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Evaluation of hemolysis during storage of red blood cell concentrates processed by centrifugation and settling method by simple gravity in Burkina Faso

Abstract

Background: Hemolysis is one of the red blood cell concentrates (RCCs) storage concerns. In Burkina Faso, hemolysis rate was not routinely assessed in RCCs. Our study aimed at assessing the degree of hemolysis in RCCs processed by centrifugation (centrifuged RCCs) and whole blood settling by simple gravity (Sedimented RCCs) in Burkina Faso.

Methods: We conducted a cross-sectional comparative study on 46 prepared by centrifugation and 46 prepared by sedimentation, matched on their collection date and initial volume of whole blood. The hemolysis percent was measured on Days 0, 7, 14, 21, 28 and 32.

Results: In the centrifuged RCCs, the hemolysis percent on D0 was 0.232% versus 0.199% for the sedimented RCCs (p = 0.046). At D32, the average hemolysis percent was 0.835% for the sedimented RCCs and 0.779% for the centrifuged RCCs (p = 0.042). The degree of hemolysis increased gradually between D0 and D32 with an average increase of 0.120% for centrifuged RCCs and 0.116% for sedimented RCCs.

Conclusion: The degree of hemolysis at D32 in both centrifuged RCCs and sedimented RCCs falls below standards. Therefore, it necessary to revisit blood components processing procedures, focusing on the centrifugation parameters and the handling conditions.

Keywords: red cell concentrate, hemolysis, storage lesions, centrifugation, storage duration

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Abbreviations: RCC, Red cell concentrate; RBC, Red blood cell; Hb, Hemoglobin; USA, United State of America; %, percent; RBTC, regional blood transfusion center; CPD, citrate-phosphate-dextrose; SAGM, saline-adenine-glucose-mannitol solution; G6PD, glucose-6-phosphate deshydrogenase; NBTC, national blood transfusion center; CIRS, internal scientific review committee; cm, centimeter; D, day; mL, milliliter; S_RCC, sedimented red cell concentrate; C_RCC, centrifuged r4ed cell concentrate; °C, degree celsius; g, gravitation symbol

Introduction

Red blood cells (RBCs) undergo multiple changes during storage known as storage lesions. Indeed, they change shapes, from the normal discoid form to echinocytes and finally to spherical form, become more rigid, shed lipid, exhibit fall in Adenosine-triphosphate and 2,3-diphosphoglycerate content before the membrane ruptures.[1] This rupture causes the release of free hemoglobin (Hb) into the suspending fluid. At visual inspection, hemolysis appears as an orange-red color, due to the presence of Hb in the supernatant.[2–5]

RBCs hemolysis can occur either during blood components processing or handling, transportation and storage.[2–5]

It is the consequence of several factors comprising blood components processing procedures (time between blood collection and separation, the type of anticoagulant and preservative solution in the blood bag, the centrifugation speed), handling and storage conditions, RBCs intrinsic abnormalities, blood donors characteristics.[4,6,7]

As one main indicator of the amount of RBC storage lesions, hemolysis in stored red cell concentrate (RCC) units raises many issues. Firstly, it can affect the dose of oxygen-carrying capacity (ie Hb within erythrocytes) that the blood unit must provide if transfused. Secondly, given the toxicity of free Hb, harmful consequences, such as kidney damage could occur if several RCC units with significant hemolysis were transfused to the patient.[8]

Several techniques are used to assess the measurement of hemolysis in RCCs, with the tetramethylbenzidine method, based on spectrophotometry that is considered as the "gold standard". Other techniques use photometry or microplate methods.[9–11] But these tests are not well suited to a blood manufacturing and delivery settings. They are cumbersome and expensive. The most common technique used in blood services is the visual inspection. Before delivery, the blood unit is inspected for a hemolytic supernatant.[5] However, this qualitative method has limitations even with colorimetric scales as a visual comparator for estimating hemolysis levels.[12,13]

It also lacks adequate standardization making inter-laboratory comparison difficult.[9] So, according, to the experience of the health worker who inspects the blood unit, the degree of hemolysis could be either overestimated, leading to unjustified destruction of RCC units, or underestimated, with the risk of transfusion of RCC units which contain high levels of free Hb.

In this context, blood services must implement quality systems with proven procedures to control blood components manufacturing, handling and storage processes. Such a quality system must include

quality controls on ready-to-use blood products with at-end-storage hemolysis thresholds not to be exceeded. These thresholds are less than 1% in USA and 0.8% in Europe.[3,14] Recently, the US Food and Drug Administration added the "95/95 rule" which requires that 95% of units meet the standard with 95% statistical certainty.[3] Some studies showed degree of hemolysis between 0.2 and 0.4% and 96.7 to 99.5% of units with an hemolysis percent less than 1%[3,14] at day 42.

