Comparative Assessment of the Haematological Viability of Blood Under DifferentStorage Temperature in RSUTH Blood Bank In Port Harcourt, Nigeria.

ABSTRACT

INTRODUCTION: Maintenance of adequate temperature is considered a key factor in the viability and quality of stored blood in healthcare institutions. Evaluating the haematological viability of blood at different storage temperature is therefore imperative for improving patient care and resource utilization in Rivers State University Teaching Hospital (RSUTH) blood bank in Port Harcourt.

METHODS: In an experimental study design, a total of sixteen (16) male and female donors in equal proportion of sex and ABO blood groups, who were of 18 to 59 years age bracket were randomly selected from the Port Harcourt blood donors' population and recruited as study subjects for this research. A wellstructured questionnaire was used to assess the donors' health, including a physical assessment of vital signs, pulse rates, blood pressure, and body weights. Their serological status, including hepatitis B (HBsAg), hepatitis C (HCV), Syphilis (VDRL), and human immunodeficiency virus (HIV), were also assessed using immunoassays.Samples collected were processed, stored at various temperatures (25^0C, 4-6^0C an and -60^0C and analysed by automation at the Rivers State University Teaching Hospital Port Harcourt blood bank for the haematological parameters. Data obtained were statistically analyzed using ANOVA.

RESULTS: Highlighting the impact of temperature on stored blood haematological parameters, the result of this study showed a significant decrease of 2.9 x 10^9/L and 80.2 x 10^9/L in WBC and platelets, respectively (p=0.00). This decrease progressed by 4.5 x 10^9/L and 135.9 x 10^9/L from their respective baselines of 2.01±1.4 x 10^9/L and 147.2±53.7 x 10^9/L, as the temperatures shifted from 25°C to 4±2°C and

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significantly dropped 0.45±0.39 at -60°C. In contrast, at 4±2°C, the levels of red blood cells, hemoglobin, and hematocrit increased slightly, but dropped significantly to 0.65±0.40 x 10^12~~9~~/L, 1.03±0.58 g~~x 10^9~~/dL, and 2.146±1.52%~~x 10^9/L~~, respectively, at -60°C.

CONCLUSION AND IMPLICATIONS FOR TRANSLATION: Lower temperatures, especially freezing at -60°C, enhanced the loss of stored blood viability especially the depletion of white blood cells and platelets (p=0.00).

KEY WORDS: Viability, frozen temperature, Oxidative stress,

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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 temperatures impact the viability of blood types is essential for improving patient care and optimizing the use of blood resources(1).

Allogeneic blood transfusion is a common medical therapy used to treat various disorders, including anemia, blood synthesis disorders, drug-related cytotoxicity, and cases of massive blood loss due to accidents, childbirth, or medical procedures (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).

Questions arise regarding the availability of required blood constituents and their integrity, storage conditions, and the implications of using alternative blood products (3). This underscores the need to evaluate the viability of blood products before transfusion, with a focus on pre-analytical variables such as storage time and temperature (4).This factor can impact the measurement of critical hematological, biochemical, and microbiological parameters during different storage periods (4).

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 temperatures. These changes involve hematological indices, including hemoglobin levels, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), as well as white blood cell and platelet counts (5).

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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 (6). These changes mostly occurs due to change in temperature and hence decrease the efficacy of transfused blood products which may lead to transfusion-related complications, including acute lung injuries, longer hospital stays, and higher mortality rates (7).

1.2 Objectives of the Study

This study experimented to assessthe change in haematological parameters of stored bloodat different storage temperatures.

1.3 Specific Aims and Hypothesis

The aim of this study was to determine~~access~~ the Haematological Viability of Blood Under Different Storage Temperature in Rivers State University Teaching Hospital blood bank, Port Harcourt.

2. METHODS

2.1 Study Variables

An experimental study design was employed in this research, involving both freshly donated blood and stored blood at different temperature ranges. 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

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immunodeficiency virus (HIV), hepatitis B surface antigen (HbSAg), hepatitis C and Syphilis by the enzymatic method, using ELISA. The samples obtained were analyzed by automated method using the Sysmex KX-1N Haematology analyzer.

2.2 Statistical Analysis

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

Ethical Approval

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

3. RESULTS

3.1 *Percentage Frequency Distribution of the Demographic Details* of the Study Population~~.~~

Table 1 displays the composition of the study population, which consisted of a total of 16 blood donors recruited for the study. Out of these 16, 8 (50%) were males, and 8 (50%) were females.

Among the subjects, 8(50%) subjects, were in the 18 – 28 years ~~age~~ bracket. 5 (31.25%) subjects, were in the 29 – 38 years ~~age bracket,~~ 2 (12.5%) subjects, were in the 39 – 48 years ~~age bracket~~ and 1 (6.25%) subject, was in the 49 – 58 years agegroups(Table 1)~~bracket~~.

