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Measurement of Free Hemoglobin Percentage in Stored Packed Red Blood Cells Units by Colorimetric Method

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AEE and SAA conceptualized and designed the study. Author SAA conducted the research and wrote the original draft. Authors TAMH and AEE revised the final version, then all authors approved the final version of the manuscript.

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ABSTRACT

Aims: To measure free hemoglobin percentage in stored packed red blood cells units by colorimetric method.
Study Design: This is a descriptive, hospital based case study.
Place and Duration of Study: Khartoum central blood bank during the period from June to September 2021.
Methodology: A total of thirty-six packed red blood cells blood bags with Citrate-Phosphate-Dextrose-Adenine-one (CPDA-1) stored at 2-6°C for 35 days were withdrawn aseptically on days one; 14, and day 35. Free hemoglobin concentration was measured with Drabkin's method through digital photoelectric colorimeter in which the percentage of hemolysis was calculated, and PH values were measured by a PH meter (ADWA). Mean and standard deviation of each day were calculated by statistical package for social science (SPSS) computer program version 22.0.
Results: The results showed statistically significant elevation in percentages of free plasma hemoglobin with prolongation of storage duration periods; day one compared with day 14 (*P* =

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0.001), day one compared with day 35 (*P* = 0.001) and day 14 compared with day 35 (*P* = 0.001). No significant correlation was observed between the degree of hemolysis in day 35 with PH values (*P* = 0.9).

Conclusion: This study concluded that prolonged storage is associated with elevation of free plasma hemoglobin level indicative of progressive hemolysis.

Keywords: Colorimetric method; free hemoglobin concentration; packed red cells.

1. INTRODUCTION

Transfusion is a lifesaving treatment with more than 100 million units of blood are collected worldwide each year. safe and adequate supply of blood and products is major public health issue faced globally especially in critically ill patients [1]. Transfusion of red blood cells (RBCs) is one of the most commonly used potentially lifesaving medical therapy; by increasing RBCs mass in patients who have low oxygen carrying capacity due to increased RBCs loss (traumatic, surgical hemorrhage), decreased bone marrow production (aplastic anemia), defective hemoglobin (hemoglobinopathies) or decreased RBCs survival (hemolytic anemias) [2]. Whole blood can be then used for preparation of Packed red blood cells (PRBCs) or it can be stored for up to 35 days if collected in CPDA-1 [3]. Changes that occur during RBCs processing and refrigerated storage of blood result in structural and functional defects, these series of biochemical and morphological changes are referred to as storage lesions [4—7]. These lesions have been associated with the post-transfusion morbidity and mortality, mainly includes decreasing of adenosine triphosphate (ATP), pH levels, depletion of 2,3 diphosphoglycerate (2,3-DPG) and biomechanical changes such as membrane deformability [8]. These membrane changes will make RBCs fragile; increase osmotic fragility, intracellular viscosity, oxidative injury that cause damage to major macromolecules such as protein and lipids on cells, loss of membrane carbohydrates and reduced RBCs lifespan [5,9]. The storage lesion results in increased adhesion of RBCs to endothelial cells and hemolysis with release of cell free hemoglobin (CFH) into the plasma [4].

Food and Drug Administration (FDA) regulations only stipulate that at the end of the storage period 75% of the cells remain in the circulation at 24 hours after transfusion and that hemolysis in the storage bag does not exceed 1% (the corresponding free Hb concentration is at least not more than 2 g/L) as the standard to evaluate the quality of blood product [8,10,11]. According to Chinese national standard quality requirements for whole blood and blood components, hemoglobin concentration in blood must be greater than 100 g/L, to ensure the quality of blood product [11]. Therefore, hemolysis of different degrees is an important parameter to evaluate the quality of blood cells [9]. So rapid and safe technique is needed to be developed in order to assess the quality of the RBCs prior to usage. Although some clinical studies suggest that there is no harm associated with the transfusion of relatively older RBCs units [12]. This study was conducted to evaluate the quality of PRBCs in Khartoum central blood bank based on the assessment of free hemoglobin percentages at different intervals of storage.

2. MATERIALS AND METHODS

This descriptive-case study was conducted in Khartoum central blood bank, during the period from June to September 2021.The study was approved by Haematology department, Faculty of medical laboratory sciences at Al-Zaiem Al-Azhari University and administration of Khartoum central blood bank. Thirty-six blood bags were included in this study. Samples (5 ml) from PRBCs were collected in three vacuum tubes (day one, 14 and day 35) for measurement of free Hemoglobin level as described by Weinberg et al. [13] using the following formula:

> Percent hemolysis = [Supernatant Hb] x [100- Hct] ⁄ [Total Hb].

Where Hemoglobin g⁄dl = absorbance x 36.77 [14]

PH level was measured for day 35 sample by using PH meter (AD 1030) PH⁄Mv; adwa.

Mean and standard deviation were calculated for numerical data using descriptive statistics. Free Hemoglobin percentages were compared between days by using paired sample T test and Pearson's correlation was used to test the correlation between free Hb percentages and pH

level at day 35. For both tests, *p*. values of less than 0.05 was considered statistically significant. SPSS version 22.0 was used for all data analysis.