In Burkina Faso, RCCs are processed from whole blood using the classic centrifugation technique. However, in some Regional Blood Transfusion Centers (RBTCs) without suitable facilities for centrifugation, the alternative settling method by simple gravity as previously described .[15] was used. The quality control on ready-to-use RCCs performed in RBTCs was limited to the measurement of the unit volume and the Hb content (i.e the entire amount of Hb in the blood unit). Changes occurring during the storage, mainly hemolysis, were not covered.[16,17]

Our study aimed at assessing the degree of hemolysis in RCC units processed both by centrifugation and settling methods in Burkina Faso.

Material and methods

Study setting

We conducted a cross-sectional comparative study at the RBTCs of Ouagadougou (RBTC-O) and Koudougou (RBTC-K). These RBTCs represent respectively the biggest and the third blood centers in Burkina Faso and collect annually around 40,000 and 12,000 blood units.

Burkina Faso is a low-income country that experiencing blood shortage and poor quality and unsafe blood transfusions.[18] T ropical infections such as malaria, dengue fever and others transfusion transmitted diseases are endemic. Blood products are issued mainly for anemia resulting from malaria, genetic red blood cell abnormalities and obstetrical hemorrhages.[19] Blood is collected from 18-60 years' voluntary unpaid blood donors of both genders. Each donor underwent a pre-donation interview conducted by trained healthcare workers using standardized selection questionnaire. Beside the donors' behaviors and other viral infectious risk factors, signs of acute infections as malaria or bacterial infections and chronic diseases comprising hemoglobinopathies, chronic inflammations are assessed.[20] Blood is collected in triple bags system, the main bag containing 63 mL of CPD-anticoagulant (citrate, phosphate, dextrose) and one of the two satellite bags containing 100 mL of SAGM additive preservative solution (saline, adenine, glucose, mannitol). Units of less than 300 mL or more than 510 mL are not suitable for therapeutic use.[16] Apart from the screening of serological markers for human immunodeficiency virus infection, hepatitis B and C and syphilis as well as the pre-donation Hb measurement, no other biological analysis is performed on the collected blood units.[18]

At the RBTC-O, whole blood is typically processed within 48 hours of collection into lecukocyte containing RCCs. They are centrifuged at 2490 g for 20 minutes and then the plasma is extracted using manual presses. Less than 15% of whole blood are processed into platelet concentrates and fresh frozen plasma. In the RBTC-K, the whole blood bags are stored vertically at a temperature of 4±2 °C for at least 72 hours, then the plasma is extracted and destroyed.[15] The centrifuged RCCs (C_RCCs) and the sedimented ones (S_RCCs) are re-suspended in 100 mL SAGM additive preservative solution and stored in temperature-controlled refrigerators between 2 - 6 °C for maximum 42 days. Upon the blood unit delivery, it is visually inspected for hemolysis signs or other macroscopic abnormalities.

Study materials

Selection of RCC units: Our study randomly included 92 ready-to-use RCCs comprising 46 units of C_RCCs and 46 units of S_RCCs, matched each other on the date of collection and the initial volume of whole blood ± 50 mL. The units that presented macroscopic abnormalities on visual inspection (presence of clots, loss of the bag integrity, greenish or blackish coloration) were not included. The RCCs included in the study were kept under standard storage conditions.[16] The day of blood collection was considered as day 0 (D0). On D0, D7, D14, D21, D28 and D32, the RCCs were visually inspected and blood samples taken (as described below) for the measurement of supernatant hemoglobin (supernatant Hb).

Blood sampling and biological analyzes: A blood sampling method that preserved RCC unit for delivery to patients who needed blood transfusion at the end of study, was carried out. After blood collection and processing, a total length of 100 - 120 centimeters (cm) of the bag tubings (tubing connecting the main bag to the needle and to the satellite bags) was kept for quality controls and compatibility tests (before delivery) purposes. These tubings are stripped and clamped in order to mix tubing blood with the blood in the bag. The bag is homogenized by successive inversions and then the tubing is refilled and 10 – 15 cm is sealed and cut for blood sampling in an anticoagulant-free tube. Blood samples was collected by this way on D0, D7, D14, D21, D28 and D32. These samples were centrifuged for 5 minutes at 2500 rpm and the supernatant was used for supernatant Hb measurement.

