# ASSESSMENT OF BIOCHEMICAL AND HAEMATOLOGICAL CHANGES THAT OCCUR IN BLOOD STORED WITH CPDA-1 AS AN ANTICOAGULANT IN A TERTIARY HOSPITAL IN NIGERIA

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## ABSTRACT

Transfused blood must be of required quality to meet body metabolic requirements. This study monitored variations in biochemical and hematological parameters following blood transfusion. Twenty blood donors age range 18-55yr were recruited. About 450mls blood was drawn into blood bag containing citrate phosphate dextrose adenine (CPDA-1) and stored at 2-8$^0$C in blood bank. Ten millilitres blood was withdrawn on different days, centrifuged to obtain the plasma which was analysed using standard methods.

Result shows a significant decrease ($p<0.05$) in plasma antioxidants, sodium, chloride, urea, lipid, protein, alkaline phosphatase, haematological parameters; and significant increase ($p<0.05$) in potassium, malondaaldehyde, bilirubin, and transaminases on day 35 when compared to values at day 0.

In conclusion, storing of blood using CPDA-1 as an anticoagulant is associated with variations in biochemical and hematological parameters which were noted to occur after the seventh day of storage. Blood for transfusion is thus best used on or before the seventh day of donation.

***Key words:*** *Transfusion, blood storage, anticoagulant, biochemical parameters, haematological parameters.*

## INTRODUCTION

The importance of blood in the body cannot be underestimated. Arterial blood carries oxygen and nutrients to all cells of the body while venous blood carries away waste products formed as a result of cellular metabolic activities for excretion. Blood transfusion is needed in situations such as anaemia; internal bleeding, excessive bleeding during surgery, cancer and cancer treatments, postpartum haemorrhage, sepsis, blood disorders and bone marrow failure, treatment for severe liver problems such as cirrhosis; prematurity, and severe trauma during accidents. To address the emergency associated with these conditions blood are usually collected and stored or processed and separated into various components before being stored in blood bank. Blood banking is highly regulated to ensure safety of both donors and recipients such that adequate and safe blood is provided to recipients and at no risk to donors[1]. Nigeria, with a population of over 150 million people uses about 1.5 million units of blood every year to satisfy the health demand of her citizens[2].

Because red blood cells (RBCs) are more exposed to oxidative stress than other cells, they are naturally armed with enzymatic (superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase) and non-enzymatic chain-breaking antioxidant systems which neutralise oxidative stress and protect it against oxidative damage[3]. These antioxidant enzyme systems are more sensitive and powerful than those present in other cells[4]. They function to scavenge free radicals and modify redox-sensitive metabolic pathways[5,6].

Red blood cells stored over a period of time undergo changes in biophysical, biochemical and immunological properties termed RBC storage lesions[7]. Storing blood for a long period of time generates a lot of free radicals which are initially scavenged by the antioxidants. As more free radicals are generated, the protective capacity of the antioxidants against oxidative damage is exceeded thereby resulting in loss of RBC membrane integrity, an important factor responsible for RBC hemolysis during storage.

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Despite the immediate restoration of blood volume achievable with blood transfusion during blood loss (acute and chronic), there is the need to ensure that the transfused blood is of required quality to meet the metabolic requirements of the body. Alterations in the quality of transfused blood may be related to the length of storage and the type of anticoagulant used for preservation. There is thus the need to monitor variations in biochemical and hematological parameters that occurred during storage.

## MATERIALS AND METHODS

### Study design and subjects

The study is a longitudinal study and comprises of 20 apparently healthy male blood donors within the age of 18-55yr that attended Ibeju-Lekki General Hospital Lagos state. Subjects were all of Yoruba tribe. Included in the study are blood donors within the age of 18-55yr who gave their consent, having a packed cell volume (PCV) of 40-55% and donors that are negative for hepatitis B, hepatitis C, syphilis, and HIV infections. Excluded from the study are subjects that are chronic alcoholics, persons who did not give their consent, persons having PCV less than 40% or above 55%; donors that are positive for hepatitis B, hepatitis C, syphilis, HIV infection; and donors that have liver related medical conditions, chronic renal disease, hypertensive, diabetics, and endocrine disorders.

