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Evaluation of Chemokine Profile in Stored Whole Blood

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Abstract
Bizarre immunological changes have been known to occur when whole blood is stored. This study investigated the changes that could occur in chemokine profile in whole blood stored in CPDA-1 anticoagulant/preservative for 28 days. This was an observational longitudinal study. The study was carried out at Nigerian National Petroleum Corporation Medical services, Akpajo, Rivers State. The effect of storage on these parameters were assessed and compared with a control. A total of 50 samples divided into 5 sets (1, 7, 14, 21, 28 days) of 10 samples each, gotten from 10 apparently healthy voluntary donors with day 1 samples used as control. The blood donors were between the ages of 24 to 57 years. Chemokine (IL-6, IL-8, IL-10 and TNF-α) were analyzed with reagents from Elabscience, Nuhan, China, using ELISA machine. The mean chemokine values for Day 1 and Day 28 were respectively IL-6: 14.61 ± 8.88pg/ml and 1064 ± 232.8pg/ml IL-8: 46.96 ± 8.32pg/ml and 769 ± 196.3pg/ml IL-10: 18.45 ± 14.56pg/ml and 1101 ± 249.4pg/ml and TNFα: 30.38± 26.52 pg/ml and 1096 ± 281.4 pg/ml. There was a significant increase in the mean values of chemokines at P-values <0.0001.Increase in the concentration of chemokines (IL-6, IL-8, IL-10 and TNFα) could probably be due to WBC degradation, which could induce pro-inflammatory response upon blood transfusion. It is therefore necessary to put into consideration the need for whole blood to undergo leukodepletion before storage and if it must be stored beyond one week. .
Keywords: *Chemokine profile, stored whole blood.*

Introduction

Blood is a fluid in the body that consists of cells which are suspended in plasma. The blood cells includes: red blood cells (RBC), white blood cells (WBC), and platelets. The liquid portion of the blood cell is the plasma consisting of hormones, water, gases proteins, glucose and nutrients. Blood has many functions which are grouped into transportation, regulatory and immunological functions [1].

Cytokines are collection of minute glycoproteins which are assembled by many cells, such as leukocytes which are regulatory cells for hematopoiesis, inflammation, and immunity [2]. Examples of cytokines include: chemokines, interferons, tumor necrosis factors, lymphokines and interleukins. Chemokines can be reported as chemotactic cytokines which are controlled by the locating and drifting immune pattern of cells [3]. Chemokines are arranged into the following

classes: C, CC, CXC, and CX_3C. In recent time, chemokines have been found to play an important role in priming of naïve, and cell fate decisions like effector memory cell differentiation and T cells regulatory function [4].

Stored whole blood encounter sequence of inflammatory, physiological, biochemical, structural, changes [5]. The advantages of stored whole blood for longer period of time is the reduction of the tendency of transmitting transfusion-associated graft-versus-host disease (GVHD) and syphilis while the disadvantages of storing whole blood for a longer period of time makes the red blood cell less viable, multiplication of bacterial cells growth, increase potassium concentration, and the release of toxins in the lipids [6].

Leukocytes in stored blood upon their exposure to the acidic conditions of storage and refrigeration become activated and release cytokines, leading to their delivery at high concentrations, therefore producing an inflammatory microenvironment transfusion during transfusion of stored whole blood. It remains to be determined whether these breakdown products have an effect on the monocyte and macrophage structure. Monocytes and macrophages hold plasticity of the differentiation potential, switching between proinflammatory and anti-inflammatory states depending on the microenvironment [7].

Blood donation is useful in reducing the risk of heart and liver illnesses triggered by the iron overload in the body. Consumption of iron-rich diet may raise the iron levels in the body, and meanwhile only limited proportions can be absorbed, extra iron gets stored in heart, liver, and pancreas. Hence, increases the risk of cirrhosis, liver failure, damage to the pancreas, and heart abnormalities like irregular heartbeats. Blood donation helps in sustaining the iron levels and lessens the risk of several health illnesses. After donating blood, the body works to replace the blood loss. This fuels the production of new blood cells and, also helps in maintaining good health of the donors [8].

