African Journal of Laboratory Haematology and Transfusion Science

Vol 2, Issue 3, page 183 - 193 | September 2023 | www.ajlhtsonline.org
ARTICLE DOI: 10.59708/ajlhts.v2i3.2317
DOI URL: doi.org/10.59708/ajlhts.v2i3.2317

ORIGINAL ARTICLE

In-Vitro Assessment of Platelets Survival in Stored Platelet Concentrates in Ile-Ife, Nigeria

Abdulwaheed Adewale Ademosun[1,2], Musa Abidemi Muhibi[1*], Tesleemah Oluwakemi Davies-Folorunso[1], Olufemi David Olaniyi[1], Nuryn Abdulganiy[1], Fatimat Adewumi Adedapo-Ismail[1], Yekeen Adebisi Kosamat[3] And Mathew Folaranmi Olaniyan[1]

[1]Department of Medical Laboratory Science, Faculty of Applied Health Sciences, Edo State University, Uzairue, Edo State.

[2]Department of Haematology and Blood Transfusion Science, Obafemi awolowo University Teaching Hospital Complex, Ile-Ife, Osun State.

[3]Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, Osun State University, Osogbo, Osun State.
* Email;muhibimm@gmail.com

Submitted:14-03-2023
Accepted: 18-08-2023
Published 30-09-2023

Abstract

Introduction: Platelets are fragments of megakaryocytes circulating in the blood and its concentrates are therapeutic in substantial bleeding disorders. Efforts to ensure adequate product quality are required due to their short lifespan and lack of robustness. A descriptive longitudinal laboratory-based study was adopted in this study. The study aimed at determining platelets survival in stored platelet concentrates and evaluating thromboxane A2 for platelets function.

Materials and Methods: Platelet concentrates were prepared manually using buffy coat, where about 50ml of concentrates suspended in plasma were allowed to rotate and agitate continuously on platelet agitator at room temperature (20-240C). Aliquots of 4ml each was collected serially for 9 days (day 0 to day 8) from 10 different platelet concentrates collected from 7 male blood donors and 3 female blood donors with age (mean± SD: 36 ± 7.14 years), weight (mean± SD: 66.8 ± 6.01kg), height (mean± SD: 163 ± 4.57cm). The samples were analyzed for platelets count, platelet distribution width (PDW), mean platelet volume (MPV), platelet-large cell ratio (P-LCR), using Automated Haematology analyzer (Sysmex XP-300) and thromboxane A2 using standard ELISA technique. Data analysis was carried out using mean, standard deviation as descriptive statistics and logistic regression as inferential statistics; and $p < 0.05$ was considered as evidence of statistical significance.

Results: Socio-demographic characteristics had no effect on all parameters estimated. There is variation in platelet count and platelet indices values in stored platelet concentrates compared with the baseline values and the survival of platelets in stored platelet concentrates was relatively stable till day 4 after preparation but depreciation surfaced from day 5 to day 8 compared to baseline values. The study also showed that the degree of deterioration of thromboxane A2 was highly significant at day 3 ($p<0.05$) while the

best duration of storage for platelet concentrates in the study area is the first 3 days, though storage up to day 5 is acceptable ($p>0.05$). Conclusion: This study confirms thromboxane A2 as a marker for platelet functionality. The best duration of storage for platelet concentrates in the study area is the first two (2) days when no significant deterioration was observed.

Keywords: Platelet count, Platelets survival, Platelet concentrates, In-vitro assessment, Thromboxane A2.

Introduction

Blood transfusion, being a life saving procedure, plays a vital role in the management of patient in both medical and surgical practices. The challenge of inadequate voluntary blood donors, poor storage facilities and irregular power supply makes availability of sufficient safe blood products difficult in developing countries such as Nigeria(1).

