ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Assessment of Hematological Profile among Adult Clients Visiting for Wellness Service at International Clinical Laboratories, Addis Ababa, Ethiopia

By: Etalemahu Ayalew

Advisors: **Aster Tsegaye (MSc, PhD)**
Jemal Alemu (MSc, PhD Candidate)
Mesfin Nigussie (MD, Pathologist)

A thesis submitted to the Department of Medical Laboratory Sciences, College of Health Science, Addis Ababa University, in partial fulfillment of Master of Science Degree in Clinical Laboratory Sciences.

September, 2021

Addis Ababa, Ethiopia

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by Etalemahu Ayalew, entitled "Assessment of Hematological Profile among clients visiting for Wellness Service at International Clinical Laboratories, Addis Ababa, Ethiopia" and submitted in partial fulfillment of the requirements for the master of science degree in clinical laboratory sciences (Hematology and Immunohematology specialty track) complies with the regulations of the university and meets the accepted standards concerning originality and quality.

Signed by the examining committee

External examiner _____Signature_____Date_____

Internal examiner _____Signature_____Date_____

Advisor_____ Signature_____Date_____

Advisor_____ Signature_____Date_____

Advisor_____ Signature_____Date_____

_____

Chairperson of the Department of Graduate Program Coordinator

Acknowledgements

First, and foremost I would like to thank the almighty God. Secondly, I would like to express my deepest gratitude to my advisers Dr. Aster Tsegaye, Mr. Jemal Alemu and Dr Mesfin Nigussie, for their intellectual advice and unreserved suggestions during the preparation of this research thesis. I am also grateful to Addis Ababa University College of Health Sciences, Department of Medical Laboratory Sciences for facilitating the study and International Clinical Laboratories for their great willingness to conduct this research.

Moreover, my deepest gratitude also goes to all study participants for their cooperation during data collection. Without their willingness, this thesis would have been hardly realized. Finally, I would like to acknowledge my friends and families for their continuous encouragement in preparation of this research thesis.

Table of contents

Contents **Page no**

List of Tables

List of figures

Abbreviations

CBC	Complete blood count
CHF	Congestive heart failure
COPD	Chronic obstructive pulmonary disease
EDTA	Ethylene diamine tetra acetic acid
EQC	External quality control
HB	Hemoglobin concentration,
ICL	International Clinical Laboratory
IQC	Internal quality control
IU	International Unit
LYM	Lymphocyte
MCH	Mean Corpuscular Hemoglobin,
MCHC	Mean Corpuscular, Hemoglobin Concentration
MCV	Mean Corpuscular Volume,
NEUT	Neutrophils,
PCV	Packed Cell Volume
PLT	Platelets
QA	Quality assurance
RBC	Red Blood Cells
SPSS	Statistical Package for Social Sciences
WBC	White Blood Cell

Abstract

Background: Hematological profiles are affected by commonly known pathological factors. However, nutritional, behavioral and life style factors may vary those parameters among apparently healthy individuals. Little is known about hematological profile of apparently healthy individuals seeking wellness service in resource limited settings like Ethiopia.

Objective: To assess the hematological profile of individuals visiting International Clinical Laboratory (ICL) Wellness service in Addis Ababa, Ethiopia

Methods: Institutional based cross-sectional study was conducted from February to April 2020 among 422 apparently healthy study participants. Demographic data were collected using structured questionnaire. Blood samples collected in EDTA tubes were analyzed using Cell-Dyn Ruby 5-Diff hematology analyzer. Data were entered and analyzed using SPSS version 20. Results were compared between categorical variables (age and sex) using parametric tests (Independent t test and ANOVA) for continuous variables. Chi-square and binary logistic regression were used to see the relation between independent variables and hematological abnormalities. P-value less than 0.05 was considered as statistically significant.

Result: A total of 422 apparently healthy clients (226 males, 53.6%) were included. Their age ranged from 18 – 87 years, with a median (IQR) age of 43 (35-54) years. The mean±SD of WBC, RBC and PLT counts among males were 5.87±1.73, 5.65±0.62 and 250.38±71.58; while the counts among females were 5.95±1.99, 5.09±0.49 and 276.89±74.13, respectively Hematological parameters RBC ($p<0.001$), HGB ($p<0.001$), HCT ($p<0.001$), and absolute lymphocyte count ($p=0.028$) were significantly higher in males than females, whereas platelet ($p<0.001$) and absolute neutrophil count ($p=0.012$) were significantly higher in female counterparts. There was no statistically significant difference in hematological profiles among the age groups. There was statistically significant variation between mean values in the current study and the reference range in current clinical use. Among the total study participants, 12 (5.3%) males and 11 (5.6%) females had anemia. On the other hand, 7 (3.1%) males and 12 (6.1%) females, 3 (1.3%) males and 2 (1%) females, 1 (0.4%) male and 4 (2%) females were found to be leucopenic, thrombocytopenic and lymphopenic, respectively

Conclusion: Hematological parameters of study participants have shown significant variation from the established ones. Thus, further investigation needs to be done to confirm our results and establish reference range applicable for local regions.

Key words: *Hematological profile, Apparently healthy, ICL Wellness service, Addis Ababa*

1. Introduction

1.1 Background

Hematological profiles are evident for diagnosis of numerous diseases. Complete blood cell examination is important for health examination (1). A hematologic profile is a standard test that evaluates a blood sample for a variety of basic measurements of blood cells which gives a tally for each different type of cell in a given blood sample. It includes determination of hemoglobin and the counts of red blood cells, white blood cells and platelets. The tests for hemoglobin and red blood cells are essential ways to identify anemia, a condition often caused by insufficient iron in the patient. Testing for white blood cells can reveal some different conditions. Fewer or higher white blood cells than expected can be an indication of infections, failure of production, hematologic pre-malignant and clonal disorders. Generally, a bacterial infection may be suspected among patients with increased total white blood cell counts than normal and hemostatic disorders may be accompanied by low or high platelet counts. On the other hand, white blood cells are indispensable for fighting infections in the bloodstream either directly by phagocytosis or via mediated immunity (2–4).

White blood cells (WBC) make up the body's primary defense system, and knowing their number is an important tool in diagnosing and monitoring infection and leukemic disorders including neutrophils, lymphocytes, monocytes, eosinophils, and basophils (5).

Neutrophil is commonly seen in patients with bacterial infection. The most severe infections are associated with more marked neutrophil and often a degree of myeloid left shift (the presence of immature myeloid cells in peripheral blood) with ‘toxic’ neutrophil granulation. Neutrophil may also be seen in non-infective disorders. It is a common response to steroid therapy, severe exercise, and following surgery or splenectomy, but can also occur in systemic vasculitis, in the presence of tissue necrosis/burns, and as a response to certain tumors (3,6). Eosinophilia is a much less common finding in clinical practice caused by parasite infestation, drugs, comorbid conditions such as asthma and other allergic conditions (4).

Lymphocytosis is commonly seen as a result of viral infection often with a mild self-limiting neutropenia. Stress lymphocytosis is a relatively common phenomenon in hospital patients and is precipitated by acute onset illnesses such as myocardial infarction, major trauma, and status

epilepticus (7). Monocytosis can be a feature in chronic infection with tuberculosis and syphilis, as part of the inflammatory reaction. Response to certain carcinomas. A persistent monocytosis that is unexplained, particularly if associated with anemia or thrombocytopenia, may be a feature of myelodysplastic and myeloproliferative disorders, so a hematology assessment is advised in these cases (5).

Red blood cells (RBC) are responsible for the transport and exchange of oxygen. Measurement of RBCs is important in monitoring the effects of blood loss and the progression of chronic disease RBC values are increased in those with anxiety or stress, bone marrow failure, and dehydration. A decreased RBC value will be found in those with chronic inflammatory diseases, chemotherapy patients, anemia, blood loss, and many cancers (8). Hemoglobin (HGB) is the oxygen-carrying protein in red blood cells. Hemoglobin levels are a direct reflection of the amount of oxygen in the blood increased HGB is seen in those with dehydration, chronic obstructive pulmonary disease, or COPD, and congestive heart failure, or CHF, and those at high altitude. A decreased HGB value is seen in anemia, blood loss, liver disease, as well as leukemia and lymphomas (9).

Hematocrit (HCT) is the proportion of red blood cells to plasma, the fluid component of your blood the increase and decrease levels of HCT mirror those of hemoglobin. RBC, HGB, and HCT tests parallel each other and are frequently used together to evaluate anemia (10). Platelets (PLT) have an essential function in blood clotting. An increased platelet value is seen in conditions that involve inflammation, such as acute infection, trauma, and some malignant cancers. A decreased platelet count is found in alcohol toxicity, anemia, blood loss, infection, many congenial conditions, and coagulation disorders (11).

The critical need for the development of normal hematological values specific for every population for interpretation of laboratory test results and provision of quality services in the health care delivery cannot be over emphasized. However, reference values being used in most laboratories in African countries have been obtained from the literature, reagent inserts accompanying the reagent kits or instrument manuals. These values have been derived from Caucasian populations of industrialized countries. Published literature has indicated that many of the reference values obtained from the developed countries differ significantly from those in most African localities (12).

Laboratory parameters vary considerably between healthy people from different geographical locations, mostly driven by ethnic, genetic, demographic, nutritional, economic and environmental differences. Factors affecting hematological parameters include age, weight, degree of physical normal temperature, pulse rate, systolic and diastolic blood pressures activity, sex and environmental factors including physiological as well as environmental conditions (13).

Factors can affect a healthy person's measured RBC indices, including ethnicity, smoking status, alcohol consumption, nutritional deficiency, and altitude of residence. Anemia is a condition in which the number of red blood cells (and consequently their oxygen-carrying capacity) is insufficient to meet the body's physiologic needs. Specific physiologic needs vary with a persons' age, gender, residential elevation above sea level (altitude), smoking status, and different stages of pregnancy. Iron deficiency is thought to be the most common cause of anemia globally, but other nutritional deficiencies (including folate, vitamin B12 and vitamin A), acute and chronic inflammation, parasitic infections, and inherited or acquired disorders that affect hemoglobin synthesis, red blood cell production or red blood cell survival, can all cause anemia (14).

Apparently healthy individuals sometimes give blood to know their status concerning infectious diseases, organ function tests and hematological parameters. Complete blood cell counts (CBC) results interpreting especially when encountered with abnormal result for intellectually rewarding practice and to recognize when a subspecialty consultation is reasonable and when it may be circumvented. A complete blood cell count is routine hematology tests in medicine useful for the differential diagnosis of anemia and other medical conditions (4). ICL among its services has a wellness clinic where apparently healthy individuals are coming to check their health status. Medical checkup while feeling healthy is a common practice in the developed countries and the practice is coming up in our country slowly. This study examines hematological profile of individuals visiting the wellness clinic of ICL. It will determine the magnitude of any abnormality of the tested parameters.

1.2. Statement of the problem

A complete blood cell count is routine hematology tests in medicine useful for the differential diagnosis of anemia and other medical conditions. The habit of going to health facilities for medical checkup while feeling healthy is not a common practice in Ethiopia. Few private clinics and laboratories are availing such a service in recent years. On the other hand, studies from southwest Ethiopia have shown that about 20.1% of apparently healthy urban residents were anemic (15). These individuals could have been early identified if there was a practice of visiting wellness clinic.

Almost all laboratories in Ethiopia use reference values which were established in countries manufacturing the complete blood count machines. Despite this fact, hematological parameters most frequently show variation depending on factors including sex, age, ethnic background, nutrition, demography, behavioral factors, genetics and environmental factors (16). Thus, using the reference values established for other countries (developed nations in this case) which have socio-demographic differences may lead to incorrect diagnosis (14). As there are very few studies conducted on current issue of the problem mentioned, the present study produces a lot of information regarding the clear picture of the problem area at large.

Previously, very few studies were conducted on assessment of hematological profile among apparently healthy individuals and there is limited information in the study area. Therefore, it is every clinician's interest to have some understanding of the specific basic test as it is important for structured action plan when confronted with abnormal results (5). Hence this study aimed to assess the hematological profile of apparently healthy adult clients coming to ICL, Addis Ababa, Ethiopia.

1.3. Significance of the study

The present study, despite its study target is specific, contributes significantly towards understanding the trend of hematological parameters; and it aims at providing additional information on hematological values among apparently healthy clients. Assessing hematological profile help to investigate complete blood count values of apparently healthy individuals, which in turn serve as a tool to investigate the extent of hematological parameter and the presence of hematological abnormalities from people who were assumed to be apparently healthy.

