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COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Establishing Reference Interval for Hematological Parameters for Apparently Healthy Adult Men and Pregnant Women in Arbaminch, South Ethiopia

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This is to certify that the thesis prepared by Adisu Nedu, entitled:

"Establishing Reference Interval for Hematological Tests Parameters for Apparently Healthy Adult Men and Pregnant Women in Arbaminch, South Ethiopia'' and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Hematology and Immunohematology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abbreviations

AAU	Addis Ababa University
ARVs	Anti-retroviral drugs
BMI	Body mass index
CBC	Complete blood count
CI	Confidence interval
CLSI	Clinical and laboratory standard institute
CSA	Central statistical agency
dL	decilitre
EDTA	Ethylene diamine tetraacitic acid
gm	gram
Hct	Hematocrit
Hgb	hemoglobin
HIV	Human immunodeficiency virus
IFCC	International federation of clinical chemistry and Laboratory Medicine
Km	kilometre
MCH	Mean cell hamoglobin
MCHC	Mean cell hamoglobin concentration
MCV	Mean cell volume
MOH	Ministry of Health
MPV	Mean platelet volume
NCCLS	National committee of clinical laboratory standard
PCT	Platecrit
PDW	Platelet distribution width
PLT	Platelet
RBC	Red blood cell
RDW	Red cell distribution width
SOP	Standard operating procedure
SPSS	Statistical package for social science
WBC	White blood cell
WHO	World Health Organization

Abstract

Background: Hematological reference intervals (RIs) are important in routine assessments for the diagnosis of blood disorders, infectious diseases, immune diseases, diseases progression and assessments of ant-retroviral treatments. However, most of Africans including Ethiopia use RIs which are adopted from textbook, manufacturer of machines or Western countries while several factors are affecting these parameters.

Objective: To establish hematological reference intervals for apparently healthy adult men and pregnant women at Arbaminch, Southern, Ethiopia, 2020/21.

Methods: A cross-sectional study design was applied from Nov 2020 to Sep 2021. A systematic sampling technique was used to recruit a total of 312 men and pregnant women aged 18 years and above. Individuals who are apparently healthy(fulfill the eligibility criteria) and are voluntary to participate in the study were included. A structured questionnaire was used to collect data about socio-demographic, health status, drug usage, and nutritional habit of the participants after getting the ethical clearance. The laboratory specimens (blood, urine, stool) were collected according to standard operating procedures (SOPs) and the laboratory investigation was performed. Quality control material for hematological tests was used according to the SOPs. Age and sex specific 2.5^{th} and 97.5^{th} reference interval was determined using the non-parametric method. Data were entered and analyzed using SPSS version 23.0 software.

Results:- The median and 95% RI for RBC parameters were, 4.92(4.32-5.79)*10^{12}/L, 14.8(13.2-17.08)g/dl and 45.2(41.3-54.18)% for RBC, Hgb and Hct respectively for adult men and 4.39(3.56-5.16)*10^{12}/L, 13(9.3-15.9)g/dl and 41(32.6-46.2)% for RBC, Hgb and Hct, respectively for pregnant women. The median and 95% RI for WBC parameters and platelet count were 7.5(4.14-11.5)*10^{9}/L, 2.2(1.2-5.21)*10^{9}/L, 0.7(0.3-1.2)*10^{9}/L, 4.8(2.06-7.88)*10^{9}/L, and 227(152-353.3)*10^{9}/L WBC count, absolute lymphocyte, absolute mixed cell and absolute neutrophil and platelet count for adult men and 7.5(4.55-12.4)*10^{9}/L, 2.2(1.2-3.9)*10^{9}/L, 0.7(0.3-1.2)*10^{9}/L, 4.9 (1.9-10.1)*10^{9}/L and 202(142-362)*10^{9}/L WBC, Lymphocyte, Mixed cell, Granulocyte and platelet count for male and pregnant women, respectively.

Conclusion:- The result from the current study identified that there is variation in most of the reference interval of hematological parameters especially red cell parameters with reference intervals conducted in other parts of the country, Africans as well as western countries.

Key words: Reference interval, Hematological Parameters, Arbaminch, Ethiopia

1. Introduction

1.1 Background

Reference Intervals(RIs) are referred as the interval between two reference limits; thait is, from the lower reference limit to the upper reference limit (1)observed/measured in the sample group/reference population(2). Hematological reference intervals are necessary in routine assessments for the diagnosis of blood disorders, infectious diseases, immune diseases, diseases progression and assessments of anti-retroviral treatments(2).

Grasbeck and Saris looked at the establishment of normal values in 1969, immediately after Fellman and Grasbeck published a paper entitled "Normal Values and Statistics" as an initial study in the field of reference intervals. Using the term “normal values“ was in many cases incorrect instead “reference values“ was used until the Clinical Laboratory Standard Institute and the International Federation of Clinical Chemistry published the guideline “Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory” (3)

In Africa clinical trials are significantly increased in the last decades especially in Sub-Saharan to identify and effective prevention and treatment to combat the burden of diseases. Even though reference intervals are critical for correct diagnosis and accurate management of patients in most regions of African countries the clinical decision relied on European generated automated instrument values, US established reference intervals and toxicity grading tables(4).

Hematological parameters including white blood cell (WBC), red blood cell (RBC), mean cell hemoglobin (MCH), mean cell hempglobin concentration (MCHC), hematocrit(Hct), hemoglobin (Hgb)are highly variable due to sex, age, genetics, dietary patterns, pregnancy, ethnic origin and environmental factors. Due to these factors CLSI recommends that the laboratory have to establish its own reference intervals from local population or validate those obtained from different setting(3).

Ethiopia has great geographical and population diversity(5), but in the national context, reference values for Ethiopians have never been established despite a very few attempts in few localities which showed variation from others(6).

Such valuable data at a local population level are scanty in the Ethiopian situation(7). The current study is part of a national effort to establish RIs and to fill existing gaps. The RIs are obtained by the observation or measurement of a particular type of quantity on an adequate number of persons (reference sample group) selected to represent the general population defined by a specific percentage (usually 95%) (2). They are constructed using 2.5 th and 97.5th percentiles as lower and upper limits at 95% confidence interval in accordance with Clinical and Laboratory standards Institute (CLSI) (formerly National committee of clinical laboratory standard (NCCLS) guideline for determining reference intervals (8).

Multicenter trials, including Phase I and/or Phase II vaccine studies and global use of antiretroviral drugs (ARVs), have increased the need for regional and locally established reference values (9). This is especially true in hematology, where multiple parameters are measured simultaneously and diagnosis is usually dependent upon the coordinated interpretation of more than one test (10).

It should be taken into mind that the comparison of a laboratory result to a reference or decision limits is not the only way of interpretation of laboratory tests, but if it is properly validated for each quantitative result, RIs are also the main criteria for medical decision taken using clinical examination. In some cases, only one reference limit may be used, usually an upper limitThe determination of the reference interval is based on statistical calculations and it is purely descriptive of a given population(11)..

According to the CLSI standards, the laboratory has to collect minimum of 120 samples per partition from healthy reference individuals. RIs are established by assaying specimens from a sample group of people who meet carefully defined criteria. Producing RIs for the general population is a major challenge, as it requires selecting the appropriate reference population and recruiting individuals who represent relevant demographic groups that meet the inclusion

criteria, collecting, processing and testing specimens; and finally calculating reference values with possible stratification of the data into different subgroups(8,11,12).

There are physiological changes during pregnancy that may affect the RI of hematological parameters. Leukocytosis occur during pregnancy which may lead to an increase in neutrophil counts, this is due to physiological stress induced by the pregnant state.(14) Gestational thrombocytopenia may occur which gives decrease in platelet count, that is due to hemodilution and partly due to increased platelet activation and clearance durin pregnancy. Dilutional or physiological anemia occur during pregnancy leading an increase in plasma volume and give rise to higher plasma volume to red cell ratio.(15)

1.2. Statement of the problem

Several factors including age, sex, pregnancy status, dietary patterns, genetics, ethnic origin and environmental factors influence hematological parameters of individuals. It is with this consideration that organizations working on clinical laboratory parameters standardization recommend the establishment of RIs by each laboratory for a specific locality and population group (8,13).

Hematological parameters reference intervals also vary considerably from laboratory to laboratory due to difference in health setting, geographical locations, differences among laboratories in clinical service needs, analytic platforms, set criteria to define populations of healthy individuals, differences in methods used to establish RIs, techniques and timing of blood collection, subject's posture when the sample is taken, and physical activity of individuals(12,13).

In most African countries, reference intervals have not been adequately addressed. Instead, clinicians adopt the textbook reference intervals or from instrument manuals or publicationsthat were mainly obtained from European and other western populations (16). Moreover, published literatures have confirmed that many of the reference values obtained from the developed countries differ significantly from what pertains in most African localities(15).It is because of these variations that a study on adult African volunteers tried to characterize laboratory reference intervals to be used in the recruitment of volunteers for HIV vaccine clinical trials(9).

However, hematological reference intervals which are currently in use in Ethiopia are adopted from textbook or company provided values. Thus, adopting non-Ethiopian reference values might be misleading as ethnic origin, genetics, geographic differences and other environmental factors may differ from others suggesting that the development of reference intervals for the Ethiopian population may contribute to the quality of health care(6,7). For appropriate diagnosis, treatment, and follow-up of patients, correct interpretation of the laboratory results is mandatory.The present study was, therefore, aimed to establish hematological reference intervals

in apparently healthy adult men and pregnant women among the age of 18 years and above in Arbaminch town.

1.3. Significance of the study

Hematological reference intervals in a population are essential for accurate interpretation of hematological test results for that population. It is crucial to improve the quality of health care and provision of quality services in the health care delivery which may otherwise lead to unnecessary expenditure or denying care for the needy. Male patients and pregnant women will benefit as their laboratory result will be interpreted with locally established values. Laboratory professionals will benefit as it avoids the unnecessary high and low flagging based on company provided values and hence repeating of a test or reviewing a stained smear. Physicians can confirm the validity of the proposed RIs with clinical colleagues based on their experience when using the test to manage their patients. Researchers can also benefit by using a published data for the interpretation of the results in the locality to screen participants for vaccine or other clinical trials and it will be a baseline data in the area for other researchers in the future.

2. Literature Review

2.1. Hematological Parameters Reference Interval studies

Some comparative studies conducted in Asia and Africa showed lower values for some of the hematological parameters compared to the established Western reference intervals. According to the study done in Iran it showed that Hgb and total WBC count were similar to US but higher than some African countries. Whereas neutrophil count was similar to the values that were reported in the US but higher compared to African countries; platelet count was lower than the reference ranges described in the textbook(16). A group of male population in Eastern India was found to exhibit lower Hgb and platelet count as compared to the international reference values according to a study among healthy males(17).

Differences were observed in some laboratory parameters between African and Western populations, most notably in Hgb, Hct, platelet, total WBC count and neutrophil values as evidenced by a study from Nigeria (18).Previous studies from Eastern and Southern Africa population indicated lower values for Hgb, Hct, RBC count, platelets, MCV than the reference intervals from the US(19). Similarly as shown by a study from Ghana the red blood cell parameters Hgb, Hct, and RBC count were lower than values set as standards on the clinical hematology machines being used for clinical trials assessments (20).

