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Assessing Haemoglobin Concentration and Red Cell Morphology in Stored Blood Units

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Authors' contributions

This work was carried out in collaboration among all authors. This study was designed and supervised by authors SBB and LQ. Authors KB and SJR drafted the manuscript. Authors PPD and BG contributed to the draft of the manuscript. Authors KB, SJR and FB participated in the recruitment and sampling of study subjects. Authors MB and YA made financial contributions towards the study and were involved in the laboratory analysis of the samples. Authors MB, CN and KM made contributions to the study design and also helped draft the manuscript. Author SBB supervised and made intellectual contributions to the manuscript. Author MB participated in subject recruitment. Authors PPD, BM, YA and LQ were involved with the statistical analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study assessed and compared the haemoglobin concentration and red cell morphological changes in stored blood units at the Tamale Teaching Hospital located in the Northern Region of Ghana.
Methods: This is experimental research conducted from November, 2019 to June, 2020. Thirty blood samples were collected (450ml) from voluntary donors at the blood bank unit of the Tamale

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Teaching Hospital for this study. The samples were collected into blood bags that contained CPDA-1 preservative and stored at 2-6℃ for thirty-five days. The haemoglobin concentration and red cell morphology of the samples were assessed at collection and every seventh day (weekly) till the thirty fifth day using the URIT-15 hemoglobin meter and Leishman-stained thin films.
Results: Microscopic examination of Leishman-stained thin films indicated significant degenerative changes in red cell morphology as the storage duration increased. The red cell morphology showed changes from normocytic cells through to echinocytes, spheroechinocytes and spherocytes. ANOVA with a Greenhouse-Geisser correction revealed the mean of haemoglobin (Hb) values as statistically significant between storage days (day 0, 7,14, 21, 28 and 35) ($F(3.264, 94.657) = 18.967$, $p < 0.05$). Post hoc analysis revealed significant differences between storage days. There were statistically significant differences in haemoglobin concentrations between days 7 and 35 of storage, as well as days 21 and 35 and between days 28 and 35 [$p < 0.05$]. Precisely, at day 0 (12. 0633± 1.10812, M±SD) the haemoglobin concentrations were higher than the other storage days. Conclusion: There is gradual reduction in haemoglobin concentration as well as gradual significant degeneration in red cell morphology as storage age of blood increased. The study therefore recommends that, blood with less storage duration should be preferred for transfusion for best therapeutic improvement.

Keywords: Stored blood; cpda-1; haemoglobin; lesion; morphology; red blood cell; storage and transfusion.

1. INTRODUCTION

Allogenic transfusion of blood or any of its components for therapeutic reasons is a relatively common treatment modality employed in modern-day healthcare in dealing with diverse medical conditions, thus making blood the most frequently demanded and applied medicine. Transfusion aims not only to provide blood but ensure it is safe and efficacious post transfusion. However, despite the major scientific breakthroughs acclaimed over the years, there is yet to be produced any substitute for blood in terms of its therapeutic value, thus making blood and many of its components one of the most frequently demanded and applied medicine globally [1]. It has been reported that more than a half million women die each year from severe bleeding as a result of childbirth and pregnancy related complications [2]. This underscores the need for transfusion to prevent some of these mortalities [3]. Among 46 member states of the WHO Africa region with total population of 836,969,536, the demand for blood transfusion is over eight (8) million units per year [4]. Although being the most frequently applied medicine worldwide, it remains one of the major public health concerns [5]. Storage and preservation of blood is therefore required to ensure readily available safe blood supply for transfusion. However, despite the efforts made, blood tends to undergo certain biochemical, molecular and morphological changes termed ''storage lesions'' during storage which can contribute to post transfusion complexities in some medical conditions.

Red Blood Cells (RBCs), are the most abundant cells among the 3 types of blood cells (Red blood cells, White blood cells and Platelet). The key role of RBC is delivery of oxygen to tissues in the body. The delivery of the oxygen is facilitated by an iron containing metalloprotein called haemoglobin found in red cells. The lifespan of RBC is about 120 days before it is aimed for destruction by the reticuloendothelial system, usually in the spleen and liver. It is understood that the ageing of RBCs is prone to entail many structural changes on the cell surface, which finally triggers macrophage recognition and phagocytosis [6].

