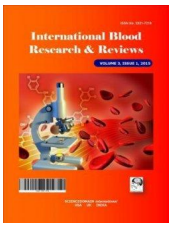

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Assessment of Blood Storage Effect Using CPDA-1 on Packed Cell Volume, Oxyhaemoglobin and Methaemoglobin in Different ABO/Rhesus Blood Types

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Authors' contributions

This work was carried out in collaboration among all authors. Author SGC designed the study, wrote the protocol, wrote the first draft of the manuscript and performed the statistical analysis. Authors EME and NEN managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study is to determine the effect of blood storage using CPDA-1 on packed cell volume, methaemoglobin and oxyhaemoglobin in different ABO/Rhesus blood types donated by some residents of Port Harcourt, Rivers State, Nigeria.
Study Design: This is a comparative study aimed at evaluating the effect of storage on the levels of methaemoglobin, oxyhaemoglobin and packed cell volume using CPDA-1. A total of eight donors were recruited with each sample obtained from the eight (8) known blood groups A+,B+,O+,AB+, A-,B-,O-,AB- and analysis of samples were in triplicate. The donors were adult males with age ranging from 35-45 years and they were apparently healthy and free from transfusion transmissible infections. The different blood group samples were stored for 30 days and samples for analysis were collected at 5 days interval.

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Place and Duration of Study: The study was conducted in Port Harcourt, Rivers State, Nigeria. All blood donors were residents of Port Harcourt. Blood donated was stored at Military Hospital Blood Bank, Port Harcourt, in a blood bag of 450 ml containing 63 ml of citrate phosphate dextrose adenine-1 (CPDA-1). The analysis was carried out at Rivers State University, Post Graduate Laboratory within March 1st to May 27th, 2019.
Methodology: A total of eight (8) different ABO/Rhesus blood types (A+,B+,AB+,O+,A-,B-,AB- and O-) were collected and stored using a blood bank refrigerator at temperature of 4°C. Day 0 was taken to be control and 5 days intervals in-between to day 30 acted as the test. Packed cell volume was estimated using micro-haematocrit method while oxyhaemoglobin and methaemoglobin levels were estimated spectrophotometrically as described by Evelyn and Malloy.
Results: The result showed a significant decrease in mean packed cell volume, oxyhaemoglobin and methaemoglobin levels compared to a higher mean of these parameters in the control; and these differences were statistically significant ($p<0.05$) across all blood groups under study. The decrease in values were as a result of haemolysis that occurs during storage.
Conclusion: Storage of blood irrespective of the blood group type using CPDA-1 for 30 days indicates that there are "storage lesions". This is attributed to red cell haemolysis and ageing of red blood cells. In general, all blood types showed no significant difference in their haematological (packed cell volume, methaemoglobin, oxyhaemoglobin) characteristic deterioration or storage lesion based on blood type differences. It is therefore necessary to state that storage lesion characteristics are the same irrespective of the blood type, and that fresh blood be transfused, and if blood is stored, prolonged storage beyond 10 days should be avoided.

Keywords: Packed cell volume; oxyhaemoglobin; methaemoglobin; ABO/Rhesus blood group; storage lesions; CPDA-1; blood storage.

1. INTRODUCTION

Blood is a body tissue albeit in fluid form that is found in human's circulatory vessels which helps in transportation of substances such as nutrients and oxygen to the body cells and organs, and at the same time transport the body's metabolic waste away from the cells, protect the body against foreign substances and regulate body homeostasis. It is a connective tissue made up of cells [red blood cells (RBC), white blood cells (WBC), and platelets (thrombocytes)] with the most abundant being the red blood cells. The red blood cells contains haemoglobin, an iron containing protein which aids in oxygen transport or a carrier of oxygen [1].

The main function of red blood cells (RBC) is uptake, transport, and delivery of oxygen. Also, red blood cells contributes to the colloid osmotic pressure, to platelet-endothelium interactions necessary for normal haemostasis, and transport of several molecules such as drugs or immune complexes. The oxygen binding capacity of the red blood cells is dependent on the blood volume (haematocrit) and the amount of haemoglobin present in the red blood cells. Haemoglobin not capable of binding oxygen may occur in the form of methaemoglobin. Methaemoglobin is a reduced form of haemoglobin in which the iron in the heme group is in the ferric form. Methaemoglobin does not carry and distribute oxygen and when in high concentration than normal, it causes cyanosis. Oxyhaemogobin is the form in which oxygen is transported after binding to haemoglobin [2].

Blood types are classified based on the presence and absence of naturally occurring antibodies and inherited antigenic substances found on the surface of red blood cells. These antigens may be proteins, carbohydrates, glycoproteins, or glycolipids, depending on the blood group system. Some of these antigens are also present on the surface of other types of cells of various tissues. Blood types are inherited and represent contributions from both parents. The two most important ones are ABO and the Rhesus blood group systems; they determine someone's blood type (A, B, AB and O, with +, − or null denoting Rhesus D status) for suitability in blood transfusion. For instance, B Rhesus "D" Positive has the B antigen and the Rhesus "D" antigen, whereas someone who is B Rhesus "D" Negative lacks the Rhesus "D" antigen. The terms Rhesus factor (Rh factor), Rhesus positive, and Rhesus negative refer to the Rh "D" antigen only [3].

Blood donation entails the process of voluntarily allowing one's blood to be withdrawn for the purposes of blood transfusion. Blood donation could be commercial, voluntary, autologous or by relatives. In developed countries, donations are usually anonymous to the recipient, but products

in a blood bank are always individually traceable through the whole cycle of donation, testing and separation into components, storage, and administration to the recipient [4].

