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Assessment of peripheral blood blast count and selected hematological parameters before and during chemotherapy of acute leukemia patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

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This is to certify that the thesis prepared by Selam Shibru, entitled: **Assessment of peripheral blast count and selected hematological parameters before and during chemotherapy of acute leukemia patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Hematology and Immunohematology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abbreviations

AML - acute myeloid leukemia

ALL – acute lymphoid leukemia

BM – bone marrow

CBC- complete blood cell count

CLL – chronic lymphoid leukemia

CML – chronic myeloid leukemia

CT – computerized tomography

D7PBBC- Day 7 peripheral blood blast count

D14PBBC- Day 14 peripheral blood blast count

EFS- event free survival

FMOH- federal ministry of health

GBD – global burden of disease

MRD- minimal residual disease

MRI- magnetic resonance imaging

OS- overall survival

PCR – polymerase chain reaction

PET- positron emission tomography

PI- principal investigator

SPM-specimen processing module

VCs- volume conductivity scattering

WBC – white blood cell

Abstract

Background: the assessment of peripheral blood blast count, some hematological parameters in acute leukemia is not researched well in developing countries although, nowadays leukemia is the main concern in these countries.

Objective: to assess the value of peripheral blood blast count, selected hematological parameters before and during chemotherapy of acute leukemia patients at Tikur Anbessa specialized hospital (TASH), Addis Ababa, Ethiopia.

Methods: A prospective cohort study was conducted in acute leukemia patients at Tikur Anbessa Specialized Hospital from February to December 2020to assess the change in selected hematological parameters at three different times after taking first induction treatment. Complete blood cell count (CBC) and peripheral blood morphology of those patients were investigated in three different time points which starts in initial diagnosis, next at 7 and 14 days of induction treatment. Bone marrow analysis data extracted from pathology department at the beginning of patient diagnosis. Data were entered and analyzed using SPSS version 23. Descriptive statistics were used to describe socio-demographic factors, chi square test of association was run for categorical variables and paired t-test and ANOVA was also used to compare pre and during induction blast counts and selected hematological parameters. P values less than 0.05 was used to declare statistical significance.

Result: 30 newly diagnosed acute leukemia patients aged 2 to 69 years were enrolled in this investigation. In this study age group is related with that of leukemia type. Some of the hematological parameters are assessed during the three time period. White blood cell (WBC), Blast count, Platelet, but not the other hematologic parameters before the administration of chemotherapy are statistically different with those values of day 7 and day 14 after starting chemotherapy. However, the two parameters WBC (50700, 5200, 2180 cells/ul) and Blast cell count (29700, 3150, 150cells/ ul) at the three time points show a significant effect for time. The respective values for Platelets were 92700, 37100, 55900 cells/ul.

Conclusion: blast count and WBC which was counted (mean ± SD) at day 7 and 14 of induction treatment has a significant value to assess the response of the patient by the treatment. Therefore it should be used as a surrogate marker in the course of induction treatment. In contrast other hematological parameters did not show an effect on the response of the patient by the treatment. Analysis of larger sample size with other additional tests like immunological, biochemical, cytogenetic and flowcytometry should be done on this area of study.

Key words: ***blast count, hematological parameters, acute leukemia, Ethiopia***

1. Introduction

1.1. Background

Leukemia's are clonal proliferations of malignant leukocytes that starts initially in the bone marrow before disseminating to the peripheral blood, lymph nodes, and other organs. They are generally classified by the type of blood cell giving rise to the clonal proliferation (lymphoid or myeloid) and by the clinical course of the disease (acute or chronic). The four main leukemia categories are acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL), and chronic myelogenous leukemia (CML) (1).

Acute leukemia is defined as the presence of over 20% of blast cells in the blood or bone marrow at clinical presentation. It can be diagnosed with even less than 20% blasts if specific leukemia-associated cytogenetic or molecular genetic abnormalities are present (2). They are usually aggressive diseases in which malignant transformation occurs in the hemopoietic stem cell or early progenitors. Genetic damage involves several key biochemical steps resulting in: (i) an increased rate of proliferation; (ii) reduced apoptosis and (iii) a block in cellular differentiation. Together these events cause accumulation of the early bone marrow hemopoietic cells which are known as blast cells. The dominant clinical feature of these diseases is usually bone marrow failure caused by accumulation of blast cells although organ infiltration also occurs. If untreated these diseases are usually rapidly fatal but, in contradiction, they are also easier to cure than chronic leukemia's. It is further subdivided into acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) on the basis of whether the blasts are shown to be myeloblasts or lymphoblasts (3).

AML is the most common type of leukemia in adults, and the incidence increase with age. AML is less common in children. The clinical presentation of AML is nonspecific but imitates decreased production of normal bone marrow elements. , although the WBC count may range from 1 to 200 $*10^9$/L Most patients with AML have a total WBC count between 5 and 30 * 10^9/L. Myeloblasts are present in the peripheral blood in 90% of patients. Acute lymphoblastic leukemia (ALL) is mostly a disease of childhood and adolescence, accounting for 25% of childhood cancers and up to 75% of childhood leukemia. The peak incidence of ALL in children is between 2 and 5 years of age. ALL is rare in adults but, risk increases with age; most adult patients are older than 50 years of age. The subtype of B-cell ALL is a good prognostic indicator for survival. Adults have a poorer outlook: 80% to 90% experience complete remission, but the cure rate is less than 40 % (4).

Decline in platelet count as well as function is common in acute leukemia. Platelets are produced by fragmentation of the cytoplasm of megakaryocytes, one of the largest cells in the body which is found in the bone marrow. The precursor of the megakaryocyte-the megakaryoblast-arises by a process of differentiation from the hemopoietic stem cell (5). The main function of platelets is the formation of mechanical plugs during the normal hemostatic response to vascular injury. Spontaneous leakage of blood through small vessels may occur due to the absence of platelets. The normal platelet count is approximately 250 x 10^9/L (range 150-400 x 109/L) and the normal platelet life span is 7-10 days (6).The mean platelet volume, MPV, is expressed as the average volume of individual platelets derived from the platelet histogram multiplied by calibration factor expressed in femtolitre (FL) and platelet distribution width (PDW) shows how variable platelets size are (7).

The global cancer burden is estimated to have risen to 18.1 million new cases and 9.6 million deaths in 2018. Worldwide, the total number of people who are alive within 5 years of a cancer diagnosis, called the 5-year prevalence, is estimated to be 43.8 million. This figure is for all cancer types. Regarding Leukemia, in 2018, it is estimated there were a total of 437.0 thousand new cases of and 309.0 thousand cancer deaths from leukemia worldwide (8). Globally, the incidence rate and mortality rate of AML were gradually increased. Males and elder people had a higher risk to develop AML. The incidence rate of AML in the developed region was significantly higher than in the developing region. Smoking, high body mass index, occupational exposure to benzene, and formaldehyde were mainly risk factors which contribute to AML-related mortality (9). The number of acute lymphoblastic leukemia (ALL) cases worldwide also increased from 49.1 thousand in 1990 to 64.2 thousand in 2017 (10). Leukemia's are diagnosed and treatment responses monitored using range of techniques including conventional routine complete blood count (CBC), peripheral blood smear and bone marrow smear analysis as well as molecular techniques including flow cytometry, cytochemistry and cytogenetic studies (11).

The rationale of this study relies much on patients' treatment response. There are limited published studies made on the relevance of blood counts and peripheral blood blast counts in the treatment response of acute leukemia patients. Although it is well known that the cells which are found in acute leukemia patients in large amount are blast cells, they are not well studied in the treatment response of the disease in limited resource settings like our country. Besides, leukemia is now the

main concern in developing countries including Ethiopia. We need more relatively affordable and simple ways to diagnose as well as monitor patient prognosis as well as treatment responses of leukemia patients.