Transfusion of blood can help in saving a patient's life as well as limiting the complications of severe blood loss as severe bleeding can lead to very low haemoglobin level thereby causing harm to major organs of the body due to a deficiency of oxygen. If bleeding persist the body's supply of platelets and plasma will as well reduced. Hence, blood transfusion aids in treating and preventing excessive blood loss [9], prompting the question are there any significant differences in the hematological indices of fresh and stored whole blood (at 7th, 14th, 21st and 28th days)? The aim of this study was to determine the chemokine profiles of stored whole blood.

Materials and Methods
Study Design
This was an observational longitudinal study.
Study Area
The study was carried out at Nigerian National Petroleum Corporation (NNPC) Medical Services, Akpajo, Port Harcourt, Rivers State with a coordinates of 4.8129° N, 7.0900° E. Port Harcourt, capital of Rivers state, southern Nigeria. It lies along the Bonny River (an eastern distributary of the Niger River) 41 miles (66 km) upstream from the Gulf of Guinea. Founded in 1912 in an area traditionally inhabited by the Ijaw and Ikwere people, it began to serve as a port (named for Lewis Harcourt, then colonial secretary) after the opening of the rail link to the Enugu coalfields in 1916. Now one of the nation's largest ports, its modern deep water (23 feet) facilities handle the export of palm oil, palm kernels, and timber from the surrounding area, coal from Anambra state, tin and columbite from the Jos Plateau, peanuts (groundnuts) from the northern states, and, since 1958, petroleum from fields in the eastern Niger River delta. Port Harcourt has bulk storage facilities for both palm oil and petroleum. In the 1970s the port was enlarged with new facilities at nearby Onne[10].
Study Population
Ten (10) voluntary blood donors were recruited and 10 units of whole blood was drawn from them

at NNPC medical center Port Harcourt, Rivers State of Nigeria.

Inclusion criteria

The voluntary blood donors were apparently healthy individuals, within the ages of 24 to 57 years, both male and female were tested to be sero-negative for Human Immunodeficiency Virus 1 and 2, Hepatitis B virus, Hepatitis C Virus, and *Treponema palladium*. Also, the donors met all the other requirements for blood donation.

Exclusion criteria

Sick volunteers or those who did not meet the requirements for participation were excluded from this study.

Ethical Consideration and Consent

Ethical approval was obtained from the Department of Medical Laboratory Science. Permission was obtained from the NNPC hospital management and informed consent of the participants was sought for verbally

Laboratory Analysis

Sample Collection: About 450 milliliters of whole blood were aseptically collected from each donor into a CPDA-1 blood bag and stored in a blood bank refrigerator maintained at 2-8°C. The chemokine analysis was carried out on the first day of collection, and subsequently on the 7th, 14th, 21st and 28th day of blood collection [11].

Determination of Interleukin 6, 8, 10 and Tumor Necrotic Factor alpha (TNFα)

These were done using Sandwich Enzyme Linked Immunosorbent Assay (ELISA) kit (Elabscience Biotechnology Inc. Principle is based on antigen-antibody reaction of a pre-coated antibody and the unknown antigen.

Statistical Analysis

The data generated was coded, entered and analyzed using the graph pad prism, Microsoft window 7 and Microsoft excel, all result obtained were presented in a tabular form. Normality of data was tested by the Kolmogorov–Smirnov test. The descriptive data was presented as means ± standard deviation (SD). The difference in mean of the parameters for fresh whole blood and each of the samples collected at 7th, 14th, 21st and 28th day of blood collection was determined with ANOVA.

Results

Demographic Information of Subjects

A total of ten (10) donors were recruited for this study and a total number of fifty (50) samples were analyzed.

Table 4.1 Demographic Information

Parameters	Values
Total Number of Donors	10
Age Range (Years)	24-57
Days of Analysis	5 Days (Day 1, 7, 14, 21, 28)
Total Number of Samples	50
Total Number of Controls	10 (Day 1)

Comparison of Chemokine Profile of Fresh and Stored Whole Blood at 7, 14, 21, and 28 days

Comparison of chemokine profile from freshly collected whole blood was made with stored whole blood that have been preserved for 7, 14, 21, and 28 days, and represented in Table 4.2a. The difference in mean and standard deviation for each parameter from day 1 to day 28 was done using Analysis of Variance and Turkey's Multiple Comparison Test (Table 4.2b).

