Prevalence of leukemia among patients who have abnormal hematological parameters in Jimma Medical Center (JMC) from January 1 to April 30 2019

By; Woldeteklehaymanot Kassahun (MSc candidate)

A RESEARCH THESIS SUBMITTED TO JIMMA UNIVERSITY, INSTITUTE OF HEALTH, SCHOOL OF MEDICAL LABORATORY SCIENCES FOR THE PARTIAL FULFILMENT OF DEGREE OF MASTER OF SCIENCES IN CLINICAL LABORATORY SCIENCES-SPECILITY IN HEMATOLOGY AND IMMUNOHEMATOLOGY.

June, 2019

Jimma, Ethiopia

Jimma University

Institute of health

Faculty of Health Sciences

School of medical laboratory sciences

Prevalence of leukemia among patients who have abnormal hematological parameters in Jimma Medical Center(JMC) from January 1 to April 30 2019

By**;** Woldeteklehaymanot Kassahun (MSc candidate)

Advisors;

1. Dr. Tilahun Yemane (MD, MSc)
2. Mr. Girum Tesfaye (MSc)

June, 2019

Jimma, Ethiopia

Declaration

This is to certify that the Thesis prepared by Woldeteklehaymanot Kassahun entitled: **Prevalence of leukemia among patients who have abnormal hematological parameters in Jimma Medical Center(JMC) from January 1 to April 30 2019** and submitted in the partial fulfillment of the requirements for the Degree of Master of Science (Clinical Laboratory Science-Hematology and Immunohematology) complies with regulation of the university and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

Principal Investigator;Woldeteklehaymanot Kassahun Sign…....…….Date…….

Advisors;

1. Dr. Tilahun Yemane Sign……...Date…….

2. Mr. Girum Tesfaye Sign……..…..Date……

Examiners;

1. Dr. Driba Fufa Sign ……...…...Date……

2. _____ Sign ……...…...Date……

Abstract

Background; Leukaemia is a heterogeneous group of haemopoietic cancers that comprise a number of diverse and biologically distinct subgroups. It is a global issue which addresses all regions of the world. Overall, these hematological cancers represent the 11th and 10th most frequent cause of cancer occurrence and death worldwide respectively. In Ethiopia, there is no adequate data regarding the current status on its prevalence and associated factors.

Objective; This study was aimed to determine the prevalence of leukemia among patients who have abnormal hematological parameters in Jimma Medical Center from January 1 to April 30 2019

Methodology; A cross-sectional study was conducted among patients who have abnormal hematological parameters in Jimma Medical Center from January 1 to April 30, 2019. A consecutive sampling method was used. Ethical clearance was obtained from Jimma University institutional review board and consent from the study subjects or their attendants or guardians. Sociodemographic data was collected by using structured and pretested questionnaire. A total of 332 patients with abnormal hematological parameters were included and their CBC parameters as well as peripheral blood morphology and Bone marrow aspiration with Wright's stain and cyochemistry study with Sudan black B stain was performed. data was entered with Epidata v4.4.31 and Descriptive statistics for theprevalence of leukemia was analyzed with IBM SPSS v25.

Results and Conclusion; A total of 332 patients were studied. The overall prevalence of leukemia was 9.3% while prevalence by type of leukemia was AML = 3.6%, ALL=2.7%, CML=1.8% while CLL, MDS and undifferentiated Leukemia had a prevalence of 0.6% and 0.3% respectively. The prevalence of leukemia is significant amount that needs further investigations in regard to the associated factors and predictors.

Key words; Leukemia, prevalence, patients, abnormal hematological parameters, Ethiopia

Acknowledgments

In this place where words of gratitude are needed, I would call Dr. Tilahun Yemane, thank you for your worthy inspiring words, comments and unaffordable experience sharing. Mr. Girum Tesfaye, your critical comments shaped the way this work shall go and it was right to call you for help because you did it. Mr. Wondimagegn Addissu and Dr. Driba Fufa, I feel your hands in this work, it was great to go through your suggestions. The last but not the least thank shall be for Mr. Lealem Gedefaw, it was heart filling to get advice from you. And those who are not mentioned here due to my forgetfulness thank you for your hands!

Acronyms and Abbreviations

- ALL = Acute Lymphocytic/lymphoid Leukemia
- AML = Acute Myeloid Leukemia
- ASR = Age adjusted incidence rate
- BASO = Basophil
- CLL = Chronic Lymphoid leukemia
- CML = Chronic Myeloid Leukemia
- EDTA = Ethyldiaminetetra acetic acid
- EOS= Eosinophil,
- FAB= French-American- British Classification
- GLOBOCAN = Global Burden of Cancer Study
- Hgb = Hemoglobin,
- IARC = International Agency for Research on Cancer
- JMC = Jimma University Medical Center
- JMCL= Jimma University Medical Center Laboratory
- LYM=Lymphocyte,
- MONO= Monocyte,
- NEU=Neutrophil,
- PLT = platelets,
- RBC= Red Blood Cell,
- SBB= Sudan B black stain
- SOP = Standard Operating Procedure
- WBC= white blood cell,

List of tables and figures

Table of Contents

Introduction

I. Background

Leukaemia is a heterogeneous group of haemopoietic cancers that comprise a number of diverse and biologically distinct subgroups. It is a clonal neoplasm of hematopoietic cells which are resulted from arrays of factors leading to somatic mutations in pluripotent stem cells and progenitor cells. This mutated neoplastic cell behaves like a hematopoietic stem cell in that it can self-replicating, can differentiate, and feed progenitor cells into the various hematopoietic lineages. These leukemic, unipotential stem cells can undergo varying degrees of maturation to phenocopies of mature blood cells(1, 2).

Even though the exact cause of malfunctions in the hematopoietic stem cells that result in malignant transformation are not exactly understood, several risk factors for the development of leukemia have been identified. The clonal neoplasms result from acquired driver and cooperating mutations within a pluripotent stem cell or a multi-potential progenitor cells (3).

The drivers of neoplasm are of varying arrays which mainly include hereditary inheritance, ionic radiation, chemical and other occupational exposures, therapeutic drugs, and some viral agents have been implicated in the development of Leukemia(4).

A mutation is a change that occurs in our DNA sequence, either due to mistakes when the DNA is copied or as the result of environmental factors. This genetic change can cause translocations, inversions, duplications (e.g., trisomy, tetrasomy), and deletions of chromosomes which result in the over or under-expression of regulatory genes and enzymes leading to mutation(5).

Two types of classification systems are commonly used for leukemia. (i)The French-American-British (FAB) classification system is based on morphology and cytochemical staining to define specific types. (ii)The World Health Organization (WHO) classification reviews information—morphology, cytochemistry, immunophenotyping, cytogenetics, and clinical features to define clinically significant disease entities (6).

Based on the clinical onset, leukemias may either be of an acute or chronic nature which further subdivided on the basis of the type of blood cell lineage affected as Acute Lymphoblastic Leukemia (ALL), Acute Myeloid Leukemia (AML), Chronic Lymphocytic Leukemia (CLL) and Chronic Myelogenous Leukemia (CML)(7).

Acute leukaemia is normally defined as the presence of over 20% of blast cells in the blood or bone marrow at clinical presentation. The lineage of the blast cells is defined by microscopic examination (morphology and cytochemical staining), immunophenotyping(flow cytometry), cytogenetic and molecular analysis(8).

The chronic leukaemias are distinguished from acute leukaemias by their slower progression. In contrast to acute leukemia, they tend to have a more protracted clinical course as the malignant cells maintain the ability to mature during the chronic phase of the disease(8, 9).

In the 1970s, a group of French, American, and British leukemia experts divided AML into subtypes, M0 through M7, based on the type of cell the leukemia develops from and how mature the cells are. This was based largely on how the leukemia cells looked under the microscope after routine staining and Cytochemistry. Subtypes M0(AML without differentiation), M1(AML with minimal differentiation), M2(AML without Maturation), M3(Acute promyelocytic Leukemia),M4(Acute Myelomonocytic Leukemia) and M5(Acute Monoblastic Leukemia) all start in immature forms of white blood cells. In M6 AML (Acute Erythroblastic leukemia) starts in very immature forms of red blood cells, while M7(Acute Megakaryoblastic leukemia) AML starts in immature forms of cells that make platelets dominantly(10, 11).

Even though the current and updated WHO classification of myeloid leukemia considered chronic myeloid leukemia as a distinct entity, the FAB classification classifies CML under the classification of Myeloproliferative Neoplasms(MPN) which consists the following:1. Chronic Myeloid Leukemia(CML) 2. Polycythemia Vera 3. Primary myelofibrosis and 4. Essential thrombocytosis or essential thrombocythemia(12).

Lymphoid malignancies represent a heterogeneous group of disorders divided into four categories based on the maturity of the neoplastic cells and the distribution of disease as: Acute lymphoblastic

leukemia(ALL), Chronic lymphoblastic leukemia(CLL), Malignant lymphoma and Plasma cell neoplasms(13).

Based on the FAB classification divides ALL into three types of L1, L3 & L3. Whereas the chronic lymphoid leukaemias are broadly separated into those of Chronic Lymphoid leukemia(CLL) and Hairy Cell leukemia(13, 14). The other type of myeloid leukemia group is the myelodysplastic syndromes (MDS). The FAB classification of MDS divided into five subgroups; Refractory anemia (RA), Refractory anemia with ringed sideroblasts (RARS), Refractory anemia with excess blasts (RAEB), Refractory anemia with excess blasts in Transformation (RAEBt), and Chronic Myelomonocytic leukaemia (CMML)(15).

Most patients with a myelodysplastic syndrome (MDS) are over the age of 60 years. The clinical course varies with some patients having a more indolent course and longer life expectancy, while others present with aggressive disease that evolves rapidly into acute myeloid leukaemia (AML). Overall, approximately 40% of patients will transform to AML during the course of their disease(15).

Acute myeloid leukaemia (AML) can occur at all ages but its peak incidence is in the seventh decade. On the other hand, median age at diagnosis for ALL is 13 years and nearly 60% of cases are diagnosed under the age of 25. There is a sharp peak in ALL incidence among 2–3 year olds which decreases for 8–10 year olds. The incidence is higher in boys than in girls. Although ALL is rare in adults, there is an increasing incidence with age after the age of 40 years(15).

CML is predominantly a leukemia of middle-aged adults. Males have a slightly greater rate of disease occurrence. CLL is the most common form of leukemia in adults with median age of onset 65 years. More males than females (1.5 to 2.1:1) are afflicted by the disorder(12).