Blood is collected into blood bags. The blood bags are sterile and pyrogen-free. The labels indicate the type, quantity of anticoagulant and the quantity of blood it can contain. Normally, blood is collected into either a 450mL or 500mL blood bag, which contains 63 mL of anticoagulant [1].The type of anticoagulant or additive in the blood bag determines the shelf life of the RBCs during storage. The most common anticoagulant used is the Citrate Phosphate Dextrose Adenine(CPDA-1), which has a storage time of 35 days. The essence of the appropriate storage is to counteract damages to the metabolic machinery and cell membrane of the red cells in order to maintain viability of the red cells and functional efficacy post-transfusion [7].

Many studies have shown that a group of disorders called 'storage lesion' affect red cells during storage [8-11]. Storage lesions are morphological and metabolic anomalies which affect red cells during storage, and these are associated with reduced survival of cells post-transfusion, increasing the risk of transfusion related mortality, morbidity and these lesions significantly increase with increasing duration of storage time [12,13].

The storage medium contains dextrose which provides glucose for glycolysis in stored blood. The medium also provides ATP, 2,3DPG and NADH needed to maintain red blood cells functionality and viability. Glycolysis results in the accumulation of byproducts as storage duration increases. Lactic acids in the supernatant result in acidosis which in turn inhibits glycolysis through a negative feedback mechanism leading to the reduction in ATP, 2,3DPG and NADH [14]. The reduction in metabolic activity results in depletion of ATP, 2,3DPG [15]. The progressive accumulation of byproducts of glycolysis and lactic acid result in decreased pH. These changes affect red cell membrane and shape, thus leading to loss of deformability with the formation of reversible echinocytes, irreversible spheroechinocytes and spherocytes [16].

This study aimed to assess the haemoglobin concentrations and red cell morphologies before storage and during storage at a regular interval of seven days till the 35^{th} day in order to ascertain the association between storage time, haemoglobin level and red cell morphologies. This study revealed the association between storage duration, haemoglobin concentration and red cell morphologies to help develop strategies or guidelines for prescription of blood.

2. MATERIALS AND METHODS

2.1 Study Design

An experimental research design was used to evaluate and comparatively assess the relationship between haemoglobin concentrations, red cell morphologies and storage duration at the blood bank of the Tamale Teaching Hospital (TTH).

2.2 Inclusion Criteria

Healthy participants between the ages of 18 to 65 years were enrolled for the study. Healthy participants in this context are those individuals that passed the blood donor selection criteria; healthy donor appearance, not showing any sign of febrile and persistent coughing, weight not less than 50 Kg and body temperature not more than 37.6 $^{\circ}C$.

2.3 Exclusion Criteria

Subjects out of the 18 to 65 age range were excluded from the study. Blood from individuals who did not pass the blood donor selection criteria were also excluded from the study.

2.4 Sample Collection and Processing

After obtaining informed consent from the participants, they were subjected to the donor selection criteria, donor interview, donor health and risk assessments, and pre-donation counselling. Participants were physically examined and haemoglobin concentration checked using the copper sulphate method [17].

Successful participants were prepared for phlebotomy following all quality control and safety procedures. Exactly 450ml of blood was collected from each participant into a blood bag containing Citrate-Phosphate Dextrose Adenine (CPDA-1) anticoagulant. The blood units were labelled with the donor identification number, date of collection and expiration date. Aliquot of 3ml of the gently mixed blood was collected into a plain tube and haemoglobin was measured and thin film for red cell morphology was prepared for day 0. The blood units were stored in a refrigerator at 2-6℃.

2.5 Haemoglobin Measurement

Aliquot (3 ml) of whole blood samples was drawn from each unit of blood into plain tubes. Haemoglobin concentrations were measured using URIT-15 haemoglobin meter manufactured by URIT medical electronic company limited.

2.6 Preparation of Thin Films

Clean dried slides were labelled with the participant's identification number. Micropipette was used to transfer 20µL of the whole blood to the center of the slide and another clean slide with smooth, flat edge used as a spreader. The spreader was placed in front of the blood at an angle of 30^0 - 45^0 allowing the drop to spread to the contact line of the two slides and the spreader pushed forward rapidly and gently. The slides were allowed to air-dry and then fixed in

absolute methanol and allowed to air-dry completely before staining with Leishman and morphology observed using light microscopy.

