Original Research Article

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

ABSTRACT

Aims: The aim of the study is to determine the effect of blood storage using CPDA-1 on packed cell volume, methaemoglobin and oxyhaemoglobin in different ABO and Rhesus blood types in 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- .The donors were adult males with age ranging from 35-45 years and they were apparently healthy and free from any transfusion transmissible infections. Samples from the stored blood was stored for 30 days and sample for analysis were collected at 5 days interval.

Place and Duration of Study: 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. All blood donors are resides in Port Harcourt. Blood donated was stored at Military Hospital Blood Bank, Port Harcourt in a blood bag of 450ml containing 63ml 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 blood group and Rhesus factor (A +,B+,AB+,O+,A-,B-,AB- and O-). Day 0 was taken to be control and a five day intervals in-between to day 30 acted as the test. Packed call volume was estimated using micro-haematocrit method while oxyhaemoglobin and methaemoglobin levels were estimated spectrophotometricically as described by Evelyn and Malloy.

Results: The result showed a significant decrease in mean packed cell volume, Oxyhaemoglobin and Methaemoglobin level 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 or Rhesus factor 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 the blood groups showed no significant difference in their haematological characteristic deterioration or storage lesion based on blood type differences. It is therefore necessary 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, RBC contributes to the colloid osmotic pressure, to platelet-endothelium interactions necessary for normal haeostasis, 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 (RBCs). 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 (BBR) 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 and Citrate Phosphate Dextrose Adenine-1. The first two anticoagulants were replaced with the last (CPDA-1) which is now widely recommended and used. Citrate phosphate dextrose adenine -1 (CPDA -1) adult blood bag has a total volume of about 450ml containing 63mls 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 (RBC) 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 (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. MATERIAL 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-.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 involved 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 PCV value 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 540nm.

Procedure: Fresh ammoniated water was prepared by adding 0.04 of ammonia to 100ml of distilled water. 4ml of ammoniated water was pipetted in to a test tube. The sample was mixed and 20ul (0.02) 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 540nm.

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 630nm.

Procedure: 0.02µl of blood was analysed in a mixture of 4ml phosphate borate buffer of pH 6.8 and 6ml of non-ionic detergent. The lysate was divided into 2 equal volumes A and B. The absorbance 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 to be 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 be added to the 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 analysed 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 difference that was significant, 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, HCV). Details are shown in Table 1.

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

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 levels, and oxyhaemoglobin levels 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.

Table 2a: Analysis of Variance of Studied Parameters of Blood Group A Rhesus "D" Negative

	Control (M±SD)	Day5 (M±SD)	Day10 (M±SD)	Day15 (M±SD)	Day20 (M±SD)	Day25 (M±SD)	Day30 (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	Day5 versus	Day10 versus	Day15 versus	Day20 versus	Day25 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 application to all Tables.

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 levels and oxyhaemoglobin levels 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.

Table 3a: Analysis of Variance of Studied Parameters of Blood Group A Rhesus "D" Positive

	Control (M±SD)	Day5 (M±SD)	Day10 (M±SD)	Day15 (M±SD)	Day20 (M±SD)	Day25 (M±SD)	Day30 (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	Day5 versus	Day10 versus	Day15 versus	Day20 versus	Day25 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}

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 levels and oxyhaemoglobin levels 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.

Table 4a: Analysis of Variance of Studied Parameters of Blood Group AB Rhesus "D" Negative

	Control (M±SD)	Day5 (M±SD)	Day10 (M±SD)	Day15 (M±SD)	Day20 (M±SD)	Day25 (M±SD)	Day30 (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	Day5 versus	Day10 versus	Day15 versus	Day20 versus	Day25 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}

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

Packed cell volume, methaemoglobin levels and oxyhaemoglobin levels of blood group AB "D" Positive were analyzed 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.

Table 5a: Analysis of Variance of Studied Parameters of Blood Group AB Rhesus "D" Positive

	Control (M±SD)	Day5 (M±SD)	Day10 (M±SD)	Day15 (M±SD)	Day20 (M±SD)	Day25 (M±SD)	Day30 (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	Day5 versus	Day10 versus	Day15 versus	Day20 versus	Day25 versus
PCV	Day5^{NS} Day10^{VS} Day15,20,25,30^{NS}	Day10^{NS} Day15^{VS} Day20,25,30^{HS}	Day15^{NS} Day20^{VS} Day25,30^{HS}	Day20^{NS} Day25^{VS} Day30^{HS}	Day25^{NS} Day30^{S}	Day30^{NS}
MetHb	Day5^{NS},10,15,20^{HS} Day25^{NS} Day30^{S}	Day10^{HS} Day15^{S} Day20,25,30^{NS}	Day15^{NS} Day20^{VS} Day25,30^{HS}	Day20,25,30^{NS}	Day25,30^{NS}	Day30^{NS}
OxyHb	Day5,10,15,20,30^{NS} Day25^{VS}	Day10,15,20^{NS} Day25^{VS},30^{HS}	Day15,20^{NS} Day25^{VS}30^{NS}	Day20,25,30^{NS}	Day25,30^{NS}	Day30^{NS}

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

Packed cell volume, methaemoglobin levels and oxyhaemoglobin levels of blood group B Rhesus "D" negative were analyzed 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 6a: Analysis of Variance of Studied Parameters of Blood Group B Rhesus "D" Negative

	Control (M±SD)	Day5 (M±SD)	Day10 (M±SD)	Day15 (M±SD)	Day20 (M±SD)	Day25 (M±SD)	Day30 (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	Day5 versus	Day10 versus	Day15 versus	Day20 versus	Day25 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}

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

Packed cell volume, methaemoglobin levels and oxyhaemoglobin levels 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.

