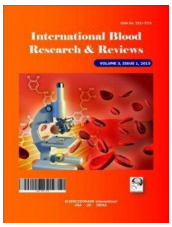

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Changes in Plasma Haemoglobin Concentration in Citrate Phosphate Dextrose Adenine-1(CPDA-1) Stored Blood

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Authors' contributions

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

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ABSTRACT

Aim: This study assessed the level of plasma haemoglobin concentration in CPDA-1 stored blood with a view to determine the extent of haemolysis during the 35 days storage period.
Study Design: This is an observational and comparative case-control study.
Place and Duration of Study: The study was conducted using healthy male donors residing in Port Harcourt. Analysis was carried out at the Blood Bank of Rivers State University Teaching Hospital, formerly Braithwaite Memorial Specialist Hospital (BMSH), Port Harcourt, Nigeria, from February 1st to March 8th, 2017.
Methodology: Blood for transfusion was collected from prospective male blood donor found to be in good health, aged between 18 and 52 years, with haemoglobin level within the range of 13.5

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g/dl – 16 g/dl, body weight within 55 kg – 75 kg, and body temperature within 37.0 to 37.5^{0}C / 99.5^{0}F, into plastic bags containing anticoagulant CPDA-1, and handled under strict sterile condition to prevent bacterial contamination. The blood was stored in a blood bank refrigerator with a constant temperature of +2 to +6^{0}C under proper inspection at intervals for colour, turbidity, haemolysis and clot formation. Two milliliters of the sample was collected aseptically at different interval days of collection from the blood bag and analyzed using the HemoCue photometer.

Results: Results showed no significant changes in plasma haemoglogin from day 1, 5, and 10, while significant increase in haemolysis occurred from day 15, 20, 25, 30, and 35 ($p = 0.000$), a significant increase ($p<0.05$) in plasma haemoglobin was observed from day 15 to day 35 of storage.

Conclusion: It is pertinent therefore to note that the use of CPDA-1 does not completely stop the changes that occur in RBC as there are several changes occurring in stored blood collectively called "storage lesions". Therefore, it is advisable that blood should be transfused within 14 days of storage to avoid transfusion of blood products that has lost most of its benefits to recipients, and where possible whole blood should be processed and components separated before storage to reduce the level of non-viable red blood cells.

Keywords: Plasma haemoglobin; haemolysis; stored blood; CPDA-1; donor.

1. INTRODUCTION

Blood transfusion has remained one of the most therapeutic ways of managing patients with anaemia and severe bleeding disorders, although not entirely without some risks ranging from immunomodulation, transmission of undetected viral and bacterial antigens, to transfusion reactions [1,2]. Haemoglobin, the most important constituent of RBC, is a chromo-protein consisting of a globin molecule attached to a red coloured haem molecule found in all red blood cells for delivery of oxygen to the cells of the body. It serves as the most significant buffer in blood with ability to resist changes in pH and play vital role in controlling erythrocytes metabolism. The normal reference range of haemoglobin for male is between 14 -18g/dl, females 12 – 16 g/dl [3].

Blood plasma is the grey part of blood constituting 55% of total body blood volume with about 95% water, and about 5% dissolved proteins (serum albumin, globulins, and fibrinogens), glucose, clotting factors, electrolytes (Na^+, Ca^{2+}, HCO_3, Cl^-), hormones and carbondioxide. Its function is mainly in the transport of excretory products, serves as proteins store of the body, regulation and maintenance of intravascular osmotic effects that keeps the electrolytes within balance, protecting the body from disease and other blood disorders. Blood plasma has a density of approximately 1025 kg/ml or 1.025 g/ml and is released from red cell when they are lysed or destroyed. Prolong contact between plasma and the red blood cell during storage leads to the outburst of haemoglobin resulting in the release of heme which potentially triggers negative effects in the blood unit leading to initiation of white blood cell and migration of adhesion molecules and cytokine, and also oxidation formation [4,5, 6,7].