1.2. Statement of the problem

A Systematic Analysis for the Global Burden of Disease Study by Global Burden of Disease Cancer Collaboration group was carried out to assess the burden for 29 cancer groups over time. The study aimed to provide a framework for policy discussion, resource allocation, and research focus. The finding revealed that in 2016, there were 17.2 million cancer cases worldwide and 8.9 million deaths. It also showed, cancer cases increased by 28% between 2006 and 2016. Also, a cross sectional study to assess the prevalence of cancer and its associated factor in patients visiting TASH, Addis Ababa, Ethiopia indicate that Cancer (CA) is an increasing public health burden for Ethiopia and Sub-Saharan Africa at large. In Ethiopia, hospital records show that there are more than 150,000 cancer cases per year and currently cancer accounts for 4% of all deaths (12,13).

Leukemia and lymphoma society facts 2018-2019 in US updated data on blood cancers from different cancer statistics results. Leukemia accounts for 35% of the new cases which is equivalent to 61,780 new cases when it is compared to the other blood cancers such as lymphoma and myeloma. Therefore, the report showed that 399,967 people in US are living with or in remission from leukemia. Males living with leukemia accounted 33% more than females. ALL accounts for 81,139 and AML accounts for 53,491 peoples from the overall leukemia patients in US (14).

Gianfaldoni G. etal in a pilot study showed whether clearance of leukemic blast cells during induction treatment is related to the response of the treatment to acute leukemia. During their investigation they showed that the clearance of blasts from peripheral blood is closely related with response to treatment. In Ethiopia this kind of investigation is not routinely practical. Blast count is reliable almost after bone marrow (BM) analysis which even makes the patient move through painful procedure. In relation to platelets and MPV Suleimman and his colleagues investigate whether MPV change through diagnosis to the course of treatment. They found that MPV was higher in the leukemia group, but it was not statistically significant. However, PDW was significantly lower. PDW was high at the time of remission. The reason they gave was it is due to platelet dysplasia. As Gianfaldoni G.etal mentioned treatment stratification is one advantage of doing blast count (15,16).

Despite these findings there is no researches made in the value of blast count, some hematological parameters in the treatment response of acute leukemia patients in Ethiopia. Therefore, this study strive to assess the treatment response of blast count, some hematological parameters in acute leukemia at Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia which until recently is the only cancer center in the country.

1.3. Significance of the study

The significance of this study which is grounded on the treatment response of peripheral blood blast count, some hematological parameters with special emphasis on platelets and MPV is for all the concerned parties. Leukemia patients will know the severity of their condition and act according to it. Patients who are not fast responsive to the treatment will be ready for the intensification of treatment early. In that case, risk of relapse will be minimized. The other parties' physicians can assess treatment options based on the condition that they addressed from the result. Researchers could get input in further investigation of this area of study. Finally policy makers and stake holders will give appropriate attention based on the information they get which may be relevant to the country.

2. Literature review

2.1. Peripheral blood blast count and Hematological parameters

A retrospective cohort study made on Hematological parameters and remission induction of childhood acute lymphoblastic leukemia for 3 years to assess for associations between hematological parameters and induction of remission in children with acute lymphoblastic leukemia in Indonesia reveal that Out of 55 patients, 33 (60%) had anemia, 6 (10.9%) had leukocytosis, and 1 (1.8%) had hyper leukocytosis, whereas 9 (34.5%) had leukopenia and 29 (52,7%) had normal leukocyte levels. Thirty-one subjects (56.4%) had thrombocytopenia, 15 (27.3%) had thrombocytosis, and only 9 (16.4%) patients had normal platelet counts. There were 29 (52.7%) with ANC > 500, whereas 26 (47.3%) had ANC level ≤ 500 (17).

A study by G.Sujun etal. revealed that the percentage of peripheral blood blasts (PBB) on day 7 of induction chemotherapy predicts response to therapy in patients with acute myeloid leukemia. According to their investigation they classify the cases by three groups as group I D7PBBP <0.43%, group II, 0.43%≤D7PBBP<0.945%, and group III D7PBBP ≥0.945%. The finding showed that out of forty six consecutive AML patients, the PBB percentage on day 7 (D7PBBP) was significantly lower in patients who achieved CR (0.03% (0.0%, 0.45%)) than in those who did not (10.85% (1.13%, 19.38%); u =–3.92, P <0.001). The CR rate was significantly higher among patients with a D7PBBP of <0.945% (84.62%, 22/26) than among those with a D7PBBP of ≥0.945% (25.0%, 5/20; $\chi 2 = 16.571$, P <0.001(18).

The Tokyo Children's Cancer Study Group on the significance of the complete clearance of peripheral blasts after 7 days of prednisolone treatment in children with acute lymphoblastic leukemia on seven hundred fifty four children for over five years study period from 1999 to 2003 showed that after 7 days of prednisolone monotherapy, 249 patients (33%) were classified as Day 8 No Blasts, 392 patients (52%) had blast counts of 1-999/μL, and 113 patients (15%) had blast counts ≥1,000/μL. The cases were classified based on the peripheral blast count; 0/μL (Day8NoBlasts),1-999/μL and ≥ 1,000/μL(19).

A quantitative reduction and qualitative dysfunctioning of platelets are the leading causes of bleeding in acute leukemia. An observational study in Bangladesh by M.Khan etal. on platelet indices to observe change of platelet indices in ALL during induction chemotherapy enrolled 52

newly diagnosed ALL patients whose age range between 1.5 and 12.It showed that Mean PLT was found to be 93442.3±29966.4 cells per cu mm before treatment, 137442.3±27217.9 per cu mm in 1st week. Mean PCT was found 0.09±0.11% before treatment, 0.16±0.11% in 1st week. Mean PDW was found to be 13.1±3.9 fl before treatment, 12.9±3.5 fl in 1st week. MPV was found 10.6±2.1 fl before treatment, 11.0±1.4 fl in 1st week. This study showed that mean PLT increased at 1st week and there is no statistically significant difference in MPV values between before treatment with first week of induction of this is probably because MPV depends on the number of new platelets. The study concluded that among four important platelet indices, PLT and PCT were significantly associated with remission in ALL during induction of remission (20).

Rapid reduction of circulating blasts with induction chemotherapy may serve as an *in vivo* marker of chemo sensitivity. A retrospective analysis of 363 patients with untreated AML patients who received induction chemotherapy was performed in order to determine the relationship between day of blast disappearance (DOBD) and complete remission (CR) rates, event-free survival (EFS), and overall survival (OS). DOBD ≤5 vs. >5 was identified as the most discriminating cutoff for OS. DOBD >5 was observed in 35 patients (9.6%). The CR rate for patients with DOBD ≤5 vs. >5 was 74.0% and 28.6%, median EFS was 9.4 months and 1.8 months, and median OS was 17.1 months and 5.8 months, respectively ($P<0.001$ for all). DOBD >5 was independently associated with a lower CR rate and shorter EFS and OS ($P<0.001$ for all) (21).

An early appreciation of treatment efficacy could be very useful in acute myeloblastic leukemia (AML), and a prognostic value has been suggested for the morphological assessment of decrease in blasts during induction therapy. A study on early clearance of peripheral blasts measured by flow cytometry during the first week of AML induction therapy as a new independent prognostic factor on 130 non M3 AML <60 age patients show that Slope thresholds (< 25, 25 to 15 and <15), or the time required to reach 90% depletion of the peripheral blast load (o5, 5 or 45 days), was strongly associated with the achievement of complete remission ($P<0.0001$).(22)

In a retrospective analysis of a cohort of 86 adult patients with AML receiving uniform induction demonstrate that the time to clearance of circulating blasts during induction chemotherapy is an independent prognostic marker of relapse free survival. Early assessment of response to therapy

provides an in vivo assessment of chemo sensitivity and may be a useful means to define the prognosis in individual patients according to treatment administered. (23)

Although leukemia is among the common cancer types in Ethiopia, the laboratory diagnostic facility is at its limited stage. And simple markers like peripheral blood blast count is not given enough emphasis on the treatment response of acute leukemia, a gap to which this study is trying to contribute.

3. Objectives

3.1. General objective

- To assess the treatment response of peripheral blood blast count, selected hematological parameters in acute leukemia patients from February 2020 to December 2020 at TASH, Addis Ababa, Ethiopia.

3.2. Specific objectives

- To determine treatment response of peripheral blood blast count in acute leukemia patients from February 2020 to December 2020 at TASH, Addis Ababa, Ethiopia.
- To determine the treatment response of some hematological parameters with special emphasis on platelets and MPV in acute leukemia patients from February 2020 to December 2020 at TASH, Addis Ababa, Ethiopia.

