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DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Assessment of platelet transfusion outcome and Factors associated with platelet refractoriness among platelet transfused adult patients at Saint Paul Hospital Millennium Medical Collage Addis Ababa, Ethiopia.

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This is to certify that the thesis prepared by **Gizachew Kelil entitled: Assessment of platelet transfusion outcome and Factors associated with platelet refractoriness among platelet transfused adult patients** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Hematology and Immunohematology Track) complies with the regulations of the University and meets the accepted standards concerning originality and quality.

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Abbreviations

Ab	Antibody
AML	Acute Myeloid Leukaemia
BMSA	Body Mass Surface Area
CBC	Complete Blood Count
CCI	Corrected count Increment
DIC	Disseminated Intravascular Coagulation
EDTA	Ethylene Diamine Tetracetic Acid
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
HPA	Human Platelet Antigen
ITP	Immune Thrombocytopenic Purpura
Kg	Kilogram
M	Meter
PLT	Platelet
PPI	Post transfusion Increment
RBC	Red Blood Cell
SHPMMC	Saint Paul Hospital Millennium Medical College
WBC	White Blood Cell

Abstract

Background: Platelet refractoriness is a clinical condition in which a patient's platelets count does not increase as expected following platelet transfusion and associated with both immune and non-immune mechanisms. It is a significant clinical outcome in platelet transfusion.

Objective: To assess Platelet transfusion outcome and factors associated with platelet refractoriness among platelet transfused adult patients at Saint Paul Hospital Millennium Medical College Addis Ababa, Ethiopia.

Methods: Hospital based cross sectional study was carried out at Saint Paul Hospital Millennium Medical College from November 01/2022 to June 30/2023. Convenience sampling techniques and structured questionnaires used to collect patient demographic and clinically relevant data. Blood sample was collected in Ethylediaminetetracid acid tube and run on Beckman coulter heamatolgy analyzer. Platelet refractoriness was identified using corrected count increment 1 hour, 18 to 24 hrs of transfusion and also descriptive statistics was used to present patient characteristics and clinical data. *Paired t-test* was used to compare platelet count, after and before platelet transfused to the patient and also factors associated to platelet refractoriness were analyzed by Multivariate logistic regression. *A P value* of less than 0.05 was considered as statistically significant.

Results: Of the total 216 patients, 28% had platelet refractoriness. we identified that platelet refractory patients associated with 1hr post platelet refractoriness , had previous history of transfusion(AOR:95%Cl,3.61,38, p=0.001), were carried on chemotherapy(p=0.001) prolonged hospital stay (one month and above) p=0.025, fever (P=0.001),Splenomegaly (AOR:95% Cl,17.92 (5.44,59.03), p=0.025) and similarly, post platelet increment 18-24hrs were associated with ABO non identical platelet(AOR:95% Cl 1.49,119.91, p=0.022).On the other hand platelet storage were lower significant with platelet refractory patients.

Conclusion: This study demonstrated that statistical different independent variables associated with platelet transfusion refractoriness among platelet transfused adult patients. Factors found to be correlated with platelet refractoriness were previous history of transfusion, Chemotherapy, prolonged hospital stay for longer time, fever, splenomegaly and ABO non identical platelet.

Key words: Refractoriness, Platelet transfusion, Platelet

Introduction

1.1 Background

1.1.1 Blood Transfusion

Blood transfusions are one of the most common practices in the healthcare systems. The oldest recorded evidence of blood transfusion experiments was from 1666 in Oxford University, where the intellectual milieu was especially conducive to such physiological studies(1,2).

In 1910, Duke performed the first demonstration and effectiveness of platelet transfusions, and also Platelet transfusion treatment has been used to treat thrombocytopenia in haematological and oncologic patients since 1960s(3,4).

Blood Transfusion has played an important role in the advancement and practice of modern medicine. However, some patients, such as members of the Jehovah's Witnesses church, may refuse transfusion for personal or religious reasons. Blood should be transfused only when absolutely essential to save a life. The decision to transfuse is based on the patients' likelihood of developing complication(5,6).

Transfusions of blood are used to treat haemorrhage and enhance oxygen delivery to tissues. the clinical status of the patient should guide blood transfusion. Symptomatic , acute sickle cell crisis, and acute blood loss of more than 30% of blood volume are all indications requiring transfusion(7).

Platelet transfusion first became routinely available since 1970s and have been a mainstay of the treatment of thrombocytopenic patients, decreasing the frequency and severity of bleeding sequence. Patients benefit from the therapeutic transfusions and in a life-saving manner. However, due to limit resources, the platelet product does not always reach the patient at the proper time (8,9).

Platelets are widely transfused blood components in a variety of clinical contexts, either therapeutically to treat acute haemorrhage or prophylactically to prevent bleeding when platelet counts fall below a given threshold or before a surgery. Platelets circulate in the body for around one to two weeks before being destroyed by the spleen and liver and then activated when there is bleeding and release the compounds held inside the granules, forming clots to stop the bleeding (10).

Platelets are generated from the cytoplasm of bone marrow megakaryocytes and are exceedingly tiny (2 to 3um), discoid in form, and anucleated. Each megakaryocytes generates 5,000 to 10,000 platelets and a normal platelet count ranges are from $150 – 450 \times 10^9$ /L(11).

Platelets are blood cell, fragments that play a vital role in the clotting process. They are usually suspended in the plasma, and they can be separate from red blood cells. A unit of whole blood has only a small volume of platelets and patients take platelets from several units of whole blood to prevent bleeding. Platelets, unlike red blood cells, do not have a red blood cell antigen, hence patients can usually acquire platelets from any qualified donor using pooled or aphaeresis procedures(12).

Platelets that are RhD compatible and ABO matching are commonly preferred for receivers. Since platelets are kept at room temperature at a Ph of between 6.2 and 7.8; they are vulnerable to bacterial development. To ensure adequate gas transfer and reduce platelet aggregation, platelets are kept on a single-layer agitator or platelet incubator with continuous agitation. Concentrate Platelets are extracted from whole blood using either pooled or aphaeresis techniques. Approximately, a single unit bag of platelet contains of 50ml plasma and more than 5.5×10^{10} platelet cells (5,13,14)

Platelet transfusion refractoriness is a clinical condition in which a patient's platelet count does not increase as expected after the patient receiving platelet transfusion. Frequently defined as a series of 2-3 post-transfusion platelet count increases below 4500–5000 at 1hr and below 2,500 platelets per microliter at 18–24 hours. In the post-platelet transfusion increment, the calculation of corrected count increment (CCI) is employed to check whether a patients develop platelet refractoriness(15–17).