Table 4.2a Comparison of Mean ± Standard Deviation of Chemokine Profile at Day 1, 7, 14, 21 and Day 28

	IL6 (pg/ml)	IL8 (pg/ml)	IL10(pg/ml)	TNFα(pg/ml)
Day 1	14.61 ± 8.88	46.96 ± 8.32	18.45 ± 14.56	30.38± 26.52
Day 7	108.8 ± 44.37	115.3 ± 28.95	133.4 ± 39.2	150.1± 41.74
Day 14	377.9 ± 152	242.2 ± 43.87	393.5 ± 132.3	379.1 ± 86.57
Day 21	705.5 ±182.1	527.5 ± 154.6	826.8 ± 266.3	776.4 ± 161.8
Day 28	1064 ± 232.8	769 ± 196.3	1101 ± 249.4	1096 ± 281.4
p-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001
F-value	85.63	69.91	67.28	117.5

Table 4.2b Turkey's Multiple Comparison Test

IL6 (pg/ml)	IL6 (pg/ml)	IL8 (pg/ml)	IL10(pg/ml)	TNFα(pg/ml)
Day 1 vs Day 7	Ns	Ns	Ns	Ns
Day 1 vs Day 14	***	**	***	***
Day 1 vs Day 21	***	***	***	***
Day 1 vs Day 28	***	***	***	***
Day 7 vs Day 14	**	Ns	*	**
Day 7 vs Day 21	***	***	***	***
Day 7 vs Day 28	***	***	***	***
Day 14 vs Day 21	***	***	***	***
Day 14 vs Day 28	***	***	***	***
Day 21 vs Day 28	***	***	**	***

Key: *= Significant at < 0.05; **= Significant at < 0.01; ***= Significant at < 0.001; Ns = Not significant at > 0.05

Discussion

When whole blood is stored, there will definitely be storage lesions which are changes that occur in blood components over time. According to [12], there are depletion of intra erythrocytic energy sources which results ultimately to a reduced structural integrity of red blood cell membrane, and thus they become less deformable or loss deformability and also more fragile as they continue to age.

The storage lesions that take place is not restricted to only red cells; it also affects chemokines, which are critical in determining the effective outcome of transfusion of whole blood to patients in demand for blood. Several studies as cited in literatures have found that transfusion of older blood are often associated with adverse clinical outcomes that may end up contributing to some complications in the recipient,[11]. In this study, the chemokine profile of stored whole blood collected from apparently healthy donors into

CPDA-1 blood bag which were stored in a blood bank at 2-8^{0}C was analyzed. Samples were collected on the 1st, 7th, 14th, 21st and 28th days of storage.

This current study undertaken on stored blood in a standard blood bank investigated the changes in some chemokines (interleukines), and compared with values from a freshly collected blood sample, in order to ascertain any change. In this study some of the parameters were increased as compared to their initial values on the first day of collection while others decreased.

Chemokines are not part of a routine investigation like other haematological parameters; therefore normal range of chemokines has not been established in this region. Test kits manufacturers tend to give the expected values as per their samples tested after the manufacture. Due to paucity of information on the chemokines in stored blood, comparing the levels of chemokines with that of other researchers is not thorough. This

study has also now established normal ranges of IL6, IL8, IL10 and TNF-α in whole blood of healthy donors. This first step was necessary to compare chemokine levels in the stored blood, so as to study the time-dependent storage effect. All the chemokines - IL6, IL8, IL10 and TNFα, had very significant variations in mean from day 1 to day 28, they all progressively increased in concentration upon longer duration of storage. This is in accordance with the study conducted by[13], in which they noted steady increase in IL8 concentration during prolonged storage. Also a study carried out on the comparative levels of cytokines showed an increase in IL-8, TNF-α.

TNF-α, IL-6 (pyrogen and acute phase reactant) they are often secreted by immune cells in response to pathogens and they act as signaling molecules. The transfusion of older red blood cells have the tendency to induce pro-inflamatory cytokines which have been observed in mice, but well tolerated in humans according to [14]. So increase in IL-6, IL-8, IL-10 and TNF-α progressively from day 1 to day 28 with statistically significant values goes a long way in telling us the role white blood cells plays in causing the increase, as white blood cells releases bio reactive substances such as histamine, lipids, and cytokines, which may exert direct effects on recipients.