Platelets are megakaryocytic fragments of circulating in the blood and they play significant roles in prevention of bleeding. Platelet concentrates (PCs) are irreplaceable therapeutics and their clinical benefit is clear. However, transfusion refractoriness was reported as an encountered problem occurring in 27% of patients. Underlying causes can be patient-associated factors, such as fever, medication, sepsis, or patient anti-PLT antigen (HPA, human PLT antigen) antibodies, or can otherwise be due to the increased consumption of PLTs within the scope of the desired hemostasis (2).

In contrast, PLT refractoriness can be product-related and associated with PLT-inherent functional variations due to the habitual and physiological conditions of the donors. The manufacturing process may also impact PLT functionality (3) Platelets play a key role over the natural history of many malignant neoplasms and contribute to local tumour growth, dissemination, and metastasis. At the site of a tumour, activated platelets release cytokines which facilitate tumour growth and angiogenesis (2).

Globally, 10 units donation per a 1000 population are required to meet a nation's blood supply demand. Nearly 120 million units of blood are donated every year and this is not sufficient to meet the global need. Physicians requesting for blood often depend on haemoglobin threshold level of the patient, anticipation of blood loss during surgery which is often subjective rather than evidence based (1)

Platelet concentrates play an important role in transfusion medicine. Their short lifespan and lack of robustness require efforts to ensure adequate product quality. The transfusion of selected single-donor Apheresis Platelets Concentrate (APCs) in the case of individual immunological requirements is beyond question; however, in cases which do not require special donor selection, quality and usage of the different products are controversially discussed (3).

Contamination of platelets may result from bacterial inoculation into the blood bags via skin plug at the time of phlebotomy, unrecognized bacteraemia in the donor, contamination from

the environment or during platelet concentrate preparation. Blood contamination will most commonly occur during blood collection because of insufficient disinfection of the venepuncture site. Bacterial contamination of platelet components occurs because of the storage temperature for platelets (22-24° C) which is optimal for bacterial growth (4).

Basically, platelets concentrates can be prepared from single or pooled platelet concentrate (PPC) from whole blood donation and platelet concentrate from single-donor apheresis (3), while platelet concentrates could be prepared from buffy coat or apheresis and stored for 5 days at 20-240C in many climes(5).

Platelets (PLTs) are usually stored for up to 5 days prior to transfusion; although in some blood services the storage period is extended to 7 days and reduced to 3 days in some centers in Nigeria. During storage, changes occur in both PLT and storage medium, which may lead to PLT activation and dysfunction. The clinical significance of these changes remains uncertain(6). According to current practice, platelet concentrates are usually stored in sterile oxygen-permeable bags of different plasticizers like polyvinylchloride and 2-(diethylhexyl) phthalate or polyvinylchloride at 22 °C ± 2 °C. Storage at temperatures outside this range should be avoided, as this can damage the platelets. Since temperatures at approximately 22°C favors the growth of bacteria and other microorganisms, platelet concentrates can be stored for up to five days. Furthermore, room temperature increases the platelet metabolic rate and leads to a reduction in platelet functionality, a process called "platelet storage lesion". To achieve the best clinical outcome, the shortest possible storage period should be targeted (7). The safety of stored PLTs can be evaluated from adverse events following PLT transfusion, such as febrile non-haemolytic transfusion reactions, transfusion-transmitted infection and overall morbidity and mortality. Difficulties in determining whether stored PLTs are as safe and as effective as fresher PLTs in critically ill patients are related to the fact that most of these outcomes are affected by other factors, including population characteristics, severity of underlying illness, cause of thrombocytopenia, concomitant bleeding, administration of other blood products and other co-morbidities impacting on these endpoints(8)

Thromboxane A2 (TXA2) is the predominant product of human platelet cyclooxygenase. It shows a strong pro-aggregation effect and is the main factor constricting blood vessels. This compound plays a key role in the patho-physiological mechanisms of stroke, promoting inflammation, apoptosis, excitotoxicity, and peri-infarct depolarization. The literature has indicated the significant role of thromboxane in myocardial infarction, in which it induces platelet aggregation and narrows blood vessels. Thromboxane, along with isoprostanes and prostaglandin 12 (PGI2), also promotes the initiation and progression of atherosclerosis by controlling platelet activation and the interaction of leukocytes with endothelial cells. A correlation has also been demonstrated between the increase in circulating plasma TXA2 and the hypersensitivity of the coronary arteries to ergonovine maleate in patients with angina. This suggests the possible role of increased TXA2 production in the exacerbation of coronary spasticity (9).