The findings of this study will also create awareness about the wellness service.

Besides, it also serves policy makers as an input in making decisions regarding the reference intervals of hematological parameters by looking what the trend looks like in these apparently healthy individuals. The study will also be used as a base line reference for further studies.

2. Literature review

A study conducted in China regarding the hematological parameters of healthy Han Chinese adult individuals reported that median and mean platelet counts from the Chengdu center were significantly lower than those from other centers. Red blood cell count (RBC), hemoglobin (HGB), and hematocrit (HCT) values were higher in males than in females at all ages. The study further revealed that other CBC parameters showed no significant instrument-, region-, age-, or sex-dependent differences (17).

Similar study on the impacts of demographic and laboratory parameters on key hematological indices among 26,497 adult population of southern Taiwan reported increasing age and male gender negatively affected the number of platelets. According to the study, gender and serum albumin level were the major determinants of variation in hemoglobin level. A modestly increased white cell count was seen in men as well as individuals with elevated apolipoprotein B levels, but it was inversely correlated with changes in age and serum albumin levels. Conversely, some variables, although statistically significantly associated with the hematological indices, only provided a trivial explanation for the heterogeneity observed (18).

A study conducted in Middle Eastern Countries in healthy Omani population reported that red blood cell (RBC), hemoglobin (HGB), hematocrit (HCT) and platelet (PLT) counts of the healthy blood donors were significantly different between males and females at all ages, with males having higher mean values of RBC, HGB and HCT than females. Other complete blood count parameters showed no significant differences between genders, age groups, instruments, or blood groups. Furthermore, the study showed a lower hemoglobin limit for the normal reference interval in males and females than the currently used in Oman (10).

Another study conducted to determine the reference interval for selected hematological and biochemical parameters among apparently healthy adults in different eco-geographical zones in Ghana reported that there were statistically significant differences in most of the hematological parameters including RBC, HGB, HCT, MCV, PLT and WBC based on gender. Moreover, significant inter eco-geographic (intra-population) variations and substantial differences between

the established reference interval (RI) and the RIs accompanying the analyzers used were also observed (19).

According to a study intended to assess some hematologic parameters of blood donors at the National Blood Transfusion Service (NBTS), Jos, Nigeria, the packed cell volume (PCV), total differential white blood cell counts and platelet count were significantly different compared to local reference ranges. Moreover, the study reported that evaluation of the parameters between genders, locations, age groups and occupations of donors, the platelet, PCV and eosinophil counts differed significantly. The average white blood cells (WBC) count was lower among donors in the rural area (2).

Hematological parameters in apparently healthy blood donors from different parts of Kaduna state, Northwestern Nigeria has reported significantly higher mean values of WBC, PLT, MCV, MCH and MCHC but insignificantly higher values of WBC and PLT among female adult study participants. The study also projected that the findings can serve as an important tool in the interpretation of laboratory results for clinical management of patients as well as for research purposes (12).

According to study conducted by International Scholarly Research Network in Togo among a total of 1379 blood donor volunteers, 1047 (77.6%) males and 302 (22.4%) females; in the total age range of 17 to 58 year. This study revealed that median hemoglobin level was higher in males than females (15.1 g/dL versus 13.0 g/dL). Median total WBC (4.2×109/L) and absolute neutrophil counts (1.6×109/L) were similar by gender. The median lymphocyte counts in males and females were, 2.1 × 109/L and 2.2 × 109/L, respectively. The median platelet count was lower in males than females (236 × 109/L versus 247 × 109/L. The authors noted that the median values for RBC parameters differ from those of African countries probably because of their inclusion criteria which eliminate most cases with iron deficiency and/or thalassemia (20).

Study conducted to assess hemogram abnormalities in apparently healthy first-time blood donors in Libreville, Gabon to determine the abnormalities of leukocyte, platelet, and erythrocyte counts in blood donors. Leukopenia and thrombocytopenia were significantly more common in men than women (29.02% vs. 24.4%, and 16.2% vs. 7.5%). Only 1.0% of women and 0.84% of men

had leukocytosis, and 0.7% of women and 0.2% of men had thrombocytosis. Anemia was significantly more common in women compared to men (69.4% vs. 45.0%). Normocytic normochromic and normocytic hypochromic anemia were most common among Libreville blood donors with 39.4% and 23.6%; followed by microcytic normochromic (18.7%) and microcytic hypochromic (13.2%) anemia. Normocytic normochromic and normocytic hypochromic anemia were significantly more common in men than in women, whereas microcytic normochromic anemia was more prevalent among women compared to men (34.6% vs. 13.9%) (21).

Similar cross-sectional study conducted in Mozambique to establish the reference values for clinical laboratory parameters among young adults reported that there were statistically significant differences between males and females in most hematological parameters, with the exception of PDW, MPV and absolute LYM. The males had higher values of RBC, Hb, HCT, MCV, MCH, MCHC and percentage LYM than females. The females had higher values of WBC, PLT, absolute neutrophils and percentage neutrophils than males (22).

A cross-sectional study in Tanzania reported a median hemoglobin level of 15.1 g/dL [10.5-23.8], erythrocytes of $5.3x10^6$/µL [4.1-8.3 $x10^6$], hematocrit of 44.0% [32.4-71.4], total leucocytes of 4300 cells/µL [1700-8500], lymphocytes 1700/µL [800-3000], neutrophils 2100/µL [300-5300]; mid-sized cells (monocytes, eosinophils and basophils) of 400/µL [100-1400] and platelets of $194x10^3$/µL [55.2-379.0 $x10^3$]. Moreover, the study revealed a significantly higher hemoglobin level as well as erythrocytes and hematocrit level among males than females (23).

Based on the study conducted to determine white blood cell counts in apparently healthy Sudanese blood donors in Gezira State (Sudan), the mean values of white blood cells were found to be 5.696 ±1.7989 with minimum count 2.1 x 109 /L and maximum count 14.1 x 109 /L, a considerable numbers of donors were leucopenic,77 cases ranged from 2.1 to 3.9 which represents 15.4% and a few numbers with leukocytosis, 5 cases ranged from 11.1 to 14.1 which represents 1%, dominated by neutrophils which suggestive of pyogenic infection, high eosinophil percentage more than 4% observed in 121 donors (24.2%), the bulk of this eosinophilia probably reflects asymptomatic parasitism (e.g. schistosomiasis). Monocyte

percentage more than 8 % observed in 6 donors (1.2%) and high lymphocyte percentage with the reactive forms of lymphocyte also detected most probably due to chronic infection. The study revealed that significant numbers of donors with low and high white blood count, which subsequently indicate that they were not eligible for blood donation (24).

Study conducted in Sudanese apparently healthy male donors in which hemoglobin level was measured using an automated cell counter (System KN21), accompanied by peripheral blood films were assessed to detect any abnormalities. The study revealed that the mean hemoglobin values were 14.5 g/dl ±1.2076, with minimum count (10.1 g/dl) and maximum count 17.8 g/dl. Hemoglobin less than 12.5 g/dl was obtained in 30 donors (6%) and they were reported as fit for blood donation using copper sulphate for hemoglobin estimation. Those 30 donors found to be unfit for blood donation because their hemoglobin concentration must be more than 12.5 g/dl (25).

Another study conducted in southwest Ethiopia reported the reference interval of red blood cell, white blood cell, and platelet count in adults was 5.19 × 1012/L (4.08–6.33 × 1012/L), 6.35 × 109/L (3.28–11.22 × 109/L), and 282.00 × 109/L (172.50–415.25 × 109/L), respectively. The reference interval of red blood cell, white blood cell, and platelet count in geriatrics were 5.02 × 1012/L (4.21–5.87 × 1012/L), 6.21 × 109/L (3.33–10.03 × 109/L), and 265.50 × 109/L (165.53–418.80 × 109/L), respectively. Most of the hematological parameters showed significant differences across all age groups (26).

Another cross-sectional study conducted in Dire Dawa, Ethiopia among adult individuals revealed that Males had significantly higher reference value for most of red cell parameters (Hgb, RBC, HCT, MCH and MCHC) than females ($p < 0.05$), while most of the WBC parameters were significantly higher in females than males. Moreover, non-pregnant women had higher values for most of red cell parameters than pregnant women. Pregnant women had higher WBC parameters than their non-pregnant counterparts (27).

Similar study was conducted in Amhara Regional State, Ethiopia with a total of 967 (55.2% males) The established 95% reference intervals (2.5th–97.5th percentile) were: for WBC: 3–11.2 9 109/l; for platelet: 90–399 9 10^9/l; for RBC: 4–6 x 10^{12}/l for males and 3.5–5.6 x 10^{12}/l for females; for hemoglobin: (Hgb) 12–18.9 g/dl for males and 10.7–17.5 g/dl for females; for

PCV:35.7–55.3% for males and 32.2–50.1% for females; for MCV: 81–100 fl; for MCH: 25.3–34.6 pg.; MCHC: 28.8–36.9%; for RDW: 11.6–15.4% and for MPV: 8–12.3 fl. Males had significantly higher RBC, Hgb and PCV than females (28).

Another study was conducted in Addis Ababa to establish immunohematological reference values among 485 healthy adult Ethiopians. The study reported that the mean values were as follows: leukocyte (WBC) counts, 6.1 x 10^9/liter (both genders); erythrocyte counts, 5.1 x 10^{12}/liter (males) and 4.5 x 10^{12}/liter (females); hemoglobin, 16.1 (male) and 14.3 (female) g/dl; hematocrit, 48.3% (male) and 42.0% (female); platelets, 205 x 10^9/liter (both genders); monocytes, 343/ml; granulocytes, 3,057/ml; lymphocytes, 1,857/ml. Moreover, the study reported that the WBC and platelet values of healthy HIV-negative Ethiopians are lower than the adopted reference values of Ethiopia (29).

Similar study conducted among blood donors in Addis Ababa, Red Cross Center revealed that red blood cell count, mean corpuscular volume and platelet count were significantly higher in the present study than the reference range. RBC indices, white blood cell, hemoglobin and hematocrit were higher in the reference range used in clinical practice. Significantly higher red blood count, hemoglobin, hematocrit and mean corpuscular hemoglobin concentration were observed among males. Significantly higher platelet count was observed among females. However, the study reported no significant change in hematological laboratory values across different age groups (30).

3. Objectives

3.1. General Objective

- To assess hematological profile among adult clients visiting International Clinical Laboratory (ICL) wellness service, Addis Ababa, Ethiopia

3.2. Specific objectives

- To assess the overall Complete Blood Cell count profile of study participants
- To assess hematological abnormalities among adult clients visiting the wellness service

4. Materials and methods

4.1 Study area

This study was conducted at International Clinical Laboratories, Addis Ababa, Ethiopia. ICL opened its doors in 2004. ICL is the largest independent clinical laboratory in East Africa and accredited by Joint Commission International five times in a row. ICL participates in international external quality assurance programs on monthly and quarterly proficiency testing agreements with the College of American Pathologists (CAP) and Randox international quality assessment scheme (RIQAS). All tests are done in the central laboratory located in Addis Ababa and it follows standard operating procedures for all its activities. Wellness services are available in 3 sites of ICL branch in Addis Ababa. The service is available at the main branch which is located at Bulgaria mazoria around the Africa union, the second branch is located at CMC around Michael church and the last collection site located around Minilik hospital. There are about 400 clients per month that visit the laboratory for wellness service (31). Hematology department is the one which is equipped with different automated analyzers (abbot Cell-Dyn Ruby) used for blood cell counting and sorting.

4.2 Study design and period

Institution-based prospective cross-sectional study design was conducted from February to April 2020.

4.3 Population

4.3.1 Source population

All clients who visited International Clinical Laboratories wellness clinic during the study period were the source population

4.3.2 Study population

The study population were adult clients who came to International clinical laboratories wellness service during the study period and those who fulfilled the inclusion criteria.

4.4 Inclusion and exclusion criteria

4.4.1 Inclusion criteria

- Apparently healthy adult client visiting wellness service.
- Clients who are willing to participant in this study.