Several factors associated with platelet refractoriness following transfusion. It might be due to immune or non-immune mechanisms that contribute to platelet refractoriness.Non-immune factors include fever, splenomegaly, diffuse intravascular coagulation (DIC), haemorrhage, and medications and Immune mechanisms are Human leukocyte antigen or a platelet-specific antigen which inherent concern from patients receiving continuous platelet transfusion. In other words, Platelet increment related with ABO-compatible platelets, storing platelets for 48 hours, and administering large quantities of platelets. If refractoriness develops, it occurs within any range following the initial transfusion(18,19). The purpose of platelet transfusions is to improve the platelet count. However, various factors such as the amount of transfused units, the duration of time from collection, and the pH during storage all have an impact on platelet recovery and survival after transfusion(20).

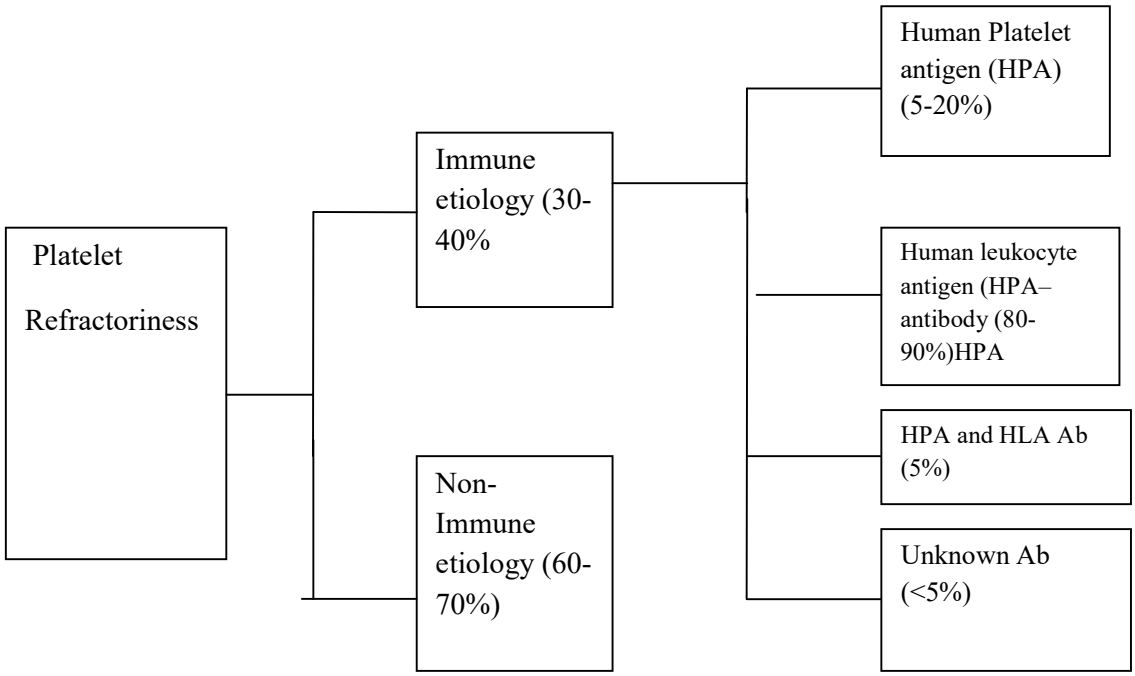

Figure 1.1 Identification of the cause of susceptibility to platelet refractoriness

The agreement between Platelet increment and corrected count increment are common for the diagnosis of platelet transfusion refractoriness(21).

The platelet count should increase after a platelet transfused, within 10 minutes to one hour and then gradually declining over three days. Broadly speaking, for an adult of average size, platelet transfusion of six units of pooled platelets or one aphaeresis unit should boost the platelet count by roughly $30x10^6$/ul(22).

Platelet features such as platelet dose, platelet source (aphaeresis versus pooled), platelet donor-recipient ABO compatibility, and platelet storage period can all have an impact on post transfusion platelet increments and Platelet increments are usually higher for aphaeresis rather than pooled platelets, ABO-identical platelets, and platelets stored three days versus four to five days(23).

Platelet transfusion increment can be calculated by different formula. however, There is no an agreement in the literature on which formula to apply or which cut-offs for successful platelet transfusion increment .According to British committee for standards in haematology the most formula used to calculate platelet post transfusion increment includes three formulae. Namely, post-transfusion increment (PI), percentage platelet recovery, and corrected count increment (CCI)(3).

Post-transfusion platelet increment (PPI) is the most basic calculation and is most commonly used in routine practice due to a lack of available data for the actual number of platelets transfused or body surface area by the health care practitioner assessing the patient(3).

PPI = (Post transfusion platelet count) _ (pre transfusion platelet count)----------(24)

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1.2 Statement of the Problem

A blood transfusion could save a person's life and it's the exclusive patient management However, during transfusion there is a great challenge due to potential of blood transfusion-transmissible infections, such as HIV, hepatitis viruses, syphilis, malaria, and Chagas disease. So it may have immediate or long-term side effects. Bacterial contamination of blood components is an infrequent complication of transfusion, however, if it occurs it's has potential risk for symptomatic sepsis in which recipient's is associated with high mortality (25,26).

Repeated transfusions make patients more susceptible to problems such as iron overload and antibody induce. especially, repeated platelet transfusion is common platelet refractoriness so, this study provides information on how these risks can be reduced as well as any available treatment options (27,28).

Platelet refractoriness can be a serious clinical concern that complicates platelet transfusion administration and may be related with negative clinical outcomes and higher health-care expenses. Platelet refractoriness is a complex process and a significant challenge during transfusion in thrombocytopenic patients, and multiple platelet transfusions cause refractoriness in 30-70% of patients with bone marrow diseases. (3,24,29).

According to research mini-review by *Rebulla P et al* on platelet refractoriness, showed that platelet transfusion refractoriness is an expensive part of a patient's treatment. More precisely, it is very expensive to manage a patient who develops platelet refractoriness and also recently published studies suggested that as no effective method (17,30).

Globally, a bibliometric analysis on Current Status and Global Trends in Platelet Transfusion Refractoriness conducted in Guangzhou, China, showed that the number of published studies increased steadily and peaked in 2020, exhibiting a strong growth trend. However, there is insufficient research on platelet transfusion in Africa, including Ethiopia(31).

According to research study reviews, there is a lack of adequate research and data in the tertiary hospitals related to platelet refractoriness; hence this study intends to investigate the level of platelet refractoriness and factors associated with platelet refractoriness at SPHMMC, Addis Ababa.

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1.3 Significance of the study

In the study area, factors related with platelet refractoriness have not yet been investigated in the Saint Paul Millennium Medical Hospital. So the results of this study will be usefully to health care professional in order to manage platelet refractory patients as well as it enables hospitals to modify prevention and guidelines on platelet transfusion refractoriness. Addition to this, it gives base for further research investigation related platelet refractoriness.

