ESTABLISHMENT OF HEMATOLOGICAL PARAMETERS REFERENCE INTERVAL FOR APPARENTLY HEALTHY INDIVIDUALS ≥ 5 YEARS OF AGE IN SOUTHWEST ETHIOPIA

BY: -

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RESEARCH THESIS SUBMITTED TO THE SCHOOL OF MEDICAL LABORATORY SCIENCES, FACULTY OF HEALTH, JIMMA UNIVERSITY FOR THE PARTIAL FULFILMENT OF THE REQUIREMENT FOR MASTER OF SCIENCE DEGREE IN CLINICAL LABORATORY SCIENCES, SPECIALITY IN HEMATOLOGY AND IMMUNOHEMATOLOGY.

JANUARY, 2018

JIMMA ETHIOPIA

JIMMA UNIVERSITY
INSTITUT OF HEALTH, FACULITY OF HEALTH
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JANUARY, 2018
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ABSTRACT

Background: *Clinical laboratory reference intervals are an important tool for identifying abnormal laboratory results and for ultimately guiding patient management decisions. The setting of hematological parameters reference intervals for local population is very crucial to improve quality of health care.*

Objective: *To determine hematological parameters reference interval for apparently healthy individuals in southwest Ethiopians.*

Methodology: *A community based cross-sectional study was conducted from March 13, 2017 to May 30, 2017. A total of 998 apparently healthy individual were included in the study. Complete blood count was done by Sysmex XS-500i hematology analyzer (Sysmex Corporation Kobe, Japan). The data were first entered in to Epidata, cleaned and exported to SPSS-version 20 statistical software for analysis. The non-parametric independent Kruskal-Wallis test and Wilcoxon rank-sum test (Mann-Whitney U test) were used to compare the distribution of the parameters among age groups and between genders.*

Result: *Most of the hematological parameters show significant age differences between all age group reference intervals for both male and females. Significant differences by gender were not detected for many of the indices in children age group. In adult and geriatric age groups males had significantly higher values of red blood cell, hemoglobin and hematocrit compared to females ($p<0.001$). And also statistically significance sex differences were observed among adult age group by having adult female participants higher platelet and lower eosinophil count than adult male participants ($p<0.05$).*

Conclusion: *This study provided local reference interval which can be used to guide patient management and interpretation of laboratory findings and potentially improve the quality of health care in southwest Ethiopia.*

Key words: *Reference interval, Hematological parameters, apparently healthy, southwest Ethiopians*

ACKNOWLEDGEMENT

First and above all I need to thank the almighty of God who helped me in all situations from beginning of my life up to today my hope of all my future life.

I would like also to express my great thanks to Jimma University Faculty of Health Sciences, School of Medical Laboratory Science and the mega research project team for giving me this opportunity. And also I am very grateful to the volunteers who participated in the study.

My sincere and deepest gratitude to my Advisor Mr Lealem Gedefaw, Mr Wondimagegn Adissu and Mr Girum Tesfaye for their motivation, unreserved assistance, giving me timely comments and relevant guidance for the development of this final research thesis.

I would also like to acknowledge Dr Daniel Yilma and Mr Yaregale Asrese for their support in all situations during my work.

My special gratitude also goes to Millione Eskinder, Meseret Lemma and Endashawe Solomon. These accomplishment would not have been possible without them.

Finally, I express my very profound gratitude to my families for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this final research thesis paper.

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ABBREVIATIONS AND ACRONYMS

CBC:	Complete blood count
CLSI:	Clinical and Laboratory Standards Institute
CRP:	C-Reactive protein
EDTA:	Ethylenediamine tetraacetic acid
Hb:	Hemoglobin
HBsAg:	Hepatitis B Surface Antigen
HCG:	Human Chorionic gonadotropin
Hct:	Hematocrit
HCV:	Hepatitis C Virus
HIV:	Human Immunodeficiency Virus
JUMC:	Jimma University Medical Center
MCV:	Mean corpuscular volume
MCHC:	Mean corpuscular hemoglobin concentration
MCH:	Mean corpuscular hemoglobin
PLT:	Platelet
RBC:	Red blood cell
RI	Reference Interval
SOP:	Standard operating procedure
SPSS:	Statistical package software for social science
WBC:	White Blood Cells

CHAPTER ONE: INTRODUCTION

1.1. Background

The most important single aspect of laboratory test interpretation is the concept of a normal range, where test values that fall inside the range are considered normal and those occurring outside the range are considered abnormal (1). The concept of reference interval (RI) was introduced in 1969 by Grasbeck and Saris to describe fluctuations of blood analyte concentrations in well-characterized groups of individuals (2).

A reference interval is defined by threshold values between which the test results of a specified percentage (usually 95%) of apparently healthy individuals would fall. (3).The first step usually employed to establish normal ranges is to assume that all persons who do not demonstrate clinical symptoms or signs of any disease are normal(4).

The two statistical methods for determining such limits are the parametric and the nonparametric procedures. The parametric method assumes that the observed values, or some mathematical transformation of those values, follow a Gaussian probability curve. The nonparametric method depends only on the ranks of the reference data arrayed in order of increasing size (5).

The Clinical and Laboratory Standards Institute (CLSI) recommended that RI should be established for each region (6).Determining local RI is more laborious and costly because it entails literature review, selection of reference individuals, application of detailed questionnaires, and analysis of biological variables, such as gender, age and genetic variability, among other tasks. Furthermore, the characteristics of the population in which the reference range was determined and the population to which it is applied must be compatible (7).

Hematology refers to the study of the numbers and morphology of the cellular elements of the blood and the use of these results in the diagnosis and monitoring of disease. Hematological studies are useful in the diagnosis of many diseases as well as in the investigation of the extent of damage to blood. Hematological parameters are those parameters mainly related to the formed elements of blood (8).

1.2. Statement of the problem

Modern medicine relies extensively on the clinical laboratory as a key component of health care. It is estimated that, in current practice, at least 60–70% of all clinical decisions rely on a laboratory result. For many diseases, the clinical laboratory provides essential diagnostic information (9).

The hematological RI was determined many years ago for the Caucasian populations. These RI were obtained from a majority population living in North America and Europe (10). It is well known that hematological parameters RI are often influenced by individual variables, such as race, age, gender, dietary habits and exposure to pathogens. In addition, ecological factors such as climate, altitude and also condition of assay, variations in instrumentation techniques and laboratory personnel might affect the parameters (11). They vary not only between individuals but also between populations. Thus, there is not a universal definition of 'normal' hence it is important to define RI that are suited to the particular population of interest (12).

RIs are very useful for providing medical information that ensures correct medical decisions. RI is the most common decision support tools used for interpretation of numerical pathology reports. As laboratory results may be interpreted by comparison with these intervals, the quality of the RI can play as large a role in result interpretation as the quality of the result itself (13,14).

One of the main problems in most non-industrialized nations, however, RI have not been adequately addressed is the use of textbook RI that were mainly developed in Western countries predominantly with Caucasian populations by the clinicians, without consideration of potential differences in the RIs of laboratory parameters. Because inappropriate RI may increase the risk of either unnecessary additional investigations, failure to detect underlying disease or mismanagement of patients (15,16).

Absences of appropriate local RI for hematological parameters are challenges in interpreting results for management of patients and other decision making. Usually health professionals use textbook RI which is made for different population to compare the reported values. Abnormal laboratory result based on non-indigenous laboratory parameters and medical abnormalities were

reported to be the main reasons volunteers were excluded from two Kenyan HIV vaccine clinical trials (16,17).

Therefore locally determined RIs have large clinical importance for the correct clinical interpretation of laboratory results. Also it can reduce disease misdiagnosis and improve patient care. The hematological RI are very important for diagnostic screening, orientation ,treatment and decision for anemia, infections, and other blood disorders in general for ultimately interpreting laboratory data and guiding patient management decisions. Also RIs are very important in screening participants for enrolment into clinical trials and for monitoring the onset of adverse events during these trials. (15,18–20).

Detailed survey of literature shows that many population based studies have been carried out in developed countries especially from Caucasians, while limited studies are available on RI in resource limited settings (21). Some studies conducted in Asian and African countries indicated lower values compared with those from populations in developed countries. Moreover, another study in Africa showed lower WBC, neutrophil counts and Hb values in healthy adults (19).

Previous studies from Eastern and Southern African populations indicate differences in hematologic values, including lower values for Hb, Hct, RBC, PLT, MCV and neutrophils and increased values for monocytes and eosinophil (22).

In Ethiopia, where heterogeneous population and different geographic areas are found, there is no nationally established RI for hematological parameters, although a few attempts at determining hematological parameters reference range in some populations have been made. The hematological RI which are currently used in the country are adopted from textbooks which is not representative for the populations(23,24).

As discussed above the ethnic origin, genetics, gender, age, geography, and environmental factors, may influence hematological profiles suggesting that the development of RI for local population is very crucial to improve quality of health care. Also as these populations are increasingly participating in clinical trials establishing hematological RI also play an important role. Such valuable information is limited in Ethiopia. Therefore this study was carried out to establish hematological parameter RI in southwest Ethiopia.

1.3. Significance of the study

Locally established RI are useful for providing medical information that ensures correct medical decisions for the patient in the area. Hematological RI estimated from apparently healthy subjects in a population are imperative for accurate interpretation of hematological test results for that population.

Therefore finding of this study will be used as hematological RI for evidence based clinical practices for the population in the area. Moreover, this study along with similar large scale researches, may serve as a basis recommendation for policy making. This research may also be used as a spring board for future similar researches.

CHAPTER TWO: LITERATURE REVIEWS

2.1. Literature review

RIs are ranges of upper and lower limits based on which values are interpreted as normal and abnormal(23). RI is used to describe the dispersion of variables in healthy individuals. They are usually reported as population-based RI comprising 95% of the healthy population (2). Several studies shows that hematological parameters are different for different populations based on factors such as age, sex, ethnic background, social, nutritional and environmental factors (25).

Several studies have recognized ethnic differences in RI of various laboratory tests, mainly between blacks and whites. Compared with whites, blacks show significantly lower total WBC, neutrophil counts, PLT counts, Hct, MCHC, MCH, and Hb and significantly higher mononuclear and lymphocyte percent (15).

A study done at Curitiba, PR, Brazil showed that, there were statistically significant differences (p-value < 0.05) between men and women for most hematology parameters. Men had higher red blood cell, Hb, eosinophil, basophil and monocyte counts and higher Hct, MCH, MCHC and RDW values compared to women. Women had higher neutrophil and PLT counts than men. The red cell distribution width for both men and women was higher than the values commonly found in the literature(26).

A case control study done in Turkey, also showed that men had significantly higher values in complete blood counts with exception of total WBC count, neutrophil count and PLT counts than women ($p<0.001$). (10).