4.4.2 Exclusion criteria

- Clients who had known chronic illness though they look apparently healthy

4.5 Study variables

4.5.1 Dependent variables

- Hematological profile

4.5.2 Independent variables

- Socio demographic characteristics (age, sex, residence, educational status,)
- Stress
- Allergy
- Family history of diseases
- Cigarette smoking
- Alcohol consumption
- Parasitic infections

4.6 Sampling procedure and sample size

4.6.1 Sample size determination

Sample size calculation was performed using single population proportion formula;

$n = z2\ P\ (1\text{-}P) / d2$

Where n = sample size,

Z = Z statistic for a level of confidence (95% level of confidence; z=1.96),

d = precision (in proportion of one; if 5%, d = 0.05). D is the margin of error, here it is 0.05.

P = expected prevalence or proportion of abnormal findings 50%

n = 1.962 0.5(1-0.5)/0.052

n= 384

With 10% Non-response rate; (384*10%) + 384

The sample size will be **422.**

4.6.2 Sampling technique

Systematic random sampling technique were used.

Study population (N) = 750 clients visited the wellness service during the data collection period

Sample size (n) = 422

Sampling fraction=n/N

=422/750=0.56 (i.e., 1 in 2 hematological profile)

n/ N=1/K i.e., = 2

Where K is sampling interval or skip

4.6.3. Measurement and data collection

With the aid of a structured questionnaire, relevant socio demographic information was gathered from each participant. The questionnaires were face to face interview by study participants; any participants who needed help in filling the questionnaires were assisted by health professionals. Laboratory related data were collected from ICL wellness service polytech (laboratory information system). Specimen collected on the customers venous blood were collected using vacuum collection in tube containing a salt ethylene diamine tetra acetate (EDTA) anticoagulant. Vacuum collection tubes or micro collection containers were filled to their proper capacity. Specimen were stored at 25-30°C and was not processed if it exceeds more than 24 hours.

4.6.4 Data entry and analysis

Responses were gathered from the structured questionnaire and laboratory results were obtained from ICL wellness service polytech (laboratory information system). Data were analyzed using SPSS version 20 software according to the study objectives. The descriptive summaries were presented with tables and graphs. In order to establish the type of distribution, Skewness and Kurtosis normality test were done. Based on the distribution of the data, results were compared between categorical variables (age and sex) using parametric tests (Independent t test and ANOVA) for continuous variables. Chi-square and binary logistic regression analysis were used to see the relation between independent variables and categorical dependent variable (hematological abnormalities). P-value less than 0.05 was considered as statistically significant.

4.7 Quality assurance and quality control

The collected data were checked for its completeness, accuracy and clarity on a regular base by the primary investigator during the data collection period. All specimens were collected according to the standard operating procedure of specimen collection and the quality of the specimen were checked and all laboratory procedure were done based on the manufacturer's instructions. Quality assurance in the pre-analytical stage including appropriate use of hematological investigations, collection, storage and transport of blood specimens were performed. Moreover, analytical stage quality control checks including quality control materials (Normal, Low and High) were analyzed along with patient samples to determine if analytic errors have occurred. In the post-analytical phase, the accuracy and completeness of the collected data were checked every day by the principal investigator. The remaining blood samples were disposed of safely.

4.8 Ethical considerations

The study proposal was reviewed and approved by the departmental research and ethics review committee (DRERC) of the Medical Laboratory Sciences, College of Health Sciences; Addis Ababa University. Official permission from the study site was obtained. Moreover, prior to commencing the study, a written informed consent was obtained from each participant. All results were kept confidential; the participants were not identified by their name or other personal identifier; rather appropriate coding system was used.

4.9 Dissemination of result

The finding of this study was submitted to Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University. In addition, a copy of this material will be given to International Clinical Laboratories. The results will be disseminated through publication in peer-reviewed journal and will be presented in relevant workshops and seminars.

4.10 Operational definitions

Apparently healthy: refer to the absence of disease based on clinical sign and symptoms and function normally assessed by physical (clinical) evaluation.

Wellness services at ICL: Wellness program that encourages individuals to take an active role in managing their health; and it all begins with ICL's health assessment. Participants are interviewed about their lifestyle choices, including nutrition, physical activity and tobacco use. Through a combination of biometric screening and laboratory testing, a personal level of care

and required oversight is determined. By coordinating the appropriate program, based on the unique health status of each customer, the ICL wellness program can help persons to get healthy, stay healthy and live the best life possible.

Conceptual framework

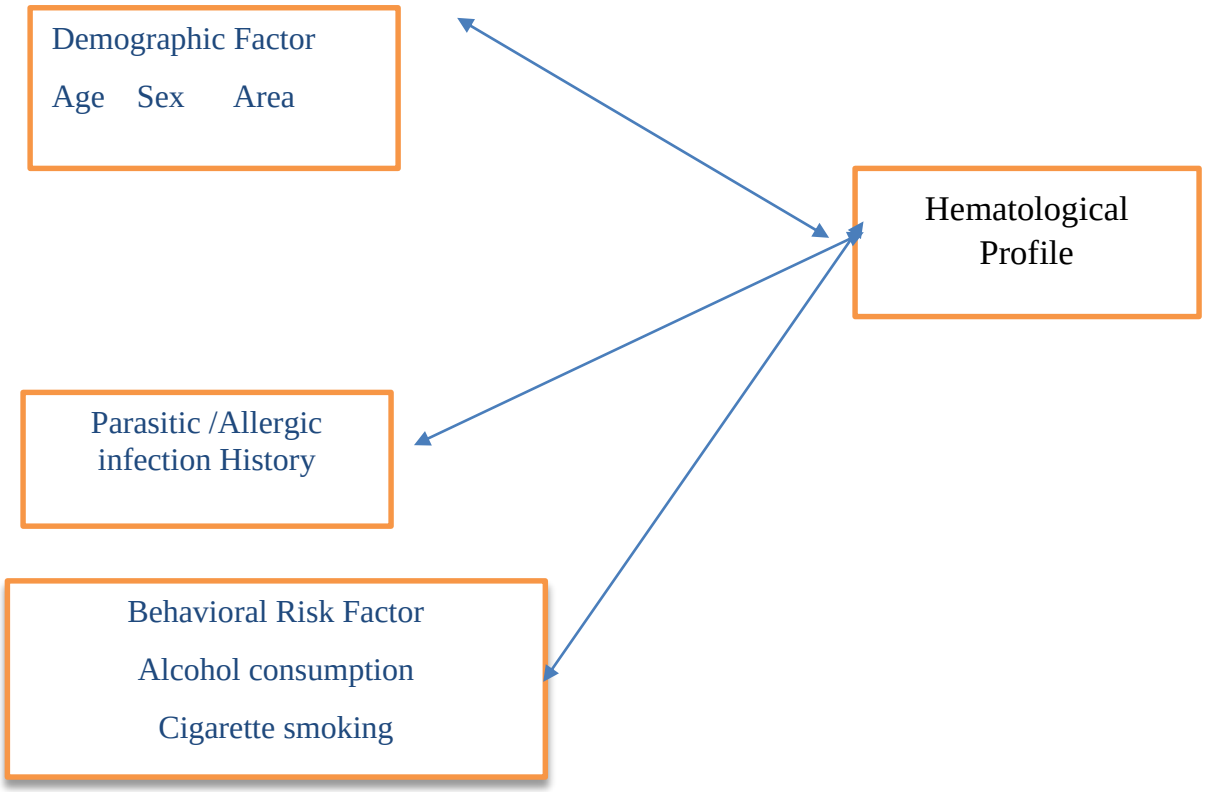

Figure 1: **Conceptual frame work of the study.**

5. Work Flow

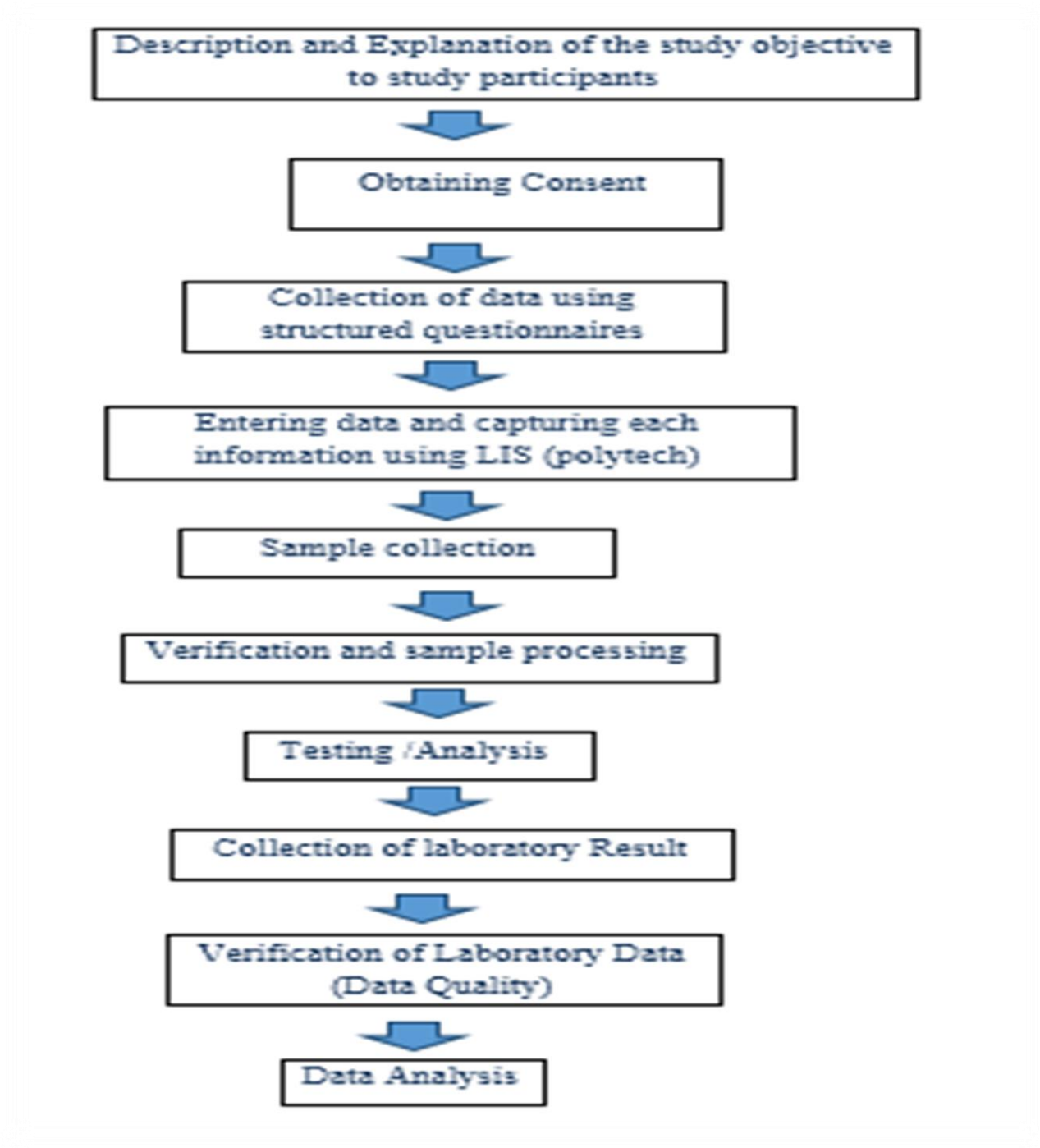

Figure 2: **Work flow used in this study.**

6. Result

6.1. Sociodemographic characteristics of study participants.

In this study, a total of 422 apparently healthy clients were included. The age of study participants ranged from 18 – 87 years, with a mean (±SD) age of 45.16±13.86 years while the median (IQR) age was 43 (35-54) years. Age generally grouped in to four categories: Category 1 = (18-35 years), Category 2 = (36-50 years), Category 3 = (51-60) and Category 4 = (>60) based on biological factors and previous studies. Majority 171 (40.5%) of the study participants were in the age group of 36-50 years. Of the total 422 study participants, 226 (53.6%) were males, whereas nearly all (97.4%) of them were urban dwellers. Regarding the educational status, majority of the study participants 67.8% had an educational level of college and above.

Nearly half (44.8%) of the study participants had family history of chronic diseases, while 35.8% had reported to have stress. Besides, highest proportion of the clients 80.6% were alcohol consumers while none of them were cigarette smokers. On the other hand, few of the participants in this study (5%) were found to be asymptomatically infected with parasites (**Table 1).**

Table 1: Sociodemographic, behavioral and clinical characteristics of apparently healthy clients visiting wellness service (n=422) at ICL, Addis Ababa, Ethiopia, 2021.