For each RCC unit, the total hemoglobin (Total Hb) and hematocrit were measured at D0 using an ABX MICROS 60 ES hematology analyzer (HORIBA ABX SAS, Kyoto, Japan). The supernatant Hb was measured at D0, D7, D14, D21, D28 and D32 on a spectrophotometer UV-Visible Cecil Aquarius CE 7400 (Cecil Instruments Limited, Cambridge CB24 6AZ, Royaume-Uni) using the photometric method with determination at end point at a wavelength of 540 nm. The degree of hemolysis expressed in percentage of free Hb in relation to the total Hb (i.e. free and intraerythrocytic Hb) of the unit was calculated at each measurement using this formula:

$$Hemolysis\ percent\ (\%) = \frac{[(100 - Hematocrit) \times Supernatant\ Hb(g.dl^{-1})]}{\text{Total } Hb(g.dl^{-1})}$$

Data management and statistical analysis

Data was entered using Epi-Data 3.1 and analyzed using Stata 15. The minimum, mean and maximum values of the hemolysis percent were calculated for each type of RCCs at D0, D7, D14, D21, D28 and D32. The average hemolysis percent for each type of RCCs from D7 to D32 was compared to D0 hemolysis using paired t-test. The average variation in hemolysis between D0 and D32 for C_RCCs and S_RCCs were compared using a paired t test at a significant level of $p < 0.05$.

Ethical considerations

The study protocol was approved by the Internal scientific review committee (CIRS) of the National blood transfusion center (NBTC).

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Whole blood was collected from voluntary non-remunerated blood donors. All have signed an informed consent so that the collected blood and samples undergo mandatory analyzes and be used for research. The study was conducted with respect to the quality system requirements of the NBTC. So, we carried out a blood sampling method that preserved the integrity and quality of the RCC units. RCC units with macroscopic hemolysis or a hemolysis rate greater than 0.8% were discarded.

Results

The characteristics of the 92 RCCs included in our study are stated in Table 1. At baseline, there were difference in volume, Hb content and hematocrit between the centrifuged RCCs and sedimented ones. The hemolysis percent at D0 was 0.232% and 0.199% respectively for C_RCCs and S_RCCs (p = 0.04). The maximum hemolysis was observed on D32 for the two groups, with 1.460% for C_RCCs versus 1.137% for S_RCCs. A hemolysis percent greater than 1% were observed in C_RCCs on D14 and on D28 for S_RCCs (Table 2).

Table 1 Overview of the baseline characteristics of the 92 red cell concentrates included in the study Burkina Faso

Parameter	Centrifuged RCC (mean ± sd)	Sedimented RCC (mean ± sd)	Reference values
Volume (mL) Þ	240.4±35.7	320.30±48.9	130 – 380
Hemoglobin content (g) Þ	55.1±8.5	49.3±7.6	> 40
Hematocrit (%) Þ	56.2±4.5	48.2±6.3	50 - 70

RCC, red cell concentrates; mL, milliliter; g, gram; %, percentage; sd, standard deviation; Þ: p < 0.05

Table 2 Mean, minimum and maximum hemolysis percent in centrifuged and sedimented red cell concentrates according to storage duration, Burkina Faso

Storage duration	Centrifuged RCC			Sedimented RCC			p-value
	Min (%)	Average (%)	Max (%)	Min (%)	Average (%)	Max (%)	
D0	0.108	0.232	0.523	0.098	0.199	0.307	0.046
D7	0.158	0.383	0.784	0.104	0.234	0.488	< 0.001
D14	0.163	0.489	1.121	0.148	0.368	0.605	< 0.001
D21	0.176	0.534	1.269	0.197	0.525	0.875	0.125
D28	0.226	0.677	1.181	0.227	0.619	1.099	0.035
D32	0.285	0.835	1.460	0.380	0.779	1.137	0.042

D, day; RCC, red cell concentrates; Min, minimum; Max, maximum; %, percentage

At D32, the average hemolysis percent was almost equal to 0.8% for the S_RCCs and greater than 0.8% for C_RCCs. We noted that 16.7% (7/42) of C_RCCs and 7.1% (3/42) of S_RCCs had a hemolysis percent greater than 0.8% at D32. Two C_RCCs and one S_RCC presented a macroscopic hemolysis detected at visual inspection at D32.

The hemolysis percent increased gradually from D0 to D32 (Figure 1) with an average increase of 0.120% and 0.116% between two measurements respectively for C_RCCs and S_RCCs. A total average increase of 0.603% and 0.580% was noted respectively for C_RCCs and S_RCCs (p = 0.02).