On the other hand age and sex related variations in PLT count were reported in Italy. Platelet RI were estimated from 40,987 subjects with the non-parametric method computing the 2.5% and 97.5% percentiles and shows women had significantly more PLTs than men (261 vs. $237x10^9$/L, P, 0.001), and PLT count decreased with age, with a reduction from infancy to old age of 35% in men and about 25% in women. The observed age related trend was common to all investigated populations. Under 15 years of age PLT count was significantly higher compared to the age range 15-64 years (299 vs. $252x10^9$/L, P,0.001), and PLT count in the age range 15–64 years was

significantly higher relative to subjects over 64 years (252 vs. $233x10^9$/L, P,0.001). Of note, under 15 years of age there was no difference in PLT count of men and women (298 vs. $299x10^9$/L, P = 0.690), whereas women had more PLTs in the age range 15-64 years (264 vs. $238x10^9$/L, P,0.001) and over 64 years (245 vs. $220x10^9$/L, P, 0.001) (27).

Hematological tests, using an automated hematology analyzer, were carried out from Eastern India on 528 blood samples from healthy male donors. The population was found to exhibit lower Hb measurement and PLT count as compared to the standard reference values, although the difference was statistically significant only for the PLT count (11).

Blood samples from 1100 male blood donors were collected and CBC and differential was performed using an automated hematology analyzer in a cross sectional study in the central part of Iran shows that the median and 95% RI(2.5th-97.5th) for Hb and PLT counts were 15.5g/dl (14.1-17.7) and 209 $\times 10^9$ cells/L (151-322) respectively. The median for total WBC count, neutrophil, lymphocyte, monocyte and eosinophil were 6.7 $\times 10^9$cells/L (4.3-11.2), %58 (%50-%70), 40% (30-49%), 0% (0-2) and %1 (0-3%), respectively. The hematological profile of the population was different from the reports of other countries and also the reference ranges described in textbooks (19).

A multicentric cross-sectional study conducted in India from 10,665 reference individuals identified as healthy by physicians shows the 95 %RI for Hb (Males: 12.3–17 g/dL; Females: 9.9–14.3 g/ dL) (21). Similarly, normal blood cells RI of healthy adults at the Gaza strip-Palestine showed substantial differences between males and females, between smokers and nonsmokers, and between the different age groups. Moreover, RI derived from the population are markedly shifted downward as compared with Western European and American populations (28).

A study conducted on 302 adult healthy volunteers in Pakistan whose ages ranged between 20–45 years, showed that in males, the mean Hb concentration of 13.04 g/dl and Hct ratio of 0.39 l/l were significantly higher than female's value of 11.63 g/dl and 0.35 l/l, respectively. The mean RBC count of $5.3x10^{12}$/l in males was also significantly higher than the corresponding value of $4x10^{12}$/l in females ($p<0.05$). The value of MCV in males (76.30 fl) was significantly higher than in females (73.84 fl), ($p<0.05$). The MCH and Mean MCHC were significantly higher in males than corresponding values in females ($p<0.05$). On the other hand, the mean WBC count of

$8.25x10^9$/l in males was lower than mean value of $8.42x10^9$/l in females ($p<0.05$). Similarly the values for PLT count of $255x10^9$/l in males were also significantly lower than corresponding values of $255x10^9$/l in females ($p<0.05$) (29).

A cross sectional study at seven clinical centers in eastern and southern Africa showed women had lower Hct (median 39.7% vs. 45.1%, p, 0.001) and lower Hb values than men (median 13.4 vs. 15.4 g/dL, p,0.001). Generally the study population had Hct values (and to a lesser degree, Hb) and WBC count particularly among women, tended to be lower than the U.S.-based comparison interval (22).

A comprehensive reference ranges for hematology parameters derived from normal Nigerian adults showed that the RBC count, Hb and Hct had significant gender difference ($p = 0.000$) but not for total white blood count (p.0.05). Platelets were significantly higher in females than men ($p = 0.001$) (30). Similar study conducted in northern Nigeria healthy adults showed, the mean values in the males were: Hct; 47±3%; WBC count; 5.4±1.6x10 /l, PLT count; 296±66x10 /l, MCH; 27±2pg, MCV; 84±6fl, MCHC; 32±1g/dl, RDW-CV; 13.5%, and Reticulocyte of 1.2±0.6%. The mean values in the female subjects were: Hct; 39±3%, WBC count; 5.4±1.6 x10 /L, PLT count; 272.0±70 x10 /L, MCH; 27.0±2.0pg, MCV; 86.0±6.0fl, MCHC; 31.0±1.0g/dL, RDW-CV; 14.1±1.0% and Reticulocyte count of 1.4±0.8%. There were no statistically significant differences between gender for the means of WBC count and MCH, (p values; 0.865 and 0.861 respectively) (31).

In a population based cross sectional study done in the adult population of Mampong, Ghana showed Hb concentrations were significantly higher in males, 14.2 g/dL (14.0, 14.4) than in female's 12.0g/dL (11.8, 12.2).MCV, MCHC and RBC values differed by gender. Platelet counts were higher in females, mean (95%CI) = 239×10^3 /µl (227×10^3, 250×10^3) than in males, mean (95% CI) = 213×10^3 /µl (203×10^3, 223×10^3) (32).

Hematological RI was established for healthy adults in the middle belt of Ghana, the result shows males had significantly higher Hb values of 113–164 against 88–144 g/L for females (p, 0.0001), Hct of 33.2–50.5 against 26.4–45.0% (p, 0.0001) and RBC of 3.79–5.96 against 3.09–5.3061012/L (p, 0.0001) compared to females. On the other hand, PLTs were significantly higher in females with 89–403 against 88–3526109/L for males (p, 0.0001). These results were lower

than the values set as standards on the clinical haematology machines being used in the study area (20).

Similarly hematological RI for healthy adults in Togo showed higher median Hb level in males than females (15.1g/dL versus 13.0g/dL, p = 0.000). 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, respectively, 2.1 × 109/L and 2.2 × 109/L (p = 0.11). The median PLT count was lower in males than females (236 × 109/L versus 247×109/L, p = 0.004) (33).

Statistically significant differences between males and females in most hematological parameters were found in a cross-sectional study conducted in young adults in Maputo, Mozambique in which males had higher values of RBC, Hb, Hct, MCV, MCH, MCHC and percentage lymphocyte than females. And the females had higher values of WBC count, PLT, absolute neutrophils and percentage neutrophils than males. These RI showed significant differences from the US population (17). Also statistically significant differences in Hb and Hct by gender, with males having higher values than females were observed in Botswana (34).

Hematological RI determination in healthy adult Ugandan blood donors showed statistically significant gender differences ($p<0.05$) in most hematological parameters, with the exception of the RBC indices (MCV, MCH, MCHC), absolute monocyte count and absolute basophil count. Several differences were observed when compared to previously established values from the United States, most notably in neutrophils and eosinophil's (35). Another study was conducted on hematological indices from healthy volunteers who participated in a clinical trial in Lira, northern Uganda. As per the finding study participants had low WBC and PLT counts. Ranges for RBC count, Hct and Hb values were generally broad when compared to Western RI obtained from Pathology Associates Medical Laboratories (36).

Hematologic RI and their respective intervals were established for the Ugandan children the lower limits of Hb, Hct levels, MCV and PLT counts for the Ugandan children were found to be less than conventional RI of Caucasian children, the WBC count RI were higher than the international intervals (37).

A cross sectional study conducted on healthy Malian adults shows a Median WBC count of 5200 cells/µL [3237.5-11900], RBC count of 4.94 10^6 [3.56-6.17], Hb of 14.2 g/dL [12.2-17.38],

PLT count of 275 103^9/µL [145.4-614.4], lymphocytes 2050/µL [1200-3800], neutrophils 2200/µL [1040-6220]; monocytes 200/µL [100-660]; eosinophil's 131/µL [0-1026] (38).

Another population based study on hematologic RI was done for a healthy Ugandan population and the result shows erythrocyte, Hb, and Hct levels and MCV all significantly increased with age ($P < 0.001$) and were independent of gender until the age of 13 years, after which the levels were higher in males than in females ($P < 0.001$). White blood cell, neutrophil, lymphocyte, basophil, and monocyte counts significantly declined with age until the age of 13 years ($P < 0.001$), with no differences by gender, while PLT counts declined with age ($P < 0.001$) and showed differences by gender only among adults older than age 24 years. The absolute values for many of these parameters differed from those reported for populations outside Africa (39).

Population based study on hematological RI for adolescents and young adults in a rural population in western Kenya showed statistically significant differences in RBC, Hb and HCT by gender, with males having higher values than females in both age groups. Also differences were observed in the hematological indices among males by age, with the young adults having higher levels of Hb, Hct, RBC, and PLT as compared to adolescents ($P= 0.001$). PLT counts were significantly higher among young adult females compared to the males in the same age group ($P = 0.0222$) and also differed between adolescent and young adult males ($P = 0.0094$). No gender or age differences were observed in absolute lymphocytes, basophil, eosinophil and monocytes counts. But there were significant differences in neutrophil counts between male and female young adults, with the females having higher counts than males (40).

Another cross sectional study on hematological reference range in apparently health adult blood donors at Gondar university hospital, Northwest Ethiopia shows that the median and 95th percentile RI for RBC, Hct, Hb and MCHC were 5.01 (3.53–6.93 × 10^{12}/l), 46.9 (36.2–58.6%), 14.2 (11.5–18 g/ dl) and 31.3 (29.5–34.4 g/dl) respectively for males and 4.8 (3.45–6.25 × 10^{12}/l), 45.2 (32.1–56.6%), 12.9 (11–16 g/dl) and 30.8 (28.5–34.4 g/dl) respectively for females. And the median and 95^{th} percentile of WBC, absolute neutrophils, absolute lymphocytes, absolute mixed cell, PLT, MCV and RDW were 5.1 (3.2–8.8 × 10^9/l), 2.7 (1.6–5.1 × 10^9/l), 1.9 (1–3.5 × 10^9/l), 0.5 (0.2–1 × 10^9/l), 264 (128–432 × 10^9/l), 92 (85–100 fl) and 14 (12–17%) respectively for the general population (41). Another study in Bahir Dar town, Ethiopia showed significantly lower Hb, Hct and RBC values in females than in males (p=0.001). There was no

statistically significant difference between the sex for hematological indices of MCV, MCH and MCHC (23).

A cross sectional study was carried out in factory workers in Akaki, Ethiopia. The result shows leukocyte (WBC) counts, $6.1x10^9$/liter (both genders); erythrocyte counts, $5.1x101^2$/liter (males) and $4.5x10^{12}$/liter (females); Hb, 16.1 (male) and 14.3 (female) g/dl; Hct, 48.3% (male) and 42.0% (female); PLTs, $205x10^9$/liter (both genders); monocytes, 343/µl; granulocytes, 3,057/µl; lymphocytes, 1,857/µl. The distributions of the RBC parameters (median Hb, Hct, and RBC) were statistically different by gender; females had lower values than males (P, 0.001). No gender-specific differences were observed for WBC or PLTs. The various WBC subset values are not statistically different between males and females (24).