A Kenyan study also showed lower Hgb, Hct, RBC MCV values as compared with company provided reference ranges derived from western population (4).Similar study from rural population of Kenya also revealed lower Hgb concentrations (9.5 g/dL; range 6.7-11.1g/dL) and neutrophil counts (1850 cells/µl; range 914-4715cells/µl) compared to North Americans(15). A high proportion of participants in African studies have WBC counts below the lower range of Massachusetts General Hospital US-derived values. This phenomenon is consistent with the number of studies that have reported lower WBC counts in African populations and those of African ancestry, including African Americans, than in Caucasian populations(21). The WBC and Hgb values of healthy HIV negative adults from the central African Republic are lower than the reference values currently used in the Central African Republic(22).

A study conducted in Ethiopia showed that low values for WBC (3.0×10^9/ to 10.2×10^9/liter) and platelets (98×10^9/ to 337×10^9/liter) were found in Ethiopians regardless of gender compared with textbook and other reference values established in Europe and the United States which are being used by hematology laboratories in Ethiopia(6). Other study in Hgb ranges in the Gambo General Rural Hospital, Ethiopia noted that the Hgb values in women (11.8-12.7 gm/dL) and men (12.9-14.0 gm/dL)from Gambo tended to be similar to Caucasian population intervals or higher than other African population(23). In the contrary, a previous study done in apparently healthy and HIV-negative populations in factory workers at Akaki Addis Ababa, Ethiopia showed that the RBC and Hgb values for Ethiopians are higher than those for residents of other Sub-Saharan African countries. Altitude-induced erythropoiesis and/or dietary factors could pay a role in causing these variations (6).

A hemato-immunological study conducted at Gilgel Gibe field research center showed that the RBC, Hgb, Hct and WBC are higher than the findings of other studies in other parts of Africa (24). Hematological reference intervals established from adult healthy populations of Northwest Ethiopia (Gondar and surrounding areas) were also different from Caucasian and African countries. The hematological reference intervals were also different from previous results obtained in other part of Ethiopia. Intervals for RBC count, Hct, Hgb, MCH, and MCHC were different for male and female but there was no difference in WBC parameters, PLT, MCV and RDW. Cognizant of this, further studies on hematological reference intervals for all age groups were recommended by the study for locally derived standard hematological reference intervals(25).

2.2. Hematological Parameters Reference Interval among pregnant women

Limited published studies are available regarding separate reference interval values among pregnant women while pregnancy is associated with physiological changes that influence hematological parameters. For example a study from west Kenya studied 120 clinically healthy, pregnant women seeking antenatal care services at either of two public hospitals in western Kenya. Hemoglobin and neutrophils of their study participants showed significant variations compared to reference intervals for non-pregnant women.

Hemoglobin values were significantly lower during pregnancy but were comparable to the values in non-pregnant women by 6 weeks postpartum. Platelets were significantly elevated in early postpartum but declined gradually, reaching normal levels by 24 weeks postpartum. The study also reported significant variations by comparing their results to reference intervals from US and UK(26).

A study from the northern part of Ethiopia recruited a total of 200 healthy, HIV-seronegative pregnant women at Gondar University Hospital. The study found changes in platelet count, and hematocrit values with advancing of pregnancy. The combined reference interval for platelet count was 221.25–240.14cells/μL. The overall 95% reference interval for HB concentration was 12.99–13.36 g/dL, HCT 40.19%–41.49%, MCV 93.33–94.63 fL, and MCH 28.88–34.81 pg. Compared with the reference ranges derived from other studies, the study found remarkable differences in HB, HCT, and MCV values(7).

2.3. Factors influencing reference values

The values of hematological parameters are affected by a number of factors even in apparently healthy populations. These factors include age, sex, ethnic background, genetics, exercise, nutritional status, environmental factors and altitude(11).

2.3.1. Sex

Studies showed that there were highly statistically significant differences for hematological values between females and males. Most studies showed mean values of RBC, Hct, Hgb, MCV, MCH, and MCHC were higher in males. However, RDW, and platelet count were higher in females(4). Sex difference in RBC parameters is a general phenomenon also confirmed by a study frpm Ethiopia (6).

2.3.2. Age

Various studies reported association of hematological changes with aging particularly observed for RBC, Hgb, Hct, MCH, and MCHC levels. These values for the healthy population showed tendency of decreasing while the MCV value showed slight increase with aging. No changes were observed for WBC differential and platelet counts(27).

2.3.3. Ethnicity and Genetics

Several studies revealed that racial/ethnic differences in RIs of various hematological values mainly between blacks and whites(28). Hct, MCHC, MCH, RBC, PLT counts and Hgb were lower in Africans compared to whites(29).Previous studies conducted in Britain showed that blacks had significantly lower WBC and neutrophil counts than whites and Indians(30).

2.3.4. Altitude

Elevated Hct and Hgb at high altitude have long been noted as a hallmark response to high altitude hypoxia(31). The unique stress at high altitude is hypobaric hypoxia caused by the fall in barometric pressure with increasing altitude and consequently fewer oxygen molecules in a breath of air as compared with sea level and hence requiring more molecules of the oxygen carrier hemoglobin(32).

2.3.5. Exercise

Physical exercise induces several hematological changes in humans(33). It has been ascertained that exercise induced hematological changes are dependent on the type, intensity, and duration of exercise (34). Acute exercise increases RBC, WBC, PLT, Hct, and Hgb concentration significantly as compared to pre-exercise values and these increments depend on plasma losses caused by the exercise(33).

2.3.6. Dietary Habit

A comparative study conducted on vegetarian with omnivorous adults in terms of iron status showed that vegetarians had significantly lower Hct and Hgb than omnivorous(34).

3. Objectives

3.1. General objective

To determine hematological reference intervals for apparently healthy adult men and pregnant women from the age of 18-60 years in Arbaminch, Southern Ethiopia from Nov to June, 2020/21.

3.2. Specific Objectives

To establish hematological reference intervals for apparently healthy adult men in Arbaminch

To determine hematological reference interval for apparently health pregnant women

4. Methods

4.1. Study area

This study was conducted at Arbaminch town. Arbaminch is one of the emerging cityin Southern Nations Nationalities and Peoples Regional state of Ethiopia. It is located 454 km from south of Addis Ababa the capital of Ethiopia and 275 km from Hawassa. There are 4 sub-cities namely, Secha, Sikella, Abaya, and Nechsar each sub-cities include eleven Kebeles. According to CSA (5)the city has total population of 74,843 among them 39192 are males and the remaining are females. It is found at an altitude of 1200-1300 meters above sea level with an average annual temperature of 29.7^0C.

4.2. Study design and period

A cross-sectional study design was implemented from November, 2020 to June, 2021

4.3.Population

4.3.1. Source population

All men and pregnant women living in Arbaminch with age of 18-60 years were source population for this study.

5.3.2. Study Population

The study population was all volunteering men and pregnant women aged18-60 years that live in Arbaminch during the study period and fulfill the eligibility criteria.

4.4. Eligibility

4.4.1. Inclusion criteria

Apparently healthy volunteering individuals aged 18-60 years(18-45 for pregnant women) and lived at least for 1 years in the study area were included in the study.

4.4.2. Exclusion criteria

- Individuals with recent illnesses, chronic diseases(diabetes mellitus, cancer and hypertension), intestinal parasites and hemoparasites, under weight participants in which their body mass index (BMI) was below 18.5kg/m2, alcohol and tobacco users.

- People with obesity, drug abuse, prescription drugs, drugs over the counter, vitamin supplement, and recent/current hospitalization.
- Individuals who donated blood within the previous 3 months and recent blood transfusion.

4.5. Study variables

4.5.1 Dependent variables

Hematological Parameters (including WBC, differential, RBC, Hgb, HCT, MCV, MCH, MCHC, red cell distribution width (RDW), mean platelet volume (MPV), platecrit (PCT), platelet d istribution width (PDW), and Platelet count

4.5.2. Independent variables

Sex

Age

BMI

Ethnicity

Nutritional factors

Other Socio-demographic variables

4.6. Sample size determination and sampling technique

4.6.1. Sample size determination

The sample size was calculated according to the Clinical Laboratory Standards Institute (CLSI) guideline for the global application. The guideline recommends to collect a minimum of 120 samples for analysis, by non-parametric method for each partition with power of 90(2).

In the current proposed study, the maximum partition needed was for hemoglobin determinations which was as follows, pregnant women 18years and Men 18years and above. Since this study focused on adult men and pregnant women, twopartition groups are needed (2x120=240).

By considering population that do not qualify the pre-determined criteria for various tests a 30% exclusion was used based on the previous studies in African countries(9). To reach the CLSI recommended total sample size of 240 for the reference interval determination, a total of 312 individuals were enrolled (i.e, 30 % x 240=72 assumed to be excluded during data analysis; 312-72=240); thus giving a total minimum sample size of 312. Thus, 312 participants were recruited from Arbaminch.

4.6.2. Sampling technique

Systematic random sampling method was employed. The K value was calculated by dividing the eligibility in the selected kebeles(1450) to the sample size 312 which was 5. The first individual was selected by lottery method, then every 5^{th} value was recruited. Accordingly, all the sub-cities in the town are considered/selected to be the participants of the study. To recruit participants, in every 5^{th} individuals were approached at their households through the study team. Once volunteering participants fulfilling the eligibility criteria are identified, they were invited to go to nearby health facilities to facilitate biological sample collection. To recruit students and employees who were not available at home were called to the teaching laboratory in Arbaminch University College Medicine and Health Science and campgain was prepared in the secondary school.

4.7. Measurement and Data collection

The study aim, risks, benefits of study participation and right to withdraw from the study at any time were explained by the study team. From those consenting participants, demographic information and a brief medical history were collected. Then physical examination was performed by health professionals(health officers and nurses). Blood specimen was collected for hematological parameters, and screening for major diseases (HIV and hemoparasites). Laboratory results were given to participants upon their requests according to the local Ministry of Health guidelines. HIV testing was performed following the national guideline.

4.7.1. Socio-demographic and clinical data

Socio -demographic and clinical data were collected using structured questionnaire by trained data collectors and physical examination and anthropometric measurements were carried out by clinicians. The data collection tool has 6 parts; part I is about genral information on address; part II is personal information; part III socio-demographic characteristics; part IV clinical information; part V Nutritional habit and life style; and part VI is Anthropometric measurement.

4.7.2. Sample collection for laboratory analysis

Etheylene diamine tetraacetic acid (EDTA) anti- coagulated whole blood for hematological tests To ensure the allowable volume of blood 5ml from adults was collected before 11:00 am to minimize the diurnal variation of some analytes. The stool sample was used for parasitological and the urine for urinalysis and HCG testing. Leak proof clean containers were used to collect urine and stool samples.

Blood type for ABO antigen were tested using direct slide method. Direct wet mount was performed for stool examination on site in the respective health facilities. Thick and thin blood film were performed to check hemoparasites since Arbaminch is an endemic area for malaria. HIV screening was performed according to the current algorism(Stat pack, Abon and SD) with their respective guidlines by trained personnel in the health center. The hard copies for cross checking was sent to Department of Medical Laboratory Sciences, AAU.

4.7.3. Laboratory testing and analysis

A complete blood count (CBC) was performed using Sysmex KX-21 automated hematology analyzer as per the manufacturer's instruction (Sysmex Corporation Kobe, Japan). The aanalyzer enumerates white blood cells (WBC), red blood cells (RBC), hemoglobin concentration (Hgb), hematocroit (HCT), platelets and their indices (PCT, MPV, PDW), absolute and relative lymphocytes, neutrophils and mid populations, and the red cells indices (MCV, MCH, MCHC, RDW). It uses the impedance principle for cell counting and photometry for hemoglobin determination (35).

