


Original Article
In-Vitro Variations in Erythrocyte Ageing Among Different Categories of Blood Donors Due to Donor-Related Factors

Sikiru Adetona Lawal¹, Daniel Ohilebo Ugbomoiko¹, Musa Abidemi Muhibi², Luqman Olayinka Olatunbosun^{3*}, Abiola Samuel Babatunde³, Fatai Dayo Olalere³, Tajudeen Kolawole Ogunwale⁴, Sulaeman Eleha Ibrahim⁴, Ameenah Ayodeji AbdulRaheem³, Rasheed Oladimeji Lambe³ and Ibrahim Kehinde Lawal⁵

¹Department of Medical Laboratory Science, Igbinedion University Okada, Edo State, 302110, Nigeria.

²Department of Medical Laboratory Science, Edo State University, Uzairue. Edo State, 312107, Nigeria.

³Department of Hematology and Blood Transfusion, University of Ilorin Teaching Hospital, Ilorin, 240001, Nigeria.

⁴Department of Chemical Pathology and Immunology, University of Ilorin Teaching Hospital, Ilorin, 240001, Nigeria

⁵Department of Science Laboratory, Osun State Polytechnic, Esa Oke, Osun State, 233130, Nigeria.

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Corresponding Author

Dr. Olatunbosun, Luqman Olayinka,
Department of Hematology and
Blood Transfusion, University of
Ilorin Teaching Hospital, Ilorin,
PMB 1459, Kwara State, Nigeria.

P.O BOX 4173

Phone Number: +2348030467510

Email: deluy008@gmail.com

Zip code: 240001

ABSTRACT

Blood storage lesion is a rapidly growing area of biomedical research which leaves several questions unanswered. This study assessed blood donation frequency, as it affects hematological and biochemical parameters among different categories of blood donors longitudinally over a period of 35 days at 2-6°C. Ethically approved cross-sectional research involving family (n=30), remunerated donors: mild (n=30), moderate (n=30) and high frequency (n=30). One hundred milliliters (100 mL) of well-mixed blood was transferred into the satellite bag from 450mls of blood from different categories of blood donors. Haemogram, plasma Ferritin, Erythropoietin (EPO), 2, 3-diphosphoglycerate, Sodium (Na⁺) and Potassium (K⁺) assessed at 0, 7, 14, 21, 28 and 35 days of storage were analyzed. This study revealed acceptable normal high values in Haemogram, erythropoietin and ferritin in family donors while Remunerated donors had mild to moderate anemia, high total WBC count, higher platelet count, a very low ferritin content, low red cell indices, high EPO, hypernatremia, hyperkalaemia and high 2, 3. Diphosphoglycerate (2, 3, DPG). Significant iron deficiency anemia and electrolyte imbalance among moderate to high frequent blood donors.

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Introduction

Blood donors represent a genetically diverse population with inherited differences in red blood cell (RBC) characteristics that may modulate

predisposition to hemolysis and RBC recovery in response to various stress conditions, including cold storage of RBC units (Kanas and Gladwin, 2012). Genetic or biological factors that modulate RBC