A study at Gilgel Gibe field research center, southwest Ethiopia showed that the mean RBC count for men and women was 4.55 x 10^{12}/L and 4.34 x 10^{12}/L (95 percentile range between 2.9 and 5.7 x 10^{12}/L) and 4.34 x 10^{12}/L (95 percentile range between 2.8 and 5.2 x 10^{12}/L), respectively. On the other hand, the RBC count of 95% of the men and women lied between 2.9-5.7 X 10^{12}cells/L and 2.8-5.2 X 10^{12} cells /L, respectively. The mean Hb value for men was 13.6 gm/dl and for women 12.7 gm/dl. The MCV for men and women was 90.2 fl and 90.8 fl, respectively. The mean PLT value for men was 229.1 $x10^9$ cells/L and for women 241.3 $x10^9$ cells/L. The mean white blood cells count for men and women was 6.08 $x10^9$ cells/L and 6.12 $x10^9$cells/L, respectively (16).

A review from literatures confirmed that there is a considerable variation in hematological parameters RI by several variables. These suggest that the use of imported reference value generated for dissimilar population may misguide hematological laboratory results on the other population. Therefore, it is clear that the uses of locally established reference value for hematological parameters are very important for proper diagnosis of the population in the area. However such comprehensive data at population level are limited in Ethiopian situation. Also there are limited data in Southwest Ethiopia. Therefore this study were carried out to establish hematological parameters RI for Southwest Ethiopians.

CHAPTER THREE: OBJECTIVES

3.1. General objective

- To establish hematological parameters RI for apparently healthy individuals ≥ 5 years of age in Southwest Ethiopians.

3.2. Specific objectives

- To evaluate potential variables that affects the hematological RIs in southwest Ethiopians.
- To compare the hematological RIs of southwest Ethiopians with other hematological RIs.

CHAPTER FOUR: METHODS

4.1. Study area

This study was conducted in Southwest Ethiopia including Jimma, Bonga and Mattu Towns by considering their population diversity and convenience to the study.

Jimma, is the largest town in Southwest Ethiopia. It is located in Oromia regional state, Jimma Zone. It has an elevation of 1780m above sea level. Based on the 2007 Census conducted by the Central Statistical Agency of Ethiopia (CSA), the town has a total population of 120,960, of which 60,824 are men and 60,136 women With an area of 50.52 square kilometers, Jimma has a population density of 2,394.30 all are urban inhabitants. A total of 32,191 households were counted in this town, which results in an average of 3.76 persons to a household, and 30,016 housing units (42).

Bonga town is located in the Kaffa zonea of the SNNPR, an elevation of 1,714 meters above sea level. Based on the 2007 Census conducted by the CSA, this town has a total population of 20,858, of whom 10,736 are men and 10,122 women (42).

Mettu Town is the capital city of Illu Ababora Zone of the Oromia Region in south-west Ethiopia. It is located around 265Km away from Jimma city and its altitude of 1605 meters above sea level. The 2007 national census reported a total population for Metu of 28,782, of whom 14,400 were men and 14,382 were women (42).

4.2. Study design and study period

A Community based cross sectional study was conducted from March 13 to May 30, 2017.

4.3. Participants

4.3.1. Source participants

All individuals ≥ 5 years of age living in Southwest Ethiopia were considered as source participants.

4.3.2. Study participants

The study participants were school children (primary and secondary school), University students and staff, government and non-government employees and any individuals who will fulfill the eligibility criteria.

4.4. Eligible criteria

4.4.1. Inclusion criteria

- Apparently healthy individuals aged five years and above
- Resident in the area for at least one year.
- Volunteer to participate and available during the study period.

4.4.2. Exclusion Criteria

- Individuals who had a positive results for the screening testes (CRP, HBsAg, HCV).
- Individuals who has any known acute and chronic disease.
- Recent history of blood loss and any blood donation in the last 6 months.
- Individuals who received blood transfusion within the previous 1 year.
- Recent past immunization in the last 6 months.
- Surgical therapy in the past 6 months.
- Intake of pharmacologically active agents including oral contraceptives, replacement or supplementation therapy e.g. Insulin.
- Intake of alcohol and tobacco.
- For women who are pregnant.

4.5. Sample size determination and sampling technique

The sample size was determined based on the recommendation of Clinical and Laboratory Standards Institute (CLSI) guideline (6). According to CLSI, well - defined exclusion and partitioning criteria is required before the selection of the reference individual. CLSI recommends that the best means to establish a RI is to collect samples from a sufficient number of reference individuals to yield a minimum of 120 samples for analysis, by parametric and non-parametric means for each partition (e.g. sex, age range). The maximum partition was needed for Hb determinations which will be as follows 5-14 years; Non-pregnant women 15 years and above and Men 15 years and above and older age group (>50 years) (43). Thus, six age and sex partition groups were needed (6x120=720).

According to previous studies in other African countries, in such large scale studies about 30%(44) of the participants do not qualify for RI determination for various reasons when tested for the common viral infections and syphilis. To reach a total sample size of 720, ($n * 70\% = 720 \Rightarrow n = 720/0.7 = 1028.6 \approx 1030$) thus, a total of 1030 individuals (30% x 1030=309; 1030-309 = 721) was needed.

By non-probability convenient sampling technique because of lack of volunteers a total of 998 study participants (626 from Jimma 247 from Bonga and 125 from Metu) sites that fulfill the eligibility criteria were included in the study. As described above based on Hb determination in the first age group between 5-14 years of age we had 166 male and 188 female children's and in the second age group between 15-49 years of age we had 165 male and 178 female adults. In the third age group we included 162 male and 139 female geriatrics individuals.

Individuals were approached through the schools, Universities administrators/deans and the respective institutions administrators. Physical examination for exclusion was done before sample collection.

4.6. Study variable

4.6.1. Dependent variable

- Hematological RI

4.6.2. Independent variable

- Age
- Sex
- Altitude
- Body mass index (BMI)

4.7. Data collection

4.7.1. Socio demographic and related data collection

Structured questionnaire was used to collect socio-demographic and other related data. Physical examination and interview with study participants were done by a trained clinical nurse.

4.7.2. Anthropometric measurement

Height and weight was taken in duplicate using calibrated equipment's and standardized techniques.

4.7.3. Sample collection

This study was a part of mega research entitled "Establishment of laboratory RI for southwest Ethiopians", so that sample was collected for both hematological and clinical chemistry RI determination. For this study about 4 ml of blood sample was collected into EDTA vacutainer tubes. The indicated volume is within allowable blood volume that can be collected from children 5-14 years. The blood sample collected from the study participants was transported to Jimma University Medical Center (JUMC) laboratory by ice box without ice from the sample collection area and processed within 4 hrs of collection.

Whole blood was used for hematological analysis and plasma samples for screening CRP, HBsAg, and HCV. Hematological analysis was performed according to the keeping time for each test. All samples were labeled with a unique identification number (0001).

4.8. Laboratory analysis

- **Screening of study participants**

The semi-quantitative determination of C-reactive protein (CRP) was done by HumaTex CRP testes (Human, Germany) for screening the participants for the presence of inflammation. Hepatitis B virus was screened by One Step hepatitis B surface antigen (HBsAg) test (Guangzhou Wondfo Biotech Co., Ltd, China), which is a rapid test for the qualitative detection of HBsAg in human plasma. Hepatitis C virus (HCV) was screened by One Step HCV antibody (Guangzhou Wondfo Biotech Co., Ltd, China), which is a rapid test for the qualitative detection of antibodies to HCV in human serum, plasma or whole blood. (Annex 2: 2.4,2.5,2.6)

- **Hematological analysis**

Four (4) ml of EDTA containing whole blood was used for complete blood count: Hb, MCH, MCHC, RBC, Hct, MCV, RDW, total WBC count, WBC differential count and PLT count. Complete blood counting was done by Sysmex XS-500*i* hematology analyzer (Sysmex® Corporation Kobe, Japan). The Sysmex XS-500*i* is an automated hematology analyzer that can analyze and output the results of 24 parameters of a blood sample. And perform analysis of WBC and differential with an optical detector block based on the flow cytometry method, using a semiconductor laser. The RBC's and PLTs are analyzed by the RBC detector

using the Hydro Dynamic Focusing method. Analysis data is displayed on the Information Processing Unit (IPU). Hb is analyzed by the Hb detector based on the SLS Hb detection method. (Annex 2; 2.3)

4.9. Operational definitions

- Children: an individual aged between 5-14 years of age.
- Adult: An individual aged between 15-49 years of age.
- Geriatric: An individual aged between ≥ 50 years of age.
- Apparently healthy: An individual who has no sign and symptoms and history for any disease and negative result for the screening tests.
- Hematological parameters: Hematological parameters are those which consist red blood cell (RBC), hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH),mean corpuscular hemoglobin concentration (MCHC), total white blood cell (WBC), WBC differential and Platelet (PLT).
- Reference Interval: The 95 percentile interval between the 97.5 percentile and 2.5 percentile form the upper and lower reference limit.

4.10. Statistical analysis

All the data from the questionnaires and laboratory results were coded and checked for completeness. Then entered to Epidata and analyzed using SPSS-version 20 statistical software for windows.

The data were tested for normality distribution by Kolmogorov Smirnoff and most of the RI parameters were not normally distributed. Therefore, the non-parametric methods for determination of RI were adopted as recommended by CLSI (6). Median, central 95 percentile and 95% confidence interval (CI) was calculated. The 97.5 percentile and 2.5 percentile were form the upper and lower reference limit to the population. The significance difference between sex among age groups were determined by using Wilcoxon rank-sum test (Mann-Whitney U test) and significance difference between age groups among sex were determined by using Independent Kruskal-Wallis test. p value <0.05 was considered as statistically significant.

4.11. Quality assurance

In order to obtain reliable and valid data in this study the following quality assurance measures were taken.

- To ensure the quality of data, training /orientation were given to data collectors prior to data collection.
- The questionnaire was translated to the Amharic and Afan Oromo and back to English.
- Standard operating procedures (SOP) for pre-analytical, analytical and post-analytical procedures were prepared implemented and followed.
- Blood specimen were rejected if there was any hemolysis and clot (partial or full), tubes not filled with minimum volume, and improperly labeled specimen.
- For Sysmex XS-500*i* hematology analyzer three levels (Tri level) commercially available quality controls (High, Medium, and Low) where used daily on start up.
- Repeated analysis of randomly selected specimens for reproducibility check (delta check) was carried out to evaluate instrument performance consistently and accurately.
- Always routine maintenance of equipment was checked prior to analysis on daily basis for background check, vacuum error, proper washing, start up and turning off of the instrument according to the manufacturers' instruction.
- The results of all laboratory examinations were recorded on standardized report format carefully and attached to questionnaire according to subject's unique identification number.
- JUMC laboratory service participated on quarterly basis on external quality assessment (EQA) scheme through the Ethiopian Public Health Institute at One World accuracy for automated hematology analysis and the laboratory had >95% of the acceptability limit of the assessor.

4.12. Ethical consideration

Ethical clearance was obtained from Jimma University, Institute of Health Ethical Review Committee. Support letter from Health Science Research coordinating office was written to concerned body and the permission was obtained from concerned office. A written informed consent was obtained from the study participants (for school children from their parents through the school). The collected data were kept confidential. The specimen collected from the

participants was analyzed only for the intended purposes. Laboratory results were given to participants and those who have positive laboratory result during the screening process, were sent to communicate the clinician.