4.8. Quality Assurance

To maintain the quality of the data, Standard Operating Procedure (SOP) was used and followed in each steps of the test procedures and structured questionnaires and check list were used to collect data. The three phases of quality control; pre-analytical, analytical and post-analytical were maintained according to the CLSI guideline to establish reference intervals. All laboratory assays were carried out following standard operating procedures by experienced medical laboratory technologists. Three level whole blood controls were used to ensure the quality of hematological parameters.

4.9. Data Analysis and Interpretation

Data after collection was entered and analyzed using SPSS version 23.0 software packages. Descriptive statistics were used to describe the study variables and summaries were presented in terms of frequencies and percentages. The reference interval was calculated from the 2.5^{th} percentile and 97.5^{th} percentile and to establish lower limit and upper limit at 95% CI. The box and whisker plots were used to illustrate the difference in median value of hematological parameters between the trimesters and the significance of the difference was analyzed using one way ANOVA. Variables with a p-value < 0.05 were considered as statistically significant.

4.10. Ethical Considerations

Ethical clearance was obtained from ethical committee of Medical Laboratory Department, College of Health Sciences, AAU. Letter of permission was obtained from the respective health administrative to conduct the study. The study participants that are above 18years were agreed with written consent form. Moreover, all the study participants were informed that they have a full right to participate or decline from participating in the study and the study participants were assured for the data obtained from them was recorded with specific code number for each subject and attainment of confidentiality for the information obtained from them. Individuals who were positive for any screening test were made to get appropriate treatment in the health facilities. The result was recorded using specific code for each study participant.

4.11. Data Dissemination

The finding of this study will be presented to AAU, College of Health Science, Department of Medical Laboratory Science. It will also be presented at different scientific conferences and published on different peer reviewed journals.

4.12. Operational Definition

Reference interval: The interval between two limits which are the lower and upper limits of which is obtained from 2.5^{th} and 97.5^{th} percentile.

Reference individual:a person selected on the basis of the inclusion criteria.

Apparently Healthy: Study participants fulfilling the eligibility criteria for health related assessments designed for this study

Hematological Parameters:a parameter is a numerical or other measurable characteristics forming one of a set that defines a system or sets a condition of the operation. In this study hematological parameters referes to WBC, RBC, Hgb, HCT, platelets and their indices (PCT, MPV, PDW), absolute and relative lymphocytes, neutrophils and mid populations, and the red cells indices (MCV, MCH, MCHC, RDW).

5. Results

In this study 312 individuals aged 18-60 years (18-45 for pregnant women) participated for screening tests, among them 50%(156), 50%(156) were adult men and pregnant women, respectively. Based on the questionnaire and other laboratory tests 14%(46) individuals were excluded due to intestinal parasites like *Hookworm, A.lumbricoide, G.lamblia*, and *Taenia species* and hemoparasites *P.falciparum* and *P.vivax*. The remaining individuals were excluded for long term drug prescription for diabetes mellitus and hypertension. All participants were HIV negative. As a result 266 individuals were eligible for the final analysis.

Table.1. Frequency and percentage based on screening test for some disease, periscription drug, and BMI of study participants, Arbaminch, south, Ethiopia, Nov 2020 to June 2021.

Category		Freguency	Percentage
Intestinal parasite	*A. lumbricoide*	5	1.87
	Hookworm	3	1.12
	Taenia species	7	2.63
	G. lamblia	7	2.63
Hemoparasites	*P. falciparum*	4	1.5
	P. vivax	2	0.75
Drug perscription	DM(diabete mellitus)	8	3
	HTN(hypertension)	5	1.87
BMI	Low BMI(<18.5kg/m2	2	0.75
	High BMI	3	1.12
HIV		_	_

5.1. Socio-demographic characteristics

Table.1. describes the socio-demographic characteristics of the study participants, 266 individuals were included for the final analysis of the study among them 52.3%(139) were pregnant women and the remaining 47.7%(127) were adult males. The majority were in the age

group18-30 years, 80%(215)married 207 (77.8%), college and above133(50.0%), government employee87(32.7%), and protestant religion followers 136 (51.1%).

Table 2. Socio-demographic characteristics of study participants in Arbaminch, South Ethiopia, November 2020-June 2021

Variables	Ctaegories	Frequency	Percentages
Sex	Male	127	47.7
	Pregnant	139	52.3
Age group	18-30	215	80,0
	31-45	42	15.8
	46-60	9	3.4
Marital status	Married	207	77.8
	Unmarried	54	20.3
	Other(divorced & widowd)	5	1.9
Education level	Cannot read and write	20	7.5
	Read and write	15	5.6
	Primary and secondary	98	36.9
	College and above	133	50.0
Occupation	Student	43	16.2
	House wife	61	22.9
	Government employee	87	32.7
	Private	50	18.8
	Farmer	25	9.4
Religion	Orthodox tewahdo	113	42.5
	Muslim	17	6.4
	Protestant	136	51.1

5.2. The nutritional status of the adult male and pregnant women at Arbaminch, south, Ethiopia, 2020/21

The table below summarizes the nutritional status of 266 individuals those who were eligible for the last analysis in the study. Among them 57.9%(154) consume potato, enset, or cassva 2-3 day in a week, large percentages of 83.5%(222) usually consume Legumes, bean, pea, 229(86.1%) concume corn and corn flour 2-3 days in a week,52%(140) consume Teff and wheat 2-3 days in a

week, and 88%(234) usually take egg, 207(77.8%) consume vegetables(tomato, cabbage) 2-3 day in week, 157(59%) consume fruits(orange, apple, banana)2-3 days per week,203(76.3%) consume meat(hen, cattle, fish)2-3 times in a week, 129(48.5%) consume milk and milk products 2-3 times in a week while 117(43.98%) usually consume milk and milk products (Table 2).

Table 3. Dietary pattern of the adult male and pregnant women at Arbaminch, south, Ethiopia, November 2020-June 2021

Types of food		Frequency	Percentage
Potato, enset, cassva	2-3 day in week	154	57.9
	Once a week	92	34.6
	Never	20	7.5
Legumes, bean, pea	2-3 day in week	11	4.1
	Once aweek	222	83.5
	Never	33	12.4
Teff wheat	2-3 day in a week	140	52
	Once a week	115	43.2
	Never	11	4.1
Corn	2-3 day in week	229	86.1
	Once aday	26	9.8
	Once a week	11	4.1
Vegetables(tomato, cabbage)	Once aday	13	4.9
	2-3 day in week	207	77.8
	Once a week	46	17.3
Fruit(orange, banana)	Once aday	91	34.2
	2-3 day in a week	157	59
	Once a week	18	6.8
Meat(hen, fish)	2-3 day in a week	203	76.3
	Once a week	61	22.9
	Once a aday	2	0.8
Milk and milk product	2-3 day in a week	129	48.5
	Once a week	117	43.98
	Never	20	7
Egg	2-3 day in a week	29	10.9
	Once a week	234	88
	Never	3	1.2

5.3.The blood type, frequency and percentage of adult men and pregnant women between the age 18-60 in Arbaminch, south, Ethiopia, 2020/21

Figure 1 illustrates the frequency and percentages of each blood types for both adult men and pregnant women in Arbaminch. Among the 266 participants who were eligible for the last analysis in the study. the blood type distribution was, 42.9%(114) O+, 28.6%(76) A+, 15.4%(41) B+, 7.1%(19) AB+, 4.5%(12) A-, and 1.5%(4) were B-..

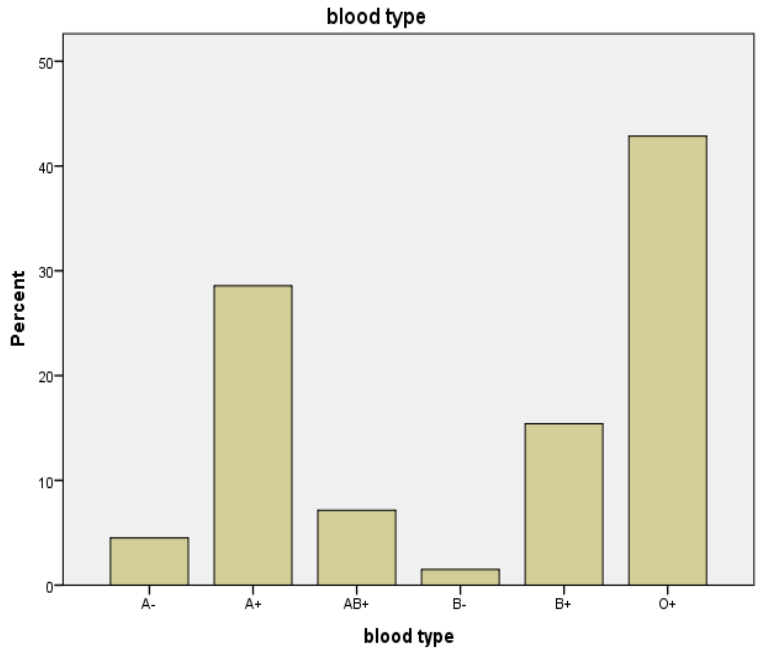

Figure 1. Blood type, frequency and percentage of adult men and pregnant women in Arbaminch, South Ethiopia, November 2020-June 202.

5. 4. The 95th percentile reference intervals of hematological parameters for adult male and pregnant women, Arbaminch, South, Ethiopia, 2020/21

Table 3 displays the calculated median value with the lower 2.5th and upper 97.5thpercentile RI of hematological parameters with 95% for LLC and ULC among two groups adult men and pregnant women.

The findings of these values were not compared between the two groups statistically because they are in different physiological states. The median and RI was 7.5(4.14-11.5)*10^9/L &7.5(4.55-12.4)*10^9/L, 2.2(1.2-5.21)*10^9/L & 2.2(1.2-3.9)*10^9/L, 0.7(0.3-1.2)*10^9/L & 0.7(0.3-1.2)*10^9/L and 4.8(2.06-7.88)*10^9/L &4.9 (1.9-10.1)*10^9/L for WBC count, absolute lymphocyte, absolute mixed cell and absolute neutrophil count for male and pregnant women, respectively.

The median and 95% RI for RBC parameters as shown in Table 3,were, 4.92(4.32-5.79)*10^{12}/L, 14.8(13.2-17.08) g/dl and 45.2(41.3-54.18)%for RBC, Hgb and Hct, respectively for adult men and 4.39(3.56-5.16)*10^{12}/L, 13.0(9.3-15.9)g/dl and 41(32.6-46.2)% for RBC, Hgb and Hct, respectively for pregnant women. The median value and the 95th percentile RI(2.5th &97.5th percentile) of platelet parameters were, 227(152-353.3)*109/L, 9.8(7.56-12.02)% and 0.21(0.18-0.36)% platelet, mean platelet volume and platcrit respectively for adult male and 202(142-362)*109/L, 9.9(7.9-12.5)fL and 0.21(0.18-0.35) platelet, mean platelet volume and platcrit respectively for pregnant female.