2.7 Data Analysis

Data for haemoglobin concentration was analyzed using Statistical Package for Social Sciences (SPSS) software. Data entry was cross-checked individually and compared. One-way Repeated means Analysis of Variance (ANOVA) was used to determine any statistical significance between storage periods (Day: 0, 7, 14, 21, 28 and 35). P-value of ≤ 0.05 was considered as statistically significant.

3. RESULTS

The mean haemoglobin concentration (g/dl) decreased gradually from 12.06 ± 1.11 g/dl at collection day 0 to 8.96 ± 2.09 g/dl at storage day 35 (Table 1). In this study, blood stored for one week (day 7) showed no significant reduction in haemoglobin concentration when compared with whole blood stored for 14 days (Table 3). Similarly, blood stored for 14 days showed no significant declined in haemoglobin concentrations as compared with blood stored for 21 days and 28 days. Blood stored for 21 days showed no significant decrease in haemoglobin concentration when compared with blood stored for 28 days (Table 3).

One-way Analysis of Variance (ANOVA) with a Greenhouse-Geisser correction showed that the mean haemoglobin (Hb) is significantly different between storage days (day 0, 7,14, 21, 28 and 35) (F 27.459, p =.000) (Table 2). Post hoc analysis revealed that the mean Hb is significantly higher on day 0; M = 12. 06g/dL, SD = 1.11 (Tables 1,3). Hb was significantly higher on day 7 compared with day 35 of storage. Similarly, Hb was significantly higher on day 21 compared with days 28 and 35 (Table 3).

Assessment of RBC morphology at collection day 0 generally showed normocytic normochromic cells with no notable changes in the shape of the RBCs. Storage day 7 generally showed normocytic normochromic cells with few echinocytes (Fig. 2). Morphological assessment of RBCs on the various storage days (14^{th}, 28^{th} and 35^{th}), revealed significant degenerative changes. The RBCs gradually changed from normocytes through echinocytes, spherocytes and spheroechinocytes (Fig. 2). Day 35 of storage revealed marked reduction of normocytes with marked spheroechinocytosis and spherocytosis.

Table 1. General characteristics

	Samples	Minimum	Maximum	Mean	Std. Deviation
Age of participants (years)	30	18	40	25.17	6.01
Hb (g/dL) of Day 0 of storage	30	8.80	13.60	12.06	1.11
Hb (g/dL) of Day 7 of storage	30	5.70	17.50	10.57	2.10
Hb (g/dL) of Day 14 of storage	30	6.20	16.50	10.01	2.07
Hb (g/dL) of Day 21 of storage	30	5.60	14.90	9.69	2.12
Hb (g/dL) of Day 28 of storage	30	5.30	17.70	9.52	2.31
Hb (g/dL) of Day 35 of storage	30	5.40	15.20	8.96	2.09

Hb: Haemoglobin, Std. Deviation: Standard Deviation

Table 2. Results of the ANOVA for within subject effects for haemoglobin (Hb)

Source		Type III Sum of Squares	Df	Mean Square	F	Sig.
Day	Sphericity Assumed	176.554	5	35.311	27.459	.000
	Greenhouse-Geisser	176.554	2.788	63.322	27.459	.000
	Huynh-Feldt	176.554	3.115	56.681	27.459	.000
	Lower-bound	176.554	1.000	176.554	27.459	.000
Error (Day)	Sphericity Assumed	186.466	145	1.286		
	Greenhouse-Geisser	186.466	80.858	2.306		
	Huynh-Feldt	186.466	90.331	2.064		
	Lower-bound	186.466	29.000	6.430		

df: Degree of freedom, F: F-Statistic or Ratio, Sig: Significance level at .05

Table 3. Post hoc comparisons of haemoglobin concentrations (g/dl) between storage days

(I) Day	(J) Day	Mean Difference (I-J)	Std. Error	Sig.
0	7	1.493	.352	.003
	14	2.057	.401	.000
	21	2.373	.388	.000
	28	2.547	.429	.000
	35	3.103	.385	.000
7	0	-1.493	.352	.003
	14	.563	.241	.394
	21	.880	.210	.004
	28	1.053	.239	.002
	35	1.610	.234	.000
14	0	-2.057	.401	.000
	7	-.563	.241	.394
	21	.317	.253	1.000
	28	.490	.268	1.000
	35	1.047	.265	.007
21	0	-2.373	.388	.000
	7	-.880	.210	.004
	14	-.317	.253	1.000
	28	.173	.219	1.000
	35	.730	.165	.002
28	0	-2.547	.429	.000
	7	-1.053	.239	.002
	14	-.490	.268	1.000
	21	-.173	.219	1.000
	35	.557	.154	.017
35	0	-3.103	.385	.000
	7	-1.610	.234	.000
	14	-1.047	.265	.007
	21	-.730	.165	.002
	28	-.557	.154	.017