### Blood Bag

Blood bag having capacity of 450 ± 10 mls containing Citrate Phosphate Dextrose Adenine (CPDA-1) solution with a shelve life of 35days for whole blood storage at 2-8$^{o}$C was used to store the collected blood.

### Collection procedure:

Four hundred and fifty milliliters (450 mL) of blood was drawn from 20 healthy volunteer donors (age range from 18-55years) into blood bag containing CPDA-1 anticoagulant. Blood was collected with adequate safety precautions to avoid contamination and infection. Blood donors were screened according to National Guideline of Federal Government of Nigeria (NBTS, 2006). All subjects were examined for anemia and screened for hepatitis B virus, hepatitis C virus, Syphilis and HIV before blood donation. Blood collected were stored in the blood bank at temperature range of 2$^{0}$C to 8$^{0}$C.

### Parameters:

The effect of storage was analysed at 0, 7, 14, 21, 28 and 35 days of storage by withdrawing 10ml blood each time from the blood bag. The blood samples collected were centrifuged at 5000 rpm for five minutes to obtain the plasma which was analysed. The sample analysed on day 0 served as baseline control. Plasma biochemical parameters were determined using standard methods. Glutathione (GPx), superoxide dismutase (SOD), Catalase, alkaline phosphatase (ALP), alanine amino transferase (ALT), aspartate amino transferase (AST), and gamma glutamyl transferase (GGT) activities were determined by methods described by Leopold and Wolfgang (1984)[8], Weydert and Cullen (2009)[9], Deisseroth and Dounce (1970)[10], Rosalki *et al.* (1993)[11], Schumann and Klauke (2003)[12], and Persijn and van der Silk (1976)[13] respectively**.** Also, triglycerides, total cholesterol, low-density lipoprotein cholesterol and lipid peroxidation were determined by methods described by Nagele *et al.* **(**1984)[14], Schettler and Nussel (1975)[15], Friedewald *et al.* (1972)[16] and Varshney and Kale (1990)[17] respectively. **H**igh-density lipoprotein cholesterol and Very low density lipoprotein cholesterol computations were done using the method of Sniderman *et al.* (2003)[18] and Obineche *et al.* (2002)[19]**.** Bilirubin, urea, creatinine, uric acid, total protein, and albumin determination were done using methods described by Jendrassik and Gróf (1938)[20], Wybenga *et al.* (1971)[21], Henry *et al.* (1974)[22], Fossati *et al.* (1980)[23], Henry *et al.* (1974)[22], and Doumas *et al.* (1971)[24] respectively. Magnesium, potassium, sodium and chloride were measured using methods described by Baaij (2015)[25], Terri and Sesin (1958)[26], Maruna (1958)[27], and Schale**s** and Schales (1941)[28] respectively. Haematological studies were done using a haematology automated analyzer (Swelab alpha hematology analyzer, Sweden).

**Ethical consideration**
Ethical approval was obtained from ethical committee of Ibeju-Lekki General Hospital, Akodo, Lagos state. Informed consent was also obtained from each participant before the commencement of the study.

**Statistical analysis**
The statistical analysis was done using SPSS software version 21. Descriptive statistic, were used to describe and represent variables. Independent t-test was used to compare differences in mean between two groups while ANOVA was used to compare differences in mean between more than two groups. The level of statistical difference was set at $p < 0.05$.

**RESULTS**
Results of plasma antioxidant levels obtained from this study revealed a non-statistically significant decrease ($p > 0.05$) in plasma GPx, SOD and CAT activities and a statistically significant decrease ($p < 0.05$) in plasma Uric acid during the storage period when compared to the values of those antioxidants at donation state. Reduction in plasma GPx activity was noted to be statistically significant ($p < 0.05$) on days 28 and 35.

