Prepared blood smears were stained using Wright-Giemsa stain and 1000 erythrocytes were examined under a light microscopy by a single pathologist. Morphologic changes observed in more than 10% of cells were considered significant.

## Results:

Paraclinical evaluations were carried out in 60 fresh (less than 7 days old) packed blood cells. Male to female ratio of donors was 3:2. Blood groups used had the following pattern: O (45%), B (25%), A (21.9%), AB (8.3%) and among them 75% had positive Rh antigen. After assessing the apparent condition of blood bags, free hemoglobin

**Table 1:** Blood destruction indexes before and after the blood going through the volumetric pump.

Blood destruction index	Before going through the volumetric pump	After going through the volumetric pump	P-Value
Hemoglobin (g/dL)	190.4 ± 7.9	189.9 ± 7.9	<b>0.06</b>
Potassium (mmol/L)	40.0 ± 12.3	41.3 ± 13.4	<b>0.08</b>
LDH (Unit/L)	64.0 ± 39.7	74.8 ± 55.7	<b>0.07</b>
Bilirubin (μmol/L)	1.5 ± 0.9	2.1 ± 1.8	<b>0.06</b>

level, potassium level, LDH and total bilirubin levels were evaluated before and after going through the pumps, which indicated no statistically significant difference (Table 1). The mean hemoglobin level in blood bags was  $190 \pm 7.9$  gram per deciliter, with a mean hematocrit of 61%, which was consistent with the standard supply of the blood bags (hematocrit less than 80%)<sup>11</sup>. Morphologic appearance of erythrocytes was assessed and compared too. No significant morphologic changes in erythrocytes (more than 10% of evaluated cells) such as acanthocytes, poikilocytes, anisocytes and schistocytes were observed in this evaluation (Table 2).

## Discussion:

Paraclinical indicators of red cell damage, including serum potassium, bilirubin, LDH and free hemoglobin levels and red cells' morphology, did not indicate any significant changes in the present study. By increasing age and decreasing erythrocytes' adenosine triphosphate, the required energy for maintaining cellular potassium is reduced, so senescent cells which lose their potassium are more susceptible to lysis<sup>12</sup>. Due to the non-significant outflow of hemoglobin from fresh packed cells during the passage through the infusion pump, it can be concluded that peristaltic pumps do not damage packed cells' erythrocytes. Observed erythrocyte changes may occur due to storage and processing but it is not significant. Not significant Bilirubin elevation in this study is consistent with a study by Frey et al. and may have occurred due to erythrocytes' damage<sup>4</sup>. Physical damage to erythrocytes, leads to their abnormal forms, such as: acanthocytes, poikilocytes,

anisocytes and schistocytes or any broken red cells (fragmented RBC). If these abnormal cells are seen in more than 10% of the examined erythrocytes, it is considered that traumatic damage has been significant<sup>13,14</sup>. On the other hand, with increasing the transit time of blood from the pump, blood cell damage increases<sup>15,16</sup>. The appearance of high number of broken cells in the peripheral blood smear is a sign of cell damages or defect in erythrocyte precursors<sup>17</sup>. Due to the lack significant increase of cell damage in smear preparations, it can be concluded that no significant blood cell damage did occur. Anisocytes, which mark the difference in the size of red blood cells, are not directly implicated in acute hemolysis, but may indicate manufacturing problems or long term corpuscular damage. Another peripheral smear finding is acanthocytes, which may be seen in storage process damage or underlying hepatic, renal or hereditary problems. The presence of these cells in this study, were not significantly high neither before nor after passing through the infusion pumps. Schistocytes are fragmented erythrocytes that make helmet-shaped or discrete fragments of cells and are critical signs of mechanical damage to red blood cells. In the present study and some similar studies these abnormal cells showed no significant increase after blood passing through the volumetric pumps<sup>18</sup>. Clinical manifestations will start when free hemoglobin is at least 2 grams per deciliter so, before the onset of clinical symptoms; laboratory investigations can detect intravascular hemolysis<sup>19</sup>. Some studies have not confirmed hyperkalemia following destruction of erythrocytes, but the release of hemoglobin had been shown, although the clinical significance of these elements has not

**Table 2:** The frequency of abnormal red blood cell morphology before and after the blood going through the volumetric pump.

Morphology	Before the the blood going through the volumetric pump (number in 1000 RBC)	Before the the blood going through the volumetric pump (number in 1000 RBC)	P-Value
Acanthocytes (Spur cell)	10	13	0.06
Poikilocytes	8	10	0.07
Anisocytes	3	4	0.09
Schistocytes	1	1	1

been definitely determined yet. In addition, high packed cell shelf life correlates with high potassium levels <sup>12</sup>. It should be noticed that although we could not see any significant changes in blood going through the volumetric pumps in 60 samples more elaborate studies with higher number of samples is needed to ensure our results.