4. Hypothesis

Null: Blast count and some hematological parameters do not have change at baseline, day 7 and day 14 of induction treatment.

Alternate: Blast count and some hematological parameters do have change at baseline, day 7 and day 14 of induction treatment.

5. Materials and methods

5.1. Study area

The study is conducted at Tikur Anbessa tertiary level specialized hospital which is found in Addis Ababa the capital city of Ethiopia. The hospital started its service as site for training medical doctors in 1972. In1998 Tikur Anbessa status showed that it has 700 beds and transferred to School of Medicine of Addis Ababa University by FMOH. Then, it becomes a university teaching hospital. It is now the main teaching hospital for both preclinical and clinical of most disciplines including specialized services which are not available in other public and private institutions.

TASH is the first and the largest hospital to start hematology-oncology services in the country. Hematology clinic which specifically diagnose leukemia cases is found in the first floor of the building. The unit accepts cases which are sent from all over the country (24).

There are about 100 beds in internal medicine department, 38 -40 of this beds is dedicated to hematology patients. The wards are organized in a way that they accommodate 6-7 beds in a room where most pre chemotherapy patient share rooms with other hematology patients like lymphoma. Whereas the others rooms which accommodate two patients in a room and two rooms with isolated beds are preserved for the most flail acute leukemia patients on induction chemotherapy.

There is also a separate hematology/oncology center located some 1.5 km away from the main compound around “amestegna” which gives service to relatively stable patients on consolidation therapy.

5.2. Study period

- The study is conducted from February 2020 to December 2020.

5.3. Study design

- A prospective cohort study design is employed.

5.4. Population

5.4,1. Source population

- All acute leukemia patients in TASH diagnosed for the first time from February 2020 to December 2020.

5.4.2. Study population

- All confirmed acute leukemia patients in TASH who registered for the first time from February 2020 to December 2020 and fulfill the eligibility criteria.

5.5. Inclusion and exclusion criteria

5.5.1. Inclusion criteria

- All confirmed acute leukemia patients diagnosed and enrolled in TASH during the time period were included.

5.5.2. Exclusion criteria

- Previously treated AML patients, patients with morphologically absence of blast cells in the initial diagnosis, AML from precursor hematologic malignancy and other cardiac, pulmonary, hepatic, renal which are not connected to the disease are excluded.

5.6. Study variables

5.6.1. Dependent variable

- Absolute blast count
- Hematological parameters

5.6.2. Independent variables

- Age of patient at diagnosis
- Sex
- Disease sub type

5.7. Measurement and data collection

5.7.1. Sample size determination

- All acute leukemia patients who attend TASH for the first time from February to December 2020.

5.7.2. Sampling method

- Convenient non probability sampling method was employed.

5.7.3. Data collection procedure

The data was collected after permission is granted from the patient for the procedure. Then clinical history was collected without breaching the confidentiality of the patient, bone marrow result was collected from pathology department which is collected in the beginning of first diagnosis and at the end of first induction treatment. About 3 ml peripheral blood was taken from the patient using EDTA tube. Complete blood count (CBC) for WBC and platelet parameters was carried out; lastly, morphology of the blood was examined at three time points. That means examination was done first at the arrival before treatment (pretreatment), second seven day after first induction and lastly at the fourteenth day.

5.7.4. Laboratory analysis

- **Complete Blood Cell Count**

Complete Blood Count was analyzed using Beckman Coulter UniCel® DxH 800 COULTER@ The COULTER VCS (volume conductivity and scattering) established WBC differential technology using three measurements: individual cell volume, high-frequency conductivity and laser- light scatter. The combination of low-frequency current, high-frequency current and light-scattering technology provided abundant cell-by-cell information that is translated by the specimen processing module (SPM) into data plots.

Volume Analysis: Electronic Leukocyte Volume Analysis using low-frequency current, It has been evaluated as a possible adjunct to the differential white cell count.

Conductivity Analysis: Cell walls act as conductors to high frequency current. The current, while passing through the cell walls and through each cell interior, detects differences in the insulating properties of the cell components. The current characterizes the nuclear and granular constituents and the chemical composition of the cell interior.

Light Scatter Analysis: Coulter's experience in flow cytometry dates back decades to Fulwyler's pioneering use of light scatter for cell analysis (7).

- **Peripheral Blood Morphology**

A drop of blood is thinly spread over a glass slide and air dried. Smear was prepared by using slide and flooded with Wright'sstain for about 5-10 minutes, then twice diluted with buffered water and allowed for another 5–10 minutes for the cells to pick the stain. After this, the slide is properly rinsed under running water. The underside of the slide was wiped with cotton wool to remove excess stain. Finally, the slide is placed on a rack with the feathered end sloping upwards to dry. Stain artifact such as debris and precipitates which may be caused by over-staining (excess stain contact time) and inadequate washing under running water were avoided to ensure quality of the stained slide (3,4). Two hundred cells were then counted and classified. The absolute blast count was calculated by multiplying the total WBC count obtained from the automated hematology analyzer by the % blast count and divided by 100(25).

5.8. Data quality assurance

The data for the purpose of this research was collected by the principal investigator from hematology ward for clinical information, pathology department for bone marrow result and laboratory clinical hematology department for CBC and morphology result. The PI performed the CBC and peripheral blast count which was also examined by senior Hematology &Immunohematology specialist and a third year pathology resident. Data were collected from both the computer system of pathology department and laboratory information system (LIS).

In the **pre analytical** phase patient sample was collected by experienced phlebotomist and transported to analysis section by trained porters. During **analytical** phase quality control sample (High, Normal, and Low) was run and checked, sample was run and investigated by trained laboratory technologist. In **post analytical** phase data was checked from the machine, LIS system and print out. All laboratory analysis was performed by strictly following standard operating procedures (SOP). After analysis data entry was made twice.

5.9. Data analysis and interpretation

The data was coded, entered, edited and transferred to SPSS 23 for analysis. Basic descriptive analysis like mean, median and others was done. Chi square test was done to determine the relationship between basic characteristics and student t-test was also used to compare pre and during treatment blast counts, WBCs. Lastly a one way repeated measure of ANOVA and post hoc test was done to see a significant effect for time. P values less than 0.05 was taken as statistically significant.

5.10. Ethical considerations

The study protocol was reviewed and ethical clearance obtained from the Department of Medical Laboratory Sciences. Support letter was written to TASH to get permission to undertake the study. Informed consent was taken from the individuals; for children consent was obtained from parents/guardians while assent was obtained from children aged 12-17 years old. All the confidentiality of the subjects was kept in private. Since bone marrow data will be taken from their records, no invasive procedure was performed.

5.11. Dissemination of result

Result will be disseminated to Addis Ababa University through presentation and hard copy; then, after presentation efforts will be made to publish the paper in peer reviewed journals. The findings will be communicated and be utilized to improve service at TASH. The responsible government parties could use this material in the improvement of community health as part of implementing plan.

5.12. Operational definition

Absolute blast count; is the total WBC count multiplied by % blast count and divided by 100(26).

Hematological parameters ;,((WBC, Red blood cells (RBC), Hemoglobin (HGB), Platelet, mean platelet volume (MPV),Hematocrit (Hct), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), red cell distribution width (RDW))

Pediatric; at TASH patients who are categorized between the age of 1 and 13 are seen by pediatric hematologists

Adult; patients who are categorized above the age of 13 are treated in adult hematology clinic as per TASH routine clinical care.

Treatment response; clearance of blasts from peripheral blood within a week after starting chemotherapy.

Induction therapy for AML; cytarabine (Ara-c), 100 mg/m^2 /day by continuous IV infusion for 7 days and daunorubucin 45-60 mg/m^2 IV bolus on days 1to 3 or idarubicin 12 mg/m^2 IV bolus on days 1to 3 or mitoxantrone 12 mg/m^2 IV bolus on days 1 to 3.

Induction therapy for ALL; combination of vincristine, dexamethasone or prednisolone, daunorubicine and L- asparginase for 4 weeks.