Table 4. Median, 95th percentile reference intervals with 95%CI of hematological parameters for adult male and pregnant women in Arbaminch, South Ethiopia, November 2020-June 2021

Parameters	Category	N	Median	Min-Max	RI(2.5th, 97.5th percentile	95%CL	
						LLC	ULC
WBC*10^9/L	Male	127	7.5	3.8-11.8	4.14-11.5	3.8-5	11-11.8
	Pregnant	139	7.5	4.2-14	4.55-12.4	4.2-4.9	11.5-14
Mid absolute*10^9/L	Male	127	0.7	0.3-1.2	0.3-1.1	0.3-0.4	1-1.2
	Pregnant	139	0.7	0.3-1.3	0.3-1.2	0.3-0.4	1.1-1.3
Lymphocyte absolute *10⁹/L	Male	127	2.2	1.1-5.4	1.2-5.21	1.1-1.3	4.3-5.4
	Pregnant	139	2.1	1.1-4.3	1.2-3.9	1.1-1.3	3.4-4.3
Neutrophil absolute*10^9/L	Male	127	4.8	1.8-8.4	2.06-7.88	1.8-2.5	7.7-8.4
	Pregnant	139	4.9	1.9-10.1	2.25-8.8	1.9-2.6	8.3-10.1
Mid %	Male	127	9.2	4.46-13.1	4.56-12.5	4.46-5.5	12-13.1
	Pregnant	139	9.6	5.5-13.8	6.2-13.2	5.5-6.3	12.8-13.8
Lymphocyte %	Male	127	27.4	17.6-48	18.4-45.9	17.6-19	42-48
	Pregnant	139	25.2	17.6-46.8	18.3-44.5	17.6-18.8	41.1-46.8
Neutrophil %	Male	127	63.9	43.8-74.8	45.9-73	43.8-48	72-74.8
	Pregnant	139	64.6	41.3-75.3	45.5-73.4	41.3-48.1	72.3-75.3
RBC*10^{12}/L	Male	127	4.92	4.23-5.88	4.32-5.79	4.23-4.39	5.6-5.88

	Pregnant	139	4.39	3.37-5.38	3.56-5.16	3.37-3.7	4.98-5.38
Hgb gm/dL	Male	127	14.8	12.7-17.7	13.2-17.1	12.7-13.8	16.4-17.7
	Pregnant	139	13	9.3-15.9	10.1-15.2	9.3-10.8	14.9-15.9
Hct %	Male	127	45.2	41-54.7	41.3-54.2	41-43	51.5-54.7
	Category	N	Median	Min-Max	95%RI	LLC	ULC
	Pregnant	139	41	31.6-47.4	32.6-46.2	31.6-35.3	46.2-47.4
MCV fL	Male	127	92.6	80-101.2	80.6-100	80-83.7	99.1-101
	Pregnant	139	93.7	78.2-102	78.9-100	77.6-83.7	100.4-102
MCH pg	Male	127	29.7	25.2-33.4	26-33	25.2-27.4	32.8-33.4
	Pregnant	139	29.6	23-32.8	23.5-32.5	23-25.5	32-32.8
MCHC %	Male	127	32.3	28.1-36.4	29.4-35.8	28.1-30.5	34.8-36.4
	Pregnant	139	31.6	27.8-34.9	28.3-34.5	27.8-28.9	33.2-34.9
RDW-CV	Male	127	15	12-18.5	12.3-17.8	12-13	16.9-18.9
	Pregnant	139	16.1	13-19.5	13.6-18.5	13-14.3	18.5-19.5
PLT*10⁹/L	Male	127	227	151-363	152-353	151-154	342-363
	Pregnant	139	202	142-405	145-362	142-148	329-405
MPV fL	Male	127	9.8	7.3-14.7	7.6-12.02	7.3-8	11.4-14.7
	Pregnant	139	9.9	7.4-14.7	7.9-12.5	7.4-8.5	11.9-14.7
PDW%	Male	127	15.1	9.3-19.2	10.3-15.8	9.3-11.1	15.6-19.2
	Pregnant	139	13.6	9.15-22	9.3-18.4	9.15-10.2	16.4-22
Pct	Male	127	0.22	0.16-0.34	0.19-0.32	0.16-0.15	0.29-0.34
	Pregnant	139	0.21	0.18-0.4	0.18-0.35	0.15-0.19	0.32-0.4

5.5. Comparison of WBC, RBC, HCT, and Hgb between trimesters

The box and whisker plots are used to illustrate the difference in median value of selected hematological parameters between the trimesters (in weeks) and the significance of the difference was analyzed using one way ANOVA.

An increasing value of median value of white blood cell was shown from the 1^{st} to 2^{nd} and 3^{rd} trimesters, $6.6(4.2\text{-}9.08)*10^9/L1^{st,}$ $7.8(4.7\text{-}12.4)*10^9/L2^{nd}$&$8.4(4.5\text{-}13.5)*10^9/L3^{rd}$. But there was statistically significant difference between 1^{st}&2^{nd} trimester p=0.001, and 1^{st}&3^{rd} p=0.000 but not between 2^{nd}& 3^{rd}trimesters p=0.943.The median value of the RBC was decreasing across the trimesters, $4.62(3.58\text{-}5.37)*10^{12}/L1^{st}$, $4.28(3.67\text{-}5.07)*10^{12}/L$ 2^{nd}& $4.15(3.38\text{-}4.97)*10^{12}/L3^{rd}$ trimester. also there was statistically significant difference between 1^{st}& 2^{nd} p=0.002, 1^{st}& 3^{rd} p=0.000 and 2^{nd}& 3^{rd} p=0.014.

The hematocrit and hemoglobin showed significant decrement when gestation period was increased. Accordingly the median of hemoglobin was 14(11.5-15.87) g/dl, 12.6(9.6-14)g/dl and 11.9(9.8-14.7)g/dl 1st, 2nd and 3rdtrimesters respectively. Statistically significant difference was observed between the trimesters, 1st& 2nd p=0.000, 1st& 3rd p=0.000 and 2nd&3rd p=0.008. The median value of hematocrit in percent across the trimesters were 44(33.8-48.9) 1st, 40.2(33-46) 2ndand 37.9(31.6-43) 3rd trimesters. The ANOVA showed significant dfference between the groups in trimesters, 1st& 2nd p=0.000, 1st&3rd p=0.000 and 2nd&3rd p=0.002 (table).

Table 5. The median and the Oneway Anova test for comparing median value of hematological parameters between the trimesters for pregnant women at Arbaminch, Ethiopia, Nov 2020 to June 2021.

Parameters	**Trimesters**			**P-value**		
	Trimester(T1)	Trimester(T2)	Trimester(T3)	T1 versus T2	T1 versus T3	T2versus T3
WBC(10*9/L)	6.6	7.8	8.4	0.001	0.013	0.943
RBC(10*12/L)	4.62	4.28	4.15	0.002	0.000	0.014
Hgb(g/dl)	14	12.6	11.9	0.000	0.000	0.000
Hct%	44	40.2	37.9	0.000	0.000	0.002
Plt(10*9/L)	212	214	209	0.611	0.663	0.153

5.6. Comparison between the 95th percentile reference interval of the current study with other studies in Ethiopia and African nations

Table4 shows comparison of the current study with the currently used RIs provided by the company and studies from Dire dawa, Asmara, Kenya and Ghana. As shown in the table, most of the hematological reference intervals in the current study overlap with the currently used values in the study area. No out of range values (OOR) values were detected for MID in pregnant women, while for RBC, Hb and platelet values in male participants. The highest OOR was detected for Lym% (12.9%) where the current male study participants have slightly lower Lym% range both in the upper and lower limit (18.5- 45.8 vs 20-50%). Comparison with the other studies from Ethiopia (Dire dawa) and other African countries showed inconsistency.

Table 6. Comparison between the 95th percentile reference interval of the current study with other studies in Ethiopia and other African nations

Parameters	Category	This study	Current practice (35)	Out of range%	Dire Dawa (36)	Other African countries		
						Asmara (37)	Ghana (16)	Kenya (4)
WBC(*10^9/L	Male	4.14-11.5	4-11	7.8%(10/127)	3.5-10.3	3.7-9.3	3.28-11.2	3.3-9.3
	Pregnant	4.55-12.4	5.6-15.9	11.5%(16/139)	3.6-11.8	NA	NA	4.4-15.3
Lymp#(*10^9/L	Male	1.2-5.21	NA	NA	1.2-3.8	NA	0.77-4.8	1.2-3.4
	Pregnant	1.2-3.9	0.9-3.9	1.4%(2/139)	1.1-2.6	NA	NA	1-4.4
MID#(*10^9/L	Male	0.3-1.1	NA	NA	NA	NA	0.21-1.02	NA
	Pregnant	0.3-1.2	0.3-1.4	No OOR	NA	NA	NA	0.11-1.1
Gran#(*10^9/L	Male	1.8-8.4	NA	NA	1.4-6.8	NA	0.65-5.5	1.3-5.2
	Pregnant	2.2-8.18	3.6-12.3	4.3%(6/139)	2.3-9.1	NA	NA	2.3-12.5
Lymp%	Male	18.5-45.8	20-50	12.9%(18/127)	18-55	22-59.9	NA	NA
	Pregnant	18.3-44.5	NA	NA	14-39.6	NA	NA	10.8-44
MID%	Male	4.56-12.5	4-11	7.9%(11/127)	NA	3.1-11.6	NA	NA
	Pregnant	5.9-13.2	NA	NA	NA	NA	NA	1.8-10.05
Neutrophil%	Male	45.9-73	40-70	12.2%(17/127)	33-72.8	31.7-73.6	NA	NA
	Pregnant	45.5-73.4	NA	NA	49-79.8	NA	NA	39-89
RBC(*10^{12}/L	Male	4.32-5.79	4.2-6.3	No OOR	4.5-6.15	4.2-6.07	3.61-6.97	4.6-6.6
	Pregnant	3.56-5.16	3.28-5.2	2.1%(3/139)	3.67-5.1	NA	NA	3.2-5.3
Hgb(g/dl)	Male	13.1-17.1	12-18	No OOR	12.4-17.5	12.5-17.8	10.7-18.8	12.6-17.2
	Pregnant	10.1-15.2	9.5-15.1	2.9%(4/139)	9.5-13.5	NA	NA	7.7-14.5
Hct%	Male	41.2-54.2	37-51	6.2%(8/127)	44-58.5	40.5-55	31.8-61.8	38.1-51.6
	Pregnant	32.6-47.4	31-45	11.5%(16/139)	32.2-46	NA	NA	30-45
MCV(fl)	Male	80.8-100	80-96	12.5%(16/127)	86-104	85.7-100	69.7-103	67.4-93.6
	Pregnant	78.9-101	79-99	7.9%(11/139)	85-103	NA	NA	61.3-94.8
MCH(pg)	Male	26-33	26-32	10.2%(13/127)	24.6-31	28-33	23.3-34.2	NA
	Pregnant	23.5-32.7	27-33	4.3%(6/139)	23.7-33	NA	NA	22.5-33.5
MCHC(g/	Male	29.4-35.8	31-36	1.6%(2/127)	27.6-31	30.4-33.7	29.7-37.2	NA

dl)	Pregnant	28.3-34.4	30-35	7.9%(11/139)	27.4-33	NA	NA	28-36.5
RDW-CV%	Male	12.3-17.8	NA	NA	12.3-15.3	12.3-15.5	11.7-18.6	NA
	Pregnant	13.6-18.5	NA	NA	12.5-17.6	NA	NA	11.3-20.3
PLT(*10^9 /L)	Male	152-353.6	140-440	No OOR	164-447	128.4-318	86-348	126-356
	Pregnant	145-362.5	146-429	6.4%(9/139)	157-421	NA	NA	128-388.4
MPV%	Male	7.6-12	NA	NA	7.7-10.9	NA	NA	NA
	Pregnant	8-12.5	NA	NA	7.6-10.3	NA	NA	NA
PDW%	Male	10.3-15.6	NA	NA	15-16.3	NA	NA	NA
	Pregnant	9.3-18.4	NA	NA	15.1-16.3	NA	NA	7.5-11.4
Pct	Male	0.19-0.32	NA	NA	0.15-0.36	NA	NA	NA
	Pregnant	0.18-0.35	NA	NA	0.15-0.35	NA	NA	0.12-0.34

6. Discussion

The WBC reference interval in male participants is almost overlapped with the currently in use value. While in pregnant women, the WBC RI was low both in the lower and upper limit than the RI in the current practice(35). The upper RI limit for platelets was also lower than the currently in use company provided value for both males and pregnant women signifying the importance of locally appropriate population specific reference interval.