Leukemia is diagnosed in the laboratory by using peripheral blood or bone marrow aspirate of the patient. The sample can be analyzed by using different methods of laboratory diagnosis such as full blood count, peripheral morphology examination with routine stains(Wright's stain), cytochemistry, immunohistochemistry, flow cytometry, immunophenotyping and molecular analysis techniques(16).

II. Statement of the problem

Leukemia is the 8th to 12th most common cancer disease of the globe. It affects all nations and peoples of the world without indiscrimination based on their sociodemographic background, even though it has greater risk in urbanized populations(5).

Leukemia causes about 265,471 deaths worldwide in 2012, where 24,400 were in Europe, 1,840 in Australia, 21,121 in Africa (by 2012) , and 3,230 deaths in Ethiopia by 2014(6, 17-19).

Leukemia affected 200,676 males and 151,289 females (with an ASR 5.6 and 3.6 respectively) globally. In Europe there were 46,449 males and 35,880 female patients diagnosed for leukemia in 2012. About 233,451 populations were diagnosed as leukemic patient in Australia, Asia and USA by 2017. While the it has an incidence of 23,928 cases (ASR 3.0 per 100,000) in Africa by 2012(6, 19, 20).

In the Western countries of the world, leukemia is dealt in depth and there is sufficient data regarding the health statistics of their population. In Ethiopia, even though leukemia and generally cancer is becoming the globe's great deal in the 21st century, there is little evidence on the current status of the disease among the population.

The impact of leukemia in developing countries is enormous due to premature death of children, loss of parents, loss of productivity due to disability and high medical cost which affects the socioeconomic and health welfare of the population. Therefore, the need to conduct this research on the prevalence of leukemia among patients is a big worth giving an insight and serving as a baseline data for further investigation(21-23).

III. **Significance of the study**

Conducting this kind of research is mandatory for the good and evidence based management of the healthcare and the wellbeing of the community since there is little step put before. This study will provide the baseline evidence of the issue by providing the current prevalence of leukemia in the study area which may represent the section of the population. For health policy makers, this study will help to plan and allocate resource in the context of the urgency and bold health problem.

Literature review

There were an estimated 14.1 million cancer cases around the world in 2012, of these 7.4 million cases were in men and 6.7 million in women. And accordingly, leukemia takes the position 11th cancer disease worldwide having an incidence of about 352,000(which accounts 2.5 % of all cancers) cases diagnosed in 2012. Leukaemia incidence rates are highest in Australia/New Zealand and lowest in Western Africa, but this partly reflects varying data quality worldwide(5, 24).

In United Kingdom(UK), 9,900 new leukemia case were diagnosed which cover 3% portion of all cancer cases diagnosed in 2015. It is the 12th most common cancer in the UK and 59% of leukaemia cases are in males while the 41% are in females. Incidence rates also have increased overall in all broad age groups in males and females combined in the UK since the early 1990s. Rates in 0-24s have increased by 10%, in 25-49s have increased by 14%, in 50-59s have increased by 17%, in 60-69s have increased by 21%, in 70-79s have increased by 23%, and in 80+s have increased by 12%(25).

Accordingly, research data from Ireland, put that Leukaemia made up about 1.7% of all registered and 2.0% of malignant cancers diagnosed between 1994 and 2008. CLL was the commonest type in both sexes, comprising 41% of cases in men and 37% in women. 30% of ALL was diagnosed before the age of 5, and 55% before age 15 the rest 15% diagnosed after age 15. The other types were more common in older people - 54% of CLL was diagnosed in those aged 70 and over. By subtypes, CLL accounts 39% followed by AML- 20%, ALL-12%, CML 9% and then other leukemias specified and unspecified 10% each(26).

In USA 62,130 new cases of leukemia were diagnosed in 2017. Among the subtypes of leukemia, cases of AML were 21,380 (34.4%), CLL were 20,110 (32.36%), CML were 8,590 (14.4 %), ALL were 5,970 (9.6%) and other unspecified were 5,720 (9.2%). The majority (92%) of the cases were diagnosed among adults 20 years and older. Among these cases, the most common were CLL (37%) and AML (31%). In children aged 0-19, Leukemia accounts 29% all of childhood cancer in which of these ALL comprises 75%(20).

Leukaemia was the 8th most commonly diagnosed cancer in Australia in 2017. In 2013, there were 3,359 new cases of leukaemia diagnosed in Australia (2,045 males and 1,313 females). In 2017, it is estimated that 3,875 new cases of leukaemia were diagnosed in Australia (2,358 males and 1,517 females). Leukemia accounts 2.9% of all cancer cases. The incidence rate by age standardized rate is from 1.9 to 93.8 person-years (age groups of 20-24 and 85+ respectively)(27). Based on the Australian 2011 total population, the total burden of the disease was 3.7%(28).

An estimated 138,100 people in Canada are living with, or are in remission from, different forms of blood cancers which of these 22,510 (16.3%) peoples (13,040 males and 9,470 females) are living with or are in remission of leukemia in 2016. And also in 2016 approximately 22,340 Canadians of all ages were diagnosed with a form of blood cancer of which 5,900 (26.4%) cases of leukemia were identified with an incidence rate of 15 per 100,000 person-years. The most common types of leukemia in adults are AML and CLL. The median age at the time of diagnosis is 67 for AML and 71 for CLL. One in 53 men and one in 72 women will develop leukemia in their lifetime. From a data developed in 1991-2008, the distribution of leukemia by subtypes shows that the higher proportion were taken by CLL (44%), then AML (24%), CML (12%), ALL (5%) and other unspecified subtypes with 15%(29).

In the same manner, in China, from a five years (2003-2007) cancer registry data, there were 65,000 new cases of various types of leukemia. The overall incidence rate was 5.4 in men and 4.2 in women while the age standardized incidence rate(world) were 5.0 in men and 3.7 in women. Of the subtypes of the disease, ALL in males was the highest with an incidence rate of 1.5 followed by AML of 1.4 per 100,000 rate in males. The subtypes of ALL, CLL, AML, CML and unspecified types had a value of incidence 1.2 in females, (0.3 in males & 0.2 in females), 1.2 in females, (0.7 in males and 0.4 in females) and 0.1 per 100,000 in each sex groups respectively(5, 6).

A cross-sectional retrospective study by morphological subtyping of 196 cases of leukemia in a tertiary teaching hospital in Nepal between January1997 to December 2002 showed that 121 cases were of acute leukemia and 75 of chronic leukemia. Amongst the acute leukemia, there was preponderance of AML 56 cases (28.57%) with ALL 39cases (19.9%) following it. Of 75 cases of chronic leukemia, 69 were of CML (35.20%) and 6 of CLL (3.06%). Undifferentiated leukemia

constituted 7 cases (3.57%). The distribution of cases in male and female ranges from 1.42;1 to 2.72:1 in acute and chronic leukemia respectively(2).

From a one year (from January 2016 to March 2017) observational cross-sectional study in Pakistan- Peshawar, 145 cases of leukemia were found. Seventy-two (49.7%) patients were having ALL, Thirty-nine (26.9%) were having AML, fourteen (9.7%) patients were detected with CLL and CML was found in twenty (13.8%) patients. The gender wise distribution of leukemia among males and females were 1.67;1(30).

In Albania, an observational cross-sectional study conducted from January 2015 to October 2016 revealed that there were 277 cases of Leukemia with that 33.3% are women and 66.7% are male. Of the total 277 cases, 71.41% of the patient had lymphoid leukemia, (61.01% are chronic and 10.4% are acute) and Myeloid Leukemia was represented with 28.51%, (8.3% are chronic and 20.21% are acute)(31).

On the other hand, in a cross-sectional research performed in Haryana- India, 650 cases were analyzed with leukemia during the period 2008-2012. 33.8% of patients were affected with AML, 39% patients with CML, 17.2% patients with ALL and 10% with CLL. There were 71.4% male patients with chronic leukemia and 62.6% male patients with acute leukemia. Additionally, Acute and chronic leukemia had 28.6% and 37.4% female patients respectively. The sex distribution of the disease was 2:1 between male and female while the age distribution shows that, ALL was more commonly noted in children (<15 years) whereas AML was more commonly noted in adults (>15 years). Both CML and CLL were observed only in adults (>15 years)(32).

In comparison, in Africa, data are limited regarding cancer incidence and prevalence specially on blood cancers. Eventhough the fact is that, based on the 2012 GLOBOCAN analysis and estimation of leukemia, 23,928 leukemia cases were diagnosed between 2003-2007. From this statistic the highest (5,037 cases with 5.6 ASR per 100,000 in men and 3,731 cases with 3.9 ASR per 100,000 in women) was recorded from Northern African region followed by the Eastern African region with 4,854 cases with 3.8 ASR per 100,000 in men and 4,116 cases with 3.4 ASR per 100,000 populations(5).

A retrospective study of childhood malignancies diagnosed, in Nigeria, there were 116 Acute leukemia cases diagnosed between January 2001 to December 2010. Out of these 83 were males and 33 were females. While from 2003-2007, as of the GLOBOCAN data, in Egypt the Age Standardized rate per 100,000 of leukemia subtypes in male and females sex shows ALL:1.3 & 1.0, CLL: 1.0 & 0.9, AML: 0.9, CML : 0.7 &0.8, other specified lymphoid undifferentiated leukemia 0.1 and other undifferentiated myeloid leukemia 0.3 each respectively(5, 33).

In similar way, in Sudan Khartoum, 140 acute myeloid leukemia cases were diagnosed between December 2013 to September 2015, of which 70 were males and 70 were females with male to female ratio1:1. 47 cases (33.6%) were in age range of 3-18 years comprising of 27 males and 20 females and 93 cases (66.4%) were adults more than 19 years. The subtypes of the disease based on FAB Classification showed that M3 was frequently observed with a 36.2% incidence in patients <18 years of age and 26.9% in adults >18 years of age. While the lowest was seen in M6 with 2.1% and 2.2% in children <18 and adults >18 years of age respectively(34).