2. Literature review

2.1 Overview of platelet transfusion outcome and refractoriness

According to study in 2005 by *Slichter SJ et al* on factors affecting post-transfusion platelet increments, platelet refractoriness, and platelet transfusion intervals in thrombocytopenic patients done at the University of Washington , USA, showed that out of 533 patients, 27% of patients acquire platelet refractoriness. The findings of this research indicated that platelet refractoriness is linked to the presence of lymphocytotoxin antibodies, administration of heparin, fever, haemorrhage, an increase number of platelet transfusions, and male gender(32)

A research conducted by *Ferreira AA et al* in 2011 Sao Paulo, Brazil, on sixteen prospective oncohematological patients and nine of them were female (56%),and Unsatisfactory platelet increases were observed in 43% of transfusion events. however 19 % of patients had confirmed platelet refractoriness According to the findings of this research, unsatisfactory platelet improve was linked to previous transfusion and alloantibody (33)

A prospective study was conducted by *Sahoo D et al* on analysis of Platelet Refractoriness in a Tertiary Care Hospital in 2020 at the Institute of Medical Sciences and Hospital Bhubaneswar, India. The study observed that overall of 155 patients, 93 patients received single platelet units whereas 62 patients were received more than one transfusion, out of 62 patients who received more than one transfusion , 28 patients developed platelet refractoriness , sixteen (57.14%) of the 28 patients were male, whereas 12 (42.86%) were female. This study's findings showed that , out of 28 refractory patients, after one hour post-transfusion, 23 patients showed no signs of refractoriness, but the 24-hour CCI confirmed refractoriness and also several factors are related with refractoriness, including fever (60.71%), chemotherapy (46.43%), bleeding (50%), pharmacological therapy (42.86%), splenomegaly (10.71%), and others (64.29%)(16).

Hospital based a prospective study carried out by *Kumawat et al in 2015* North India on the prevalence of risk factors for platelet transfusion refractoriness in multitransfused hematooncogical patients at tertiary care centers, showed that out of 30 patients, 17 (56.66%) patients did not show expected platelet count improvement. Among nonimmunological causes, bleeding ($P < 0.019$, odd ratio 8.7), fever ($P < 0.08$, odd ratio 5.2), and infection ($P < 0.07$, odd ratio 5.4) were found to associated with refractoriness. as shown in this research Platelet refractoriness is correlated with multitransfusion patients(34).

A study in 2021 on by *Arabi S et al* a retrospective cohort analysis on the prevalence, risk factors, and outcomes of patients with platelet refractoriness in Riyadh, Saudi Arabia, showed that platelet refractoriness is highly prevalent among patients in intensive care units with prevalence of over 50%. But it was not linked to a higher mortality rate. However, the prevalence of platelet transfusion refractoriness, on the other hand, was 54.8% based on PI 10,000 10^6/L and 57.0% based on CCI 5,000 x10^6/L. (21).

Another study on the effectiveness of pooled platelet transfusion in concordant and discordant groups among dengue patients was carried out at Sapthagiri Institute of Medical Sciences and Research Canter, Bangalore in 2022 showed that out of 203 patients enrolled in the study, 159 (78.32 percent) patients encountered an increase in platelet counts. 60 (37.73%) of these patients received ABO compatible platelet units, while 99 (62.26%) of these patients received ABO identical platelet units. blood type identical platelets are strongly linked to platelet increment (35,36)

According to a 2020 cross-sectional investigation by *Ahmed DA et al* on platelet refractoriness cases after transfusion of fresh and stored platelet concentrate in all children at tertiary Hospital, Dhaka, Bangladesh. Showed that Platelet refractoriness were identified in 15 (18.5%) of 81 patients. As a result of this study, there were no significant differences ($p<0.05$) between fresh platelet concentrate and stored platelet concentrate(37).

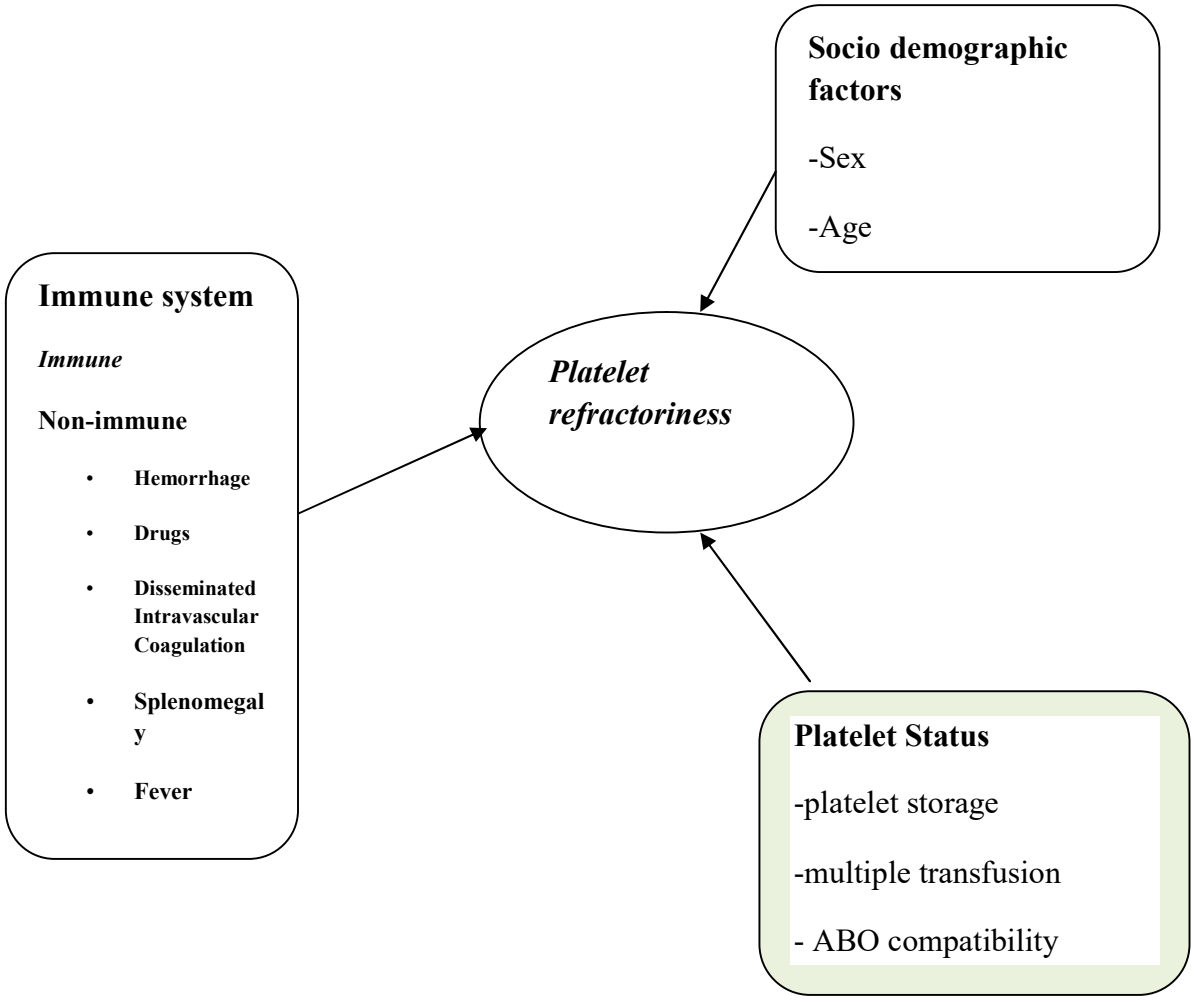

Figure 2.2 Conceptual framework

3. Objectives

3.1 General Objective

To assess platelet transfusion outcome and factors associated with platelet refractoriness among platelet transfused adult patients at SPMMC, Addis Ababa, Ethiopia.