In this study there was collection of blood samples that might contain potential dangerous pathogens. Hence all specimens were handled with strict precaution following safety rules and good laboratory practice guidelines not to harm the health personnel and the community at large. All specimen containers and specimen transporting materials are leak proof to prevent spillage of specimen to the environment. Needles, syringes, and other sharp materials were discarded in sharps container and left over specimens was disposed as per the recommendation for laboratory wastes disposal.

CHAPTER FIVE: RESULT

5.1. Socio demographic characteristics

A total of 998 individuals were enrolled in the study of which, 11.5% (115/998) of them were positive for the screening tests therefore excluded from the analysis. Data from the remaining 883 apparently healthy individuals were analyzed for hematological parameter RI determination. Out of them 48.7% (430/883) were males and 51.3% (453/883) were females with a mean age of 27.61 years (male=28.5 and female=26.7) which ranged from 5 to 71 years (Table 5.1).

Table 5.1: Socio demographic characteristic of participants in South waste Ethiopia from March 13 To May 30, 2017.

Variable	Socio demographic characteristic	Frequency	Percentage (%)
Sex	Male	430	48.7
	Female	453	51.3
Age group	Children	334	37.8
	Adult	289	32.7
	Geriatrics	260	29.4
Ethnicity	Oromo	356	40.3
	Amhara	187	21.2
	Tigre	26	2.9
	Keffa	150	17.0
	Dawero	76	8.6
	Others	88	10
Religion	Orthodox	458	51.9
	Muslim	192	21.7
	Protestant	173	19.6
	Catholic	56	6.3
	Others	4	0.4
Educational status	Illiterate	3	0.3
	Read and write	3	0.3
	Grade 1-4	263	26.7
	Grade 5-8	182	20.6
	Grade 9-12	133	15.1
	College/university	326	36.9

5.2. Hematological RI for children's

The median and 95% RI of RBC, Hb, WBC and PLT in this age group were 5.04x10^{12}/L (4.06x10^{12}/L-6.57 x10^{12}/L), 141g/L (120g/L-196g/L), 7.05x10^{9}/L (4.04x10^{9}/L-11.72x10^{9}/L) and 326.5x10^{9}/L (158.5x10^{9}/L-469.9x10^{9}/L) respectively for males and 4.96x1012/L (4.32x10^{12}/L-5.63x10^{12}/L), 140g/L (115.7g/L-159.4g/L), 7.02x10^{9}/L (3.74x10^{9}/L-11.42x10^{9}/L) and 321x10^{9}/L (197.7x10^{9}/L-460.4x10^{9}/L) respectively for females. Significant differences by gender were not detected for many of the indices in children age group. (Table 5.2).

Table 5.2: Hematological profile in children aged 5-14 years in southwest Ethiopians.

Parameter	Sex	Unit	N	Median	Min	Max	95%		P-Value
							2.5	97.5	
WBC	M	10^{9}/L	152	7.05	3.29	14.10	4.04	11.72	
	F	10^{9}/L	182	7.02	3.21	12.18	3.74	11.42	0.933
RBC	M	10^{12}/L	152	5.04	3.48	8.28	4.06	6.57	
	F	10^{12}/L	182	4.96	3.49	6.61	4.32	5.63	0.177
Hb	M	g/L	152	141.0	111.0	208.0	120.4	196.0	
	F	g/L	182	140.0	97.0	182.0	115.7	159.4	0.409
Hct	M	%	152	41.40	33.1	60.6	35.60	55.19	
	F	%	182	41.50	31.3	52.7	35.97	46.92	0.820
MCV	M	fl	152	82.35	72.1	95.5	75.03	93.01	
	F	fl	182	83.20	69.4	94.3	74.51	91.08	0.104
MCH	M	pg	152	27.95	24.2	32.0	25.18	31.05	
	F	pg	182	28.0	21.5	31.7	25.08	30.8	0.675
MCHC	M	g/L	152	340.0	315.0	364.0	321.0	362.0	
	F	g/L	182	338.0	310.0	368.0	320.7	354.4	0.073
PLT	M	10^{9}/L	152	326.5	122.0	494.0	158.5	469.9	
	F	10^{9}/L	182	321.0	110.0	483.0	197.7	460.4	0.834
RDW-CV	M	%	152	13.85	12.30	18.80	12.70	16.07	
	F	%	182	13.70	9.40	19.30	12.30	15.97	0.021*
Neutrophil	M	10^{9}/L	152	3.34	0.90	8.71	1.26	7.39	
	F	10^{9}/L	182	3.41	0.80	8.31	1.00	6.99	0.633
Lymphocyte	M	10^{9}/L	152	2.62	1.00	4.86	1.50	4.25	
	F	10^{9}/L	182	2.60	1.15	4.78	1.41	4.47	0.501
Monocyte	M	10^{9}/L	152	0.54	0.17	1.61	0.27	1.05	

	F	10^9/L	182	0.53	.22	1.47	0.27	1.06	0.431
Eosinophil	M	10^9/L	152	0.43	0.04	1.81	0.048	1.49	
	F	10^9/L	182	0.36	.02	1.96	0.055	1.31	0.370
Basophil	M	10^9/L	152	0.02	0.0	0.07	0.01	0.051	
	F	10^9/L	182	0.02	0.0	0.4	0.01	0.06	0.220

WBC: White blood cell, RBC: Red blood cell, Hb: Hemoglobin, Hct: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, PLT: Platelet, RDW-CV: Red cell distribution width-Coefficient of variation,
M: Male, F: Female, N: Number of participants
*p<0.05 by (Mann-Whitney U test) for comparison of medians between genders

5.3. Hematological RI for adults

In adult age group males had higher value in most of the RBC parameters. The median and 95% RI of RBC, Hb, and Hct in males were 5.32x10^{12}/L (4.26x10^{12}/L-6.68 x10^{12}/L), 155g/L (120.6g/L-187.6g/L), 45.2% (36.7%-54.5%) and respectively and in females were 5.02x1012/L (4.02x10^{12}/L-6.15x10^{12}/L), 146g/L (123g/L-178.6g/L), 43.1% (36.8%-51.5%) and Similarly the eosinophil counts of males' 0.28x10^9/L (0.05x10^9/L-1.21x10^9/L) were significantly higher than the corresponding females' 0.22x10^9/L (0.04x10^9/L-1.12x10^9/L). The median and 95% RI of WBC count shows no significance difference between genders in which males had 6.36x10^9/L (3.31x10^9/L-11.62x10^9/L) and females had 6.34x10^9/L (3.24x10^9/L-10.05x10^9/L) (p>0.05). On the other hand the median and 95% RI of MCV and PLT values in males 84.3fl (74.8fl-93.8fl) and 275x10^9/L (164x10^9/L-403.4x10^9/L) were lower than the corresponding females 86.15fl (77.3fl-98.8fl) and 288x10^9/L (202.3x10^9/L-444.5x10^9/L) (Table 5.3).

Table 5.3: Hematological profile in adult aged 15-49 years in southwest Ethiopians.

Parameter	Sex	Unit	N	Median	Min	Max	95%		P-Value
							2.5	97.5	
WBC	M	10^9/L	143	6.36	2.66	12.14	3.31	11.62	
	F	10^9/L	146	6.34	2.86	13.22	3.24	10.05	0.826
RBC	M	10^{12}/L	143	5.32	3.26	8.00	4.26	6.68	
	F	10^{12}/L	146	5.02	3.67	6.75	4.02	6.15	0.000*
Hb	M	g/L	143	155.0	111.0	233.0	120.6	187.6	
	F	g/L	146	146.0	91.0	190.0	123.0	178.6	0.001*
Hct	M	%	143	45.2	34.2	65.40	36.72	54.48	

	F	%	146	43.1	30.4	56.10	36.86	51.59	0.001*
MCV	M	fl	143	84.3	57.9	111.3	74.8	93.94	
	F	fl	146	86.15	70.7	114.2	77.3	98.82	0.003*
MCH	M	pg	143	29.0	18.8	38.0	24.86	32.84	
	F	pg	146	29.4	21.20	39.90	26.3	33.58	0.098
MCHC	M	g/L	143	343.0	303.0	370.0	320.6	365.0	
	F	g/L	146	339.5	299.0	368.0	320.0	360.0	0.084
PLT	M	10^9/L	143	275.0	134.0	637.0	164.0	403.4	
	F	10^9/L	146	288.0	144.0	508.0	202.3	444.5	0.021*
RDW-CV	M	%	143	13.7	12.1	21.0	12.46	17.56	
	F	%	146	13.6	12.1	27.3	12.4	15.59	0.032*
Neutrophil	M	10^9/L	143	3.3	0.69	7.96	1.01	7.22	
	F	10^9/L	146	3.3	0.57	9.29	1.08	6.69	0.760
Lymphocyte	M	10^9/L	143	2.14	0.84	4.26	1.1	3.84	
	F	10^9/L	146	2.16	0.86	4.73	1.2	3.98	0.462
Monocyte	M	10^9/L	143	0.48	0.19	1.1	0.24	0.88	
	F	10^9/L	146	0.48	0.23	1.12	0.27	0.87	0.865
Eosinophil	M	10^9/L	143	0.28	0.03	1.53	0.05	1.21	
	F	10^9/L	146	0.22	.02	1.38	0.04	1.12	0.011*
Basophil	M	10^9/L	143	0.02	0.0	0.07	0.01	0.05	
	F	10^9/L	146	0.02	0.0	0.07	0.0	0.05	0.190

WBC: White blood cell, RBC: Red blood cell, Hb: Hemoglobin, Hct: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, PLT: Platelet, RDW-CV: Red cell distribution width-Coefficient of variation,

M: Male, F: Female, N: Number of participants

*p<0.05 by (Mann-Whitney U test) for comparison of medians between genders

5.4. Hematological RI for geriatrics

In geriatric age group males had significantly higher median and 95% RI of RBC 5.16 x10^{12}/L (4.25x10^{12}/L-5.99x10^{12}/L) against 4.92x10^{12}/L (3.91x10^{12}/L-5.72x10^{12}/L), Hb of 151 g/L (126.4g/L-179g/L) against 142g/L (119.1g/L-177.8g/L) and Hct of 44.5% (38.3%-52.4%) against 42.6 (36.2-51.4) compared to females (p<0.001). The other hematological parameters show no significance between genders (p>0.05) (Table 5.5).

Table 5.4: Hematological profile in aged geriatrics >50 years in southwest Ethiopians.

