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An Assessment of the Viability of Haematological and Haemostatic Parameters of Blood under certain Storage Conditions at the Rivers State University Teaching Hospital Blood Bank in Port Harcourt, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Despite significant advances in transfusion medicine, concerns about the inherent risks of blood transfusion persist, even under optimal temperature and duration of storage. This makes the evaluation of blood viability a global task (1). Maintenance of adequate temperature is considered a key factor in the viability and quality of stored blood in healthcare institutions. Evaluating the haematological and haemostatic functionality of blood elements at different storage

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temperature and duration is therefore imperative for improving patient care and resource utilization in Rivers State University Teaching Hospital (RSUTH) blood bank in Port Harcourt.

Methods: In this cohort study design, a total of sixteen (16) male and female blood donors in equal proportion of sex and ABO blood groups were randomly selected from the Port Harcourt blood donors' population and recruited as study subjects for this research. A well-structured questionnaire and the immunoassays were used to assess the donors' health and serological status respectively. Also the sample obtained were analayzed by automation and data statistically analyzed using ANOVA.

Results: The results of this study shows a statistically significant decrease in white blood cell count from 4.93×10^9/L ± 0.33 to 2.79×10^9/L ± 1.68 (p=0.00) and platelet count from 227.38×10^9/L ± 32.17 to 153.75×109/L ± 58.39 (p=0.00) at day 7. Also, a significant decrease in platelet count from 227.38×109/L ± 32.17 to 141.50±60.92 at day 14. A significant decrease in Fibrinogen from 340.75mg/L±18.69 to 281.2575 mg/L ±46.41 at day 1 and day 14 respectively, and rise in PT and aPTT from 17.02s ±1.28 to 24.31s ±6.67 and 41.25s±3.23 to 46.63s±6.28 at day 14th to day 21 respectively (p=0.00).

Conclusion and Implications for Translation: Pooled plasma at 4-6^{0}C contain clinically significant amount of coagulation factors up to day 21 in storage. The WBC and platelet is lost within seven day of storage at 4-6^{0}C. Lower temperatures, especially freezing at -60°C accelerate the loss of haematological viability of blood especially the depletion of white blood cells and platelets (p=0.00). Antihaemophilic factor and fibrinogen is maintained in FFP at 180 day in storage at -60^{0}C.

Keywords: Viability; frozen temperature; oxidative stress.

1. INTRODUCTION

1.1 Background of the Study

Blood transfusion plays a critical role in modern medicine, ensuring the availability of safe and viable blood products for patients in need. The maintenance of blood quality during storage is a primary concern for blood banks and healthcare institutions. Understanding how different storage durations and temperatures impact the viability of blood parameters is essential for improving patient care and optimizing the use of blood resources [1].

To ensure the effectiveness of transfusion, specific guidelines especially as regards the condition of storage have been established. Adherence to these guidelines is crucial for safe blood and blood components for transfusion [2]. To enhance best practices in blood preparation and release, blood banks must closely monitor changes that occur in blood intended for transfusion at its various storage durations and temperatures.

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 [3]. Anaerobic metabolism increases, leading to lactate accumulation, these changes mostly occurs due to change in temperature and aging of the stored blood, hence decrease the efficacy of transfused blood which may lead to transfusion-related complications, including acute lung injuries, longer hospital stays, and higher mortality rates [4]. Moreover, haemolysis, which a reflection of red blood cell integrity, could increase significantly in samples stored at room temperature, particularly after Day 7, with a decrease in platelet count [5] Prothrombin Time (PT), which is often use to detect deficiencies in certain coagulation factors [6] could be prolong under the wrong storage condition and duration [7].

Determining viability is best achieved by considering the crucial constituents of blood, and any changes can significantly impact its viability [8].

The goal of appropriate storage methods is to preserve the biological function of blood constituents, slow down their metabolic activities, and minimize bacterial growth in the blood components. This approach aligns with the objectives of transfusion medicine in a hospital setting, which aims to guarantee that 'the right blood is given to the right patient at the right time and in the right place (…)

1.2 Objectives of the Study

This study experimented the change in haematological and haemostatic parameters of

blood at different storage durations and temperatures.

1.3 Specific Aims and Hypothesis

The aim of this study was to access the viability of haematological and haemostatic parameters of blood under different storage temperature in Rivers State University Teaching Hospital blood bank, Port Harcourt.