Platelet concentrates are transfused to treat bleeding or prevent bleeding in patients with quantitative or qualitative platelet defects as reported by Jones et al. (2020)(10). It is of great interest that studies have indicated the importance of platelet indices as biomarkers of platelet activation (11).

Aim of the study

The aim of the study was to determine the survival of platelets in stored platelet concentrates and evaluate specific platelet

marker (Thromboxane A2) for platelets functionality in the study area.

Materials And Methods

Study Design

This study is a descriptive, longitudinal, laboratory-based study.

Study Area

The study was conducted in Haematology and Blood Transfusion unit of Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Osun State. Ile- Ife is located within latitudes 7.28N and 7.46N and longitudes 4.36E and 4.56E. Ile-Ife is an ancient Yoruba town in south western Nigeria. It is situated at the geographical centre of the Yoruba-speaking states of Nigeria.

Duration of study

The study was conducted between December, 2022 and February, 2023.

Sampling Technique

A purposive and convenient technique was adopted for the study, through standard donor recruitment followed by aseptic phlebotomy and standard platelet concentrates preparation.

Sample Size Determination

In this study, sampling of convenience was adopted and a sample size of 90 was used.

Laboratory Procedure

Whole blood was collected by venepuncture in CPDA blood bag and allowed to stand at room temperature before centrifugation.The blood was centrifuged using a 'soft' spin (1500 rpm for 15 mins) and the supernatant containing platelets was transferred into another sterile bag (without anticoagulant).Then, this was centrifuged at a higher speed (3000 rpm for 15 mins) and platelet concentrate was obtained. The concentrate was suspended in a little volume of plasma and the concentrate rotated gently and continuously on platelet agitator at room temperature while the ambient temperature was monitored. Aliquots of the concentrate was taken serially to evaluate parameters such as platelet count, platelet distribution width (PDW), mean platelet volume (MPV), platelet large cell ratio (P-LCR) using Automated Haematology Analyzer (Sysmex-XP 300) for platelet quality and survival while standard ELISA technique was used to evaluate thromboxane A2. A digital thermometer was used to monitor the ambient room temperature throughout the study.

Data Management and Analysis

Data collected was checked for completeness, accuracy, consistency and completeness. The data collected was carefully entered into the Statistical Package for Social Science (SPSS IBM version 22) statistical software and analysed using mean, standard deviation as descriptive statistics and logistic regression as inferential statistics.

Results

This study considered the impact of socio-demographic characteristics of blood donors that participated in the study and has shown that age, sex, weight, height, occupation has no effect on platelet count and platelet indices as there is no significant statistical difference in the values got from both sexes with variation of weight and height; age (mean± SD: 36 ± 7.14 years), weight (mean± SD: 66.8 ± 6.01kg), height with mean± SD: 163 ± 4.57 (Table 1).
Comparison of baseline parameters across socio-demographic variables as shown in table 2 clarified that there is no statistical difference with $p>0.05$ in this study. Table 4.2 also revealed that the baseline values for all parameters estimated (platelet count, MPV, PDW, P-LCR and thromboxane A2) were relatively stable for all the donors, there is no significant difference ($p>0.05$).

The study also revealed as shown in table 3 that there is variation in platelet count and platelet indices values in stored platelet concentrates compared with the baseline values. There is drastic fall in platelet count by day 2 after preparation ($p<0.05$) but platelet indices were still stable between day 3 and day 5 ($p>0.05$).