Table 7a: Analysis of Variance of Studied Parameters of Blood Group B Rhesus "D" Positive

	Control (M±SD)	Day5 (M±SD)	Day10 (M±SD)	Day15 (M±SD)	Day20 (M±SD)	Day25 (M±SD)	Day30 (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	Day5 versus	Day10 versus	Day15 versus	Day20 versus	Day25 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}	Day10,15,20^{HS}	Day15^{NS}	Day20,25,30^{HS}	Day25,30^{HS}	Day30^{NS}

	Day10^{S},15^{VS} Day20,25,30^{HS}	Day25,30^{HS}	Day20,25,30^{HS}			

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

Packed cell volume, methaemoglobin levels and oxyhaemoglobin levels of blood group O Rhesus ''D'' Negative were analyzed 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.

Table 8a: Analysis of Variance of Studied Parameters of Blood Group O Rhesus "D" Negative

	Control (M±SD)	Day5 (M±SD)	Day10 (M±SD)	Day15 (M±SD)	Day20 (M±SD)	Day25 (M±SD)	Day30 (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	Day5 versus	Day10 versus	Day15 versus	Day20 versus	Day25 versus
PCV	Day5^{NS} Day10^{S},15^{VS} Day20,25,30^{HS}	Day10^{NS} Day15^{VS} Day20,25,30^{HS}	Day15^{NS} Day20^{VS} Day25,30^{HS}	Day20^{NS} Day25,30^{HS}	Day25^{NS} Day30^{VS}	Day30^{NS}
MetHb	Day5,10,15,20^{HS} Day25,30^{HS}	Day10,15^{HS} Day20,25,30^{HS}	Day15^{HS} Day20,25,30^{HS}	Day20^{NS} Day25,30^{HS}	Day25,30^{HS}	Day30^{HS}
OxyHb	Day5^{HS} Day10,15^{NS} Day20,25,30^{NS}	Day10,15,20^{HS} Day25,30^{HS}	Day15^{NS} Day20,25,30^{NS}	Day20,25,30^{NS}	Day25,30^{NS}	Day30^{NS}

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

Packed cell volume, methaemoglobin levels and oxyhaemoglobin levels of blood group O Rhesus "D" Positive were analyzed 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.

Table 9a: Analysis of Variance of Studied Parameters of Blood Group O Rhesus "D" Positive

	Control (M±SD)	Day5 (M±SD)	Day10 (M±SD)	Day15 (M±SD)	Day20 (M±SD)	Day25 (M±SD)	Day30 (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	Day5 versus	Day10 versus	Day15 versus	Day20 versus	Day25 versus
PCV	Day5^{NS} Day10^{S} Day15,20,25,30^{HS}	Day10^{NS} Day15^{VS} Day20,25,30^{HS}	Day15^{NS} Day20,25,30^{HS}	Day20^{NS} Day25,30^{HS}	Day25^{NS} Day30^{HS}	Day30^{NS}

MetHb	Day5,^{HS}10^{NS},15,25^{VS} Day20^{S},30^{HS}	Day10,15^{HS} Day20^{S},25,30^{NS}	Day15,30^{HS} Day20^{S},25^{VS}	Day20,25,30^{HS}	Day25^{NS},30^{HS}	Day30^{HS}
OxyHb	Day5^{S},10,15^{NS} Day20,25,30^{HS}	Day10^{NS},15^{HS} Day20,25,30^{HS}	Day15^{VS} Day20,25,30^{HS}	Day20^{S},25^{VS} Day30^{HS}	Day25,30^{NS}	Day30^{NS}

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, showed statistically significant decrease ($p<0.0001$). Blood analyzed immediately after donation (control), and the stored donated blood analysed at 5 days interval for 30 days showed a steady decrease in PCV. This implies that the higher the number of days of the storage, the lower the level of PCV. 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 he 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 the PCV in CPDA-1 anticoagulated blood stored for 28 days (P -value 0.080). Also, the findings in this work's PCV was in agreement with that of Leo et al. [17], where they stated that PCV values has a decline as a result of increased period of storage.

Furthermore, PCV levels drastically decreased after one (1) week of storage and this was in support with the work done by Ahmed et al. [18], which in his 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 dissociation curve to the right thereby impairing oxygen delivery.

Also in this research work, methaemoglobin level showed a significant decrease on the test days mean value compared to the mean value of the control. 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 was followed suit. For haemoglobin to reversibly bind oxygen within the red 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 .This 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 blood group. 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 in at 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 cognisance 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 PCV 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.

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