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Research Article

Haematological and Biochemical Viability of Stored Whole Blood, Packed Red Cells and Fresh Frozen Plasma

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Abstract:

Aim: This study aimed at evaluating the haematological and biochemical viability of whole blood, packed red cells and fresh frozen plasma under storage. **Methods:** This study adopted the venipuncture blood collection method, sedimentation method for components preparation and the manual, automated and enzymatic method for analysis. Data obtained were statistically analyzed using ANOVA, descriptive analysis, frequency distribution, and mean standard deviations with a statistical significance set at $P < 0.05$. **Results:** The result showed (1.6, 3.7, and 4.5) × 10^9/L and (56.1, 86.0, and 135.9) × 10^9/L WBC and platelets reduction, in whole blood (WB), packed red cells (PRC) and fresh frozen plasma (FFP), respectively. There occurred a RBC and HCT reductions of (0.8 and 4.1) × 10^{12}/L and (4.6 and 39.5) % in whole blood (WB) and FFP, respectively. Also, a 6.8 g/dL increase in Hb. No significant difference was observed in MCH and MCHC in whole blood and PRC. An ANOVA analysis revealed a pH stability in the fresh frozen plasma, 9.4 mmol/L decrease in sodium and 7.9 mEq/L increase in potassium in PRC.

Keywords: Packed red cells (PRC), fresh frozen plasma (FFP), Viability, blood products.

Introduction

To ensure the effectiveness of transfusion, specific guidelines have been established and adherence to these guidelines is crucial before accepting blood and blood products for transfusion [1]. Blood banks play a pivotal role in ensuring the availability of viable blood and its components to address medical emergencies requiring transfusions [2]. This may involve the processing, preservation and selection of these products for transfusion when indicated. Questions arise regarding the availability of required blood constituents, their integrity, storage conditions, and the implications of using alternative blood products [3,4]. This underscores the need to evaluate the viability of blood products before transfusion, with a focus on pre-analytical variables such as storage duration and temperature.

Physical and biochemical alterations also take place during blood storage. Red blood cells (RBCs) experience changes in shape due to oxidative damage to their membranes. Anaerobic metabolism increases, leading to lactate accumulation, pH reduction, and decreased 2,3-diphosphoglycerate (2,3-DPG) levels, resulting in increased potassium levels [5]. These factors decrease the efficacy of transfused blood products and may lead to transfusion-related complications. Those changes could be best be determined by considering those vital constituents that the blood is made of, which changes can significantly alter its viability [6]. A viable blood is expected to have the normal haemoglobin, with an active oxygen-binding capacity of 1.34 mL O_2 per gram, functioning as an antioxidant and a regulator of iron metabolism [7]. Although most stored blood have red cells that are less deformable and tend to breakage as their storage age increases [8], however, lack of focused studies on the effect of the loss of viability in blood components under storage other than whole blood is a signaling gap in the blood transfusion practice [9,10].

To enhance best practices in blood preparation, storage and issuance for transfusion, blood banks must closely monitor changes that occur in blood intended for transfusion with keen regards to their temperature and storage period. These changes in hematological and biochemical parameters is observed in the behavior of white blood cells, (WBC), red blood cells (RBC), haemoglobin (HGB), haematocrit (HCT), platelets (PLT), lymphocytes (LYM) and neutrophils (NEUT) and mixed cells (MIXED), as well as sodium, potassium, pH, etc under storage. So the evaluation of the blood viability should involve the observation of the changes that occurs in these parameters, which is achieved through their laboratory assessment.

Although challenges exist in managing component inventories efficiently [13], the viability of blood and its components depends significantly on the protocols for collection, preparation, and their indications for transfusion [10,11]. The aim of this study was to evaluate the Haematological and biochemical viability of whole blood, packed red cells and fresh frozen plasma under storage in Rivers State University Teaching Hospital blood bank, Port Harcourt.