responses to stress and favor hemolysis may impact the efficacy of RBC transfusions and increase the risk of transfusion-related complications via enhanced destruction of stored RBCs in the patient's circulation. The quality of RBC products can be greatly affected by the characteristics of donors' health status, age, sex, blood groups, lifestyle, etc. Identification of donor characteristics associated with transfusion recipient outcomes may lead to optimal selection of blood donors and donor-recipient matching. Red blood cells undergo a series of biochemical fluctuations during a 35–42-day storage period at 2°C to 6°C. Stored red blood cells undergo morphologic changes, metabolic alterations, and some degree of hemolysis during storage. During storage, red blood cells undergo depletion of potassium, 2, 3-diphosphoglycerate, ATP, lipids and membrane, with increased red cell rigidity and impaired oxygen delivery (Kim-Shapiro *et al.*, 2011). The stored units accumulate products of glycolysis, primarily protons, leading to a decrease in pH and hence, acidosis. This slowly causes a decrease in the rate of glycolysis and ATP formation, which in turn leads to loss of deformability with the formation of reversible echinocytes and eventually, the formation of irreversible spherocytocytes. Further changes involve loss of membrane by vesiculation (Rubin *et al.*, 2008). A decrease in 2, 3 DPG (diphosphoglycerate) is observed, which translates to increased affinity of hemoglobin to oxygen, consequently leading to decreased capacity of the erythrocytes to release oxygen into tissues (D'Alessandro *et al.*, 2010). The accumulation of immune-modulatory factors in stored RBC concentrates has been implicated as a potential cause of transfusion reactions (Caroline Sut *et al.*, 2017). US FDA standards requirement for stored blood is such that hemolysis must be <1% at the end of storage and at least 75% of allogeneic erythrocytes still circulating in the recipient's blood 24 h after transfusion. To achieve this, appropriate donor selection is pivotal among other conditions. Storage duration alone is just one of the many factors that impact on RBC component properties and quality. It has long been known that RBCs from some donors store well while others store poorly (Dumont and AuBuchon, 2008; McAteer *et al.*, 2010). Consequently, energy metabolism through glucose consumption leads to lactate production, which accumulates in the RBC storage solution (Blasi *et al.*, 2012) and induces its acidification (Hess, 2010). This metabolism-related decrease of pH inhibits the glycolysis rate, and affects the 2, 3-diphosphoglycerate (2, 3-DPG) level through the

activities of diphosphoglycerate mutase and phosphatase. After two weeks of storage, RBCs reportedly show 2, 3-DPG depletion (Bennett-Guerrero *et al.*, 2007). Low 2, 3-DPG levels increase the affinity of hemoglobin for oxygen, inhibiting its release and thus altering the oxygenation capacity of stored RBCs. However, 2, 3-DPG depletion appears to recover within a few hours post-transfusion.

Cold storage of RBCs induces inactivation of membrane ionic pumps, as shown by Na⁺ uptake and K⁺ loss throughout the storage period. Moreover, low-temperature storage slows metabolic enzyme activities, inducing a progressive decrease in ATP levels (Bennett-Guerrero *et al.*, 2007).

WBCs in non-leukoreduced RBC concentrates have been documented to induce adverse immune effects as a result of microbial risks, transfusion-associated GVHD, and recipient immune reactions such as alloimmunization and non-hemolytic febrile transfusion reactions (Hendrickson and Hillyer, 2009). It is believed that these WBCs, upon exposure to the acidic conditions of storage and refrigeration, become activated and release cytokines, which can lead to their delivery at high concentrations during transfusion (Vamvakas and Blajchman, 2007). In addition, during routine storage of RBCs, lipids accumulate in the plasma fraction that can prime neutrophils, causing neutrophil-mediated cytotoxicity of human pulmonary endothelial cells, which has been implicated as the mechanism of lung injury in transfusion-related acute lung injury (TRALI) (Silliman *et al.*, 1994).

The efficacy of transfusing stored, refrigerated erythrocytes from remunerated commercial blood donors in improving oxygen-carrying capacity and delivery to the tissues has not been very well studied in the past. A growing number of studies have discussed the potential impact of donor characteristics on RBC storage lesions and post-transfusion outcomes, which led to the scrutiny of genetic and biological factors in blood donors that may contribute to variations in the quality of RBC units (Tamir Kanas *et al.*, 2017). Optimal selection of blood donors is critical for ensuring the safety of blood products.

Materials and Methods

The study is an ethically approved random, cross-sectional research comprising 120 apparently healthy male prospective blood donors within the age of 21-55 years residents in Ilorin metropolis, Kwara state using the serial recruitment method.

A total number of 120 male donors were recruited as blood donors, family donors (n=30) and non-family donors (n=90). Non-family remunerated donors were

grouped into three (3) based on the frequency of donation per year as mild (n=30), moderate (n=30) and high frequency (n=30) donors.

Blood donors screening

Satellite double blood bag having a capacity of 450 ± 10 mls containing Citrate Phosphate Dextrose Adenine (CPDA-1) solution with a shelf life of 35 days for whole blood storage at 2-6⁰ C was used to store the collected blood.