Most of the results obtained from this study are different from studies conducted in other parts of the world. For instance study conducted in Turkey(38) reveals higher reference values for red cell parameters(RBC, Hgb, Hct and the red cell indicies). Another study in USA(39) strengthen this idea in which there is higher red cell parameters(RBC and Hgb) this is due to high altitude, genetics, better nutritional status of the population especially a high content of iron and a better life style in these countries affect the reference values of these parameters(RBC, Hgb and Hct). In other way there was a comparable median value of RBC, Hgb, Hct, MCV, MCH, MCHC, with study conducted in Brazil(40) and India, with the exception of ethinicity these population have comparable life style, nutritional status and altitude below the sea level(41).

The reference intervals for red blood cell, hemoglobin and hematocrit in the current study are not comparable with other studies in other parts of Africa except studies conducted in Asmara which shows comparable results for RBC, Hgb Hct, MCV, MCH and MCHC for adult male(39). This is due to the population of Asmara have comparable life style, nutritional status ethinicity The other studies in Africa(Ghana)(16)shows lower reference values of RBC, Hgb and Hct. Another study in western Kenya(4)shows a different reference values for red cell. The hemoglobin RI of pregnant women was also higher than what has been reported from Kenya(4).Also lower red cell parameters(RBC, Hgb, Hct), and platelet were reported from Uganda(42).

The findings of this study especially the red cell parameters (RBC, Hgb and Hct) in both adult male and pregnant women are relatively comparable with the study conducted in the eastern part of Ethiopia this is due to the comparable similarity between Arbaminch and Dire Dawa with altitude(1160m)above sea level. But there is a remarkable difference in the reference intervals of

red cell parameters(RBC,Hgb and Hct) with the research conducted in the suburbs of Addis Ababa(6). The reference intervals for these parameters are higher in Addis Ababa; it may be because of the high altitude (2355m) above sea level for Addis Ababa which can stimulate erythropoitin hormone that causes a high production of red cells when the human body gets hypoxic condition in high altitude. Another study conducted in the northern parts of the country illustrated higher median value and RI for RBC, Hgb and Hct(25). The population in the north uses injera as a daily meal which has a high content of iron but in the southern part the people mostly uses inset, maize, wheat and barley. In addition to this the northern parts are located at a high altitude compared to the current study area(Arbaminch)(43).

The WBC reference interval in male participants which almost overlapped with the currently in use company provided value (35),was higher compared to the RI determined for males in Dire Dawa(36), Asmara(37), Ghana(16), and Kenya (4). While in pregnant women, the WBC RI was low both in the lower and upper limit than the RI in the current practice but higher than the values from Dire Dawa (36)and overlaps with the values from Kenya (4). On the other hand, the study conducted in Chile indicated higher median value of (8.4vs7.5*10^9/L, Chile and current study, respectively). But there were lower granulocyte and lymphocyte count(44). Another study in Mali indicated lower median value of WBC parameters compared to the current study (45). In addition, other studies in Africa (11)also indicated lower median value and RI for WBC=5.6(3.9-10.2*10^9/L, and Gran#=1.9(0.8-5.0*10^9/L while comparable median Lym#=2.2and PLT224 *10^9/L (1.1-3.1*10^9/L) (133-386)compared to the current study which are 7.5(4.14-11.5*10^9/L), 4.8(2.06-7.88)*10^9/L, 2.2(1.2-5.21)*10^9/L and 227(152-353)*10^9/L for WBC, granulocyte, lymphocyte and platelet, respectively. As can be seen from the finding, the consistently low lower WBC limit seen in other studies from Ethiopia (6) as well as other African countries (4,14) was not confirmed by the present study. For example, the difference in median and RIs value of WBC parameters and platelet count between the current study and the earlier study from the outskirt of Addis Ababa was remarkable (6). The median value and the reference interval in Addis Ababa was lower for both WBC 3-10.2x10^9/L versus 4.14-11.5x10^9/L and platelet98-337x10^9/L versus 152-363X10^9/L, respectively. On the other hand, a comparable median value

and reference interval was obtained from the previous study conducted in South-West Ethiopia both in WBC and platelet parameters(46). Research conducted in Debremarkos, Ethiopia streghten the previous finding that there was comparable median values for both WBC and platelet count(47). The heterogeneity of the Ethiopian population, socio-cultural differences might have contributed for the observed variation. Findings from Africa are difficult to generalize, as there are articles from the continent which showed differences in the median value of WBC and platelet parameters. For instance, study in Burkinafaso showed higher median value for WBC and lower RI for platelet parameters(48). Higher median value and RI was reported in Uganda vs current study $8.9*10^9$/L vs $7.5*10^9$/L $285*10^9$/L vs $227*10^9$/L WBC and Platelet, respectively(49). This may be due to the variation of methodology used, the machine used for analysis, the average age of the study participants, the epidemiology of diseases that affect the outcome of the study. This and other reasons revealed that population specific reference interval is necessary for the diagnosis and treatment for patients. The median and the upper limit of RBC was higher in male according to the research conducted in Gojjam(Ethiopia)(49). In this study the lower limit RI of WBC parameters(both neutrophil and lymphocyte) and platelet were lower compared to the current study but the upper limit of red cell parametrers(RBC, Hgb, Hct and MCV) were higher.

The other finding from the current study was the observation of a significant difference between the trimesters with the median value of WBC, RBC and Hgb which was also confirmed by other studies in Kenya(51). In the current study the median value of WBC increase from 1^{st} to 3^{rd} trimesters with significant p-value that was confirmed by the study in Kenya.RBC and Hgb were decreased across the trimester even if it was not statistically significant(50).

Study conducted in the Eastern part of Ethiopia illustrated the comparison of the parameters (WBC, RBC, Hgb and platelet across the trimesters(36). According to this study the median value of WBC increased from 1^{st} $7.7(2.8\text{-}12)*10^9$/L to $8.0\ (4.1\text{-}12)*10^9$/L in the 3^{rd}trimester without statistically significant difference but in the current study the median value of WBC increased from 1st to 3^{rd} trimester with significant p-value. The median value of RBC was decresed from the 1^{st} $4.38(3.65\text{-}5.21)*10^{12}$/L to 3^{rd} $4.09(3.22\text{-}4.98)*10^{12}$/L trimester in the

previous study in Dire Dawa with no statistically significant differencebut in the current study the difference was statistically significant. Additionally there were decreased median value of Hgb from 1st to 2nd trimester which was statistically significant confirmed the current study and to the 3rd trimester which was not significant(36). The variation in WBC parameters between the gestation age is due to physiological changes in pregnancy which may leads to neutropilia, increase in the plasma volume during pregnancy which also leads to hemodilution and hormonal changes collectively gives low level of RBC count and the decreased level of Hemoglobin when the gestation age gets higher and higher(51). Another study conducted in Hawassa(52) among pregnant women showed comparable median value white blood cell count. The lower and upper limit RI for WBC, lymphocyte, RBC, Hgb, Hct and platelet were lower in the previous study in Hawassa. The current study showed significant difference on the median value of WBC, RBC, Hgb and Hct which was comparable with study conducted in Hawassa between the trimesters.

7. Strength and Limitations

The study failed to include non pregnant women in order to compare the finding with adult male and pregnant women. This is due to most of them were non voluntary during the study period which was influenced by COVID-19 in order to collect blood sample. Males relatively were better and pregnant mothers were volunteering to get full assessment which is befecial for both the mother and the baby. However, determining the RI for the two partitions in an area where RI have never been established is among the strength. The study failed to screen some TTI(HBV, HCV and syphillis). In addition concentration technique was not considered during stool examination that may give a better outcome in the screening of the participants.

8. Conclusion and Recommendations

8.1. Conclusion

The result from the current study identified that there is variation in most of the reference interval of hematological parameters especially red cell parameters with reference intervals conducted in other parts of the country, Africans as well as western countries. WBC count of males almost overlap with current company provided RIs, though higher compared to the consistently low lower WBC limit seen in other studies from ETH and other African countries but not confirmed by the current study. Also there were statistically significant difference in the median value of some clinically important parameters by gestational age and it is important to avoid pregnancy related damages due to hematological changes. Additionally the study recognized reference interval for red cell parameters were lower in some extent when the altitude is lower. This indicated utilizing population specific reference interval is better in health facilities for quality health care, diagnosis, treatment management, monitoring disease progress of hematology related disorders.

8.2. Recommendation

It is recommended to use the result obtained from the current study in the health facilities around Arbaminch and Arbaminch Zuria Woreda for the diagnosis, treatment management, prognosis and monitoring diseases rather than using RI of the western countries as well as text book. Also it is better to conduct additional researches in the remaining sex and age groups(adolescents, pediatrics and non pregnant women) in the future.

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Annex III : Information sheet for adults (>18 years)

Research Title:Establishment of hematological Reference Intervals for apparently healthy men and pregnant women at Arbaminch, Southern, Ethiopia

Research PI: Adisu Nedu

Organization: Addis Ababa University College of Health Science Department Medical Laboratory Science

Sponsor: AAU, Ethiopia

Introduction:

Hello! My name is _____ and I am working with researchers from Addis Ababa University Department of Medical Laboratory Sciences.

Purpose of the research:

The health laboratory plays an indispensable role in the health care system. It supports diagnosis (to rule in or rule out a diagnosis), monitoring of response to treatment, epidemiological surveillance, prevention as well as Research (to understand the patho-physiology of a particular disease process). Especially there is lack of local reference interval for indigenous population. Therefore, the purpose of this proposed study is to Establish Hematological Reference Intervals for Arbaminch population.

You have been chosen for this study. Therefore, we invite you to take part in this study and contribute to the establishment of indigenous reference values which are needed for providing quality laboratory service. Thus, result from this study is anticipated to improve the health status of the adult population at large in Arbaminch, Ethiopia.

Procedures:

After agreeing that you can take part, one or more of our research staff will ask you some questions which will take up to 15 minutes. Your weight, height and vital signs will be measured. You will be asked to provide urine and fresh stool on a particular container we provide. We will also collect 13 ml venous blood (about 1 table spoon) from you by sterile-

disposable vacutainer tube and needle (9ml in plane tube and 4 ml in tube containing EDTA). We will conduct laboratory examination to determine different hematological, serological, parasitological and clinical chemistry parameters.