Methods

Study Variables

This study employed an experimental study design and conducted in the Rivers State University Teaching Hospital Blood Bank in Port Harcourt. A 32 subjects who were not frequent blood donors were recruited for this study from the Port Harcourt blood donors' population, using the multi-stage probability sampling, as described by the World Health Organization venipuncture method for sample collection [12].

Ethical Approval

Ethical approval for of this research was obtained from the Rivers State Research and Ethics Committee, Port Harcourt.

Procedure

On the selection of a prominent vein on the donors subcubital area using a pressure cuff and disinfection with 70% alcohol, a venipuncture was performed with a secured needle held in place with adhesive tape. When the blood began to flow, the pressure in the cuff was reduced to 40–60 mmHg. Once 450mls of blood had been collected, the pressure in the cuff was reduced to zero, the donation stopped and the needle removed. The unit was labeled with the donor's bag code/number, blood group, and screening details [12]. The component separation was done on the collected units by sedimentation in a cold centrifuge [10]. The PRC was first separated using a light spin, expressed off the platelet rich plasma (PRP) and stored at 2-6^{0}C. The PRP was then subjected to a heavy spin and the FFP separated and expressed of the platelet concentrate (PLC) and stored at -60^{o}C. Aliquots of the prepared samples were collected and analysed by automation using the Symex KX-1N Haematology analyzer [13] and Automatic Ion selective electrode (ISE) [14].

Statistical Analysis

With a statistical significance level set at $P < 0.05$, the data obtained was statistically analyzed using ANOVA, univariate and multivariate analysis, descriptive analysis, frequency distribution, and mean standard deviations.

Results

Table 1 displays the demography of the 32 blood donors recruited for recruited for this study, out of which 16 (50%) were males, and 16 (50%) were females. Among the subjects, 16(50%) subjects, were in the 18 – 28 years age bracket. 10 (31.25%) subjects, were in the 29 – 38 years age bracket, 4 (12.5%) subjects, were in the 39 – 48 years age bracket and 2 (6.25%) subject were in the 49 – 58 years age bracket.

Table 1. Percentage Frequency Distribution of the Demographic Details of the Study Population

Parameters	18-28 years	29-38 years	39-48years	49-58years	Total
Numberof Subjects	16(50%)	10(31.25%)	2(6.25%)	4(12.5%)	32 (100%)
Gender Males	12(37.5%)	2(6.25%)	2(6.25%)	2(6.25%)	18(56.25%)
Females	4(12.5%)	8(25%)	0(0.0%)	2(6.25%)	14(43.75%)

Table 2 illustrates the variation in hematological parameters of whole blood, PRC and FFP, showing a WBC reduction of (1.6, 3.7, and 4.5) × 109/L in whole blood (WB), packed red cells (PRC), and fresh frozen plasma (FFP), respectively, from the baseline of 4.93 × 109/L ± 0.33 showed a slight stability of white blood cells in whole blood compared to PRC and FFP. Also, a platelet deviation of (56.1, 86.0, and 135.9) × 109/L in WB, PRC, and FFP, respectively, from the baseline of 227.38 ± 32.17. A reduction of (0.8 and 4.1) × 1012/L in RBC from the baseline of 4.72 × 1012/L ± 0.37,

(1.8 and 12.9) g/dL in Hb from the baseline of 13.913 g/dL ± 0.827, and (4.6 and 39.5) % in HCT from the baseline (within the normal range) was observed in whole blood (WB) and FFP, respectively. In packed red cells (PRC), there was a sharp increase of 3.2 × 1012/L in RBC from the baseline of 4.722 ± 0.3743, 6.8 g/dL in Hb from the baseline of 13.91 ± 0.83, and 17% in HCT from the baseline of 41.63%± 2.67.