6.Result

6.1. Demographic and clinical Characteristics

In this study, a total of 30 acute leukemia patients who were newly diagnosed from February2020 to December 2020 at TASH were included. Among the study participants 20 (66.7%) were male patients the mean ± SD age of study participants was 16.5±.16.7 years ranging from 2 to 69 years. Nineteen (63.3%) were pediatric the rest were adult patients. Nineteen patients (63.3)were diagnosed as ALL and the rest were AML patients (Table 1). All had circulating blasts and received induction chemotherapy.

Table 1: Demographic and clinical characteristics of the participants at TASH, Addis Ababa, Ethiopia 2020 (n=30).

Variables		**Frequency (%)**
Sex	Male	20 (66.7%)
	Female	10 (33.3 %)
Age group*	1-13	19 (63.3 %)
	>13	11 (36.7 %)
Clinical characteristics	ALL	19 (63.3 %)
	AML	11 (36.7 %)
Total		**30**

- At TASH the age groups above 13 years old are treated in the adult hematology clinic

6.2. Hematological parameters before beginning, day 7 and day 14 of induction therapy

The complete blood cell count (CBC) and morphological analysis of the study participants show different results in the three time interval. The mean Hgb, WBCs, Plt, MPV and blast cell count in the beginning was 8.94 g/dl, 50.7 *10^3/µl,92.7*10^3/µl, 8.56% and 29.6*10^3/µl respectively. The detail of day 7 and 14 of the above listed parameters are shown in the table below Table 2.

Table 2:Initial,day 7 and day 14 induction therapy Mean and SD values of selected hematological parameters at TASH, Addis Ababa, Ethiopia, 2020 (n=30).

Parameters (Mean ± SD)	**Before starting chemotherapy**	**At day seven (7)**	**At day fourteen (14)**
Hemoglobin (g/dl)	8.94 ± 2.7	9.3 ± 2.1	8.8 ± 2.2
White blood cells (*10^3/µl)	50.75±101.4	5.21 ± 21.3	2.18 ± 4.2
Platelet count (*10^3/µl)	92.77 ± 181	37.1 ±56.2	55.9 ± 67
MPV (%)	8.56 ± 1.5	8.25 ± 1.1	8.52 ± 1.6
Absolute blast count (*10^3/µl)	29.67 ± 76.7	3.15 ± 17.2	0.15 ± 0.8
RBC (*10^6/µl)	3.06± 1.0	3.16 ± 0.8	2.9 ± 0.78
HCT (%)	26.7 ± 7.8	27.7 ± 6.4	25.8 ± 7.0
MCV (fl)	88.7 ± 7.3	88.1 ± 4.3	88.6 ±4.8
MCH (pg)	29.6 ± 3.1	29.8 ± 2.1	30 ±2.17
MCHC g/dl	33.4 ± 1.8	33.8 ± 1.5	33.9± 1.67
RDW (%)	16.0 ± 2.7	15.3 ± 2.97	15.1 ± 2.2

6.3. Association of demographic variables with leukemia type

Chi square test was done on leukemia type with that of gender showed that 13/20 (43%) of the cases are male with disease type ALL and 7/20 (23%) of the cases are also males with disease type AML with no statistical significant difference. Another test of association made on age group with that of leukemia type showed that 50 % of the cases are pediatric patients with ALL type with significant value of 0.047 (Table 3).

Table 3: Association of some categorical variables of participants at TASH, Addis Ababa, Ethiopia 2020 (n=30).

Characteristics	**Leukemia type**		**P value**
	AML n (%)	ALL n(%)	
Gender			
Male	7 (23 %)	13 (43 %)	0.98
Female	4 (13 %)	6 (20 %)	
Age group			
1-13 (pediatric)	4 (13 %)	15 (50 %)	0.047
>13 (adult)	7 (23 %)	4 (13 %)	

6.4.Comparison of hematological parameters before chemotherapy data with day 7

A paired t test of continuous variables of the data was run to find if there is difference between them. WBC, Blast count, Plt, before the administration of chemotherapy have statistically significant difference with day 7 data after starting chemotherapy. On the other hand MPV has no significant difference in the two time period (Table 4).

Table 4: Comparison of selected hematological parameters before chemotherapy result with day 7 of Acute leukemia patients at TASH, Addis Ababa, Ethiopia 2020 (n=30).

Parameters	**Before chemotherapy Mean ± SD**	**Day 7 of chemotherapy Mean ± SD**	**P value**
WBC (*10^3/µl)	50.7 ± 101.4	5.2 ± 21.3	<.001
Plt (*10^3/µl)	92.7 ± 181.0	37.1 ± 56.2	0.032
Blast count (*10^3/µl)	29.7 ± 76.7	3.15 ± 17.2	0.00
MPV (fl)	8.56 ± 1.5	8.2 ± 1.12	0.057
RBC (*10^6/µl)	3.06± 1.0	3.16 ± 0.8	0.56
Hemoglobin (g/dl)	8.94 ± 2.68	9.3 ± 2.07	0.45
HCT (%)	26.7 ± 7.8	27.7 ± 6.4	0.49
MCV (fl)	88.7 ± 7.3	88.1 ± 4.3	0.45
MCH (pg)	29.6 ± 3.1	29.8 ± 2.1	0.68
MCHC (g/dl)	33.4 ± 1.8	33.8 ± 1.5	0.16
RDW (%)	16.0 ± 2.7	15.3± 2.97	0.05

6.5. Comparison of before chemotherapy hematological parameters data with day 14

Comparison of selected hematological parameters before chemotherapy with day 14 after starting chemotherapy showed WBC and Blast count statistically significantly differ between the two time points while Plt and MPV did not show a significant difference on the two time period (Table 5).

Table 5: Comparison of selected hematological parameters of baseline data with day 14 of Acute leukemia patients at TASH, Addis Ababa, Ethiopia 2020 (n=30).

Parameters	**before chemotherapy Mean ± SD**	**Day 14 of chemotherapy Mean ± SD**	**P value**
WBC (*10^3/µl)	50.7 ± 101.4	2.18 ± 4.2	<.001
Plt (*10^3/µl)	92.7 ± 181.0	55.9 ± 67.0	0.23
Blast count (*10^3/µl)	29.7 ± 76.7	0.15 ± 0.82	0.00
MPV (fl)	8.56 ± 1.5	8.52 ± 1.66	0.41
RBC (*10^6/µl)	3.06± 1.0	2.9 ± 0.78	0.47
Hemoglobin (g/dl)	8.94 ± 2.68	8.8 ± 2.2	0.87
HCT (%)	26.7 ± 7.8	25.8 ± 7.0	0.61
MCV (fl)	88.7 ± 7.3	88.6 ±4.8	0.88
MCH (pg)	29.6 ± 3.1	30 ±2.17	0.37
MCHC (g/dl)	33.4 ± 1.8	33.9± 1.67	0.2
RDW (%)	16.0 ± 2.7	15.1 ± 2.2	0.42

6.6. One way repeated measures and post hoc test of WBC and Blast counts

A one-way repeated measures ANOVA was conducted to compare results on the counts of WBC and blast cells at time 0 (before starting of chemotherapy), time 1 (at day 7 of chemotherapy) and at time 2 (day 14 of chemotherapy). There was a significant effect for time where both WBC and blast cell count significantly decreases over the three time points (WBC= wilks lambda 0.32, $p<0.05$ and blast count = wilks lambda 0.067, $p<0.05$). Further analysis of ANOVA which is the post hoc test show that the difference lie between group 1 and 2 also between group 1 and 3 (Figure 2 and 3).

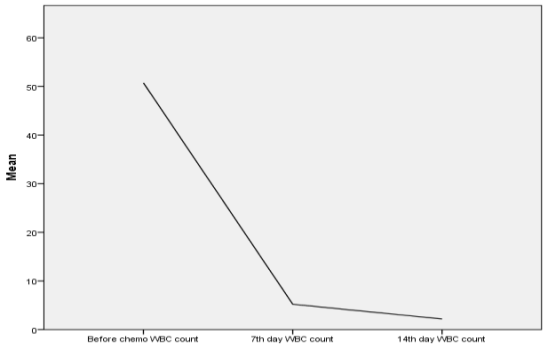

Figure 1: Patterns of WBCs responses to chemotherapy among acute leukemia patients through three time periods: Before start of chemotherapy, at the seventh day and at day 14 of chemotherapy.