Bleeding of donors

Four hundred and fifty milliliters (450 mls) of blood was drawn from each of the 120 apparently healthy donors (age range from 21-55 years) into a double blood bag containing CPDA-1 anticoagulant. Blood was collected with adequate safety precautions to avoid contamination and infection. 100 mls of well-mixed blood was transferred into the satellite bag, detached and stored in the blood bank at 2-6⁰ C for 35 days.

Sample collection

The effect of storage was analyzed at 0, 7, 14, 21, 28 and 35 days of storage by withdrawing 10 mls blood

each time from the satellite blood bag. The blood samples collected were analyzed for hematological parameters before being centrifuged at 5000 rpm for five minutes to obtain the plasma which was stored at -20 C for further biochemical investigations. The sample analyzed on day 0 served as baseline control.

Laboratory Procedures

Full Blood Count (FBC)

The blood samples were analyzed for full blood count (FBC) immediately and repeated at intervals of 7 days throughout the 35 days of storage using the hematology auto-analyzer MODEL: SYSMEX KX21. Procedures were performed according to the standard operating manuals.

Plasma Ferritin Assay, 2, 3- Disphosphoglycerate and Human Erythropoietin- EPO were done using ELISA methods.

Electrolytes were estimated, using Ion Selective Electrode (ISE) (Springer, 2012)

Results

Table 1: Haemoglobin, Haematocrit and Red Blood Cells

Categories	Baseline	Week 1	Week 2	Week 3	Week 4	Week 5
Family	13.5±0.7	12.5±.3	12.4±1.0	12.3±0.9	12.3±0.8	12.3±0.7
Haemoglobin						
Remunerated	12.6±1.4	11.5±1.5	11.4±1.4	11.4±1.4	11.3±1.5	11.3±1.6
t-test	3.376	3.264	3.613	3.296	3.483	3.312
P-value	0.001	0.014	0.004	0.013	0.007	0.012
Haematocrit						
Family	0.44±0.03	0.41±0.03	0.40±0.04	0.40±0.03	0.39±0.04	0.39±0.03
Remunerated	0.40±0.04	0.37±0.03	0.35±0.02	0.35±0.04	0.34±0.03	0.34±0.02
t-test	5.021	5.325	8.997	6.276	7.244	10.372
P-value	0.001	0.001	0.001	0.001	0.001	0.001
Red Blood Cells-RBC						
Family	5.1±0.5	4.6±0.4	4.5±0.5	4.4±0.6	4.4±0.5	4.3±0.5
Remunerated	4.4±0.5	3.9±0.5	3.9±0.6	3.8±0.5	3.8±0.6	3.7±0.5
t-test	6.641	6.956	4.932	5.407	4.932	5.692
P-value	0.001	0.001	0.001	0.001	0.001	0.001

Table 1 above shows the Hemoglobin, Hematocrit and Red Blood Cells (RBC) among different categories of donors throughout the storage of whole blood units under standard blood bank conditions. Values

obtained with independent samples t-test and expressed as Mean ± Standard deviation, bold p-value indicates statistical significance at p < 0.05.

