ORIGINAL ARTICLE

Changes in Laboratory Parameters of Washed Leucofiltered Packed Red Blood Cells Using Normal Saline With or Without 0.2% Dextrose

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ABSTRACT

Introduction: Washing of red blood cells (RBCs) removes excess proteins before transfusion. Traditionally, washing of RBCs is performed by using 0.9 % saline (normal saline; NS). This study was performed to compare the changes in laboratory parameters of leucofiltered packed RBCs washed using NS with or without 0.2% dextrose. **Methods:** Thirty-two units of leucofiltered packed RBCs were equally divided into two groups. The first group was washed with a solution that contained a mixture of NS and 0.2% dextrose, and the second group was washed with NS only. The washing process was performed at room temperature using an automated red cell washer. Samples were taken at pre-wash, immediately post-wash, and at day two, seven, and 14 post-wash. Laboratory parameters including haemoglobin, haematocrit (Hct), haemolysis, pH, and potassium level were measured at each time. **Results:** There were no significant differences in the laboratory parameters between the two types of washing solutions ($P > 0.05$). Post-wash, all parameters tested showed a significant difference over the storage duration of 14 days ($P < 0.001$). Potassium level had significantly increased to greater than the acceptable level (14.6 mmol/L for NS, and 13.9 mmol/L for NS and 0.2% dextrose solution) by day seven of storage. **Conclusion:** Both solutions are effective as RBCs washing solutions. However, the maximum storage time after washing was two days due to the significant increase in potassium level beyond that time point.

Keywords: Red blood cells, Washing, Haemolysis, Normal saline

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INTRODUCTION

As medical technologies have progressed, transfusion medicine services have greatly improved their ability to provide safe blood to the patient. Traditional blood components such as packed red blood cells (RBCs) have been modified to ensure that their safety and efficacy are not compromised during storage. One modification for packed RBCs is a washing process. Washed RBCs refer to whole blood or packed RBCs that have undergone sequential washing and resuspension in an additive solution (1).

Washing can be performed manually or using an automated cell processor (2). Washed units usually contain 10–20% fewer RBCs than the original units, and they have been depleted of 99% of the plasma proteins and 85% of the leucocytes (3). Use of a washed RBCs product is indicated for patients with severe allergic reactions to plasma-containing products, in IgA-deficient patients when IgA-deficient products are not available, in neonatal alloimmune thrombocytopenia patients for whom maternal blood that contains anti-HPA-1a is used for neonatal transfusion, and when complement must be removed from the blood products (4). Washed RBCs are also considered a safer option than non-washed RBCs for transfusion in haemodialysis patients with underlying hyperkalaemia and anaemia (5). Additionally, transfusing washed RBCs in patients undergoing coronary artery bypass graft was shown to result in a significant reduction in one-year post-surgical mortality and hospital adverse events (6). Washed RBCs product expires 24 hours after washing in an open system, but the expiry date may be prolonged if washing is performed in a closed system using a suitable additive solution (1).

Washing can be performed at any time of the RBCs storage up till 42 days, using different washing solutions

(either saline or combination of saline and glucose), and at different centrifugation speeds based on automated red cell washers the manufacturers' recommendation. (7). At the National Blood Centre (NBC) of Malaysia, washing of RBCs is usually performed using automated cell washers with a washing solution consisting of a mixture of normal saline (NS) and 0.2% dextrose (NS + 0.2% dextrose) as recommended by the manufacturer (8). However, the cost of this solution is higher than that of NS alone, which can also be used as a washing solution (9). Furthermore, NS + 0.2% dextrose solution may not be available in many blood banks. Thus, this study was performed to assess the in vitro quality of leucofiltered RBCs after washing with two different types of washing solutions (NS + 0.2% dextrose, and NS) over a storage period of 14 days. Understanding the effects of the washing process on RBCs blood products is important because the foremost goal of blood transfusion service is to provide the safest and best quality blood products for transfusion.

MATERIALS AND METHODS

Collection and production of RBCs

This was a cross-sectional study that included 32 AB RhD positive blood donors. Details about this research were explained to all donors, and consent from each donor was obtained. A 450 ± 45 mL of whole blood was collected in a triple blood bag (Teruflex®, Terumo Corporation, Shizuoka, Japan) that contained an anticoagulant-preservative solution (63 mL of the anticoagulant citrate-phosphate- dextrose (CPD) with 100 mL of OPTISOL red cell preservative solution. Subsequently, leucofiltration was performed using leucocyte filtered blood bag (BioR Max, Fresenius Kabi, Bad Homburg, Germany) within 48 hours post-collection, and the bag was stored at 2–6 °C before undergoing the washing process.