After blood is collected from a donor, the blood may be transfused immediately or stored and preserved. Storage of blood products is the act of subjecting the blood products to a low temperature at which little or no morphological or physiological changes occurs [5]. The process of blood storage is achieved using the blood bank refrigerator. A Blood bank refrigerator is a designated temperature controlled refrigeration machine specially designed to store blood bags at 4^0C. As the name indicates, it is widely used in blood banks and hospitals. Not every refrigerator is a blood bank refrigerator as temperature uniformity is the prime requirement for blood bag storage. Blood are collected mostly as units. One unit of whole blood from a donor is collected into a suitable anticoagulant-preservative solution which is a combination of citrate and dextrose to prevent coagulation from taking place and also it contains inorganic phosphate buffer to increase the production of energy-rich adenosine triphosphate (ATP) to increase red cell viability. Some anticoagulants we have are Anticoagulant Citrate Dextrose (ACD), Citrate Phosphate Dextrose (CPD) and Citrate Phosphate Dextrose Adenine-1 (CPDA-1). The first two anticoagulants were replaced with the last (CPDA-1) which is now widely recommended and used. Citrate phosphate dextrose adenine -1 adult blood bag has a total volume of about 450ml containing 63 ml of anticoagulant. This blood is stored in an approved blood bank refrigerator at 2-6^0C [6]. Shelf life of such blood is 35 days [7].

The ultimate aim of transfusing blood is the maintenance or restoration of a medium for transport of oxygen to body tissues. Reports have shown that varieties of changes are identified in red blood cells during red blood cell preservation. They are collectively termed as storage lesion and include extensive biochemical and haematological changes. Over time the glucose in stored blood is consumed, levels of 2, 3-diphosphoglycerate (2, 3-DPG) and adenosine triphosphate (ATP) decreases, leading to reduced structural integrity of cells. Thus red cells become less deformable and more fragile as they age, affecting their ability to bind, carry, and transport oxygen [8]. This fragility leads to the release of cell free haemoglobin and formation of micro particles, sub-micron haemoglobin containing vesicles and additional haemolysis [9]. It is therefore necessary to examine the effect of blood storage with respect to oxygen delivery after blood is stored for transfusion purpose, and investigate if there are variations in storage effects based on differences in blood group types, hence the need for this research.

2. MATERIALS AND METHODS

2.1 Study Design

This is a comparative study which is aimed at evaluating the effect of storage on the levels of methaemoglobin, oxyhaemoglobin and packed cell volume using CPDA-1. A total of eight donors were recruited with each sample obtained from the eight known blood groups A+, B+, O+, AB+, A-, B-, O-, AB-. Samples for analysis were collected in triplicate on each interval days of collection and analyzed respectively. The donors were adult males with age ranging from 35-45 years. Samples from the stored blood was collected at 5 days interval.

2.2 Study Area

The study was conducted in Port Harcourt, Rivers State, Nigeria. Port Harcourt, the capital of Rivers State is located at latitude 4.75^0N and longitude 7.00^0E and lies along Bonny River in the Niger Delta. Blood donated was stored at Military Hospital Blood Bank, Port Harcourt, in a blood bag of 450ml volume containing 63ml of citrate phosphate dextrose adenine-1 (CPDA-1). The analysis was carried out at Rivers State University, Post Graduate Laboratory.

2.3 Study Population

Blood (450 ml) was drawn from eight healthy volunteer donors into Citrate Phosphate Dextrose Adenine-1 (CPDA-1) anticoagulant-preservative and placed on the quarantine shelf of the blood bank refrigerator. The donors were 8 in number within the age range of 35 to 45 years. The different blood groups were 1A-, 1A+, 1B-, 1B+, 1O+. 1O-, 1AB-, 1AB+. The donors were all screened for hepatitis B and C, syphilis and HIV.

2.4 Collection of Blood Samples and Storage

Blood was collected into adult blood bag containing anticoagulant-preservative CPDA-1.

The samples were stored at Military Hospital Blood Bank, Port Harcourt. Collection of blood was performed as described by Cheesebrough [10]. Blood was collected from each of the donors with care and adequate safety precautions to avoid contamination and infection from blood transmissible pathogens. All sterile measures including protective gloves were worn during collection and syringes were sterile. Adequate care was taken to avoid injury from needles and lancets. Blood bags were carefully stored in a quarantine shelf in the blood bank, with temperature ranging from 2-6°C with proper inspection at intervals for colour, turbidity, haemolysis and clot formation.

2.5 Methodology

2.5.1 Determination of packed cell volume

Method: Microhaematocrit method.

Principle: Packed cell volume determination is based on microhaematocrit method. It involves the filling by capillary action, three-quarter of plain capillary tubes specifically 75mm long and 1mm diameter, with anticoagulated blood. The tubes were properly sealed with plasticine and centrifuged in a microhaematocrit centrifuge at 12000 rpm for 5 minutes to obtain constant packing of the red cells. The packed cell volume was then read with a microhaematocrit reader.

2.5.2 Determination of oxyhaemoglobin

Method: As described by Evelyn and Malloy [11].

Principle: The sample was diluted in a weak ammonia solution. This lyses the red blood cells. The absorbance of the solution was measured as haemoglobin in a filter colorimeter at a wavelength of 540 nm.

Procedure: Fresh ammoniated water was prepared by adding 0.04 ml of ammonia to 100 ml of distilled water. 4 ml of ammoniated water was pipetted in to a test tube. The sample was mixed and 20 ul (0.02 ml) from it was added to the test tube and stoppered with a band. The content was mixed by inversion. The standard solution and the test solutions were read using the spectrophotometer at 540 nm.

2.5.3 Determination of methaemoglobin

Method: As described by Evelyn and Malloy [11].

Principle: The test was based on the absorption of methaemoglobin by the formation of cyanmethaemoglobin read at 630 nm.

