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ORIGINAL RESEARCH

Evaluation of Changes in Levels of P-Selectin, C-Reactive Protein and Hematological Indices in CPD - A Blood Stored for 35 Days in Port Harcourt before Transfusion

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Abstract

Background: Long-term storage of whole blood results in lesions, alterations in the biophysical, biochemical, and immunological characteristics of the cells. Each year, in Nigeria, about 1.5 million units of blood are used for blood transfusions as treatment intervention to a variety of medical conditions. This cross-sectional study evaluated changes in the levels of P-selectin, C-reactive protein and Hematological indices with respect to long-time storage of whole blood meant for transfusion in Port Harcourt.

Materials and methods: Blood Samples free of hepatitis B and C, syphilis and human immunodeficiency viruses 1 and 2 were randomly withdrawn from eligible 52 healthy donors aged 18-54 years who were at the Rivers State University Teaching Hospital for blood donation. From the 450 milliliters blood withdrawn into Citrate Phosphate Dextrose Adenine -1 (CPDA-1) containing blood bag, 30 ml was taken and stored at 2 °C - 8 °C for 35 days, 5 ml was used each day for Full blood count analysis with HM - 200x autoanalyzer on days 0, 7, 14, 21, 28, and 35. P-selectin and C-reactive Proteins were assayed using Enzyme Linked Immunosorbent Assay sandwich kit from Ela science. A well-structured questionnaire served for medication history, smoking and alcohol intake. Results were analyzed using Statistical Analysis Software version 9.4 and expressed as (mean ± SEM). Comparison of means was done with One-way analysis of variance (ANOVA), significance level set at $P < 0.05$ and results were represented in tables.

Results: During the 35 days of blood storage, the study observed a consistent statistically significant increase ($p < 0.0001$) in P-selectin, reduced C-reactive protein concentration though not statistically significant ($p > 0.05$), significant decrease ($p < 0.0001$) in White blood cells indices, significant increase ($p < 0.0001$) in red blood cell, hemoglobin, hematocrit and mean cell hemoglobin. Significantly decreased ($p < 0.0001$) in Mean cell volume and mean cell hemoglobin concentration and statistically significant reduction ($p < 0.0001$) in Platelets Indies.

Conclusion: This study concludes that Storage of whole blood collected in CPDA-1 for duration of 35 days caused significant upregulation in P-selectin concentration, while the hematological constituents of the blood also showed depleted viability.

Keywords

Blood transfusion, Citrate phosphate dextrose adenine-1, P-Selectin, C-reactive protein and Storage duration

Introduction

Background of study

Blood transfusions are necessary in cases of anemia, excessive bleeding during surgery, internal bleeding, cancer and cancer treatment, postpartum hemorrhage, sepsis, and severe trauma from accidents. It is also an important therapy used to replenish a patient's blood component or components when their amount is reduced [1]. Blood to be transfused, sometimes, is kept in the blood bank at 2-8 °C for up to 35 to 42 days in preservative such as citrate phosphate dextrose adenine (CPDA) -1 [2]. P-selectin and C-reactive proteins are substances that activate leucocytes and platelets to sites of inflammation in the case of injury or when there is a need for an immune response, so, when they

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are reduced or increased, the functioning of these cells could be impaired. However, there is a paucity of information on the assessment of P-selectin, C-reactive protein and hematological indices in ABO/Rh stored whole blood for transfusion.

Long-term red blood cell storage can result in lesions, or alterations in the biophysical, biochemical, and immunological characteristics of the cells [3]. Enzymatic and non-enzymatic chain-breaking antioxidant mechanisms found in red blood cells help protect the cell from oxidative damage and naturally counteract oxidative stress [4]. Long-term blood storage also generates a lot of free radicals, which antioxidants initially neutralize. The integrity of the red blood cell membrane is destroyed when the antioxidants' ability to prevent oxidative damage is surpassed by the increased production of free radicals [1].

The maintenance of blood quality in the form of keeping its initial value during storage before blood transfusion is a primary concern for blood bank, therefore understanding how storage duration influences the stability and viability of blood parameters is crucial for improving patient care.

Materials and Methods

Study design and population

The study was a cross-sectional study conducted at the Rivers State University Teaching Hospital (RSUTH), located in Port Harcourt, Rivers State, Nigeria. The blood samples were randomly collected from eligible 52 healthy volunteer donors between the ages of 18 and 54 years of both genders.

Ethical considerations and informed consent

Ethical approval for this study was obtained from the Ethical Committee of Rivers State University Teaching Hospital, Rivers State, Nigeria. The participants voluntarily gave written and verbal consent.