3.2 Specific objectives

- To determine platelet refractoriness level among platelet transfused adult patients at SPHMMC, Addis Ababa, Ethiopia.
- To assess factors associated with platelet refractoriness among platelet transfused adult patients at SHPMMC.

4. Materials and methods

4.1 Study Area

This research was conducted at SPHMMC which is in Addis Ababa, Ethiopia and which has 650 beds and an annual average population of 700,000. It was established by Emperor Haile Selassie I in 1969 with support from the German Evangelical Church. It's catchment area includes more than 5 million people. There are different departments in St. Paul Hospital Millennium Medical College, including forensic Medicine, Toxicology, Internal medicine, Neurology, General surgery, Psychiatry, Ophthalmology, Dentistry (maxillofacial surgery), Radiology, Dermatology, Gynaecology, Obstetrics, Paediatrics, Emergency medicine, Neurosurgery, pharmacy, radiology and also under Medical Laboratory Department, There are Mini blood bank services, Chemistry room, Serology, Microbiology and Haematology room (38)

Mini Blood Services

Saint Paul Hospital Medical Millennium College's mini blood bank serves as a Blood supply for all wards in the hospital. It receives approximately 74 platelet components monthly from Ethiopian National Blood Bank. Based on request form submitted to mini blood bank from all wards of hospital.

4.2 Study design and period

Hospital based cross-sectional study design was carried out between November 30/2022 and June 30 / 2023, to gather clinically relevant patient's data.

4.3 Populations

4.3.1 Source population

Source of population were platelet transfused adult patients at SPHMMC.

4.3.2 Study population

Platelet transfused patients who fulfilled eligibility criteria

4.4. Eligibility

4.4.1 Inclusion criteria

- All platelet transfused adult patients
- Adults 18 and above

4.4.2 Exclusion criteria

- Adult Patients who transfused platelet but not admit to the Hospital

4.5 Study variables

4.5.1 Dependent variables

- proportion of patients with Platelet refractoriness

4.5.2 Independent variables

- Gender
- Clinical condition of the patient
- Previous transfusion history
- Clinical diagnosis
- Number of platelet units transfusion
- ABO platelet compatible

4.6 Measurement, and Data, collection

.4.6.1 Sample size determination

- It determined by applying the following formula. Due to a previous study, p=27%, q=1-p (0.73), and a Z-score with a 95 percent confidence interval of 1.96 was used. The sample size (n) was determined using the formula

  n=z2pq/d2

  n= $(1.96)^2(0.27)(0.73)/(0.05)^2$ P=0.27…… Slichter et al. 2005(39)

  S=302

By assuming 10% non-response rate, 332.

4.6.2 Sampling method

Hospital based Convenience sampling applied to assess the platelet transfusion outcome and factors associated with platelet of refractoriness among platelet transfused adult patients from November, 2022 to June 2023 at SPHMMC.

4.6.3 Data collection procedure

All platelet transfused patients card was reviewed and data extracted with data collection format. The questionnaire was basically consisting of relevant Sociodemographic, clinical condition and patient history.

To calculate Body surface area Physical measurements was taken with the help of expert scientific nurse equipment Adult Scale STOR digital Machine (different equipment at different wards) and from pre Evaluation data checklist. The height and weight measurement was used to compute BSA.

$$\textbf{BSA } (M^2) = \textbf{(height (cm) x weight (kg)/3600)½} \text{ ----------(40)}$$

About 4 ml of blood sample was collected into EDTA test tube. During analysis of complete blood count, the sample was collected from each targeted patient after explaining the importance of sample collection. Platelet transfusion response was evaluated by calculating the corrected count increments as the following.

$$\textbf{CCI} = [(A-B)\ x BSA]/C \times 10^{11})$$

Where **A** is post platelet count/L, **B** is the pre- transfusion platelet count/L, **BSA** is the body surface area (m^2), and **C** number platelet transfused

4.6.4 Laboratory analysis

Haematological parameters such as WBC, RBC, PLT and haemoglobin was measured by using Beckman coulter analyzer, as the manufacturer's instructions and protocols. Before and after the patient being transfused with platelets, a sample was taken. Pre-CBC analysis was performed before the patient receives platelets, and again analysis was performed at intervals one hour, as well as at 18-24 hours.

Principle of CBC analysis

The Beckman Coulter analyzer accurately counts cell size and shape by detecting and measuring changes in electrical resistance which is produced by a particle a cell it movies through a small aperture in a conductive liquid. As each cell passes through the aperture, the resistance of the electrical path between the submerged electrodes on either side of the aperture temporarily increases. This produces a detectable electronic pulse. The suction used to pull the diluted suspension of cells through the aperture must be at a specified volume for counting purposes. The number of recorded pulses represents particle count, and the electrical pulse size is related to cell volume. Furthermore, the system counts individual cells and displays cell size distribution. The number of cells counted per sample is approximately 100 times more than the standard microscope count, reducing statistical error by a factor of ten. (41,42).

4.6.5 Data analysis

Data was analyzed by using SPSS (Statistical Package for the Social Science) version 23 statistical software. Descriptive statistics used to summarize socio-demographic characteristics, variables, mean, and platelet increment was calculated. Paired t-test was used to compare pre and post platelet increment as well as association between variables and factors influencing post-platelet transfusion was analyzed by multivariate logistic regression. *P-value* less than 0.05 were considered as statistically significant

4.7 Data quality Assurance

4.7.1 Preanalytical

After the validity and completeness of the questionnaires have been verified, data was collected. Platelet units before being transfused to a patient, a platelet units was examined for bacterial growth, and expiration date and also to analysis a CBC, Quality control checks was performed for Beckman Coulter Haematology Analyzer.

4.7.2 Analytical

Prior to testing the patient samples, standard quality control protocols (Coulter 6C cell control store, Coulter LIN-X Linearity control and Coulter Latron CP-X control) be run and check to ensure the instrument's accuracy and functionality for Beckman coulter machine. After three level controls passed, the patient's sample was analyzed.

4.7.3 Post analytical

A laboratory result was reviewed for accuracy, documented in the accessible logbook, and results analysed using the appropriate statistical tests.