Variables	Socio demographic characteristics	Frequency	Percentage (%)
Sex	Male	226	53.6
	Female	196	46.4
Age group (years)	18-35	113	26.8
	36-50	171	40.5
	51-60	82	19.4
	>60	56	13.3
Residence	Rural	11	2.6
	Urban	411	97.4
Educational level	Illiterate	6	1.4
	Primary school	41	9.7
	Secondary school	89	21.1
	College and above	286	67.8
Stress	Yes	151	35.8
	No	271	64.2
Cigarette smoking	Yes	0	0
	No	422	100
Alcohol	Yes	82	19.4
	No	340	80.6
Family history of	No	233	55.2
	DM	59	14.0
	HTN	47	11.1
	Cancer	9	2.1
	DM, HTN	57	13.5
	DM, Cancer	3	0.7
	HTN, Cancer	3	0.7
	DM, HTN, Cancer	11	2.6
Allergy	Yes	18	4.3
	No	404	95.7
Parasitic infection	Yes	21	5.0
	No	401	95.0

Note: DM: Diabetic Mellitus; HTN: Hypertension.

6.2. Hematological profile of the study participants

As our variables were approximately normally distributed and fulfill the main assumptions, parametric tests were used and data is summarized by mean and standard deviation. Hematological profile of the study participants is summarized in Table 6.2. As shown in the table, the mean ±SD of WBC, RBC and PLT counts among males were 5.87±1.73, 5.65±0.62 and 250.38±71.58; while the counts among females were 5.95±1.99, 5.09±0.49 and 276.89±74.13, respectively.

Independent samples t-test was conducted to check statistical differences between the means of the two groups (males and females). In this study, there was statistically significant difference in some hematological profiles between males and females. The hematological parameters RBC ($p<0.001$), HGB ($p<0.001$), HCT ($p<0.001$), and absolute lymphocyte count ($p=0.028$) were significantly higher in males than females, whereas platelet ($p<0.001$) and absolute neutrophil count ($p=0.012$) were significantly higher in female counterparts. No significant difference was observed in the rest of the hematological parameters across gender groups (**Table 2**).

Table 2: Hematological profile of male and female clients (N=422) visiting wellness service at ICL, Addis Ababa, Ethiopia, 2021.

Parameters	Sex	Mean±SD	t	Mean difference	95% CI		P-value
					Lower bound	Upper bound	
WBC (10^3/µL)	Male	5.87±1.73	0.42	0.077	-0.28	0.43	0.672
	Female	5.95±1.99					
RBC (10^6/µL)	Male	5.65±0.62	10.05	0.559	0.45	0.67	<0.001*
	Female	5.09±0.49					
Hgb (g/dL)	Male	16.41±1.97	10.87	1.871	1.53	2.20	<0.001*
	Female	14.54±1.47					
HCT (%)	Male	48.21±5.53	10.49	5.11	4.15	6.06	<0.001*
	Female	43.11±4.27					
MCV (fL)	Male	84.95±5.73	0.56	0.03	-0.97	1.03	0.95
	Female	84.92±4.54					
MCH (pg)	Male	29.00±2.02	1.95	0.394	-0.003	0.790	0.052
	Female	28.60±2.10					
MCHC (g/dL)	Male	34.02±1.50	1.79	0.277	-0.02	0.58	0.074
	Female	33.74±1.65					
PLT (x10^3)	Male	250.38±71.58	3.73	26.51	12.55	40.47	<0.001*
	Female	276.89±74.13					
Neu (%)	Male	50.57±11.44	2.51	2.77	0.61	4.94	0.012*
	Female	53.34±11.14					
Lymp (%)	Male	39.81±10.61	2.21	2.30	0.25	4.4	0.028*
	Female	37.51±10.80					
Mon (%)	Male	5.30±2.17	0.15	0.036	0.42	0.49	0.879
	Female	5.27±2.61					
Eos (%)	Male	3.49±3.76	1.09	0.354	-0.29	0.995	0.278
	Female	3.13±±2.92					
Baso (%)	Male	0.70±0.73	1.72	0.117	-0.016	0.251	0.086
	Female	0.82±0.66					
Abs Neu (10^3/µL	Male	3.03±1.34	1.71	0.236	-0.04	0.51	0.089
	Female	3.27±1.50					
Abs Lymp (10^3/µL)	Male	2.26±0.79	1.21	0.092	-0.057	0.240	0.225
	Female	2.17±0.74					
Abs Mon (10^3/µL)	Male	0.30±0.18	0.06	0.001	-0.032	0.035	0.949
	Female	0.30±0.17					
Abs Eos (10^3/µL)	Male	0.19±0.20	0.52	0.011	-0.029	0.050	0.600
	Female	0.18±0.21					
Abs Bas (10^3/µL)	Male	0.04±0.05	1.82	0.009	-0.001	0.020	0.069
	Female	0.05±0.06					

Note: Abs: Absolute; CI: Confidence interval; SD: Standard deviation; * P < 0.05: statistically significant difference between males and females.

One sample t-test was conducted to compare the mean hematological parameters of the study participant with that of a reference value in current clinical use. In almost all cases absolute value of calculated- t value is greater than tabulated -t value. This indicates that there was statistically significant variation between values in the current study and reference range at 95% confidence interval; $p<0.05$ is assumed significant in all tests. However, for platelet count among females |Calculated- t| < |t-tabulated| which indicates that there was no statistically significant difference between values in the current study and the reference range in current clinical use at 95% confidence interval **(Table 3).**

Table 3: Comparison of the mean of hematological parameters of the present study with that of a reference value in current clinical use (Cell-Dyn Ruby CBC analyzer).

Hematological parameter	Sex	Means of present study	Means of Reference	t-tabulated	t-calculated	p-value
RBC (10^6/µL)	Male	5.66	5.11	1.96	13.16	<0.001*
	Female	5.10	4.51	1.97	16.58	<0.001*
Hgb (g/dL)	Male	16.42	15.5	1.96	6.95	<0.001*
	Female	14.54	13.7	1.97	7.99	<0.001*
HCT (%)	Male	48.22	46	1.96	6.03	<0.001*
	Female	43.11	40.9	1.97	7.23	<0.001*
PLT (x103)	Male	250.39	277.5	1.96	-5.69	<0.001*
	Female	276.89	277.5	1.97	-0.11	0.909
WBC (103/µL)	Male	5.88	8.3	1.96	-20.94	<0.001*
	Female	5.95	8.3	1.97	-16.47	<0.001*
Granulocytes (103/ µL)	Male	54.73	62	1.96	-10.50	<0.001*
	Female	57.29	62	1.97	-6.09	<0.001*
Lym (103/µL)	Male	39.81	30	1.96	13.89	<0.001*
	Female	37.51	30	1.97	9.73	<0.001*
Mon (103/µL)	Male	5.30	8.5	1.96	-22.16	<0.001*
	Female	5.27	8.5	1.97	-17.31	<0.001*

* |Calculated- t| > |t-tabulated| is assumed significant.

According to the one sample t-test conducted to compare the mean hematological parameters of the study participants with that of the Ethiopian reference value, in majority of the cases, absolute value of calculated- t value is greater than tabulated -t value; indicating that there was statistically significant difference between values in current study and the Ethiopian reference

range at 95% confidence interval; $p<0.05$ is assumed significant in all tests. Whereas, for means of HCT, WBC and Granulocyte counts among males; and WBC and Lymphocyte counts among female counterparts, |Calculated- t| < |t-tabulated| which indicates that there was no statistically significant difference between values in the current study and the Ethiopian reference range at 95% confidence interval **(Table 4).**

Table 4: Comparison of the mean of hematological parameters of the present study with that of an Ethiopian reference value.

Hematological parameter	Sex	Means of present study	Means of Ethiopian Reference**	t-tabulated	t-calculated	p-value
RBC (10^6/µL)	Male	5.66	5.1	1.96	13.4	<0.001*
	Female	5.10	4.5	1.97	16.86	<0.001*
Hgb (g/dL)	Male	16.42	16.1	1.96	2.39	0.018*
	Female	14.54	14.3	1.97	2.31	0.022*
HCT (%)	Male	48.22	48.3	1.96	-0.22	0.081
	Female	43.11	42	1.97	3.63	<0.001*
PLT (x103)	Male	250.39	207	1.96	9.11	<0.001*
	Female	276.89	202	1.97	14.14	<0.001*
WBC (103/µL)	Male	5.88	6	1.96	-1.06	0.290
	Female	5.95	6.2	1.97	-1.73	0.086
Granulocytes (103/ µL)	Male	54.73	55.1	1.96	-0.54	0.590
	Female	57.29	54.3	1.97	3.87	<0.001*
Lym (103/µL)	Male	39.81	35.2	1.96	6.53	<0.001*
	Female	37.51	36.4	1.97	1.43	0.154
Mon (103/µL)	Male	5.3	6.7	1.96	-9.69	<0.001*
	Female	5.27	6	1.97	-3.93	<0.001*

* |Calculated- t| > |t-tabulated| is assumed significant.

** Tsegaye et al (29)

One-way ANOVA was conducted to compare the means of the four age categories to check if there were statistically significant differences between the means of each of the age groups (18-35, 36-50, 51-60 and >60 years). From one-way ANOVA analysis, absolute lymphocyte, monocyte and eosinophil counts between mean groups had statistically significant difference but, in the Tukey HSD post hoc multi comparison test there was no statistically significant difference observed between mean groups for absolute lymphocyte, monocyte and eosinophil counts of

each age category. In all cases, there was no statistically significant difference in hematological profiles among the four age groups.

A descriptive data for the risk factors of hematological parameters is shown in the table below. In this study, minor differences in mean of hematological parameters with no statistical significance were observed among the different risk factors (**Table 5**).

Table 5: Descriptive statistics of hematological parameters across risk factors among apparently healthy clients attending ICL for wellness services, Addis Ababa, Ethiopia, 2021.