Haemoglobin is found inside the red blood cell and not inside plasma and if found in plasma there are physiological disturbances it will cause. In the blood cells (red blood cells), it binds with oxygen and transport oxygen to body cells and tissues and also to the brain, making it a very important physiological molecule of life because without it there will be no life! When red cells complete their lifespan in the body, haemoglobin are not released into the plasma by the scenescent red cells but are rather broken down physiologically to produce biomolecules that are excreted in the urine as urobilinogen and in the faeces as stercobilinogen.

In apparently normal conditions, plasma does not contain haemoglobin, the presence of haemoglobin in plasma is an indication of the occurrence of haemolysis from conditions such as sickle cell disease, paroxysmal nocturnal haemoglobinuria (PNH), hereditory spherocytosis, microangiopathic haemolytic anaemia, blood transfusion reactions, cardiopulmonary bypass, and prolong storage of blood [8]. In stored blood, haemolysis gradually set in as red blood cells are damaged due to ''storage lesion'' arising from a range of biochemical changes such as decline in pH, reduction in glucose utilization, depletion in ATP and 2,3 diphosphoglycerate (2,3-DPG) level

needed to maintain its shape and viability. Some researchers have suggested that more than 70% of red blood cell transfused remain viable in circulation 24 hours following transfusion of stored whole blood in CPDA-1 for 35 days [9, 10].

The presence of haemoglobin in plasma causes the haemoglobin tetramer to be in equilibrium with the alpha and beta haemoglobin dimer and helps in the binding of these haemoglobin dimers to haptoglobin with resultant binding to haptoglobin receptors on macrophages when haemoglobin is degraded [11,12,13]. In case of massive or chronic haemolysis, free haemoglobins are released in plasma when haptoglobin/CD163 are overwhelmed. The oxyhaemoglobin in plasma are recruited to act in response with nitric oxide to form nitrate and ferric haeamoglobin, as haemoglobin is not bound to haptoglobin.

Storage lesions are sequence of biochemical and structural changes that occurs in the red blood cell units during storage in an anticoagulated blood bag containing CPDA-1. These storage lesions reduce subsequently the red cell survival and function in-vivo when the blood is being transfused. The improvement on CPDA-1 over the years by addition of phosphate, adenine and various other nutrient solutions has improved the storage of CPDA-1 to 42 days mean length 15 days [9]. The citrate prevents coagulation by binding or chelating to calcium, phosphate acts as a buffer thus maintaining the pH of the blood, dextrose serves as the substrate for energy needed for the blood cell, and adenine maintains the high ATP level in red blood cells [9]. Research has shown that long storage of blood has a negative effect on the red blood cell oxygen delivery capacity of blood, long storage cause blood to become more acidotic arising from high concentration of free haemoglobin and biologically active lipids in plasma containing high amount of negatively charged micro vesicle with pro-inflammatory and pro-coagulant activity [14].

Studies by Gladwin et al., [15], shows that methaemoglobin in steady state can attain level as high as 5% in human inhaling 80 parts per million (ppm) nitric oxide for four hours. This methaemoglobin in its unstable state, releases ferric heme from the globin chain. Steady state methaemoglobin level does not reveal precisely the kinetics of methaemoglobin in plasma [16].

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Braithwaite Memorial Specialist Hospital (BMSH), now Rivers State University Teaching Hospital, located in the city of Port Harcourt, Rivers State, Nigeria. Port Harcourt is located between latitudes 4°2′ North and 4°47′ North and longitudes 6°55′ East and 7°08′ East. Braithwaite Memorial Specialist Hospital (BMSH) is a 346 bed specialist hospital owned by Rivers State Government with a standard blood bank.

2.2 Study Population

Four hundred and fifty milliliters (450 ml) of blood was collected from ten (10) apparently healthy volunteer male donors aged 20-52 years into CPDA-1 blood bag using standard venipuncture technique and placed on quarantine shelf of the blood bank refrigerator. The donors were all screened for hepatitis B and C, syphilis and HIV.

