ORIGINAL ARTICLE

A Pilot Study to Assess Serum Potassium Levels and Haemolysis in Red Cell Units

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ABSTRACT

Introduction: Percentage of haemolysis is widely used as a quality parameter to assess red blood cell viability in blood banking. In certain blood banks, serum potassium level is used due to the unavailability of the former test. The relationship between these two tests, however, is still unclear. The objective of this study is to determine the association between haemolysis measured using two different methods for quality control. **Methods:** A total of forty-four samples of packed red cell in citrate-phosphate-dextrose with optisol were randomly selected from donation drives. Nine millilitres of blood was collected weekly starting from day-2 of storage, followed by day-7, 14, 21, 28, 35 and 42 for assessment of red blood cell haemolysis by measuring serum potassium level and percentage of haemolysis. **Results:** These two parameters were correlated significantly with a positive moderate linear relationship on day 7, 21 and 28 with r = 0.393, 0.448 and 0.425, respectively and p-values less than 0.01. The linear regression analysis showed there was a significant regression equation which could be used to predict the serum potassium level from the percentage of haemolysis. **Conclusion:** There were significant increases in the percentage of haemolysis and serum potassium level in the packed red cell units with storage. The serum potassium level would be able to be predicted from the percentage of haemolysis using the regression equations on day 7, 21 and 28. The serum potassium measurement could be used as an alternative test to the percentage of haemolysis before issuing blood.

Keywords: Blood transfusion, Potassium level, Haemolysis

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INTRODUCTION

Red blood cells (RBCs) degrade progressively during refrigerated storage and affects the storage media. This phenomenon is called storage-related RBC lesion. It is partially due to the bio-reactive substance released by leukocytes in the storage medium, such as histamine and cytokines which directly affect structural and biochemical changes on RBC associated with senescence (1) and haemolysis. Biochemical changes related to RBC storage lesions include depletion of 2,3-diphosphatidylserine (2,3-DPG) and adenosine triphosphate (ATP). Depletion in intracellular RBC ATPs affects the RBCs cellular metabolism including maintenance of sodium-potassium (Na+-K+) ATPase activity, RBC membrane stability, glucose transport and oxidative stress defence mechanisms. During storage of RBCs, there is a slow but constant leakage of potassium following the concentration gradient into the surrounding storage medium due to a failure of Na+-K+ ATPase pump activity (2).

In blood banking, proper storage of RBC is a critical and crucial step to ensure the quality product is given to patients. To store RBCs, anticoagulant and preservatives solutions are needed. The anticoagulant solution comprises citrate either in the form of sodium citrate or citric acid, phosphate and dextrose. The purposes of preservative are to allow red cells to be stored up to 42 days without having structural and functional changes and also to provide nutrients for the red blood cells (3).

The quality of packed red cell unit must be regularly assessed to ensure it is in good quality to serve its purpose of red cells transfusion. In Pusat Darah Negara (PDN), Malaysia, the referral centre for blood banking, the quality check on the packed red cell units is the percentage of haemolysis, haematocrit and haemoglobin level which are usually done on the day of expiry (4).

Other blood banks in the country, however, may use other parameters such as potassium level as their quality parameter due to the unavailability of the equipment to measure the percentage of haemolysis. This study was done to evaluate the changes in the percentage of haemolysis and potassium level of packed red cell units at various time points of storage and to compare these two parameters.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Quality and Haematology laboratory of National Blood Centre, Kuala Lumpur, Malaysia and Core Laboratory of Pathology Department, Hospital Kuala Lumpur, Malaysia from June 2014 to December 2016.

Ethical clearance to conduct this study was obtained from Medical Research Ethics Committee (MREC), The Ethics Committee for Research of University Putra Malaysia (JKEUPM) and Research and Development Committee of Pusat Darah Negara (R&D PDN). MREC Ref. No.: NMRR-14-1825-22589; JKEUPM Ref. No.: FPSK (EXP15) P064; R&D PDN Ref No.: bil (20) dlm. PDN/07-24 Jld 2.