Eligibility criteria

Only participants who tested negative for HCV, HBsAg, Syphilis and HIV 1 and 2 respectively; had hemoglobin (Hb) value of 12 g/dl for females and 13 g/dl and above for males were included. Menstruating and pregnant female, donors unwilling to give consent and those who tested positive to either HCV, HBsAg, Syphilis or HIV 1 and 2 respectively were excluded.

Sample collection

A total of 450 milliliters of venous blood was aseptically drawn from each of the donors into each blood bag containing Citrate Phosphate Dextrose Adenine-1 (CPDA-1). Blood collecting materials were discarded safely to avoid injury from needles and lancets. The blood bags were placed on the quarantine shelf of the blood bank refrigerator maintained at 2-8 °C. From each of the blood bags, 30 ml of whole blood was collected into plain vials with screwed caps and stored at 2 °C - 8 °C for 35 days in the blood bank.

Determination of full blood count

The hematological parameters were measured using HM-200x auto-analyzer. The hematologic indices measured were hemoglobin concentration (HB), hematocrit (HCT), white blood cells (WBC), red blood cells (RBC), Platelets (PLT), lymphocytes (LYM), mixed cells (MID), granulocytes (GRAN), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red blood cell distribution width-coefficient of variance (RDW-CV), red blood cell distribution width-standard deviation (RDW-SD), mean platelet volume (MPV), platelet Rit (PCT), platelet distribution width (PDW). The results displayed on the LCD screen after automatic analyses were printed out.

Determination of P-selectin level using Ela science

ELISA kit as described by Albescence Biotech Co. Ltd, China

Samples were assayed for P-selectin using quantitative sandwich Ela science ELISA kits. The ELISA micro-plates were provided with the kits pre-coated with antibody specific to Human P-selectin and when the standard samples were added to the micro-ELISA plate wells in combination with the specific antibody, and a biotinylated detection antibody specific for Human P-selectin and Avidin-Horseradish Peroxidase (HRP) conjugate are added to each microplate well successively and incubated; then followed by the washing away of free components and the addition of the substrate solution that causes the Avidin-HRP conjugate to appear blue in color. The series of enzyme-substrate reaction taking place is stopped by the addition of stop solution with the product resulting in a yellow coloration. The optical density (OD) is then measured spectrophotometrically at a wavelength of 450 nm. The optical density (OD) value obtained from the reading is directly proportional to the concentration of Human P-selectin the sample.

Determination of C-reactive protein level using Ela science

ELISA kit as described by Albescence Biotech Co., Ltd, China

Samples were assayed using Ela science ELISA kits that utilize the sandwich-ELISA as method. The ELISA micro-plates were provided with the kits pre-coated with antibody specific to Human C-reactive protein molecule and when the standard samples were added to the micro-ELISA plate wells in combination with the specific antibody, and a biotinylated detection antibody specific for Human C-reactive protein molecule and Horseradish Peroxidase (HRP) conjugate are added to

each microplate well successively and incubated; then followed by the washing away of free components and the addition of the substrate solution that causes the Avidin-HRP conjugate to appear blue in color. The series of enzyme-substrate reaction taking place is stopped by the addition of stop solution with the product resulting in a yellow coloration. The optical density (OD) is then measured spectrophotometrically at a wavelength of 450 nm. The optical density (OD) value obtained from the reading is directly proportional to the concentration of Human C-reactive protein molecule in the sample.

Statistical analysis

The results are expressed as mean ± standard error of the meaning. The data were analyzed using Statistical Analysis Software version 9.4. Descriptive statistical tools such as mean and standard deviation (SD) were used. Analysis of variance ANOVA was used to compare means of more than two groups for inferential evaluation, with Turkey's multiple comparison test to check for mean difference between multiple groups. Student t-test was used for comparison of means and statistical significance set at $P < 0.05$.

Results

Comparison of p-selectin and c-reactive protein by duration of storage prior to transfusion

The mean ± SEM of C-reactive protein showed consistent decreasing trend from day 0 having a concentration of 54.71 ± 5.547 to day 28 having a concentration of 47.58 ± 5.594 but on day 35, an increase in concentration 51.15 ± 5.461 was observed yet these variations were not statistically significantly different ($P > 0.05$) at the various storage durations. The mean ± SEM of P-selectin protein showed a consistent increasing trend with day 0 having a concentration of 4160.65 ± 84.411 and day 35 was observed to be 6578.77 ± 100.523. The results statistically were significantly different ($p < 0.0001$) indicating that P-selections are increased for 35 days storage period. These observed results are shown in Table 1 below.