4.8 Ethical consideration

Issue of patient ethics was reviewed by Research Committee of the Department of Medical Laboratory Sciences, College of Health Sciences, and Addis Ababa University. An ethic approved was signed by Head of Department of Medical laboratory Sciences, Letters of cooperation was written to the SPMMC administrators, a place where study was carried out and volunteered. Admitted patients were informed about study's goals and objectives, and verbal were given. Furthermore, all participants told that they have an unrestricted right to join or refuse to engage in the study.

4.9 Dissemination

The outcomes of the thesis presented and discussed with the Medical Laboratory Science department's faculty and students. Additionally, the findings will be published in journals and discussed at scientific conferences.

4.10 Operational definition

Apheresis Platelet: a blood component product which is extracted a single donor's whole blood

Pooled platelet: a platelet that is pooled from different donors or unit of blood.

Platelet Refractoriness: is a clinical condition in which the post-transfusion count increments after 1 hour and 24 hours are less than 4.5-5x10^3 and 2.5x10^3/ul, respectively following platelet delivery to patients.

Platelet transfusion outcome: it is clinical outcomes after platelet transfusions

5. Results

5.1 Sociodemographic of patients

Total of 216 patient's characteristics summarized as the following in **Table** 5.1. Out of the total platelet transfused adult patients admitted to the hospital, 55.6% were male and 44.4% were female. The majority of patients had thrombocytopenia 64(29.6%) with platelet count less than (150x109/L and had bleeding 61(28.2%).

Table: 5.1 Sociodemographic of among platelet transfused adult's patients at Saint Paul Millennium Medical College, Addis Ababa, Ethiopia.

Variables	Categories	Adult patients	Percent
Sex	Male	120	55.6%
	Female	96	44.4%
Clinical condition	Thrombocytopenia	64	29.6%
	Bleeding	61	28.2%
	Pancytopenia	11	5.1%
	Infection	45	20.8%
	ITP	2	.9%
	DIC	22	10.2%
	Others	11	5.2%
Occupation	Student	26	12.0%
	Government employed	15	6.9%
	Private employee	47	21.8%
	Farmer	66	30.6%
	Unemployed	36	16.7%
	Self employee	26	12.0%
Educational; status	Illiterate	26	12.0%
	Elementary	15	6.9%
	high school	47	21.8%
	Diploma	66	30.6%
	Degree	36	16.7%
	MSc and above	26	12.0%

5.2 Characteristics of the transfused platelets

A total of 469 pooled platelets were transfused to adult patients admitted to the hospital. Transfused platelets were prepared by only one method at Ethiopian National Blood Bank from individual volunteer blood donors.

Of these transfused platelets 146(67.6%) were ABO-identical platelet and 70(32.4%) ABO non identical platelet. Addition to these 94% platelets were stored between 3 up to 5 days and 13(6%) were stored for less than 3 days before transfused to patients.

Table: 5.2 Characteristics of platelet storage of received by adult patients at Saint Paul Millennium Medical College, Addis Ababa, Ethiopia, 2023

Variables	Numbers	
Platelet storage	< 3days	25
	3-5 days	195
ABO identical		146
ABO non identical		70

5.3 Response to platelet transfusions

A paired samples t-test was used to analyze pre and post platelet count after a patient receives platelet units. The association of Preplatelet count and post platelet count transfusion after 1hr responses are given **Table** 5.3. The result showed a significant increment 1 hr after platelet transfusion compared to pre-transfusion count (M=39, SD=17.46), t=-26 $p<0.001$(M= 23.19, SD=16.19) respectively. (Two tailed). The mean increase in the test scores was 15.81 with a 95% confidence Interval ranging from -16.95 to -14.69.

Similarly, the association between pre platelet count and post platelet count after 18-24hrs carried out (**Table** 5.3). The result showed a significant increase of pre platelet count (M=23.19 SD=16.91) to post platelet count after 18-24hrs(M=40.68 SD=17.89),t=-27.73 $p<0.001$(two tailed).the mean increases in the test scores was 17.48 with a 95% confidence interval test scores from -18.72 to -16.24.

Furthermore, the relationship between post platelet 1hr and post platelet count 18-to-24rs was investigated (**Table** 5.3). The result showed a significant increase of Post platelet count after 1hr(M=39 SD=17.46) to post platelet count after 18-24hrs(M=40.68 SD=17.89),t=-4.99 p<0.001(two tailed).the mean test scores was 1.6 which significance difference with a 95% confidence interval test scores from -2.32 to -1.00.

Table: 5.3 Paired t test Analysis of platelet transfusion outcome among platelet transfused adults patients at Saint Paul Millennium Medical College, Addis Ababa, Ethiopia.

Platelet count	Mean	Std dev	P value
Preplatelet count	23.19	16.91	0.001
Post Platelet cunt after 1hr	39.19	17.46	
Preplatelet count	23.19	16.91	0.001
Post Platelet count after 18 to 24hrs	40.68	17.89	
Post Platelet cunt after 1hr	39.19	17.46	0.001
Post Platelet count after 18 to 24hrs	40.68	17.89	

5.4 Selected factors associated with platelet refractoriness

The results of patients analyzed by Multivariate logistic regression and platelet-related variables showed that a number of factors were related to either a statistically rise the platelet count or a decrease the platelet count post platelet transfusion of 1 hour, and 18 - to 24 hours . According to this study, factors associated with both 1-hour and 18-to-24-hr increment of post platelet count 24hrs platelet storage and short time admission to hospital (less than one month) p value above 0.05.

In contrast, patient related factors that were associated with significantly decrement in post transfusions after 1hr were previous history of transfusion(AOR: 95%Cl, 3.61,38.05 , p=0.001), Chemotherapy (AOR: 95%Cl 3.82,34.85,p=0.001),stay at hospital for longer time (One month and above) (AOR :95%Cl 1.22,21.35,p=0.025) and fever(AOR:95%Cl 1.22,21.35, P=0.001). See **Table** 4.a .Similarly, post platelet decrement observed at 24hrs related with ABO non identical platelet (AOR: 95%Cl 1.49, 119.91, p=0.022), see **Table** 5.4b

Table: 5.4a Factors associated with platelet refractoriness at 1hr among platelet transfused patients at Saint Paul Hospital Millennium Medical College, Addis Ababa,Ethiopia,2023(n=216)

Independent Variables	Categories	Refractoriness 1hr		COR (95%CL)	AOR (95%CL)	P-Value
		Yes	No			
Fever	Fever	44	68	16.17(5.57, 47.10)	6.26(1.52, 25.30)	0.011*
	No fever	4	100	1	1	
Duration hospitality	less one month	6	67	1	1	
	1month and above	42	101	4.64(1.87, 11.53)	5.10(1.22, 21.35)	0.025*
History of transfusion	Yes	42	37	24.78(9.78, 62.81)	11.72(3.61, 38.05)	<0.001*
	No	6	131	1	1	
Splenomegaly	Yes	44	29	52.27(17.56, 158.23)	17.92(5.44, 59.03)	0.025*
	No	4	139	1	1	
Chemotherapy	Yes	41	43	16.89(7.05, 40.45)	11.55(3.82, 34.85)	<0.001*
	No	7	124	1		
ABO platelet compatibility	Identical	15	131	1	1	
	Nonidentical	33	37	7.60(3.83, 15.87)	3.38(1.45, 9.97)	0.027*