Procedure: 0.2 ml of blood was lysed in a mixture of 4 ml phosphate borate buffer of pH 6.8 and 6 ml of non-ionic detergent. The lysate was divided into 2 equal volumes “A” and “B”. The absorbance of “A”, was read at 630nm and recorded as D1 followed by the addition of a drop of potassium cyanide and reading of the second absorbance was recorded as D2. Then a drop of potassium ferricyanide was added to the “B” and the mixture was allowed to stand for five minutes and absorbance read as D3, a drop of potassium cyanide was then added to “B”, mixed and read as D4.

Calculation: The percentage of methaemoglobin was calculated using:

$$\text{MetHb (\%)} = \frac{D1 - D2}{D3 - D4}$$

2.6 Statistical Analysis

Data collected was statistically analyzed using Graph-pad prism 5.0 to determine the statistical inference and to obtain mean and standard deviations of the data. Analysis of variance (ANOVA) was done, and where there was statistical significant difference, Tukey’s multiple comparison test was used to identify where the differences was. $p<0.05$ was considered statistically significant.

3. RESULTS

3.1 Demographic Details of Study Population

A total of eight (8) blood donors were used for this study. All were male subjects and also residents of Port Harcourt, Rivers State. Each subject donated a pint of blood respectively (A+, B+, AB+, O+, A-, B-, AB-, O-). All were potentially healthy donors with no history of any transmittable disease (HIV, Syphilis, HbSAg, and HCV). Details are shown in Table 1.

3.2 Comparison of Packed Cell Volume, Methaemoglobin and Oxyhaemoglobin of Blood Group A Rhesus “D” Negative as a Result of Storage Using Citrate Phosphate Dextrose Adenine-1 (CPDA-1)

Packed cell volume, methaemoglobin level, and oxyhaemoglobin level of blood group A Rhesus

"D" Negative were analysed using Analysis of Variance (ANOVA). Mean ± standard deviation of their different day's analysis are shown in Table 2a. $p<0.05$ was considered statistically significant. Tukey's multiple comparison test was done in-between the 30 days interval and statistical inference are shown in Table 2b.

3.3 Comparison of Packed Cell Volume, Methaemoglobin and Oxyhaemoglobin of Blood Group A Rhesus "D" Positive as a Result of Storage using Citrate Phosphate Dextrose Adenine-1 (CPDA-1)

Packed cell volume, methaemoglobin level and oxyhaemoglobin level of blood group A Rhesus "D" Positive were analysed using analysis of variance (ANOVA). Mean ± standard deviation of their different day analysis are shown in Table 3a. $p<0.05$ was considered statistically significant. Tukey's multiple comparison test was done in-between the 30 days interval and statistical inference are shown in Table 3b.

3.4 Comparison of Packed Cell Volume, Methaemoglobin and Oxyhaemoglobin of Blood Group AB Rhesus "D" Negative as a Result of Storage Using Citrate Phosphate Dextrose Adenine-1 (CPDA-1)

Packed cell volume, methaemoglobin level and oxyhaemoglobin level of blood group AB Rhesus" D" Negative were analysed using analysis of variance (ANOVA). Mean ± standard deviation of their different day analysis are shown in Table 4a. $p<0.05$ was considered statistically significant. Tukey's multiple comparison test was done in-between the 30 days interval and statistical inference are shown in Table 4b.

3.5 Comparison of Packed Cell Volume, Methaemoglobin and Oxyhaemoglobin of Blood Group AB Rhesus "D" Positive as a Result of Storage using Citrate Phosphate Dextrose Adenine-1 (CPDA-1)

Packed cell volume, methaemoglobin level and oxyhaemoglobin level of blood group AB "D" Positive were analysed using analysis of variance (ANOVA). Mean ± standard deviation of their different day analysis are shown in Table 5a. $p<0.05$ was considered statistically significant. Tukey's multiple comparison test was done in-between the 30 days interval and statistical inference are shown in Table 5b.

3.6 Comparison of Packed Cell Volume, Methaemoglobin and Oxyhaemoglobin of Blood Group B Rhesus "D" Negative as a Result of Storage Using Citrate Phosphate Dextrose Adenine-1 (CPDA-1)

Packed cell volume, methaemoglobin level and oxyhaemoglobin level of blood group B Rhesus "D" negative were analysed using analysis of variance (ANOVA). Mean ± standard deviation of their different day analysis are shown in Table 6a. $p<0.05$ was considered statistically significant. Tukey's multiple comparison test was done in-between the 30 days interval and statistical inference are shown in Table 6b.

Table 1. Social demographic characteristics of study population

Parameters	Frequency	Percentage (%)
Total number of donors	8	100
Total number of blood group A+	1	12.5
Total number of blood group A-	1	12.5
Total number of blood group B+	1	12.5
Total number of blood group B-	1	12.5
Total number of blood group AB+	1	12.5
Total number of blood group AB-	1	12.5
Total number of blood group O+	1	12.5
Total number of blood group O-	1	12.5

Table 2a. Analysis of variance of studied parameters of blood group A Rhesus "D" negative

	Control (M±SD)	Day 5 (M±SD)	Day 10 (M±SD)	Day 15 (M±SD)	Day 20 (M±SD)	Day 25 (M±SD)	Day 30 (M±SD)	p-value	F-value	Remark
PCV	40±1.0	39±1.0	37±1.0	36±1.0	33±1.0	31±1.0	29±1.0	<0.0001	51.00	S
MetHb	1.18±0.01	3.77±0.01	1.81±0.01	1.51±0.01	1.10±0.01	0.50±0.01	0.50±0.10	<0.0001	2534	S
OxyHb	11.5±0.10	13.9±1.67	11.5±0.30	10.1±0.11	9.9±0.00	9.3±0.20	9.1±0.00	<0.0001	21.14	S

Table 2b. Tukey's multiple comparison test for packed cell volume, methaemoglobin and oxyhaemoglobin results of blood group A Rhesus "D'' negative