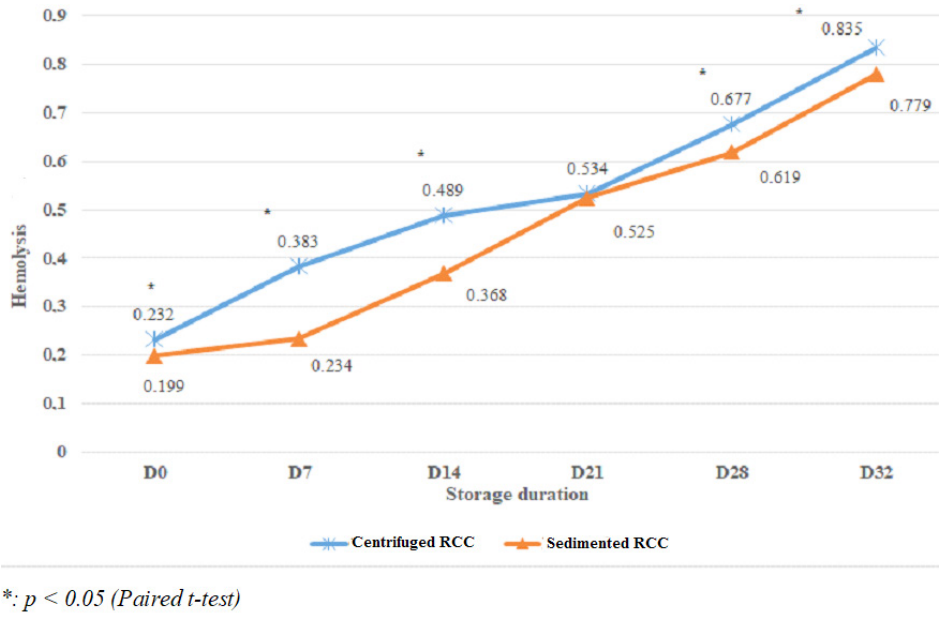

Figure 1 Evolution of degree of hemolysis in centrifuged and sedimented red cell concentrates during storage, Burkina Faso.

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Discussion

Our study aimed at assessing the degree of hemolysis in RCCs units prepared by two methods, centrifugation and sedimentation. We found that the hemolysis percent increased gradually between D0 and D32 with an average increase of 0.120% for centrifuged RCCs and 0.116% for sedimented RCCs. At D32, the average hemolysis percent was 0.835% for the sedimented RCCs and 0.779% for the centrifuged RCCs ($p = 0.042$).

These findings give actual information on an aspect of the quality of RCCs that is not took into account in routine quality controls. Indeed, the quality control on the ready-to-use blood components in Burkina Faso is limited to the control of volume and hemoglobin content. However, the document on the standard and characteristics of blood components in Burkina Faso[16] as well as the standards for the steps-by-steps accreditation program of the Africa Society of Blood Transfusion[21] prescribe the control of hemolysis rate. However, our study has some limitations. We did not assess that at-end-storage (at D42) hemolysis levels; patient demand for blood was not fully covered and it would not be ethical to store blood units longer than 32 days just for our study purposes. Our study assessed only the hemolysis percent that is certainly an important parameter of the RBCs storage lesions, but it is not the only one. The dosage of potassium and lactate dehydrogenases in plasma could be informative. Moreover, since we have not tested the presence of plasmodium or other hematophagous parasites in the blood units as well as any abnormal hemoglobin such as Hb S or C (which are frequent in our context), we were therefore unable to analyze their influence on the degree of hemolysis of the RCCs.

The D32 hemolysis percent (0.835% and 0.779% respectively for C_RCCs and S_RCCs) was higher than those noted by other authors at D42. Indeed, Makroo et al.[2] and Arif et al.[1] in India noted at-end-storage hemolysis percent of 0.553% and 0.359% respectively in RCCs containing a SAGM additive solution. Gkoumassi et al.[3] in the Netherlands found at-end-storage hemolysis percent varying from 0.267% to 0.304%. Several factors can explain these differences including inappropriate handling during processing, improper storage conditions, high centrifugation speed, rapid addition of additive preservative solution, variation in the quality of blood bags, bacterial hemolysins, antibodies causing complement mediated lysis, red cell membrane defects.[1]

The studies in India and Netherlands included leukoreduced SAGM RCCs, contrary to our context where leukoreduction was not practiced. Leukocytes contain numerous chemical components and enzymes like proteases, which are involved in RBCs hemolysis. Also, leukoreduction halves the occurrence of hemolysis as showed by Hess et al., with a reduction of the hemolysis percent at D43 from 0.34±0.29% to 0.16±0.07% on more than 10,000 RCCs.[14] In another study, authors noted that all the RCC units with more than 1% of hemolysis concerned non-leukoreduced units.[22]

Furthermore, the centrifugation speeds can induce a physical stress on RBCs and predisposes them to hemolysis. In our study, C_RCCs were obtained after a centrifugation at 2490 g for 20 minutes (approximately 2225 rpm), unlike the study by Arif et al.[1] where the whole blood units were centrifuged at 1750 rpm for 9 minutes. We found a significant difference in hemolysis percent between RCCs obtained by centrifugation and decantation, supporting the hypothesis of an impact of the centrifugal force.