Confidentiality:

The information obtained during the study will remain confidential. Disclosure of any of the data tothird parties other than those allowed in the Informed Consent form will not be permitted. The results of the research study may be published, but participants' names or identities will not be revealed. To maintain confidentiality, the investigator will keep records in locked cabinets in a locked room at the office and the results of the tests will be coded to prevent identification of the volunteers. Access to data entered into computerized files will be permitted only for authorized personnel directly involved with the study and will be password protected. Individual-specific information may be provided to responsible local medical personnel only with your permission. Urine, stool and blood collected will not be used for other purposes. The leftover samples will be stored at the Department of Medical Laboratory Sciences of AAU in a secure place for additional tests as needed. Finally, all the biological wastes, after analysis will be safely disposed in an environmentally friendly manner.

Risks and Discomfort:

There will be minimal discomfort in giving urine and stool samples. However, there might be some minimal risk and discomfort when we take venous blood. Nevertheless, we will try to minimize the discomfort as much as possible, as the blood samples will be taken by experienced laboratory professionals.

Safety:

The venous blood sample will be collected using sterile vacutainer tube/syringe and needle by experienced health professional after disinfecting the site of picture by 70% ethanol. Moreover, leftover stool, urine and blood sample (that is not stored) will be discarded following the guideline of bio-safety.

Benefits:

By participating in the study, you will directly benefit by being investigated for any pathogenic organisms and other clinical and hematological abnormalities. Establishing the reference interval

and developing the in-house quality control materials will be used in the future to improve the general health status of Arbaminch, Ethiopians.

Incentives:

Any positive finding in your stool/urine/blood will be taken care of by referring you to the nearby health institution; you will get all the laboratory investigation results for free. However, we will not pay you for taking part in this study as well as your treatment costs. But, we will thank you for your participation.

Right to refuse or withdraw:

We assure you that our best care will be taken if you agree to take part in the study. You should also know that you are free to withdraw from the study at any time and that you will not be discriminated in any form of service like health.

Whom to contact:

If you have any questions, you may ask the person whom you are giving your urine, stool and blood or the principal investigator (PI) of the study or the investigators/focal persons using the following addresses:

1. Dr Aster Tsegaye, Lead PI: AAU 09 11 696085
2. Jemal Alemu AAU/Regional Lab 0911429989
3. Adisu Nedu Investigator(AMU academic staff 0924038607

IRB address: Addis Ababa University, College of Health Science, Department of Medical Laboratory Science 0911 107099

Code No._____

Annex IV. Consent form for adults (≥18 years)

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

To give my stool☐

To give my urine☐

To collect my blood☐ and be a participant in this study and understand that I have the right to withdraw from the study at any time☐.

Print name of participant, date and signature or thumb impression of participant

_____ (dd/mm/yy)_____

If illiterate;

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

_____ (dd/mm/yy) _____

Phone number_____

Print name of researcher, date and signature of researcher

_____ (dd/mm/yy) _____

Annex IX: Information sheet for adults (≥18 years old) (18 ዓመትና ከዚያ በላይ ለሆኑ አዋቂዎች መረጃ)

የጥናቱርዕስ፡“እድሜአቸው አምስት ዓመትና ከዚያ በላይ ለሆኑ የአርባምንጭ የጤናማ ሰው ደም ውስጥ የሚገኙ የክሊኒካል ላቦራቶሪ ምርመራዎች መጠን ሪፈረንስ ኢንተርቫል መስራት “፡በበርካታ ማዕከላት የሚሰራ ጥናት “

የጠናቱ ዋና ተመራማሪ: አዲሱነዱ(ማስተርስተመራቂ)

ተባባሪ ተመራማሪዎች አርባምንጭ ዩኒቨርሲቲ የህክምና ላቦራቶሪ ት/ት ክፍልና የክልልዊ ላቦራቶሪ

ስፖንሰር (ወጪውንየሸፈነው)፡ አዲስ አበባ ዩኒቨርሲቲ ጤና ሳይንስ ኮሌጅ

መግቢያ:

ጤና ይስጥልኝ! ስሜ _____ ነው። የህክምና ላቦራቶሪ ሳይንስ ትምህርት ከሚያስተምሩ ዩኒቨርስቲዎች፣ሪጅናል ላቦራቶሪዎች፣ጋር እየሰራሁ ነው። በላቦራቶሪ ውስጥ የጤናማ ሰው ደም ውስጥ የሚገኙ የሄማቶሎጂ ምርመራዎች መጠን ሪፈረንስ ኢንተርቫል እድሜአቸው አምስት ዓመትና ከዚያ በላይ ለሆኑ የአርባምንጭ ነዋሪዎች ጥናት እያካሄድኩ ነው።

የምርምርጥናቱአላማ:

የህክምናላቦራቶሪበጤናውአገልግሎትውስጥከፍተኛሚናይጫወታል።ምርመራንለማረጋገጥ፣ህሙማንለ መድሃኒቶችምላሽመስጠታቸውንክትትልለማድረግ፣የበሽታዎችንስርጭትለማጥናት፣በሽታለመከላከልእናስ ለበሽታዎችምንጭምርምርለማድረግአስተዋፅያያደርጋል።በተለይምበአገራችንየጤናማሰውየላቦራቶሪው ጤትማወዳደሪያሪፈረንስኢንተርቫልየለም።ስለሆነምየዚህጥናትዓላማበአገርውስጥበላቦራቶሪውስጥየጤና ማሰውየሄማቶሎጂናየክሊኒካልኬሚስትሪውጤትማወዳደሪያሪፈረንስኢንተርቫልእድሜአቸውአምስትናከዚ ያበላይለሆኑየአርባምንጭነዋሪዎችመሥራትነው።

አንተም/አንቺምበዚህጥናትእንድትሳተፍ/ፊእየጋበዝኩወላጆችሽ/ወላጆችህፈቃዳቸውንገልፀዋል።።ስለዚህበዚ ህጥናትበመሳተፍበአርባምንጭበላቦራቶሪውስጥየሚመረትየጤናማሰውየክሊኒካልላቦራቶሪውጤትማወዳ ደሪያሪፈረንስኢንተርቫልለመስራትአስተዋፅዎእንድታደርግ/ጊተጋብዘሃል/ሻል።ይህምጥራትያለውየላቦራቶሪ

አገልግሎትለመስጠትአስፈላጊነው፡፡ስለዚህየዚህጥናትውጤትአርባምንጭውስጥአምስትእናከዚያበላይለሆ ኑሰዎችጤናንለማሻሻልይረዳል፡፡

የጥናቱአካሄድ:

በጥናቱለመሳተፍከተስማማህ/ሽየጥናቱአባል/አባላት 15 ደቂቃየሚወስድጥያቄይጠይቁሃል/ሻል፡፡ክብደት፣ቁመት፣የክንድእናየደምግፊትልኬትይወሰዳል፡፡ሽንትናሰገራ በምንሰጠውእቃእንድትሰጠን/ጭንእንጠይቃለን፡፡፣በተጨማሪም 10 ሚሊሊትር (አንድየሾርባማንኪያየሚሆንደም) በንፁህቫኩቴይነርብልቃጥእናመርፌእንቀዳለን (7 ሚሊሊትርበባዶቲዩብ፣ 3 ሚሊሊትርደምእንዳይረጋየሚያደርግንጥረነገር፣ኢዲቲኤ፣ባለበትቲዩብ)፡፡የሄማቶሎጂ፣ሴሮሎጂ፣ፓራሲቶሎ ጂእናየክሊኒካልኬሚስትሪምርመራዎችንእናካሂዳለን፡፡

ሚስጥርስለመጠበቅ:

በዚህጥናትየሚሰበሰብመረጃበሙሉበሚስጥርይጠበቃል፡፡መረጃበዚህየስምምነትቅፅከተፈቀደውውጪለሶ ስተኛወገንተላልፎአይሰጥም፡፡የዚህጥናትውጤትሊታተምይችላልነገርግንየጥናቱተሳታፊዎችስምናማንኛው ምመለያአይገለፅም፡፡ሚስጥራዊነቱንለመጠበቅየዚህጥናትአባላትመረጃዎችንበተቆለፈክፍልበተቆለፈካቢኔ ትውስጥያስቀምጣሉ፣የፈቃደኛተሳታፊዎችንማንነትንላለማሳወቅውጤቶቸምበኮድይቀመጣሉ፡፡በኮምፒዩ ተርውስጥለተቀመጡፋይሎችለጥናቱተመራማሪዎችብቻየሚፈቀዱናበሚስጥርቁልፍየሚጠበቁይሆናል፡፡የ ተሳታፊውጤትለህክምናባለሞያሊተላለፍየሚችለውበተሳታፊውፈቃድብቻነው፡፡የተሰበሰበውሽንት፣ዓይነ ምድርናደምለሌላአገልግሎትአይውልም፡፡የሚተርፉትናሙናዎችበአዲስአበባዩኒቨርስቲህክምናላቦራቶሪትም ህርትክፍልደህናቦታተቀምጠውለተጨማሪምርመራዎችእንደአስፈላጊታቸውጥቅምላይይውላሉ፡፡በመጨረ ሻምተሰርቶባቸውየተራረፉየሚደፉናሙናዎችአካባቢንበማይበክልመልኩበጥንቃቄይወገዳሉ፡፡

ጥናቱየሚያስከትላቸውየጤናችግሮችናአለመመቸት:

ሽንትናዓይነምድርበመስጠትየሚደርስመጠነኛአለመመቸትሊኖርይችላል፡፡ሆኖምደምበሚቀዳበትጊዜመጠ ነኛመጎዳትናየተወሰነአለመመቸትሊኖርይችላል፡፡ይሁንእንጂበተቻለመጠንልምድያለውየላቦራቶሪባለሞያበ መጠቀምአለመመቸቱንለመቀነስእንሞክራለን፡፡

ደህንነት:

የደምናሙናበሚወሰድበትጊዜበንፁህየደምመቅጃበመጠቀምየሚቀዳውንቦታበ70% አልኮልበማፅዳትልምድባለውባለሞያይከናወናል።በተጨማሪምጥቅምላይከዋሉበኋላለማስቀመጥየማይሆ ኑየሚደፉየዓይነምድር፣ሽንትእናደምትራፊዎችየላቦራቶሪደህንነትመመሪያበመከተልይወገዳሉ።

ጥቅማጥቅሞች፡

በዚህጥናትበመሳተፍለበሽታአምጪተህዋስያን፣ደምናሽንትምርመራበማድረግየጤንነትሁኔታማወቅይቻላ ል።በአገርውስጥበላቦራቶሪውስጥየሚመረትየጤናማሰውየሄማቶሎጂናየክሊኒካልኬሚስትሪውጤትማወዳ ደሪያሪፈረንስኢንተርቫልእድሜአቸውአምስትናከዚያበላይለሆኑበአርባምንጭመሰራቱየአርባምንጭነዋሪዎ ችንየጤናሁኔታለማሻሻልይረዳል።

በጥናቱለመሳተፍማትጊያ:

ከዓይነምድር፣ሽንትእናደምምርመራጤናማያልሆነውጤትከተገኝበአቅራቢውወደሚገኝጤናተቋምትላካለህ /ትላኪያለሽ፣የላቦራቶሪውጤቶቹንበነፃታገኛለህ/ታገኚያለሽ።ይሁንእንጂበዚህጥናትለመሳተፍምሆነለመድሃ ኒትክፍያአይሰጥም።ስለተሳትፎህ/ህግንእናመሰግናለን።

ያለመሳተፍመብት:

በዚህጥናትከተሳተፍክ/ሽየቻልነውንሁሉእንክብካቤእናደርጋለን።በማኛውምሰዓትከጥናቱመውጣትእንደሚ ቻልናይህምበምታገኘው/ኚውአገልግሎትላይ (ለምሳሌየጤናአገልግሎት) ምንምአይነትልዩነትአይደረግም።

ጥያቄካለለማነጋገር:

ምንምዓይነትጥያቄካለየዓይነምድር፣ሽንትእናየደምናሙናየሰጠኸውን/የሰጠሽውንሰውመጠየቅይቻላልወ ይምየጠናቱዋናተመራማሪንወይምተባባሪዎችበሚከተለውአድራሻመጠየቅይቻላል።

1. ዶክተርአስቴርጸጋዬየጥናቱዋናመሪአ.አ.ዩ. 09 11 696085
2. ጀማልአለሙየአ.አ.ዩ. ሪጅናልላቦራቶሪ0911429989
3. አዲሱነዱተመራማሪናየአምዩመምህር0924038607

የምርምርስነምግባርቢሮአድራሻ፡በአዲስአበባዩኒቨርስቲየጤናሳይንስኮሌጅየህክምናላቦራ ቶሪሳይንስት/ክፍልስልክ0911 107099

Annex X. Laboratory test

Sysmex KX-21 Hematology Analyzer

Purpose/Definition:

The KX-21 performs speedy and accurate analysis of 18 parameters in blood (Whole WBC, LYM%, MXD%, NEUT% , LYM# , MXD#, NEUT# , RBC count, Hemoglobin, HCT, MCV, MCH, MCHC, RDW-CV, RDW-SD, PLT, PDW, MPV, P-LCR) The KX-21 employs three detector blocks and two kinds of reagents for blood analysis. The WBC count is measured by the WBC detector block using the DC detection method. The RBC count and platelets are taken by the RBC detector block, also using the DC detection method. The HGB detector block measures the hemoglobin concentration using the noncyanide hemoglobin method.

PRINCIPLE:

The Sysmex KX-21N is a quantitative automated hematology analyzer for in vitro diagnostic use for determining 17 hematological parameters. Examination of the numerical and/or morphologic findings of the complete blood count are useful in diagnosis of such disease states as anemias, leukemias, allergic reactions, viral, bacterial, and parasitic infections. The Sysmex KX-21N analyzer directly measures the WBC, RBC, HGB, HCT, PLT, LYM#, MIXED# and NEUT#. The remaining parameters are calculated or derived, MCV, MCH, MCHC, MPV, RDW-CV and RDW-SD, and differential percentages LYM%, MIXED%, NEUT%. The KX-21N counts and sizes red blood cells (RBC) and platelets (PLT) using electronic resistance detection. Hematocrit (HCT) is measured as the ratio of the total RBC volume to whole blood using cumulative pulse height detection. Hemoglobin (HGB) is converted to methemoglobin, and read photometrically at 555 nm. Blood cells (WBC) are analyzed by direct current and discriminated into a three-part differential using Particle Distribution Analysis (PDA). The resulting WBC histogram is discriminated into lymphocyte, neutrophil and mixed cell populations. The mixed cell population contains monocytes, basophils and eosinophils.

SPECIMEN:

1. Required specimen: Whole blood anticoagulated with EDTA preferred.
2. Specimen volumes required: Optimal draw is a tube drawn to capacity. The collection tube

should be filled to a minimum of one-half full for acceptable results. An EDTA micro-container filled above the 250 uL line is adequate for testing in the whole blood mode.

3. Unacceptable specimens including those listed below must be redrawn:

a. Clotted samples or those containing clots, fibrin strands, or platelet clumps. All specimens will be checked visually for obvious clots prior to sampling by the analyzer.

b. Check capillary tubes manually with a toothpick for clots.

c. Grossly hemolyzed samples.

d. Samples drawn above an IV.

4. Characteristics that may affect test results are: lipemia, icterus, and cold agglutinins.

Reagent Storage Conditions

Sysmex KX-21 diluent 5 - 30ºC

Stromatolyser- KX 21 5 - 30ºC

CellClean (detergent) 1 - 30ºC

Procedures:

1. Check to see that the reagents needed for the number of the samples to be processed for the day are available.

2. Turn ON the power switch on the right side of the unit. Self-check, auto rinse, and background check will be automatically performed, and the "Ready" (ready for analysis) will appear.

3. When auto rinse and background check are normally completed, "Ready" is displayed. 4. Perform quality control analysis on 3 levels of control blood material (low, normal and high) to verify that the instrument is performing within the specified ranges of the quality control material.

5. If the result of quality control in acceptable range input your blood samples.

6. Input from the panel keyboard.

7. Press [SAMPLE No.] key in the Ready status.

8. Entering patient ID, sample ID, Patient name, etc

9. Press [ENTER] key, This will fix the sample No. and the status becomes ready for analysis.

10. Mix the sample sufficiently before analysis.

11. Remove the plug while taking care not to allow blood scatter.

12. Set the tube to the sample probe, and in that condition, press the start switch.

13. when the LCD screen displays "Analyzing," remove the tube.

14. After that, the unit executes automatic analysis and displays the result on the LCD screen.
15. Then the unit turns to the ready status, becoming ready for analysis of the next samples.

Quality control procedures:

1. At the beginning of each work shift, all parameters are tested with blood control.

2. The 3 levels include: Abnormal Low, Normal, Abnormal High

3. Controls are stored at 2-8ºC and brought to room temperature on a roller mixer before use .

4. Controls are gently inverted eight times according to the manufacturer's instruction before use.

5. From the RUN screen, press [SPECIMEN TYPE].

6. Use the arrow key on the keyboard to move the cursor to the appropriate QC file (i.e., low, normal or high) and press the [QC SPECIMEN] key. 9.

Control values must be within three standard deviations, otherwise the measurement has to be repeated, if the control still out of range:

a. Check operation of the machine, ensuring it is clean and that all required supplies are present in sufficient quantities.

b. Check reagents for expiration dates and lot numbers. Ensure that all machine lines are in appropriate receptacle where applicable, If this does not solve the problem: Contact Medical Maintenance where applicable, or servicing engineer.

7. All control data are managed using software that provides graphical reports (LeveyJennings graphs, and monthly cumulative histograms).

8. Dilute the sample if White blood cell counts ≥100,000 /mm3 and platelet counts ≥1,000,000 /mm3 are outside the linearity specifications of the instrument.

Annex X.Consent form for adults (≥18 years)(18 ዓመት እና ከዚያ በላይ ለሆኑ አዋቂዎች የስምምነት ቅፅ)

ከላይየተገለፀውንመረጃአንብቤአለሁ

/ወይምተነበልኛል።ጥያቄለመጠየቅዕድልተሰጥቶኝጠይቄበሚያረካመልኩተመልሰልኛል።በዚህጥናትለመሳ

ተፍበፈቃደኝነትተስማምቻለሁ።

የዓይነምድርናሙናለመስጠት☐

የሽንትናሙናለመስጠት☐

ደምለመቀዳት ☐

እናበዚህጥናትተሳታፊለመሆን፣በማንኛውምሰዓትከጥናቱለመውጣትመብትእንዳለኝምተረድቻለ

ሁ☐.

የተሳታፊስም፣ቀንእናፈርማ (ወይምአሻራ) ከዚህበታችይፃፉ

_____ (ቀን/ወር/ዓመተምህረት)

ያልተማሩከሆኑ;

የተማሩገለልተኛእማኝሰውስም፣ቀንናፊርማ(ከተቻለይህሰውበተሳታፊውቢመረጥናከተመራማሪአባላትግኑ

ኝነትየሌለውቢሆን)

_____ (dd/mm/yy) _____

ስልክቁጥር_____

የተመራማሪውስም፣ቀንናፊርማ

_____ (dd/mm/yy) _____

Annex XIII. Questionnaire

Questionnaires to be filled by health professionals

Part I. General information

Code Number_____ Region _____ Zone _____

Woreda_____ / city /_sub city _____ Kebele _____

Part II. Personal information

Age (in years)_____

Sex _____

Place of Birth_____

For how long (years) did you live in the birth place?_____

How long do you live in this specific area? (If different from the birth place) _____years

No.	Questions	Responses
	Part III. SOCIO-DEMOGRAPHIC INFORMATION	
	Educational status	Illiterate Read and write Primary (1-8) Secondary (9-12) College diploma/degree and above

6.	Occupation	Student House wife Government employee Private employee Farmer Others (specify) _____
	Marital status	Single Married Divorced Widowed Not applicable (children)
	Religion	Orthodox Christian Muslim Protestant Catholic Others (Specify) _____
	Ethnicity	_____If mixed, specify_ _____
	Residence	Rural

		Urban
	Questions 7-12 are additional questions to Students	
	Father's Age	_____
	Mother's Age	_____
	Father's Educational Level	Illiterate Read and write Primary (1-8) Secondary (9-12) College diploma/degree and above
	Mother's Educational Level	_____
	Father's Occupation	
	Mother's Occupation	
	Monthly income (in birr collected from salary, rent, and other income)	_____Birr
	Family Size (Number of People)	_____
	Source of water	Pipe Spring water Well water River

		Other sources (specify)
	Type of house	Mud Cement Wood Bricks others/specify_____
	Presence of or contact with Pet animals (e.g. Cat, Dog)	Yes No
	Presence of domestic animals	Yes No
	Part IV. Clinical information	
	Questions 24-28 for female participant who are pregnant specify	
	Gestation _____(weeks)	
	Parity _____	
	Iron supplementation:	Yes No
	Folate supplementation	Yes No
	Iron and folate combined supplementation	Yes No
	Did you take any type of drug for any illness for the last three month?	Yes No
	If yes to Q29, what type of drug? (more than one answer possible)	Anti-protozoa Anti-helminthic Anti-allergy

		Birth control pills Anti-bacterial Anti-TB Other (specify) _____
	History of common diseases	
	History of diabetes	Yes No
	History of Hypertension	Yes No
	History of Blood transfusion for the last 1 year	Yes No
	Any history of blood transfusion	Yes No
	History of Hospital Admission for the last 1 year	Yes No
	History of Surgical procedure for the last three years?	Yes No
	History of chronic gastritis	Yes No
	History of Malaria for the last 6 month	Yes No
	History of TB for the last two years	Yes No
	History of Cancer	Yes No
	History of Cardiac illness	Yes No
	History of Bleeding disorders	Yes No

	History of allergy	Yes No
	History of Wheezing	Yes No

Part V. Nutritional habit and your life style

How often do you eat the following food? (put a "√ " mark)							
No.	**Food type**	**A Once/day**	**B More than Once/ day**	**C 2-3 times/week**	**D Occasionally (e.g holidays, special ceremonies)**	**E Never**	**Remarks**
	Roots and Tuber (Potato, sweet potato, Enset, Cassava)						
	Legumes (Beans, peas, chicken pea, etc)						
	Cereals (Corn, Teff, Wheat, sorghum, etc)						
	Vegetables (Tomato, cabbage, etc)						
	Fruits (Orange, banana, etc)						

	Meat (including poultry, fish, etc)						
	Milk and Milk products (Butter, yoghurt, cheese, etc)						
	Egg						
	Tea and/or coffee						
How frequent do you consume/use the following (put a √ mark)							
		Once/day (Regular)	More than once/day	2-3 times/week	Once a week	Occasionally (holiday, special ceremony)	Never
	Alcohol						
	Khat						
	Cigarettes						

	Part V. Life style/Habit Continued…	
	Do you have Fasting habit?	Yes No
	If Yes, How is your fasting habit?	Eating vegetable food only Complete abstinence from food then eating all kinds of food Complete abstinence from food then eating

		vegetable food only
	Did you eat undercooked/raw meat?	Yes No
	Do you have the habit of physical Exercise?	Yes No
	If yes, how many times do you do the exercise per week?	
	Any sexual contact	Yes No Not applicable (children)
	If yes to Q45, condom use`	Yes No
	Part VI. Anthropometric measurement	
	Height (in cm)	
	Weight (in kg)	
	MUAC	_____in cm (will be interpreted later)
	Blood pressure (mm Hg)	

We thank you for your cooperation!