2. METHODS

2.1 Study Variables

A cohort design was employed in this research. A random recruitment technique based on multi-stage probability sampling, as described by [8] was used. Following donors' consents and their responses to the study's questionnaire, a pre-donation serological screening of the study subjects was conducted for human immunodeficiency virus (HIV), hepatitis B surface antigen (HbSAg), hepatitis C and Syphilis by the enzymatic method, using ELISA. The samples obtained were analyzed by automation [9].

2.2 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.

3. RESULTS

3.1 Effect of Change in Temperature (25ºC, 4±2ºC and -60ºC) on Some Haematological Parameters (WBC, PLC, RBC, HB, HCT and fHB) of the Study Subjects

Table 1 shows the effect of Change in Temperature (25ºC, 4±2ºC and -60ºC) on Haematological Parameters (WBC, PLC, RBC, HB, HCT and fHB) of the Study Subjects. With a change in storage temperatures from 25ºC to 4±2ºC, there occurred a sharp decrease of 2.9 x 10^9/L and 80.2x 10^9/L in WBC and platelet respectively (p=0.000). At 4±2ºC the red cells, haemoglobin and haematocrit dropped significantly to 0.653±0.40, 1.030±0.576, and 2.146±1.516 respectively at -60ºC frozen temperature (p=0.000).

3.2 Effect of change in temperature (25ºC, 4±2ºC and -60ºC) on the red cells indices (MCV, MCH, MCHC) of the study subjects

Table 2 shows the effect of Change in Temperature (25ºC, 4±2ºC and -60ºC) on the Red Cells Indices (MCV, MCH, MCHC) of The Study Subjects. At -60ºC, a very significant drop of 70.1% and 53% in MCV and MCH was observed (p=0.000).

3.3 Effect of Changes in Storage Duration on Haematological Parameters

Table 3 presents the effect of changes in storage duration on hematological parameters. The results show a statistically significant decrease in white blood cell count from 4.93×10^9/L ± 0.33 to 2.79×10^9/L ± 1.68 (p=0.00) and platelet count from 227.38×109/L ± 32.17 to 153.75×109/L ± 58.39 (p=0.00) at day 7. Also, a significant decrease in platelet count from 227.38×109/L ± 32.17 to 141.50±60.92 at day 14.

3.4 Effect of Change in Temperature on Hemostatic Parameters

Table 4 presents the effect of temperature changes on hemostatic parameters. An examination of temperature variations (25°C, 4±2°C, and -60°C) on hemostatic parameters revealed an elongation of Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT), but a decrease in fibrinogen (FIB) as the temperature changed from 25°C to 4±2°C. However, clotting factor VIII (FVIII) deviated from the baseline with a 0.4% increase but remained viable and significantly stabilized at -60°C (p = 0.909).

3.5 Effect of Changes in Storage Duration on Haemostatic Parameters

There was a significant decrease in Fibrinogen from 340.75mg/L±18.69 to 281.2575 mg/L±46.41 at day 1 and day 14 respectively and a rise in in PT and aPTT from 17.02s ±1.28 to 24.31s ±6.67 and 41.25s±3.23 to 46.63s±6.28 from day 14th to day 21 respectively (p=0.00).

4. DISCUSSION

Since transfusion of viable blood and blood products serve as active measures to restore the expected functionality of blood, Comparative Assessment of the Haematological Viability of Blood Under Different Storage Temperature

in Rivers State teaching Hospital in Port Harcourt, assessed the haematological viability of those elements that ensure this functionality.

In the demographic view point, the equal number of males and females subjects is as a result of the study design which prescribed a recruitment selection based on the inclusion and exclusions criteria that gave a sex and blood group stratified donor representing males and females, in equal proportion of their blood groups.

In the hematological assessment of blood viability at the various temperatures of 25°C, 4-6°C and -60°C, the significant decrease in white blood cells and platelets indicates a loss of viability in stored blood in CPDA-1 within 24 hours. These findings are in agreement with [9], who reported an accelerated decrease in WBC over time. This could be a process facilitated by room temperature storage. Nevertheless, the non-statistically significant changes in red blood cell count, hemoglobin, hematocrit, and red cell indices demonstrate their stability during storage and their viability for transfusion for up to 35 days. This is also aligned with research on red cell viability conducted by [10]. According to their research, the HCT, Hb, and RBCs remained relatively unchanged compared to the baseline. Although granulocytes decreased over time, this could be a process accelerated by room temperature storage, improper storage, anticoagulants used and microbial contamination [11].