Comparison of hematological parameters of study participants blood by duration of storage prior to transfusion

Assessment of ABO/Rh stored whole blood for transfusion at Day 0, 7, 14, 21, 28 and 35 respectively followed a decreasing though inconsistent in the level white blood cells count (WBC) hence it decreased from 4.08 ± 0.278 × 10^9/L on day 0 to 3.00 ± 0.339 × 10^9/L on day 7 and 1.83 ± 0.104 × 10^9/L on day 14, but days 21 to 35 fluctuated between 0.83 ± 0.053 × 10^9/L and 0.92 ± 0.062 × 10^9/L. This variation in values of WBC level were statistically significantly different $P < 0.0001$ and indicates that white blood cells count was reduced during the 35 days storage of blood in citrate phosphate dextrose adenine (CPDA)-1. The rest of the WBC indices (lymphocytes, Granulocytes, and mixed cells also showed reduced variations in means ± SEM value at the various storage durations at baseline (day 0), Day 7, 14, 21, 28 and 35, and these variations were statistically significantly different ($P < 0.0001$). These results are shown in Table 2a below. The duration of storage results of hemoglobin value showed reducing variations at the different days during the period of storage. The mean ± SEM value was Day 0 (baseline) having (7.54 ± 0.523 g/dl), Day 7 had (7.92 ± 0.558 g/dl), Day 14 had (7.62 ± 0.530 g/dl), Day 21 had (7.46 ± 0.549 g/dl), Day 28 had (7.28 ± 0.532 g/dl). Day 35 had (7.03 ± 0.495 g/dl), these values started reducing only from Day 14, but these values were not statistically significantly different from each other ($P > 0.05$) as seen in the Table 2b.

Hematocrit though increased from the day 7 and never reduced to the baseline value, but it was observed to have fluctuating values on the different days during the blood storage period, 22.79 ± 1.570 (%) on Day 0, 24.18 ± 1.725 (%) on Day 7, 23.11 ± 1.603 (%) on Day 14, 24.71 ± 1.822 (%) Day 21, 26.77 ± 1.397 (%) on Day 28 and 23.46 ± 1.689 (%) on Day 35. The values were significantly different ($p = 0.0095$). The results indicate that hematocrit during the 35 days storage slightly increased. Mean Cell Volume (MCV)

Table 1: P-selectin and C-reactive protein in study participants' blood by duration of storage prior to transfusion.

Characteristic	N	C-Reactive Protein (Iu/mL)	P-Selectin (Iu/mL)
Duration of Storage			
0 (Baseline)	52	54.71 ± 5.547	4160.65 ± 84.411^{d}
7	52	52.28 ± 5.187	4416.39 ± 102.946^{cd}
14	52	49.64 ± 5.432	$4763.365 \pm 102.303^{bc}$
21	52	49.60 ± 5.559	4963.923 ± 199.84^{b}
28	52	47.58 ± 5.594	6725.31 ± 115.428^{a}
35	52	51.15 ± 5.461	6578.77 ± 100.523^{a}
F-Ratio		0.1988	80.483
P-value		0.9628	$< 0.0001^{****}$

Significance Level: *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001

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Table 2a: Hemoglobin before sample collection and hematological parameters of study participants by duration of stay prior to transfusion (Mean ± SEM).