Comment [AA1]: The method section is not properly elaborated. The authors have to deliver sufficient information on how the study participants were selected, how the experiment was conducted, what is the outcome variables and the factors (independent variables), when was the study conducted, what type of statistical tool (SPSS, STATA or other) was used.

Comment [AA2]: Put the reference of the letter

Comment [AA3]: It will be better if you put Tables at the end of each section in bracket.

Comment [AA4]: What does it mean?

Comment [AA5]: It needs rewriting of the description of table 1.

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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
Number of Subjects	84(25%),	54(25%),	14(25%),	24(25%)	16 (100%)
Gender					
Males	6(37.5%)	1(6.25%)	1(6.25%)	1(6.25%)	9(56.25%)
Females	2(12.5%)	4(25%)	0(0.0%)	1(6.25%)	7(43.75%)

Comment [AA6]: The total frequencies and the percentage distribution of the age group classification is incorrect.

3.2 Effect of Change in Temperature (25^{o}C , 4±2^{o}C and -60^{o}C) on Some Haematological Parameters (WBC, PLC, RBC, HB, HCT and fHB) of the Study Subjects.

Table 2 shows the effect of Change in Temperature (25^{o}C , 4±2^{o}C and -60^{o}C) on Haematological Parameters (WBC, PLC, RBC, HB, HCT and fHB) of the Study Subjects.

With a change in storage temperatures from 25^{o}C to 4±2^{o}C, there occurred a sharp decrease of 2.9 x 10^{9}/L and 80.2x 10^{9}/L in WBC and platelet respectively. Then significantly reduced by 4.5 x 10^{9}/L and 135.9 x 10^{9}/L (p=0.000). On the contrast, at 4±2^{o}C the red cells, haemoglobin and haematocrit slightly increased instead but dropped significantly to 0.653±0.40, 1.030±0.576, and 2.146±1.516 respectively at -60^{o}C frozen temperature (p=0.000). Although there is less impact of temperature changes between 25^{o}C and 4±2^{o}C on the plasma haemoglobin, a significant effect occurred at frozen temperature (p=0.000)

Comment [AA7]: It must be 2.86 when you find the mean difference

Comment [AA8]: It must be 78.87 instead of 135.9

Comment [AA9]: These values are not seen in table2. Where did you get them?

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Table 2. 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 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]

Δ^s= Significant at P < 0.05, compared with the baselines at room temperature (RMT);

Δ^n= Not significant at P < 0.05, compared with the baselines at room temperature (RMT)

Δ^a= strongly significant at P < 0.05 (Post hoc); Δ^b= moderately significant at P < 0.05 (Post hoc)

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3.3 Effect of Change in Temperature (25^{o}C, 4±2^{o}C and -60^{o}C) on the Red Cells Indices (MCV, MCH, MCHC) of the Study Subjects.

Table 3 shows the effect of Change in Temperature (25^{o}C, 4±2^{o}C and -60^{o}C) on The Red Cells Indices (MCV, MCH, MCHC) of The Study Subjects. Although like other haematological parameters, the red cells indices were stable at room temperature with only 22%, 3.6% and 5.6% drops at 4±2^{o}C in MCV, MCH and MCHC respectively. However, at -60^{o}C, a very significant drop of 70.1% and 53% in MCV and MCH was observed (p=0.000), contrary to MCHC in which 17.3 % increase was observed at the same frozen temperature (-60^{o}C).

Table 3. Effect of Change in Temperature (25^{o}C , 4±2^{o}C and -60^{o}C) on The Red Cells Indices (MCV, MCH, MCHC) of the Study Subjects.

Parameters / Temperature	MCV (fl) MEAN±SD	MCH (pg) MEAN±SD	MCHC (g/dL) MEAN±SD
RMT (25^{o}C)	90.06±10.59	28.29±3.71	35.86±6.58
4±2^{o}C	70.49±22.63	27.03±4.46 ^{ns}	33.79±2.41
-60^{o}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}

Δ^{s}= Significant at P < 0.05, compared with the baselines at room temperature (RMT);

Δ^{n}= Not significant at P < 0.05, compared with the baselines at room temperature (RMT)

Δ^{a}= strongly significant at P < 0.05 (Post hoc); Δ^{b}= moderately significant at P < 0.05 (Post hoc)

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3.4 Relationship Between Weights of The Study Subjects And Changes in Their Haematological Parameters.

Table 4 Relationship Between Weights of The Study Subjects And Changes in Their Haematological Parameters (WBC, PLC, RBC, HB, HCT and fHB). With a P-Value of P > 0.05, there is no observed statistical significance in the white blood cells count, platelets counts, red blood cells count, haemoglobin and Haematocrit of the study subjects with respect to weight. But there is an observed statistical significance in the Plasma haemoglobin with a P-Value of P = 0.000

Comment [AA10]: Unnecessary subsection, not mentioned in the objective of the study. Therefore, you have better to remove it or include the in your objectives.