Sample size was calculated based on the mean estimation using percentage standard deviation of haemolysis. A total of forty-four packed red cell units were collected after informed consent from blood donors selected by convenience sampling from sequential blood donation days. All selected packed red cell units were quarantined until blood grouping (ABO and Rh D) and serology testing for transfusion-transmitted diseases [(TTD); Human Immunodeficiency Virus (HIV), Hepatitis B and Hepatitis C] were cleared.

The packed red cell units were stored at 4^0C in a cold room. On each sampling day (2nd, 7th, 14th, 21st, 28th, 35th and 42nd day of storage), the selected blood bags were brought to the quality laboratory for blood sampling. Before blood sampling was done, blood bags were placed on a rotator for at least 5 minutes by gentle inversion to ensure blood is well mixed. Nine ml of blood was syringed out aseptically from needle-free bag spike device. Two ml of blood were placed into ethylenediaminetetraacetic acid (EDTA) bottle; 3.5 ml of blood were put into a plain bottle and 3.5 ml of blood were drawn into spray coated silica vacutainer (SST). Blood in plain bottles and vacutainer SST were centrifuged for 10 min at 1000 rpm.

The free-plasma haemoglobin (Hb) was measured using HemoCue plasma Hb analyser (HemoCue AB, Sweden). Briefly, a large drop of the plasma from a plain bottle was dropped onto a special paper. The sample was drawn into the cuvette by capillary action and spontaneously mixed with dry reagents containing sodium nitrite and sodium azide which resulted in the conversion of Hb to azidemethaemoglobin. A measurement was taken at 570 nm and 880 nm.

Blood samples in the EDTA bottles were used to measure full blood count using Sysmex XE 5000 (Sysmex Corporation, Kobe, Japan) automated haematology analyser. The plasma from the vacutainer SST was used to measure the potassium level using Cobas 8000 (Roche Diagnostic, USA) automated biochemistry analyser

The percentage of haemolysis in a packed red cell unit was then calculated using the formula [(100-haematocrit) x free plasma haemoglobin] / total haemoglobin.

Mean potassium level and percentage of haemolysis at seven point duration were determine. Repeated measures of analysis of variance (ANOVA) and Bonferroni's part-wise multiple comparison tests were used to assess the effect of storage duration on potassium level and percentage of haemolysis with a *p-value* less than 0.05 was considered significant. Correlation between parameters in this study was analysed with Pearson correlations test with a *p-value* less than 0.01 as significant. Linear regression analysis was carried out between the percentage of haemolysis and potassium level to predict the potassium level based on the percentage of haemolysis.

RESULTS

A total of 44 blood donors consented to the study. One sample had a percentage of haemolysis of more than 0.8% which is more than the permissible limit as recommended by national guidelines and was excluded from subsequent analysis.

Mean percentages of haemolysis at seven time-points of storage
The measurements for the percentage of haemolysis were done at seven times points during storage on 2nd, 7th, 14th, 21st, 28th, 35th and 42nd day. The mean percentages of haemolysis for the 43 samples are shown in Figure 1. There was a statistically significant effect
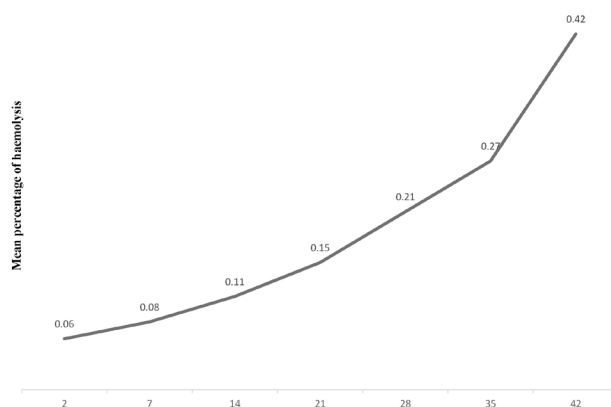

Figure 1: Mean percentage of haemolysis at seven points of duration

of storage time on the percentage of haemolysis with a p-value of less than 0.05. The longer the duration of packed red cell storage was associated with an increased in the percentage of haemolysis.