2.3 Collection of Blood Samples and Storage

Blood for storage was collected from prospective blood donor found to be in good health into plastic bags containing anticoagulant CPDA-1 and handled under strict sterile condition to prevent bacterial contamination. Collection time was 8 am to 10 am. The blood was stored in a refrigerator with a constant temperature of +2 to +6°C under proper inspection at intervals for colour, turbidity, haemolysis and clot formation.

2.4 Methodology

Two milliliters of the blood sample were collected aseptically from each of the blood bag and analysed using the HemoCue photometer. The HemoCue Plasma/low Hb system is a cyanmethaemoglobin based method of analysis used for the quantitative determination of low levels of haemoglobin in plasma and serum specimens, aqueous solutions, stored or banked erythrocytes using specially designed microcuvettes called the hemocue plasma/low Hb microcuvettes.

The HemoCue works on the principle of determining the haemoglobin concentration as azidemethaemoglobin utilizing a microcuvette with a dry reagent system and a dual wavelength photometer. When haemoglobin is present, the

membranes of erythrocytes are disintegrated by sodium deoxycholate, releasing haemoglobin. Sodium nitrite converts the haemoglobin iron from ferrous state to ferric state to form methhaemoglobin which then combines with sodium axide to form xidemethaemoglobin and measured at 570 nm and at 880 nm.

2.5 Statistical Analysis

All data in this study were analyzed using SPSS version 20. The various groups were compared for significance using ANOVA (Post Hoc). The level of significance was pegged at 95%. $P < 0.05$ was considered statistically significant.

The percentage of haemolysis was calculated by measuring the total haemoglobin, haematocrit and plasma haemoglobin using the formula:

> Percentage Haemolysis = (100-Haematocrit) × (Plasma Haemoglobin) g/dl / (Total Haemoglobin) g/dl

3. RESULTS

3.1 Mean Values of Haematocrit, Whole Blood Haemoglobin, Plasma Haemoglobin Level and Percentage Haemolysis from Day 0 to Day 35

The mean values of haematocrit, whole blood haemoglobin, plasma haemoglobin and their respective level and percentage haemolysis from Day 0 to Day 35 were analysed and details are shown in Table 1.

3.2 Comparison of Plasma Haemoglobin Levels based on Days of Storage

Comparison was made based on storage days and a post-hoc outcome of the comparison to show where statistical significance was observed are shown in Tables: 2, 3, 4, and 5.

4. DISCUSSION

Storage of whole blood and/or components are necessary therapeutic criteria needed to arrest most cases of accident emergencies, obstetric bleeding and post-partum haemorrhage. Provision and storage of blood and blood component is therefore important in the hospital setting. This study shows that for red blood cells, as storage time increases, haemolysis increase in the stored blood and this is in agreement with most studies done by several investigators. Transfusion of red blood cells stored longer than 4 weeks, considerably increased plasma free haemoglobin [17]. A study done by Houxiang et al., [18] also showed that free haemoglobin and percentage of free to total haemoglobin in the storage medium were also significantly increased after storage as ATP and 2,3-DPG level were significantly decreased when compared to fresh red blood cells.

In line with other studies, Sani et al., [19], this study also showed major increase in proportion of haemolysis, plasma haemglobin and plasma potassium level in all the groups in the cause of storage ($p > 0.001$). It has been described by different authors that the presence of leukocytes in the red cell units contributes notably to increase in red cell haemolysis for the period of storage [20,21]. This occurs as a result of leukocytes breakdown and release of a number of chemicals and enzymes such as hydrogen peroxide and proteases. These proteases release during storage has been reported to cause red blood cell lysis during storage [22].