Table 2. Variation in Haematological Parameters Whole Blood, PRC and FFP

Parameters Component Types	WBC ($\times 10^9$/L) MEAN±SD	PLT ($\times 10^9$/L) MEAN±SD	RBC (10^{12}/L) MEAN±SD	HB (g/dl) MEAN±SD	HCT (%) MEAN±SD	MCHC (g/dL) MEAN±SD
Baseline	4.93±0.32	227.38±32.17	4.72±0.37	13.91±0.82	41.63±2.66	38.10±8.83
Whole Blood	3.30±1.27	171.27±59.80	3.93±1.43	12.09±2.10	37.02±5.96	33.33±3.88^{ns}
Packed Red Cells	1.24±1.27^{s}	141.38±51.28^{s}	7.94±1.42	20.75±2.453	58.77±4.15	33.61±1.79^{ns}
Fresh Frozen Plasma	0.45±0.39^{s}	91.52±62.76^{s}	0.65±0.40^{s}	1.03±0.58^{s}	2.15±1.52^{s}	35.53±4.92^{s}
f-value	104.28	25.72	429.38	1274.44	1951.78	7.89
df	159					
P-value	0.00^{s}	0.00^{s}	0.00^{s}	0.00^{s}	0.00^{s}	0.00^{s}

^{s}= Significant at P < 0.05, across the sample types compared with the baselines;
^{ns}= Not significant at P < 0.05, from the baselines (post hoc) in specific sample types

Table 3 shows the variation in Red Cells Indices among different blood components – (WB, PRC & FFP) of the study subjects. No significance was observed in MCH and MCHC in whole blood and PRC. The 86.2 ± 11.5 fL MCV and up to 27.7 ± 3.9 pg MCH of WB and PRC indicate a normocytic red cell shape. However, in FFP, there is a significant decrease of up to 27% in MCV and 22% in MCH values from their baselines (P =0.000).

Table 4 displays the variation in biochemical parameters of different blood samples – whole blood (WB), packed red cells (PRC), and fresh frozen plasma (FFP) of the study subjects. An ANOVA analysis revealed a the pH remained stable between the baseline and fresh frozen plasma, but there was an increase of 9.4 mmol/L in sodium. Between whole blood and the baseline, there was a moderate decrease of 0.3 mmol/L in pH and 3.4 mmol/L in sodium, which then decreased progressively by 0.5 mmol/L in pH and 5.9 mmol/L in sodium in the PRC from the baseline values of 7.42±0.04 and 142.75±4.59, respectively. Furthermore, there was a sharp increase of 7.9 mEq/L in potassium in whole blood, which progressed significantly to 16.1 mEq/L in PRC, and then moderately increased to 9.5 mEq/L in FFP from a baseline of 3.71 mEq/L

Table 3. Variation in Red Cells Indices in Whole Blood, PRC and FFP

Parameters	MCV (fl)	MCH (pg)	MCHC (g/dL)
Component Types	MEAN±SD	MEAN±SD	MEAN±SD
Baseline	94.125±8.288	29.488±2.462	38.100±8.830
Whole Blood	86.234±11.246^{ns}	27.666±3.879 ^{ns}	33.333±3.882 ^{ns}
Packed Red Cells	86.000±11.576 ^{ns}	27.088±4.479 ^{ns}	33.613±1.795 ^{ns}
Fresh Frozen Plasma	68.167±28.354 ^{s}	22.796±8.767 ^{s}	35.533±4.922 ^{s}
f-value	180.468	99.339	7.887
df	159		
P-value	0.000^{s}	0.000^{s}	0.001^{s}

^{s}= Significant at P < 0.05, across the sample types compared with the baselines;
^{ns}= Not significant at P < 0.05, from the baselines (post hoc) in specific sample types

Table 4. Variation in Biochemical Parameters Based on Sample Types

Parameters	Na (mmol/L)	K (mEq/L)	pH
Component Types	MEAN±SD	MEAN±SD	MEAN±SD
Baseline	142.75±4.59	3.71±0.36	7.424±0.04
Whole Blood	139.34±9.59	11.58±11.36^{a}	7.162±0.55^{a}
Packed Red Cells	136.81±4.26	19.76±13.05^{a}	6.909±0.47^{a}
Fresh Frozen Plasma	152.17±6.02^{a}	13.16±6.76^{a}	7.46±0.06^{a}
f-value	62.79	8.43	18.93
df	159		
P-value	0.00^{s}	0.00^{s}	0.00^{s}