Meanwhile, table 4 simply revealed that

beyond reasonable doubt, thromboxane A2 is a marker to determine functionality of platelets, though thromboxane A2 is independent of quantity of platelets, the concentration of thromboxane A2 dropped sharply after day 2 with p-value less than 0.05 despite the indication that platelet indices are stable till day 5 (p>0.05) as shown in table 3.

Table 5 revealed a weak influence of thromboxane A2 as a platelet maker in addition to platelet count of the platelet donors. It showed that only 47% of the variation in platelet count is explained by thromboxane A2. The result indicates that thromboxane A2 does not significantly associate with platelet count as a platelet maker at B = 0.477, p = 0.168(p> 0.05).

Therefore, the null hypothesis is accepted that there is no association between screening for thromboxane A2 as platelet marker and platelet count in platelet donors.

All results obtained from this study were analyzed using appropriate statistical tools and are presented as below.

Table 1: Socio-Demographic Characteristics of Blood Donors

Variables	**Frequency n = 10**	**Percentage (%)**
Age		
21 – 30 Years	2	20
31 – 40 Years	4	40
41 – 50 Years	4	40
mean±SD: 36 ± 7.14		
Sex		
Male	7	70
Female	3	30
Weight		
60 – 64 Kg.	4	40
65 – 69 Kg.	3	30
70 – 74 Kg.	1	10
75 – 79 Kg.	2	20
mean±SD: 66.8 ± 6.01		
Height		
158	2	20
160	2	20
162	1	10
164	1	10
168	2	20
169	2	20
mean±SD: 163 ± 4.57		

Body Mass Index		
0 – 18.4 Underweight	-	-
18.5 – 24.9 Normal weight	4	40
25.0 – 29.9 Overweight	6	60
30.0 – 39.9 Obesity	-	-
40.0 and above Morbidly Obese	-	-
Blood Pressure		
Less than 90/60mmHg	-	-
90/60mmHg – 120/80mmHg	10	100
140/90mmHg and above	-	-
Educational Level		
No Formal Education	-	-
Primary Education	-	-
Secondary Education	4	40
Tertiary Education	6	60
Ethnicity		
Hausa	-	-
Ibo	-	-
Yoruba	10	100

Table 2: Comparison of Baseline Parameters across Socio-Demographic Variables

Age	**21 – 30 Years**	**31 – 40 Years**	**41 – 50 Years**	**p-value**
	Mean ± SD	Mean ± SD	Mean ± SD	
Platelet	812.0 ± 45.25	954.7 ± 238.89	1039.7 ± 283.19	0.581
Mean Platelet Volume	9.0 ± 0.00	9.0 ± 0.081	9.2 ± 0.244	0.254
Platelet Distribution Width	15.3 ± 0.07	15.4 ± 0.355	15.3 ± 0.150	0.973
Platelet-Large Cell Ratio	0.2 ± 0.002	0.2 ± 0.002	0.2 ± 0.002	0.474
Thromboxane A_2	993.0 ± 39.59	1001.2 ± 267.79	848.7 ± 291.20	0.684

Gender	Male	Female	p-value
	Mean ± SD	Mean ± SD	
Platelet	1028.8 ± 248.43	800.0 ± 38.15	0.064
Mean Platelet Volume	9.1 ± 0.19	8.9 ± 0.05	0.185
Platelet Distribution Width	15.4 ± 0.24	15.2 ± 0.15	0.512
Platelet-Large Cell Ratio	0.2 ± 0.00	0.2 ± 0.00	0.955
Thromboxane A_2	877.7 ± 255.22	1080.6 ± 154.40	0.183