Results of plasma electrolyte levels showed a statistically significant decrease ($p < 0.05$) in plasma sodium, and chloride concentrations and a statistically significant increase ($p < 0.05$) in plasma potassium concentrations during the storage period when compared to the values of those electrolytes on day 0. A progressive reduction in plasma $Mg^{2+}$ was observed to occur as the storage days increases.

Results of plasma lipid, lipid peroxidation, and index of renal function parameters during the storage period showed a significant reduction ($p < 0.05$) in plasma urea, total cholesterol, HDLC, and LDLC concentrations and a significant increase ($p < 0.05$) in plasma MDA level over the storage period while the changes in plasma creatinine is not significant ($p > 0.05$).

Results of liver function test parameters revealed reduction in plasma total protein and albumin concentrations and reduction in plasma ALP and GGT activities on day 35 when compared to values at day 0. Also, an increase in the plasma concentrations of bilirubin (total and conjugated), AST and ALT activities were observed over the storage period. The observed changes in these parameters were not statistically significant ($p > 0.05$) except that of AST that was noted to be significant on days 28 and 35.

Results of hematological parameters showed a reduction in concentrations of all the parameters on day 35 when compared to values at donation.

**Table 1: Plasma antioxidants levels during the storage period**

| Parameters | GPx (IU/gHb) | SOD (IU/gHb) | CAT (U/mL) | Uric acid (mg/dL) |
|---|---|---|---|---|
| Day 1 | 83.77±3.12 | 111.34±17. 33 | 742.68±68.71 | 3.23±0.55 |
| Day 7 | 71.68±15.79 | 100.97±13.26 | 753.27±57.41 | 2.46±0.38 * |
| Day 14 | 81.26±11.73 | 106.03±12.52 | 705.49±45.72 | 1.52±0.38 * |
| Day 21 | 79.68±5.19 | 106.48±10.63 | 689.49±41.69 | 1.04±0.11 * |
| Day 28 | 73.47±5.56 * | 105.96±13.45 | 677.78±46.53 | 0.82±0.13 * |
| Day 35 | 59.67±28.95 * | 93.52±28.51 | 381.67±338.02 | 0.64±0.13 * |
| T value | 1.793 | 0.645 | 4.502 | 49.735 |
| p value | 0.152 | 0.668 | 0.005 | 0.000 |

Table 1 above shows the plasma antioxidant levels in the stored blood. Values are expressed as mean standard deviation, and statistica lly significant at $p < 0.05$.

**Table 2: Plasma electrolyte levels during the storage period**

| Parameters | $Mg^{2+}$ (mEq/L) | $K^{+}$ (mEq/L) | $Na^{+}$ (mEq/L) | $Cl^{-}$ (mEq/L) |
|---|---|---|---|---|
| Day 1 | 0.75±0.12 | 4.02±0.28 | 138.91±3.38 | 96.55±2.45 |
| Day 7 | 0.72±0.11 | 4.88±0.37 * | 136.15±4 .52 | 94.18±1.47 |
| Day 14 | 0.68±0.05 | 6.56±0.65 * | 134.32±3.92 | 91.73±2.38 * |
| Day 21 | 0.68±0.07 | 8.02±0.99 * | 133.18±3.99 * | 89.79±3.06 * |
| Day 28 | 0.55±0.25 | 9.52±0.53 * | 130.45±0.80 * | 87.66±2.14 * |
| Day 35 | 0.45±0.06 * | 13.26±1.03 * | 132.97±4.91 * | 82.98±1.96 * |
| T value | 3.937 | 114.857 | 2.903 | 22.145 |
| p value | 0.010 | 0.000 | 0.035 | 0.000 |

Table 2 above shows the plasma electrolyte levels in the stored blood. Values are expressed as mean standard deviation, and statistically significant at $p < 0.05$.