When we come to Ethiopia, there is little information on previously published data regarding the rate of prevalence of leukemia among Ethiopians. A retrospective study conducted in adult Ethiopians admitted to Tikur Anbessa specialized Hospital, from January 1982 to December 1987 the pattern of leukaemias was analyzed. There were a total of 7,969 medical admissions, of which 180 (2.3%) were for leukaemia. The age range was 14 to 80 years, with a mean of 37.6 years. The male to female ratio was 2.3:1. The commonest type of leukaemia was CML 57.8%, acute leukaemias and CLL accounted for 21.1% each. Of the acute leukaemias, 53.3% were ALL while 46.7% were AML. The overall incidence of leukaemia in Addis Ababa population was estimated at 1.95 +/- 0.79/100,000/year at 95% confidence interval.(35-37)

Similarly, Eighty-two adult Ethiopians were admitted to Tikur Anbessa Specialized Hospital with cases of acute leukaemia from January 1982 to December 1992. AML and ALL occurred in 53.7% and 46.3%, respectively with age range 13-78 (mean 29.6) years. The male to female ratio was 1.6:1. On the other hand, 102 cases of CLL were seen at the Hospital from January 1982 to December 1994 who were in age range between 35-91 (mean 55.6 +/- 11.08) years. The male to female ratio was 3.6:1(38, 39).

By the statistical data of WHO country profile for Ethiopia in 2014, the rate of incidence of leukemia, 1,947 in males and 1,558 in females were recorded accounting 12.7% and 5.3 % of cancer deaths in males and females respectively among 14,500 men and 26,200 female deaths. Whereas a prospective cross-sectional study carried out in patients of all age groups visiting oncology unit of Tikur Anbesa specialized hospital between November 2015 to June 2016, thirteen leukemia cases were diagnosed out of 142 cancer cases with 1.6:1 male to female ratio. Of these leukemias, 6 were AML (4 males and 2 females), 6 were ALL (3 males and 3 females) while 1 case were CML in male(17, 40).

Additionally, a retrospective study of 3 year period carried out among children aged below 15 years old admitted into the pediatric wards of Gondar University Hospital, Northwest Ethiopia resulted a total of 71 cancer cases were diagnosed in which 43 (60.6 %) were hematological malignancy out of these 13 (33%) were leukemia(41).

Recently, a population based cancer registry data analysis and estimation revealed that about 1,386 and 1,886 leukemia cases have diagnosed with an age adjusted incidence rate of 2.8 & 3.8 in men and women respectively. It also stated that leukemia was the most commonest cancer among children (accounting 29% of 3,707 pediatric cancer cases) and Acute leukemia accounted for 89% (of which 91% was acute lymphocytic leukemia and 9% was acute myeloid leukemia) of all the leukemia cases in children(42).

Objective

I. General objective

To assess the prevalence of leukemia and associated factors among patients who have abnormal hematological parameters in Jimma Medical Center(JMC) from January 1 to April 30 2019.

II. Specific objective

- To determine the prevalence of leukemia among patients who have abnormal hematological parameters in JMC from January 1,2019 to April 30,2019.
- To identify the types of leukemia among patients who have abnormal hematological parameters in JMC from January 1,2019 to April 30,2019.

Methodology

I. Study area

Jimma Medical Center(JMC) is one of the university teaching hospitals found in Southwest Ethiopia in Oromia regional state, Jimma town about 350 Kms southwest of the capital, Addis Ababa. It was stablished in 1930 as Ras Damitew Hospital. The medical center provides different arrays of clinical services which relays on laboratory tests and procedures results produced by the JMC laboratory(JMCL). JMCL performs different sets of laboratory tests and procedures, of these Hematology Laboratory is the one which can be distinguished. In Hematology Laboratory, Complete blood count, peripheral blood morphology assessment and other hematologic diagnostic tests are performed. In the medical center about 200 pediatric cancer patients have been admitted in the period from August 2016 to August 2018. From these patients, 55 patients were diagnosed with leukemia.

II. Study period

This study was conducted from January 1 to April 30, 2019.

III. Study design

A facility based cross-sectional study design was applied.

IV. Population

A. Source population

The source population of the study were all patients who have abnormal hematological parameters.

B. Target population

Target population of the study are those patients who have abnormal hematological parameters in in JMC during the study period.

C. Study units(population)

Patients who have abnormal hematological parameters and fulfilled the inclusion criteria was the study units (population)

V. Sampling method

Consecutive sampling method was applied.

VI. Sample size determination

The size of the study sample was calculated by using the P value 0.315(31.5%) from a study By Solomon T.M. and colleagues in Ethiopia(42) with 95% confidence interval;

$$n = \frac{\left(Z\alpha/_2\right)^2 * P(1-P)}{d^2} = \frac{1.96^2 * 0.315(1-0.315)}{(0.05)^2} = 332$$

where *n*= number of sample units; *p* = prevalence and *d* = margin of error.

By considering 10% nonresponse rate, the total sample size was 366.

*During the data collection there were no non-respondents.

VII. Variables

A. Dependent variables

- Leukemia

B. Independent variables

- Age
- Sex
- Residency
- Occupation
- CBC values

VIII. Inclusion and exclusion criteria

A. Inclusion criteria

- Blood samples with Completed Clerical data such as, date of sample collection, sex and age of the patient on the request paper as well as on the collection tube accordingly
- Blood sample collected with EDTA vacutainer tube and no more than 2 hours ago
- Complete blood count & analysis with abnormalities

B. Exclusion criteria

- Blood samples with clot or hemolysis

IX. Sample collection and Laboratory methods

Sample was obtained from patients' blood samples ordered for routine CBC analysis and the sociodemographic information was collected by using structured, pretested questionnaire. The laboratory methods which were implemented include complete blood count, peripheral morphology and Bone marrow aspirate study with Wright's stain and cytochemical study with Sudan Black B stain.

Laboratory diagnosis procedures were done according to the standard operation procedures (SOPs). Automation on Sysmex XS-500i and XT-1800 CBC analyzers (Sysmex Corporation Kobe, Japan), peripheral blood morphology staining with Wright's stain and cytochemical study with Sudan B Black stain was done. The detailed procedures for each laboratory methods are attached at the end of the document as Annexes.

Patients' samples who have below or above the reference interval of RBC, Hemoglobin concentration, WBC, and platelet count(43);

i. For children; (4.06–6.57 × 10^{12}/L), (120–196 g/L), (4.04–11.72 × 10^{9}/L), (158.5–469.9 × 10^{9}/L) respectively for males (4.32–5.63 × 10^{12}/L), (115.7–159.4 g/L), (3.74–11.42 × 10^{9}/L), (197.7–460.4 × 10^{9}/L), respectively, for females.
ii. For adults; (4.26–6.68×10^{12}/L), (120.6–187.6g/L), (3.31–11.62×10^{9}/L) and (164 - 403.4×10^{9}/L) respectively for males and (4.02–6.15×10^{12}/L), (123–178.6g/L), (3.24–10.05×10^{9}/L) and (202.3–444.5×10^{9}/L) respectively for females.
iii. For Geriatrics; (4.25–5.99×10^{12}/L), (126.4– 179g/L), (3.31-11.62x10^{9}/L), and (164-403.4x10^{9}/L) for males respectively and (3.91–5.72×10^{12}/L), (119.1–177.8g/L), (3,24-10.05x10^{9}/L) and (202.3-444.5x10^{9}/L) respectively for females.

Their peripheral morphology was examined with Wright's stain and Sudan Black B stain. Then if it becomes difficult for diagnosis Bone marrow aspirate examination was performed.

X. Operational definitions

Hematological parameters; complete blood count profiles which include, WBC, RBC, Hgb, Platelet and their derived parameters.

Abnormal hematologic parameters; patients' blood count values which are above or below the cut of values set.

Difficulty in classification; a condition where there is no certainty to classify morphology examinations in one of the FAB classifications of leukemia.

XI. **Laboratory work flow**

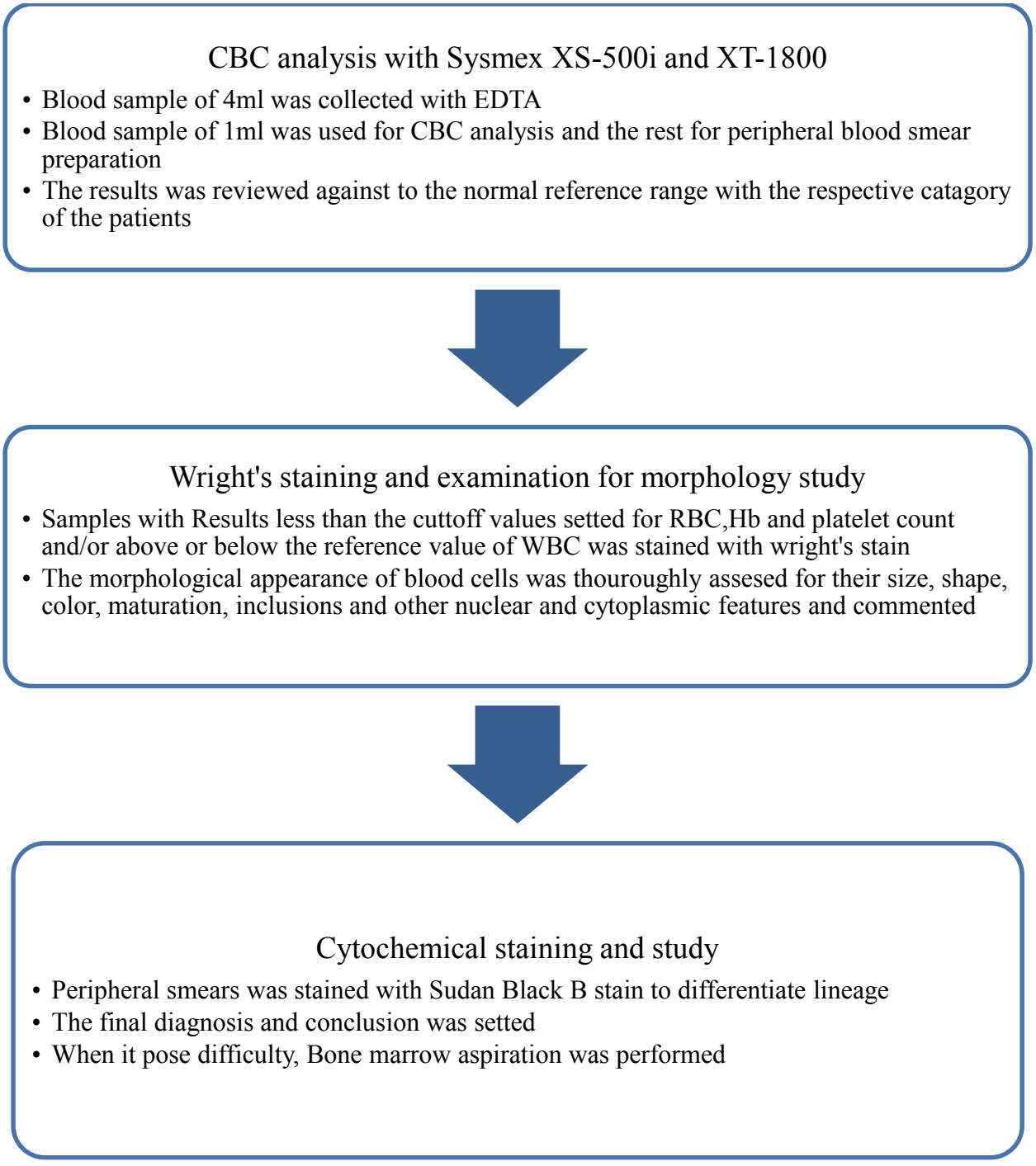

Figure 1: Labratory work flow(developed by PI)

XII. Data management and Statistical analysis

Data were entered in Epidata version 4.4.3.1 and analyzed by IBM SPSS v25. Descriptive statistical analysis was made including; Sums, proportions, percentage, mean, median and standard deviation while the relationship b/n variables was assessed by executing; Multinomial Logistic regression – with explanatory model and Odds Ratio - from the regression coefficients. Independent t-test was done to assess the mean difference of parameters which didn't show association with leukemia. At the end, Data out puts were presented with charts, tables and figures accordingly.