## Conclusion

Due to insignificant increase in free hemoglobin caused by erythrocyte destruction after passing through the volumetric peristaltic pumps in this in-vitro study, we suggest that these pumps can be safely used for transfusion of fresh packed cells. However clinical in-vivo studies on children and neonates are strongly recommended to ensure the safety and effectiveness of this new method of transfusion.

## Acknowledgment

The authors thank the pediatric hematology department staffs, particularly Mr. Mohsen Ahmadi.

## References

1. Brugnara C. Diagnostic Approach to the Anemia Patient. In: SH O, editor. Nathan and Oski's Hematology of Infancy and Childhood. 7 ed. Philadelphia: Saunders, Elsevier; 2009. p: 455 - 66.
2. Sloan SR. Transfusion Medicine. In: Orkin SH, editor. Nathan and Oski's Hematology of Infancy and Childhood. 7 ed. Philadelphia: Saunders elsevier; 2009. p: 1623-75.
3. Nightingale MJ, Norfolk DR, Pinchon DJ. Current uses of transfusion administration sets: a cause for concern? *Transfus Med*. 2010;20(5):291-302.
4. Frey B, Eber S, Weiss M. Changes in red blood cell integrity related to infusion pumps: a comparison of three different pump mechanisms. *Pediatr Crit Care Med*. 2003;4(4):465-70.
5. Carson JL, Grossman BJ, Kleinman S, Tinmouth AT, Marques MB, Fung MK, et al. Red blood cell transfusion: a clinical practice guideline from the AABB. *Ann Intern Med*. 2012;157(1):49-58.
6. Nightingale MJ, Leimbach R. An evaluation of proposed changes to International Standards for blood bags and transfusion sets to improve their compatibility. *Transfus Med*. 2008;18(5):281-6.
7. Tinmouth A, Chin-Yee I. The clinical consequences of the red cell storage lesion. *Transfus Med Rev*. 2001;15(2):91-107.
8. Gmerek K, Fabijanska-Mitek J. [Potentially pathogenic changes in red blood cells during their storage]. *Pol Merkur Lekarski*. 2011;30(177):224-7. ([Article in Polish])
9. Hod EA, Spitalnik SL. Harmful effects of transfusion of older stored red blood cells: iron and inflammation. *Transfusion*. 2011;51(4):881-5.
10. Precautions in flow rate programming of infusion pump [database on the Internet]. Medical Safety Information Group. 2011. Available from: [www.pmda.go.jp/english/service/pdf/safety/No21.pdf](http://www.pmda.go.jp/english/service/pdf/safety/No21.pdf).
11. Bergeron DA. Component Preparation. In: Rudmann SV, editor. Textbook of Blood Banking and Transfusion Medicine. 2 ed. Philadelphia: Elsevier, Saunders; 2005. p: 232- 57.
12. Sowemimo-Coker SO. Red blood cell hemolysis during processing. *Transfus Med Rev*. 2002;16(1):46-60.
13. Ding MJ, Xu SW, Zhang J, Wang Q, Chang Y, Chen F, et al. Trauma to erythrocytes induced by long term in vitro pumping using a roller pump. *Cell Biol Int*. 2007;31(8):763-7.
14. Denison M, U P, Bell R, Schuldreich R, Chaudhri MA. Effect of different pump mechanisms on transfusion of blood. *Australas Phys Eng Sci Med*. 1991;14(1):39-41.
15. Fan J, Xu S, Chen F, Ding M. [The significance of erythrocyte fragment count to predicting hemolysis in roller pumps]. *Sheng Wu Yi Xue Gong Cheng Xue Za Zhi*. 2008;25(3):632-6. (Article in Chinese)
16. Xu S, Chen F, Ding M, Chen R, Lu S, Zhong H. [The study of erythrocyte fragility and morphological changes caused by roller pump in vitro]. *Sheng Wu Yi Xue Gong Cheng Xue Za Zhi*. 2002;19(3):419-22. (Article in Chinese)
17. Stanley L Schier EAP. Extrinsic nonimmune hemolytic anemia. In: Hoffman R, editor. Hematology Basic principles and Practice. 5 ed. Philadelphia: Churchill Livingstone Elsevier; 2000. p: 659-67.
18. Burch KJ, Phelps SJ, Constance TD. Effect of an infusion device on the integrity of whole blood and packed red blood cells. *Am J Hosp Pharm*. 1991;48(1):92-7.
19. Parfitt HS, Davies SV, Tighe P, Ewings P. Red cell damage after pumping by two infusion control devices (Arcomed VP 7000 and IVAC 572). *Transfus Med*. 2007;17(4):290-5.