Washing of packed RBCs

All the 32 leucofiltered blood bags were divided into two groups; 16 bags in group I and 16 bags in group II. Group I bags were washed with a solution containing a mixture of NS+0.2% dextrose supplied by Haemonetics (Haemonetics Corporation, Braintree, MA, USA) whereas group II bags were washed with NS. The washing process was performed at room temperature using an automated red cell washer (ACP 215, Haemonetics Corporation, Braintree, MA, USA) according to the procedures recommended by the manufacturer. Each bag was connected to the disposable washing set using a sterile tubing connection welder (TSCD II, Terumo BCT, Lakewood, CO, USA). A 0.2 µm hydrophobic bacterial barrier filter was integrated into the system to prevent any potential contamination. The haematocrit (Hct) and weight of the bag were then entered into the system. If the bag weighed < 270 g, the machine would wash for four cycles within 20 minutes. If the bag weighed > 270 g, it would wash for eight cycles in 40 minutes. Upon completion of the washing process, the bag was automatically resuspended into Additive Solution-3 (Haemonetics Corporation, Braintree, MA, USA), which contains dextrose, sodium citrate, sodium chloride, sodium phosphate, citric acid, and adenine. The washed bags were stored at 2–6 °C for 14 days.

Sampling

For each bag, a 10 mL aliquot was taken at pre-wash, immediately post-wash (day 0), and at day two, seven, and 14 post-wash for laboratory testing. Haematocrit (Hct), haemoglobin (Hb), supernatant (plasma) Hb, pH, and potassium were measured. The Hct and Hb levels were measured using haematology analyser (Coulter LH750, Beckman Coulter, Miami, FL, USA); supernatant Hb content was measured using Hb device (HemoCue Ltd, Angelholm, Sweden); potassium level was measured using Cobas ISE device (Roche Diagnostics, Mannheim, Germany); and pH was measured using pH meter (Mettler Toledo Delta 320 pH meter, Zurich, Switzerland). The haemolysis percentage was calculated using the following formula (10):

$$\text{Haemolysis \%} = \frac{(100 - \text{Hct}) \times \text{plasma Hb}}{\text{Hb (g/dL)}}$$

Acceptance criteria

Table I lists the acceptance criteria used for washed RBCs in this study based on NBC guidelines and previous studies (11–13).

Table I: Acceptance criteria for haemoglobin, haematocrit, haemolysis, potassium and pH level.

Parameter	Acceptance criteria*
Haemoglobin (g/dL)	10.0
Haematocrit (%)	50–70
Haemolysis (%)	< 0.8%
Potassium (mmol/L)	≤ 10.2
pH level	> 6.5

*Acceptance criteria of the parameters were based on NBC guidelines and previous studies (11-13)

Statistical analysis and ethical approval

Data were analysed using IBM Statistical Package for the Social Sciences (SPSS) version 22 for Windows software (SPSS, Chicago, IL, USA). Repeated measures analysis of variance (ANOVA) was used to compare results between the two groups and within groups over the test duration period. The results were expressed as mean ± standard deviation (SD). Statistically significant was set at $P < 0.05$.

Ethical approvals were obtained from both National Blood Centre, Malaysia; (7)dlm.PDN/07-24 jld.2, and from the Research Committee, Advanced Medical and Dental Institute, Universiti Sains Malaysia (USM/IPPT/200/G-3/i).

RESULTS

The weight for all 32 leucofiltered blood bags was

between 250 g to 280 g. Table II shows the parameter differences and changes that occurred during this study. None of the parameters measured differed significantly between the two types of washing solutions ($P > 0.05$). However, there were significant changes in all parameters over 14 days of storage for both groups ($P < 0.001$). Nevertheless, all values remained within the acceptance criteria except for potassium level, which increased from 8.8 to 24.3 mmol/L (in NS washing solution) and from 8.9 to 23.2 mmol/L (in NS+0.2% dextrose washing solution) by day 14 of storage.

Figure 1 shows the percentage changes of the parameters from pre-wash to day 14 post-wash. Potassium level changed the most from the pre-wash to post-wash period, followed by haemolysis. For all parameters, the bags that were washed with NS+0.2% dextrose solution exhibited lower percentage changes compared to those bags washed with NS alone.

DISCUSSION

In this study, the two washing solutions produced comparable results and were effective as washing solutions. No significant differences in any of the parameters measured were detected between the two washing solutions. One potential explanation is due to the low concentration of dextrose (0.2%) in that mixed solution. A previous study also reported that co-infusion of saline and dextrose-containing fluid had no adverse effect on in vitro RBC product quality (14).

Over the storage period of 14 days, significant changes were detected in all parameters tested ($P < 0.001$). These biological changes to stored RBCs are collectively referred to as storage lesions, and they include membrane vesiculation, which results in Hb loss from circulating RBCs (15), morphological alteration of RBCs to non-discocyte phenotypes (16), decreased adenosine triphosphate levels, pH, and 2,3-diphosphoglycerate content, increased potassium and lactate levels, and released of cytokines and oxygen radicals (17). Storage lesions occur as early as within a few hours of storage, and normally about 0.2–0.4% of stored RBCs in additive solutions undergo haemolysis after five to six weeks of storage (18). Furthermore, prolonged storage is accompanied by an increase in RBCs osmotic fragility, which subsequently leads to haemolysis (19).