The guidelines of the council of Europe have established 0.8% quantity of haemolysis within red blood cells (RBC) products for transfusion as the acceptable limit of hemolysis [23]. Most RBC unit shows haemolysis level of about 0.3 - 0.4% for the period of storage. In this study, the minimum percentage haemolysis 0.0% was observed on day 0 (day of collection before storage) while the maximum haemolysis was observed on the 35^{th} day of storage (1.72%). Stored blood units on day 25^{th}, 30^{th} and 35^{th} showed percentage haemolysis of more than 0.8% which is more than the maximum acceptable units of haemolysis as stated by the council of Europe guideline for blood stored in CPDA-I.

This could be due to the epileptic power or electricity in Nigeria. Only a small number of unit displayed levels higher than the maximum level but not often outside the control of Haptoglobin to scavenge [24]. Studies conducted by different researchers to enumerate the level of plasma in packed red cell units throughout storage showed significant increase in free haemoglobin [23]. Human RBC deformability decrease notably by 34% following four (4) weeks of storage as shown by D'Almeida et al., [25], prolong storage also causes increase in potassium and free haemoglobin concentration in the suspending fluid plasma, resulting in a fall in pH level.

Table 1. Mean values of haematocrit, whole blood haemoglobin, plasma haemoglobin level and percentage haemolysisfrom day 0 to day 35

Storage time (Days)	Haematocrit (%)	Whole blood haemoglobin (g/dl)	Plasma haemoglobin (g/dl)	Percentage haemolysis (%)
0	43.0	14.30	0.±0.0	0.0
1	42.9	14.30	0.01±0.03	0.04
5	42.7	14.20	0.03±0.05	0.12
10	42.4	14.10	0.05±0.05	0.20
15	41.9	13.96	0.11±0.06	0.46
20	40.9	13.60	0.14±0.08	0.61
25	39.5	13.20	0.20±0.08	0.92
30	38.0	12.70	0.27±0.08	1.32
35	35.0	11.70	0.31±0.09	1.72

Table 2. Comparison of plasma haemoglobin levels from day 0 to day 35

Storage time (days)	p-values
0 vs 5	0.734 Ns
0 vs 10	0.310 Ns
0 vs 15	0.093 Ns
0 vs 20	0.000 s
0 vs 25	0.000 s
0 vs 30	0.000 s
0 vs 35	0.000 s

Table 3. Comparison of plasma haemoglobin level from day 1 to day 35

Storage time (days)	p-values
1 vs 5	0.498 Ns
1 vs 10	0.177 Ns
1 vs 15	0.001 s
1 vs 20	0.000 s
1 vs 25	0.000 s
1 vs 30	0.000 s
1 vs 35	0.000 s

Table 4. Comparison of plasma haemoglobin level from day 5 to 35

Storage time (Days)	p-values
5 vs 10	0.498 Ns
5 vs 15	0.008 s
5 vs 20	0.000 s
5 vs 25	0.000 s
5 vs 30	0.000 s
5 vs 35	0.000 s

Table 5. Comparison of plasma haemoglobin level from day 10 to day 35

Storage time (days)	p-values
10 vs 15	0.044 s
10 vs 20	0.003 s
10 vs 25	0.000 s
10 vs 30	0.000 s
10 vs 35	0.000 s

Table Keys: S=Significant; Ns=Non Significant

The overall effect leads to decreased fraction of RBCs that survive after being returned to circulation through transfusion as discussed by Hess, [26,24]. A study by Tinmouth and Chin Yee [27] showed also that, during RBC storage, metabolism slows down as RBCs are stored in the blood bank shelf.

5. CONCLUSION

The use of CPDA-1 does not completely stop the changes that occur in RBC as there are several changes occurring in stored blood collectively called storage lesions. Therefore, it is advisable that blood should be transfused within 14 days of storage to avoid transfusion of blood products that has lost most of its benefits to recipients, and where possible whole blood should be processed and components separated before storage to reduce the level of non-viable RBC.

CONSENT AND ETHICAL APPROVAL

Informed consent was obtained from apparently healthy subjects prior to enrolment upon ethical clearance by the Ethics Committee of the Department of Medical Laboratory Science, Rivers State University and Ethical Committee of Rivers State University Teaching Hospital, Port Harcourt.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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