**Table 3: Plasma lipid, lipid peroxidation, and index of renal function parameters during the storage period.**

| Para meters | T Chole (mg/dL) | TG (mg/dL) | HDL (mg/dL) | LDL (mg/dL) | VLDL (mg/dL) | MDA (U/mgprot) | Urea (mg/dL) | Creatin (mg/dL) |
|---|---|---|---|---|---|---|---|---|
| Day 1 | 149.40±28.0 | 115.50±25.27 | 45.80±3.21 | 80.50±28.35 | 23.10±5.05 | 12.89±0.80 | 15.36±3.39 | 0.82±0.05 |
| Day 7 | 71.46±7.53* | 73.70±19.59* | 20.00±0.91* | 16.72±8.07* | 14.74±3.92* | 17.31±3.39* | 6.82±1.04 | 0.85±0.09 |
| Day 14 | 65.70±10.63* | 87.48±30.09 | 27.40±1.88* | 20.80±9.61* | 17.49±6.02 | 17.15±1.42* | 6.84±0.10 | 0.84±0.10 |
| Day 21 | 69.28±11.99* | 110.34±9.62 | 22.14±2.78* | 25.07±12.11* | 22.07±1.92 | 22.38±1.95* | 4.88±1.12 | 0.78±0.11 |
| Day 28 | 68.38±3.42* | 95.46±7.81 | 22.64±3.78* | 26.63±7.48* | 19.09±1.56 | 27.94±1.81* | 4.78±0.80 | 0.75±0.09 |
| Day 35 | 69.28±11.99* | 87.16±8.18 | 18.86±1.90* | 32.99±12.12 | 17.43±1.64* | 68.78±48.99* | 4.94±1.05 | 0.78±0.05 |
| T value | 21.877 | 2.887 | 74.681 | 10.674 | 2.887 | 4.495 | 34.740 | 0.991 |
| p value | 0.000 | 0.035 | 0.000 | 0.000 | 0.035 | 0.005 | 0.000 | 0.444 |

Table 3 above shows the plasma lipid, lipid peroxidation, and index of renal function parameters during the storage period. Values are expressed as mean standard deviation, and statistically significant at $p < 0.05$

**Table 4: Plasma liver function parameters during the storage period**

| Para meters | T protein (g/L) | Albumin (g/L) | T Biliru (mg/dL) | C Biliru (mg/dL) | ALP (IU/L) | AST (IU/L) | ALT (IU/L) | GGT (IU/L) |
|---|---|---|---|---|---|---|---|---|
| Day 1 | 7.22±0.86 | 3.52±0.55 | 8.24±2.44 | 2.26±0.48 | 75.00±18.47 | 29.12±11.65 | 24.16±8.88 | 35.76±4.93 |
| Day 7 | 6.76±0.64 | 3.88±0.29 | 12.02±4.99 | 7.77±1.89 | 84.80±28.77 | 31.62±10.42 | 25.12±11.80 | 36.00±7.59 |
| Day 14 | 6.68±0.84 | 3.34±0.48 | 13.03±5.27 | 7.14±1.63 | 62.92±34.57 | 30.76±3.69 | 32.22±9.39 | 37.16±13.33 |
| Day 21 | 6.90±0.79 | 3.46±0.53 | 13.26±6.66 | 5.24±0.68 | 37.12±12.98 | 34.74±11.89 | 30.22±17.78 | 35.28±15.32 |
| Day 28 | 6.74±0.90 | 3.42±0.90 | 10.20±2.40 | 5.68±0.51 | 31.54±12.93 | 38.70±9.01* | 34.40±5.07 | 33.38±6.24 |
| Day 35 | 6.60±0.54 | 2.66±0.59 | 11.72±6.50 | 5.80±1.46 | 17.68±6.58 | 40.24±5.70* | 27.74±3.99 | 27.66±6.26 |
| T value | 0.408 | 5.737 | 5.289 | 8.477 | 0.746 | 0.602 | 0.550 | 0.619 |
| p value | 0.839 | 0.001 | 0.002 | 0.000 | 0.560 | 0.699 | 0.737 | 0.687 |

Table 4 above shows plasma liver function test parameters during the storage period. Values are expressed as mean standard deviation, and statistically significant at $p < 0.05$.