XIII. Ethical considerations

Ethical clearance was obtained from the Jimma University Institute of Health, Post graduate and research Director office, Institutional Review Board(IRB).

Letter of support was obtained from Health Research and Postgraduate director's office and School of medical laboratory sciences and letter of permission from JMC laboratory manager for the access of patient data and blood samples.

Informed consent was obtained from patients or their attendants or guardians of patients who have abnormal hematological parameters prior to morphological and cytochemical examinations.

No names were used in the data collection as well as analysis and dissemination.

Results

I. Sociodemographic characteristics

A. Age and sex distribution of the study units

In this study a total of 332 study subjects were included. Among these 180(54.2%) were female. With respect to the age category, most of them 179(53.91%) were within the age range of 15-49 years. The median age of the patients was 23 with a range 73 years.

Table 1: Age and Sex distribution of Patients who have abnormal hematological parameters in Jimma Medical Center from January 1 to April 30 2019

Age category(years)		Sex of the patient		Total
		Male	Female	
0-14	Count	63	64	127
	Percent	49.6%	50.4%	38.25%
15-49	Count	75	104	179
	Percent	41.9%	58.1%	53.91%
50+	Count	14	12	26
	Percent	53.8%	46.2%	7.83%
Total	Count	152	180	332
	Percent	45.8%	54.2%	100.0%

B. Residency distribution of study subjects

Of the total 332 patients who have abnormal hematological parameters 188(57%) were Urban residents.

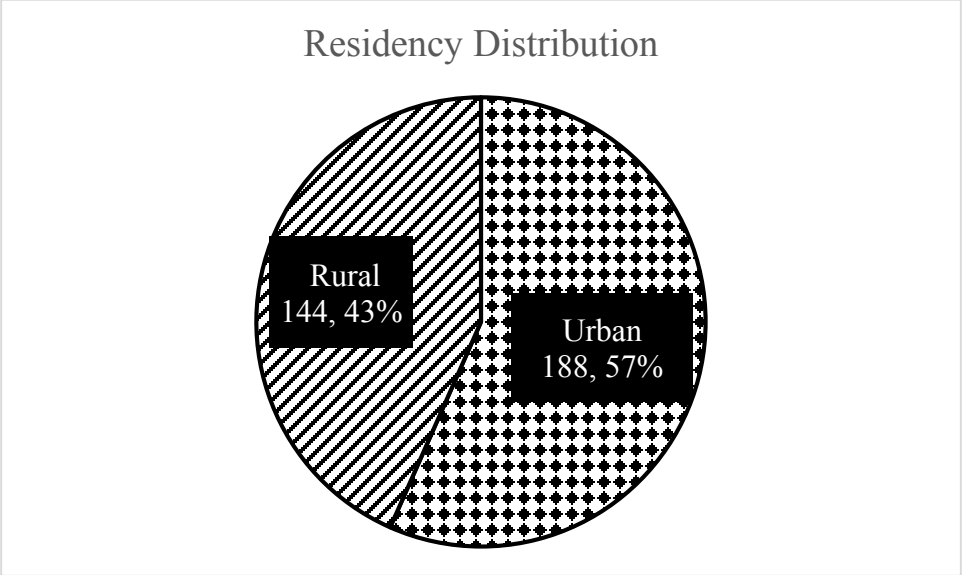

Figure 2: Residency distribution of Patients who have abnormal hematological parameters in Jimma Medical Center from January 1 to April 30 2019

C. Occupation distribution

From the total of 332 study patients who have abnormal hematological parameters, 232(69.88%) have provided their occupational status whereas 100(30.12%) didn't comply any occupation. Of these patients, government employees and students have the highest figure (66,19.88% each) while the least frequency were observed on private workers (39,11.75%).

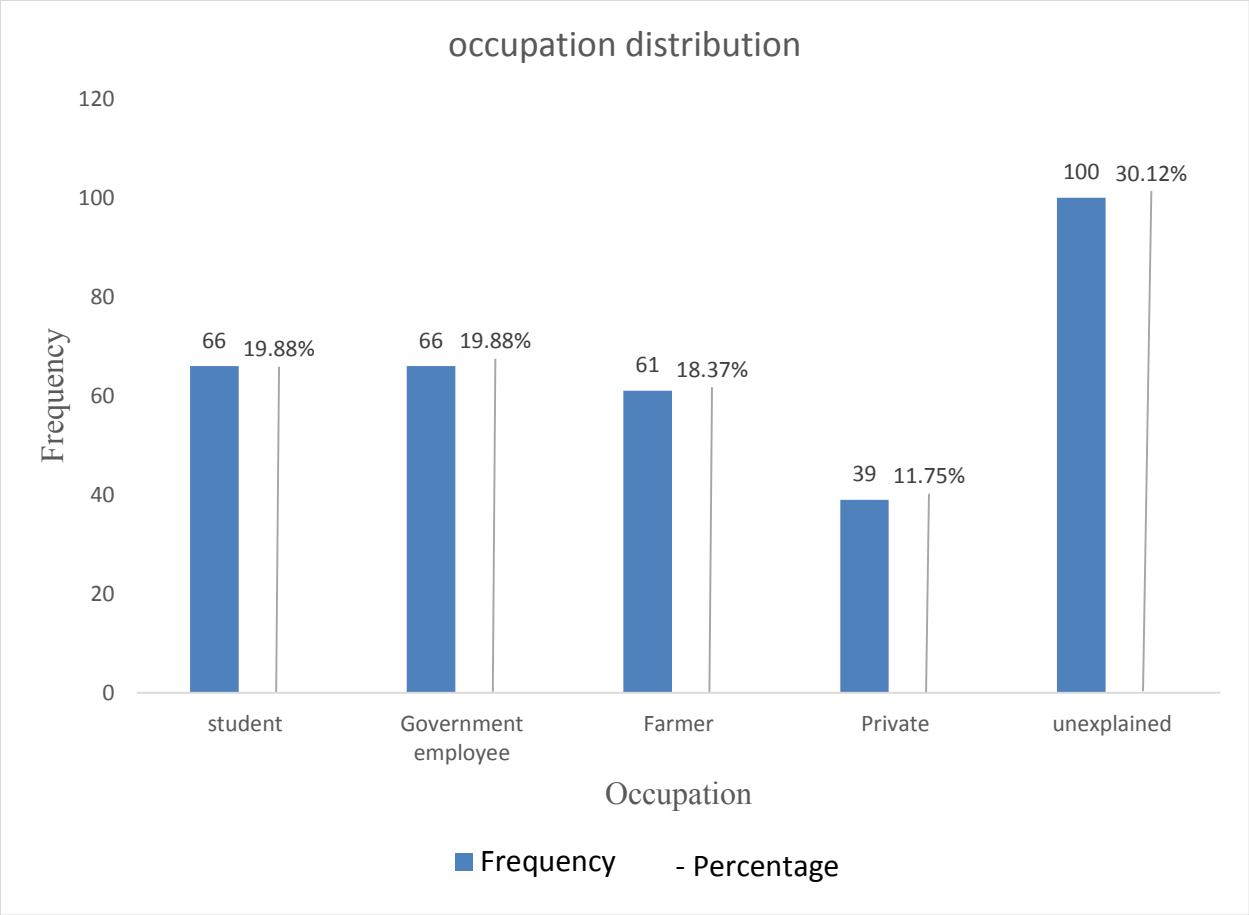

Figure 3: Occupation distribution of Patients who have abnormal hematological parameters in Jimma Medical Center from January 1 to April 30 2019

II. Prevalence of leukemia

In this study, from the total of 332 patients studied, 31(9.3%) have leukemia. The highest prevalence of leukemia was seen in males 4.8%(n=16). The prevalence of Acute Leukemia was 6.6% while Chronic Leukemia and Myelodysplastic Syndrome account 2.4% and 0.3% respectively. The most frequent type was Acute Myeloid Leukemia(AML) with a prevalence of 3.6%(n=12). As compared to Urban residents, the highest prevalence of leukemia was seen in patients who came from rural area which is 6.9%(n=23).

By leukemia subtype, from the total of 332 study patients who have abnormal hematological parameters, 3.6% had AML, 2.7% had ALL, 1.8% had CML while CLL, MDS and undifferentiated Leukemia had a prevalence of 0.6%, 0.3% and 0.3% respectively.