	Control versus	Day 5 versus	Day 10 versus	Day 15 versus	Day 20 versus	Day 25 versus
PCV	Day5^{NS} Day10^{S} Day15^{VS} Day20,25,30^{HS}	Day10^{NS} Day15^{S} Day20,25,30^{HS}	Day15^{NS} Day20^{VS} Day25,30^{HS}	Day20^{S} Day 25,30^{HS}	Day25^{NS} Day30^{VS}	Day30^{NS}
MetHb	Day5,10,15,25,30^{HS} Day20^{NS}	Day10,15,20,25,30^{HS}	Day15,20.25,30^{HS}	Day20,25,30^{HS}	Day25,30^{HS}	Day30^{NS}
OxyHb	Day5,20^{S} Day10,15^{NS} Day25,30^{VS}	Day10^{VS} Day15,20,25,30^{HS}	Day15,20^{NS} Day25^{S} Day30^{VS}	Day20,25,30^{NS}	Day25,30^{NS}	Day30^{NS}

Key: HS=Highly Significant; VS=Very Significant; NS=Non Significant; PCV=Packed Cell Volume; MetHb=Methaemoglobin; OxyHb=Oxyhaemoglobin; M±SD=Mean ± Standard Deviation

Note: The abbreviations are applicable to all tables

Table 3a. Analysis of variance of studied parameters of blood group A Rhesus "D" positive

	Control (M±SD)	Day 5 (M±SD)	Day 10 (M±SD)	Day 15 (M±SD)	Day 20 (M±SD)	Day 25 (M±SD)	Day 30 (M±SD)	p-value	F-value	Remark
PCV	41±1.0	40±1.0	38±1.0	36±1.0	35±1.0	33±1.0	31±1.0	<0.0001	39.71	S
MetHb	0.15±0.01	1.46±0.0	2.70±0.10	2.50±0.10	2.20±0.0	1.80±0.1	1.60±0.1	<0.0001	378.2	S
OxyHb	12.4±1.0	15.1±0.1	11.8±0.01	11.7±0.03	10.9±0.0	10.9±0.01	10.6±0.2	<0.0001	46.97	S

Table 3b. Tukey's multiple comparison test for packed cell volume, methaemoglobin and oxyhaemoglobin results of blood group A Rhesus "D'' positive

	Control versus	Day 5 versus	Day 10 versus	Day 15 versus	Day 20 versus	Day 25 versus
PCV	Day5^{NS} Day10^{S} Day15,20,25,30^{HS}	Day10^{NS} Day15^{VS} Day20,25,30^{HS}	Day15^{NS} Day20^{S} Day25,30^{HS}	Day20^{NS} Day25^{S} Day30^{HS}	Day25^{NS} Day30^{HS}	Day30^{NS}
MetHb	Day5,10,15,20^{HS} Day25,30^{HS}	Day10,15,20^{HS} Day25^{VS} Day30^{NS}	Day15^{NS} Day20.25,30^{HS}	Day20^{VS} Day25,30^{HS}	Day25,30^{HS}	Day30^{NS}
OxyHb	Day5^{HS} Day10,15^{NS} Day20,25,30^{VS}	Day10,15^{HS} Day20,25,30^{HS}	Day15,20,25^{NS} Day30^{VS}	Day20,25,30^{NS}	Day25,30^{NS}	Day30^{NS}

Table 4a. Analysis of variance of studied parameters of blood group AB Rhesus "D" negative

	Control (M±SD)	Day 5 (M±SD)	Day 10 (M±SD)	Day 15 (M±SD)	Day 20 (M±SD)	Day 25 (M±SD)	Day 30 (M±SD)	p-value	F-value	Remark
PCV	40±1.0	39±1.0	37±1.0	35±1.0	34±1.0	30±1.0	29±1.0	<0.0001	53.43	S
MetHb	0.89±0.02	1.60±0.1	2.10±0.1	1.40±0.1	1.30±0.1	1.10±0.1	1.10±0.06	<0.0001	62.30	S
OxyHb	14.2±0.0	14.8±0.02	11.3±0.11	10.7±0.22	10.0±0.90	9.9±0.01	9.7±0.22	<0.0001	43.81	S

Table 4b. Tukey's multiple comparison test for packed cell volume, methaemoglobin and oxyhaemoglobin results of blood group AB Rhesus "D'' negative

	Control versus	Day 5 versus	Day 10 versus	Day 15 versus	Day 20 versus	Day 25 versus
PCV	Day5,10,15,20^{HS} Day25^{NS} Day30^{S}	Day10,25,30^{HS} Day15^{NS} Day20^{S}	Day15,15^{HS} Day25,30^{HS}	Day20^{NS} Day25,30^{HS}	Day25^{NS} Day30^{NS}	Day30^{NS}
MetHb	Day5,10,15,20^{HS} Day25^{NS} Day30^{S}	Day10,20,25,30^{HS} Day15^{NS}	Day15,20^{HS} Day25,30^{HS}	Day20^{NS} Day25,30^{S}	Day25,30^{NS}	Day30^{NS}
OxyHb	Day5^{NS} Day10,15^{HS} Day20,25,30^{HS}	Day10,15^{HS} Day20,25,30^{HS}	Day15,20,25^{NS} Day30^{S}	Day20,25,30^{NS}	Day25,30^{NS}	Day30^{NS}

Table 5a. Analysis of variance of studied parameters of blood group AB Rhesus "D" positive