Table 1. Effect of change in temperature on haematological parameters (WBC, PLT, RBC, HB, HCT and fHB)

Parameters	WBC ($\times 10^9$/L)	PLT ($\times 10^9$/L)	RBC (1012/L)	HB (gm/dl)	HCT (%)	fHb (mg/dl)
Temperature	**MEAN±SD**	**MEAN±SD**	**MEAN±SD**	**MEAN±SD**	**MEAN±SD**	**MEAN±SD**
RMT (25°C)	4.87±0.36	226.06±31.20	4.73±0.35	13.81±0.81	41.56±2.68	0.94±0.32
4±2°C	2.01±1.38 [b]	147.19±53.66 [b]	5.79±2.62	16.14±5.16	47.14±12.77	0.97±0.25
-60°C	0.45±0.39 [a]	91.52±62.76 [a]	0.65±0.40 [a]	1.03±0.58 [a]	2.15±1.52[a]	0.97±0.15[a]
f-value	100.41	39.12	101.55	227.34	329.88	0.15
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, compared with the baselines at room temperature (RMT);
[ns]= Not significant at P < 0.05, compared with the baselines at room temperature (RMT)
[a]= strongly significant at P < 0.05 (Post hoc); Δ[b]= moderately significant at P < 0.05 (Post hoc)

Table 2. Effect of change in temperature (25°C, 4±2°C and -60°C) on the red cells indices (MCV, MCH, MCHC) of the study subjects

Parameters	MCV (fl)	MCH (pg)	MCHC (g/dL)
Temperature	MEAN±SD	MEAN±SD	MEAN±SD
RMT (25°C)	90.06±10.59	28.29±3.71	35.86±6.58
4±2°C	70.49±22.63	27.03±4.46 [ns]	33.79±2.41
-60°C	26.92±15.82 [a]	13.13±8.27[a]	42.06±21.11[a]
f-value	97.98	99.03	7.79
df	159		
P-value	0.00[S]	0.00[S]	0.00[S]

[S]= Significant at P < 0.05, compared with the baselines at room temperature (RMT);
[ns]= Not significant at P < 0.05, compared with the baselines at room temperature (RMT)
[a]= strongly significant at P < 0.05 (Post hoc);
[b]= moderately significant at P < 0.05 (Post hoc)

Table 3. Effect of changes in storage duration on haematological parameters

TIMING	WBC ($\times 10^9$/L)	PLC ($\times 10^9$/L)	RBC (10^{12}/L)	HB (g/dl)	HCT (%)	fHb (mg/dl)	MCV (fl)	MCH (pg)	MCHC (g/dL)
Storage Duration	**MEAN±SD**	**MEAN±SD**	**MEAN±SD**	**MEAN±SD**	**MEAN±SD**	**MEAN±SD**	**MEAN±SD**	**MEAN±SD**	**MEAN±SD**
0HR	4.93±0.33	227.38±32.17	4.72±0.37	13.91±0.83	41.63±2.67	0.90±0.37	94.13±8.29	29.49±2.46	38.10±8.83
1HR	4.81±0.42	224.75±32.40	4.74±0.36	13.70±0.84	41.50±2.88	0.98±0.27	86.00±11.58	27.09±4.48	33.61±1.79
24HR	2.79±1.68	216.33±31.26	4.08±2.52	11.19±7.59	33.13±22.53	0.96±0.17	68.17±28.35	22.79±8.77	35.53±4.92
Day 7	2.04±1.18[b]	153.75±58.39	4.18±2.69	11.13±7.56	32.58±22.51	1.03±0.25	66.17±28.73	23.09±8.61	37.15±5.81
Day 14	1.60±1.09[a]	141.50±60.92[b]	4.44±3.06	11.05±7.61	32.96±22.80	1.02±0.26	61.88±29.78	21.85±8.35	37.29±8.56
Day 21	1.09±1.11[a]	92.92±23.12[a]	4.32±3.34	11.15±8.37	32.50±24.86	0.89±0.23	51.92±25.99	23.27±8.04	38.38±14.43
Day 28	0.74±0.86[a]	86.46±26.19[a]	4.30±3.42	11.29±9.46	31.58±25.44	0.99±0.16	47.71±23.45	22.87±8.04	41.06±11.97
Day 35	0.67±0.77[a]	80.83±22.49[a]	3.17±4.29	10.81±9.79	30.08±26.24	0.92±0.22	39.96±29.51	20.51±11.62	29.86±21.77
f-value	28.86	42.29	0.48	0.24	0.39	1.11	6.48	1.25	1.71
df	159								
P-value	0.00[s]	0.00[s]	0.86[ns]	0.98[ns]	0.91[ns]	0.36[ns]	0.00[s]	0.28[n]	0.11[n]