Our study showed that both Hb and haemolysis levels differed significantly over 14 days of storage. As haemolysis is calculated from Hb level, any factors that cause Hb decrement might also contribute to the net haemolysis percentage. Additionally, variation in blood donor's race, age, and gender may influence the degree of haemolysis in the stored RBCs products (18). Furthermore, frequent blood donation was also found to have an impact on the haemolysis susceptibility of stored blood (20). Besides, RBCs haemolysis also can be induced by processing methods, such as rapid mixing with anticoagulant or additive solutions, shear-induced damage during stripping of RBCs into sample tube segments, forcing through a leucocyte filtration filter, high centrifugation speed during packed RBCs preparation, and improper temperature during transportation or storage (21,22).

Table II: Laboratory parameters of washed packed red blood cells over a storage period of 14 days (mean ± SD)

Washing solution	Time	Hb (g/dL)	Haematocrit (%)	Haemolysis (%)	Potassium (mmol/L)	pH
NS (n = 16)	Pre-wash	19.7 ± 1.42	62.3 ± 2.13	0.4 ± 0.18	8.8 ± 2.15	7.5 ± 0.25
	Day 0	16.7 ± 1.13	53.0 ± 3.29	0.2 ± 0.55	1.6 ± 0.46	6.6 ± 0.14
	Day 2	16.6 ± 1.16	52.3 ± 3.30	0.2 ± 0.06	5.6 ± 1.02	6.6 ± 0.14
	Day 7	16.6 ± 1.29	52.0 ± 3.30	0.3 ± 0.09	14.6 ± 1.94	6.7 ± 0.58
	Day 14	16.5 ± 1.32	51.9 ± 3.44	0.4 + 0.12	24.3 ± 3.48	6.5 ± 0.96
NS+0.2% dextrose (n = 16)	Pre-wash	19.9 ± 0.92	63.1 ± 2.24	0.4 ± 0.18	8.9 ± 2.51	7.5 ± 0.25
	Day 0	16.3 ± 1.37	51.7 ± 4.02	0.2 ± 0.08	1.7 ± 0.73	6.5 ± 0.11
	Day 2	16.3 ± 1.35	51.1 ± 3.73	0.2 ± 0.04	5.3 ± 1.23	6.5 ± 0.13
	Day 7	16.2 ± 1.37	50.9 ± 3.93	0.3 ± 0.05	13.9 ± 2.38	6.6 ± 0.09
	Day 14	16.1 ± 1.42	50.6 ± 4.21	0.4 ± 0.11	23.2 ± 4.15	6.5 ± 0.09
*Between subject effect		F (df) = 0.370 (1,30) $P = 0.547$	F (df) = 0.702 (1,30) $P = 0.409$	F (df) = 0.014 (1,30) $P = 0.907$	F (df) = 0.381 (1,30) $P = 0.542$	F (df) = 1.195 (1,30) $P = 0.283$
*Within subject effect: time effect		F (df) = 41.483 (4,27) $P < 0.001$	F (df) = 99.265 (4,27) $P < 0.001$	F (df) = 21.332 (4,120) $P < 0.001$	F (df) = 314.783 (4,27) $P < 0.001$	F (df) = 364.349 (4,27) $P < 0.001$
*Within subject effect: time and group interaction		F (df) = 0.623 (4,27) $P < 0.650$	F (df) = 0.623 (4,27) $P = 0.650$	F (df) = 0.348 (4,120) $P = 0.731$	F (df) = 0.354 (4,27) $P = 0.839$	F (df) = 1.209 (4,27) $P = 0.330$

NS:normal saline, Hb: haemoglobin
* Repeated measures ANOVA, $P < 0.05$ indicates statistically significant