Parameter	Sex	Unit	N	Median	Min	Max	95%		P-Value
							2.5	97.5	
WBC	M	10^9/L	135	6.59	2.70	13.54	3.18	10.18	0.108
	F	10^9/L	125	6.09	2.77	11.12	3.34	9.98	
RBC	M	10^{12}/L	135	5.16	3.9	6.28	4.25	5.99	0.000*
	F	10^{12}/L	125	4.92	3.72	5.82	3.91	5.72	
Hb	M	g/L	135	151.0	124.0	184.0	126.4	179.0	0.000*
	F	g/L	125	142.0	109.0	184.0	119.1	177.8	
Hct	M	%	135	44.5	37.8	57.7	38.34	52.46	0.000*
	F	%	125	42.6	33.9	52.9	36.27	51.41	
MCV	M	fl	135	87.8	70.8	98.5	79.34	97.08	0.220
	F	fl	125	87.0	72.7	103.0	77.13	99.31	
MCH	M	pg	135	29.6	25.6	34.1	26.62	32.56	0.136
	F	pg	125	29.3	23.0	37.4	25.21	34.1	
MCHC	M	g/L	135	338.0	314.0	363.0	319.0	356.8	0.125
	F	g/L	125	335.0	310.0	363.0	314.3	358.8	
PLT	M	10^9/L	135	262.0	43.0	423.0	145.4	399.2	0.152
	F	10^9/L	125	273.0	148.0	477.0	182.0	439.5	
RDW-CV	M	%	135	14.0	11.6	16.9	12.6	15.5	0.972
	F	%	125	14.0	12.6	21.2	12.81	17.93	
Neutrophil	M	10^9/L	135	3.27	0.75	9.49	1.13	6.53	0.180
	F	10^9/L	125	3.01	0.89	7.38	1.06	5.62	
Lymphocyte	M	10^9/L	135	2.25	0.30	4.18	0.96	3.74	0.631
	F	10^9/L	125	2.28	1.13	5.23	1.22	3.94	
Monocyte	M	10^9/L	135	0.48	0.23	0.86	0.24	0.84	0.330
	F	10^9/L	125	0.48	0.17	1.01	0.21	0.87	
Eosinophil	M	10^9/L	135	0.23	0.02	1.77	0.04	1.15	0.634
	F	10^9/L	125	0.22	0.01	1.53	0.05	1.03	
Basophil	M	10^9/L	135	0.02	0.0	0.08	0.004	0.06	0.224
	F	10^9/L	125	0.02	0.0	.150	0.001	0.078	

WBC: White blood cell, RBC: Red blood cell, Hb: Hemoglobin, Hct: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, PLT: Platelet, RDW-CV: Red cell distribution width-Coefficient of variation,

M: Male, F: Female, N: Number of participants

*p<0.05 by (Mann-Whitney U test) for comparison of medians between genders

5.5 Comparison of hematological parameters between age groups by sex

Independent Kruskal-Wallis test was used to compare the distribution of hematological parameters between age groups by sex (Table 5.5). There were statistically significant differences among children and adult age groups, adults had higher RBC, Hb Hct and MCV in male and Hb Hct and MCV in female. Similarly significance difference was observed within children and geriatrics age groups in Hb and Hct in both sexes. And also adult participants had higher Rbc and MCV in male and RBC and Hb in female than geriatrics.

There were statistically higher WBC, lymphocyte, monocyte and eosinophil count in children than adult and geriatrics in both sexes. Between adult and geriatrics except eosinophil in male participants none of the values for the WBC subset showed any differences. There were significance differences in platelet counts between all age groups in both sexes. Platelet counts declined steadily with age increment.

Table 5.5: Independent Kruskal-Wallis test to compare the distribution of hematological parameters between age groups by sex in South waste Ethiopians.

Age group	Sex		WBC	RBC	Hb	Hct	MCV	MCH	MCHC	PLT	RDW-CV	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil
Children's and adult	male	Chi-Square	11.453	26.708	50.264	52.357	19.648	30.732	7.387	21.816	2.245	.968	35.972	10.459	5.213	.017
		df	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		Asymp. Sig.	.001	.000	.000	.000	.000	.000	.007	.000	.134	.325	.000	.001	.022	.895
	female	Chi-Square	12.276	1.713	34.926	35.313	37.715	54.096	4.788	17.746	2.585	1.269	25.785	10.584	19.604	5.884
		df	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		Asymp. Sig.	*.000	.191	*.000	*.000	*.000	*.000	*.029	*.000	.108	.260	*.000	*.001	*.000	*.015
Adults and geriatrics	male	Chi-Square	.532	11.756	3.779	.552	30.943	6.455	19.264	3.696	6.557	.201	2.921	.731	4.623	1.469
		df	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		Asymp. Sig.	.466	.001	.052	.458	.000	.011	.000	.055	.010	.654	.087	.393	.032	.226
	female	Chi-Square	1.061	4.937	7.922	2.794	1.730	.452	14.246	8.342	19.053	2.256	.208	.000	.000	1.599
		df	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		Asymp. Sig.	.303	*.026	.005	.095	.188	.501	*.000	*.004	*.000	.133	.648	.989	.994	.206
Children's and geriatrics	male	Chi-Square	5.934	2.373	40.322	54.778	84.051	70.342	4.367	41.162	1.822	.007	24.323	5.232	17.286	3.411
		df	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		Asymp. Sig.	.015	.123	.000	.000	.000	.000	.037	.000	.177	.933	.000	.022	.000	.065
	female	Chi-Square	20.932	2.836	6.849	12.486	52.940	42.060	3.268	44.735	9.315	6.693	23.079	8.237	19.278	.789
		df	1	1	1	1	1	1	1	1	1	1	1	1	1	1
		Asymp. Sig.	*.000	*.092	*.009	*.000	*.000	*.000	.071	*.000	*.002	*.010	*.000	*.004	*.000	.374

WBC: White blood cell, RBC: Red blood cell, Hb: Hemoglobin, Hct: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, PLT: Platelet, RDW-CV: Red cell distribution width-Coefficient of variation, df: degree of freedom.

*p<0.05 by Kruskal-Wallis test between age groups

CHAPTER SIX: DISSCUSION

6.1. Discussion

In the present study adults have higher RBC, Hb and Hct than children's in in males and Hb and Hct in females ($p<0.001$). It could be because of the gradually increase of Hb and RBC through childhood to reach almost adult levels by puberty. Thus with the aging process, modest changes in red blood cell mass may occur in adults (45,46).

No significant gender difference was observed in children's for RBC, Hb and Hct ($p>0.05$), in this study which is similar with other previous reports from Tanzania and Uganda(39,47). On the other hand male participants have higher values in RBC, Hb and Hct than female participants in adults ($p<0.001$) which supports the well-established fact that males have higher values for RBC, Hb, and Hct than females. It could be caused by a direct stimulatory effect of androgen in adult men in the bone marrow in association with erythropoietin, a stimulatory effect of androgen on erythropoietin production in the kidney, and an inhibitory effect of estrogen on the bone marrow in women. Apart from a hormonal influence on hemopoiesis, iron deficiency is likely to be a factor influencing the difference in which menstrual blood loss may lead to iron depletion (46,48).

In the current study adult participants showed higher RI value of RBC, Hb and Hct parameters and lower RI of MCV than the Caucasian populations and other studies done in Africa with lower altitude (20,38,49). The median values in adult males of this study for Hb and Hct were lower as compared with a study done in Ethiopia (Akaki) with higher altitude (24). This may be due to difference in altitude. The effect of altitude is to reduce plasma volume, increase the Hb and Hct and raise the number of circulating red cells with a lower MCV. These differences appear to be the result of both increased erythropoiesis which is secondary to the hypoxic stimulus and the decrease in plasma volume that occurs at high altitudes (46).

The current study showed no difference among male and female with respect to total WBC count ($p>0.05$) which is comparable with the Mali and Ethiopia (Akaki) reports (24,38). The current study in children showed lower WBC and neutrophil count than the Caucasian RI and higher than a report from Tanzania (47,50). In adult age group the 95% RI of WBC and

neutrophil count in adult males was higher and females had lower 95% RI than the Mali and Mozambique report (17,38). In adult age group the 95% RI of WBC and neutrophil compared with the Caucasians population males had higher value in the upper limit of the 95% range and lower value in the lower limit of the 95% range and females had lower 95% range (49). The cause of WBC and neutrophil count variation may be partly explicable on the basis of diet and other extraneous influences, but there might be also a true biological difference (51).

In the current study higher eosinophil and lower basophil 95% RI was observed in children than the Caucasians (50). Adult participants in the present study show almost similar lymphocyte 95% RI with the Caucasian population and Ethiopia (Akaki). Monocyte RI of adult participants in the present study was slightly higher than Caucasians population, Mali and Ethiopia (Akaki) studies. And also this adult subject has higher eosinophil count than the Caucasian populations (24,38,49,51). These observed differences may be suggestive of different factors such as environmental difference, dietary role, ethnic variation and subclinical illnesses. Higher eosinophil count may be due to disease related causes particularly parasitic infection (52,53).

In the present study PLT count decreased with age is consistent with Italian and Ugandan reports (27,39). The mechanisms that are responsible for the age- related changes might be, the sharp decrease of platelets during infancy may be related to the thrombopoietin that have been reported to decline from birth to adulthood. While the reduction in elderly levels people may reflect a reduction in hematopoietic stem cell reserve during aging or a survival advantage in subjects with lower platelet counts.

In the current study, adult female participants have higher PLT count ($p<0.05$) than adult male participants which is similar with other studies done Italy, Ghana and Ethiopia (Bahir Dar) (27,32,41). The observation that women begin to have PLT count higher than men only after the age of 14 supports the hypothesis that puberty makes the difference. The reduction of body iron in menstruating women probably related to their higher PLT count in that moderate iron deficiency is known to stimulate PLTs production (27).

The RI of PLT count of this study in children age group was almost similar with Caucasians and compared to a study done in Tanzania the lower limit was higher and the upper limit was lower (47,50). Adult participants in the present study have higher PLT count RI than other studies done in Ethiopia (Akaki) and the Caucasian population. And also adult participants have higher lower

limit and lower upper limit 95% RI than a study done in Mali (24,38,49,51). Also geriatric participants has higher PLT count than a report from Ethiopia (Gilgel gibe) (16). The etiology of these differences is unknown, although undetected illness, environmental and genetic factors have been proposed (27).

The strengths of this study were first, it was the first study in the community setting in Southwest Ethiopia and confirm previous findings that regional differences exist for hematological RI which are critical for patient management and clinical research. Second, time of blood sampling was in the morning, so the influence of diurnal variation was minimized. Third, all the laboratory procedures were done based on the SOPs and qualified personnel.

This study has also some limitation the participants were not screen for all medical conditions which may affect hematological parameters, such as helminthic infections and HIV. Participants with parasitic infections or with other subclinical conditions may have been included, which may have influenced the results. The second limitation was most of the participants were from urban area so that these RI may not be representative for peoples from rural area.

CHAPTER SEVEN: CONCLUSION AND RECOMMENDATION

7.1. Conclusion

This study established hematological parameter RI from apparently healthy individual's ≥ 5 years of age living in Southwest Ethiopia. There was difference in hematological parameters RI of Southwest Ethiopian from other Africa countries and the Caucasian populations. Therefore, this study provided region-specific hematological parameters RI which can be used to guide patient management and interpretation of laboratory findings, screening participants for enrolment into clinical trials and potentially improve the quality of health care in the area.

7.2. Recommendations

- This region specific RI needs to be used in health care facilities in southwest Ethiopia.
- Further similar study needs to be done for peoples living in rural area in southwest Ethiopians.
- RI for infants and children less than 5 year need further studies.
- Further investigation by screening all medical conditions needs to be done.