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Table 4. Relationship Between Weights of The Study Subjects And Changes in Their Haematological Parameter.

Parameters	**WBC ($\times 10^9$/L)**	**PLC ($\times 10^9$/L)**	**RBC (1012/L)**	**HB (g/dl)**	**HCT (%)**	**fHb (mg/dl)**
Weights (Kg)	**MEAN**±SD	**MEAN**±SD	**MEAN**±SD	**MEAN**±SD	**MEAN**±SD	**MEAN**±SD
<70kg	1.750±1.792	117.250±43.774	3.353±0.749	11.955±8.137	34.450±23.511	0.768±0.224
70 - 79kg	1.881±1.696	141.663±66.477	3.072±0.344	11.158±7.651	32.675±22.303	0.994±0.184
80 - 89kg	1.729±1.522	138.88±71.591	3.076±0.401	11.421±8.374	33.000±23.422	0.991±0.249
90kg	4.800±.1.322	267.000±.1.042	4.400±.1.122	14.000±.1.370	43.000±.1.079	1.550±.
f-value	1.201	2.007	0.116	0.092	0.095	8.807
df	159					
P-value	0.311^{ns}	0.115^{ns}	0.950^{ns}	0.965^{ns}	0.963^{ns}	0.000^{s}

Δ^s= Significant at P < 0.05, compared with the baselines at room temperature (RMT);

Δ^{ns}= Not significant at P < 0.05, compared with the baselines at room temperature (RMT)

Δ^a= strongly significant at P < 0.05 (Post hoc); Δ^b= moderately significant at P < 0.05 (Post hoc)

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4.1 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 agrees with research on red cell viability conducted by (9). 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.

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 (10), 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. 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.

Comment [AA11]: You used single reference for this much information. So, better to add more references supporting the stated information.

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

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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.

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.

Concerning the effect the study subjects' demography on haematological, the non-effect of sex on the studied parameters disagrees with the findings of (16), which observed that storage hemolysis was higher in males' RBCs compared to that of females. This difference may be attributed to the significance of sex hormones in the study subjects and improvements in the storage process. The findings of this study also disagree with similar study conducted by (17), on the evaluation of 18 donors BMI, which revealed a positive association of donor's weight with the storage and osmotic hemolysis of such blood units from them. They argued that BMI was a significant modifier for all hemolysis measurements, concluding that obese donors' units were associated with significant changes in RBC metabolism and increased susceptibility to hemolysis under routine storage of RBC units. This disagreement could be traced to the non inclusion of obese donors in this current study, as no obese passed the donation eligibility test during the random recruitment exercise.

The observation of the non effect of age on the tested parameters in this study agrees with the findings of (18), that sex hormones have shown no correlation with the oxygen affinity of hemoglobin. On the contrary, it disagrees with a retrospective cohort study by Roubinian *et al.,*(2019), which showed that prolong hospital stay was lower for the recipients of RBC

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units from young donors (<20 years old). This observed difference could be due to male hemoglobin having a higher affinity for oxygen than female hemoglobin, hence more efficient oxygen distribution, resulting in quick recovery of patients receiving such pints from the younger age. This reason agrees with (19), that wide-ranging variation in hemoglobin-oxygen affinity among humans has been attributed to sex.

Furthermore, the observed difference in platelet count between the male and female subjects agrees with a gender-based study on the differences in platelet function by (20), which affirmed that women have a larger platelet count than men. This may be due to a trend towards higher platelet reactivity in females than males. Also, women typically have higher estrogen levels than men, especially during their reproductive years, which can lead to increased platelet production. Women also tend to have lower iron stores due to menstrual blood loss, which may result in the body compensating by producing more platelets. Some autoimmune disorders, which are more common in women, may also contribute to higher platelet counts in this population. Female donors' units could therefore be considered most viable for patients with thrombocytopenia, hematologic malignancy, bone marrow failure, or other platelet function disorders. More so, female donors therefore could stand a better chance of being chosen for platelet concentrate component preparation for transfusion.

In agreement with (21), 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.

Comment [AA12]: The discussion part is poorly stated and correlated with the references. So, for the better understanding of the readers, the discussion needs to be discussed in way below forwarded.
1.First describe your finding
2.Compare your findings with previous research findings
3.Put your justification if differences have been observed between your research and the previous similar research.

4.2 Limitations

This study was greatly challenged with finacial constraints.

Small sample size can also be the limitation of your study

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4.3 Recommendation for further studies

The study of blood viability under different storage temperatures should be extended to the haemostatic and biochemical parameters and the various blood components as well as its various duration of storage.

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).

Competing Interest???

Comment [AA13]: What about RMT for Red cells?
Your conclusion must comprehensive, It could indicate at what different temperature for WBC, Platelet and RBCs viability is good and bad.

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