Characteristic	N	Hb (g/dl)	WBC (10^9/L)	LYMT (10^9/L)	MID (10^9/L)	GRAN (10^9/L)	RBC (10^{12}/)	Hb (g/dL)	HCT (%)	MCV (fL)	MCH (pg)
Duration (Baseline)	52	13.84 ± 0.147	4.08 ± 0.278^{a}	2.68 ± 0.179^{a}	0.41 ± 0.064^{ab}	0.96 ± 0.068^{a}	2.39 ± 0.154	7.54 ± 0.523	22.79 ± 1.570^{a}	84.61 ± 0.667^{a}	28.87 ± 0.325^{abc}
7	52	13.84 ± 0.147	3.00 ± 0.339^{b}	2.43 ± 0.284^{ab}	0.27 ± 0.034^{bc}	0.31 ± 0.068^{b}	2.89 ± 0.210	7.92 ± 0.558	24.18 ± 1.725^{a}	84.99 ± 0.742^{a}	28.26 ± 0.291^{c}
14	52	13.84 ± 0.147	1.83 ± 0.104^{c}	1.74 ± 0.219^{bc}	0.46 ± 0.076^{a}	0.16 ± 0.068^{b}	2.75 ± 0.171	7.62 ± 0.530	23.11 ± 1.603^{a}	80.18 ± 0.756^{b}	28.76 ± 0.285^{bc}
21	52	13.84 ± 0.147	0.83 ± 0.053^{d}	1.27 ± 0.111^{cd}	0.21 ± 0.037^{c}	0.20 ± 0.068^{b}	2.50 ± 0.180	7.46 ± 0.549	24.71 ± 1.822^{a}	75.63 ± 0.669^{c}	29.70 ± 0.339^{ab}
28	52	13.84 ± 0.147	0.83 ± 0.053^{d}	097 ± 0.041^{d}	0.09 ± 0.004^{c}	0.06 ± 0.068^{b}	2.38 ± 0.168	7.28 ± 0.532	26.77 ± 1.397^{b}	77.53 ± 0.669^{bc}	30.07 ± 0.316^{a}
35	52	13.84 ± 0.147	0.92 ± 0.062^{d}	0.74 ± 0.054^{d}	0.10 ± 0.000^{c}	0.06 ± 0.068^{b}	2.42 ± 0.169	7.03 ± 0.495	23.46 ± 1.689^{a}	75.29 ± 0.669^{c}	29.44 ± 0.314^{abc}
F-Ratio		0.000	52.148	20.905	11.619	25.336	1.486	0.3223	3.103	38.406	4.609
P-value		1.000	< 0.0001****	< 0.0001****	< 0.0001****	< 0.0001****	0.1941	0.8994	0.0095**	< 0.0001****	0.0005***

Significance Level: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$

Table 2b: Hemoglobin before sample collection and hematological parameters of study participants by duration of stay prior to transfusion (Mean ± SEM).

Characteristic	N	MCHC (g/dL)	PLT (10^9/L)	MPV (fL)	PDW	PCT (mL/L)	PDW	PCT (mL/L)
Duration								
Baseline	52	33.83 ± 0.163^{a}	205.37 ± 11.583^{a}	8.31 ± 0.140^{a}	16.07 ± 0.307^{a}	3.71 ± 1.989	16.07 ± 0.307^{a}	3.71 ± 1.989
7	52	33.23 ± 0.190^{a}	159.58 ± 14.205^{b}	7.14 ± 0.115^{b}	43.63 ± 5.105^{b}	2.24 ± 5.095	43.63 ± 5.105^{b}	2.24 ± 5.095
14	52	31.06 ± 0.217^{b}	104.25 ± 8.12^{c}	4.72 ± 0.062^{c}	11.37 ± 0.096^{a}	0.75 ± 0.064	11.37 ± 0.096^{a}	0.75 ± 0.064
21	52	30.45 ± 0.21^{bc}	52.00 ± 2.854^{d}	2.72 ± 0.150^{d}	2.49 ± 0.066^{c}	0.16 ± 0.102	2.49 ± 0.066^{c}	0.16 ± 0.102
28	52	29.99 ± 0.130^{c}	37.462 ± 2.126^{d}	2.74 ± 0.168^{d}	2.72 ± 0.108^{c}	0.18 ± 0.098	2.72 ± 0.108^{c}	0.18 ± 0.098
35	52	30.18 ± 0.176^{c}	39.15 ± 2.088^{d}	3.02 ± 0.161^{d}	2.68 ± 0.099^{c}	0.15 ± 0.083	2.68 ± 0.099^{c}	0.15 ± 0.083
F-Ratio		81.008	70.429	312.145	58.314	148.7	58.314	148.7
P-value		< 0.0001****	< 0.0001****	< 0.0001****	< 0.0001****	< 0.0001****	< 0.0001****	< 0.0001****

Significance Level: $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$

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significantly decreased from 84.61 ± 0.667 fL on Day 0 to 75.29 ± 0.669 fL on Day 35 of storage, while Mean Cell Hemoglobin (MCH) demonstrated a slightly significant difference (p = 0.00005) by increasing trend from 28.87 ± 0.325 (pg.) to 30.44 ± 0.314 indicating that, mean cell hemoglobin during the 35 days storage slightly increase; Mean Cell Hemoglobin Concentration demonstrated a significant decreasing trend from 33.83 ± 0.163 to 30.48 ± 0.176 indicating that, mean cell hemoglobin concentration during the 35 days storage reduce; Platelets demonstrated a significant decreasing trend from 205.37 ± 11.583 to 39.15 ± 2.088 indicating that, platelets during the 35 days storage reduce; and Platelet indices (Mean Platelet Volume, Platelet Distribution Width and Platelet crit) all demonstrated significant decreasing trend from the baseline during storage. Loss of viability due to ATP depletion, improper storage, anticoagulants used and microbial contamination (Daniel, et al*.,* 2021). Red blood Cells demonstrated insignificant increase from the baseline value from 2.39 ± 0.154 to 2.42 ± 0.169; Hemoglobin showed insignificant decrease from the baseline values from 7.54 ± 0.523 to 7.03 ± 0.495, indicating that during the 35 days storage, hemoglobin reduced. This result can be seen in Table 2a.