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ANNEXES

ANNEX-1: QUESTIONNAIRE

JUMMA UNIVERSITY

INSTITUTE OF HEALTH

SCHOOL OF MEDICAL LABORATORY SCIENCE

QUESTIONNAIRE FOR SCREENING

1. Have you ever practiced and/or exposed to the following (if yes thick in the specific cases)
    A. Chronic illness
    B. History of transmitted diseases
    C. Any allergy
    D. On any medication (including contraceptive)
    E. Jaundice
    F. Resent blood transfusion
    G. Pregnancy
    H. Lactation
    I. Menses
    J. Family planning devices
    K. Drug abuse
    L. Cigarette smoking
    M. Alcohol consumption

JUMMA UNIVERSITY

INSTITUTE OF HEALTH

SCHOOL OF MEDICAL LABORATORY SCIENCE

QUESTIONNAIRE FOR SCHOOL CHILDREN

1. Identification

1.1 Code _____

1.2 Address _____

2. Physical examination

2.1 Describe what you observe

_____

_____

_____

_____

2.2 Do you have any sign and symptoms for a disease? 1. Yes 2. No

2.3 If yes describe it

_____

_____

3. Anthropometric measurement

3.1 weight in the nearest 0.1 Kg _____

3.2 Height in the nearest 0.1 cm _____

4. Socio-demographic information

4.1 Age in years _____

4.2 Sex

1. Male 2. Female

4.3 Site

1. Jimma 2. Bonga 3. Metu

4.4 For how money years have you been here _____

4 Socio-demographic information

4.1 Age in years _____

4.2 Sex

1. Male 2. Female

4.3 For how money years have you been here _____

4.4 Mother's Occupational status

1. Merchant 3. Former 5. Student
2. Employee 4. Daily laborer 6. Others -------

4.5 Father's Occupational status

1. Merchant 3. Former 5. Student
2. Employee 4. Daily laborer 6. Others -------

4.6 Mother's Educational status

1. Illiterate 3. Grade 1-4 5. Grade 9-12
2. Read and write 4. Grade 5-8 6. College/university

4.7 Father's Educational Status

1. Illiterate 3. Grade 1-4 5. Grade 9-12
2. Read and write 4. Grade 5-8 6. College/university

4.8 Educational of the child

1. Illiterate 3. Grade 1-4 5. Grade 9-12
2. Read and write 4. Grade 5-8

4.9 Religion

1. Muslim 3. Orthodox 5. Others_____
2. Catholic 4. Protestant

4.10 Ethnicity

1. Oromo 3. Amhara 5. Dawuro
2. Kaffa 4. Tigrea 6. Others_____

4.11 Family size _____

5 Life style factors and nutritional habit

5.1 What is your staple food?

1. Injera 3. Fish
2. Porridge 4. Other, specify _____

5.2 Fruit, fruit juice consumption

1. No
2. Every day
3. Every other day
4. Once a week
5. Once a month
6. Others specify _____

5.3 Vegetables consumption (weekly)_____

1. No
2. Every day
3. Every other day
4. Once a week
5. Once a month
6. Others specify _____

5.4 Frequency of consumption of foods from animal sources (weekly)_____

1.No

2.Every day

3.Every other day

4.Once a week

5.Once a month

6.Others specify _____

6. Laboratory data

Laboratory results will be attached with this questionnaire.

JUMMA UNIVERSITY

INSTITUTE OF HEALTH

SCHOOL OF MEDICAL LABORATORY SCIENCE

QUESTIONNAIRE FOR ADULTS

1. **Identification**

1.1 Code _____

1.2 Address (Phone number) _____

2. **Physical examination**

2.1 Describe what you observe

_____

_____

_____

_____

2.2 Do you have any sign and symptoms for a disease? 1. Yes 2. No

2.3 If yes describe it

_____

_____

3. **Anthropometric measurement**

3.1 weight in the nearest 0.1 Kg _____

3.2 Height in the nearest 0.1 cm _____

3. **Socio-demographic information**

3.1 Age in years _____

3.2 Sex

3. Male 4. Female

3.3 Site

1. Jimma 2. Bonga 3. Metu

3.4 For how money years have you been here _____

3.5 Occupational status

1. Merchant
2. Employee
3. Former
4. Daily laborer
5. Student
6. Others -------

3.6 Educational Status

1. Illiterate
2. Read and write
3. Grade 1-4
4. Grade 5-8
5. Grade 9-12
6. College/University

3.7 Marital status

1. Unmarried
2. Widowed
3. Married
4. Divorced

3.8 Religion

1. Muslim
2. Catholic
3. Orthodox
4. Protestant
5. Others_____

3.9 Ethnicity

1. Oromo
2. Kaffa
3. Amhara
4. Tigrea
5. Dawuro
6. Others_____

3.10 Family size _____

3.11 Annual household income (in ETB)_____

4. Life style factors and nutritional habit

4.1 Regular exercise (at least once per week for 1 year)

1. yes
2. No

4.2 What is your staple food?

1. Injera
2. Porridge
3. Fish
4. Other, specify _____

4.3 Vegetables consumption (weekly)_____

1. No
2. Every day
3. Every other day
4. Once a week
5. Once a month
6. Others specify ____

4.4 Frequency of consumption of foods from animal sources (weekly)_____

1. No
2. Every day
3. Every other day
4. Once a week
5. Once a month
6. Others specify __

6. Laboratory data

Laboratory results will be attached with this questionnaire

ANNEX-2: LABORATORY PROCEDURES

2.1 Blood collection procedure

- **Purpose:** To give general guidance to obtain a venous specimen from participants.
- **Material required:**
    - Purple top (EDTA) blood tubes
    - Vacutainer needle and holder
    - 70% alcohol swab
    - Tourniquet
    - Plastic gloves
    - White coat
    - Sharps box and clinical waste bag
    - Permanent marker
- **Test procedure:**
    1. Assemble the necessary materials and equipment.
    2. Thread the short end of the double-pointed needle into the holder and push the tube forward until the top of the stopper meets the guide mark on the holder. The point of the needle will thus be embedded in the stopper without puncturing it and loosing the vacuum in the tube.
    3. Identify the participant and allow him/her to sit comfortably preferably in an armchair stretching his/her arm.
    4. Apply a tourniquet at a point about 6-8cm above the bend of the elbow making a loop in such a way that a gentle tug on the protruding ends will release it. It should be just tight enough to reduce venous blood flow in the area and enlarge the veins and make them prominent and palpable.
    5. Using the index finger, feel for a suitable vein, selecting a sufficiently large straight vein that does not roll and with a direction that can be felt.
    6. Prepare the arm by swabbing the antecubital fossa with a gauze pad or cotton moistened with 70% alcohol. Allow it to dry in the air or use a dry pad or cotton. The area should not be touched once cleaned.
    7. Using the assembled needle, enter the skin first and then the vein.
    8. When entered in to the vein, the vacuum tube is pushed into the needle holder all the way so that the blood flows into the tube under vacuum.
    9. When sufficient blood has been collected, release the tourniquet and instruct the patient to open his or her fist. Remove the needle and immediately press on the

puncture site with a piece of dry cotton wool. Remove the tourniquet completely. Instruct the patient to continue pressing on the puncture site until the bleeding has stopped.

10. Remove the tube from the vacutainer holder and gently mix the blood with the anticoagulant by inverting the tube 8-10 times.
11. Carefully label the tubes with participants unique identification number.
12. Reinspect the venipuncture site to ascertain that the bleeding has stopped. Do not let the patient go until the bleeding stops.

(Source:- Cheesbrough M. District Laboratory Practice in Tropical Countries. 2nd Edition Part 2. 2006)

2.2 Sample transportation and storage procedures

- **Purpose:** To give general guidance for participants sample transportation and storage.
- **Material required:**
  - Ice box without ice
  - Nunc tube
  - Pasture pipet
  - Labels
- **Test procedure:**
  - Blood samples collected from the participants will be transported to JUMC laboratory by ice box without ice with in 4 hr of sample collection.
  - After CBC analysis centrifuge the remaining blood sample to separate the plasma from the blood cells.
  - Aliquot 1000 µl plasma to nunc tube (label them with the participants unique identification number) and put the sample at -20 freezer.

(Source:- Cheesbrough M. District Laboratory Practice in Tropical Countries. 2nd Edition Part 2. 2006)

2.3 Complete blood counting (CBC) by Sysmex XS-500*i* hematology analyzer

- **Material required:**
  - CELLPACK: Diluent for use in hematology analyzers.
  - STROMATOLYSER-4DL: Lysing reagent for use in blood analyzers.
  - STROMATOLYSER-4DS: Used to stain the leukocytes in diluted and lysed blood samples. It serves for the determination of 5-part differential count (Neut, Lymph, Mono, Eo, Baso) Celldyn 1800 tri- level control reagents.
  - SULFOLYSER: Used for the determination of hemoglobin.

- CELLCLEAN: A strong alkaline detergent to remove lysing reagents, cellular residuals and blood proteins remaining in the hydraulics of Sysmex Automated Hematology Analyzers.
- e-CHECK (XS): A quality control material. Quality Control is performed in order to monitor an instrument's performance over time.
- Peripheral Equipment
  - List Printer (LP)
  - Graphic Printer (GP)
  - Data Printer (DP)

➢ **Supplies:**

- EDTA anticoagulant tube(Vaccutainer tube)
- Dry gauze
- Cotton Swab
- Vaccutainer needle with holder
- 70% Ethanol alcohol or similar antiseptic
- Tourniquet
- Glove

➢ **Specimen type:** EDTA anticoagulated whole blood.

➢ **Specimen stability:** A well-mixed whole blood specimen, collected in EDTA anticoagulant and run within eight hours after collection, provides the most accurate results for hematological parameters.

➢ **Checks prior to turning power on**: Be sure to check following items 1-3 before turning the power on to obtain correct analysis results.

1. Reagent inspection:
   - The amounts of reagent used vary between analysis modes.
   - Estimate the volume which will be required for the day, and get it ready, allowing an extra margin.
   - The instrument will stop automatically if it runs out of a reagent during analysis.
   - In that case, replace the reagent that ran out.
   - Re-start analysis once replacement is complete.

2. Instrument inspection
    - Check the tubing and cable connections.
    - Make sure that the tubing is not bent nor kinked.
    - Make sure the power cord is securely plugged into the outlet.
3. Waste fluid
    - Check waste containers if it is more than ¾ full discard it and replace it

➢ **Analysis of samples**

- Turn on power to the printer, IPU (personal computer) and main unit in order.
- Enter the password and click on OK.
- Self-tests
    - The instrument performs a self-check automatically.
    - When the Main Unit power is turned ON, the following operations are performed in this order: Self-Check, Main Unit control program download, initialization of mechanical and hydraulic parts, a rinsing sequence, waiting for temperature stabilization, and a background check.
    - Analysis starts after the temperature inside the instrument reaches the required value. The temperatures of the reaction chamber and reagent heater are displayed in the Temperature Monitoring dialog box. The system waits for these to stabilize at their target temperatures. When they have stabilized at their target temperatures, the Temperature Monitoring dialog box is closed automatically.
- Background check
    - Once temperature stabilizes, the Background check dialog box appears.
    - Background analysis is performed up to three times for the background check.
    - If the background value is at or below the values shown in the table below, the background check is completed.