Mean potassium level at seven time-points of storage

Measurements of potassium levels were done at seven times points during storage at 2nd, 7th, 14th, 21st, 28th, 35th and 42nd day. The mean potassium levels of the 43 samples at each time-point of storage are shown in Figure 2. An increasing trend of plasma potassium level was observed with the storage period. There was also a statistically significant effect of the storage duration on the potassium level with a p-value < 0.05 for the seven time-points.
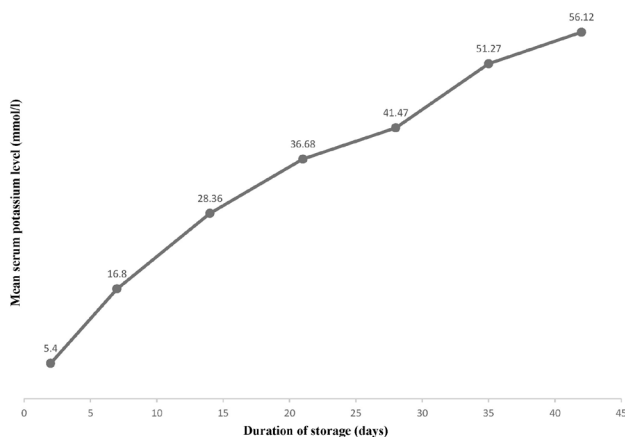

Figure 2: Mean serum potassium level at seven points of duration

Correlations between the percentages of haemolysis and potassium levels packed red cell unit at different time-points of storage

There was a statistically significant positive moderate linear relationship (p-value < 0.01) between the percentage of haemolysis and potassium level at day 7, day 21 and day 28 of storage with r = 0.393, 0.448 and 0.425 respectively. However, there was no significant correlation between the percentage of haemolysis and potassium level at day 2, day 14, day 35 and day 42 of storage (p-value > 0.01). The findings are summarised in Table I.

Table I: Correlation between percentage of haemolysis and serum potassium level of packed red cell units according to the duration of storage

Storage duration	Pearson correlation (r)	p value
Day 2	-0.152	0.332
Day 7	0.393	0.009
Day 14	0.353	0.020
Day 21	0.448	0.003
Day 28	0.425	0.005
Day 35	0.351	0.021
Day 42	0.340	0.025

Linear regression analysis to predict potassium level from a percentage of haemolysis

Statistically significant regression equations with a p-value less than 0.05 were obtained for day 7, day 21 and day 28 of storage. At these particular storage periods, the prediction of potassium level from the percentage haemolysis could be calculated. Results are summarised in Table II.

Table II: Linear regression analysis at different time-point of storage

Storage duration	R	R²	p value (regression)	Regression equation
Day-7	0.393	0.155	< 0.05	y = 15.3 + 19.8*(x)
Day-21	0.448	0.201	< 0.05	y = 32.3 + 29.5*(x)
Day-28	0.425	0.180	< 0.05	y = 36.9 + 21.9*(x)

DISCUSSION

Plasma Hb is free Hb in the plasma following red cell haemolysis and gradually increases during red cell storage as the consequences of storage-related RBC lesions. Various methods are available for the assessment of haemolysis. The visual method is the simplest and cheapest, but it is inaccurate and result in overestimation of haemolysis as well as interobserver variations (5,6,7). It is more accurate to use a validated quantitative test (8), such as a photometric method using HemoCue plasma haemoglobin analyser, which was used in this study.

The International Council for Standardization in Haematology (ICSH) guideline recommended cynomethaemoglobin method as a standard reference method in measuring free Hb in whole blood. Several studies had proven that measurement of plasma Hb using HemoCue was comparable to this standard method. Janatpour et al. reported that the HemoCue method correlated extremely well with the standard spectrophotometric method based on oxidation 3, 3′, 5, 5′ tetramethylbenzidine (TMB) (6).