Table 2: Prevalence of leukemia among Patients who have abnormal hematological parameters in Jimma Medical Center from January 1 to April 30 2019

Characteristics		Diagnosis							Total
		Acute Leukemia			Chronic Leukemia		MDS		
		AML	ALL	Undifferentiated	CML	CLL	MDS	Non-malignant	
Sex	M	7(4.6%)	3(1.97%)	1(0.7%)	1(0.7%)	2(1.3%)	1(0.7%)	136(89.5%)	152
	F	5(2.2%)	6(3.3%)	0	5(2.8%)	0	0	165(91.7%)	180
Age group (years)	0- 14	2(1.6%)	7(5.5%)	0	1(0.8)	0	0	117(92.1%)	127
	15 – 49	4(2.2%)	2(1.1%)	1(0.6)	2(1.1%)	0	1(0.6%)	169(94.4)	179
	50+	6(23.1%)	0	0	3(7.7%)	2(7.7%)	0	15(57.7%)	26
Residency	Urban	2(1.1%)	3(1.6%)	0	2(1.1%)	1(0.5%)	0	180(95.7%)	188
	Rural	10(6.9%)	6(4.17%)	1(0.7%)	4(2.8%	1(0.7%)	1(0.7%)	121(84.0%)	144
Occupation	Student	3(4.5%)	4(6.1%)	0	1(1.5%0	0	0	58(87.9%)	66
	Gov't employee	0	0	0	1(1.5%)	1(1.5%)	0	64(97.0%)	66
	Farmer	7(11.5%)	1(1.6%)	1(1.6%)	4(6.56%	1(1.6%)	1(1.6%)	46(75.4%)	61
	Private	1(2.6%)	1(2.6%)	0	0	0	0	37(94.9)	39
	unexplained	1(1%)	3(3%)	0	0	0	0	96(96%)	100
Total		12(3.6%)	9(2.7%)	1(0.3%)	6(1.8%)	2(0.6%)	1(0.3%)	301(90.7%)	332
		22(6.6%)			8(2.4%)		1(0.3%)	301(90.7%)	332

III. Distribution of some hematological Parameters between types of Leukemia.

The distribution of some Hematological parameters with respect to leukemia types with reference values of WBC count (3.31-11.62x10^9/L), Platelets counts (145.5-444.5x10^9/L), Neutrophil count (1.01-7.22x10^9/L), Eosinophil count (0.05-1.21x10^9/L) and Basophil count (0.01-0.05x10^9/L) were analyzed and their proportion were calculated. In Acute leukemia, Neutropenia (60.0%)and thrombocytopenia (76.2%) were found while in Chronic Leukemia Neutrophilia (71.4%), Basophil leukocytosis (100.0%) and Eosinophilia (85.7%) were found. Leukocytosis was observed in all types of leukemia.

Table 3: Distribution of hematological parameters counts with respect to Leukemia type of patients who have abnormal hematological parameters in JMC from January 1,2019 to April 30,2019.

Hematological parameters		Leukemia Types			
		Acute Leukemia(N,%)	Chronic Leukemia(N,%)	MDS (N,%)	non-malignant(N,%)
WBC count	Leucopenia	4(19.0)	0	1(50.0)	37(12.3)
	Normal	3(14.3)	0	0	104(34.6)
	Leukocytosis	14(66.7)	8(100.0)	1(50.0)	160(53.2)
Platelet count	Thrombocytopenia	16(76.2)	2(25.0)	2(100.0)	153(50.8)
	Normal	5(23.8)	4(50.0)	0	118(39.2)
	Thrombocytosis	0	2(25.0)	0	30(10.0)
Neutrophil count	Neutropenia	12(60.0)	2(28.6)	2(100.0)	73(27.5)
	Normal	2(10.2)	0	0	100(37.7)
	Neutrophilia	6(30.0)	5(71.4)	0	92(34.7)
Basophil count	Low	7(38.9)	0	1(50.0)	78(28.7)
	Normal	4(22.2)	0	0	118(43.4)
	High	7(38.9)	6(100.0)	1(50.0)	76(27.9)
Eosinophil count	Low	11(64.7)	0	1(50.0)	111(39.6)
	Normal	5(29.4)	1(14.3)	1(50.0)	168(60.0)
	High	1(5.9)	6(85.7)	0	1(0.4)

Discussions

Knowledge of prevalence of leukemia in a population may predict etiologic hypotheses for disease control and help for effectual management. In developing countries, especially in Africa there is little information on the burden and pattern of hematological malignancies specifically-leukemia. In this study the overall prevalence of leukemia was 9.3% (95% CI= 8.8-9.8%). As per the GLOBOCAN estimate for 2018, the prevalence of leukemia in Ethiopia was estimated as 7.4%, while the result of this study shows relatively higher figure(5). The differences may arise due to differences in source population.

The prevalence of leukemia is thought to be different across sex because of biological factors. There were 332 patients in this study where the male to female ratio is almost equal even though the female slightly dominates (1:1.06) but with respect to leukemia preponderance, males slightly overweight (1.06:1). This figure is in line with data from Australia, Ireland, Canada, China and study by Solomon Tessema and colleagues in 2015, in Ethiopia(19, 26, 42, 44, 45). The preponderance of leukemia was observed in males with 51.6% while Females comprise 48.4% in which it was in agreement with a report from Albania; 66.7% were male and 33.3% were women(31). The reason why males are more affected than females is not clearly defined but from the sociodemographic picture of this study, most of the cases of leukemia are observed in farmers, in which males are the main players. The other reason which contribute for the cancer development may be due to mutation in a tumor suppressor gene called *Lysine Demethylase 6A*(*KDM6A*), located on the X chromosome. Frequent mutation of *KDM6A* gene are observed in males than females(46, 47).

Leukemias may present at all ages, from the newborn to the very old, but different forms have different age distributions(48). The mean age of this study was 34 years ±21.59 with a range of 73 years. Comparing within each age group, the highest proportion of leukemia (35.5%) was seen among patients older than 50 years while there was similar proportion between age groups 0-14 and 15-49(32.3% each). This high preponderance of older adults (p<0.001) for leukemia may be mainly due toageing where malignant mutations occur frequently.

Environmental factors, even though not well articulated, have an influence on the chance of developing leukemia. In this study, the highest proportion 74.2% of leukemia was observed among rural residents(p<0.0003). This may be related to use of chemicals such as pesticides, herbicides and fertilizersused for agricultural activities and/or the working environment(49).

On the other hand, regarding the occupation distribution of patients who have leukemia, the highest proportion 55.6% was recorded in patients who were farmers (p<0.001). This result corresponds with the casethat they were from rural residency. The second highest proportion 29.6% was seen among patients who stated they were students; even though being students doesn't have any biological relation withleukemia, this may be due to the age distribution that most of the children are students.

The types of leukemia were identified by using the FAB- classification method, morphological examination stained with Wright's stain and cytochemical staining with Sudan Black B stain to differentiate, between cell lineage. In this study, the leukemia was classified as Acute Leukemia, Chronic Leukemia and Myelodysplastic Syndrome, which then further classified in to specific types as Acute Myeloid Leukemia(AML), Acute Lymphoid Leukemia(ALL), Chronic Myeloid Leukemia(CML), Chronic Lymphoid Leukemia(CLL) and Myelodysplastic Syndrome(MDS). The prevalence of Acute Leukemia was 6.6% while Chronic Leukemia and Myelodysplastic Syndrome account 2.4% and 0.3% respectively. Out of the total leukemia cases, the proportion of Acute and Chronic Leukemia showed 70.96% and 25.80% respectively. This was in line with the finding from Nepal and Pakistan(2, 7) while it was in contrast with Albania(31).

Regarding the distribution of some hematological parameters with types of leukemia, In Acute leukemia, Neutropenia (60.0%) and thrombocytopenia (76.2%) were found while in Chronic Leukemia, Neutrophilia (71.4%), Basophil leukocytosis (100.0%) and Eosinophilia (85.7%) were observed. This result is supported by biological process that in Acute Leukemia, due to the infiltration of malignant immature cells in the bone marrow and maturation arrest, there will be neutropenia anemia and thrombocytopenia in the peripheral circulation(50, 51). On the other hand, Chronic leukemia is characterized by markedly increased bone marrow activity, resulting in increased number of white blood cells in the bone marrow as well as in the circulation, and for CLL in the lymph nodes. In CML, morphologically and functionally normal granulocytes, predominantly

comprised of neutrophils and myelocytes are present. Basophil leukocytosis is a distinct feature of CML, although Eosinophilia also usually present(52).

Of the types, the most frequent one was AML,38.7%(n=12) whereas the rarely found leukemia type was MDS(3.2%,n=1). There was one case which cannot be classified as AML or ALL and grouped as Undifferentiated. The rest types of leukemia; ALL, CML and CLL have accounted, 29.0%, 19.4% and 6.5% respectively. This result was quite different from American Cancer society's finding which showed, CLL were 32.36%, CML 14.4 %, ALL 9.6% , from C. S. Hodgson's finding from Canada which rivaled that the highest proportion of leukemia were taken by CLL (44%), then AML (24%), CML (12%), ALL (5%) and other unspecified subtypes with 15%, and Albania (61.01% CLL ,10.4% ALL, 20.21% AML and 8.3% CML) (7, 31, 45, 53). On the other hand it was in line with the report from Nepal(2). This discrepancy may be due to the difference in demographic structure and low life expectancy in Ethiopia compared the Western countries(54).

AML was the commonest type in both sexes, comprising 66.7% of cases in men and 33.3% in women. It was more common in patients older than 50 years (50%). 77.8% of ALL was diagnosed before age of 15 years. CML and CLL were more common in older people – half of CML and all of CLL was diagnosed in those aged 50 and over. This finding was in line with the result from Ireland and Haryana- India(26, 55).

Limitation of the study

This study is limited due its facility-based study design, short study period with respect to the rareness of the case and the diagnostic methods applied are not absolute for diagnosis. Additionally, bone marrow aspiration procedure for adults was not regularly practiced.

Conclusions and Recommendations

Understanding the prevalence and distribution of leukemia is a fundamental prerequisite for the design of sound clinical and public health programs, as well as research programs, to support the health-care effort required in order to cure these afflictions. This study showed overall prevalence of leukemia among patients who have abnormal hematological parameters is significant amount. Which reflects that the need for comprehensive investigation about the status of the general population is mandatory for the planning and management of providing healthcare modality which helps the diagnosis and effectual treatment of leukemia.

The investigator suggests any other associated factors should be investigated for the complete understating of the distribution and pattern of leukemia in the population.

Therefore, Health policy makers and planners should facilitate and design researching opportunities and allocate resources which help in the diagnosis of leukemia. Health care practitioners should provide their services in evidence-based manner considering the rising of non-communicable chronic diseases specifically leukemia.

Laboratory professionals, the first who encounter the patients' results, should perform peripheral morphology assessment as a reflex test for those who have abnormal hematological parameters.

References

1. Kenneth K Marshall A, Josef T, Marcel Levi, Oliver W, Linda J, Michael A. Williams Hematology 9th ed: McGraw-Hill education 2016.

2. Kulshrestha R Sah SP. Pattern of Occurrence of Leukemia at a Teaching Hospital in Eastern Region of Nepal - A Six Year Study. J of Nepal Medi Ass. 2009;48(173; 35-40).