RBC	0.02 [x10^6/µL]
HGB	0.1 [g/dL]
PLT	10 [x10^3/µL]
WBC-C	0.30 [x10^3/µL]
WBC-D	0.10 [x10^3/µL]

- QC analysis
    - Quality control analysis can be carried out in the manual analysis mode.
    - Control blood is analyzed by the X-bar or L-J Control programs, and the data is stored in the specified quality control file.
    - Follow the manufacturer's instructions for handling the control blood samples.

➢ **Sample analysis**

- There are two types of sample analysis: Manual/ Capillary mode and Sampler mode.
- Either mode is run when the instrument's status is Ready or Manual Aspiration Ready.

a. Manual mode analysis: XS-500i: Analysis in manual mode can be performed when the Main Unit is in Manual Aspiration Ready status.

- Select the Manual Mode icon on the Controller Menu, then double-click or press the Enter key to start the Manual Mode screen.
- Input the required information.
- The READY LED on the Main Unit changes to green, indicating that it is in Manual Aspiration Ready status.
- Mix sample thoroughly by inverting the sample tube.
- Taking care to avoid blood spattering, remove the cap from the sample tube, insert the probe to the bottom of the sample tube, and press the Start switch.
- The green READY LED flashes during sample aspiration, then the buzzer beeps to indicate the end of aspiration, and the READY LED goes out.
- Remove the sample tube carefully so as not to bend the probe.
- The fact that the READY LED is not lit indicates that analysis is in progress.
- When the READY LED lights green again, the next sample can be analyzed.

➢ **Source of Error:** If the sample is

- Hemolysed
- Clotted
- Collected in improper tube
- Not mixed well
- Small volume

(Source:- Sysmex XS-500i hematology analyzer user manual)

2.4 C - reactive protein test:

- **Principle:** HumaTex CRP is based on the reaction between human CRP of a specimen and the corresponding anti-human CRP antibodies bound to latex practice. The positive reaction is indicated by a distinct visible agglutination of latex particles in the test cell of the slid.
- **Material required:**
    - CRP latex reagent
    - Positive control
    - Negative control
    - Stick
    - Pipette tips
    - Gloves
    - Timer or clock
    - Micropipette
- **Specimen:** Plasma
- **Reagents stability and storage:** Stable up to the expiry date when store at 2 to 30oC. Do Not Freeze!
- **Test procedure:**
    1. Bring the reagent and the sample to room temperature.
    2. Mix the latex reagent carefully prior to use to suspend the latex particles completely.
    3. Drop 40 µl of sample and controls onto a separate cell of the slide.
    4. Mix with separate sticks and spread the fluid over the entire area of the particular cell.
    5. Place the slide in automated rotator at 100 revolutions per minute.
    6. Read the slide under bright artificial light.
- **Interpretation of the result:**
    - Distinct agglutination indicates a CRP content of more than 6 mg/l.
- **Quality control:** The positive and negative controls are to be used with each test. Their result should be compared with those of the unknown specimens to distinguish possible granularity from agglutination.

(Source:- HumaTex manual)

2.5 Hepatitis BsAg Testing:

- **Principle:** As a test sample flows through the membrane assembly of the test device, the coloured monoclonal anti-HBsAg-colloidal gold conjugate complexes with the HBsAg in the sample. This complex moves further on the membrane to the test region where it is immobilized by another monoclonal anti-HBsAg antiserum coated on the membrane leading to formation of a pink-purple coloured band which confirms a positive test result. Absence of this coloured band in the test region indicates a negative test result. The unreacted conjugate and unbound complex if any move further on the membrane and are subsequently immobilized by the anti-rabbit antiserum coated on the membrane at the control region, forming a pink-purple band. This control band serves to validate the test results.
- **Material required:**
    - Timer.
    - Specimen collection container.
    - Buffer
    - General laboratory equipment.
- **Storage:** Should be store at 2-30^{o}C.
- **Specimen:** Serum or plasma only.
- **Procedure:**

    1. Allow test device, serum or plasma to equilibrate to room temperature prior to tasting.
    2. Place the test device on a clear and level surface.
    3. Label each device with the appropriate patient information / ID.
    4. Using the provided disposable pipette, transfer 2-3 drop of specimen (about 60-90 µl) to the specimen well (S) of the device and start the timer.
    5. As the test begins to work, color will migrate across the membrane.
    6. Wait for the color band to appear. The result should be read at 15 minutes. Do not read result after 20 minutes.
- **Interpretation of the result:**
    - **Positive:** Two distinct red lines appear. One line appears in the control region (C) and another line appears in the test region (T).
    - **Negative:** Only one color line appears in the control region (C).

- **Invalid:** control line fails to appear.

- **Quality control:** A colored line appearing in the control region (C) is considered an internal procedural control.
- **Limitation:** Do not use hemolyzed, turbid or contaminated samples. Turbid samples should be centrifuged and clear supernatant must be used for testing.

(Source:- One step HBsAg manual)

2.6 Hepatitis C Virus Test

- **Principle:** The test involves capturing of antibodies to HCV by immunodominant proteins of the virus immobilized onto a porous membrane. After washing, the presence of antibodies is revealed by treatment with conjugate which will bind to absorbed HCV antibodies, forming a red spot on the membrane.
- **Material required:**
    - Timer.
    - Specimen collection container.
    - HCV buffer
    - General laboratory equipment.
- **Storage:** Should be store at 2-30^{o}C.
- **Specimen:** Plasma.
- **Procedure:**

    1. Allow test device, specimen and buffer to equilibrate to room temperature prior to tasting.
    2. Place the test device on a clear and level surface.
    3. Label each device with the appropriate identification number.
    4. Dispense 2 drops of buffer solution from the blue top dropper bottle into the device
    5. Add 1 drop of sample using the pipette provided.
    6. Dispense 2 drops of buffer solution into the device.
    7. Dispense 2 drops of wash solution from the red top dropper into the device.
    8. Dispense 2 drops of Protein-A gold conjugate from the white top dropper bottle into the device.

9. Dispense 3 drops of wash solution into the device.
10. Read results within 10 minutes for easiest interpretation.

- **Interpretation of the result:**
  - **Positive:** Two distinct red lines appear. One line appears in the control region (C) and another line appears in the test region (T).
  - **Negative:** Only one color line appears in the control region (C).
  - **Invalid:** control line fails to appear.
- **Quality control:** A colored line appearing in the control region (C) is considered an internal procedural control.
- **Limitation:** Lipemic, hemolyzed, icteric or heat inactivated sera may cause erroneous results.

(Source:- One step HCV manual)

ANNEX-3 INFORMATION SHEETS

3.1 information sheet English version

This information sheet is prepared for individuals who are volunteer to participate in the study. The detailed explanation about what will be undertaken in the study is presented as follows and it is after reading the description that informed consent is obtained.

Title of the project:- Establishment of hematological parameters RI for Southwest Ethiopia**.**

Name of Principal Investigator:- Kaleab Eskinder

Telephone number: 0961928304

E-mail: eskinderkaleab@gmail.com

Description and Purpose of the study:- Clinical laboratory RIs (RI) are an important tool for identifying abnormal laboratory results and for ultimately guiding patient management decisions. The development of hematological parameters RI together with clarification of the interval of distribution for local population is very crucial to improve quality of health care. Also it plays an important role for clinical trials. This study therefore will be carried out to establish hematological parameters RI for apparently healthy individuals in Southwest Ethiopia.

Procedures:- Following your willingness you are asked to sign a consent form (for school children from parents/guardians and assent from them) and the following procedures will be undertaken

- A physical examination will be undertaken
- You will provide us 10 minutes interview
- Blood sample (4ml) will be collected
- The blood sample will be analyzed for screening and hematological tests..

Risks and discomforts:- During sample collection we will follow Standard operational procedures. The blood drawing may cause minor pain, at the place where blood is taken. However, this pain will no longer appear.

Benefits:- This study will be of benefit to the entire community since its success will aid in proper clinical decision making and treatment of patients. There is no direct financial benefit you get by participating in this study but the test result will be delivered timely and appropriate intervention will be pointed.

Confidentiality:- Any information obtained during this study will be kept confidential. This is assured by avoiding use of any identifier and information will be recorded with code number. Should we release the result obtained from the study, it is in the way that avoids any identifier of you and if there is any identifier, there should be signed confirmation of you.

Voluntary participation:- Participation on this study is voluntary and you have the right to refuse participation at any time. Your decision will not result in any penalty or loss of benefits to which you are entitled. Your decision will not put you at risk any present or future medical care or other benefits to which you otherwise entitled.

You may ask questions now and in the future if you do not understand something that is being done contact the investigator on above address.

For the success of the study, i will be asking you to give the correct answer for the respective questions. Thank you for your assistance.

3.2 የጥናቱ ተሳታፊዎች መረጃ ቅፅ(የአማርኛ ግልባጭ)

ይህ የመረጃ ቅጽ የተዘጋጀው በዚህ ጥናት ለሚሳተፉ ፍቃደኛ ሰዎች ይሆናል ። ፍቃደኝነታቸው እንዴት እንደሚወሰድ ከተገለፀ በኋላ የሚከናወኑት ተግባራት በግልፅ ይብራራሉ ።

የጥናቱ ርዕስ ፡- የ Hematology ኖርማል የላብራቶሪ ማጣቀሻ ውጤቶች ለደቡብ ምዕራብ ኢትዮጲያ ።

የዋና ተመራማሪ ስም፡- ቃለአብ እስክንድር

ስልክ ቁጥር፡ +251961928304

ኢሜይል፡ eskinderkaleab@gmail.com

የጥናቱአላማ፡- የህክምና ላብራቶሪ ኖርማል ውጤት ችግር ያለባቸው የላብራቶሪ ውጤቶች ለማወቅ እና በትክክል ታማሚውን ለማከም ጉልህ አስተዋፅኦ አላቸው ;; Hematology ኖርማል ውጤት ለአካባቢው ህብረተሰብ ማዘጋጀት የህክምናውን ጥራት በእጅጉ ያሳድገዋል ;; እንዲሁም ለተለየያየዩ የህክምና ምርምር ስራዎች በጣም ጠቃሚ ሚና ይጫወታል ስለዚህ ይህ የምርምር ስራ Hematology ኖርማል የላብራቶሪ ውጤት በደቡብ ምዕራብ ኢትዮጵያ ለሚገኙ ሰዎች ይሰራል ;;

የጥናቱ ዝርዝር ሂደት፡- በጥናቱ ለተሳተፉ ፍቃደኝነትዎን በፊርማዎ ስለገለፁልን ለት/ቤት ህፃናት ከወላጆቻቸው ወይም ከአስተማሪዎቻቸው እንዲሁም ከራሳቸው ቃል በኋላ የሚከተሉትን መረጃዎችና ናሙናዎች አንወስዳለን ;;

- የአካል ምርመራ ይደረግሎታልራሉ
- ከራስዎ አንደበት የአስር ደቂቃ ቃለ መጠይቅ ይደረግሎታል
- 4 ሚሊ ሌትር የደም ናሙና ይወሰዳል ;;
- የደም ናሙናው የScreening እና Hematology ምርመራ ይደረግለታል ።