[s]= Significant at P < 0.05, compared with the baselines at 0Hr;
[ns]= Not significant at P < 0.05, compared with the baselines at 0Hr
[a]= strongly significant at P < 0.05 (Post hoc);
[b]= moderately significant at P < 0.05 (Post hoc)

Table 4. Effect of change in temperature on hemostatic parameters

Parameters	PT (s)	INR	APTT (s)	FIB (mg/dl)	FVII (%)
Temperature	**MEAN±SD**	**MEAN±SD**	**MEAN±SD**	**MEAN±SD**	**MEAN±SD**
RMT (25^{o}C)	14.49±1.05	1.16±0.11	39.84±2.99	341.38±18.19	0.94±0.06
4±2^{o}C	22.96±6.95	3.57±6.88	47.06±7.43^{a}	207.73±117.01^{b}	1.32±7.60
-60^{o}C	15.33±0.76^{b}	1.24±0.08^{b}	39.95±2.88^{n}	341.15±18.14^{ns}	0.89±0.05
f-value	40.16	3.72	26.54	40.65	0.09
df	159				
P-value	0.00^{s}	0.03^{s}	0.00^{s}	0.00^{s}	0.91^{ns}

^{s}= Significant at P < 0.05, across all temperatures across the sample types baselines at room temperature (RMT)
^{ns}= Not significant at P < 0.05, across all temperatures across the sample types baselines at room temperature (RMT)
^{ns}= Not significant at P < 0.05, specific temperature (post hoc);
^{a}= strongly significant at P < 0.05 (Post hoc);
^{b}= moderately significant at P < 0.05 (Post hoc)

Table 5. Effect of changes in storage duration on haemostatic parameters

Parameters	PT (s)	INR	APTT (s)	FIB (mg/dl)	FVII (%)
Duration	MEAN±SD	MEAN±SD	MEAN±SD	MEAN±SD	MEAN±SD
0HR	14.50±1.07	1.15 ±0.12	39.51±3.26	342.00±18.94	0.94±0.06
1HR	14.49±1.10	1.16±0.10	40.16±2.89	340.75±18.69	0.95±0.06
24HR	14.69± 0.76	1.17±0.09	40.40±2.75	340.79±18.27	0.91±0.05
Day 7	15.99±0.88	1.29±0.08	40.73±3.16	328.54±26.69	0.77±0.19
Day 14	17.01±1.28	1.37±0.09	41.25±3.23	281.25±46.41	0.76±0.17
Day 21	24.31±6.67^{a}	4.25±7.95^{a}	46.63±6.28	227.96±84.13^{b}	0.62±0.22
Day 28	25.25±7.17^{a}	4.34±7.92^{a}	49.42±8.54^{a}	221.04±88.55^{a}	0.53±0.28
Day 35	25.25±7.17^{a}	4.34±7.92^{a}	49.71±8.59^{a}	113.63±164.49^{a}	0.48±0.29
Day 180	0.00	0.00	0.00	338.63±35.51	0.86±0.05
f-value	22.03	1.85	11.47	19.99	0.81
df	159				
P-value	0.00^{s}	0.01^{s}	0.00^{s}	0.00^{s}	0.58^{n}

^{s}= Significant at P < 0.05, compared with the baselines at 0Hr;
^{ns}= Not significant at P < 0.05, compared with the baselines at 0Hr
^{a}= strongly significant at P < 0.05 (Post hoc) at specific storage duration.
^{b}= moderately significant at P < 0.05 (Post hoc) at specific storage duration.

The significant low MCV observed in the study subjects before the expiration of the storage duration is an indication of microcytic anaemia due to iron deficiency. This could be traceable to the high percentage (50%) of adolescence and youth within (18-28 years) age bracket in the study population. Reasons could be that, teenagers are prone to choosing foods and beverages high in substances like calcium or tannins, which can inhibit iron absorption. Also, rapid cognitive development could be the cause, since iron is crucial for cognitive development, and teenagers' brains are still maturing during this period, which places an additional demand on their iron stores. This agrees with (12), that donors in this age bracket tend to have high demand for iron, coupled with their active lifestyle and meddling of physical exercise. This could be to the fact that, adolescence is a period of rapid growth and development, which increases the demand for iron to support the production of new blood cells and muscle tissues. Consequently, that could cause the blood from them to undergo up-regulation of pro-inflammatory cytokines, as also agreed with [11].

Furthermore, blood units especially from donors within 18 -23 years age bracket may not be viable enough for transfusion to pregnant women and children with vitamin C deficiency, since patients in that category are prone to iron deficiency hypochromic microcytic anaemia due to disruption in their iron supply. That also, agrees with [12]. That vitamin C deficiency impairs the absorption of iron from plant-based sources, potentially leading to anemia.

A drastic decrease in platelet count RBC, HB, HCT, also shows a reduction in the haematological viability of blood under different storage conditions. This agrees with [13], that in the transfusion of 28 days old red cells, there occurred a decrease in microvascular flow in oxygen levels, compared to the transfusion of freshly donated blood. That led to the conclusion of [13], that a patient transfused with 4 units of blood approaching outdate may have received one full unit of dead cells, that must definitely affect the normal functioning of the reticuloendothelial system. This could be because as blood components age, the red blood cells can break down and may lose their ability to carry oxygen, platelets can lose their ability to clot, and the risk of bacterial contamination may increase. Thus the blood units could become less effective and potentially unsafe over time. Therefore, it's important to ensure that blood used for transfusions is fresh and meets safety standards. It is therefore worth noting that, though stored blood may be stable in its red cells viability at older age, such blood may not be too good for certain clinical conditions that may be of high oxygen demand. A freshly donated or less older blood is most preferably viable in such cases. The principle of first in-first out in blood banking may therefore be discouraged in such scenario as much older blood might be the target of this principle.

Regarding the evaluation of plasma hemoglobin for the rate of hemolysis, the significant increase in plasma hemoglobin depicts a mark of hemolysis and a loss of viability, which is associated with age, weight, and storage temperature. This increase in hemolysis may be due to enhanced oxidative stress in older blood. This finding aligns with research by [14] on changes in plasma hemoglobin in CPDA-1 stored blood, which showed a major increase in the proportion of hemolysis and plasma hemoglobin due to storage temperature. This could also result from leukocyte breakdown and the release of various chemicals and enzymes, such as hydrogen peroxide and proteases, causing red blood cell lysis during storage.

Although this study observed a significant effect of temperature on the viability of white blood cells, platelets, red blood cells, hemoglobin, and hematocrit, as well as their red cell indices, it disagrees with the findings of [15]. Their findings on the use of frozen and deglycerolized red blood cells concluded that red blood cells could be frozen for 10 years with no significant change in their hemoglobin or hematocrit. The reason for this contradiction could be because the red cells in their study were treated with glycerol prior to freezing, which protects RBCs during freezing and thawing. Since glycerolized red cells can still exhibit the same efficacy and viability as freshly collected units, even after 10 years, it is recommended that teaching hospitals in sub-Saharan Africa adopt this for efficient transfusion services [15].

The sharp depletion in white blood cells and platelets could be attributed to their poor survival rate at such a frozen temperature of -60°C. The normal MCV values in WB and PRC could be a function of the donor selection criteria that favor normal donors with normal hemoglobin levels, as frequent donation could result in iron deficiency and microcytic cells [16].

In agreement with [17], the highest percentage of study subjects falling within the 29-38 years age bracket could be attributed to the readiness and spirited enthusiasm of individuals in this age group to donate blood, especially among students. This age bracket has consistently shown a high degree of prosocial motivation, often referred to as "altruism," which has made it a desirable target group for blood centers, encouraging their ongoing recruitment efforts. Furthermore, the willingness of the youth in this age group to donate blood is often driven by a sense of responsibility and self-determination, as it is not influenced by parental or peer pressure.

5. CONCLUSION AND IMPLICATIONS FOR TRANSLATION

Lower temperatures, especially freezing at -60° led to significant alterations in haematological parameters with white blood counts and platelet counts showing a very high decrease ($p=0.000$). The weight of the donor of a unit of blood does not affect the general haematological parameters of such blood units if stored at the ideal temperature ($p>0.115$). However, extremely higher weigh could contribute to the haemolysis of such units at 4-6^{o}C ($p=0.000$).

6. LIMITATIONS

This study was greatly challenged with finacial constraints.

7. RECOMMENDATION FOR FURTHER STUDIES

The study of blood viability under different storage conditions should be extended to the biochemical parameters and the various blood components.

CONSENT

As per international standards or university standards, patient(s) written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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