	Control (M±SD)	Day 5 (M±SD)	Day 10 (M±SD)	Day 15 (M±SD)	Day 20 (M±SD)	Day 25 (M±SD)	Day 30 (M±SD)	p-value	F-value	Remark
PCV	40±0.1	38±1.0	37±1.0	35±1.0	33±1.0	31±1.0	30±1.0	<0.0001	41.43	S
MetHb	1.25±0.1	0.95±0.01	1.95±0.04	1.50±0.04	1.20±0.1	1.00±0.5	1.01±0.01	0.0002	9.882	S
OxyHb	13.1±0.7	13.5±0.11	12.8±0.1	10.2±0.2	9.8±0.1	9.1±4.6	8.9±0.0	0.0021	6.352	S

Table 5b. Tukey's multiple comparison test for packed cell volume, methaemoglobin and oxyhaemoglobin results of blood group AB Rhesus "D" positive

	Control versus	Day 5 versus	Day 10 versus	Day 15 versus	Day 20 versus	Day 25 versus
PCV	Day5^{NS}	Day10^{NS}	Day15^{NS}	Day20^{NS}	Day25^{NS}	Day30^{NS}
	Day10^{VS}	Day15^{VS}	Day20^{VS}	Day25^{VS}	Day30^{S}	
	Day15,20,25,30^{NS}	Day20,25,30^{HS}	Day25,30^{HS}	Day30^{HS}		
MetHb	Day5^{NS},10,15,20^{HS}	Day10^{HS}	Day15^{NS}	Day20,25,30^{NS}	Day25,30^{NS}	Day30^{NS}
	Day25^{NS}	Day15^{S}	Day20^{VS}			
	Day30^{S}	Day20,25,30^{NS}	Day25,30^{HS}			
OxyHb	Day5,10,15,20,30^{NS}	Day10,15,20^{NS}	Day15,20^{NS}	Day20,25,30^{NS}	Day25,30^{NS}	Day30^{NS}
	Day25^{VS}	Day25^{VS},30^{HS}	Day25^{VS}30^{NS}			

Table 6a. Analysis of variance of studied parameters of blood group B Rhesus "D" negative

	Control (M±SD)	Day 5 (M±SD)	Day 10 (M±SD)	Day 15 (M±SD)	Day 20 (M±SD)	Day 25 (M±SD)	Day 30 (M±SD)	p-value	F-value	Remark
PCV	43±1.0	42±1.0	39±1.0	37±1.0	35±1.0	33±1.0	31±1.0	<0.0001	60.43	S
MetHb	2.77±0.02	2.52±0.01	3.17±0.01	3.21±0.01	1.38±0.1	1.60±0.1	1.2±0.0	<0.0001	734.9	S
OxyHb	11.9±1.0	15.5±1.0	11.9±0.01	10.6±0.2	10.2±0.1	9.9±0.0	9.9±0.2	<0.0001	41.11	S

Table 6b. Tukey's multiple comparison test for packed cell volume, methaemoglobin and oxyhaemoglobin results of blood group B Rhesus "D'' negative

	Control versus	Day 5 versus	Day 10 versus	Day 15 versus	Day 20 versus	Day 25 versus
PCV	Day5^{NS} Day10^{VS} Day15,20,25,30^{HS}	Day10^{S} Day15,20,25,30^{HS}	Day15^{NS} Day20^{VS} Day25,30^{HS}	Day20^{NS} Day25^{VS} Day30^{HS}	Day25^{NS} Day30^{VS}	Day30^{NS}
MetHb	Day5,10,15,20^{HS} Day25,30^{HS}	Day10,15,20^{HS} Day25,30^{HS}	Day15^{NS} Day20,25,30^{HS}	Day20,25,30^{HS}	Day25^{VS},30^{S}	Day30^{HS}
OxyHb	Day5^{HS} Day10,15^{NS},20^{S} Day25,30^{VS}	Day10,15,20^{HS} Day25,30^{HS}	Day15^{NS} Day20^{S} Day25,30^{VS}	Day20,25,30^{NS}	Day25,30^{NS}	Day30^{NS}

Table 7a. Analysis of variance of studied parameters of blood group B Rhesus "D" positive

	Control (M±SD)	Day 5 (M±SD)	Day 10 (M±SD)	Day 15 (M±SD)	Day 20 (M±SD)	Day 25 (M±SD)	Day 30 (M±SD)	p-value	F-value	Remark
PCV	40±1.0	39±1.0	37±1.0	35±1.0	33±1.0	31±1.0	29±1.0	<0.0001	50.43	S
MetHb	0.10±0.0	3.48±0.01	3.10±0.1	2.30±0.1	2.1±0.1	0.8±0.1	0.4±0.1	<0.0001	746.7	S
OxyHb	13.0±0.05	14.9±0.1	12.4±0.4	12.2±0.25	11.4±0.02	10.1±0.1	9.7±0.2	<0.0001	238.1	S

Table 7b. Tukey's multiple comparison test for packed cell volume, methaemoglobin and oxyhaemoglobin results of blood group B Rhesus "D'' positive

	Control versus	Day 5 versus	Day 10 versus	Day 15 versus	Day 20 versus	Day 25 versus
PCV	Day5^{NS} Day10^{S} Day15,20,25,30^{HS}	Day10^{NS} Day15^{VS} Day20,25,30^{HS}	Day15^{NS} Day20^{VS} Day25,30^{HS}	Day20^{NS} Day25^{VS} Day30^{HS}	Day25^{NS} Day30^{VS}	Day30^{NS}
MetHb	Day5,10,15,20^{HS} Day25^{HS},30^{VS}	Day10^{VS} Day15,20,25,30^{HS}	Day15^{HS} Day20,25,30^{HS}	Day20^{NS} Day25,30^{HS}	Day25,30^{HS}	Day30^{HS}
OxyHb	Day5^{HS} Day10^{S},15^{VS} Day20,25,30^{HS}	Day10,15,20^{HS} Day25,30^{HS}	Day15^{NS} Day20,25,30^{HS}	Day20,25,30^{HS}	Day25,30^{HS}	Day30^{NS}