Table 3: Comparison of Platelet Parameters Baseline Values with Successive Days

	Platelet	p-value	MPV	p-value	PDW	p-value	PLCR	p-value
Baseline	960.2 ± 231.71	NA	9.0 ± 0.18	NA	15.4 ± 0.23	NA	0.2 ± 0.00	NA
Day 1	900.3 ± 235.84	0.009	9.2 ± 0.25	0.010	14.0 ± 4.46	0.381	0.2 ± 0.01	0.108
Day 2	791.3 ± 182.06	0.000	9.9 ± 1.59	0.112	15.5 ± 0.47	0.183	0.3 ± 0.10	0.070
Day 3	726.3 ± 176.96	0.000	10.2 ± 1.38	0.025	15.6 ± 0.48	0.094	0.3 ± 0.10	0.044
Day 4	648.3 ± 178.92	0.000	10.5 ± 1.32	0.005	15.6 ± 0.50	0.093	0.3 ± 0.08	0.001
Day 5	586.3 ± 245.31	0.000	10.9 ± 1.26	0.001	15.7 ± 0.24	0.005	0.3 ± 0.10	0.000
Day 6	505. 6 ± 235.91	0.000	11.4 ± 1.32	0.000	15.8 ± 0.24	0.001	0.4 ± 0.07	0.000
Day 7	459.4 ± 215.52	0.000	12.0 ± 1.11	0.000	15.8 ± 0.24	0.000	0.4 ± 0.06	0.000
Day 8	402. 4 ± 174.77	0.000	13.1 ± 0.88	0.000	16.0 ± 0.21	0.000	0.5 ± 0.02	0.000

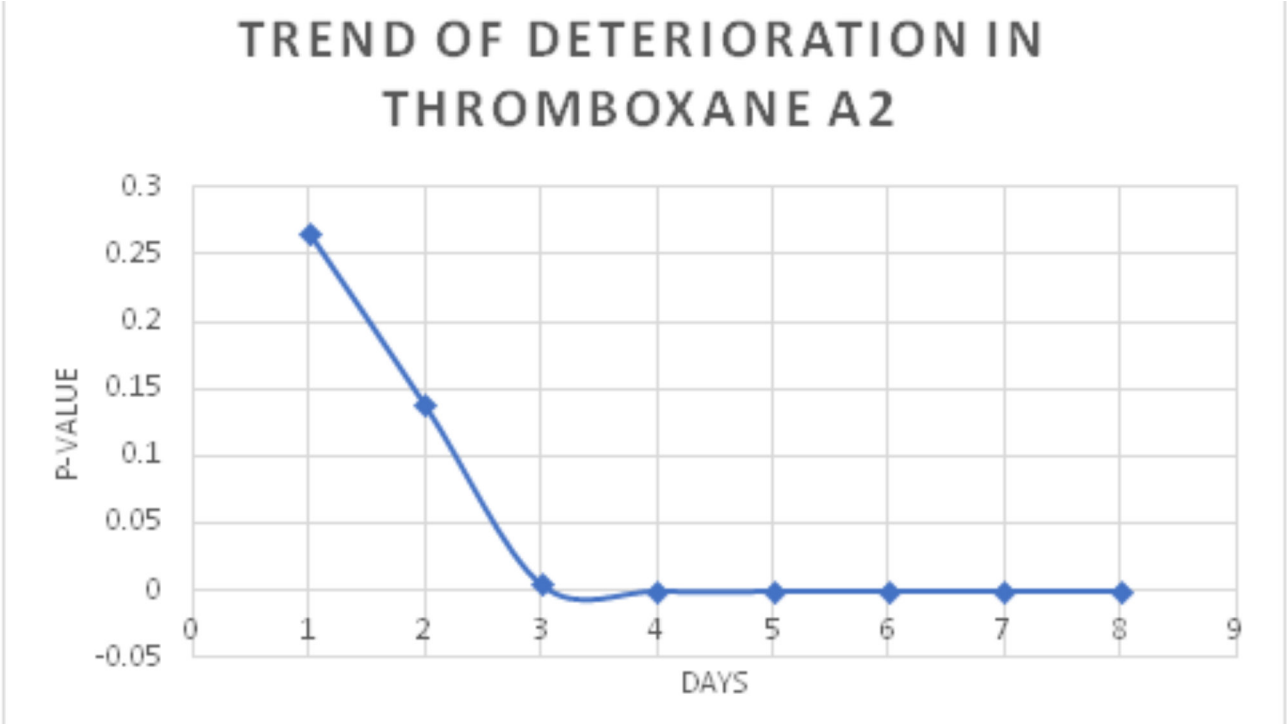
Table 4: Comparison of Thromboxane A_2 Baseline Values with Successive Days