Table 2: Red Blood Cells Indices, Ferritin and Erythropoietin-EPO

Categories	Baseline	Week 1	Week 2	Week 3	Week 4	Week 5
Mean Cell Volume-MCV						
Family	84.2±4.9	82.2±6.7	80.1±6.3	78.4 ±5.7	78.2±6.1	78.9±6.3
Remunerated	81.7±5.6	79.6±5.7	78.1±4.3	76.5±4.2	75.9±5.2	75.2±5.6
t-test	2.181	2.069	1.949	1.953	2.007	3.037
P-value	0.031	0.041	0.054	0.053	0.047	0.003
Mean Cell Haemoglobin-MCH						
Family	25.5±2.3	23.4±3.1	22.9±2.2	22.6±1.5	22.6±1.3	22,5±1.4
Remunerated	24.5±2.2	22.3±2.4	22.0±2.1	22.0±1.4	21.9±1.7	21.9±1.3
t-test	2.132	2.015	2.009	1.997	2.061	2.148
P-value	0.035	0.046	0.047	0.048	0.042	0.034
Mean Cell Haemoglobin Concentration-MCHC						
Family	30.8±1.6	29.9±2.3	29.7±1.7	29.6±1.6	29.9±1.1	30.0±1.2
Remunerated	30.0±1.5	28.5±2.3	28.5±2.0	28.7±1.5	29.0±1.8	29.3±1.4
t-test	2.488	2.887	2.948	2.799	2.579	2.453
P-value	0.014	0.005	0.004	0.006	0.011	0.016
Ferritin						
Family	43.6±2.2	43.6±2.2	43.6±2.2	43.7±2.1	45.9±2.0	47.9±2.2
Remunerated	28.3±11.2	29.0±11.1	29.3±10.7	30.5±10.0	32.4±10.1	34.5±9.9
t-test	7.401	7.118	7.238	7.208	7.284	7.414
P-value	0.001	0.001	0.001	0.001	0.001	0.001
Erythropoietin-EPO						
Family	4.4±0.6	4.3±0.8	3.8±0.6	3.2±0.6	2.3±0.5	1.2±0.5
Remunerated	6.3±1.2	5.7±1.0	5.4±1.2	4.6±1.1	3.5±1.0	2.2±0.7
t-test	-9.094	-6.956	-7.002	-6.637	-6.302	-6.693
P-value	0.001	0.001	0.001	0.001	0.001	0.001

Table 2 above shows the Red Blood Cells Indices, Ferritin and Erythropoietin-EPO among different categories of donors throughout the storage of whole blood units under standard blood bank conditions.

Values obtained with independent samples t-test and expressed as Mean ± Standard deviation, bold p-value indicates statistical significance at $p < 0.05$.

Table 3: White Blood Cells-WBC and Platelets

Categories	Baseline	Week 1	Week 2	Week 3	Week 4	Week 5
White Blood Cells						
Family	5.3±0.7	4.2±1.0	3.9±1.0	3.7±1.1	3.5±1.1	3.6±1.0
Remunerated	6.0±1.3	4.8±1.1	4.5±1.2	4.3±1.3	4.0±1.2	4.1±1.2
t-test	-2.811	-2.644	-2.466	-2.270	-2.016	-2.055
P-value	0.006	0.009	0.015	0.025	0.046	0.042
Platelets-PLT						
Family	238.6±75.1	190.2±37.4	171.9±37.8	168.7±37.8	166.2±34.7	169.5±76.0
Remunerated	228.8±61.2	194.0±49.5	180.9±48.8	173.4±46.0	177.2±46.2	179.7±54.4
t-test	0.716	-0.385	-0.921	-0.505	-1.195	-0.801
P-value	0.475	0.701	0.359	0.614	0.234	0.425

Table 3: above shows the White blood cells and Platelet Counts among different categories of donors throughout the storage of whole blood units under standard blood bank conditions. Values obtained with

independent samples t-test and expressed as Mean ± Standard deviation, bold p-value indicates statistical significance at $p < 0.05$.

Table 4: 2, 3, Diphosphoglycerate (2, 3 DPG), Sodium and Potassium

Categories	Baseline	Week 1	Week 2	Week 3	Week 4	Week 5
Family	29.4±1.4	28.1± 1.3	26.7±1.1	21.8±1.5	15.9±1.1	10.7±0.9
Remunerated	43.4±11.3	42.4±11.1	40.4±10.8	32.9±8.2	24.0±5.9	15.7±4.0
t-test	-6.750	-6.992	-6.915	-7.294	-7.430	-6.798
P-value	0.001	0.001	0.001	0.001	0.001	0.001
Sodium (Na⁺)						
Family	143.5±1.2	140.5±1.9	137.3±2.8	126.8±0.9	121.3±1.3	116.6±1.5
Remunerated	150.7±8.6	149.4±8.9	145.1±9.2	137.0±9.4	129±11.4	122.3±14.3
t-test	-4.558	-5.422	-4.562	-5.918	-3.681	-2.173
P-value	0.001	0.001	0.001	0.001	0.004	0.032
Potassium (K⁺)						
Family	4.1±0.5	7.2±0.7	10.2±1.1	13.0±1.6	16.7±1.3	20.6±1.6
Remunerated	4.3±0.6	8.0±2.3	10.5±2.9	13.3±3.8	16.7±4.5	20.2±5.6
t-test	-1.644	-1.872	-0.552	-0.419	0.000	0.385
P-value	0.103	0.064	0.582	0.676	1.000	0.701

Table 4: above shows the potassium (K⁺) among different categories of donors throughout the storage of whole blood units under standard blood bank conditions. Values obtained with independent samples

t-test and expressed as Mean ± Standard deviation, bold p-value indicates statistical significance at p < 0.05.

Discussion

In this current study, out of a total number of 120 adult male recruited as blood donors, family donors were 30 and remunerated donors were 90. In the recruited family donors, majority were above 40 years of age; had secondary education, government employee; feed majorly on carbohydrates and were none alcohol drinkers, none cigarette smokers with history of donating more than four years before the study and less than four times donation frequency per year. These findings on family donors aligned with previous studies (Lownik *et al.*, 2012). This study corroborates why the International Society of Blood Transfusion and WHO regarded blood donation as voluntary in all circumstances and financial benefit must never be a motive.

Contrarily, remunerated, blood donors were majorly young individuals of age between 31-40 years having primary education, self or unemployed, moderate alcohol drinkers and moderate cigarette smokers donating more than four years before the study and more than four times a year. These observations probably can be due to the increasing rate of poverty, joblessness and bad social life. This study is in support of the evidence from previous studies that alcohol and cigarette consumption have associated risks of addiction which in turn have adverse effects on the metabolic quality of blood from remunerated high-frequency blood donors.

In the assessment of haemogram biomarkers in the studied groups, Hb, Hematocrit and RBCs in family

donors from baseline to week 5 were found to be higher than remunerated blood donors and progressively decreasing during cold storage. The decrease that showed statistically significant p-value < 0.05 along the weeks. These imply family donors have high values in these parameters all through the weeks of storage compared with remunerated donors with unacceptably low values as donation frequencies increase. Hence, neither the socio-economic status nor the frequency of donation of the family replacement donor would adversely affect their hemoglobin, hematocrit and RBCs count. In contrast, commercial donors are usually of low socio-economic status who are in most cases involved in drug abuse with consequent nutritional neglect. Hence, commercial donors often suffer from nutrient depletion and iron deficiency anemia. This result agreed with the study reported by (Amballi *et al.*, 2020). Also, several studies have reported anemia in blood donors (Rajab *et al.*, 2005) with higher frequencies in Africa than in Western countries (Tayou Tagny *et al.*, 2006), (Erharbor *et al.*, 2007). Erharbor *et al.* in a similar study reported that hemoglobin levels are lower in remunerated blood donors than replacement donors in Nigeria (Gomez-Simon *et al.*, 2007). Anemia is the most common reason for donor ineligibility (Ukaejiofor *et al.*, 1979). White Blood Cells counts in the stored blood from baseline values to week-5 for family donors were found to be lower than that of remunerated donors, with progressive decrease that in

overall showed statistical significance $p\text{-value} < 0.05$ thereby aligned with the mean values that were previously reported among healthy Nigerians (Dale, 1991). However, the mean values of total WBC count of the commercial donors were significantly higher than that of family replacement donors. This result may suggest that the higher total WBC count seen among the commercial donors may be due to elevated neutrophil count, which may be due to excessive blood loss resulting from high frequency of blood donation since hemorrhage is an important cause of elevation in neutrophil count (Hsieh *et al.*, 2007). In addition, commercial donors are often involved in drug and substance abuse including cigarette smoking, which is known to be associated with elevation in neutrophil count (Essien *et al.*, 2004). Platelets in the stored blood at baseline values for family donors were found to be higher than in remunerated donors, but moderately higher in remunerated donor from week 1 to week 5 with a progressive decrease that showed no statistically significant $p\text{-value}$ when compared with family donors. The mean values of platelet count seen among the family replacement donors were comparatively similar and consistent with normal values that were previously reported among healthy Nigerians (Schafer, 2004). In addition, in this study high frequent remunerated donor had very low ferritin content among others which is indicative of iron deficiency, which is also known to cause thrombocytosis (Ahmed *et al.*, 2010).