Table 8a. Analysis of variance of studied parameters of blood group O Rhesus "D" negative

	Control (M±SD)	Day 5 (M±SD)	Day 10 (M±SD)	Day 15 (M±SD)	Day 20 (M±SD)	Day 25 (M±SD)	Day 30 (M±SD)	p-value	F-value	Remark
PCV	40±1.0	39±1.0	37±1.0	35±1.0	33±1.0	31±1.0	29±1.0	<0.0001	50.43	S
MetHb	0.61±0.1	2.77±0.01	2.5±0.02	2.1±0.02	2.1±0.05	1.6±0.01	1.2±0.1	<0.0001	508.7	S
OxyHb	11.2±0.2	14.5±0.03	11.7±0.1	11.5±0.05	11.0±0.01	10.9±0.0	10.8±1.0	<0.0001	33.70	S

Table 8b. Tukey's multiple comparison test for packed cell volume, methaemoglobin and oxyhaemoglobin results of blood group O Rhesus "D" negative

	Control versus	Day 5 versus	Day 10 versus	Day 15 versus	Day 20 versus	Day 25 versus
PCV	Day5^{NS}	Day10^{NS}	Day15^{NS}	Day20^{NS}	Day25^{NS}	Day30^{NS}
	Day10^{S},15^{VS}	Day15^{VS}	Day20^{VS}	Day25,30^{HS}	Day30^{VS}	
	Day20,25,30^{HS}	Day20,25,30^{HS}	Day25,30^{HS}			
MetHb	Day5,10,15,20^{HS}	Day10,15^{HS}	Day15^{HS}	Day20^{NS}	Day25,30^{HS}	Day30^{HS}
	Day25,30^{HS}	Day20,25,30^{HS}	Day20,25,30^{HS}	Day25,30^{HS}		
OxyHb	Day5^{HS}	Day10,15,20^{HS}	Day15^{NS}	Day20,25,30^{NS}	Day25,30^{NS}	Day30^{NS}
	Day10,15^{NS}	Day25,30^{HS}	Day20,25,30^{NS}			
	Day20,25,30^{NS}					

Table 9a. Analysis of variance of studied parameters of blood group O Rhesus "D" positive

	Control (M±SD)	Day 5 (M±SD)	Day 10 (M±SD)	Day 15 (M±SD)	Day 20 (M±SD)	Day 25 (M±SD)	Day 30 (M±SD)	p-value	F-value	Remark
PCV	40±1.0	39±1.0	37±1.0	35±1.0	33±1.0	31±1.0	29±1.0	<0.0001	50.43	S
MetHb	3.6±0.1	1.24±0.1	3.59±0.02	5.0±1.0	2.44±0.1	2.2±0.01	0.8±0.1	<0.0001	43.98	S
OxyHb	12.4±0.41	13.9±0.04	12.9±0.03	11.4±0.95	9.9±0.11	9.5±0.50	9.28±0.1	<0.0001	52.27	S

Table 9b. Tukey's multiple comparison test for packed cell volume, methaemoglobin and oxyhaemoglobin results of blood group O Rhesus "D" positive

	Control versus	Day 5 versus	Day 10 versus	Day 15 versus	Day 20 versus	Day 25 versus
PCV	Day5^{NS}	Day10^{NS}	Day15^{NS}	Day20^{NS}	Day25^{NS}	Day30^{NS}
	Day10^{S}	Day15^{VS}	Day20,25,30^{HS}	Day25,30^{HS}	Day30^{HS}	
	Day15,20,25,30^{HS}	Day20,25,30^{HS}				
MetHb	Day5,^{HS}10^{NS},15,25^{VS}	Day10,15^{HS}	Day15,30^{HS}	Day20,25,30^{HS}	Day25^{NS},30^{HS}	Day30^{HS}
	Day20^{S},30^{HS}	Day20^{S},25,30^{NS}	Day20^{S},25^{VS}			
OxyHb	Day5^{S},10,15^{NS}	Day10^{NS},15^{HS}	Day15^{VS}	Day20^{S},25^{VS}	Day25,30^{NS}	Day30^{NS}
	Day20,25,30^{HS}	Day20,25,30^{HS}	Day20,25,30^{HS}	Day30^{HS}		

3.7 Comparison of Packed Cell Volume, Methaemoglobin and Oxyhaemoglobin of Blood Group B Rhesus "D" Positive as a Result of Storage using Citrate Phosphate Dextrose Adenine-1 (CPDA-1)

Packed cell volume, methaemoglobin level and oxyhaemoglobin level of blood group "D" Positive were analysed using analysis of variance (ANOVA). Mean ± standard deviation of their different day analysis are shown in Table 7a. $p<0.05$ was considered statistically significant. Tukey's multiple comparison test was done in-between the 30 days interval and statistical inference are shown in Table 7b.

3.8 Comparison of Packed Cell Volume, Methaemoglobin and Oxyhaemoglobin of Blood Group O Rhesus "D" Negative as a Result of Storage using Citrate Phosphate Dextrose Adenine-1 (CPDA-1)

Packed cell volume, methaemoglobin level and oxyhaemoglobin level of blood group O Rhesus ''D'' Negative were analysed using analysis of variance (ANOVA). Mean ± standard deviation of their different day analysis are shown in Table 8a. $p<0.05$ was considered statistically significant. Tukey's multiple comparison test was done in-between the 30 days interval and statistical inference are shown in Table 8b.

3.9 Comparison of Packed Cell Volume, Methaemoglobin and Oxyhaemoglobin of Blood Group O Rhesus "D" Positive as a Result of Storage Using Citrate Phosphate Dextrose Adenine-1 (CPDA-1)

Packed cell volume, methaemoglobin level and oxyhaemoglobin level of blood group O Rhesus "D" Positive were analysed using analysis of variance (ANOVA). Mean ± standard deviation of their different day analysis are shown in Table 9a. $p<0.05$ was considered statistically significant. Tukey's multiple comparison test was done in-between the 30 days interval and statistical inference are shown in Table 9b.