Thromboxane A_2	Mean	SD	p-value
Baseline	938.6	241.52	NA
Day 1	836.1	419.90	0.266
Day 2	750.7	447.87	0.138
Day 3	525.8 `	446.40	0.005
Day 4	374.7	343.23	0.000
Day 5	192.2	176.09	0.000
Day 6	145.7	172.16	0.000
Day 7	73.7	83.17	0.000
Day 8	56.7	68.02	0.000

Table 5: Association between thromboxane A2 as platelet marker and platelet count in platelet donors

R=0. 473 R^2= 0.224 Adjusted R^2 =0.127 F=203.85		Unstandardized Coefficients		Standardized Coefficients		
		Beta	Std. Error	Beta	T	Sig.
1	(Constant)	0.289	0.959		4.791	0.001
	Thromboxane A_2	0.457	0.301	0.477	1.518	0.168
Dependent Variable: Platelet count in platelet donor						

Figure 1: Trend of deterioration in Thromboxane A_2



Discussion

The alterations of platelet indices in this study indicate that the indices are more potent as indicators of platelet activities, rather than platelet count alone. The results of this study also provide valuable insights into the relationship between various demographic and physiological factors and platelet count and indices. The findings suggest that age, sex, weight, height, and occupation do not have a significant effect on platelet count and platelet indices. This is consistent with the lack of statistical difference observed across different socio-demographic variables in this study. These results are in line with the work of Biruk et al. (12), who also reported that these factors do not play a significant role in platelet parameters(12).The findings of this study is also overtly in agreement with the report of Mittal et al (13) where platelet indices have roles to play in causing thrombocytopenia (13). The values of platelet indices (MPV, PDW and P-LCR) increase on storage and they are related to platelet morphology and proliferation kinetics. The survival of platelets in stored platelet concentrates was relatively stable till day 4 after preparation but depreciation surfaced from day 5 to day 8, where about 50% of the platelets were lost compared to baseline values, though the best in terms of quantity could be achieved in the first two days of preparation. This finding is closely related to the report of Aubron et al.(8) with which reported that platelets can be stored and stable for 5 days (14). The degree of deterioration of thromboxane A2 was highly significant at day 3, which is indication for its half-life. This is in agreement with statement of Rucker and Dhamoon (15) that thromboxane A2 has a short half-life and can serve as marker for platelet function (15). A reference range of 697 to 1180 pg/ml was generated for thromboxane A2 in adults in the study area as a representative for Osun State.

In this study, it is revealed that there is no association between thromboxane A2 as a marker and platelet count in platelet donors. Hence, this finding is not in agreement with the report by Szczuko et al. (9) which suggested the possible role of increased TXA2 production in the exacerbation of coronary spasticity.

Conclusion

This study has shown that thromboxane A2 is a marker for qualitative assessment of platelet function in-vitro but it is independent of platelet count while evaluation of platelet indices is also strong tool for assessment of platelet viability on storage. Meanwhile, thromboxane A2 as a maker of platelet viability, cannot be used to predict platelet count, since presumably, not all platelets that are countable in-vitro are truly active. The best duration of storage for platelet concentrates in the study area is the first two (2) days when no significant deterioration would have taken place. However, storage up to day 5 is acceptable where more than 70% of the survival rate is guaranteed and the study was able to generate a reference range of 697 to 1180 pg/ml. Given the decline in platelet count and the stability of platelet indices during storage, further research encompassing a more diverse population is needed to develop improved storage techniques that can extend the shelf life of platelet concentrates. The study confirms thromboxane A2 as a marker for platelet functionality. Further research could explore its implications for various clinical contexts, such as transfusion medicine and platelet-related disorders.

Conflict of Interest

There is no conflict of interest.