3. RESULTS

A total of thirty-six PRBCs blood bags preserved with CPDA-1 and stored at 4±2 °C, were included in this study. Samples were collected from bags in day one, day14 and day 35. The Hemolysis markers in thirty-six PRBCs bags are shown in Table 1.

4. DISCUSSION

In this study, colorimetric is proposed as a diagnostic tool for the quality assessment of erythrocyte suspensions units under storage. Results showed significant differences in the percentages of free plasma hemoglobin between storage days. There is significant elevation in the mean level of percentage of hemolysis in relation to storage periods in which the packed red blood cells units exceeded the acceptable quality limit of in-bag hemolysis levels above 1.0%, (at free plasma hemoglobin level of 0.8% and 1.0% hemolysis corresponding to 233 and 291 mg⁄dl respectively calculated based on assumption that whole blood has 45% packed cell volume and total hemoglobin 16g⁄dl) [1]. This finding agrees with a previous study that showed increased percentage of free hemoglobin with storage [15]. Also the finding is in agreement with Can, et al. study that showed statistically significant elevation in erythrocyte suspension with presence of hemolysis [11]. Also in accordance to study conducted by Mustafa and Hadwan, this study showed statistically significant elevation in oxidative damage to RBCs lysis with an increase in storage time (*p.* value **<** 0.05) [6], and agrees with Hess, et al. which showed increase storage from 35 to 42 days' lead to increased hemolysis by 30% [12]. This study also agrees with Tzounakas, et al. study which exhibited higher average in bag hemolysis in PRBCs compared with whole blood at every time point of stored period (*P* value >0.05) [3].

Another finding of this study is that is no significant correlation between the percentages of free hemoglobin with PH level at day 35. This indicates that, the acidity of the blood doesn't elevate the degree of red blood cells hemolysis in blood units.

A limitation of this study is the small sample size, so authors recommend larger sample size studies for more accurate conclusions.

Table 1. Hemolysis markers in thirty-six packed red blood cells bags

	Range	Mean	SD
Total hemoglobin (g⁄dl)	12.4-16.0	14.7	0.78
Packed cell volume (%)	33.0-56.0	43.0	5.18
Day 1-free hemoglobin (g⁄dl)	0.70-3.60	1.83	0.74
Day 14- free hemoglobin (g⁄dl)	4.70-15.9	10.5	2.82
Day 35- free hemoglobin (g⁄dl)	10.6-19.1	14.9	1.93
PH value (Day 35)	5.10-7.40	6.34	0.45

Table 2. Comparison between the percent of hemolysis and duration of storage period in day 1, 14 and day 35 of blood units

	Percent of hemolysis (Mean ± SD)	*P* value
Day1	7.12±2.75	
Day 14	42±13.43	0.001
Day1	7.12±2.75	
Day 35	57.58±11.42	0.001
Day 14	42±13.43	
Day 35		0.001
	57.58±11.42	

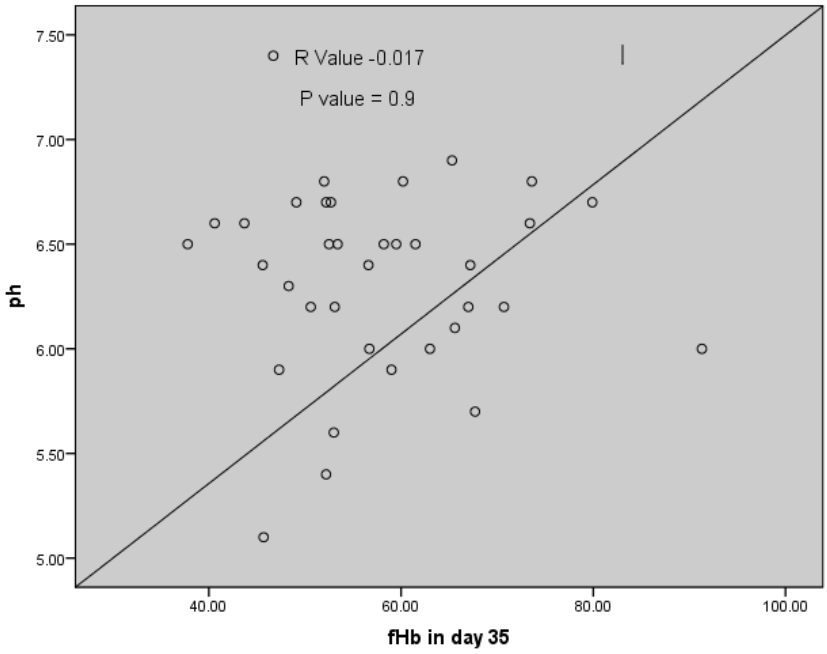

Fig. 1. Correlation between percentages of free hemoglobin in day 35 with PH level

5. CONCLUSION

This study concluded that prolonged storage is associated with increased free hemoglobin level indicative of progressive hemolysis.

CONSENT

All authors declare that 'written informed consent was obtained from the participants for this study.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the faculty of medical laboratory sciences at Alzaiem Alazhari University and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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