4. DISCUSSION

The research carried out on the effect of blood storage using CPDA-1 on packed cell volume, oxyhaemoglobin level and methaemoglobin level in different ABO and Rhesus blood types showed some fluctuations in the above mentioned parameters when day 0 (Control) of all the blood groups was compared to other days (Test) irrespective of the blood group. This alterations were statistically significant ($p<0.05$).

In this study, the packed cell volume in all the different blood groups donated and stored, showed statistically significant decrease ($p<0.0001$). Blood analysed immediately after donation (Control), and the stored donated blood analysed at 5 days interval for 30 days showed a steady decrease in packed cell volume. This implies that the higher the number of days of the storage, the lower the level of packed cell volume. The report from this study may be attributed to the fact that storage of the whole blood might have induced some sort of haemolysis, as confirmed by Osaro et al. [12], where they observed that stored red blood cells undergo a progressive degradation which results in depletion of the energy source required to operate the physiological processes, which in turn affects the structural integrity of the red cell membrane, the deformability that allows it to pass through microcirculation to perfuse tissues and its fragility is distorted, resulting in some level of haemolysis and thus the release of haemoglobin contained in its cytoplasm into the plasma.

Also the result obtained in this study is in agreement with Muhammed et al. [13], in a work done on the effect of storage time on haematological and biochemical parameters in blood bags containing CPDA-1 anticoagulant during 30 days of storage where they stated that the decrease was due to ATP depletion leading to red blood cell deformability and change in shape to echinocytes. Also, the report from this study agrees with that from similar studies carried out by Saleh and Bashi [14] and Arif et al. [15], but disagrees with that of Adias et al. [16], stating that there was no significant change in packed cell volume in CPDA-1 anticoagulated blood stored for 28 days (p-value = 0.080). Also, our findings on packed cell volume was in agreement with that of Leo et al. [17], where they stated that packed cell volume values declined as a result of increased period of storage.

Furthermore, packed cell volume levels drastically decreased after one (1) week of storage and this was in tune with the work done by Ahmed et al. [18], which in their own case was as a result of storage lesion. A decrease in packed cell volume will affect haemoglobin and

this will cause a shift in 2-3 DPG which will lead to a shift on oxygen/haemoglobin dissociation curve to the left thereby impairing oxygen delivery.

Also in this research work, methaemoglobin level showed a significant decrease on test days mean values compared to the mean values of the controls. The same way packed cell volume was analysed where the freshly donated samples acted as control and the subsequent days with 5 days interval till day 30 followed suit. For haemoglobin to reversibly bind oxygen within the red blood cell, its iron content must be maintained in the reduced or ferrous state. Sometimes, a little quantity of oxyhaemoglobin undergoes spontaneous oxidation (auto-oxidation), generating methaemoglobin (in which the iron becomes oxidised to the ferric state, and cannot bind oxygen) and reactive oxygen species are generated [19]. Methaemoglobin is unstable and breaks down into haemin and globin [20]. Free haemin and iron, together with reactive oxygen species, can generate highly reactive hydroxyl radicals that can induce oxidative injury to membrane lipids and proteins [21]. Under normal circumstances, red cells are protected against this oxidative injury because the rate of the auto-oxidation of the haemoglobin is slow [22], the NADH-dependent cytochrome-b5 reductase (CYTb5) reduces methaemoglobin back into oxyhaemoglobin, and cytosolic antioxidants (primarily reduced glutathione or GSH) and membrane anti-oxidants (primarily ascorbic acid or vitamin C) neutralise the generated reactive oxygen species. However, during storage, all of these protective mechanisms are impaired [23]; this impairment might be responsible for the report obtained from this study.

A statistically significant difference in oxyhaemoglobin levels for all the different blood groups donated, was also noted amongst the various groups. It was however observed that in the various blood groups, oxyhaemoglobin increases significantly within the first week as compared to the control, and then begins to decrease as the number of days of blood storage increases. The main aim of blood transfusion is the ability of red blood cells to deliver oxygen and the continuous decrease in this parameter after storage for a long time makes this aim questionable.

Metabolic changes in red blood cells during liquid storage increases the affinity of haemoglobin for oxygen by depleting 2, 3-diphosphoglycerate (2,3-DPG). This change reduces the partial pressure of oxygen gas where the oxygen tension of haemoglobin is 50 percent [24]. The report from this study agrees with that from a similar study carried out by Bunn et al. [25], stating that during the first week of blood storage in acid-citrate-dextrose (ACD), a progressive increase in oxygen affinity was observed, after which little further change was noted. Also Ahmed et al. [26], in their work on effect of blood storage on certain haematological parameters showed that there was a drastic increase in oxyhaemoglobin within the first week and a drastic significant decrease after that. This may be as a result of erythrocyte haemolysis due to improper storage, not mixing the blood periodically which will cause decrease in 2,3 DPG and old red blood cell haemolysis. The increase in oxyhaemoglobin level within the first week may be as a result of the red blood cells still fresh and no haemolysis has taken place yet. These findings were the same as that of Elemchukwu et al. [27]. In general, all the blood groups showed no significant difference in their haematological characteristic deterioration or storage lesion based on blood type differences.