Interview Date:_____

Interviewer's Name_____ Signature_____

Annex XIV: Questionnaire Amharic version (ቃለመጠይቅ)

በጤናባለሞያዎችየሚሞላቃለመጠይቅ

መመሪያ፡

በቅድሚያይህንንቃለመጠይቅለመሙላትለሰጡንጊዜናትብብርአድናቆቴንእገልፃለሁ።የዚህቃለመጠይቅአላማ

“በላቦራቶሪውስጥየጤናማሰውደምውስጥየሚገኙየሄማቶሎጂምርመራዎችመጠንሪፈረንስኢንተርቫልእድሜአቸው 18 ከዚያበላይለሆኑኢትዬጵያውያንለመስራት“ መረጃለመሰብሰብነው።የዚህጥናትሃሳቡንያመጡትየጥናቱዋናተመራማሪበአዲስአበባዩኒቨርስቲየህክምናላቦራቶሪትምህርትክፍልማስተርስተመራቂየሆነውአዲሱነዱሲሆንአዲስአበባዩኒቨርሲቲጤናሳይንስኮሌጅየህክምናላቦራቶሪት/ክፍልያስተዳድረዋል።የጥናቱንወጪየሸፈነውአአዩጤናሳይንስኮሌጅነው።ስለሆነምየእርስዎቅንትክክለኛመልስበሰዓቱመስጠትየዚህንጥናትስኬትይወስናል።የአርባምንጭዩኒቨርሲቲየህክምናላቦራቶሪተ/ክፍልእናሪጅናልላቦራቶሪጥናቱንለመደገፍዝግጁነታቸውንገልፀዋል።ስለሆነምይህንንቃለመጠይቅሃላቀኝነትናሃላፊነትበተሞላውመንገድእንዲሞሉበትህትናእጠይቃለሁ።

አመሰግናለሁ!!!

ክፍል 1.አጠቃላይመረጃ

ኮድ_____ ክልል _____ ዞን_____

ወረዳ_____ ከተማ/ክፍለከተማ _____ቀበሌ _____

ክፍል 2.የግልመረጃ

እድሜ_____

ጸታ_____

የትውልድቦታ_____

በትውልድቦታዎለምንያህልጊዜኖረዋል?_____

አሁንያሉበትቦታለምንያህልጊዜኖረዋል? (ከትውልድቦታዎየተለየከሆነ)_____ዓመት

ክፍል 3.ማህበራዊናኢኮኖሚያዊመረጃ

ቁጥር.	ጥያቄ	ምላሽ
	የትምህርትደረጃ	ያልተማሩ ማበብናመፃፍ አንደኛደረጃ (1-8) ሁለተኛደረጃ (9-12) ኮሌጅዲፕሎማ/ዲግሪእናከዚያበላይ
	ሥራ	ተማሪ የቤትእመቤት የመንግስትሠራተኛ የግልተቀጣሪ ገበሬ ሌላካለይግለፁ _____
	የጋብቻሁኔታ	ያላገቡ ያገቡ የተፋቱ ባል/ሚስትየሞተባቸው አይመለከታቸውም (ህፃናት)
	ሃይማኖት	ኦርቶዶክስክርስቲያን ሙስሊም ፕሮቴስታንት ካቶሊክ ሌላካለይግለፁ _____
	ብሄረሰብ	_____ድብልቅከሆኑይግለፁ

	መኖሪያቦታ	ገጠርከተማ
	ጥያቄ 7-12 ለተማሪዎችተጨማሪጥያቄዎች	
	የአባትእድሜ	
	የእናትእድሜ	
	የአባትየትምህርትደረጃ	ያልተማሩ ማንበብናመፃፍ አንደኛደረጃ (1-8) ሁለተኛደረጃ (9-12) ኮሌጅዲፕሎማ/ዲግሪእናከዚያበላይ
	የእናትየትምህርትደረጃ (ከተ/ቁ 14 ይምረጡ)	
	የአባትሥራ	ተማሪ የቤትእመቤት የመንግስትሠራተኛ የግልተቀጣሪ ገበሬ ሌላካለይግለፁ
	የእናትሥራ (ከተ/ቁ 16 ይምረጡ)	
	ወሃዊገቢ (በብርከደሞዝ፣ኪራይ፣እናሌሎችገቢዎች)	_____ብር
	የቤተሰብብዛት	
	የውሃምንጭ	ቧንቧ

		የምንጭ የጉድጓድ የወንዝ ሌላካለይግለፁ _____
	የቤትአይነት	ጭቃ ሲሚንቶ እንጨትጡብ/ሸክላ ሌላካለይግለፁ
	የቤትውስጥለማዳእንስሳመኖርወይምንክኪ (ለምሳሌድመት፣ውሻ)	አለየለም
	የቤትእንስሳትመኖር	አለየለም
	ክፍል 4. የጤናመረጃ	
	ከ 24-28 ያሉትጥያቄዎችለነፍሰጡርሴቶችብቻነው	
	ከፀነሱስንትጊዜዎነው?	_____(ሳምንት)
	ለስንተኛጊዜነውየፀነሱት?	
	ተጨማሪብረትንጥረነገር	አዎንየለም
	ተጨማሪፎሌትንጥረነገር	አዎንየለም
	ተጨማሪየብረትንጥረነገርናፎሌት	አዎንየለም
	ባፉትሶስትወራለማንኛውምዓይነትህመምማንኛውን ምዓይነትመድሃኒትወስደኋል?	አዎንየለም

	ለተራቁጥር 29 መልስዎወስጃለሁከሆነየትኛውንዓይነትመድሃኒትነው ወሰዱት? (ከአንድበላይመልስይቻላል)	ፀረ-ፕሮቶዞአ ፀረ-ሄልሚንትስ ፀረ-አለርጂ የወሊድመከላከያኪኒን ፀረ-ባክቴሪያ ፀረ-ቲቢ ሌላካለይግለፁ _____
	የሚከተሉትየህመምዓይነቶችአሞዎትያውቃል?	
	የስኪርህመም?	አዎንየለም
	የደምግፊትከፍማለት?	አዎንየለም
	ባለፈው 1 ዓመትደምተሰጥቶዎያውቃል?	አዎንየለም
	ማንኛውምጊዜደምተሰጥቶዎያውቃል?	አዎንየለም
	ባለፈው 1 ዓመትሆስፒታልተኝተውያውቃሉ?	አዎንየለም
	ባለፉት 3 ዓመታትየቀዶህክምናተደርጎልዎያውቃል?	አዎንየለም
	የቆየየጨጓራህመምአለብዎት?	አዎንየለም
	ባፉት 6 ወራትየወባህመምአጋጥሞዎትያውቃል?	አዎንየለም
	ባለፉት 2 ዓመታትየቲቢህመምኖሮዎትያውቃል?	አዎንየለም
	ካንሰርህመም	አዎንየለም
	የልብህመም	አዎንየለም

	የመድማትችግር/ህመም	አዎንየለም
	አለርጂ (የሰውነትመቆጣት)	አዎንየለም
	የመተንፈስችግር (ሲተነፍሱሲርሲርየሚልድምፅ)	አዎንየለም

ክፍል 5.የአመጋገብእናየህይወትልምድዎ

የሚከተሉትንየምግብዓይነቶችምንያህልጊዜይመገቢቸዋል ? (“√ “ ይህንምልክትያስቀምጡ)							
ተ/ቁ	የምግብዓይነት	1 በቀንአን ድጊዜ	2 በቀንከአንድ ጊዜበላይ	3 በሳምንትከ 2 እስከ 3 ጊዜ	4 አልፎአልፎ(ለምሳሌ፣ለበዓል፣ልዩ ዝግጅቶችሲኖሩ)	5 ተጠቅሜአላውቅም	ማብራሪያ
	ሥራሥር (ድንች፤ስኳርድንች፣እንሰት፣ካሳቫወዘተ)						
	አባዝርት (Legumes፡ባቄል፣አተር፣ሽንብራወዘተ)						
	ጥራጥሬ (በቆሎ፣ጤፍ፣ስንዴ፣ማ						

	ሽላ)						
	አትክልት (ቲማቲም፣ጎመን፣ወዘተ)						
	ፍራፍሬ (ብርትኳን፣ሙዝ፣ወዘተ)						
	ሥጋ (የዶሮ፣የአሳንጨምሮ)						
	ወተትናየወተትተዋፅዎ (እርጎ፣ቅቤ፣አይብ፣ወዘተ)						
	እንቁላል						
	ሻይእና/ወይምቡና						
የሚከተሉትንምንያህልይበላሉ/ይጠቀማሉ (√ይህንምልክትያስቀምጡ)							
		በቀንአንድ ጊዜ (ሁልጊዜ)	በቀንከ1 ጊዜበላይ	በሳምንትከ 2 እስከ 3 ጊዜ	በሳምንት 1 ቀን	አልፎአልፎ (ለምሳሌ፣ለበዓል፣ልዩዝግጅቶችሲኖሩ)	ተጠቅሜ አላውቅም
	አልኮል						

	ጫት						
	ሲጋራ						

	ከክፍል 5 የቀጠለ. የህይወትአመራርናልምዶች	
	የመፅምልምድአለዎት?	አዎንየለም
	መልስዎአዎንከሆነ፣የመፅምልምድዎእንዴትነው?	አትክልቶችንብቻመመገብ በአጠቃላይከምግብመታቀብከዚያምያገኙትንመመገብ በአጠቃላይከምግብመታቀብከዚያምአትክልቶችንመ መገብ
	በደንብያልበሰለወይምጥሬሥጋይመገባሉ?	አዎንየለም
	የሰውነትእንቅስቃሴየማድረግልምድአለዎት?	አዎንየለም
	መልስዎአለኝከሆነበሳምንትለምንያህልጊዜይንቀሳቀሳ ሉ?	
	የግብረሥጋግኙነትአድርገውያውቃሉ	አዎንየለም አይመለከትም (ለህፃናት)
	ለተ/ቁ 66 መልስዎአዎንከሆነ፣ኮንዶምይጠቀማሉ?`	አዎንየለም
	ክፍል 6. ክብደት፣ቁመት፣የክንድናየደምግፊትልኬት	
	ቁመት	_____ሴንቲሜትር
	ክብደት	_____ኪሎግራም

	የክንድመሃለኛውክፍልዙሪያው (MUAC)	_____ሴንቲሜትር
	የደምግፊት (በሚሊሜትርሜርኩሪ)	_____(mm Hg)

ስለትብብርዎእናመሰግናለን!

ቃለመጠይቅየተደረገበትቀን:_____

ቃለመጠይቁንያካሄደውስም_____ ፊርማ_____

Declaration

I, the undersigned, declwere 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.

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Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: **Aster Tsegaye (MSc, PhD)**

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Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: **JemalAlemu (MSc, PhD candidate)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

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