Variable (n)		WBC	RBC	HGB	HCT	MCV	MCH	MCHC	PLT	LYM	MON	Granulocyte
Education level	No (6)	5.62±1.52	5.51±0.49	14.32±2.09	45.28±6.36	82.67±10.84	26.0±3.63	31.67±1.97	320.5±84.51	40.0±14.64	5.67±2.07	54.33±12.82
	Primary school (41)	5.88±2.0	5.12±0.82	14.74±2.44	43.47±7.09	84.73±3.12	28.78±1.46	33.88±1.33	269.98±73.1	40.1±10.02	5.12±2.63	54.51±10.25
	Secondary school (89)	6.02±2.11	5.37±0.63	15.5±1.82	45.68±5.67	85.64±3.94	28.93±1.85	33.8±1.49	266.95±82.0	38.71±10.22	5.67±2.83	55.61±10.70
	College & above (286)	5.89±1.77	5.45±0.60	15.70±1.95	46.25±5.26	84.79±5.62	28.84±2.14	33.97±1.60	259.12±70.8	38.53±10.97	5.18±2.19	56.25±10.71
Stress	No (271)	5.92±1.89	5.4±0.63	15.54±2.0	45.83±5.63	84.88±5.49	28.79±2.04	33.89±1.58	260.82±69.85	38.54±10.08	5.28±2.47	56.15±10.01
	Yes (151)	5.89±1.80	5.39±0.64	15.55±1.98	45.87±5.56	85.03±4.62	28.85±2.13	33.91±1.58	266.06±80.76	39.09±11.88	5.30±2.22	55.50±11.79
Alcohol	No (340)	5.85±1.85	5.36±0.64	15.39±1.97	45.43±5.59	84.80±5.34	28.76±2.05	33.87±1.58	263.47±74.2	39.01±10.8	5.29±2.42	55.67±
	Yes (82)	6.15±1.89	5.58±0.6	16.19±1.99	47.58±5.32	85.49±4.6	29.05±2.14	33.98±1.58	259.51±73.1	37.61±10.53	5.27±2.25	56.93±10.76
Allergy	No (404)	5.9±1.89	5.4±0.64	15.54±2.01	45.85±5.63	84.9±5.27	28.78±2.08	33.86±1.58	263.0±74.97	38.82±10.83	5.3±2.4	55.82±10.75
	Yes (18)	6.13±0.98	5.35±0.57	15.75±1.69	45.69±4.9	85.72±3.4	29.5±1.69	34.61±1.46	255.89±43.79	37.0±8.97	4.94±2.1	58.06±8.76
Parasitic infection	No (401)	5.92±1.85	5.4±0.63	15.55±1.99	45.84±5.58	84.88±5.29	28.8±2.09	33.91±1.58	262.8±73.78	38.74±10.75	5.23±2.36	55.98±10.69
	Yes (21)	5.76±2.13	5.35±0.7	15.5±2.06	45.99±6.08	86.05±3.26	29.1±1.7	33.67±1.59	260.71±77.71	38.67±10.96	6.38±2.56	54.71±10.36
Family history of	No (233)	5.89±2.0	5.43±0.69	15.69±2.1	46.22±6.03	85.17±5.56	28.94±1.93	33.88±1.5	258.13±77.6	39.19±10.87	5.33±2.51	55.36±10.72
	DM (59)	6.06±1.9	5.39±0.58	15.55±1.81	45.72±4.87	84.66±4.82	28.69±2.18	34.07±1.53	264.44±80.7	38.97±9.07	5.12±2.09	55.9±8.97
	HTN (47)	5.79±1.55	5.33±0.55	15.38±1.77	45.45±4.82	85.36±4.79	28.98±2.27	33.87±1.64	261.68±58.9	38.3±10.64	5.34±2.52	56.36±10.91
	Cancer (9)	5.21±1.44	5.41±0.63	14.78±2.99	44.23±6.39	81.44±6.89	27.0±3.61	33.0±2.45	248.33±463	37.56±15.69	6.0±2.45	56.44±14.68
	DM, HTN (57)	5.96±1.71	5.39±0.56	15.52±1.72	45.81±5.09	84.96±3.95	28.81±1.72	33.98±1.65	262.87±52.5	37.84±12.12	5.23±2.13	56.96±12.19
	DM, Cancer (3)	6.3±0.95	5.48±0.99	13.53±3.73	42.73±11.18	77.67±8.02	24.67±4.16	31.67±2.52	407.3±66.58	28.67±8.08	6.67±1.16	64.33±8.74
	HTN, Cancer (3)	6.37±0.76	5.23±0.44	15.03±1.59	43.53±3.07	83.33±4.93	29.0±2.65	34.67±1.53	302.3±107.7	37.33±9.07	2.33±2.31	60.33±6.81
	DM, HTN, Cancer (11)	6.26±2.34	5.11±0.4	14.61±1.07	43.24±2.76	84.73±3.47	28.64±1.43	33.91±1.45	314.9±74.38	38.55±5.96	5.0±1.95	56.64±6.58

6.3. Hematological abnormalities among study participants

In this study, despite the study participants were apparently healthy, some hematological abnormalities were observed. The distribution of hematological abnormalities among gender is shown in the figure below. Among the total study participants, 12 (5.3%) males and 11 (5.6%) females had anemia. On the other hand, 7 (3.1%) males and 12 (6.1%) females, 3 (1.3%) males and 2 (1%) females, 1 (0.4%) male and 4 (2%) females were found to be leucopenic, thrombocytopenic and lymphopenic, respectively (**Figure 3**).

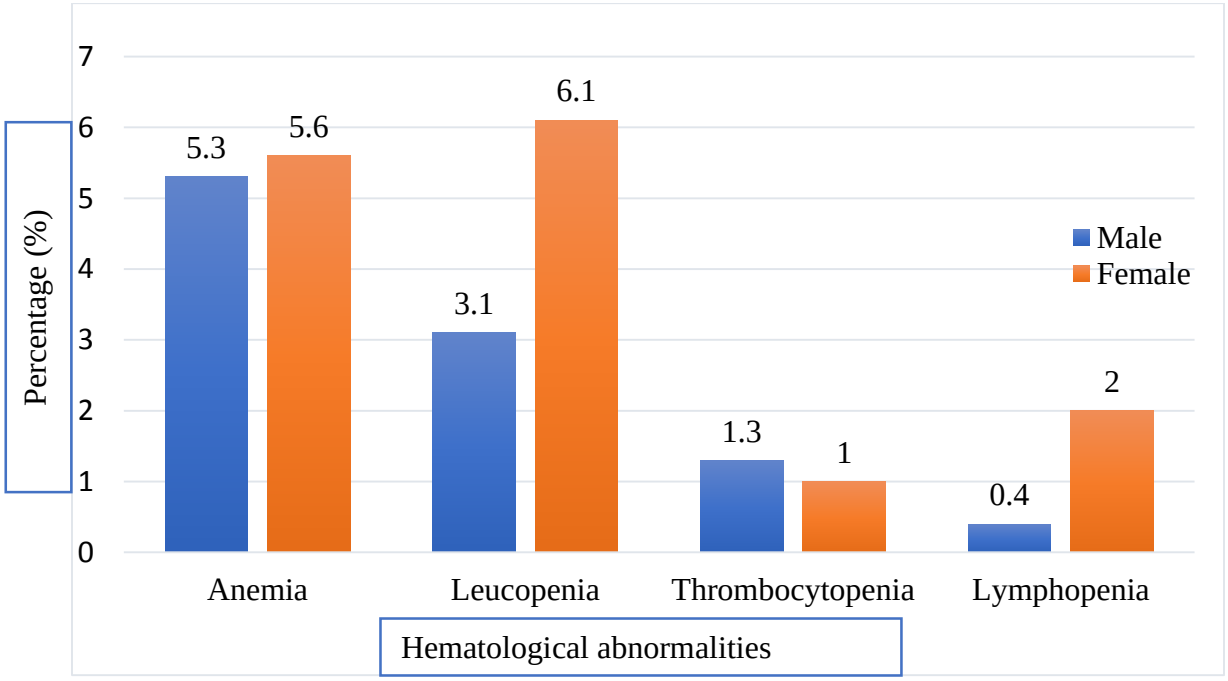

Figure 3: Hematological abnormalities among male and female study participants.

Bivariate and multivariate logistic regression analysis was done to identify factors that significantly affect the occurrence of each of the hematological abnormalities. In this study, 9 independent variables including sex, age, residence, educational status, presence of stress, allergy, alcohol consumption habit, cigarette smoking habit, chronic diseases and parasitic infections were considered during the bivariate logistic regression analysis of risk factors for hematological abnormalities. Relatively higher magnitude of hematological abnormality was found among the age group of >35 years, females, among study participants who had family history of multiple chronic diseases (diabetes mellitus, hypertension, cancer) and those who had

allergy. However, in multivariate analysis, none of the risk factors had statistically significant association with any of the hematological abnormalities including anemia, leucopenia, thrombocytopenia and lymphopenia.

7. Discussion

Hematological profile studies obtained from smaller groups of healthy adults have contribution towards understanding range of values in the country. Furthermore, regular assessment of hematological parameters in apparently healthy segment of the population is essential and serves as an input in revising and updating the national hematological parameter reference ranges. Hence, the present study was carried out to determine hematological parameters in apparently healthy adult clients attending wellness service in International Clinical Laboratories (ICL), Addis Ababa region.

Among the investigated hematological parameters, mean ± SD values of RBC (males: 5.65±0.62, females: 5.09±0.49), HGB (males: 16.41±1.97, females: 14.54±1.47), HCT (males: 48.21±5.53, females: 43.11±4.27), MCV (males: 84.95±5.73, females: 84.92±4.54), MCH (males: 29.00±2.02, females: 28.60±2.10) and MCHC (males: 34.02±1.50, females: 33.74±1.65) were obtained. These findings are found to be relatively higher compared to other similar studies done in Sudan (25) and Ghana (32). This discrepancy might be attributable to the fact that Ethiopia is found within high altitude plateau which induce polycythemic changes by enhancing erythropoiesis. Moreover, dietary factors, especially the common use of *'teff'* in Ethiopia, which high iron content might contribute to enhanced red cell production (33).

In this study, hematological parameters RBC, HGB and HCT were significantly higher in males than females. This finding is a well-established fact and is in line with study reported from Bahir Dar (34), Southwest Ethiopia (26,35), Gondar (36), and Tanzania (23). The significant difference in RBC, HGB and HCT values between males and females might be attributed to the physiological and biological factors such as the direct effect of sex hormones estrogen and androgen on erythropoiesis. This effect mostly takes place in the kidney which occur as a result of renal microvasculature dilation and vasoconstriction effect of estrogen and androgen, respectively, which in turn increase and decrease the hematocrit in blood venules, arterioles and capillaries resulting in varying red cell mass. Another justification for such variation might be due to the lower iron storage in females as a result of iron losses during regular menstruation cycle (37).

This study showed that platelet counts were significantly higher in females compared to male counterparts. Similar findings were reported by previous studies from Ethiopia in Addis Ababa (30), Dire Dawa (27), Mekelle (38) and other areas of the world including Mozambique (22), China (17) and Italy (39). This finding is also in agreement with similar study conducted in Ghana (19) which reported that females had elevated platelet count than males. Such gender-based variations in platelet count are mainly a result of hormonal differences, in which females have higher level of estrogen hormone, which has triggering effect on platelet production from megakaryocytic progenitor cells (18). Another justification for the higher platelet count of females than males is the stimulation of platelet production by the reduction in body iron in females which occurs mainly as a result of menstruation. However, this finding of our study was not in agreement with the study conducted in Addis Ababa (29), which has reported no gender specific differences in platelet counts. This suggests that gender-specific hematologic parameter reference ranges should be used for blood count result interpretations in order to enhance accurate diagnosis of disease and reduction of unnecessary healthcare cost.

Likewise, males had significantly higher absolute lymphocyte count than females. This finding is different from a study conducted in Togo which revealed that there was no gender differences in absolute lymphocyte count among females than their male counterparts (40). The possible explanation for females to have lower absolute lymphocyte values might be due to the fact that estradiol hormone in females has been shown to reduce lymphocyte production in the bone marrow (41).

Females had significantly higher absolute neutrophil counts than men. This finding is in line with a study report from Kenya which revealed that there was statistically significant difference in neutrophil count between female and male adults (42). Such variation in Neutrophil count between male and females might be attributed to gender differences in which females had higher values related to estrogen hormone since a reduction in count has been reported after menopause (43). On the contrary, a study conducted in Togo revealed that there was no significant gender difference in absolute neutrophil count (40). Moreover, a study conducted in China revealed that there is significant variation in absolute neutrophil count in which absolute neutrophil count were found to be increased in males than females (17). This might be attributed to genetic, dietary and environmental variations among the study subjects.

In the present study, there was no statistically significant difference in all tested hematological parameters among different age groups. This finding is in agreement with a study done in the Middle Eastern countries (10). However, it is contrary to a study reported from China (17) which stated that there is significant variation in RBC, HGB and HCT in different age groups among males with the elderly having a higher mean as compared to the young adults while these values were reported to increase with age among females. Moreover, the study revealed an increase in WBC and Neutrophil values with age which might be associated with chronic infection in the elderly individuals. It is further depicted from the study that mean values of MCV, MCH and MCHC did not show variation among age categories (17). Another study from Kenya (42) reported significant variations in the hematological parameters among males by age, with the young adults having a higher median of RBC as compared to adolescents, which could be attributable to an accelerated growth. The possible reason for lower hematological parameters among the elderly might be reduction in the numbers of hematopoietic stem cells, lack of hormonal stimulation and defective progenitor cell proliferation (44).

Reference ranges for almost all laboratory parameters including hematological values are traditionally being obtained from European and North American populations, from which the laboratory kits and machines are manufactured. Hematologic parameter values of apparently healthy individuals in the present study was found to be different from the reference values currently being used in clinical practice. Except WBC, platelet and absolute granulocyte counts, the rest of the hematological parameter values were higher in the present study than the reference ranges currently in clinical use. This finding is inconsistent with other studies which reported lower hemoglobin, red blood cell counts, hematocrit, platelets, mean corpuscular volume and neutrophils, and higher monocyte and eosinophil levels for African population compared to their Western counterparts (16). Genetics, gender, age, dietary patterns, ethnic origin, and environmental pathogens, all of which are known to influence and alter hematological parameters, are considered to be the cause of these disparities.

The majority of the assessed hematological parameters in this study, including RBC, HGB, HCT, platelet, lymphocyte and monocyte counts, were found to have statistically significant difference from that of the Ethiopian reference range (29). Such significant variation shows the need to conduct similar studies in other parts of the country and establish an updated reference interval.