3. Wolfgang Hiddemann. Handbook of Acute Leukemia. Munich,Germany: Springer International Publishing, 2016.

4. Dennis L Anthony S, Dan L, Stephen L, Larry J, Joseph L. Harrison's principle of Internal medicine. 19th ed. USA: McGraw-Hill, 2015.

5. Adalberto MF Marion P, Jacques F, Isabelle S, Alain M, Freddie B. Epidemiological patterns of leukaemia in 184 countries: a population based study. Lancet Haematol. 2018;5:14–24.

6. Karen Seiter. Acute Myeloid Leukemia Staging. Medscape 2018 [cited 2018 Sept 26]; Available from: https://emedicine.medscape.com/article/2006750-overview

7. Khan AR Ahmad M, Khan SA. Analysis of clinicoepidemiological features of leukemiaat a tertiary care facility in Peshawar. . J Med Sci 2018;26(1): 41-5.

8. Hoffbrand AV Moss P Essential haematology 6th ed. London: McGraw-Hill, 2011.

9. Robert S Kenneth A, Michel L, Henry M. Hematology in Clinical Practice. 5th ed: McGraw-Hill, 2010.

10. American Cancer Society. Acute Myeloid Leukemia (AML) Subtypes and Prognostic Factors: American Cancer Society Inc.; 2018.

11. Abdul-Hamid Gamal. Classification of Acute Leukemia.pdf. The Scientist's Perspective and Challenge. 2011.

12. Turgeon Mary Louise. Clinical hematology : theory and procedures 5th ed: Lippincott Williams & Wilkins, a Wolters Kluwer business., 2012.

13. McKenzie Shirlyn B. Clinical Laboratory Hematology. England: Pearson Education Limited 2014.

14. Barbara J.Bain. Leukaemia diagnosis 3rd ed. India: Blackwell Publishing Ltd, 2003.

15. Hoffbrand AV Douglas RH, David MK, Atul BM. Postgraduate haematology. 7th ed: John Wiley & Sons, Ltd, 2016.

16. Steffen K Utz K. Myeloid Leukemia – Clinical Diagnosis and Treatment. Croatia: InTech publishers, 2011

17. WHO. Cancer Country Profiles - Ethiopia: World Health Organization 2014.

18. M. Malvezzi G. Carioli, P. Bertuccio, P. Boffetta, F. Levi C., La Vecchia, E. Negri. European cancer mortality predictions for the year 2017, with focus on lung cancer. Ann Oncol. 1 May 2017;28(5: 1117–1123).

19. Australia Cancer. Leukaemia statistics. Cancer Australia [Internet] 2017 Tue, 14/08/2018 - 13:57 [cited 2018 Aug 5]; Available from: https://leukaemia.canceraustralia.gov.au/statistics

20. American Cancer Society. Cancer facts and Figures. USA: 2017.

21. Zeidan AM, Mahmoud D, Kucmin-Bemelmans IT, Alleman CJ, Hensen M, Skikne B, et al. Economic burden associated with acute myeloid leukemia treatment. Expert Rev Hematol. 2016 Jan;9(1):79-89.

22. Mutuma J., Wakhungu J.andMutai C. The socio economic effect of cancer on patients' livelihoods in Kenyan house holds. 2016;14:37.

23. American Cancer Society. The Global Economic cost of cancer. USA; 2010.

24. Cancer Research UK. World Cancer Research fund Interantional. Worldwide data | World Cancer Research Fund International 2015 [cited 2018 6 Aug]; Available from: https://www.wcrf.org/

25. Cancer Research UK. Leukaemia (all subtypes combined) incidence statistics. Cancer Research UK. UK: Cancer Research UK, 2018.

26. Ireland National Cancer Registry. Cancer Trends; Leukaemia. Dublin: National Cancer Registry Ireland; 2010.

27. Australian Institute of Health and Welfare. Leukaemia statistics. [Internet] 2015 Aug 18 [cited 2018 Aug 5]; Available from: https://leukaemia.canceraustralia.gov.au/statistics

28. Australian Institute of Health and Welfare. Cancer In Australia. Canberra: Australian Institute of Health and Welfare; 2017. Report No.: Cat. no. CAN 100.

29. Hodgson Corinne S. Blood Cancer in Canada facts and statistics 2016: Leukemia And Lymphoma society of Canada; 2016.

30. Khan AR Ahmad M, Khan SA. Analysis of clinicoepidemiological features of leukemia at a tertiary care facility in Peshawar. J Med Sci January 2018;26(1):41-5.

31. Pepa E. . The prevalence of leukemia in Albania in the recent year Interdisplinary Journal of Research and Development. 2017;4(2).

32. Radha Rathee Minakshi Vashist, Ashok Kumar, Sunita Singh. Incidence of acute and chronic forms of leukemia in Haryana. Int J Pharm Pharm Sci. Mar 2014;6(2): 323-5.

33. Shehu UA, Adegoke SA, Abdulsalam U, Ibrahim M, Oyelami OAandAdeodu OO. Pattern of childhood malignant tumours in two tertiary teaching hospitals in Nigeria: comparative study. Niger J Paed. 2013;40(2):175 - 8.

34. Sitelbanat H.Omer. Prevalence of the different FAB sub type of Acute Myeloid Leukemia related to hematological parameters in Sudanese. J Hematol Blood Disord 2017;3((1): 102).

35. Stefan Cristina. Cancer of childhood in sub-Saharan Africa. ecancer. 2017;11(755).

36. Shamebo M. Leukaemia in adult Ethiopians. Ethiop Med J January, 1990;28(1):31-7.

37. Bruno de B, Erin McL, Ines EandMary C. Worldwide prevalence of anaemia 1993–2005 : WHO global database on anaemia. Spain: WHO, 2008.

38. Shamebo M. Acute leukaemias in adult Ethiopians in a teaching hospital. Ethiop Med J. January, 1994;32(1):17-25.

39. Shamebo M Gebremedhin A. Chronic lymphocytic leukaemia in Ethiopians. East Afr Med J. 1996 October;73(10):643-6.

40. Woldu MA, Legese DA, Abamecha FEandAB. Berha. The Prevalence of Cancer and its Associated Risk Factors among Patients Visiting Oncology Unit, Tikur Anbessa Specialized Hospital, Addis Ababa-Ethiopia. . J Cancer Sci Ther 2017;9:414-21.

41. Sisay Yifru Dagnachew Muluye. Childhood cancer in Gondar University Hospital, Northwest Ethiopia. BMC. 2015;8(474).

42. Solomon Tessema M, Mahlet Kifle H, Mathewos Asefa, Biniyam Tefera D, Getamesay Abayneh, Tsegaye Biniam, et al. Estimates of Cancer Incidence in Ethiopia in 2015 Using Population-Based Registry Data. American Society of Clinical Oncology March 2018.

43. Lealem Gedefaw, Tesfaye Demie, Kaleab Eskinder, Aklilu Getachew, Shiferaw Bekele, Waqtola Cheneke, et al. Reference intervals for hematology test parameters from apparently healthy individuals in southwest Ethiopia. SAGE open medicine. 2018;6:2050312118807626-.

44. Bao-An Chen, Zhi-Hu Huang, Xiao-Ping Zhang, Jian Ou-Yang, Jian-Yong Li, Yong-Ping Zhai, et al. An epidemiological investigation of leukemia incidence between 2003 and 2007 in Nanjing, China. Journal of Hematology & Oncology Letters. 2010(21).

45. Hodgson Corinne S. Blood Cancer in Canada facts and statistics 2016. Canada: Leukemia And Lymphoma society of Canada; 2016 2016.

46. National institute of Health. KDM6A gene: lysine demethylase 6A. USA: U.S National Library of Medicine, 2019.

47. DANNA- FABBER Cancer instuitute. Why are Men More Likely Than Women to Develop Cancer Over the Course of Their Lives? 2018 [cited 2019 May 10]; Available from: https://blog.dana-farber.org/insight/2018/10/men-likely-women-develop-cancer-course-lives/

48. Juliusson G.andHough R. Leukemia. Progress in tumor research. 2016;43:87-100.

49. Sinner P. J., Cerhan J. R., Folsom A. R.andRoss J. A. Positive association of farm or rural residence with acute myeloid leukemia incidence in a cohort of older women. Cancer Epidemiol Biomarkers Prev, 2005 Oct;14(10):2446-8.

50. Döhner Hartmut, Weisdorf Daniel J.andBloomfield Clara D. Acute Myeloid Leukemia. 2015;373(12):1136-52.

51. De Kouchkovsky I.andAbdul-Hay M. 'Acute myeloid leukemia: a comprehensive review and 2016 update'. Blood Cancer J. 2016 Jul 1;6(7):e441.

52. Ogasawara A. , Matsushita H. , Tanaka Y. , Shirasugi Y. , Ando K. , Asai S. , et al. A simple screening method for the diagnosis of chronic myeloid leukemia using the parameters of a complete blood count and differentials. Clinica chimica acta;. 2019 Feb;489:249-53.

53. American Cancer Society. Cancer facts and figures. Atlanta: American Cancer Society Inc.; 2017.

54. The World Bank. Life expectancy at birth, total (years). 2019 [cited 2019 May 17]; Available from: https://data.worldbank.org/indicator/SP.DYN.LE00.IN?locations=ET&view=chart

55. Radha R, Minakshi V, Ashok KandS Sunita. Incidence of acute and chronic forms of leukemia in Haryana. Int J Pharm Pharm Sci. 2014;6(2):323-25.

56. Sysmex. Lab-Policies-Sysmex-XS-500i-Procedure. In: America S, ed: Sysmex America Inc., 2012.

57. McPherson Richard A.and Pincus Matthew R. Henry's clinical diagnosis and management by laboratory methods 23rd ed. China: St. Louis, Missouri : Elsevier, , 2017.

58. Sigma-Aldrich. Sudan B Black Stain. UAS: Sigma-Aldrich Co. LLC., 2014.

Annexes

I. Information sheet: English Version

I, the principal investigator Woldeteklehaymanot Kassahun, am doing a research on prevalence of leukemia and associated factors among patients who have abnormal hematological parameters in Jimma Medical Center(JMC) from January 1 to April 30, 2019. The study is aimed to assess the prevalence and associated factors of leukemia. And you are selected for the research. If you are willing to participate your blood sample was analyzed further for the presence of leukemia and the result of analysis was given to you. If the result shows that you need medical help, it was immediately announced to your medical care provider (Physician or Nurse on duty) and you was benefitted from the result without any extra cost. But if you are not volunteer you are not obligated to participate.