*N.B: **1** shows reference group,*Shows significant association **COR**= Crude Odds Ratio, **AOR**= Adjusted Odds Ratio*

Table :5.4b Factors associated with platelet refractoriness at 24hr among platelet transfused patients at Saint Paul Hospital Millennium Medical College, Addis Ababa ,Ethiopia,2023(n=216)

Independent Variables	Categories	Refractoriness 24hr		COR (95%CI)	AOR (95%CI)	P value
		Yes	No			
ABO platelet compatibility	Identical	1	145	1		
	Nonidentical	12	58	30(3.81, 235.89)	13.18(1.49,119.91)	0.022*
Platelet storage time	<3days	1	89	1	1	-
	3-5days	12	114	9.36(1.19, 73.41)	-	-
History of Previous transfusion	Yes	12	67	24.35(3.10,191.27)	8.67(1.00, 78.54)	0.005
	No	1	136	1	1	
Duration of hospitality	<One month	1	115	1		
	≥ Month	12	88	15.68(2.00,122.89)	8.76(1.00, 78.54)	0.060

*N.B: **1** shows reference group,*****Shows significant association **COR**= Crude Odds Ratio, AOR= Adjusted odd Ratio.*

6. Discussion

Platelet refractoriness is a clinical condition in which platelet counts do not increase after a patient receiving at least two consecutive platelet transfusions. During our study 216 platelet transfused adult patients included in the study, of the 216 patients analyzed; Platelet refractory condition was developed in 28% (61 0f 216) patients. Out of all refractory patients, 48 patients became refractory during post platelet transfusion of 1hr (Cut value CCI $<5x10^9$/L).The rest of 13 patients was developed post platelet refractoriness after 18 to 24 hrs (Cut value $<2.5x10^9$/L) of platelet units received.

Platelet refractoriness is currently a major health problem in developed countries as different researches showed but in developing countries no adequate studies available(28). So this study provided valuable finding regarding proportion of platelet refractory patients and factors associated with platelet refractoriness among adult patients platelet transfused. During the study's period, all adult patients received platelet units were enrolled in the study. But, adult patients on follow up who received platelet units were not included in the study.

The results of study showed that several factors, including fever, chemotherapy, splenomegaly, a prolonged hospital stay, and a history of blood transfusions had an impact on post platelet transfusion increments. Moreover, ABO non identical platelet, which is crucial, had an impact at 1 hr post-platelet transfusion refractoriness. Among adult patient's had received ABO non identical platelet were 3.38 more likely vulnerable for platelet refractoriness as compared with patients received ABO identical platelet refractoriness. It could be because of ABO-antigen on the platelet surface. This study's findings are comparable with the results reported by *Chien et al* in Taiwan, which showed that total of 58 refractory patients, 25 refractory patients had ABO nonidentical platelets (36).

We found that 28% (61of 216) platelet transfused adult patients admitted to the hospital showed indications of platelet transfusion refractoriness. This might be due to adult patients only received pooled platelets, which could raise the risk of allo immunization when compare with Apheresis platelet. According to the findings of this study, roughly one-fourth (28%) of the patients had a chance of becoming refractory. The result was comparable to the study of earlier reported by *Slichter* 27% (143 of 528) were refractory patients(39). However, from north India

and Saudi Arabia found higher proportion platelet refractoriness, with 57% (152 of 267) and 56.60% (17 of 30) respectively(21,34).

According to this study, patients who had fever were 6.26 times more likely acquired platelet refractoriness as compare to who have no fever at 1hr platelet refractoriness. It might be due to fever causing pathogenic infectious such as viruses which activate platelets .since platelet once activated decrease in terms efficiency. The results of this study were the similarly with the results reported by *Slichter et al* (39) and also according to *Sahoo D et al* reported, several factors correlated with platelet refractoriness, among these fever were one (16).

A patient who received chemotherapy were more likely acquired platelet refractoriness 1hr as compare to who have no receive chemotherapy. It could be happening when a patient is exposed to chemotherapy multiple times, which result in a cell is damaged and platelet decrement. *Sahoo* also proved that as chemotherapy patients associated with platelet refractoriness (16).

A patient who had history of previous transfusion were 11.7 and 8.67 more likely developed platelet refractoriness as compared with patients had no previous history transfusion at interval of 1hr and 18-24hrs respectively. This might be due to antibodies immunized from both recipients and donors which likely resulted in developing alloimmunization. Similarly, previous study noticed the effects of alloimmunization on the platelet transfusion refractoriness (43).

This study also indicated that platelet transfusion refractoriness lower association with platelet storage time at 1hr platelet refractoriness. This finding is comparable with study reported by *Aubron* which showed that Platelet storage time has not much impact on clinical outcomes (platelet refractoriness) and also study carried out recently by *Ahmed* in 2022, Dhaka, Bangladesh, showed that platelet stored 3 upto 5 days were equally effective as platelet stored 24hrs in terms their quality after post platelet increment (37,44).

Results of this study showed that patients who admitted to hospital for more than one month in the hospital were 5.10 and 8.76 times more likely developed platelet refractoriness as compare to who stay less than one month admitted to hospital for platelet refractoriness at 1hr and 24hr respectively. It could be due to exposure of patients for multiple transfusions and chemotherapy as stay in the hospital. Especially, haematological patients .Similarly, long time stay in the ICU room also reported by *Saeed ,* as it associated with platelet refractoriness(21).and also from this

study, splenomegaly patients were significantly associated with 24hr platelet refractoriness as compare with non splenomegaly patients. This might be due to sequestration of platelet in spleen as it causes low platelet count in blood circulation. Similarly, *Slichter* in 2005 reported that splenomegaly patients as correlated with platelet refractoriness (39).

7. Strength and limitation of the study

7.1 Strength

- Data and sample collection was collected by skilled nurses and laboratory technologists with strictly guiding to SOPs..
- Fully automated Beckman coulter were used to analyse CBC laboratory tests..

7.2 Limitation

- The good response to platelet transfusion was arbitrarily .some studies have used 7,500 or 10,000 as threshold.
- This study was limited to only patients admitted to hospital not include outpatients.
- The study only included pooled platelets, which were not enough to provide adequate information concerning refractoriness..

8. Conclusion and Recommendation

8.1 Conclusions

Platelet refractoriness is commonly encountered in platelet transfusion patients. Specifically, fever. Splenomegaly, ABO-non identical, chemotherapy and previous history transfusion were related to platelet transfusion refractoriness. Whereas ABO identical platelets were lower significant in platelet refractoriness.