**Table 5: Plasma hematological parameters during the storage period**

| Parameters | WBC ($x 10^9$/L) | RBC ($x 10^{12}$/L) | Hb (g/dL) | HCT (%) | MCV (fl) | MCHC (g/dL) | MCH (pg) | PLT ($x10^4$/L) |
|---|---|---|---|---|---|---|---|---|
| Day 1 | 4.12±0.52 | 3.96±0.23* | 10.88±1.00 | 32.64±3.01 | 79.33±7.96 | 33.130.00 | 26.44±2.65 | 112.6±2.30 |
| Day 7 | 2.90±0.48 | 3.65±0.43 | 10.12±0.92 | 30.36±2.79 | 83.19±8.63 | 33.33±0.00 | 27.73±2.88 | 112.20±2.77 |
| Day 14 | 2.78±0.58* | 3.72±0.97 | 10.64±0.86 | 31.92±2.59 | 91.54±5.56 | 33.30±0.00 | 30.51±1.85 | 81.20±3.19* |
| Day 21 | 2.48±0.64* | 3.50±0.40 | 9.92±1.33 | 29.76±3.98 | 86.89±35.34 | 33.13±0.00 | 28/96±11.78 | 100.60±5.32 |
| Day 28 | 2.14±0.63* | 3.26±0.30 | 9.94±0.82 | 29.82±2.46 | 69.74±9.56 | 33.33±0.00 | 23.25±3.19 | 93.20±9.12* |
| Day 35 | 2.16±0.61* | 3.32±0.48 | 9.10±1.42 | 27.3±4.26 | 53.56±8.47* | 33.12±0.00 | 17.85±2.82* | 108.40±3.36 |
| T value | 8.009 | 1.912 | 1.663 | 1.663 | 3.622 | 0.000 | 3.622 | 31.520 |
| p value | 0.000 | 0.230 | 0.216 | 0.182 | 0.014 | 1.000 | 0.014 | 0.000 |

Table 5 above shows the plasma hematological parameters during the storage period. Values are expressed as mean standard deviation, and statistically significant at $p < 0.05$.

## DISCUSSION

The electrochemical balance between the intracellular and extracellular environment is controlled by the $Na^+/K^+$ ATPase enzyme system present in the cell membrane. For every $2K^+$ ions pumped out of the cells, $3Na^+$ ions are pumped back into the cell[29]. These pumps thus maintain a higher intracellular and extracellular concentration of $K^+$ and $Na^+$ respectively.

Few hours after blood for transfusion or storage is drawn into blood bag, hemolysis began resulting in the release of intracellular contents into the surrounding plasma, affecting the concentrations of different parameters[30]. Also, storing of blood under universal standard blood banking conditions causes some biochemical changes (qualitatively and quantitatively) in RBCs which are related to the length of storage time. These changes include loss of energy in the form of ATP, glutathione peroxidase antioxidant, membrane lipids, and membrane and structural proteins due to oxidative damage from oxidative stress encountered during storage.

Results from this study revealed that the activities of GPx and SOD enzymes are significantly decreased throughout the period of storage. This result corroborates similar findings earlier reported by Ogunro *et al.* (2010) [7] and Deyhim *et al.* (2014) [31] but disagree with that of Vani *et al.* (2015)[32] who reported increase in the activities of SOD and CAT in stored blood. This result showed that GPx appears to provide the primary antioxidant defence in stored RBCs. The observed decrease in GPx activities indicates increase in free radicals generation during storage of blood and increase oxidative modification of membrane lipid and proteins, thereby destabilizing RBC membrane skeleton and compromising RBCs' survival.

During blood storage there is a slow but constant leakage of potassium from red cells into surrounding plasma resulting in

increased plasma potassium. This fact was established in this study where increase in plasma potassium was observed. This finding corroborates the earlier work of Wallas (2010)[33], Vraets *et al.* (2011)[34], and Opoku-Okrah *et al.* (2015)[35]. Potassium loss is recognized to be secondary to the changes in metabolic activity and decreasing pH with cooling (Hess, 2006)[36]. Sodium on the contrary was observed to progressively reduce throughout the storage period. These findings corroborate the earlier reported increase and reduction in plasma potassium and sodium respectively in stored blood[35,37]. The mean sodium concentration over the period was observed to have dropped from an initial level of 138.9 mEq/L across the days to a level of 132.9mEq/L at the end of the study on day 35. This progressive reduction holds possible adverse clinical effects on recipients of such units.

Magnesium is involved in virtually every metabolic processes in the cell, including maintenance of energy balance, protein synthesis, and DNA stability[25]. A progressive reduction in plasma $Mg^{2+}$ was observed to occur as the storage days increases. The observed reduction in plasma $Mg^{2+}$ during blood storage will result in derangement of energy homeostasis thereby causing reduction in ATP generation.

Blood is rich in polyunsaturated fatty acids and esterified cholesterol and can easily undergo peroxidation to give hydroperoxides and malondaaldehyde (MDA)[38].

Malondaaldehyde is an indirect marker of lipid peroxidation. It modifies membrane proteins and lipid causing damage to erythrocyte membranes and subsequent hemolysis[38]. Result of plasma lipid parameters and index of lipid peroxidation during the storage period showed a significant reduction ($p < 0.05$) in plasma total cholesterol, HDLC, LDLC, VLDLC and triglycerides; and a significant increase ($p < 0.05$) in plasma MDA. This result is in agreement with that of Abdoljalal (2006)[39] who reported increase plasma MDA concentrations during storage. The observed increase in MDA is an indication of on-going oxidative damage to membrane phospholipids. There is resultant increased production of ROS and modification of membrane lipids which plays a major role in the process of membrane damage during blood storage.

Concerning plasma liver function test parameters, a reduction in plasma total protein; albumin, ALP and GGT; and increase in the concentrations of bilirubin (total and conjugated), AST and ALT were observed over the storage period in this study. This finding corroborates the earlier reported findings from similar studies[40]. Red blood cells contain higher concentrations of AST (20 fold as high as plasma) than ALT[40]. The observed change in the activities of AST is as a result of hemolysis that occurred during storage. The observed little changes in the activities of ALP, ALT, and GGT may be due to preservation of the enzymes by refrigeration and freezing. Also, a significant reduction ($p < 0.05$) in plasma urea and a non-significant ($p > 0.05$) change in plasma creatinine was observed over the storage period.

Results of hematological parameters revealed a decrease in the mean values of RBCs and white blood cells (WBC) as the storage days increases when compared to day 0. Similar reduction in RBC count was reported[39]. The decrease in WBC is most likely due to the sum effects of the loss of individual cell characteristics from degenerative changes that occur as the cell ages. Clinical implications collectively known as the RBC storage lesion, is in part related to bioreactive substances (such as histamine, lipids, and cytokines) released by leucocytes in the storage medium, which may exert direct effects on recipients[36].
Concerning haematological parameters an initial increase in mean cell volume (MCV) was observed in the first 21 days followed by reduction throughout the storage period. This observation may be due to high intracellular sodium content which affects cell volume and shape such that the MCV of stored red cells is

increased after 3 weeks of storage[41]. This effect is more pronounced in older RBCs, impairing their deformability and viability[42].

## CONCLUSION

The result obtained from this study suggests that storing blood in blood bank using CPDA-1 as anticoagulant is associated with variations in plasma electrolyte concentration, RBC antioxidant enzyme activities, and plasma biochemical and hematological parameters. These changes were noted to occur after the seventh day of storage. Thus, Blood for transfusion is best used on or before the seventh day of donation. Whenever transfusion with stale blood is done, the recipients of such blood should be given prophylactic antioxidant therapy to counter or prevent associated oxidative damage to macromolecules.

**Conflict of interest**

Authors declare that there is no competing interest in this research.

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**Contribution of authors**

AAA and OWE conceived the experiment and supervised laboratory work; OOD and OAA designed and performed laboratory work; ADFA drafted the manuscript for publication. All authors critically revised the article and gave final approval.

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