The mean difference is significant at the .05 level

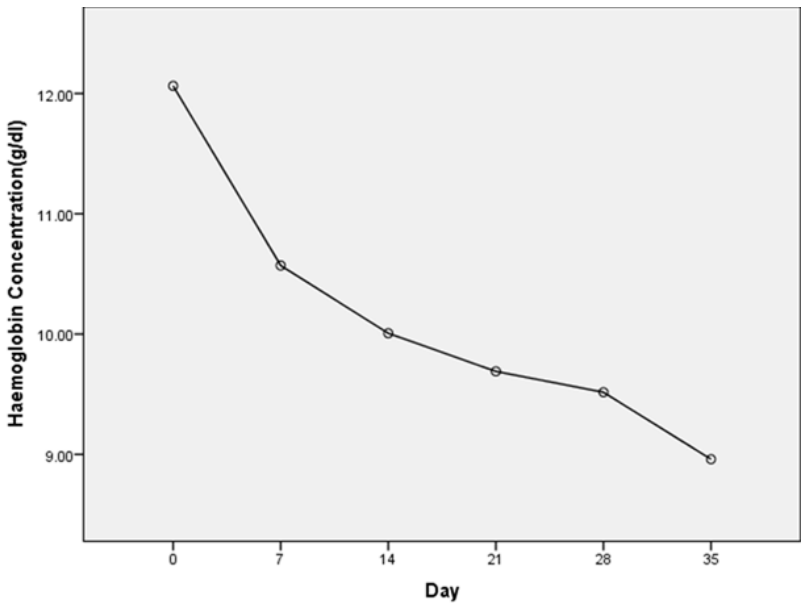

Fig. 1. Profile plot of estimated marginal means of haemoglobin

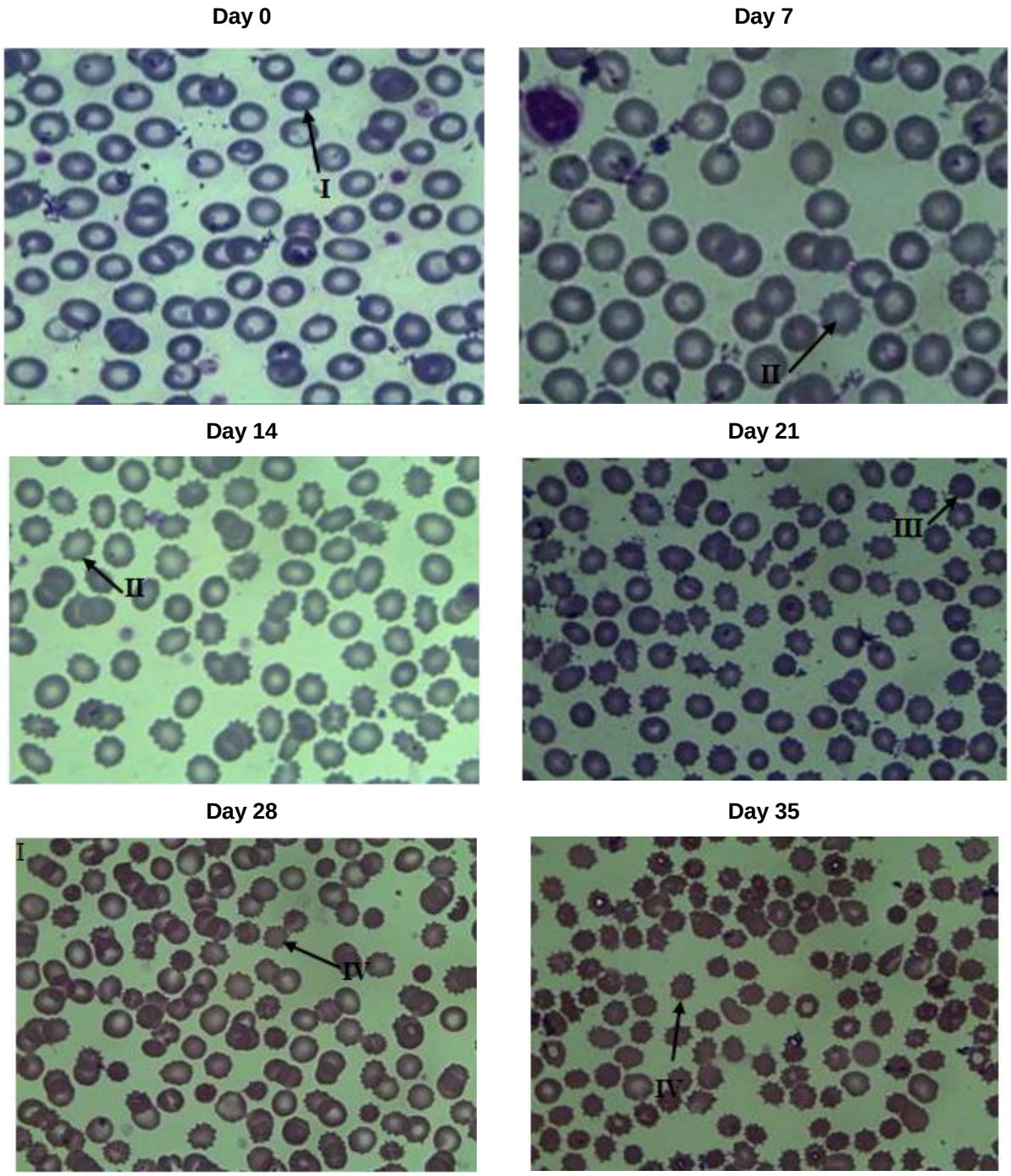

Fig. 2. Red cell morphology at the assessment days

Photomicrographs of representative slides showing the morphological abnormalities on the assessment days; day 0, 7, 14, 21, 28 and 35. Keys: I: Normocytic cell, II: Echinocytic cell, III: Spherocytic cell, IV: Spheroechinocytic cell

3.1 Morphological Assessment of Red Blood Cells

Microscopic examination of Leishman-stained thin films suggested significant degenerative changes in red cell morphology as the storage duration increased. The red cell morphology showed changes from normocytic through to chinocytes, spheroechinocytes and spherocytes.

4. DISCUSSION

The 30 voluntary blood donors in good health and within the ages of 18 to 65 years recruited into this study is in conformity with recommendations from the World Health Organization for allowable safe age range for blood donation [18,19]. The minimum, mean and maximum ages of 18, 25.17 and 40 years documented among donors respectively in this study is similar to the findings of Antwi-Baffour [20] which recruited 10 healthy donors between the ages of 20 to 52 years. Participants younger than 18 years were excluded in order to reduce the possibility of adverse effect which is common amongst younger people [21,22]. Younger adolescents also have an increased requirement for iron for their own growth and development, especially in adolescent menstruating females [23-25]. All the study participants were males, many of the female blood donors did not meet the criteria for blood donation because many of the females had haemoglobin concentration below recommended levels. These results are similar to those reported by Pavord, Myers [26], Salvin, Pasricha [27].

The decrease in haemoglobin as storage time increased could possibly be due to haemolysis of red blood cells in the blood bag. Blood undergoes gradual haemolysis in the suspending medium due to prolonged contact with plasma resulting in release of haemoglobin into the plasma, increasing the plasma haemoglobin while reducing the total haemoglobin within the RBCs. Also, as the storage duration increase there is haemolysis of the oldest red blood cells in the stored blood. This observation is consistent with studies conducted by MI Al Nuaimy [28] and Eze et al. [29] which documented decreases in haemoglobin concentration as storage duration increase. In contrast, similar study conducted by Adias, Moore-Igwe [30] did not find any decreases in Haemoglobin concentration as storage duration increase. The decreases in Hb observed in this study could be attributed to micro-environmental changes during storage. Normally, few RBCs haemolyse under storage conditions unless the RBC membrane becomes compromised. The decrease in haemoglobin concentration is indicative of membrane instability due to the fact that during storage, metabolism activities slow down resulting in reduce ATP and pH concentrations [31,32]. Reduced pH in RBC metabolic activity reduces the production of 2, 3-DPG, leading to shape change [20,33]. This leads to the development of a knobbed appearance and progresses into blunt echinocytic projections resulting in ultimate haemolysis [20,33].