5. CONCLUSION

This study demonstrated the effect of blood storage using CPDA-1 on packed cell volume, methaemoglobin and oxyhaemoglobin in different ABO/Rhesus blood groups. From the results obtained in this study, it can be concluded that storage of blood in a blood bank refrigerator at temperature of about 4^0C using CPDA-1 anticoagulant induced a decrease in packed cell volume by causing haemolysis, and that the longer the storage duration, the higher the haemolysis. Oxyhaemoglobin level also showed a decrease after the first one week of storage. Methaemoglobin as well showed decrease as it is a part of haemoglobin but its decrease is advantageous in terms of oxygen delivery since it is a non–oxygen carrying haemoglobin; this decrease is an advantage for oxyhaemoglobin in normal condition. All the stored blood irrespective of their ABO group or Rhesus factor undergo haematological changes that are unavoidable and this leads to decrease in the oxygen carrying capacity and reduction of red blood cell. It is therefore necessary to take into cognizance the transfusion of fresh blood or blood that have not been stored for a long period of time. Since storage of blood induces haemolysis and reduces the packed cell volume level (and thus haemoglobin level), blood for transfusion in

severe anaemic patients should be collected fresh and transfused. Blood for transfusion in the management of sickle cell patients, and other disorders related to anaemia should also be transfused fresh, and if blood is stored, prolonged storage beyond 10 days should be avoided.

CONSENT

Informed consent was given by all blood donors.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Robert B, Talhitsch M, Fredrick T, Michael J. Blood - Human anatomy, 5th edition, San Francisco: Pearson/Benjamin Cummings; 2006.
2. Saladin KS. Anatomy and physiology. 3rd edition, New York: McGraw-Hill; 2004.
3. Reid ME, Lomas-Francis C. Blood group antigen facts book, 2nd edition, New York: Elsevier Academic Press; 2004.
4. Goldman M, Fournier E, Cameron-Choi K, Steed T. Effect of changing the age criteria for blood donors. Vox Sanguis. 2007;92(4): 368–372.
5. Ochei JA, Kolhatkar A. Medical laboratory science theory and practice, New Delhi: McGraw-Hill Limited; 2004.
6. Hess JR, Greenwalt TG. Storage of red blood cells: New approaches. Transfusion Medicine Reviews. 2002;16(4):283-295.
7. Kawthalkar T. Most frequent procedures performed on US hospitals. Hospital Statistical Agency for Healthcare and Quality Rockville. 2012;3(1):321–328.
8. Kim-Shapiro DB, Lee J, Gladwin MT. Storage lesion, role of red cell breakdown. Transfusion. 2012;51(4):844-851.
9. Kor DJ, Camilie M, Van Buskirik A, Ognjen G. Red cell storage lesion. Bosnian Journal of Basic Medical Science. 2009; 9(1):21–27.
10. Cheesbrough M. District laboratory practice in tropical countries, 2nd edition, Part 2. Cambridge: Cambridge University Press; 2010.
11. Evelyn KA, Malloy HT. Microdetermination of oxyhaemoglobin methaemolobin, and sulphaemoglobin in a single sample of Blood. Journal of Biological Chemistry. 1938;126:655.
12. Osaro E, Lukman H, Abiodun E, et al. A review of the pathophysiology and consequences of red cell storage - fresh versus stored red cells - implication for optimum use of scarce allogenic blood. American Association for Science and Technology Journal of Medicine. 2018; 4(2):32-50.
13. Muhammad AZ, Fahama P, Mujahida M, et al. Effect of storage time on haematological and biochemical parameters on blood bags containing CPDA-1 anticoagulant during 30 days of storage. Indology American Journal of Pharmaceutical Sciences. 2018;5(10):32–38.
14. Saleh BM, Bashi AYD. Effect of blood storage on certain hematological parameters. Tikrit Medical Journal. 2009; 15(1):171-180.
15. Arif SH, Yadav N, Rehman S, Mehdi G. Study of hemolysis during storage of blood in the blood bank of a tertiary health care centre. Indian Journal of Haematology and Blood Transfusion. 2017;33(4):598–602.
16. Adias TC, Moore IB, Jeremiah ZA. Storage related haematological and Biochemical changes of CPDA-1 whole blood in a resource limited setting. Journal of Blood Disorders and Transfusion. 2012;3:124-128.
17. Leo MG, van de Watering A, Anneke B. Effects of storage on red cells. Transfusion Medicine and Haematology. 2008;4(1):23–28.
18. Ahmed SG, Orakah JA. Cellular changes in stored whole blood and the implication on efficacy of transfusion therapy in Nigeria. The Internet Journal of Third World Medicine. 2008;8(2):312–317.
19. Lutz HU, Bogdanova A. Mechanisms of tagging senescent red blood cells for clearance in healthy humans. Frontiers in Physiology. 2013;4:387-392.
20. Greer P. Wintrobe's clinical hematology. 13th edition. Philadelphia, PA: Lippincott Williams & Wilkins Publishers; 2014.
21. Kanias T, Acker JP. Biopreservation of red blood cells-the struggle with hemoglobin oxidation. The Federation European Biochemical Societies Journal. 2010;277: 343-56.

22. Reeder BJ. The redox activity of hemoglobins: From physiologic functions to pathologic mechanisms. Antioxidants & Redox Signaling. 2010;13:1087–1123.
23. Orlov D, Karkouti K. The pathophysiology and consequences of red blood cell storage. Anaesthesia Public Medicine. 2015;70:29–37.
24. Inglut C, Kausch K, Gray A, Landrigan M. Rejuvenation of stored red blood cells increases oxygen release capacity. Blood. 2016;128(22):4808.
25. Bunn HF, May MH, Kocholaty WF, Shields CE. Haemoglobin function in stored blood. The Journal of Clinical Investigation. 1969;48:311-321.
26. Ahmed Y, Dallal B, Bashar MS. Effect of blood storage on certain haematological parameters. Tikrit, Medical Journal. 2009; 15(1):171–180.
27. Elemchukwu Q, Obeagu EI, Ochei KC. Effect of storage on full blood count on different anticoagulants. Journal of Dental and Medical Science. 2014;13(9):128–131.

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