The findings in this study is somehow in agreement with the study carried out by [15] however, in cats, where they noticed an increase in IL-6 and IL-8 after 4weeks of storage, but their levels were below the lower limit of detection of the assay used. When leukocytes were reduced in blood before storage, [16], observed a reduced level of IL-8. It can therefore be recommended that as much as possible, white blood cells should be reduced in whole blood before storage so as to avoid an increase in cytokines which could trigger febrile non-haemolytic transfusion reaction.

Conclusion

The demand for blood is high as a result of several emergencies like gunshot injuries, surgeries, accidents and other conditions that results to loss of blood. The study have confirmed other findings by previous researchers that there are storage lesion in stored blood which cannot be compared with the beneficial clinical outcome when fresh blood is transfused, hence the campaign should be the transfusion of fresh blood that have not been stored, or if it must be stored, the days should be shortened as much as possible.

Specifically, this study has revealed that when whole blood is stored in CPDA-1 for 28days, there is increase in the concentration of chemokines (IL-6, IL-8, IL-10 and TNF-α).

The increase in cytokines as observed in this study could induce pro-inflammatory response as the blood ages, even though humans can well tolerate it, but individuals who are immuno-compromised may not. It is therefore necessary to put into consideration the need for whole blood to undergo leukodepletion before storage if it must be used beyond one week.

References

1. Hall, J. E. (2015). Guyton and Hall textbook of medical physiology e-Book. Elsevier Health Sciences.
2. Khan, M. M. (2008). Immunopharmacology. New York: Springer Science and Business Media.
3. Jackson, D. G. (2014). Lymphatic regulation of cellular trafficking. Journal of Clinical & Cellular Immunology, 5(1), 221 - 228.
4. Griffith, J. W., Sokol, C.L., Andrew, D. & Luster, A.D. (2014). Chemokines and chemokine receptors: Positioning cells for host defense and immunity. Annual Review of Immunology. 32, 659–702.
5. Triulzi, D. J. &Yazer, M. H. (2010). Clinical studies of the effect of blood storage on patient outcomes. Transfusion and Apheresis Science, 43(1), 95-106.
6. Zimrin, A. B. & Hess, J. R. (2009). Current issues relating to the transfusion of

stored red blood cells. VoxSanguinis, 96(2), 93-103.

7. Jiwaji, Z., Nunn, K. P., Conway-Morris, A., Simpson, A. J., Wyncoll, D. & Rossi, A. G. (2014). Leukoreduced blood transfusion does not increase circulating soluble markers of inflammation: A randomized controlled trial. Transfusion, 54(10), 2404-2411.

8. World Health Organization. (2010). Screening donated blood for transfusion-transmissible infections recommendations. World Health Organization.

9. Klein, H. G., &Anstee, D. J. (2014). Mollison's blood transfusion in clinical medicine. John Wiley & Sons.

10. Agwu, M. O. &Emeti, C. I. (2014). Issues, challenges and prospects of small and medium scale enterprises (SMEs) in Port-Harcourt City, Nigeria. European Journal of Sustainable Development, 3(1), 101

11. Adias, T. C., Moore-Igwe, B. & Jeremiah, Z. A. (2012). Storage related haematological and biochemical changes of CPDA-1 whole blood in a resource limited setting. Journal of Blood Disorders & Transfusion, 3, 1000124-1000124.

12. Gladwin, M. T. & Kim-Shapiro, D. B. (2009). Storage lesion in banked blood due to hemolysis-dependent disruption of nitric oxide homeostasis. Current Opinion in Hematology, 16(6), 515–523.

13. Grunenberg, R. &Krüger, J. (1995). Analysis of cytokine profiles of stored CPDA-1 whole blood.

14. Spitalnik, S. L. (2011). Harmful Effects of Stored RBC Transfusion: Bench-Bedsides. Retrieved from: www.arup.utah.edu Accessed: May 10, 2018.

15. Katherine, A. & Cummings, D. V. M. (2016). Effect of storage on ammonia, cytokines and chemokine concentrations in feline whole blood. Retrieved from: Willey Online Library. Accessed: May 10, 2018.

16. Nagura, Y., Tsuno, N. H., Matsuhashi, M. & Takahashi, K. (2013). The effect of pre-storage whole blood leukocyte reduction on cytokines/ chemokines level in autologous CPDA-1 whole blood. Retrieved from: www.trasci.com. Accessed: May 10, 2018.