The morphological changes observed on assessment days 0 and 7 are in consonance with the findings of Antwi-Baffour [20] and Bhargava et al. [34] which reported normocytic normochromic RBCs on assessment days 0 and 7. The morphological changes on days 14 and 21 are in contrast with the findings of Antwi-Baffour [20] who reported fairly normocytic cells with few echinocytes on day 14 and few spheroechinocytes on day 21. The findings of Bhargava et al. [34] on days 14 and 21 revealed few echinocytes, spherocytes, marked echinocytes which are in contrast with the findings of this study. The morphological changes observed on assessment day 28 were similar to the observations of Bhargava et al. [34] which revealed few normocytes with marked echinocytosis, spheroechinocytosis and spherocytosis. The morphological changes observed on assessment days 35 of storage were similar to the findings of Bhargava et al. [34] which reported marked echinocytosis and spherocytosis, but in contrast with the findings of Antwi-Baffour (2015) which reported hypochromasia with rouleaux formation and marked spheroechinocytosis.

Morphological assessment of RBCs on the various storage days revealed degenerative changes. The change in the shape of normocytes through echinocyte, spherocytes to spheroechinocytes could be attributed to fact that, RBCs depend on only glucose metabolism to generate ATP, so RBCs undergo glycolysis in the enclosed blood bag. As the storage duration increase, the bye product of glycolysis, lactic acid accumulates in the plasma in the blood bag, resulting in acidosis which inhibits glycolysis through a negative feedback mechanism. Adenosine deaminase in stored blood causes the breakdown of adenosine to produce inosine and ammonia. The changes in glycolytic metabolism, coupled with increase in protons reduce the pH of the medium. The reduction in pH leads to a decrease in 2, 3 diphosphoglycerate level. As acid accumulates, glycolytic metabolism slows down, levels of ATP, pH and 2, 3 DPG decline progressively altering the shape of RBCs [35,36]. ATP is vital to maintaining the RBC membrane and the shape of the RBCs [37,38]. The depletion of ATP in the blood bag leads to loss of membrane integrity and RBC deformability

property (which allows it to meander its way through vessels). This also leads to crenation and spiculation of the RBCs [37,38].

Decrease in ATP production directly relates to spherocytosis of RBCs [39], the reason for the observation of increase spherocytes as storage duration increase. Findings from studies have proven that storage lesions impact the post transfusion viability of RBCs through various mechanisms. During storage, glucose metabolism is altered resulting in the reduction of intracellular glucose, which leads to low ATP production and reduced 2, 3 diphosphoglycerate. These biochemical changes make RBCs prone to oxidative stress, compromising membrane integrity and also cause early haemolysis [40,41]. Again, RBCs undergo untimely exposure and the activation of elimination or removal signal on the cell membranes, which are similar to the physiological aging antigens. Removal signals when activated could lead to early immune recognition and the removal of RBCs from circulation post transfusion [40,42]. The optimum reason for transfusion of RBCs is the re-establishment of a medium to carry oxygen to body tissues. It is therefore worth noting that, efficacy of transfusion is evaluated by the survival of the donor RBCs in the recipient [43].

Also, the loss of RBC membrane integrity results in increase echinocytes, spherocytes and spheroechinocytes, thus exposes RBCs to enhanced clearance by macrophages of the reticuloendothelial system of the blood recipient [42,44]. The unique biconcave structure of RBCs is important to perform its function of delivering oxygen to tissues because of its deformability which enables it to traverse capillaries without any obstruction, thus any change in the RBC membrane affects its deformability, leads to circulatory obstruction and would increase RBC clearance post transfusion [45,46].

5. CONCLUSION

Blood stored in CPDA-1 collection bag at a temperature of 4 ± 2℃ develops lesions during the 35 days storage period. Haemoglobin concentration decreased with increase duration of storage. Again, significant degenerative changes occur in the shape of red cells from normocytic through echinocytes, spheroechinocytes and spherocytes as the storage duration increase. This could increase patients' risk of experiencing post transfusion complications and may not promote the achievement of the expected therapeutic benefits of blood transfusion. The study therefore recommends that, blood with less storage duration should be preferred for transfusion for best therapeutic improvement.

CONSENT AND ETHICAL APPROVAL

Ethical clearance was granted by the University for Development Studies, Tamale; and the Research and Ethical Clearance Committee of the Tamale Teaching Hospital. Informed consent was obtained from participant before recruiting them for the study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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