Discussion

This study observed a consistent significant increase in P-selectin during the 35 days of blood storage for transfusion. Factors that contribute to P-selectin increase during storage are macrovesicles shed from packed red blood cells [5]. C-reactive protein (CRP) was observed to be statistically insignificantly reduced during the 35 days of blood storage and this could be because of CRP being markers of inflammation. C-reactive protein as acute phase reactants naturally increases in response to the presence of inflammatory cytokines produced by the white blood cells during inflammation, however, the result of this study showed decreased level of white blood cells as storage days increased, inferring there was no inflammation or infection in the subjects who donated the blood samples and hence no inflammation in the stored blood bags. The result of red blood cells, hemoglobin and Hematocrit revealed that fluctuating changes occurred compared to day 0 but they were not statistically significant changes. These demonstrate their stability during blood storage for transfusion for up to 35 days. This research is in tandem with the study conducted by [6] which demonstrated that changes in RBCs, and Hb were not statistically significant during storage at 35 days for transfusion, however, it conflicts the results of red blood cells carried out by [7] on blood storage in CPDA-1. The statistically significant decrease in granulocytes during storage could be because of complimenting the reduction WBCs, it could also be due to degenerative changes as the cells get older, the other contributors could be improper storage, microbial contamination, or effect of preservative used. These findings are in harmony with the study conducted by [8], which demonstrated significant decrease in granulocytes overtime. According to studies by [9] on the activities of granulocytes collected for transfusion, the observed reduced granulocytes could probably be due to timed variation resulting from gene expression which could probably occur from storage. It was suggested that the cells generally remain functional for a few hours and should be transfused in less than 6 hours after collection for avoidance of some abnormalities in function due to timed variation from gene expression and storage lesion [9]. Results of mean cell volume (MCV) were observed to be statistically significantly reduced from the day 21 during 35 days storage durations and this could be an indication that increased intracellular sodium has affected the cell volume and the shape which was also reported by [10] that MCV of stored red blood cells is decreased after 3 weeks of storage. These results also agree with [5], which demonstrated significantly low MCV at 35 days storage duration and its attributed cause to donor's age which could be a factor. The direct significance of this on the patients who receive the blood could be microcytic anemia after transfusion of such blood. There were fluctuating changes in the results of mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) from the 21-day compared to the first 14 days which had stabile results. The decline in the results MCH and MCHC could be attributed to responding to the little fluctuating increase in Hematocrit value which often causes a decrease in MCHC. Platelets, during storage have been reported to be highly active in the utilization of glucose and the cells would suffer death under depletion of ATP [11]. The significant decrease in platelet and its indices counts could be due to loss of blood viability resulting from high metabolic activity which causes ATP depletion. This research agrees with the study conducted by [9], which demonstrated that as the red blood cells age, platelets lose their ability to clot, and this increases the risk of microbial contamination. It is therefore important to ensure that blood used for transfusion is fresh.

Conclusion

Duration of storage of whole blood collected in CPDA-1 in this study, caused significant regulation in P-selectin concentration during 35 days of storage. Reduced changes in the concentration of C-reactive protein were not significant within the period of storage, a proof of proper screening against inflammatory triggers. Storage also significantly alters hematological indices with white blood cells and platelets showing a very high decrease. Duration of cold storage of blood collected in CPDA-1 and stored for up to 35 days impose unhealthy and depleted viability on the constituents of the blood during storage. These changes would evidently result

in the impairment of certain blood constituents in the patients when transfused with such blood. Medical workers having this knowledge would be empowered to reject the use of aged blood for transfusion therapy.

Author(s) Contributions

SUKE and MDG conceptualized the study; SUKE and EME designed the methodology: MDG, SUKE, and EME, performed data collection; MDG performed the data analysis; MDG, SUKE, and EME, drafted and reviewed the manuscript.

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Competing Interesting

The authors declare that they have no form of conflict of interest in this work.

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