Currently, the recommended limit of the allowable percentage of haemolysis at the end of the RBCs storage is 0.8% by European guidelines and 1.0% by the FDA United States. The National Blood Centre Guidelines of Malaysia accepted the threshold for percentage of haemolysis for up to 0.8% at the end of RBC storage (4). Out of all 44 samples at the initial part of the study, only 1 sample had a percentage of haemolysis more than the permissible limit as recommended by national guidelines (0.8% haemolysis at the end of storage). The exact cause was not investigated, but may possibly be due to microorganism contamination such as bacteria

(9) or fungus, improper handling during sampling, for example, using a small-bore needle or vigorous shaking of the blood bag during mixing before the sampling procedure. The other factor such as improper temperature during storage was not possible because all the blood bags were stored in the same cold room.

The remaining 43 samples maintained percentages of haemolysis below 0.8% at the end of the storage period and therefore expected that transfusion of these blood is suitable for the recipients. In this study, the mean percentage of haemolysis progressively increased from day 2 until day 42. The increments of the percentage of haemolysis reached 30% to 40% at the end of the first five weeks. At the last week of storage, the percentage of haemolysis increased to 50%. It showed the longer storage duration, the percentage of haemolysis would be higher. These findings are also comparable to the percentage of haemolysis measured in packed red cells stored in other additive solution such as SAGM (saline-adenine-glucose-mannitol) (10,11,12,13).

With the use of anticoagulants and additive solution packed red cells could be stored up to 42 days (14). In this study, we used CPD (anticoagulant) and optisol (additive solution) and the mean percentage of haemolysis at day 42 of storage was 0.42% much below the permissible limit by the national guideline (4).

Serum potassium levels increased significantly by day 7 of storage, an increase of approximately 200% compared to the serum potassium level on day 2. These continued to increase until the end of the storage period (42 days) to 56.1 mmol/L. These findings agreed with the observations by many previous studies (16,17,26). There is also constant leakage of potassium from the intracellular to extracellular fluid at a rate of 0.5 to 1.0 mmol/L per day during the storage of blood (18,19,20). With a simple calculation, at the end of storage duration, the serum potassium level will be at least 24.5 mmol/L (limitation of this study in which we presumed the serum potassium level during collection was 3.5 mmol/L which is a lower limit of normal range). Previous studies also reported serum potassium concentrations in the blood stored more than 21 days were above 20 mmol/L (21,22). This phenomenon is partly due to the normal function of Na+ - K+ ATPase pump which is inhibited at a lower temperature (20,23, 24,) leading to leakage of the potassium from the RBCs. In addition, microvesicles release from the RBC is another contributing factor. The high level of serum potassium in the current study is most likely as a consequence of these phenomena and not only due to major haemolysis in stored RBC. These factors also might contribute to the non-significant correlation between the percentage of haemolysis and potassium level at day 2, 14, 35 and 42.

Using the equation derived from linear regression analysis at day-7, the predicted mean potassium level was 16.8 mmol/L ± 1.96 (range of 14.8 to 18.7 mmol/L) which is still within the expected range for day 7 of storage. Serum potassium level in the stored blood could be calculated from the percentage of haemolysis and vice versa, at a particularly time-point. Therefore, two different blood banks using two different methods for their quality control can be made comparable.

CONCLUSION

This study demonstrated a significant correlation in the percentage of haemolysis with serum potassium levels at day 7, 21 and 28 of storage. Serum potassium levels may be used to predict percentages of haemolysis on these days from the regression equation. Significant correlations were achievable on days 14, 35 and 42 only at higher p-value. Thus, there is a potential of using serum potassium levels to predict haemolysis. These results, however, require validation with a bigger study population. Furthermore, the acceptance limit for serum potassium has yet to be defined.

ACKNOWLEDGEMENT

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