ስጋትና ጉዳት፡- ህክምናው የሚያስገድደውን የአሰራር ሂደት ስለምንከተል ሊያጋጥም የሚችለው የህመም ስሜት በጣም አነስተኛ ነው። ቢሆንም የደም ናሙናው በሚወሰድበት ጊዜ ትንሽ የህመም ስሜት ሊያጋጥም ይችላል። ነገር ግን ህመሙ በአጭር ጊዜ ይጠፋል።

ሊያስገኛቸው የሚችሉት ጥቅሞችና የካሳ ክፍያ፡-ይህ ጥናት ለህብረተሰብ በጣም ጠቃሚ ነው ምክንያቱም የጥናቱ ውጤት ታካሚው ትክክለኛ ህክምና እንዲያገኝ ይረደዋል። በዚህ ጥናት ውስጥ በመሳትፎዎ በጥሬ ገንዘብ የሚደረግ የካሳ ክፍያ አይኖርም ነገር ግን የምርመራው ውጤት በወቅቱ የሚሠጥ ሲሆን በምርመራው ውጤት መሠረት አስፈላጊው የህክምና እርዳታ ይጠቅማል ።

የጥናቱ ሚስጥራዊነት፡- ማንኛውም በጥናቱ የሚገኙ መረጃዎች በሚስጥር ይጠበቃሉ ። የሚሰጡን መረጃዎች በሙሉ የሚቀመጡት ከእርሶዎ ስም ጋር ሳይሆን ለጥናቱ ተብሎ በሚሰጠው ሰውር ቁጥር ሲሆን ጥናቱን ከሚያካሄዱት ባለሞያዎች በስተቀር ማንም ሊያውቅ አይችልም። የእርስዎን ማንነት በሚያጋልጥ መልኩ የተዘጋጀውን መረጃ በፊርማዎ የተረጋገጠ ፍቃድ ሳናገኝ ይፋ አናደርግም ። ይህ ጥናት ሳይንሳዊ መረጃ እንደመሆኑ መጠን በወረቀት ታትሞ ቢወጣ ወይም በሚዲያ ቢነገር የእርሶዎ ስም በምንም መልኩ አይጠቀስም።

በፍቃደኝነት የመሳተፍ መብት፡- በዚህ ጥናት ውስጥ የሚኖርዎት ተሳትፎ ሙሉ በሙሉ በፍቃደኝነት ላይ የተመሠረተ ይሆናል፡፡ በማንኛውም ጊዜ ይህንን ጥናት የማቋረጥ መብትዎ ሙሉ በሙሉ የተጠበቀ ነው፡፡ በጥናቱ በመሳትፍዎ ወይም ከጥናቱ በመገለልዎ ምክንያት በአሁኑ ወይም የወደፊት የህክምና እርዳታዎ ላይ ምንም አይነት ተፅዕኖ አይኖረውም፡፡ ከዚህ በፊት ሲያገኙ ከነበሩት ጥቅሞች ላይ አንዳች ነገር አይነልቦትም፡፡

ስለ ጥናቱ ማንኛውንም አይነት ጥያቄ ካሎት በማንኛውም ጊዜ አሁንም ሆነ ወደፊት ከላይ በተጠቀሰው አድራሻ የጥናቱን ባለቤት ማነጋገር ይችላሉ ፡፡ ለዚህ ጥናት ስኬታማነት ጥያቄዎቹን በትክክል በመመለስ እንድትተባበሩ ስል በትህትና እየጠየኩ ስለትብብርዎ ልባዊ ምስጋና አቀርባለሁ፡፡

3.3 Ibsa Hirmaatota qo'annotif guca AfaanOromoo.

Guciin kun wareen qu'niichatti fe'aan hirmataniif yoo ta'u halichi adeemsa qu'anichaa irriga ibsamee booda wanti itti aanu hundu ifa godhama.

Mataduree qu'anna:- Qorannon bu'a laaboraatori Hematology warren fayyaa qaban irratti naanno kiba lixa ithopiyaatti hojjetamu.

Maqaa qorata:- Kaala'ab Iskiindir

Lak.bilbila:- 0961928304

Imeela:- eskinderkaleab@gmail.com

Dhimi qu'anicha:- Bu'aan laboratory kaneen warra fayaa qabanii hagam akka ta'ee murteesufii gara fulduraaf fayyaaf dhibamaan adaan basuuf akka toluuf yoo ta'u, bu'aan isaas warra naanotiif bay'ee gudaadha.

Haala adeemsa qu'anicha:- Qu'anichirrat fe'aan hirmaachuuf mallatoo keessanin wan nuuf ibsitaniif,ragaawan armaan gadi kanneen ni fudhanna.

- Qoranno qaama
- Ganfii afaanii dakika kudhanif siif goona
- Dhiiga 4ml ni fudhanna
- Wantootini arman oliti fudhatam hundinu qu'anichaaf kanbarbaadaman ta'usa ni ibsina.

Sodaafi miidha qabu:- Seraf namusa wal'ansa fayya waan hordufinuuf want nama sodaachisu hinjiru, hata'u malee yeroo dhigni fuudhamu dukubiin tinno bakka lilmoon seenteetti dhaga'aamu mala ,ta'us yoosu bada.

Faayida qu'anichaafi kanfalti hirmaataf godhamu:- Qu'anichi bu'aa gudda sabaaf busa innis tajajila dhugaa akka argatan tasisa. Qu'anicha irratt hirmaachuun kanfaliti tokkolle hin qabuu.haatu malee bu'aan qoranno wanta batalumatti deebi'uuf tajaajila wadhansa argachuu ni danda'a.

Icciiti qu'anicha:- Wantooni qu'anichaan argaman hundi icciitin eeggamu.akkasumas ragaawan fudhatama hundinu maqaa keesanin oso hin ta'in lakkofisa koodii addaa ta'een wanta'eef kanas kan beekan warreen ragicha guraan qofaadha.

Mirga fe'aan hirmachu:- Qu'annoo kana irratti hirmaachun fe'a gutuu kee qofa ta'usaa beektee yeroo barbaadeetti dhiisu akka dandeesu mirgi kee guututdha.qu'anno kanarati hirmaachufi hirmaachu baatun kee gara fulduraf tajaajila argaturatti rakkoo tokkolee hin fidu.

Qu'anicha ilaalate gaafille qabadan hundaa yeroo barbaadanit tisso mataarati bareefameen aba qu'anicha gafachu nidandeesan.itti annisees qu'anno kanaaf hirmana gootanif galani keya guddaadh. Galatoomaa.

ANNEX-4: CONSENT FORMS

4.1 Consent forms (English version)

Participant Code Number_____

Participant full name _____

I am informed fully in the language I understand about the aim of above mentioned research. I understood the purpose of the study entitled with **"Hematological parameters RI for Southwest Ethiopians"**. I have been informed that physical examination, blood samples will be taken and there will be minimal risk during sample collection. In addition I have been told all the information collected throughout the research process will be kept confidential. I understood my current and future medical services will not be affected if I refused to participate or with draw from the study.

Agree _____ Not agree_____

Therefore I give my consent freely for my participation in this study.

Participant's Name _____ signature _____ Date_____

Investigator's name _____signature _____Date_____

Witness

1. Name _____signature _____date _____
2. Name _____signature _____date_____

4.2 የስምምነት ቅፅ (የአማርኛግልባጭ)

የተሳታፊው ልዩ መለያ ቁጥር _____

የተሳታፊው ስም _____

እኔ ስሜ ከላይ የተጠቀሰው ተሳታፊ **“የ Hematology ኖርማል የላብራቶሪ ማጣቀሻ ውጤቶች ለደቡብ ምዕራብ ኢትዮጲያ”** በሚል ርዕስ በታሰበው ምርምር ላይ በሚገባኝ ቋንቋ በቂ መረጃ አግኝቻለሁ።።የህክምና መረጃና የደም ናሙና ምንም አይነት ጉዳት በማያደርስ መልኩ እንደሚወሰድ ተረድቻለሁ።። በተጨማሪም የሚወሰዱ ማናቸውም መረጃዎች በሚስጥር እንደሚያዙ ተነግሮኛል።። እንድሁም የምጠየቀውን መረጃ ያለመስጠትና ለጥናቱ ያለመሳተፍ ከጥናቱ በማናቸውም ወቅት ራሴን ማግለል እንደምችል የተገለፀልኝ ሲሆን ይህንንም በማድረጌ ወደፊትም ሆነ አሁን የማገኛቸው የህክምና ግልጋሎቶች እንደማይጓደሉብኝ ተረድቻለሁ።።

እስማማለሁ_____ አልስማማም_____

በመሆኑም ለዚህ ምርምር ለመሳተፍ ወስኛለሁ።።

የተሳታፊ ስም .ስም _____ ፊርማ _____ ቀን_____የተመራማሪ ስም .ስም _____ ፊርማ _____ ቀን_____

ምስክሮች

1.ስም _____ ፊርማ _____ ቀን_____

2.ስም _____ ፊርማ _____ ቀን_____

4.3 UunkaaWaliigaltee (AfaanOromoo)

Lakk.Addaa Hiirmaataa_____

Maqaa guutuu Hiirmaataa_____

Anii hiirmaataan maqaan kiyya kan armaan olitti tuuqamee, kaayyoo qo'annoo"Mata Duree **Qoranno bu'a laabooraatori Hematology Warren Fayyaa Qaban Irratti Naanno Kiba Lixa Ithopiyaatti Hojjetamu.**" Jedhuuratti afaan naa galuun odeefannoo gahaa argadheen jira. Odeefannoon fayyaa fi naamudni dhiigaa karra rakkoo hingeessinen akka fuudhatamu hubadheen jira . Dabalataaniis odeefannoon narraa argaman hunduu icciitiin akka qabaman natti hiimameera. Akkasumaas gaafileen gaafatamuuf deebii kennuu dhiisuu , hiirmachuu dhiisuu fi yeroon barbaadetti addaan kutuu akkan danda'uu bareen jira. Kana godhuu kiyyaaniis ammas ta'ee fuulduraaf fayyadamummaa tajaajila fayyaa kiyyaa irratti rakkoon tokkollee akka hinuumamanee huubadheen jira.

Waliigaleera_____ Walii hin gallee_____

Kanaafuuqoorannoo kana irattifeedhiin nan hirmaadha.

Maqaa hiirmaataa_____Mallattoo_____Guyyaa_____

Maqaaqoo'ataa_____Mallattoo_____Guyyaa_____

Ragaa

1. Maqaa_____Mallattoo_____Guyyaa_____
2. Maqaa_____ Mallattoo_____Guyyaa_____

Declaration

I, the undersigned, declare that this research thesis is my own work and it has not been presented in other universities, colleges or other institutions for similar degree or other purpose. Where other peoples work has been used, it has been carefully acknowledged and referenced in accordance with the requirements.

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_____	_____	_____

APPROVAL SHEET OF THESIS

As a member of the board of examiners of the master of science thesis open defense examination, I certify that I have read, evaluate the thesis prepared by Kaleab Eskinder, and examined the candidates as well. I recommended that the thesis be accepted by fulfilling the thesis requirements for the degree of Master of Science in Clinical Laboratory Science specialty in Hematology and Immunohematology.

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   Date_____ Signature_____

2. External Examiner_____

   Date_____ Signature_____