The sample collection was made from your blood sample collected for routine CBC analysis unless and otherwise it is insufficient for further diagnosis procedures. If you are requested to give a blood sample for the research, it will have a minimal pain and no risk of health.

For the recollection procedure you was required about 4ml blood and it has a minimum effect on your health as you will give for the routine diagnosis purpose.

Contact Address;

Phone; 0915865685

Email: teklu1142@gmail.com

የመረጃ ቅጽ፡ የአማርኛ ቅጅ

እኔ አቶ ወልደተክለሃይማኖት ካሳሁን እባላለሁ፡፡ « prevalence of leukemia and associated factors among patients who have abnormal hematological parameters in Jimma University Medical Center(JMC) from January 1,2019 to April 30,2019.›› በሚል ርዕስ ዙሪያ የሁለተኛ ዲግሪ መመረቂያ ጥናት እየሰራሁ ነው፡፡

የጥናቱ ዐላማ የደም ካንሰርን (leukemia) እና ተዛማጅ ጉዳዮችን ዳሰሳ ማካሄድ፡፡ ስልሆነም እርስዎ በጠናቱ እነዲሳተፉ ተመርጠዋል፡፡ በጥናቱ ለመሳተፍ ፍቃደኛ ከሆኑ ለምርመራ ወደ ላቦራቶሪ የላኩት የደም ናሙና ለደም ካንሰር(leukemia) መኖር ወይም አለመኖር ይፈተሻል፡፡ በመጨረሻም ውጤቱ እንዲደርስዎት ይደረጋል፡፡

የምርመራዎ ውጤት ተጨማሪ የህክምና አገልግሎት እንደሚየስፈልግዎት ካመላከተ በአፋጣኝ ለሃኪምዎ ወይም ለተረኛ ሀኪሞችና ነርሶች ይነገራቸወል፡፡ ለዚህ ስራ ምንም ዓይነት ክፍያ አይጠየቁም፡፡ ነገር ግን በጥናቱ ለመሳተፍ ፈቃደኛ ካልሆኑ አይገደዱም፡፡

ፍቃደኛ ከሆኑና ለመጀመሪው ምርመራዎ የላኩት የናሙና መጠን ካነሰ ለጥናቱ የደም ናሙና እስከ 4ሚ.ሊ ደም እንዲሰጡ ሊጠየቁ ይችላል፡፡ የደም ናሙናውን ለመውሰድ እንደማንኛውም ደም እንደሚሰጡበት ጊዜ ትንሽ የህመም ስሜት ሊሰማዎተ የችላል ፤ ነገር ግን ምንም ዓይነት የጤና ጉዳት አያስከትልበዎትም፡፡

አደራሻ፡

ስልክ፤ 0915865685

ኢሜይል፡ teklu1142@gmail.com

Waraqaa odeeffannoo: Hiikka afaan Oromoo

Ani gaggeessan qo’annoo fi qorannaa adda dureen Waldateklahaymaanoot Kaasaahuun, waa’ee baay’ina kaansarii dhiigaa fi sababoota isatti hidhata qaban dhibamtoota giddugala yaalaa yuuniivarsiitii Jimmaatti yaalaman keessaa kanneen safartuuwwan hamma seelota dhiigaa rakkoo qabanirratti sadaasa 25,2018 hanga bitootessa 25,2019tti gaggeessuu dha. Qorannichis baay’inaa fi sababoota kaansarii dhiigaatti hidhata qaban sakatta’uu irratti xiyyeeffata; atis/isinis qo’annoo fi qorannoo kanaaf filatamtee jirta/jirtu. Yoo hirmaachuuf fedha qabaattan dhiigni keessan kaansarii dhiigaa qabaachuu fi qabaachuu dhabuu isaa qoratamee bu’aan qorannaa isaa isiniif kennama. Yoo bu’aan qorannichaa gargaarsa yaalaa kan barbaachisu ta’e, hatattamaan ogeessota yaala kennaniin deeggarsa yaalaa kafaltii dabalataa tokko malee argattu. Garuu, yoo fedha kan hin qabaanne ta’e hirmaachuudhaaf dirqama hin qabdu.

Saamudni dhiigaa kan fudhamuu dhiiga qorannaa seelota dhiigaa guutuutiif waraabame irraati. Yoo ga'aa kan hin taane ta'ee dhiiga irra deebiin fudhachuun dhukkubbii xiqqoo qofa fi rakkoo fayyaa biroo kan hin qabneedha. Irra deebiidhaan saamuda dhiigaa fudhachuun yoo barbaachisee, dhiiga miilliilitira 4 qofa waan ta'eef, rakkoon fayyaa sirra gahu hin jiru.

Goddinaa;

Bilbilla; 0915865685

Email; teklu1142@gmail.com

II. Consent form : English Version

Participant code ----------

Participant full name ----------

I, the participant, here assure that I have understand the purpose of the study "prevalence of leukemia and associated factors among patients who have abnormal hematological parameters in Jimma Medical Center(JMC) from January 1 to April 30, 2019." And convinced on the benefits and risks associated with being a participant. Therefore, here I give my written consent.

Agree---------- Disagree ----------

Participant/attendant/guardian ---------- ---------- Sign ---------- date ----------

የፈቃድ መስጫ ቅጽ፡ የአማርኛ ቅጅ

የተሳታፊ መለያ ኮድ----------

የተሳታፊ ሙሉ ስም----------

እኔ የዚህ ጥናት ተሳታፊ ፤ የ “prevalence of leukemia and associated factors among patients who have abnormal hematological parameters in Jimma University Medical Center(JMC) from January 1,2019 to April 30,2019.” የጥናት ዓላማ በደንብ ተገንዝቤዋለሁ፡፡ የጥናቱን ጥቅምና ጉዳትም ተረድቼዋለሁ፡፡ ስለዚህም ፈቃዴን በፊርማዬ አረጋግጣለሁ፡፡

እስማማለሁ---------- አልስማማም ----------

የተሳታፊ/የአስታማሚ/ የቅርብ ተጠሪ ስም---------- ፊርማ ---------- ቀን----------

Uunkaa waadaa: Hiikka afaan Oromoo

Koodii hirmaataa/hirmaattuu ----------

Maqaa guutuu hirmaataa/hirmaattuu ----------

Ani hirmaataan/hirmaattuun qorannoo kanaa mata-dureen isaa baay’ina kaansarii dhiigaa fi sababoota isatti hidhata qaban dhibamtoota giddugala yaalaa yuuniivarsiitii Jimmaatti yaalaman keessaa kanneen safartuuwwan hamma seelota dhiigaa rakkoo qabanirratti sadaasa 25,2018 hanga bitootessa 25,2019tti gaggeeffamu ta’uu isaa hubachuun hirmaataa/hirmaattuu ta’een jira. Kanaafuu, waadaa barreeffama kanaan galeen jira.

Walii-galeera---------- walii-hingalle ----------

Hirmaataa/kan guddise/yaalchisaa ---------- ----------mallatoo ---------- guyyaa ----------

III. Questionnaire

The English version of the questionnaire

Structured Questionnaire for the sociodemographic and personal data collection from patients for the study the prevalence of leukemia and associated factors among patients who have abnormal hematological parameters in Jimma Medical Center(JMC) from January 1 to April 30, 2019. *I am an MSc student in Clinical Hematology and Immunohematology in Jimma University. This questionnaire is used to collect about your personal information and its only uses it only if you are volunteer to give your information. Your name is not used and your identity is kept anonymous.*

1. *Sex*
    a. *Male* ---------
    b. *Female* -------
2. *How old are you (Age)* --------
3. *Where did you came from (Residency)?*
    a. *Rural* ----------
    b. *Urban*----------
4. *What is your occupation?*
    a. *Student* --------
    b. *Government employee*---------
    c. *Private business (specify)*----------
    d. *Farmer*--------

መጠይቅ፡ የአማርኛ ቅጅ

ይህ የተጠናቀረ መጠየቅ በ « prevalence of leukemia and associated factors among patients who have abnormal hematological parameters in Jimma University Medical Center(JMC) from January 1,2019 to April 30,2019>> ለሚሳተፉ ተሳታፊዎች የስነ-ማሕበረሰባዊና ግላዊ መረጃዎች መሰብሰቢያነት የሚያገለግል ነው።

እኔ የዚህ ጥናት ከዋኒ በጅማ ዩኒቨርሲቲ የClinical Hematology and Immunohematology ሁለተኛ ዲግሪ ተማሪ ስሆን የሚሰጡኝን ማንኛቸውም መረጃ የምወሰደው ሲፈቅዱልኝ ብቻና ደህንነቱም የተጠበቀ ነው። ስምዎትን አልጠቀምበትም፣ ለማንምም አይገለጥም።

1. ዕድሜዎ ሰንት ነው----------
2. ፆታ ወንድ --------- ሴት ----------
3. የት ነው የሚኖሩት(ከየት ነው የመጡት)
    a. ገጠር
    b. ከተማ
4. ሰራዎ ምንድን ነው
    a. ተማሪ
    b. የመንግስት ሰራተኛ
    c. የግል ሰራ(በግልፅ አስቀምጥ)----------
    d. ግብርና

Waraqaa gaaffii fi deebii : Hiikka afaan Oromoo

Waraqaa gaaffii fi deebii waa'ee hawaas-dinagdee fi ragan dhuunfaa hirmaattota qorannichaa waa'ee baay'ina kaansarii dhiigaa fi sababoota isatti hidhata qaban dhibamtoota giddugala yaalaa yuuniivarsiitii Jimmaatti yaalaman keessaa kanneen safartuuwwan hamma seelota dhiigaa rakkoo qabanirratti sadaasa 25,2018 hanga bitootessa 25,2019tti gaggeeffamu *ittiin sassaabamu*

Ani barataa digrii 2^{ffaa} muummee kiliinicaal heemaatooloojii fi immunooheemaatooloojii (Clinical Hematology and Immunohematology) yuuniivarsiitii Jimmaati. Waraqaan gaaffii fi deebii kun odeeffannoo dhuunfaa sassaabuuf kan gargaaruu fi kan fayyadamuu immoo odeeffannoo kennuuf yoo hayyamamoo taatan qofaa dha. Maqaan keessan kan hin katabamnee waan ta'eef iccitii keessan eeguuf lakkoofsa fayyadama.