8.2 Recommendation

We recommend that further investigation is important to identify platelet transfusion refractoriness and ABO identical platelet were suggested for patient require more transfusion in order to prevent platelet refractoriness..

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Annexes

Annex 1: English Version of Participant Information Sheet

Addis Ababa University College of Health Sciences Department of Medical Laboratory Sciences

Title: Assessment of platelet transfusion outcome and determinant of platelet refractoriness among platelet transfused adult patients at St.paul Hospital millennium medical college, Ethiopia.

Introduction: This information sheet is prepared to clarify the importance of the study that you are going to participate. If there is any unclearity before you decide to participate or not.

Purpose: I have planned to conduct a study entitled Assessment of platelet transfusion outcome and factors associated with refractoriness among platelet transfused adults patients at Staint Paul hospital millennium medical college, Addis Ababa, Ethiopia.

Confidentiality: Any information we acquire about you during this research will be kept strictly confidential. For the purposes of this study, your name and identification on the request document will be changed to Confidentiality code. The samples and information provided by participants will only be used for this research and will not be used for any other purpose.

Benefit: You, participant, will receive no immediate benefit!. Managers, administrators, and policymakers, on the other hand, will use the study's findings to improve patient care. As a result, by participating in this quality-related laboratory study, you indirectly help yourself, other patients, and society as a whole. The finding of study ensure a sustainable blood bank service, which has been challenged by quality of blood transfusion.

Person to contact: Please direct any questions or problems you may contact me by this address.

Principal Investigator:

Gizachew kelil, Department of Medical Laboratory

Tel: +2519847833573

Email: gizachewkelil2012@gmail.com

Annex 2: English Version of Informed Consent Form

Consent Form

As I confirmed that, in giving consent to participate in the study, I have read the information page above and clearly understood the research's goal and expected benefit. I hereby certify with my signature below that I have decided to voluntarily participate in the study to contribute my part by providing information on assessment platelet transfusion outcome and factors associated with platelet refractoriness among platelet transfused Adult patients at Saint Paul Hospital millennium medical college Addis Ababa, Ethiopia.

Unique ID No._____ Signature _____ Date_____

Date of data collection _____Start time _____End Time_____
participant's Name_____ Signature_____

I thank you for your cooperation

Please direct any questions or problems you may encounter during this study to:

Gizachew Kelil Zewudie

Cell phone number +251984783573

E-mail.gizachewkelil2012@gmail.com

Annex 3: Dummy table

Variables	Patient characteristics		Numbers	Percent
Sex	Male			
	Female			
Blood components	PLT	24hrs fresh		
		2-5 days Old		
	Whole Blood			
	Packed Red Cell			
	Fresh Frozen Plasma			
Clinical dx	Pancytopenia			
	Sepsis			
	ITP			
	Fever			
	Thrombocytopenia			
	Chemotherapy			
	Bleeding			
	Splenomegaly			
	Fever			
	DIC			
	Medications			
	Others			

Annex: 4 Questionnaires

Patient code_____

Part 1

1. Age

2. Sex

3. Occupations

A) Farmer B) Government employee C) Private employee D) Businessman E) Pensioner F) Others_____

4. Do you have fever? a) Yes b) No c) frequently If Yes for how many times?---------

5. Do you've bleeding problem? a) Yes b) No

6. Do you have blood clotting problem/hemophilia? a) Yes b) No c) frequently

If your answer yes, have you take a drug? Specify a drug----------

7. Do you have a liver disease? a) Yes b) No

8. Do you have kidney infection? a) Yes b) No

9. Weight in kg_____ and Height in M___ and_____cm

10. Do you receive chemotherapy? a) Yes b) No

If your answer yes for how many times _____

11. Do you receive X-ray diagnosis? a) Yes b) No

If your answer yes how many times

12. Which type of Radiotherapy do you receive a) X-ray b) CT-Scan c) EMR d) others_____

13. Have you previously been pregnant? a) Yes b) No if your answer is yes, how many times have you been pregnant? a) Once b) twice c) three times

14. Do have any known hematology cancer? a) Yes b) no c) if yes specify----------

15. Do you have a family history of bleeding? a) Yes b) no c) specify----------

16. How long have you been admitted to hospital?_____

17. How long have you had a disease?_____

18. Do have previous blood transfusions? a) Yes b) no c) if yes how many times?_____

Annex: 5 Data collection format for platelet transfusion assessment among platelet transfused patients at Saint Paul Millennium Medical College, Addis Ababa, Ethiopia, 2023.

MRN	ABO COMP.	Plt storage	pretransfusion	Post transfusion	Plt increments		Clinical dx
					1hr	24hr	

Annex: 6 Checklist for platelet evaluation among platelet transfused Adult patients at Saint Paul Hospital Millennium Medical College, Addis Ababa,Ethiopia,2023.

MRN	No of units transfusion	Wt Kg	Height cm	BSA	Pre transfusion	Post transfusion		CCI 1hr	CCI 24hrs
						1hr	24hr		

Annex: 7 Amharic Version of Informed Consent Form

ስለጥናቱ ማረጋገጫ

እኔ ስሜ ከታች የተገለጸው የጥናቱ ተሳታፊለ መሆንስ ወስን የጥናቱ አላማ፣አሰራሮች እና ቅድመ ሁኔታዎች በግልጽ በመረዳት እና ለጥናቱ ተሳታፊነት ፍቃደኝነቴን በማንኛውም ደረጃ የማንሳት መብቴን በማረጋገጥ ነው። በመሆኑም በጥናቱተሳታፊ ለመሆን ስወስን_በጥናቱ ሳቢያ ሊከሰቱ የሚችሉ አደጋዎችን በሚገባ የተረዳሁና ከጥናቱ በማንኛውም ደረጃ እራሴን ለመሰረዝ ብወስን ተገቢ የሆኑ እገዛዎች ሁሉ እንደ ማይነፈጉኝ በማመን ነው።እነዚህን መረጃዎች ሁሉ በሚገባ በምረዳው ቋንቋ የተገለጸልኝ መሆኑን በፊርማዬ አረጋግጣለሁ።

ፊርማ **----------**

ክፍል አንድ

ቃለ መጠይቅ

ከዚህ በታች የቀረበውን መጠይቆችን ይመልሱ

1.እድሜ በአመት ----------

2. ጾታ ሀ) ወንድ ለ) ሴት

ክፍል ሁለት

ከዚህ በታችየቀረበውን መጠይቆችን ይመልሱ

1. ትኩሣት አለብዎት?

ሀ) አዎ ለ) አይ ሐ) አልፎአልፎ

መልስዎት አዎ ከሆነ ለምን ያህል _____

2. ምን ያህል ጊዜ ደም ወሰዱ?

ሀ) አንድ ጊዜ ሁለት ጊዜ ሶስት ጊዜ

ከሦስት ጊዜ በላይ አልወሰድኩም

3. የመድማት ችግር ለብዎት? ሀ) አዎ ለ) አይ

መልስዎ አዎ ከሆነ ለስንት ጊዜ?