Mean Cell Volume, Mean Cell Hemoglobin and Mean Cell Hemoglobin Concentration in the stored blood at baseline values for family category was found to be higher when compared with remunerated donors with progressive decrease that showed statistically significant $p\text{-value} < 0.05$ all through from week-1 to 5 when compared with family category. This study was in agreement with report of (Benedict *et al.*, 2012) which stated that the mean values of red cell indices (MCV, MCH, MCHC) for the commercial donors were lower than that of family replacement donors but, in contrast, the differences were not statistically significant ($p > 0.05$) for all three indices. The relatively lower mean values of red cell indices among the commercial donors would further suggest the existence of a negative iron balance in this donor category. Also, the concomitant reduction in the red cell indices further suggests that the anemia most likely resulted from too frequent blood donation (Finch and Huebers, 1982). In assessing haemopoietic biomarkers in the studied groups, ferritin in family donors from baseline to week-5 was higher than in remunerated non-family donors, and progressively

increased during cold storage. This increase showed statistical significance ($p\text{-value} < 0.05$) from baseline to week 5 when compared with family category of blood donors. This means that high frequent donation has resulted to decrease in ferritin levels. This is so because the physiologic importance of the storage iron is that, it provides a rapidly available supply in the event of blood loss (Szymczyk-Nuzka and WoÅ, 2003). Findings in this study support previous research (Badami K, and Taylor, 2008) who reported that an increase in the donation frequency was followed by a significant decrease in serum ferritin concentration.

Erythropoietin (EPO) in family donors from baseline to week 5 was lower than other remunerated donors of the non-family group and progressively decreased during cold storage. This decrease showed statistical significance ($p\text{-value} < 0.05$) all through from baseline to week-5 when compared with family category of donors. The erythropoietin (EPO) mediates the remarkable regenerative capacity of the bone marrow to compensate for blood loss or reduced oxygen tension (Aziz *et al.*, 2014). These findings agreed with the previous study by (Hess, 2006) which found that EPO was significantly higher and the ferritin was significantly lower in regular donors than in the first-time donors.

In assessing the biochemical changes in the studied groups, both sodium and potassium in family donors from baseline to week-5 were lower than remunerated non-family donors and Na^+ was progressively decreasing while K^+ was progressively increasing during cold storage. The decrease showed statistically significant $p\text{-value} < 0.05$ in Na^+ all through. While the increase in K^+ showed no statistically significant $p\text{-value}$ when compared with the family category. The high concentration of sodium (hyponatremia) reported is likely to be dehydration that occurred, as a result of high donation frequency without adequate fluid intake coupled with too much fluid loss. Potassium loss is recognized to be secondary to the changes in metabolic activity and decreasing pH with cooling (Opoku-Okrah *et al.*, 2015). Findings in this study corroborate the earlier reported increase and reduction in plasma potassium and sodium respectively in stored blood by Opoku-Okrah *et al* (Freiser, 1986).

2, 3, Diphosphoglycerate (2, 3, DPG) in the stored blood is lower in family donors than remunerated non-family donors and progressively decreased during cold storage, this decrease showed statistically significant $p\text{-value} < 0.05$ from baseline to week-5 when compared with the family category.

Conclusion

This study revealed acceptable normal high values in haemogram, erythropoietin and ferritin in family donors while non-family remunerated donors had mild to moderate anemia, high total WBC count, higher platelet count, a very low ferritin content, low red cell indices (MCV, MCH, and MCHC), high EPO, hypernatremia, low potassium and high 2, 3. Diphosphoglycerate (2, 3, DPG) reflects iron deficiency anemia and electrolyte imbalance.

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