1. *Saala*
    a. *Dhiira* ---------
    b. *Dhalaa* -------
2. *Umrii* --------
3. *Eddoo jireenyaa*
    a. *Baadiyyaa*----------
    b. *Magaalaa*----------
4. *Hojiin keessan maalii dha?*
    a. *Barataa* --------
    b. *Hojjetaa mootummaa*---------
    c. *Hojii dhuunfaa (ibsi)*---------
    d. *Qotee bulaa*-------

IV. SOP for complete blood count with Sysmex XS-500i and XT-1800i

PRINCIPLE:

The System XS-500i and XT-1800 are quantitative automated hematology analyzers for in-vitro diagnostic use for determining 21 hematological parameters. Examination of the numeric and/or morphologic findings of the complete blood count are useful in diagnosis of such disease states as anemias, leukemia's, allergic reactions, viral, bacterial, and parasitic infections. The Sysmex XS-500i analyzer directly measures the WBC, RBC, HGB, HCT, PLT, NEUT#, LYMPH#, MONO#, EO#, and BASO#. The remaining parameters are calculated or derived: MCV, MCH, MCHC, RDW-CV, RDW-SD, MPV, and differential percentages(56).

The Sysmex XS-500i and XT-1800i counts and sizes red blood cells (RBC) and platelet (PLT) using electronic resistance detection enhanced by hydrodynamic focusing. Hematocrit (HCT) is measured as the ratio of the total RBC volume to whole blood using cumulative pulse height detection. Hemoglobin (HGB) is converted to SLS-hemoglobin and read photo-metrically(56).

WBC count and differential are evaluated using flow cytometry with a semiconductor laser utilizing scattered light and fluorescence to determine the differences in cell size, complexity and nuclear content. The WBC differential channel classifies neutrophils (NEUT), lymphocytes (LYMPH), monocytes (MONO), eosinophils (EO), and basophils (BASO) by cellular complexity and nucleic acid content. The differential cell placement is then enhanced utilizing Adaptive Cluster Analysis(56).

SPECIMEN:

A. **The specimen** – Whole blood anticoagulated with potassium EDTA is preferred.
B. Specimen Volumes required:
    i. Optimal draw is a tube drawn to capacity. The collecting tube must be filled to a minimum of one-half full for acceptable results.
    ii. A minimum of 1 ml whole blood is required for running specimen

C. Unacceptable specimens including those listed below must be rejected:

i. Clotted samples or those containing clots
ii. Grossly hemolysis samples.
iii. Samples drawn before 3 hours and above

REAGENTS AND MATERIALS:

A. Supplies

1. Distilled water
2. Gauze, plastic lined wipes
3. EDTA Test tubes
4. CELLCLEAN (0.5% bleach)
5. Sysmex reagents
6. Commercial controls: e-CHECK (XS) 5 x 1.5 ml vials
7. Sysmex xs-500i whole blood calibrator

B. Sysmex Reagents

Reagent	Abbreviation	Open Expiration	Remark
Sysmex CELLPACK	EPK	60 days	Diluent
Sysmex STROMATOLYSER-4DL	FFD	60 days	Lyse reagent
Sysmex STROMATOLYSER-4DS	FFS	90 days	Staining reagent
Sysmex SULFOLYSER	SLS	60 days	Lyse reagent

C. Patient Sample Processing

1. On the IPU, click (Manual) or press (F2).
2. Enter the specimen number (alpha or numeric characters) using the keyboard
3. Click on CBC or CBC + Diff if this information is not being provided by the Host Computer.
4. Click (OK).
5. Mix the patient sample 8-10 times by end-to-end inversion.
6. Place sample in sample tube adapter.
7. Press Start switch. (Located above the sample tube position on the Main Unit)
8. When Ready LED is lit green, repeat steps 1-7 for each additional sample.

D. QUALITY CONTROL:

Use Sysmex e-CHECK (4.5 ml vials) and e-CHECK (XS) (1.5 ml vials)

1. Remove Sysmex e-CHECK/e-CHECK (XS) vials from refrigerator and allow them to come to room temperature (18-25 ºC), for approximately 15 minutes.
2. Mix vials by gentle end-to-end inversions until the cell button in the bottom of the vial is completely suspended.
3. Run with the machine.
4. Review control results each shift prior to resulting patient tests.

References

Sysmex. Lab-Policies-Sysmex-XS-500i and XT-1800i-Procedure-Lab-1501.pdf. In: America S, editor.: Sysmex America Inc.; 2012.

V. Wright's stain standard operating procedure

PRINCIPLE:

Wright's stain is a polychromatic stain consisting of a mixture of eosin and methylene Blue. When applied to blood cells, the dyes produce multiple colors based on the ionic charge of the stain and the various components of the cell. The eosin ions are negatively charged and stain basic cell components an orange to pink color. The methylene blue ions are positively charged and stain the acid cell components in varying shades of blue. The neutral components of the cell are stained by both components of the dye producing variable colors(57).

SPECIMENS

1. Thin wedge blood smears are prepared on pre-cleaned glass slides from venous blood anticoagulated with EDTA and BMA aspirate as well
2. The blood smear slide must be properly prepared, labeled and completely dry before commencing with the staining procedure.
3. For best results, prepare and stain smears within two (2) hours of specimen collection.

EQUIPMENT AND REAGENTS

1. EQUIPMENT:
    - 1.1. Staining racks
    - 1.2. Pre-clean Frosted microscopic slides
    - 1.3. Whitman Grade 41 filter paper – 110mm diameter
    - 1.4. Filtering Funnel – short neck
    - 1.5. Pasture pipettes
    - 1.6. Cotton gauze
2. REAGENTS:
    - 1.7. Commercial Wright's stain
    - 1.8. Absolute methanol as fixative
    - 1.9. Commercial buffer
    - 1.10. Distilled Water

PROCEDURE:

1. Fix the prepared slide for 2 to 5 seconds with absolute methanol.
2. Add 2 to 5ml undiluted Wright's stain solution for 2 minutes laying the slides facing up on the staining rack.
3. Without removing the stain from the horizontal slide, add equal amount of buffer and mix by blowing gently till metallic scum is observed and let to act on foe 3 minutes.
4. Flush the stain from the slide with distilled water.
5. Clean with gauze the back of the slide.
6. Allow the slide is to air dry in a tilted position the feathered up.
7. Look under microscope starting with low magnification

SOURCES OF ERROR:

I. Fixation:

A. Inadequate fixation may result in a detached specimen, indistinct nuclear detail or poor granule staining. This is especially evident in basophils and primary myeloid granules.

B. Dry freshly prepared smears for at least fifteen (15) minutes to prevent drying artifacts. Use only pre-cleaned glass slides. Slides other than pre-cleaned may result in improper fixation and the presence of haze over the slide due to acid-base disturbance. Pre-cleaned slides are necessary for quality stain appearance.

C. Use only absolute methanol for fixative. Keep it tightly closed and away from moisture or chemical fumes.

D. If fixation problems occur or increased granularity visualization is necessary, increase the fixation period to one (1) minute.

II. Staining:

A. Excessively blue stain appearance may be due to improper smear preparation, prolonged staining time or increased stain alkalinity.

B. Excessively pink stain appearance may be due to insufficient or decreased staining time, prolonged washing time or increased stain acidity.

III. Rinse:

A. Use only distilled water for the final rinse. Tap water is never acceptable as a substitute for the rinse since it can disturb the acid base balance and may contain chlorine.

B. Vigorous or prolonged washing may dislodge cell from under-fixed smears, cause nuclear clumping or under-staining.

C. Insufficient rinsing may result in a blue smear or excessive precipitate.

IV. Drying:

A. Air drying is the preferred method. Forced rapid drying may alter the color intensities by shortening the exposure time to the wash water because the red spectrum of colors continues to develop as long as the cellular elements are wet.

QUALITY CONTROL

1. Prepare one differential slide daily using a patient sample with a normal MCV, MCH, MCHC and total white count. Stain the slide as indicated in procedure section above. Review the slide for Color, Precipitation and Contamination.
2. If the color does not meet the specifications identified in the SOP as indicated above or precipitation
3. and/or contamination are present, the quality is determined to be unsatisfactory.
4. If the stain is indicated unsatisfactory, replace the stain with new filtered stain.

References;

Richard A. McPherson MRP. Henry's clinical diagnosis and management by laboratory methods 23rd ed. China: St. Louis, Missouri : Elsevier, ; 2017.

VI. Sudan B black staining SOP

Principle

Sudan black B is a lipophilic dye that stains intra cellular phospholipids and other lipids. Following fixation, blood or bone marrow films are immersed in a buffered Sudan black B solution. After rinsing, slides are counterstained with Mayer hematoxylin. Cells are examined microscopically for the presence of blue-black discrete granulation. Cells committed to the lymphoid pathway display negative staining reactions, whereas myeloid and monocytic forms display characteristic positive reactions. The Sudan black B staining pattern usually parallels the myeloperoxidase stain and is useful in the identification of Myelogenous and myelomonocytic leukemias(12, 58).

Reagents:-

- Fixative:- Absolute Methanol(99 %)
- Stain:- 0.3% SBB in absolute alcohol
- Buffer :- Phosphate buffer(pH=6.8)
- Working stain solution:- add 40ml Phosphate buffer to 60ml SBB solution.
- Counter stain :- Wright's Stain (1:2 diluted)

Procedure:-

1. Fix air dried smear in Absolute Methanol for 5 seconds.
2. Then air dry 5 min.
3. Now stain with working solution of SBB stain solution for 1 hour at 37^{o}C.
4. Wash with water and air dry.
5. Now counter stain with Wright's Stain (1:2 diluted) for 30 mins.

Results:-

The reaction product is black and granular. All nuclei are Purple-pink.

Interpretation:-

The Granules of myeloid(granulocyte) cell will stain black/dark both in normal and leukemic cells even though there are differences like; The eosinophil granules are SBB negative and in rare cases (1-2%) of ALL shows non granular smudgy positivity not seen in MPO staining e.g. in Burkett's lymphoma. Generally Lymphoid cells do not stain with SBB stain.

To say SBB positive, at least 3-4% of the granulocytes must show positive reaction.

Quality control

Apparently health individual blood sample was stained and checked for the results.

References;

Turgeon ML. Clinical hematology : theory and procedures 5th ed: Lippincott Williams & Wilkins, a Wolters Kluwer business.; 2012.

Sigma-Aldrich. Sudan B Black Stain. In: Sigma-Aldrich, editor. UAS: Sigma-Aldrich Co. LLC.; 2014.

Pangalis GA. Human Leukemias_ Cytochemical and Ultrastructural Techniques in Diagnosis and Research. Polliack A, editor. US: Springer; 1984.