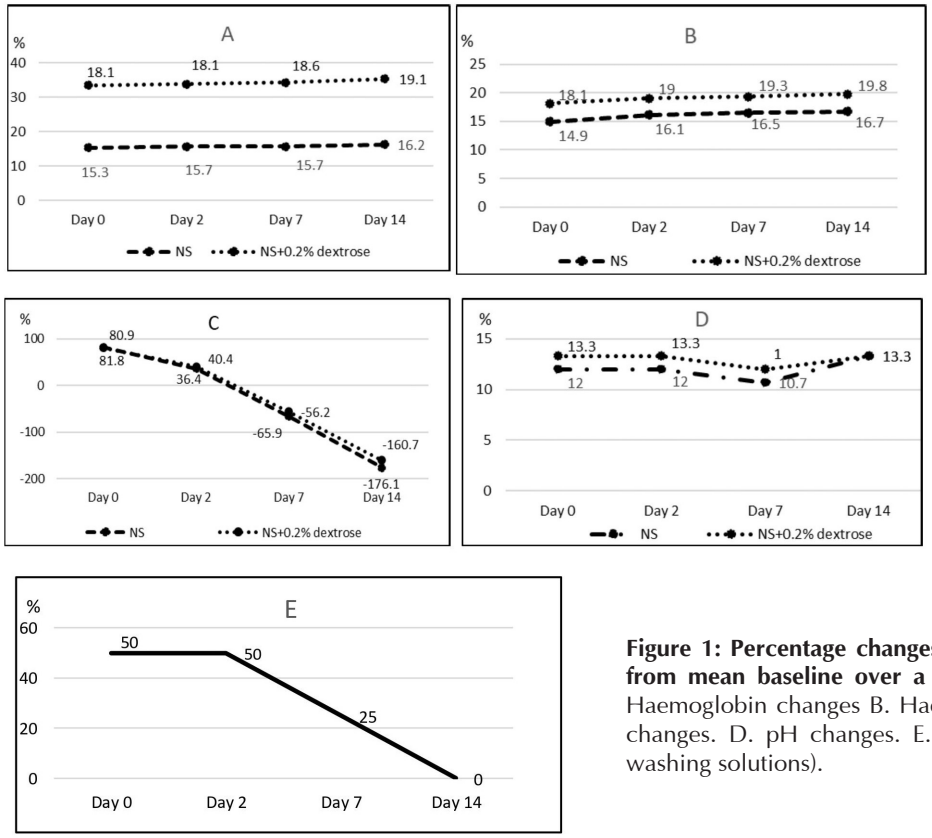

Figure 1: Percentage changes of parameters in both groups from mean baseline over a storage period of 14 days. A. Haemoglobin changes B. Haematocrit changes C. Potassium changes. D. pH changes. E. Haemolysis changes (for both washing solutions).

During RBCs haemolysis, lactate dehydrogenase is released and may further decrease the pH of stored blood (23). Haemolysis of RBCs also releases intracellular potassium and causes an increase in potassium levels. Because of the cellular transport mechanism, pH is also interrelated with potassium level; the potassium level increases during acidosis and decreases during alkalosis (24,25).

Among the parameters measured in this study, the potassium level changed most significantly over storage for 14 days. From pre-wash to day 0 of post-wash, the potassium level decreased to 81%, which indicated that washing is an effective way to remove potassium. However, a previous study reported that RBCs are fragile after washing and that haemolysis is greater than that found with other manipulation methods such as leucoreduction and irradiation (26). Nevertheless, our study showed that the potassium level had increased beyond its acceptance criterion by day 7 of storage. Thus, two days of shelf life of washed RBCs product is recommended irrespective of types of washing solution to prevent potassium accumulation.

The Hct % in our study was relatively stable and met the acceptance criterion even though it showed a significant decrease over 14 days of storage. Our result was also comparable with an earlier study performed by Grabmer et al. They studied different times of RBCs storage before washing process (6 days, 14 days, and 21 days) by using ACP 215 automated washing device and NS + 0·2% dextrose as washing solution. Their study found that the Hct level was between 49.0 % to 49.9 %, Hb level of 16.4 to 17.0 g/dL, and pH level of 6.3 to 6.8 by day 14 post-wash (27). In another study by Bennett-Guerrero et al for longer stored RBCs (40-42 days) using NS washing solution, the authors found that there were decreased in Hct, potassium, and RBCs recovery using Haemonetics Cell Saver Elite autotransfusion system as compared to Terumo Cobe 2991 cell processor device. However, Cell Saver Elite caused more haemolysis, total cell-free Hb, and RBC-derived microparticle formation. This was probably due to higher centrifugation g-force (2,034) used by Cell Saver Elite as compared to Cobe 2991 (g-force 1,245), and thus causing slightly more cell damage (28).

Nevertheless, our study had some limitations as it did not assess the protein content which is one of the quality parameters in washed RBCs. Besides, assessment of other metabolic and proteomics analyses of post-wash RBCs is also necessary to understand the effects of storage in post-wash RBCs products. Furthermore, assessment of other factors such as time of RBCs storage before washing, amount of washing solutions, centrifugation force as well as donors' characteristics are also important aspects that may contribute to the parameters changes in the washed RBCs.

CONCLUSION

The results of this study showed that packed RBCs can

be washed using either NS or a mixture of NS and 0.2% dextrose solution. During the 14 days of storage, all parameters remained within the acceptance criteria except potassium level, which showed a mark increased above acceptance criteria from day 7 onwards. Hence, it is recommended that post-wash RBCs shelf life is set at two days and the products are transfused to those clinically indicated.

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