In addition, in our context, the storage period before transformation was quite long (48 - 72 hours) while it was 24 hours maximum in most of the referred studies. Such long delays favor the degradation of leukocytes and the release of their toxic substances that alter RBC membrane. This long storage time before transformation and the hard centrifugation in our context probably explain the very high hemolysis percent at D0 (0.232% for C_RCCs and 0.199% for S_RCCS). In similar studies, the hemolysis at D0 was 0.027 - 0.057% in the Netherlands[3] and 0.81 - 0.1% in India.[1,2] The hemolysis percent at D0 in our study was sometimes higher than the at-end-storage (D42) hemolysis percent in some studies in Europe.[3,14] This testifies to the potential deleterious effects of blood collection kits, blood components processing techniques and equipment and blood storage conditions on RBCs.

Beside the impact of the factors cited above, we can discuss certain characteristics of blood donors. Indeed, the correlation between RBC defects (membrane, hemoglobin and enzymes abnormalities) and the occurrence of hemolysis is well described. RBCs of individuals with G6PD deficiency, hemoglobinopathies may be more prone to hemolysis.[23] Physiological differences related to gender could have some impact. Indeed, RCCs from male donors were more likely to hemolyze.[24,25] Female blood on average hemolyzed 21% less than male blood. The difference seems to be greatest during donor's reproductive years, suggesting a possible role of sex hormones.[25]

The hemolysis percent gradually increased from D0 to D32, both in centrifuged RCCs (+ 0.603%) and in sedimented ones (+ 0.580) with respectively an average increase between two measures of 0.120% and 0.116%. This observation is expected and was already underlined.[1–4,23] It reflects the gradually cellular changes occurring during storage.[4,6,26]

We had hemolysis percent greater than 0.8% in centrifuged RCCs and sedimented RCCs respectively at D14 and D18. At D32, there were 16.7% of centrifuged RCCs and 7.1% of sedimented RCCs had hemolysis percent greater than 0.8%. In the Indian studies, such an achievement was not observed.[1,2,27] These findings show that the RCCs produced in Burkina Faso, mainly by centrifugation, do not fulfill standards requirements that fix the hemolysis threshold at less 0.8%.[16] The evolution of hemolysis percent on Figure 1 clearly suggests that at the end of storage on D42, the hemolysis percent will be far beyond the authorized limit, both for centrifuged and sedimented RCCs. This is a major concern, since visual inspection seems to have a low sensitivity to detect hemolysis.[5] In our study, the visual inspection have detected hemolysis only in three units. This questions the safety blood units delivered after visual inspection and transfused to patients in many blood transfusion services. These cases could cause adverse reactions in patients due to the harmfulness of free hemoglobin on certain organs. Circulating free hemoglobin can bind with nitric oxide at endothelium and induce vasoconstriction, endothelial dysfunction and platelet activation, which increases the risk for thrombus formation.[27] It can also cause kidney damage.[8] Unfortunately, because of weakness of hemovigilance system in sub-saharan countries, these adverse reactions are underreported.[19]

We noted that hemolysis percent was significantly higher in C_RCCs than in S_RCCs from D0 to D14 and D28. In addition to the physical stress due to centrifugation already mentioned above, we can discuss the volume of residual plasma. Indeed, as shown on table I, the volume of sedimented RCCs was greater than the volume of centrifuged RCCs, due to a larger volume of residual plasma in sedimented RCCs (81 mL versus 25 mL) reported in previous study.[15] So, sedimented RCCs could build up lesser levels of lactate and metabolic waste products that might affect hemolysis late in storage.

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Conclusion

The RCCs during their preparation and storage undergo hemolysis. Our study shows that the percent hemolysis for both centrifuged and sedimented RCCs observed in Burkina Faso fall below the international standards. So, the National blood transfusion service in Burkina Faso needs to review and improve RCC processing methods. The major points to revisit are the storage and handling conditions, mainly the time from whole blood collection to blood components processing that must be reduced at most 24 hours, the centrifugation parameters.

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Conflicts of interest

None.

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