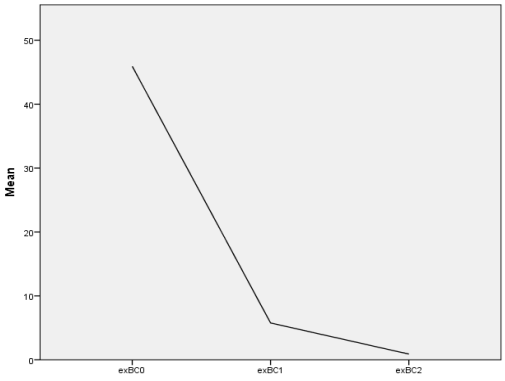

Figure 2: Patterns of blast cells responses to chemotherapy among acute leukemia patients through three time periods: Before start of chemotherapy, at the seventh day and at day 14 of chemotherapy.

7. Discussion

The aim of this study was to assess peripheral blood blast count, some hematological parameters with special emphasis on platelet count and mean platelet volume in acute leukemia patients at Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia. It starts with a hypothesis of blast count and some hematological parameters do have change at day 7 and day 14 of induction treatment as compared to the pretreatment level.

In this study there are about 30 newly diagnosed acute leukemia patients; males are dominant over female (2:1) ratio, hematological parameters are also presented at the three time period. Age group is significantly related with that of leukemia type where ALL is predominantly seen in children consistent with several reports (27, 28). WBC, Blast count, Plt, before the administration of chemotherapy are statistically different compared with the counts of day 7 after starting chemotherapy as well as day 14. Lastly, the two parameters WBC and blast cell count at the three time point show a significant decline over time.

According to this study there were thirty new acute leukemia patients enrolled in about eleven months which is too large when compared to research made by Shamebo which was published on Ethiopian Medical Journal in1994 reported 88 patients within a period of 10 years(29). The low number is also comparable with a study by Lelise Gemechu in 2019 a retrospective study made in a period of three years out of 235 newly diagnosed acute leukemia patients 68 went against medical advice from the inpatient ward, 8 disappeared without notice and 42 of them lost after they get appointment from hematology clinic so in a period of three years only 117 start to follow the therapy when compared to this eleven month research they are almost equal in number (30).

Moreover, the emergence of the global pandemic COVID 19 remarkably affected hospital visits and health seeking behavior of patients. An experience from referral hospital of wollo university for about 8 weeks before and after implementation of preventive measures show that there is a decrease in patient flow in all elements of essential health care service. Medical and surgical chronic illness follow up like malignancy is also among the affected one (31). In other cross sectional study telephone based survey made in Tikur Anbessa specialized hospital reveal that a loss to follow up (70 %), missed medication (12 %)and death (1.3 %) occurred in patients with chronic medical conditions (32).Thus this number might not be a true reflection of new acute leukemia patients.

The sex distribution show that male are dominant over female with total of 20 male patients (66.7) and 10 (33.3) female patients which is close to a retrospective study in TASH by Lelise Gemechu who reported 61.3 % male to 37.3 % female patients but higher than other researches (30).

Hematological parameters at the baseline of the investigation show differences when compared to research made on the same site with different time. Mean WBC, platelet, blast percentage at the baseline was 50755, 92770, 45.9% respectively. On the same site mean WBC, platelet and blast percentage was 57106, 47105 and 52.9 % respectively. The difference on the results may be due to age difference on the participants which range between 1 and 69 in the current study while in the other research it ranged between 13 to76. On the other hand, treatments that can suppress the over immune cells and blood transfusion in response to bleeding, given before chemotherapy may create a difference on the count of the cells (30).

In this study, leukemia type is related with that of age group which means out of 19 ALL participants 15 were pediatric (1-13 age). This study is in line with Graca M etal.(33) and Zand A.M etal. (34). Recent evidence has underscored that the difference in characteristics and biology of adult versus childhood ALL might be the result of a different origin. According to the two-hit paradigm of Knudson, to develop cancer two genetic events are necessary. It has been suggested, that in childhood ALL the first genetic event happens in the more mature lymphoid committed progenitor cells, whereas in adult ALL the first hit occurs in multipotent stem cells (35).

ALL occurs approximately five times more frequently than acute myelogenous leukemia (AML) and accounts for approximately 78% of all childhood leukemia diagnoses (36). But the leukemia sub type does not show a relation with gender which is in contrast to a study by Graca M etal (33) and Zand A.M etal (34). In both studies males are at higher risk of getting leukemia than females. The difference in the result could be due to the size of the sample.

WBC, Blast count, Plt, before the administration of chemotherapy are statistically different with both day 7 and day 14 of WBC, Blast count, Plt, respectively after starting chemotherapy. The findings of low platelet count in the current study after induction therapy is related with other studies (37,38).A study by American cancer society mention that chemotherapy decrease the count of the cell lines due to the toxicity of the drug; it affects not only the toxic but also the normal one. According to the result of the study decrease in WBC is the most serious side effect which expose

the patient to infection also transfusion may not help since WBCs last for few days. RBC and Plt also decrease during the course of treatment but these two can be maintained by transfusion (14).

Since WBC and blast count has a significant difference at different time further analysis was made which can expose the differences in the three time frame. In return, time at three point of analysis has impact on the result of the analysis. This may not help on where the difference is but post hoc of the repeated measure had made it easy to know where the exact difference between groups was. Therefore the base line result of WBC and blast count has a significant difference with both day 7 and day 14 result of WBC and blast count. In contrary, MPV does not show difference in the three time periods which is in parallel to studies by Khan M. and Sweedan SA. Their findings indicate that this is probably because MPV depends on the number of new platelets (16,20).

In sum, this study shades some light on the effect of chemotherapy in acute leukemia patients and the effect on blast count is evident already at day 7 and 14 of induction chemotherapy.

8. Strength and Limitation

8.1.Strength

- Since it use primary data information cannot be biased.

8.2.Limitation

- Patients may not survive to the end of the study. During the study period four patients died and replaced by other new leukemia diagnosed patients.
- Patients may go to isolation (COVID 19 effect) in the middle of the chemo procedure by terminating the treatment process.in this case about 5 patients started the treatment but after days of the chemo they will be suspect of COVID 19 and go to isolation.
- Patients may stop the treatment and go to home with fear. Among the selected cases 6 of them left before starting the treatment.
- Some important data needed for the research may not be found on the record or the electronic device due to negligence or work load.

9. Conclusion and Recommendation

9.1. Conclusion

Mean ±SD of WBC, Blast count, Plt, before the administration of chemotherapy are statistically significantly different from that of day 7 and day 14 after starting chemotherapy. The two parameters WBC and blast cell count at the three time points show a significant effect for time**.** which means theyhave differences when compared to each other. Blast count and WBC which is counted (mean ± SD) at day 7 and 14 of induction treatment has a significant value in assessing the response of the patient. Therefore, it could be used as a surrogate marker in the course of induction chemotherapy treatment. In contrast, MPV did not made an impact on the response of the patient to the treatment.

9.2. Recommendation

- Absolute blast count should be routinely used as marker in the followup time of induction treatment.
- The larger the sample size the more reliable the result, it is recommended to advance this research with larger size, additional tests (immunological, biochemical, cytogenetic, flowcytometry) and with enough amount of time. Because acute leukemia in our setup is not investigated with enough investigation materials and this type of study need complicated investigation.

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Annex 1

Standard operating procedure (SOP)

Standard operating procedure of blood collection (phlebotomy).

Equipment

• Needle for each participant with closed vacutainer system

• Blood collection tubes for each participant

• Tourniquet

• Box of nitrile /vinyl gloves

• 70% Alcohol wipes

• Cotton balls/swabs

• Bandages

• Pillow/pad for raising arm to comfortable elevation

• Apple/orange juice and snacks for fasting participants

• Disposable, single use materials or equipment are to be used whenever possible

• Any reusable materials or equipment must be cleaned and disinfected with Alcohol-based sanitizers before use with another participant Safeguards /safety procedures

Procedure for drawing blood

Step 1; Assemble equipment Collect all the equipment needed for the procedure and place it within safe pack which is simple for transport to collection site and place easy reach on a flat surface table ensuring that all the items are clearly visible.