Δ^{s}= Significant at P < 0.05, across the sample types compared with the baselines;
Δ^{ns}= Not Significant at P < 0.05, across the sample types compared with the baselines
Δ^{a}= strongly significant at P < 0.05 (Post hoc) in specific sample type

Discussion

Since transfusion of viable blood and blood products serve as active measures to restore the expected functionality of blood, this study, the Evaluation of the viability of whole blood, packed red cells and fresh frozen plasma under storage conditions in Rivers State University Teaching Hospital Port Harcourt, blood bank assessed the haematological and biochemical viability of those parameters that ensure this functionality. In the demographic view point, the equal number of males and females subjects observed in this study is as a result of the study design which prescribed a recruitment selection that ensures a sex and blood group stratified donors representing the males and females in equal proportion.

In regards to the observed changes in the various component types, the sharp depletion in white blood cells and platelets in FFP could be attributed to their poor survival rate at such a frozen temperature of -60°C. The high haematocrit levels in the packed red cells other than the whole blood and FFP, may be due to its high concentration of RBCs, resulting in higher hematocrit levels compared to whole blood or other components. The significant reduction in RBC, Hb, and HCT in FFP may be due to the removal of about 95% of the red cells from the FFP during component preparation. Ideally, a good practice of component preparation requires that a clean and clear FFP be void of traces of red cells.

Generally, the non-significant change shown in the post hoc analysis of FFP, whole blood, and packed red cells could be due to the absence or negligible amount of red cells in the FFP, as opposed to its presence in the whole blood and packed red cells. A common factor underlying the change in viability of the studied blood components at 4°C, could be linked to the difference in their storage temperatures.

The normal MCV values in WB and PRC could be a function of the donor selection criteria that favoured normal donors with normal hemoglobin levels. This is because frequent blood donors whose blood could result to iron deficiency and microcytic cells couldn't passed the acceptance criteria to be included in this study.

The statistically significant biochemical change among the various blood components (WB and PRC and FFP) could be due to a prolonged contact of plasma with RBCs, which has been found to cause an exchange of contents between the plasma and the RBCs. This as well could cause a dilution and change in electrolyte concentrations, hence the alteration in biochemical viability. This change in biochemical parameters could have caused the cells to undergo a red cells lesion, resulting in such observed significant change.

These findings corresponds with that of Lagerberg *et al.* (2017) [15], who showed that red cell storage lesions occur mostly in red cells and other blood products in a progressive manner. The findings also agree with (García-Roa *et al.,* (2017) [8] who proved that, biochemical viability of blood for transfusion varies among the different blood components. They further emphasized that this changes is intact in FFP at -60°C, moderate in whole blood at 4±2°C, but declines considerably in PRC at 4±2°C with prolonged storage. On a clinical note, when such older blood with its alteration in viability is transfused, its effectiveness is weakened by its increasing rate of removal from circulation by macrophages [16].

Limitations

The straneous process of components preparation was a challenging task.

Recommendation for further studies

Evaluation of haemostatic parameters should be included in study of blood viability. Also, the component under study should be extended to other blood components as platelet concentrate, platelet rich plasma and cryoprecipitate.

Conclusion and implications for translation

White blood counts and platelet counts are highly unstable PRC and FFP (p=0.000). RBC, HB, HCT and the red cells indices are stable and viable in PRC and whole blood (p=0.052). Biochemical changes occurred in whole blood, PRC and FFP (p=0.000).

Summarily, indeed other researchers have actually reported their findings on haematological and biochemical viability of blood used for transfusion, however, documentation of specific findings of these changes in regards to their respective blood components is wanting. Hence, this study, which has established and affirmed the variation in the changes in haematological and biochemical status of blood cells with regards to the respective blood components.

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