Despite the fact that our study participants were apparently healthy clients, significant proportion of hematological abnormalities were observed, especially anemia and leucopenia. Moreover, the identified hematological abnormalities did not show statistically significant association with the assessed risk factors. Our study did not go further to identify the possible cause of these hematological abnormalities among the apparently healthy study participants. However, it might possibly be due to the use of inappropriate cut points based on the right reference range; because the cut points used to identify these hematological abnormalities are still of international standards.

8. Strength and Limitations of the study

8.1. Strength of the study

- This study was conducted in apparently healthy individuals visiting a wellness clinic the first of its type and the hematological analysis was carried out in an internationally accredited laboratory.

8.2. Limitation of the study

- The study could not be able to assess the cause-effect analysis for the hematological parameters.

9. Conclusion and recommendations

9.1 Conclusion

In this study, statistically significant variations in hematological parameters were observed from the reference range used in current clinical practice. Significant difference in hematological values based on sex was also observed. Furthermore, no age-related variation in hematological parameters was noticed in our study. The hypothesized risk factors did not have impact on blood parameters and had no statistically significant association with any of the hematological abnormalities.

9.2 Recommendation

Further investigation needs to be done to confirm the current results and establish reference range applicable for local regions. Statistically significant variation in hematological parameters of the present study with the national reference range calls for conducting similar studies in other parts of the country to confirm our results and make an update to the existing national reference range.

10. References

1. Sahoo D, Gosai H, Harsoda JM, Palan BM. A comparative hematological profile study among young individuals. *Canadian Journal of Basic and Applied Science*. 2015; 3(1):178–181.

2. MD L, JB O, UY P, NY V, MJN O, OU N, et al. Some Hematologic Parameters of Blood Donors at the National Blood Transfusion Service (NBTS), Jos, Nigeria. *Journal of Blood Disorders & Transfusion*. 2019; 10(1):1–5.

3. Bain B, Bates I, Laffan M. Blood Cell Morphology in Health and Disease. Dacie and Lewis Practical Haematology 12th ed. 2012.

4. Jacob EA. Complete Blood Cell Count and Peripheral Blood Film, Its Significant in Laboratory Medicine: A Review Study. *American Journal of Laboratory Medicine*. 2016; 1(3):34–57.

5. Tefferi A. Complete Blood Cell Count in Adults. *Mayo Clinic Proc*. 2005; 80(6):923–936.

6. Mary T. Definition of Leukopenia, Neutropenia,and Monocytopenia. In: Merck Sharp & Dohme Corp, a subsidiary of Merck & Co, Inc, Kenilworth, NJ, USA. 2020.

7. Porwit A, McCullough J, Erber W. Blood and Bone Marrow Pathology. 2nd ed. Elsevier B.V.; 2011.

8. Hoffbrand V. Megaloblastic Anemia. In: Postgraduate Haematology,. 5th ed. 2015.

9. Panawala L. What is the Function of Hemoglobin in the Human Body. *Cell Biology*. 2017.

10. Al-Mawali A, Pinto AD, Al-Busaidi R, Al-Lawati RH, Morsi M. Comprehensive haematological indices reference intervals for a healthy Omani population: First comprehensive study in Gulf Cooperation Council (GCC) and Middle Eastern countries based on age, gender and ABO blood group comparison. *PLoS ONE*. 2018; 13(4):1–13.

11. Etim NN. Haematological Parameters and Factors Affecting Their Values. *Agricultural Science*. 2014; 2(1):37–47.

12. Garba DD, Aminu M, Ameh JB, Sheyin Z, Banda JM, Bako JB. Haematological Profile of Apparently Healthy Blood Donors in Kaduna State, North Western Nigeria. *International Journal of Advances in Medical Sciences and Biotechnology*. 2016; 3(1):29–38.

13. Kempf T, Horn-Wichmann R, Brabant G, Peter T, Tim A, Klein G. Circulating concentrations of growth-differentiation factor 15 in apparently healthy elderly individuals and patients with chronic heart failure as assessed by a new immunoradiometric sandwich assay. *Clinical Chemistry*. 2006; 53(2):284–291.

14. Du Y, Chen H, Sun H, Chunli W, Wang C, Li Y. Investigation and Analysis of Reference Intervals for Blood Cell Parameters in a Healthy Population from Daxingan Region Inner Mongolia. *Clinical Laboratory*. 2016; 62(1–2):129–134.

15. Tesfaye TS, Tessema F, Jarso H. Prevalence of anemia and associated factors among "apparently healthy" urban and rural residents in Ethiopia: A comparative cross-sectional study. *Journal of Blood Medicine*. 2020; 11:89–96.

16. Clement E. Zeh, Collins O Odhiambo LAM. Laboratory Reference Intervals in Africa. Blood Cell - An Overview of Studies in Hematology. 2012.

17. Wu X, Zhao M, Pan B, Zhang J, Peng M, Wang L, et al. Complete Blood Count Reference Intervals for Healthy Han Chinese Adults. *Plos One*. 2015; 10(3):e0119669.

18. Wang MC, Huang CE, Lin MH, Yang YH, Lu CH, Chen PT, et al. Impacts of demographic and laboratory parameters on key hematological indices in an adult population of southern Taiwan: A cohort study. *PLoS ONE*. 2018; 13(8):1–14.

19. Abbam G, Tandoh S, Tetteh M, Afrifah DA, Annani-Akollor ME, Owiredu EW, et al. Reference intervals for selected haematological and biochemical parameters among apparently healthy adults in different eco-geographical zones in Ghana. *PLoS ONE*. 2021; 16(1):1–16.

20. Kueviakoe IM, Segbena AY, Jouault H, Vovor A, Imbert M. Hematological Reference Values for Healthy Adults in Togo. *International Scholarly Research Network Hematology*. 2011; 2011:1–5.

21. Bisseye C. Hemogram abnormalities in apparently healthy first-time blood donors in Libreville, Gabon. Sudan. *Journal of Medical Science*. 2019; 14(3).

22. Tembe N, Joaquim O, Alfai E, Sitoe N, Viegas E, Macovela E, et al. Reference values for clinical laboratory parameters in young adults in Maputo, Mozambique. *PLoS ONE*. 2014; 9(5).

23. Faya A, Charles M, Sembajwe LF, Dika HI. Haematological profile of healthy adult blood donors in Mwanza, Tanzania. *Tanzania Journal of Health Research*. 2018; 20(3):1–7.

24. A A, A K, H, Yasir H. Hemoglobin level and red blood cell indices in apparently healthy Sudanese blood donors in Gezira state (Sudan). *Pyrex Journal of Biomedical Research.* 2015; 1(6):91–94.

25. Awad K, Bashir A, Osman A, Malek M, Alborai A, Ali I, et al. Reference Values for Hemoglobin and Red Blood Cells Indices in Sudanese in Khartoum State. *International Journal of Health Sciences and Research*. 2019; 9(1):210–214.

26. Bimerew LG, Demie T, Eskinder K, Getachew A, Bekele S, Cheneke W, et al. Reference intervals for hematology test parameters from apparently healthy individuals in southwest Ethiopia. *SAGE Open Medicine*. 2018; 6:1-9.

27. Sissay TM, Tibebu M, Wasihun T, Tsegaye A. Hematological reference intervals for adult population of Dire Dawa town, East Ethiopia. *PLoS ONE*. 2021; 16(2):1–15.

28. Enawgaw B, Birhan W, Abebe M, Terefe B, Baynes HW, Deressa T, et al. Haematological and immunological reference intervals for adult population in the state of Amhara, Ethiopia. *Tropical Medicine and International Health*. 2018; 23(7):765–773.

29. Tsegaye A, Messele T, Tilahun T, Hailu E, Sahlu T, Doorly R, et al. Immunohematological reference ranges for adult Ethiopians. *Clinical and Diagnostic Laboratory Immunology*. 1999; 6(3):410–414.

30. Eshete EA, Weldemariam TZ. Hematological and lipid profiles of blood donors at Red Cross Center in Addis Ababa. *Ethiopian Medical Journal*. 2016; 54(1):21–25.

31. International Clinical Laboratories Ethiopia. Wellness services [Internet]. 2021. Available from: https://icladdis.com

32. Addai-Mensah O, Gyamfi D, Duneeh RV, Danquah KO, Annani-Akollor ME, Boateng L, et al. Determination of Haematological Reference Ranges in Healthy Adults in Three Regions in Ghana. *BioMed Research International*. 2019; 2019.

33. Gebru YA, Sbhatu DB, Kim KP. Nutritional Composition and Health Benefits of Teff (Eragrostis tef (Zucc.) Trotter). *Journal of Food Quality*. 2020; 2020.

34. Abera B, Alem A, Cherenet A, Kibret M. Immunological and hematological reference values for apparently healthy HIV-negative adults in Bahir Dar Town, Ethiopia. *Ethiopian Journal of Health Development*. 2012; 26(3):152–159.

35. Haileamlak A, Muluneh AT, Alemseged F, Tessema F, Woldemichael K, Asefa M, et al. Hematoimmunological profile at gilgel gibe field research center, southwest Ethiopia. *Ethiopian journal of health sciences*. 2012; 22(S):39–50.

36. Yalew A, Terefe B, Alem M, Enawgaw B. Hematological reference intervals determination in adults at Gondar university hospital, Northwest Ethiopia. *BMC Research Notes.* 2016; 9(1):1–9.

37. Murphy WG. The sex difference in haemoglobin levels in adults - Mechanisms, causes, and consequences. *Blood Reviews*. 2014; 28(2):41–47.

38. Haileslasie H, Tsegaye A, Teklehaymanot G, Belay G, Gebremariam G, Gebremichail G, et al. Community based hematological reference intervals among apparently healthy adolescents aged 12-17 years in Mekelle city, Tigrai, northern Ethiopia: A cross sectional study. *PLoS ONE* [Internet]. 2020; 15(9):1–12.

39. Biino G, Santimone I, Minelli C, Sorice R, Frongia B, Traglia M, et al. Age- And Sex-Related Variations in Platelet Count in Italy: A Proposal of Reference Ranges Based on 40987 Subjects' Data. *PLoS ONE*. 2013; 8(1):1–7.

40. Kueviakoe IM, Segbena AY, Jouault H, Vovor A, Imbert M. Hematological Reference Values for Healthy Adults in Togo. *International Scholar Research Network Hematology*. 2011:1–5.

41. Medina KL, Strasser A, Kincade PW. Estrogen influences the differentiation, proliferation, and survival of early B-lineage precursors. *Blood*. 2000; 95(6):2059–2067.

42. Odhiambo C, Oyaro B, Odipo R, Otieno F, Alemnji G, Williamson J, et al. Evaluation of locally established reference intervals for hematology and biochemistry parameters in Western Kenya. *PLoS ONE*. 2015; 10(4):1–12.

43. E. C, O. C, A. L. Laboratory Reference Intervals in Africa. Blood Cell - An Overview of Studies in Hematology. 2012;

44. Broxmeyer HE, Liu Y, Kapur R, Orschell CM, Aljoufi A, Ropa JP, et al. Fate of Hematopoiesis During Aging. What Do We Really Know, and What are its Implications? *Stem Cell Reviews and Reports*. 2020; 16(6):1020–1048.

11. Annexes

Annex-I Information sheet

Principal Investigator: Etalemahu Ayalew.

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

Title: Assessments of hematological profile among Clients visiting International Clinical Laboratory (ICL) Wellness service, Addis Ababa Ethiopia.

Introduction: First of all I would like to express my great thankfulness for your participation in this study. You are invited to participate as a study participant voluntarily. The research team includes principal investigator and advisors.

Purpose: - The main objective of the study is to assess hematological profile among visitor clients.

Risk associated with the study - You will not be at any physical or psychological risk and should experience no discomfort resulting from the research procedures.

Compensation for participation: You will not receive any payment for your participation in this research study.

Confidentiality of your information- To keep the confidentiality of the study, participant's name will not be mentioned rather unique codes are used for each participant.

Person to contact: If you have any question you can contact the principal investigator and you may ask at any time you want.

Etalemahu Ayalew

Email- Etalemahua@yahoo.com

Tel- 0912068846

Annex –II. Consent Form

Here the undersigned agree on terms and the conditions and give my consent to participate in the study to assessment of Hematological Profile among client visiting International clinical laboratory (ICL) Wellness Service I understand that my participation is wellness customer and there is no serious procedure that harms.