4. የደም መርጋት ችግር አጋጥሞዎት ያውቃል?

ሀ) አዎ ለ) አይ

መልስዎ አዎ ከሆነ ምን ያህል ጊዜ አጋጠመዎት?_____

5. የኩላሊት ህመም አለብዎት? ሀ) አዎ ለ) አይ

መልስዎ አዎ ከሆነ ከመቼ ጀምሮ? _____የሚወስዱት መድሃኒት ካለ ይጥቀሱ_____

6. የደም ግፊትህመም አለብዎት? ሀ) አዎ ለ) የለብኝም

7. የስኳር በሽታ አለብዎት ? ሀ) አዎ ለ)የለብኝም

8. ኬሞቴራፒ ወስደው ያውቃሉ? ሀ) አዎ ለ) አየልወሰድኩም

መልስዎ አዎ ከሆነ ለምንያህል ጊዜ?..... .....

9. ኤክስሬይ ተነስተው ያውቃሉ? ሀ) አዎ ለ) አይ መልስዎ አዎ ከሆነ ለምን ያህል ጊዜ?

.....

10. የትኛውን ጨረር ወስደው ያውቃሉ?

ሀ)ኤክስሬይ ለ) ኤም. አር .አይ ሲቲ_ስካን ሌላ

መልስዎ ሌላ ከሆነ ይግለፁ..... .....

የሚወስዱት መድሃኒት ካለ ይጥቀሱ

11. ምንያል ጊዜ ሆስፒታልቆዩ?_____

12. የሚታወቅ የበሽታ ህመም አለብዎት? ካልዎትይጥቀሱ

13. የካንሰር በሽታ አለብዎት ወይ? ሀ/አዎ - ለ/ አይ

14. ደም ወስደው ያቃሉ ወይ? ሀ/አዎ - ለ/ አይ

Annex 8: Laboratory SOP
Instrument Name: **Beckman UniCel DxH 800 Hematology Analyzer**

Instrument Model: **UniCel DxH 800**

Company (manufacturer): **Beckman Coulter**

The Beck man coulter DxH 800 Analyzer is a quantitative, automated hematology analyzer for in vitro diagnostic use in screening patient populations found in clinical laboratories. The DxH 800Analyzer provides a:

- Complete Blood Count (CBC), Leukocyte 5 Part Differential (Diff), Reticulocyte (Retic) and
- Nucleated Red Blood Cell (NRBC) on whole blood
- Total Nucleated Count (TNC) and Red Cell Count (RBC) on Body Fluids (cerebrospinal, serous and synovial) (BF)

A complete blood count (CBC) gives important information about the kinds and numbers of cells in the blood, especially red blood cells, white blood cells, and platelets. A CBC helps to check any symptoms, such as weakness, fatigue, or bruising. It also helps to diagnose conditions, such as anemia, infection, and many other disorders. In general, the complete blood count can be done as part of routine health examination and general screen

Principles

Coulter Method (impedance)

- Accurately counts and sizes cells by detecting and measuring changes in electrical resistance when a particle (such as a cell) in a conductive liquid passes through a small aperture. Each cell suspended in a conductive liquid (diluents) acts as an insulator. As each cell goes through the aperture, it momentarily increases the resistance of the electrical path between the sub merged electrodes on either side of the aperture. This causes a measurable electronic pulse. For counting, the vacuum used to pull the diluted suspension of cells through the aperture must be at a regulated volume. While the number of pulses indicates particle count, the size of the electrical pulse is proportional to the cell volume.

CBC Analysis

In hematology, the complete blood count, the CBC, is the fundamental analytical test that evaluates the three main cellular components: white blood cells, red blood cells and platelets. The DxH 800 CBC analysis is based on the Coulter Principle.

The sample preparation and data collection occurs in the SAM and CBC modules and analysis is handled by the System Manager.

Venous blood collection procedure

1. Introduce yourself and identify the patient
2. Explain the procedure to the patient
3. Wash hands and wear gloves
4. Prepare materials (syringes, needles, test tubes etc.)
5. Prepare the patient and apply tourniquet
6. Disinfect the draw site
7. Collect 5ml of blood with either Vacutainer tubes or syringe and needle
8. Exit the pain, apply pressure and check the patient.
9. Discard the needle in safety box
10. Label the specimen in each tube

Specimen Preparation

The aspiration pump activates and aspirates 165 µL of sample. After the probe is removed from the specimen tube a second pull of the aspiration pump draws the blood through the BSV pathway, verifying a proper aspiration at the blood detectors.

With each cycle, the BSV directs the delivery of sample and DxH Diluent to the WBC and RBC triple aperture baths.

The RBC diluent and WBC diluent/Lyse dilutions enter through a port in the bath that is located at the bottom and tangential to a sloping surface for bubble free delivery and mixing.

In the WBC bath, ~6.0 mL of DxH diluent and ~28 µL of sample are combined with ~1.08 mL of DxH Cell Lyse for a final dilution of 1:251. In the RBC bath, ~10 mL of DxH diluent and ~1.6 µL of sample are combined for a final dilution of 1:6250.

Detection/Sensing

After the mixing and incubation of sample and reagents, 6 inches of vacuum and aperture current are applied to the apertures simultaneously for the measurements of cell count and cell volume. The RBC and PLT count includes the application of sweep flow to prevent the recirculation of cells behind the aperture. All pulses generated by the apertures are collected and sent to the Signal Conditioner Analyzer Card for analog to digital conversion. The process provides the following raw counts and digital measurements to the System Manager:

• Time
• Volume (pulse peak amplitude)
• Count rate
• Wait time
• Pulse width

The System Manager processes the measurements. The process includes:

• Coincidence correction
• Voting
• The generation of 256 channel histograms for WBC, RBC, and PLT and their voting pattern analysis
• Interference correction

Controls and Calibrators

Quality Control

Quality Control is the routine monitoring of performance and service using commercial or Patient controls. Controls have known characteristics when running on a given system and are analyzed periodically in the same manner that patient specimens are analyzed. The results of analyzed controls are then compared to the known characteristics using statistical methods.

- The quality control is run every working morning and whenever it is needed i.e.
    - If the instrument is calibrated
    - When a new lot of reagent opened.
    - After major instrument service maintenance

Controls

COULTER® 6C Cell Control

The COULTER 6C Cell Control is an integrated control that enables monitoring of system Performance and calibration status for all directly measured and calculated CBC, Diff and NRBC parameters.

COULTER® Retic-X Cell Control

he COULTER Retic-X Cell Control is a control recommended for monitoring system performance of the reticulocyte parameters.

DECLARATION

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been properly acknowledged.

M.Sc. candidate: **Gizachew (B.Sc.)**

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

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Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor Rahel Alemu (MSc)

Signature: _____

Date: _____