Step 2; Identify and prepare the participants and allow to sit comfortably preferably be stretching his/her arm

Step 3; Perform hand hygiene and put on gloves

Step 4; Select the site of injection

Step 5; apply the tourniquet

Step 6; prepare the arm by swabbing the antecubital fossa with a gauze pad or cotton moistened with 70% alcohol.

Step 7; insert the needle properly into the vein

Step 8; draw the required amount of blood

Step 9; Fill the laboratory sample tubes and mix properly When obtaining multiple tubes of blood, use vacutainer tubes with a needle and tube holder. This system allows the tubes to be filled directly. If this system is not available, use a syringe or winged needle set instead.

Step 10; Draw samples in the correct order and label the sample using unique code of participants

Step 11; Clean contaminated surfaces and complete patient procedure

Step 12; Prepare samples for transportation

Step 13; Clean up spills of blood or body fluids

Standard operating procedure of coulter unicel DXh 800 hematology analyzer

Purpose; The UniCel® DxH 800 Analyzer is a quantitative multi-parameter, automated hematology analyzer for *in vitro* diagnostic use in screening patient populations found in clinical laboratories. It provides a Complete Blood Count (CBC), Leukocyte Five-part Differential (Diff), Reticulocyte (RET), Nucleated Red Blood Cell (NRBC) on whole blood, Total Nucleated Count (TNC) and Red Blood Cell Count (RBC) on Body Fluids (cerebrospinal, serous and synovial) but for body fluid the machines does not do differential count rather than total cell count.

Principle ; The Coulter Principle of automated cell counting and sizing is used in the analysis of the whole blood and body fluid specimens. Each cell suspended in a conductive liquid (diluent) acts as an insulator. As each cell goes through the aperture, it momentarily increases the resistance of the electrical path between two submerged electrodes on either side of the aperture. This causes a measurable electronic pulse. While the number of pulses indicates particle count, the amplitude of the electrical pulse is proportional to the cell volume. These pulses are sent to the Signal Conditioner for analog to digital conversion. Pulse counts and digitized pulse measurements are sent to the System Manager for processing **by** the algorithms where the reported parameter values, flags and histograms are generated.

The lytic reagent used for the white cell count prepares the blood so the system can count leukocytes and measure the amount of hemoglobin. The lytic reagent rapidly and simultaneously destroys the erytbrocytes and converts a substantial proportion of the hemoglobin to a stable pigment while it leaves leukocyte nuclei intact. The absorbance of the pigment is directly proportional to the hemoglobin concentration of the sample. Hemoglobin is measured photometrically at **525** nm using the sample from the white cell analysis. Clean diluent is introduced into the cuvette during each operating cycle and is used as a blank in the calculation of the HGB.

The **COULTER@ VCSn** technology is used to determine the white cell differential, nucleatedred blood cell count and reticulocyte parameters along with associated flags, messages,histograms and dataplots.

The sample preparation and analysis uses specific reagents and analytical processes for the WBC differential, NRBC and Retic analysis. The prepared sample is delivered to the flow cell for sample detection. As the cells pass through the sensing zone, a diode laser illuminates the particles causing

light scatter and light absorption. Simultaneously to the light scalier measurements, cell volume and cell conductivity are also measured. The data collected during each of the analytical processes is transferred to the System Manager where the digital raw values are processed **by** the algorithm using mathematical approaches designed for finding optimal separation between clusters of data. The identified clusters are used to calculate the frequency of cells within each population, generate parameter values, flags, histograms and data plots.

Reagent

- COULTERe DxH Diluents
- COULTER DxH Lyse
- The COULTER® Duff Pack
- COULTER® DxH Retic Pack
- COULTER" DxH Cleaner

Procedure

Cassette presentation

I. Specimen processing module (SPM) must be online to run the sample
II. Ensure that SPM is set up for the appropriate test of analysis
III. Load the sample into the cassette.
IV. Place the cassette in the input buffer to the right of the SPM. The SPM automatically begins cycling the cassette.
V. It mix and analyze the sample by itself.
VI. Review the sample results at the system manager.

Single tube presentation

I. Select the Single -Tube presentation icon at the top of any screen to display the single-Tube presentation dialog box
II. Place the specimen in the barcode reader platform of the Single- Tube presentation station with the barcode facing the SPM to allow the Single- Tube presentation bar- code reader to scan the specimen label.

III. Verify the specimen accession number and test request.

IV. Thoroughly mix the specimen.

V. Place the specimen in the correct Single- Tube position.

Control

- COULTER® 6C Cell Control
    - An integrated control that enables monitoring of system performance for all directly measured and calculated CBC, Diff and NRBC parameters.
- COULTER® Retic-X Cell Control
    - A control product for monitoring system performance of the reticulocyte parameters
- COULTER® LIN-X Linearity Control
    - A control product for the verification of the reportable range, and calibration assessment of the WBC, RBC, HGB, and PLT parameters.
- **COULTER® Body** Fluid Control
    - A control product for monitoring system performance of the body Fluid Control fluid cycle's RBC and TNC count parameters. Additionally, COULTER Body Fluid Control can be used for verification of the measuring range of the TNC and RBC parameters in the body
- **COULTER"** LATRONTM CP-X Control
    - A control product used to monitor the volume, conductivity and light scatter parameter measurements.

Standard operating procedure of peripheral blood morphology examination.

Purpose

To outline the procedure of performing and reporting of peripheral blood morphology in a thin blood film stained by wright stain

Principle

The stained blood smear permits the study of the appearance and the identification of the different kinds of leukocytes, and the appearance of erythrocytes and thrombocytes.

Reagents and Supplies

- Wright staining solution
- Immersion oil
- gauze
- Distilled water
- Slides

Equipment

- Microscope
- Staining rack
- Timer

Quality control

Quality of the Wright's stain is checked by preparing one differential slide daily using a patient sample with a normal MCV, MCH, MCHC and total white count. The stained slides were reviewed for meeting color specifications of the different white cells, red cells and platelets under normal conditions.

Procedure

i. Label the slide with patient identification.
ii. Place a drop of blood on a center of a glass slide 1 to 2 cm from one end
iii. Place the slide on a flat surface, and hold the narrow side of the non-frosted edge between your left thumb and forefinger.
iv. Hold the spreader slide at a 30-45 angle, and draw it back against the drop of blood.
v. Allow the blood to spread almost to the edges of the slide.
vi. Allow the blood film to air dry completely before staining.

vii. Place the air dried smear film side up on the staining rack.

viii. Cover the smear with Wright stain and leave for 2 minutes.

ix. Dilute with distilled water for three minutes.

x. Wash the smear with tap water and let Air dry the smear.

Result reporting

- RBC's Morphology……..
- WBC's differential:
    - Neutrophils%.....
    - Lymphocytes%.....
    - Eosinophil's%.....
    - Basophils%.....
    - Blast cells%.....
    - Promyelocyte%.....
    - Myelocytes%.....
    - Metamyelocytes%.....
    - Bands%.....
- Platelets estimation & distribution

Result interpretation

- Red cell according to hemoglobin content: normochromic, hypochromic.
- Variation of red cell size (anisocytosis): normocytic, macrocytic, and microcytic.
- Variation of red cell shape (poikilocytosis): report the presence of sickle cells, target cells, spherocytes, ovalocytes etc.
- Red cell inclusion: Heinz bodies, Basophilic stippling, Pappenheimer bodies, Howell-Jolly bodies.
- Nucleated red blood cells and all types of erythroblasts are abnormal.
- Elevated white blood cell count may mean infection.
- Decreases in white blood cell count may occur with disease progression or may indicate bone marrow suppression.
- An increase in neutrophils may be due to an acute bacterial infection or hematological malignancies such as myeloid leukemia.
- An increase in eosinophils may be due to a parasitic infection or an allergic reaction.