References

1. Ibijola A.A., Adegbamigbe O.J., Okunlola A.I., Durowade K.A., Adebara I and Fasakin K.A. Pattern of blood component request and utilization in a tertiary hospital in Nigeria. Niger Journal of Medicine 2022;31:87-91.

2. Giannakeas V., Kotsopoulos J..Brooks, J.D., Cheung, M.C., Rosella, L., Lipscombe, L., Akbari, M.R., Austin, P.C and Narod, S.A. Platelet Count and Survival after Cancer. Journal of Cancers 2022;14:549.

3. Sarah A. F., Klaus B., Ann-Christine J., Christel K., Anneliese H., Wolfgang S., Rainer S. and Ursula S. Evaluation of the in vitro Function of Platelet Concentrates from Pooled Buffy Coats or Apheresis. Journal of Transfusion Medicine and Hemotherapy; 2020;47(2):314-324.

4. Onchaga M.K, Mbugua A, Mwamba P.M, and Mwanda W.O. Bacterial Contamination In Platelet Concentrates Prepared At Kenya National Blood Transfusion Service (NRBTC). East African Medical Journal 2018;95 No. 1: 1134- 1138.

5. Ogbonna C.N. and Thomas O.N. Challenges of Blood Transfusion in a Developing Country and the Ways Forward. Biomedical Journal of Scientific & Technical Research 2021;33(4).

6. Sullivan M.T., Cotten R., Read E.J. and Wallace E.L Blood collection and transfusion in the United States. Journal of Transfusion.2020;47(3):385–394.

7. Eisinger F., Patzelt J. and Langer H.F. The Platelet Response to Tissue Injury. Frontier Medicine 2018;5:317.

8. Aubron C., Andrew W. J. Flint, Ozier Y. and McQuilte Z. Platelet storage duration and its clinical and transfusion outcomes: a systematic review; 2018;22:185-198.

9. Szczuko M., Kozioł I., Kotlega D., Brodowski J. and Drozd A. (2021). The Role of Thromboxane in the Course and Treatment of Ischemic Stroke; Review. International Journal of Molecular Sciences 2021;22(21):11644

10. Jones J.M., Sapiano M.R.P., Savinkina A.A., Haass K.A., Baker M.L.,Henry R.A., Berger J.J., and Basavaraju S.V. Slowing decline in blood collection and transfusion in the United States. Transfusion 2020; 60 (Suppl 2):S1-S9

11. Budak Y.U., Polat M. and Huysal K. The use of platelet indices, plateletcrit, mean platelet volume and platelet distribution width in emergency non-traumatic abdominal surgery: A systematic review. Biochem Med 2016;26(2):179-193. doi:10.11613/BM

12. Biruk B. , Fikir A., Aregawi Y., and Berhanu W. Role of Platelet Indices as a Potential Marker for Malaria Severity. Journal of Parasitology Research; 2021;3:1-8

13. Mittal V, Munesh, Bali IK, Arora S, Singh I and Dadu M. Study of platelet indices and their interpretation in thrombocytopenia in a tertiary care hospital. Journal of Evolution Med Dent Sci 2021;10(07):435-439.

14. Aubron C, Flint A W J, Ozier Y and McQuilten Z. Platelet storage duration and its clinical and transfusion outcomes: a systematic review. Critical Care 2018;22:185.

15. Rucker D and Dhamoon A S. Physiology, Thromboxane A2. National Center for Biotechnology Infor-

mation. StatPearls Publishing; US National Library of Medicine; (2023).

How to cite this article

Ademosun AA, Muhibi MA, Oluwakemi T,Davies-Folorunso1,Olaniyi OD, Abdulganiy N, Adedapo-Ismail FA, Kosamat YA , OlaniyanMF In-Vitro Assessment of Platelets Survival in Stored Platelet Concentrates in Ile-Ife, Nigeria.Afr J Lab Haem Transf Science 2023;2(3):183-193.

DOI: https://doi.org/10.59708/ajlhts.v2i3.2317