Do you agree to answer the following questions to the best of your ability?

Yes_____ No_____

Thank you for participation in the study!

Participant's signature: _____ Date: _____

Participant's Code _____

Annex- III. Questionnaire

Code No.: _____

Date: _____

1. Sex: M □ F □
2. Age: _____ Years
3. Address: Addis Ababa □ outside Addis Ababa □
4. Occupation:

   Student □ private□ Government □ self-employed □
5. Do you work out physical exercise today?

   Yes □ No □
6. If there is any noticed health problem with you?
   - Diabetics Yes □ No □
   - Blood pressure Yes □ No □
   - Cancer Yes □ No □
7. Do you have treatment follow up Yes □ No □
8. Do you have cigarette smoking? Yes □ No □
9. Do you drink alcohol? Yes □ No □

If yes how often?

Rarely □ Occasionally □ Habitually □

10. Do you have previous history of intestinal parasites? Yes □ No □
11. Do you have Allergic infection? Yes □ No □

Annex-IV Amharic version of consent form

የስምምነት ውል

እኔ ከዚህ በታች የፈረምኩት የጥናቱ ተሳታፊላይ ኢንተርናሽናአል ክሊንካል ለቦራቶሪ በመልካም ፈቃድ ላይ የተመሠረተ አጠቃላይ የጤና ምርመራ በሚያደርጉት ግለሰቦች ላይ ያለውን የደም ህዋስ መጠኑን እና ይዘቱን ለማጥናት በሚደረገው ስራ ለይ አላማውና ጥቅሙን በሚገባ ተረድቻለሁ፡፡ በጥናቱ ላይ መሳተፍም ሆነ አለመሳተፍም በራሴ ፍቃድ የሚወሰን መሆኑንም ተገልጸልኛል፡፡ እንዲሁም ጥናቱ ምንም አይነት ጉዳት እንደማያደርስብኝ ተረድቻለሁ፡፡

በጥናቱ ለመሳተፍ ፈቃደኛ ነዎት?

ሀ. አዎ ነኝ ለ. አይደለሁም

በዚህ ጠቃሚ ጥናት ስለረዱኝ ከልብ አመሰግናለሁ፡፡

የጥናቱ ተሳታፊ መለያ ቁጥር

ፊርማ_____

ቀን_____

Annex V Amharic version of information sheet

የጥናቱ ማብራሪያ

የጥናቱ ርዕስ

ኢንተርናሽናል ክሊኒካል ላቦራቶሪ በመልካም ፍቃደ ላይ የተመሰረተ አጠቃላይ የጤና ምርመራ በሚያደርጉ ግለሰቦች ለይ ያለውን የደም ህዋስ መጠን እና ይዘት ማጥናት ነው

መግቢያ

በመጀመሪያ በጥናቱ ላይ ለመሳተፍ ፈቃደኛ ስለሆኑ ልባዊ ምስጋናዬን አቀርባለሁ ፡፡ እርስዎ በዚህ ጥናት ውስጥ ተሳታፊ እንዲሆኑ ሲጠየቁ ተሳታፊ የሚሆኑት ፈቃደኛ ከሆኑ

ብቻ ነው፡፡ ይህንን ጥናት የሚያካሂዱት ሰዎች የተዋቀሩት በዋና ተመራማሪ እና በጥናቱ አማካሪዎች ናቸው፡፡

ዓላማ

የዚህ ጥናት ዋናው አላማ የደም ህዋስ መጠኑን እና ይዘቱንበመልካም ፈቃደኛ ለይ የተመሠረተ አጠቃለይ የጤና ምርመራ በሚያደርጉ ግለሰቦች ለይ ለማወቅ ነው ከጥናቱ ጋር ተያይዞ የሚመጣ ጉዳት በዚህ ጥናት ዝርዝር አሰራር ሂደት ውስጥ ምንም አይነት አካላዊም ሆነ አእምሮዊ ጉዳት የለም፡፡

የጥናቱ ጥቅም

ከጥናቱ የሚገኘው ውጤት ለእርሱም ህክምና ተጨማሪ መረጃ ለማግኘት እና የጎንዮሽ ጉዳት ለመቀነስ ይጠቅማል ።

ለተሳትፎ የሚሰጥ ማካካሻ

በዚህ ጥናት ውስጥ ለተሳታፊዎች የሚሰጥ ምንም አይነት የማስማሚያ ሆነ የማካካሻ የገንዘብ ክፍያ የለም፡፡

የጥናቱ ምስጢራዊነት

የተሳታፊዎችን መረጃ ሚስጢራዊነት ለመጠበቅ ይረዳ ዘንድ የጥናቱ ተሳታፊዎች ስም በጥናቱ ላይ አይገለፅም ፡፡ በስም ፋንታ መረጃዎቹ በሚስጢራዊ ቁጥር (ኮድ) ይመዘገባለ፡፡

ለጥናቱ ተጠሪ

በማንኛውም ግዜ ስለ ጥናቱ መጠየቅ የሚፈልጉት ጥያቄ ካለ መጠየቅ ይችላለ፡፡

ተመራማሪ እታለማሁ አያሌው

ስልክ 0912068846

Annex VII Amharic version of data collection sheet

ኢንተርናሽናል ክሊኒካል ላቦራቶሪ በመልካም ፍቃደ ላይ የተመሰረተ አጠቃላይ የጤና ምርመራ በሚያደርጉ ግለሰቦች ለይ ለማጥናት የተዘጋጀ መጠይቅ፡-

የሚስጢር ቁጥር .....

ቀን .....

1. **ዖታ ወንድ □ ሴት□**
2. **ዕድሜ**
3. **የመኖሪያ አድራሻ፡ ከተማ□ □□ር□**
4. **የትምህርት ደረጃ የመጀመሪያ □ ደረጃ ቴክኒክ እና ሙያ □**
   ሁለተኛ ደረጃ □ ኮሌጅ/ዩኒቨርሲቲ □
5. **የሥራ ዘርፍ ተማሪ □ የግል መስሪያ ቤት ሠራተኛ □ የመንግሥት ሠራተኛ □ የግል ሥራ □**
6. **አልኮል መጠጥ ይጠጣለ? እጠጣለሁ □ አልጠጣም □**
   የሚጠጡ ከሆነ?
   በጣም አልፎ አልፎ □ አልፎ አልፎ □ ዘወትር □
7. **ሲጋራ ያጨሳሉ አዎ □ አይ□**
8. **በእርሶ ላይ የሚስተዋል የህመም አይነት ካለ**
   የስኳር ህመምአዎ አለ□ የለም□
   የደም ግፊት አዎ አለ□ የለም□
   ካንስር አዎ አለ□ የለም□
9. **አሁን ላይ የህክምና ክትትል እያደረጉ ነው ? አዎ□ አይ□**
10. **ከዚህ በፊት የሆድ ውስጥ ትላትል** (parasites) **ኖሮቦት ያውቃል አዎ□ አይ□**
11. **ከዚህ በፊት አላርጅ ኖሮቦት ያውቃል** አዎ □ **አይ□**

Annex- V Principle and procedure of tests

Cell-Dyn Ruby CBC analyzer

Principle: - The Cell-Dyn Ruby uses flow cytometer techniques to analyze the RBC/PLT, WBC, and NOC populations. Flow cytometer is a process in which individual cells or other biological particles in a single file produced by a fluid stream are passed through a beam of light. A sensor or sensors measure, by the loss or scattering of light, the physical or chemical characteristics of the cells or particles. Flow cytometer enables the rapid screening of large numbers of cells and provides quantitative cell analysis at the single-cell level.

The basic components of a flow cytometer include: sample collector and transporter, flow system to focus the sample flow stream, light source and focusing optics, Light collectors, signal detectors (PMT and dioids), and polarizers, Data collection and storage and data display & analysis. The PMTs amplify the weak signal of side scattered light whereas dioids detect the strongest forward scattered light.

Sample Aspiration

A sample is aspirated either in open mode or closed mode and transferred to the shear valve.

Sample Segments

The Shear Valve rotates in order to separate three volumes of the aspirated sample. The three volumes are: 20 µL for the WBC dilution

1.67 µL for the RBC/PLT dilution

12 µL for the HGB dilution

RBC/PLT Analysis

1. The Diluent/Sheath Syringe dispenses 2.79 mL of diluent through the Shear Valve where the 1.67 µL RBC/PLT volume is transferred to the RBC Mixing Chamber.

2. The segment and diluent are then routed to the RBC/PLT Mixing Chamber where the dilution is mixed. The final dilution is 1:1675.

3. The Sample Transfer Pump transfers the RBC/PLT dilution from the RBC/ PLT Mixing Chamber to the Optical Flow Cell Sample Feed Nozzle.

4. Diluent/Sheath reagent, under constant pressure in the Sheath Reservoir, is directed into the Optical Flow Cell.

5. Sequentially, the Sample Metering Syringe injects 24 µL of the RBC/PLT dilution into the flow cell at a pressure (and speed) lower than that of the diluent/sheath reagent.

6. The higher speed of the sheath, which surrounds the RBC/PLT dilution, and the special geometry of the flow cell combine to focus the RBC/PLT dilution stream so that individual cells can be counted.

7. A laser beam is focused on the flow cell. As the sample stream intersects the laser beam, the light scattered is measured at 0°, 10°, and 90° for red blood cells, and at 0° and 10° for platelets.

Hemoglobin Analysis

1. The Diluent/Sheath Syringe injects 1.7 mL of diluent through the Shear Valve where the 12 µL HGB volume is transferred to the HGB Flow Cell.

2. The HGB Lyse Syringe dispenses 0.9 mL of HGB Lyse into the line after the diluent has transferred the HGB volume to the HGB Flow Cell. The entry point for the HGB Lyse is between the Shear Valve and the HGB Flow Cell.

3. The segment, lyse, and diluent are routed to the HGB Flow Cell where the dilution is mixed. The final dilution is 1:218.

4. A low-energy LED attached to the HGB Flow Cell measures the absorbance of light at 555 nm. The absorbance is proportional to the HGB concentration of the sample.

WBC Analysis

WBC are analyzed optically as follows:

1. The WBC Lyse Syringe dispenses 0.973 mL of WBC Lyse reagent through the shear valve where the 20 µL WBC volume is transferred to the WBC Mixing Chamber/WOC Heater.

2. The segment and reagent are then routed to the WBC Mixing Chamber/WOC Heater where the dilution is mixed. The final dilution is 1:50. The diluted sample remains in the mixing chamber for 14 seconds for the lysing of the red blood cells.

3. The sample transfer pump transfers the WBC dilution from the WBC Mixing Chamber/WOC heater to the optical flow cell sample feed nozzle.

4. Diluent/Sheath reagent, under constant pressure in the Sheath Reservoir, is directed into the optical flow cell.

5. Sequentially, the sample metering syringe injects 46.5 µL of the WBC dilution into the flow cell at a pressure (and speed) lower than that of the diluent/sheath reagent.

6. The higher speed of the sheath, which surrounds the WBC dilution, and the special geometry of the flow cell combine to focus the WBC dilution stream so that individual cells can be counted.

7. A laser beam is focused on the flow cell. As the sample stream intersects the laser beam, the light scattered by the cells is measured at four different detectors located in the forward (0° and 10°) and side (90° and 90°D) angles.

Fragile WBC and Resistant RBC

When running patient samples in the CBC test selection, the operator may suspect the presence of fragile WBC when the FWBC flag is displayed or may suspect the presence of resistant RBC when the RRBC and NRBC flags are displayed. In the case of samples containing fragile WBC or resistant RBC, alternate test selections are used to measure white blood cells. The results of these test selections are referred to as the Nuclear Optical Count (NOC).

When resistant RBC specimens are rerun in the CBC+RRBC test selection, the diluted WBC sample is held in the mixing chamber 15 seconds longer than in the routine patient mode. This additional lysing time is used to break down (lyse) the resistant RBC cells and prevent them from interfering with the WBC count and differential.

Histograms:

The CELL-DYN Ruby can present the WBC scatter information as two histograms: NWBC-LYM-MONO (N-L-M) and Mono-Poly (M-P).

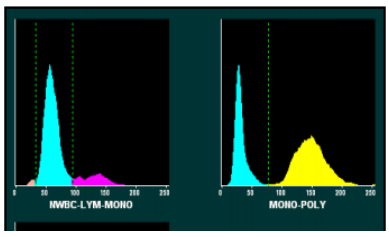

Fig: Histogram presentation of WBCs using Cell-Dyn Ruby

The WBC subpopulations are further identified by the following colors:

Neutrophils — yellow

Lymphocytes — blue

Monocytes — purple

Eosinophils — green

Basophils — white

Results Displayed: All data is transmitted to the Data Module Computer for analysis. Results are computed for all parameters and are displayed on the Run View. Results are also stored in a log format called the Data log.

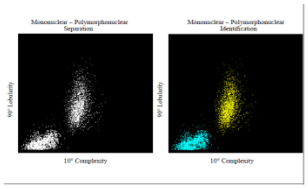

Fig: Mononuclear-Polymorphonuclear scatter

Mononuclear-polymorphonuclear Separation

The scatter information is plotted with the 90° scatter on the Y axis and the 10°

Scatter on the X axis. (The 90°/10° scatterplot is shown in the previous figure.) Two populations of cells are clearly seen on the display. The mononuclear cells fall in the cluster in the lower left corner of the scatterplot and the polymorph nuclear cells fall in the cluster above and to the right of them. The instrument uses a dynamic threshold to determine the best separation between the two populations. Each cell is then identified as a **MONO** or a **POLY**. Once each cell is identified, it retains this classification no matter where it appears on other scatterplots

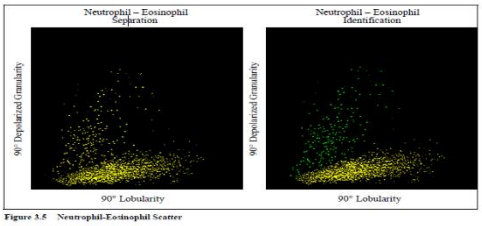

Fig: **Neutrophil- Eosinophil scatter**

Neutrophil- Eosinophil Separation

The scatter information is plotted with the 90°D scatter on the Y axis and the 90° scatter on the X axis. (The 90°D/90° scatter plot is shown in the previous figure.) Only the polymorphonuclear cells are plotted on this scatter plot. The mononuclear cells have been identified and therefore do not interfere in the further classification of the polymorphonuclear cells.

Two populations of polymorphonuclear cells are clearly seen on the display. The neutrophils fall in the lower of the two clusters. The eosinophils fall in the upper cluster. The instrument uses a dynamic threshold to determine the best separation between the two populations. Each cell is then classified as a **NEUT** or an **EOS**. All cells scatter a certain amount of 90°D light. The eosinophils scatter more 90°D light than any of the other cells because of the unique nature of granules they contain. This property of the eosinophils is used to positively identify them and thus clearly differentiate them from the neutrophil population

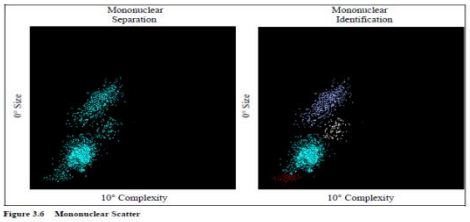

Figure 3.6 Mononuclear Scatter

Fig: Mononuclear scatter

Mononuclear Separation

The scatter information is plotted with the 0° scatter on the Y axis and the 10° scatter on the X axis. (The 0°/10° scatter plot is shown in the previous figure.) The mononuclear cells are plotted on this scatter plot. The algorithm also uses the orientation of the neutrophil cluster to aid in classifying the mononuclear. Three populations of mononuclear cells are clearly seen on the display.

There are three populations of mononuclear because basophils are included in the mononuclear cluster. Typically, basophils are granulated cells and therefore more complex than the mononuclear cells. However, the basophilic granules are water soluble and dissolve in the WBC Lyse reagent. Consequently, the degranulated basophils become a less complex cell that falls into the mononuclear cluster.

The lymphocytes fall in the lowest large cluster. (The small population of cells below the

lymphocytes contains particles that are unlikely to be WBC.) The basophils fall in the cluster above and slightly to the right of the lymphocytes. The monocytes fall in the cluster above the lymphocytes and basophils. The instrument uses dynamic thresholds to determine the best separation between the three main populations. Each cell is then classified as a **LYMPH**, a **MONO** or a **BASO** Finally, the instrument evaluates the area below the lymphocyte cluster but above the hardware threshold (channel 23). Any particles that fall in this area are separated from the lymphocytes by a dynamic threshold. The following cell types may be present in this region: NRBC, Unlysed RBC, Giant PLT, PLT clumps.

All particles in this region are excluded from the WBC count and the Differential.

Other Scatter plots 90°/0°

The scatter information is plotted with the 90° scatter on the Y axis and the 0° scatter on the X axis. 90°D/0°.

The scatter information is plotted with the 90°D scatter on the Y axis and the 0° scatter on the X axis. 90°D/10°.

The scatter information is plotted with the 90°D scatter on the Y axis and the 10° scatter on the X axis.

All scatter plots may be displayed and printed at operator request. **Nuclear Optical Count (NOC)**

Samples containing fragile WBC are difficult to measure accurately because of the rapid breakdown of cells during the measurement process. To obtain an accurate WBC count, an alternate method using the HGB segment (instead of the WBC segment) is used to measure samples containing fragile WBC. The HGB sample segment, after being measured in the HGB Flow Cell, is transferred to the Optical Flow Cell instead of being sent to a waste chamber as in the CBC test selection. While in the HGB Flow Cell, the HGB reagent lyses the cytoplasmic membrane of the white blood cells but allows the nuclear membrane to remain intact. This results in a greater stability of the white cells in the sample. The HGB segment is lysed for approximately 15 seconds before it is sent to the Optical Flow Cell.

As the HGB segment passes through the Optical Flow Cell, the nuclei of the cell are counted. The results of this measurement are stored in the Data log as NOC.

1.2. SPECIMEN TYPE AND STORAGE SPECIMEN TYPE:

EDTA-ant coagulated whole blood Specimen storage and Retention:

Specimen should be stored at 25-30°C and shouldn't be performed if it exceed more than 48 hours.

1.3. SPECIMEN CONDITIONS

- Collect specimens using venipuncture in tube containing a salt of EDTA as the anticoagulant.
- Vacuum collection tubes or micro collection containers must be filled to their proper capacity.
- Specimens should be analyzed within 48 hrs of drawing.
- For best differential results, wait 30 minutes after drawing before analyzing.
- Free of macroscopic hemolysis, clots and fibrin material

2. PROCEDURE

- Check operation of the machine, ensuring it is clean and that all required supplies are present in sufficient quantities.
- Switch the instrument on by pressing the on/off switch, located on the back of the instrument.
- Perform daily maintenance.
- Press main to return to the main menu. At the main menu, enter in the operator id and press run, next press specimen type.
- If the open mode background count results are acceptable, proceed to the next step.
- If the open mode background count results fail, press clear orifice to clear the orifice. Press main then special protocol then auto clean and put enzymatic cleaner in tube and place the sample probe in the tube and press run. When cleaning is complete, press normal background and press the plate.
- Perform control and check the calibration status.

To perform patient testing:

- Press main to return to the main menu screen. Enter in the operator ID and press run. Press specimen type then press patient specimen. Verify that run ready is displayed in the status box.

- Mix the patient sample well by automatic mixer and remove the cap.
- Place the sample probe in the tube so that the end is immersed in the sample but not resting on the bottom of the tube.
- Press the Touch Plate to start the run. The Status Box on the RUN menu indicates the stage of the run.
- When Remove Specimen is displayed in the Status Box and the probe has moved up through the wash block remove the sample tube and replace the tube cap. A beep will indicate that the probe cleaning cycle has begun.
- After the probe cleaning cycle is complete, the probe will move down into position for the next sample and the results will be displayed on the screen.

If needed, press print report for a hardcopy of the report.

Calibration is performed:

- When reagent lot number change.
- When controls are out of acceptable limits

Quality control

Control: Tri Level Controls (Low, Medium High Control)

One level of CELL-DYN controls is analyzed per day early in the morning before patient testing starts. Patient results will not be released if these values are exceeded. As new lot numbers of CELL-DYN are received, Control files containing the new Lot Numbers, Expected Values (target means) and Expected Ranges (allowable limits) must be set up in the computer (LIS).

Procedure:

- In the RUN screen, press [SPECIMEN TYPE]
- Move the cursor to the desired QC file and press [QC SPECIMEN].
- Run the control as a patient sample
- Verify that the results are acceptable.
- If the results are unacceptable, repeat the run. If the results are still unacceptable, obtain a new bottle of the control, be sure it is warmed and mixed properly and again repeat the run. If the results on all levels are unacceptable, troubleshoot as directed in section 10: Troubleshooting and Diagnostics; Subsection: Troubleshooting Guide of User's manual.

Limitations/ Interfering substance:

- WBC: platelet aggregation, giant platelets, nucleated RBCs, cryoglobulins, lyseresistant RBCs in patients with haemoglobinopathies, severe liver disease or neonates.
- RBC: Cold agglutinins, severe micryocytosis, fragmented RBCs, large numbers of giant platelets, in vitro haemolysis.
- Hgb: Lipemia, abnormal proteins in blood plasma, severe leukocytes (above 100,000/µl).
- Hct: Cold agglutinins, leukocytosis (above 100,000/µl), abnormal red cell fragility.
- PLT: Pseudothrombocytopenia, platelet aggregation, increased micrcrocytosis, megalocyttic platelets.
- Low sample volume of <1 mL may dilute patient samples with EDTA in the collection tube giving falsely low results.

RESULT INTERPRETATION

Before reporting any patient results, each CBC parameter is automatically checked against a set of abnormal criteria (see hematology confirmation criteria and procedures

CRITERIA FOR CELL-DYN CONFIRMATION

PARAMETER	CRITERIA	ACTION
WBC	< $2.0X10^3$/ µ l	Repeat Cell-Dyn
	< 2.5 or > 18.0x103/ µ l	Scan Diff
	WOC> 4.1 + Lymp >80 %	Scan Diff
RBC	RBC > 6.0 x106 /µl	Review RBC Morphology
HGB	< 7.0	Repeat Cell Dyn and Scan Diff
	< 10. Or > 18 and RDW > 18	First check specimen for clots, Review RBC Morphology
HCT	< 18	Repeat Cell-Dyn and Review RBC Morphology
	< 21 and RDW > 18	Review RBC Morphology
HCT / Hgb	< 2.7 or > 3.3	Repeat Cell-Dyn

MCV	80 or > 100 fL	Review RBC Morphology
MCHC	<29 mg/dl	Review RBC Morphology
	>39 mg/dl	Repeat Cell Dyn + Review + Review RBC Morphology
PLT	< 50 x103/ µ l	Repeat Cell-Dyn AND Review Platelet
	< 100 or > 750 x103/ µ l	Review Platelet
Neutrophils	< 0.5 or > 15 x103 / µl	Scan Diff
Lymphocyte	< 0.4 or > 8.0 x103 /µl	Scan Diff
	" Variant Lymphocytes " & LY % > 50+ LY # > 4.5 x103/µ l	Scan Diff
Monocytes	>20 % or 1.0 x103 µl	Scan Diff
Eos	Eos >20 %	Scan Diff
Basophils	Basophils >3 %	Scan Diff + Repeat Cell-dyn
Blasts	% Blast >1 % of Total WBC count	Manual Diff

CELL-DYN PARAMETER CODES AND SUSPECT FLAGS

CODE	CAUSE/SUSPECT FLAG	ACTION
<<<	<Low Event #	Scan Diff
>>>	High Event #	Scan Diff
WBC	Immature Gran./Bands Immature Neutrophils	Scan Diff if: WBC > 11.0 + Neutrophils # > 8.7 x103/ µl WBC > 11.0 + Lymphocytes # < 1.5 x103/ µ
RBC	NRBCs	Scan Diff
	RRBCs / RBC Fragment	Scan Diff
PLT	PDW > 22	Review Platelates

	PLT < 20 X 10 3/ µl	Review Platelates
	Platelate Clumps	Review Platelate

Annex-VI. 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.

MSc. candidate: Etalemahu Ayalew (B.Sc.)

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: Aster Tsegaye (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Jemal Alemu (MSc, PhD candidate)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Mesfin Nigussie (MD, Pathologist)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.