- An increase in lymphocytes may be due to viral infections or chronic infections such as tuberculosis or lymphocytic leukemia.
- An increase in monocytes is found in hematological malignancies such as chronic myelomonocytic leukemia and certain bacterial and parasitic infections.

Reference interval

WBC= 2-5WBC/10HPF

RBC= 200-250RBC/100OIF

PLT= 8-12PLT/100OIF

Annex 2
Information sheet in English Version

Title of the Research Project: Assessment of peripheral blood blast count, selected hematological parameters with special emphasis on platelet count and mean platelet volume before and after chemotherapy in acute leukemia patients at – Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

Principal Investigator: Selam Shibru (BSc, MSc candidate)
Name of the Organization: TASH, Addis Ababa University College of Health Sciences Department of Medical Laboratory Sciences.

Introduction

You are invited to participate as a study subject in a research conducted by MSc candidate, from Addis Ababa University. Your participation is voluntarily. The research teams will include one principal investigator, three advisors; one from Addis Ababa University hematology department and two from oncology department. Please take as much time as you need to read or listen in the information sheet.

Purpose of the Research Project

We are asking you to take part in this study because we will try to assess the prognostic significance of peripheral absolute blast count and platelet indices in acute leukemia.

Procedures and the expected participation

If you are willing to participate, you need to understand the purpose of the study and give your consent. Not only this but also specimen collected from you will be used for the research purpose, and the results of your sample will be exposed to some concerned professional staffs as needed. The required clinical sample will be collected by phlebotomists of laboratory department. Then, you are requested to give your consent to the sample collector. After consent, a sample will be taken from your vein. Moreover, there will be a face-to-face interview for additional questions.

Potential risks and Discomforts

During collection of blood sample from you, appropriate precaution will be taken and all samples will be collected by trained health professionals. The blood samples will be taken three times in different intervals from your vein. If anything happened, appropriate medical care will be provided to you.

Confidentiality

We respect your privacy and confidentiality. Any information that identifies you will not be shared with anyone else outside the study team. The information we will collect from you as part of the study will be kept in a locked file cabinet, or be protected by a password on the computer only accessible to personnel involved in the study.There is no sensitive issue that you will be asked related with your social appeal but any information that is obtained in connection with this study and that can be identified with you will remain confidential.

Potential benefits to subjects and/or to the society

You will not receive any payment for your participation in this research study as compensation. However, based on the result you will be treated in view of that. In addition, the result of the study will be beneficial to assessment of prognosis in Acute Leukemia. Hence, you are indirectly benefiting other patients and the society in this respect.

Participation and Withdrawal from the Study

The participation is voluntary and you have the right not to participate in this study. You may withdraw at any time and place without consequences of any kind. You may also reject to give any sample. You can ask any questions regarding to this study and you have a right to get a laboratory investigation result free.

Contact information

If you have any questions about this study you can contact the following principal investigators and advisors for further information.

Selam shibruPhone: 0922160964 **E-mail:**shibruselu@gmail.com

Dr. Aster Tsegaye Phone: 0911696085

Information sheet in Amharic

የተሳታፊዎች ፈቃድና መተማመኛ ቅፅ

በአዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ የሕክምና ላቦራቶሪ ሳይንስ ት/ክፍል በማስተርስ ድግሪ ተማሪ የመመረቂያ ጥናት ላይ እዲሳተፉ ተጋብዘዋል፡፡እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብ በጥሞና ያንብቡና ግልጽ ያልሆነልዎትን ማንኛዉም ሃሳብ ይጠይቁ፡፡

መግቢያ

የጥናቱ ርዕስ “Assessment of peripheral blood blast count, selected hematological parameters with special emphasis on platelet count and mean platelet volume before and after chemotherapy in acute leukemia patients at — Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.”.

የእርስዎ በዚህ የደም ካንሰር ጥናት ላይ የሚኖርዎት ተሳትፎ ሙሉ በሙሉ በበጎፈቃደኝነት ላይ የተመሰረተ ነዉ፡፡በዚህ ጥናት ዉስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በነላ ለማቋረጥ የሚወስኑ ቢሆንም እንኩዋ በዚህ ሆስፒታል የሚሰጠዉ ማንኛዉም አገልግሎት አይቋረጥም፡፡ በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጹሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅዎታል፡፡

የጥናቱ ተሳታፊ ለመሆን የሚጠበቅበዎት ምንድን ነው?

በዚህ ጥናት ለመሳተፍ የሚስማሙ ከሆነ ናሙናዎ ለጥናቱ እንዲሚዉል መስማማት ይጠበቅብዎታል፡፡ ከተወሰደዉ ናሙና ላይ የሚገኙ መረጃዎች ከዚህ ሆስፒታል ዉጭ ለሚገኙና ለስራዉ አግባብነት ላላቸዉ ሰዎች ቢነገር የማይቃወሙ መሆኑን መስማማት ይጠበቅብዎታል፡፡ ይሁን እንጅ ይህ አይነቱ መረጃ የርስዎን ማንነት የሚገልጡ መረጃዎችን ማለትም ስም፤አድራሻና የስልክ ቁጥር የመሳሰሉትን መረጃዎቸን አይጨምርም፡፡ይልቁንም ለዚህ አገልግሎት ብቻ የሚዉል እርስዎን ለማወቅ የሚያስችል መለያ ቁጥር ጥቅም ላይ እንዲዉል ይደረጋል፡፡በተጨማሪም

ስለርስዎ አጠቃላይ የጤና ሁኔታ ለሚቀርቡ አንዳንድ ተጨማሪ ጥያቄዎች መልስ መስጠት ይኖርብዎትአል።

በዚህ ጥናት መሳተፍ የሚያስከትላቸዉ ቸግሮች ምንድን ናቸዉ?

ናሙና በሚሰበሰብበት ወቅት ምንም አይነት የከፋ ችግር አያጋጥምዎትም።ሆኖም ግን ናሙናዉን ለመሰብሰብ ልምድ ያለዉ ባለሙያ ስለሚመደብና አስፈላጊዉ የጥንቃቄ እርምጃ ስለሚወሰድ የህመም ስሜት አይኖርም።

የህክምና መረጃ በሚስጥር ተጠብቆ መቆየት የሚችለዉ እንዴት ነዉ?

ስለራስዎ የሰጡት ማንኛዉም መረጃና ከተወሰደዉ ናሙና ላይ የተገኘዉ የላቦራቶሪ ዉጤት የሚዉለዉ ለጥናቱ አላማ ብቻ ነዉ።ይህን ማህደር ሊያገኙ የሚችሉት የተወሰኑ የጥናቱ ተባባሪ ሰዎች ብቻ ናቸው።ከዚያም በላይ ስለእርስዎ ያለውን ማንኛውንም መረጃ የተለየ የይለፍ ቃል ባለው የኮምፒውተር የመረጃ ማህደር ውስጥ እንዲቀመጥ ይደረጋል።

በዚህ ጥናት መሳተፍ የሚያስገኛቸው ጥቅሞች ምንድን ናቸው ?

ይህ ጥናት የማስተርስ ዲግሪ መመረቂያ እንደመሆኑ መጠን በዚህ ጥናት በመካፈልዎ በገንዘብ የሚያገኙት ጥቅም ባይኖርም ከጥናቱ በሚገኘው ውጤት ግን ተጠቃሚ ነዎት።የእርሶዎ ተሳትፎ የእርስዎንና የወገንዎትን የደም ካንሰር ለማወቅና ለማከታተል ከፍተኛ ጥቅም ይኖረዋል።

በዚህ ጥናት ተሳታፊ የመሆንዎ መብቶች ምንድን ናቸው ?

በዚህ ጥናት መሳተፍ ሙሉ በሙሉ በእርስዎ ፈቃደኝነት የተመሰረተ በመሆኑ በማንኛውም ሰዓትና ቦታ የማቋረጥ ሙሉ መብት የተጠበቀ ከመሆኑም በላይ እራስዎን ከጥናቱ በማግለልዎ ምክንያት የሚቀርብዎት ምንም አይነት የሆስፒታል አገልግሎት አይኖርም።ከዚህም በተጨማሪ ጥናቱን በተመለከተ ማንኛውንም አይነት ጥያቄ የመጠየቅና ገለጻ የማግኘት መብት አለዎት።የላብራቶሪ ምርመራ ውጤቱንም በነጻ ማግኘት ይችላሉ።ነገር ግን እርስዎ በሚሰጡን መረጃ የችግሩን ስፋት ለመከላከል እና ለመቆጣጠር ጠቃሚ ስለሆነ ለሚቀርብልዎት ጥያቄ ቀጥተኛ መልስ ይሰጡን ዘንድ በታላቅ አክብሮት እንጠይቃለን።

ጥያቄ ካለኝ ወይም ችግር ቢያጋጥመኝ ምን ማድረግ ይገባል?

ይህንን ጥናት በተመለከተ ወይም ከዚህ ጥናት ጋር በተዛመደ መልኩ ስለሚያጋጥሙ ድንገተኛ አደጋዎች ወይም ጥያቄ ካለዎት በሚከተለው አድራሻ ይጠቀሙ፡፡

ሰላም ሽብሩ

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Information sheet in English Version for parents and guardians

Title of the Research Project: Assessment of peripheral blood blast count, selected hematological parameters with special emphasis on platelet count and mean platelet volume before and after chemotherapy in acute leukemia patients at – Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.

Principal Investigator: Selam Shibru (BSc, MSc candidate)
Name of the Organization: TASH, Addis Ababa University College of Health Sciences, Department of Medical Laboratory Sciences.

Introduction

Your child is invited to participate as a study subject in a research conducted by MSc candidate, from Addis Ababa University. Your child participation is voluntarily. The research teams will include one principal investigator, three advisors; one from Addis Ababa University hematology department and two from lab and oncology department. Please take as much time as you need to read or listen in the information sheet.

Purpose of the Research Project

We are asking you to take part in this study because we will try to assess the prognostic significance of peripheral absolute blast count and platelet indices in acute leukemia.

Procedures and the expected participation

If you are willing on behalf of your child to participate, you need to understand the purpose of the study and give your consent. Not only this but also specimen collected from your child will be used for the research purpose, and the results of your child sample will be exposed to some concerned professional staffs as needed. The required clinical sample will be collected by phlebotomists of laboratory department. Then, you are requested to give your consent on behalf of your child to the sample collector. After consent, a sample will be taken from your vein of your child. Moreover, there will be a face-to-face interview for additional questions.

Potential risks and Discomforts

During collection of specimen from your child, appropriate precaution will be taken and all samples will be collected by trained health professionals. If anything happened, appropriate medical care will be provided to your child.

Confidentiality

We respect your child privacy and confidentiality. Any information that identifies your child will not be shared with anyone else outside the study team. The information we will collect from your child as part of the study will be kept in a locked file cabinet, or be protected by a password on the computer only accessible to personnel involved in the study.There is no sensitive issue that your child will be asked related with your social appeal but any information that is obtained in connection with this study and that can be identified with you will remain confidential.

Potential benefits to subjects and/or to the society

You will not receive any payment for your child participation in this research study as compensation. However, based on the result your child will be treated in view of that. In addition, the result of the study will be beneficial to assessment of prognosis in Acute Leukemia. Hence, your child is indirectly benefiting other patients and the society in this respect.

Participation and Withdrawal from the Study

The participation is voluntary and your child has the right not to participate in this study. Your child may withdraw at any time and place without consequences of any kind. Your child may also reject to give any sample. You can ask any questions regarding to this study and your child has a right to get a laboratory investigation result free.

Contact information

If you have any questions about this study you can contact the following principal investigators and advisors for further information.

Selam shibruPhone: 0922160964 **E-mail:**shibruselu@gmail.com

Dr. Aster Tsegaye Phone: 0911696085

Annex 3

Informed consent form in English version for adults

Card no…..……

I had been informed that the objective of this study is to assess peripheral blood blast count, selected hematological parameters with special emphasis on platelet count and mean platelet volume before and after chemotherapy in acute leukemia patients at – Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.. The results of this study have an importance to treat me and other patients, and to be used as an input for the future development of strategies or guidelines for diagnosing of acute leukemia in Ethiopia. I had been also informed about the confidentiality of this study. The principal investigator requested me to participate in the study that would require my willingness to provide the required data that include blood sample. Therefore, with full understanding of the importance of the study, I agreed voluntarily to provide the requested samples and my benefit will be only from the free laboratory investigation result/s.

I_____ hereby give my consent for providing the requested information and specimens as the doctors find best for me.

Signature: _____ Date_____

Informed consent Amharic version for adults

የተሳታፊዎች ስምምነት ማረጋገጫ

የሚስጥር ቁጥር ----------

የተሳታፊዉ ስም ----------

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ “Assessment of peripheral blood blast count, selected hematological parameters with special emphasis on platelet count and mean platelet volume before and after chemotherapy in acute leukemia patients at — Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia.”**ጥናት ላይ በቂ ገለጻ ተደርጎልኛል፡፡ ለጥናቱም የደም ናሙና እንደሚያስፈልግ ተገልጾልኛል፡፡የጥናቱንም አላማዎችም ተረድቻለሁ፡፡**

በቃለመጠይቁ ላይ የገለጽኳቸው መረጃዎች በሙሉ በሚስጥር የተጠበቁ እንደሚሆኑ ተነግሮኛል፡፡ በጥናቱ ላይ ያለመሳተፍና ማንኛውንም መረጃ ያለመስጠት እንዲሁም በማንኛውም ጊዜ ከጥናቱ ራሴን የማግለል መብቴ የተጠበቀ እንደሆነ ተገልጾልኛል፡፡

ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳትና በፍጹም ፍቃደኝነት ነው፡በተጨማሪም ጥያቄ ለመጠየቅ ተፈቅዶልኝ ለማወቅ የፈለኩትን ያህል ማብራሪያ አግኝቻለሁ፡፡የዚህ ጥናት ተሳታፊ በመሆኔ የማገኘው ጥቅም የሁሉንም ምርመራ ውጤት በነጻ ማግኘት እንደሆነ ተረድቻለሁ፡፡

በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ አንብቤዋለሁኝ፡፡ ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ፡፡

ፊርማ---------- ቀን ----/---/----------

(የስምምነት ቅጹን ማንበብ ለማይችሉ ተሳታፊዎች)

የአማካሪ ነርስ ስም ---------- ፊርማ ----------

ቀን----------

Assent form English version for under age group (12-17)

I have read the information above, or it has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. I voluntarily assent that I would participate in this study provided my parents/guardians give their consent.

Signing here means that you have read this form, or have had it read to you, and that you are willing to be in this study.

I_____ hereby give my assent for providing the requested information and specimens as the doctors find best for me.

Signature: _____ Date_____

If illiterate;

Print name of independent literate witness, date and signature of witness (if possible, this person should be selected by the participant and should have no connection to the research team)

_____ Date _____

Phone number (parents/guardians)_____

Print name of researcher, date and signature of researcher

_____ Date _____

Assent form Amharic version for 12-17 age group

ከላይ የተገለጸውን መረጃ አንብቤዋው ወይም ተነቦልኛል።ጥያቄ የመጠየቅ በቂ እድል ተሰጥቶኛል

የተሰጠኝም መልስ በቂ ነው። በዚህ ጥናት ላይ ለመሳተፍ ፍቃደኛ ነኝ ።

የተሳታፊው ስም _____

ቀን _____

ፊርማ _____

(የስምምነት ቅጹን ማንበብ ለማይችሉ ተሳታፊዎች)

የአማካሪ ነርስ ስም ---------- ፊርማ ----------

ቀን----------

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 duly acknowledged.

M.Sc. candidate: Selam Shibru (B.Sc.)

Signature:_____

Date of submission:_____

This proposal has been submitted with our approval as advisors.

Advisor: Aster Tsegaye (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Dr amha G/Medhin (MD, Hematologist)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Dr Abdulaziz Sherif (MD, Hematologist)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Dr Fissehatsion Tadesse (MD, Hematologist)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Dr Daniel Hailu (MD, Pediatric Hematologist)

Signature:

Date:

Place: Addis Ababa, Ethiopia.

Advisor: Elias Bisrat (MSc, Hematology Immunohematology specialist)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia