ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Establishment of Lipid Profile and Major Electrolytes Reference Interval in Apparently Healthy Adults and Pregnant Women in Addis Ababa, Ethiopia.

By: Fikirte Aboneh

Advisors: Samuel Kindie (MSc, PhD Candidate)

Aster Tsegaye (MSc, PhD)

A research thesis Submitted to the Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University, in Partial Fulfillment of Master of Science in Clinical Laboratory Sciences (Clinical Chemistry).

June 2022

Addis Ababa, Ethiopia

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by Fikirte Aboneh entitled: Establishment of lipid profile and electrolytes reference intervals in apparently healthy adults and pregnant women in Addis Ababa, Ethiopia and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Clinical Chemistry) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

Examiner_____ Signature _____Date _____

Examiner_____ Signature _____Date _____

Advisor_____ Signature _____Date _____

Advisor_____ Signature _____Date _____

_____

Chairman of the Department or Graduate Program Coordinator

Acknowledgment

This study would not have been possible without sustainable support and guidance from my advisors Mr Samuel Kinde and Dr. Aster Tsegaye, thus my first and biggest appreciation goes to them. In addition, my special thanks go to the Department of Medical Laboratory Sciences of the College of Health Sciences, Addis Ababa University to support me in conducting the MSc program. The former Ministry of Science and Technology (MOST) current Ministry of Innovation and Technology (MiNT) for financing this study is gratefully acknowledged. I would also like to thank EPHI for allowing us to use the equipment and clinical chemistry staff members for their patience and support during laboratory analysis. I am very grateful to the health centers, health extension workers and those who have served us as a source of information during the collection, facility and their staff for their holistic support during data collection period. I would like to recognize adults of Addis Ababa for their willingness to participate in this study, Federal Ministry of Health, Addis Ababa Health Bureau and heads of the respective health facility for facilitating the study. The Central Statistical Agency is also acknowledged for selecting the sub cities based on their population size.

Further, yet importantly, a sense of respect goes to my friends for their guidance and comment during study. In addition, I would like to give my special thanks to Arsi University for the full sponsorship opportunity. Finally, my greatest respect goes to my family especially my husband Esubalew Demissie for their regular encouragement and support in every step of the study.

Abbreviations

CLSI	Clinical and Laboratory Standards Institute
EPHI	Ethiopian Public Health Institute
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HDL	High-Density Lipoprotein
HIV	Human immunodeficiency virus
IFCC	International Federation of Clinical Chemistry
ISE	Ion-Selective Electrode
ISO	International Organization for Standardization
K	Potassium
LDL	Low-Density Lipoprotein
Na	Sodium
Cl	Chloride
PPS	Probability proportional to size
RI	Reference Interval
TC	Total Cholesterol
TG	Triglycerides
WHO	World health organization
MRA	Multiple regression analysis
CHD	Coronary heart disease
ANC	Anti natal care

BMI	Body mass index
%OOR	percentage out of range
CI	Confidence interval
RMP	Reference measurement procedure
C-RIDL	Reference Intervals and Decision Limits
RV	Reference value
NCD	Non communicable disease

Table of Contents

List of figures

List of tables

Abstract

Background: Reference Interval is value obtained by measurement of a particular type of quantity on an individual belonging to a reference group. It is very important in clinical management of patients, maternity follow-up, and monitoring of clinical trials before onset of serious adverse events. In Ethiopia, similar to most developing country laboratory test results are interpreted by using reference intervals which developed for western population .Different factors make the application of western RI during the interpretation of laboratory result leads to unnecessary exclusion of eligible participants, contribute to over reporting of adverse events and increase the number of referrals for clinical investigations. Despite its significance, there have few efforts to establish these tools. Thus, the current study aimed to address the gaps in serum lipid profile and selected electrolytes reference intervals by determining variability with gender among adults and pregnant women in Addis Ababa.

Objective: To establish lipid profile (TC, TG, HDL-C, LDL-C) and selected electrolytes (Na, K, Cl) reference interval for apparently healthy adults and pregnant women in Addis Ababa, Ethiopia.

Methods: The present community cross-sectional study was conducted from March 1 to June 30, 2020. A total of 522 individuals with age of ≥18 years and pregnant women ≥15 years that lived in the city of Addis Ababa were recruited. After demographic information and a brief medical history of the adults were collected by using questionnaires, important physical examination, and anthropometric measurement were done. Serum lipid profile and selected electrolytes values were determined using Cobas c501 chemistry analyzer. SPSS version 23 and Med Calc ver 20.008 software were used for reference limits determination for adults and pregnant women.

Results: Established reference interval for adult female and male adult were; TC [(120.4-272) and (100.1-243) mg/dl], TG [(51-164) and (46.5-170)mg/dl]; HDL-c [(25-57) and (16-52.7)mg/dl], LDL-[(51-170) and (35-162)mg/dl]; Na [(138-149)and (138-150)mmo/l]; K[(3.9-5.6)and(3.9-5.5) mmol/L] and Cl [(101-112)and (99.14-108) mmol/l]. For pregnant women established RI of lipids and major electrolyte were TC(118-275) mg/dl, TG (64 -304) mg/dl, HDL (22-62.5)mg/dl, LDL(47.8-167)mg/dl, Na(136-143) mmo/l, K(3.65-5.1) mmol/L,Cl (100.5-108) mmol/l.

. Also statistically significant difference was seen for TC, TG, HDL, LDL and Cl between male and female and for TC, TG, Na and K between adult females and pregnant women.

Conclusion: The current study established RI to be used for adults and pregnant women of Addis Ababa. There was a difference between the present established reference interval and other reported studies. Thus high or low value seen for lipids (TC,TG and LDL) and electrolyte. Also the value of HDL-c was very low. Significant differences were also seen between genders for most of the analyte, which alerts for further large scale studies, and need careful verification of test kit manufactures' given values before directly used for the desired purpose.

Key words: Adult, Reference Intervals, Lipid profile, Electrolyte, Female, Male, Pregnant.

1. Introduction

1.1 Background

Lipids are class of compound that are soluble in organic solvents, but nearly insoluble in water. Chemically, lipids contain primarily nonpolar carbon-hydrogen (C-H) bonds and typically yield fatty acids and or complex alcohols after hydrolysis. Thus, include cholesterol, triglyceride, phospholipids and cholesterylesterase (1). In the body lipids are transported through lipoproteins thus, lipoproteins constitute the body's "petroleum industry." Chylomicron, Very-low-density lipoprotein (VLDL), low density lipoprotein (LDL), high density lipoprotein (HDL) are the main lipoproteins that transport lipids throughout the body. Lipids and lipoproteins, which are central to the energy metabolism of the body, have become increasingly important in clinical practice, primarily because of their association with coronary heart disease (CHD) through derangements of one or more of the lipoproteins in blood, such as elevated in total cholesterol (TC), LDL and/or triglycerides (TG), or low levels of HDL (2).

Physiologically electrolytes are ions capable of carrying an electric charge in the human body. They are classified as anions or cations based on the type of charge that they carry. In the body, they play a role in conducting nervous impulses, contracting muscles, keeping the body hydrated and regulating body PH levels. While commonly, electrolytes encompass Potassium (K), Sodium (Na) and Chloride (Cl). Sodium, which is osmotically active cation, is one of the most important electrolytes in the extracellular fluid. It is responsible for maintaining the extracellular fluid volume, and for regulation of the membrane potential of cells (3). Intracellularcation, which plays a great role in cardiac function, is potassium. Its disorder may result in arrhythmias, weakness, fatigue, and muscle twitching muscle cramps (4). Chloride, which is extracellular anion mainly, found in association to Na. At hyper level may result in gastrointestinal bicarbonate loss and congestive heart failure at hypo level (5).

Interpretation of the laboratory test results with appropriate diagnostic accuracy requires reference range. Through this laboratory scientist aid the clinician in the interpretation of physical examination and laboratory test result (6). Thus, a reference interval (RI) is the value obtained by observation or measurement of a particular type of quantity on an individual belonging to a reference group. Theory of reference intervals was developed and published

between 1987 and 1991 by an International Federation of Clinical Chemistry (IFCC) to avoid the problems with normal values and values obtained from an individual under clinical investigations (7). In the same year Ceriotti, defined RI as “an interval that, when applied to the population serviced by the laboratory correctly, includes most of the subjects with characteristics similar to the reference group and excludes the others”. (8)

To develop a reference interval for a laboratory, a minimum of 120 samples should be collected from the normal individuals for analysis (9). No RI is completely right or wrong (IFCC) (7). Thus, reference intervals comprise the central segment with 95% of the reference values from the reference sample group and the limits of the reference interval are defined according to the use of the laboratory marker. If increased and decreased values are diagnostically relevant, the 2.5% and 97.5% percentiles are applied (7).

As defined by International Organization for Standardization (ISO)(15189) it is the responsibility of individual laboratories or laboratory networks to use reference intervals that are appropriate for their methodologies and the population they serve (10).Thus, well-defined reference population with exclusion of diseased subjects, proper portioning of sex and age, optimum control of pre analytical and analytical variables, identifying proper method to estimate reference population are the main that the laboratories should focus during the development reference interval. ISO 15189 also recommended that reference intervals should be updated periodically and when there is change in method analysis, pre analytical and analytical procedure (10).

During pregnancy the pregnant woman undergoes significant anatomical and physiological changes in order to nurture and accommodate the developing fetus. These changes begin after conception and affect every organ system in the body this is due to serum progesterone and estrogen concentrations increase progressively and reach pick during late pregnancy. There is an increase in serum lipids thus level of TC and TG increased significantly during pregnancy. The increased its synthesis by the liver and decreased lipoprotein lipase activity result in increment of TG. LDL levels also increase and reach 50% at term. High-density lipoprotein levels also increase in the first half of pregnancy and fall in the third trimester but concentrations are 15% higher than non-pregnant women. Alternation in electrolyte value is correlate well with those of estrogen hormone increment which lead to activation of the RAA system result in increased plasma levels of aldosterone and subsequent NaCl and water retention in the distal tubule and collecting duct. Also K is constant throughout pregnancy due to changes in tubular reabsorption

while total body K increases during pregnancy. For many commonly used laboratory assays, reference intervals based on a population of healthy pregnant women importance for proper interpretation of laboratory results during pregnancy. Therefore, it is advisable to establish reference intervals for lipid profile and electrolyte (11).

In spite of its significance in Ethiopia, similar to most developing country laboratory test results are interpreted by using reference intervals which have been obtained from literature, reagent inserts accompanying the reagent kits or Instrument manuals; those all are derived from western countries (12,13). While factors such as age, gender, ethnicity and environment including altitude and geo-chemicals make reference interval that are derived from western countries not appropriately applicable locally because this makes the health of an individual conceptually different in different countries' populations. Therefore, the present study aimed to address the gaps in serum lipid profile and selected electrolytes reference intervals by determining variability with gender among adults and pregnant women in Addis Ababa

1.2 Statement of the problem

Serum lipids levels are much dependent upon genetic background, ethnicity and dietary pattern of a particular population. Thus using RIs established elsewhere may not be appropriate to classify laboratory test results as normal or abnormal. For example CHD is the leading causes of mortality and morbidity throughout the world population. The incidence of coronary artery disease increases with advancing age in men and in postmenopausal women while recently the prevalence of these disorders is also reported at early adult's age (14). Relationship of lipids and other risk factors with CHD are established in different literature; thus, lipid profile variables could predict major cardiovascular outcomes and all-cause mortality in patients with CHD. As indicated earlier including our country, most of the laboratory and clinicians use the reference intervals from western study, which usually does not match with local population especially in case of lipid profile and electrolyte (14).

Studies conducted in Ghana show that there is huge difference in reference interval between Western population and African for the majority analyte including lipid profile and electrolytes (12). Several related studies reported that laboratory parameters test results vary as the health of an individual is not the same in different countries, in the same country in different regions, at different times and in the same individual at different ages. Reference interval developed for the Zimbabwe adult population showed that the value of TC, HDL cholesterol and LDL cholesterol levels were higher when compared with results from Tanzanian and Kenyan studies (15). A study conducted for the Russian population show that there was a huge difference seen for the reference interval of lipid to India, China, Asia, Turkey, and Saudi population (16). Additionally, significant regional differences were seen for the adult population of china, thus Hangzhou Na reference limit was higher than Beijing, Guangzhou, Urumqi and Chongqing region of China for both sexes (17).

In 2021, the adult population of Ethiopia accounted for around 56.9% of the total population. As a developing country, the adult population plays a great role in the sustainability of the country and the existence of the remaining population will be on the hand of this population group. Therefore, the health system of the country should struggle to offer quality service to the population. While the main health problem of Ethiopia was communicable disease, recent reports suggest that mortality rate of the adult population due to ischemic heart disease and

cerebrovascular disease rose (18). Thus, non-communicable diseases cause 42% of deaths, of which 27% are premature deaths before 70 years of age. As studies predict the future status of the country with no action, Ethiopia will be the first among the most populous nations in Africa to experience dramatic burden of premature deaths and disability from NCD by 2040 (18).

While Pregnancy is a natural gift to human, it causes physiological, anatomical and biochemical change in the female body. Thus, changes of pregnancy may mask symptoms and signs as well as the pregnancy itself being the source of the problem (19). As study show that around 15% of all pregnant women will develop a potentially life-threatening complication that calls for health care system to focus on the maternity health (20). Nowadays there is change in the laboratories through developing reference interval for their population by portioning through age group and sex. While including western laboratory most of world population lack reference interval for pregnant women. Without adequate reference intervals, there is both an increased risk of missing important changes due to pathological conditions and to erroneously interpret normal changes as a pathological event (19, 20). This and other interrelated factors result in rising pregnancy and childbirth complications thus mask economic growth of our country.

There are many stairs to develop reference intervals that represent the local population. It is time consuming, costly and demands a lot of resources and a volunteer population. To compensate for this and other difficulty transference and validation of reference intervals that are provided by manufacturer leaflet is relatively simple and economical also it is the most commonly adopted alternative. However, this may be non-representative for the general population, which leads to wellness of healthy population under risk, and exclude unhealthy population from treatment (12). Furthermore, laboratory test results influenced by several factors mainly by gender, age, sample type, pre analytical variables, analytical procedures, instruments and geographical location of the healthy individuals (7). Consequently, utilizing improper clinical reference interval result in rise number of incorrect medical decisions which leading to increased cost and unnecessary investigations and risk in patient safety. The above-interrelated factors suggested that there is a need for the development of locally derived reference values, which portioned through sex and age group for clinical parameters (lipid profile and electrolytes) to improve clinical care and for monitoring participant in clinical trials. Likewise, as our country is part of a national program,

which focuses on maternal health, the Ethiopian laboratory should develop a reference interval for pregnant women.

1.3 Significance of the study

This study can create a good environment for qualified laboratory service. For physicians the result of this study will create a common decision support tool for interpretation of numerical pathology reports beside this local population will receive adequate service without persecution and referral. Policy makers will receive appropriate and reliable information on health related issues to make decisions, which are important to create a healthy population. The current study also aids other laboratories by providing reference intervals, which developed through direct methods to transfer and validate for their population. Also this study focuses on the capital city of the Ethiopian population; regional laboratories will be motivated by results and will strive to develop reference intervals for their local population.

2. Literature review

The Clinical and Laboratory Standards Institute (CLSI) published and updated a guideline for laboratories and manufactures to perform their own reference interval. Also IFCC recently published 2 papers including a protocol and standard operation procedures (SOPs) for multicenter RI studies. As indicated by CLSI the best means to establish reference interval is by collecting sufficient number of qualified reference individual while in fact only few laboratory even manufacturer develop reference interval by direct method. Because every laboratory is more than capable of verifying the applicability of reference interval to its own population .To minimize the problem related to the establishment of RI CLSI recommended transformation and verification of RI established elsewhere is much less formidable task. Currently there is an improvement in number of laboratory that the develop RI based on the recommended protocol that represents the local population.

The study conducted in 2016 on 3148 apparently healthy volunteers in 6 cities of China by Liangyu Xia, et al. reported RIs for TG 47.17 -305.3 mg/dl and 40.94-202.92 mg/dl; for TC 124.41-240.24 and 12 mg/dl 1.7-267.93; for HDL 31.2-76 mg/dl and 35.5-85 mg/dl, and for LDL 52-149 mg/dl and 48-172.77 mg/dl for male and female, respectively. On the other hand, electrolyte reference intervals which developed for the study population were; 136-144 mmol/l, 3.7-4.7 mmol/l and 101-109 mmol/l for Na, K and Cl, respectively (17).

The study also revealed that LDL changed significantly with age in both sexes while TG increased with age only in females. In males, the level of lipids increased mainly until the age of 40, whereas in females, the increase occurred more prominently after 40 years of age. The level of standard deviations ratio for sex (SDRs) was significantly high (0.3) for TG. In addition multiple regression analysis (MRA) results revealed that test results of TG, TC, HDL and LDL in males were related to BMI. Of all lipids, only HDL-C decreased with the increase of BMI, although the findings show that no appreciable changes in test results in association with exercise level were noted in any analyte in both sexes (14). On the other hand, from the study we can understand that the UL (upper limit) of some analytes decreased significantly when stricter secondary exclusion criteria were applied. This was seen for TG, TC, HDL and LDL in females. And in males, only TG and TC were included (17).

Other study that was done in Kintampo north municipality and Kintampo south district of the Brong Ahafo region of Ghana establish RI for 22 clinical chemistry parameters in 2012. A total of 691 randomly selected adults made up of 351 males and 340 females between the ages of 18 and 59 years were included in the study. The study found RI for male and female for total Cholesterol (70-195 mg/dl) and (78- 210 mg/dl), Triglyceride(35.6-195 mg/dl) and (35.6 -196 mg/dl) also for electrolyte; Cl (101–115 (mmol/l) and (102–114 mmol/l), Na (135–151 mmol/l) and (135–150 mmol/l) and K (3.6–5.2 mmol/l and 3.4–5.1 mmol/l, respectively. As the study showed that majority of the clinical chemistry tests were also within the precision limits indicated by the reagent producer. On the other hand, females had significantly higher Cholesterol levels of 2.1–5.6 against 70-195 mg/dl for males (p value 0.0001). The column percentage out of range (% OOR) value of Cholesterol (mg/dl) and Triglycerides (mg/dl) were 27.6 % ,1.9% and 6.4 %, 6.2 % for male and female, respectively, though for electrolyte % OOR was not available (NA) (21).

Comprehensive reference interval for Hematology and Clinical Chemistry Laboratory Parameters developed for Nigerian Adults population recruited a total of 428 voluntary non-remunerated blood donors and antenatal care (ANC) attending women between the ages of 18 and 65 years. The finding showed that comparison by gender indicated no significant difference for sodium, potassium and chloride ($p>0.05$). However statistically significant difference was seen between sex for lipids. The result of this study also revealed that pregnant women reference interval of electrolyte were; Na [134 – 138, 135 –142.3 and 135–152 mmol /l], K [1.4 -4.4 , 3.9–5.3 and 3.9 –5.7 mmol /l] and Cl [105 -111, 103–115.3 and 111 –127 mmol /l] for first, second and third trimester, respectively. Cumulative RI for Total cholesterol and for Triglyceride was 159 – 175.5 mg/dl and 80 – 115.5 mg/dl, respectively. There were significant differences for lipids and electrolyte parameters between pregnant and non-pregnant women in this study ($p < 0.05$) except for TC which was ($p>0.05$) (22).

Svetlana E *et al*. in 2020 recruited 793 healthy individuals from different regions of Russia with target age range of 18–64 and the ratio of females to males was 53 to 47. In the study RIs were derived by the parametric method after normalizing data using modified Box-Cox power transformation formula and nonparametric method for both sexes and age group also 90% confidence interval (CI) for LL and UL of RIs were calculated based on the bootstrap method

through a random resampling of the same dataset 50 times. When it is necessary to ensure their traceability to the reference measurement procedures (RMP) re-calibration of RVs based on the panel test results using major axis linear regression was performed (16).

The result of this study shows that most of the analytes had positive correlation with age thus the list of analytes with positive rp in its descending order of magnitude for males was Cl, TC, LDL and for females was TC, LDL, Na and TG. BMI also indicated significant positive association with test results for both sex for TG and for LDL-C and TC in male. Reference interval of the analyte developed for the Russia population were; TC (128-297mg/dl), Na (135-141mmol/l), K (3.5-4.5 mmol/l) and Cl (99-107 mmol/l) were for both sexes also TG (37-161 and 40-238 mg/dl), HDL (39-87 and 30-69 mg/dl), LDL (77-189 and 86-229 mg/dl) were for female and male, respectively (16).

The study conducted by Heba B *et al*. in 2021 developed a reference interval for 34 analytes, which include electrolyte, and lipids for the Egyptian population. A total of 691(323 males and 368 females) apparently healthy Egyptians aged 18 to 65 were included in this study. To recruit the participant C-RIDL protocol was applied. The parametric method was used for computing reference intervals after transforming the distribution of RVs into Gaussian form using the modified Box-Cox transformation to obtain mean and SD. The RI was calculated as the mean±1.96SD, which corresponds to the central 95% limits or LL and UL under transformed scale, and then they were reverse-transformed to get the LL and UL on the original scale. Also the bootstrap method was applied to get smoothed lower and upper limits (LL, UL), mean and to predict 90% confidence intervals (CI) for the limits of the reference interval. Conditions affecting nutritional, muscular, and inflammatory markers were excluded by the LAVE method. (23).

As multiple regression analysis (MRA), results reveal that in males, with the advanced age TG was increased. Increasing BMI was associated with an increase in nutritional markers like TC and LDL. In females, age was positively associated with TG, LDL and TC, the association of BMI with nutritional markers was weak for HDL. Related to this between sex differences with significant SDR sex show that SDRsex did not exceed the threshold of 0.4 for TG. Reference interval which was developed in this study was; TC (135-271 mg/dl), LDL (55-185 mg/dl), Na (131-148 mmol/l) K (3.4-5.3 mmol/l), Cl (94-111 mmol/l) was RI for both sexes also TG (36-

311 mg/dl and 44-359 mg/dl), HDL (28-78 mg/dl and 26-71 mg/dl) was for female and male RI, respectively (23).

To our literature search no published study on RI for lipid profile and electrolytes is available for the population of Addis Ababa, the capital city of Ethiopia

3. Objective

3.1. General objective

- To establish lipid profile and major electrolytes reference interval for apparently healthy adults in Addis Ababa, Ethiopia from March 1st to June 30, 2020.

3.2. Specific objective

- To establish lipid profile reference intervals (TC, TG, HDL-C, LDL-C) for apparently healthy adults in Addis Ababa, Ethiopia.
- To establish lipid profile reference intervals for pregnant women in Addis Ababa, Ethiopia.
- To establish major electrolytes (Na, K, Cl) reference intervals for apparently healthy adults in Addis Ababa, Ethiopia.
- To establish major electrolytes reference intervals for pregnant women in Addis Ababa, Ethiopia.

4. Materials and Methods

4.1. Study area

This study was done in four sub cities found in Addis Ababa which include Arada, Akaki Kality, Yeka and Kirkos. Addis Ababa is the capital city of Ethiopia with square meter 527. The total population of the city, which estimated in 2022, was 5,227,794. The city is the diplomatic center of Africa and embodies a 130 years of development history. Its average altitude is 2,400 meter above sea level with constant moderate temperature of roughly 23^{o}C average high and 11^{o}C average low throughout the year. The average annual rainfall is about 1,200 mm, out of which close to 80% falls during the main rainy season. The population of Addis Ababa accounts for 26% of the national population and 18% of the urban population in Ethiopia. Also the population between the ages of 15 to 65 is 72%. Estimate also shows that females are slightly higher than male residents. Percent Life expectancy at birth is 65.7 years and infant mortality rate is 50.3 per 1000 live births. The city was divided into 11 sub-cities called “kifle-ketemas” and 116 woredas, which are the lowest administrative units (24). During the study period there were 10 sub-cities and the 4 were selected with the help of the central statistical agency.

4.2 Study design and period

A community based cross-sectional study design was implemented from March 1st to June 30, 2020.

4.3 Population

4.3.1. Source population

The source population for this study was individuals living in the city of Addis Ababa, with age of 18 years and above and for pregnant women 15 years and above.

4.3.2. Study Population

The study population was people with age of ≥18 and pregnant women ≥15years that live in the city of Addis Ababa and fulfill the eligibility criteria.

4.4. Inclusion criteria and Exclusion criteria

4.4.1. Inclusion criteria

Apparently healthy individuals aged ≥18 and pregnant women aged ≥15 years and lived at least for 5 years in Addis Ababa, Ethiopia were included in the study.

4.4.2. Exclusion criteria

- Individuals with chronic disease like diabetes mellitus, chronic renal insufficiency, hypertension, ischemic heart disease, anemia, thyroid, liver diseases, and cancer of any type.
- Seropositive Individuals for HIV, syphilis, Hepatitis B and C
- Tobacco smoker, alcohol individual, sport men or women.

4.5. Study variables

4.5.1. Dependent variables

- Establishment of reference intervals for lipid profiles.
- Establishment of reference intervals for major electrolytes.

4.5.2. Independent variable

- Sex
- Age
- BMI
- Pregnancy status

4.6. Sample size determination and sampling technique

4.6.1. Sample size determination

CLSI recommends that the best means to establish a reference interval is to collect samples from a sufficient number of reference individuals to yield a minimum of 120 samples for analysis, by non-parametric means for each partition (e.g. sex, age) with a power of 90% (8). As recommended by CLSI and IFCC a minimum of 120 reference individuals from each sample group shall need to estimate a nonparametric reference interval with 90% CI. This number does not consider any losses or deletion of observations. For our study population were partitioned as male 18-60, female 18-60 and pregnant women 15-49. Therefore, three portion groups were needed (3 x 120=360).

According to previous studies in other African countries, in such large scale studies about 30% of the apparently healthy population did not qualify for reference interval determination for various reasons when tested for the common viral infections and syphilis. Considering a 30% exclusion from data analysis, to reach the CLSI recommended total sample size of 360 for the reference interval determination, a total of 630 individuals were enrolled (i.e. 30 % x 360=108 to be excluded during data analysis); thus giving a total maximum sample size of 630. Thus, 630 participants were recruited from four sub-cities of Addis Ababa. The study participants were selected using systematic random sampling by considering woreda as a sampling frame and then households the final selection units.

4.6.2. Sampling technique

Probability Proportional to Size (PPS) sampling method was employed to recruit the participant of the study. Since Addis Ababa is a very large city, four sub-cities were selected based on PPS, namely Arada, Kirkos, Akaki and Yeka sub-cities; thus all woredas under the selected sub-cities were included. To recruit 630 participants, the number of households is determined by dividing the total household in the selected sub-cities by the estimated number of individuals per household. Individuals in every selected household were approached at their households through health extension workers. Once volunteering participants fulfilling the eligibility criteria were identified by the health extension workers, they were invited to go to nearby health facilities for

interviews using structured questionnaires and to facilitate biological sample collection. The Federal Ministry of Health has written letter to the Addis Ababa Health Bureaus and the Central Statistical Agency. The Bureau wrote letter of cooperation to the respective sub-cities .

Table 1: Selected site and their respective sample in 2020.

Selected sub city	**No. Households**	**Individuals per household**	**Total population**	**Number of sample**
Akaki Kality	47021	3.8	178,680	120
Kirkos	54398	4.0	217,592	145
Arada	49564	4.1	203,212	136
Yeka	90195	3.8	342,741	229

4.7. Measurement and Data collection

The study team to the participants explained the study aim, risks, benefits of study participation and right to withdraw from the study at any time. After explanation about responsibility and right of the volunteers, the aim and significance of the study, agreement to be participant were gained. Before they came to the health, facility participants were interviewed about their health status and lifestyle by extension worker. Demographic information and a brief medical history were collected. These procedures aid to be sure whether the participant can continue in the study or not. They were requested to fast overnight about ten to twelve hours before sample collection. Height, Weight and sociodemographic status were collected through questionnaire then physical examination was done by health professionals. Finally, biological samples were collected at 8:30 AM until 11:00 AM at the health facility. Ten (10ml) of blood sample was collected by SST and (EDTA) tube and properly packed in an icebox then samples were transported from health facility to the Department of Medical Laboratory Sciences to separate serum. In addition, blood sample was screened for HIV, HBV, HCV and hemoparasites. Stool sample was collected and direct wet mount examination was performed immediately on site. The leftover sample was preserved for concentration test analysis at the department's laboratory. In addition, Urine

sample was collected by leak proof clean containers then visual, microscopic and dipstick as well as pregnancy test for females was performed. Then leftover samples were stored in Addis Ababa University Department of Medical Laboratory Sciences for future additional analyses, each time going through a new ethical clearance process. All results entered into soft and hard copies for cross checking were sent to the Department of Medical Laboratory Sciences, AAU. Finally, laboratory results were given to participants upon their requests according to the local Ministry of Health guidelines.

4.7.1. Demographic and clinical data

Socio-demographic and clinical data was collected using structured questionnaires 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 general information on address; part II is personal information; part III socio-demographic characteristics; part IV clinical information; part V Nutritional habit and lifestyle; and part VI is Anthropometric measurement.

4.7.2. Sample collection for laboratory analysis

Blood samples of about 10ml from adults were collected in EDTA and serum separator tubes using a multisampling needle. To minimize diurnal variation of some analyte, blood samples were collected before 11:00 am. The serum separator tube blood sample was centrifuged within 1 hr. after collection for 2-3 minutes at 1500-3000 revolutions per minute, then immediately the serum separated and stored at -80^{o}C this made the serum sample stable for several months. Serum samples were used for measuring lipid profile and electrolytes as well as for screening HIV, HBV, HCV, and syphilis.

4.7.3. Laboratory testing and analysis

4.7.3.1. HIV, Syphilis, HBV, and HCV Serology testing: serum samples were initially screened for HIV, Hepatitis B and Hepatitis C using ELISA techniques and for syphilis using RPR test. For HIV testing, the national testing algorithm was followed (WantaiBejieng, Unigold and Vika as tiebreaker).

4.7.3.2 Test principle and factors that influence analysis of lipids and electrolytes

Lipid profiles

Total cholesterol was measured through enzymatic methods by using CHOL2 reagent kit thus esterified cholesterol is converted to cholesterol by cholesterol esterase. The resulting cholesterol is then acted upon by cholesterol oxidase to produce cholest-4-en-3-one and hydrogen peroxide. The hydrogen peroxide then reacts with 4- aminophenazone in the presence of peroxidase to produce a colored product that is measured at 505 nm (λ^{max}) and 2^{ndry} wavelength at 700 nm. The final step is known as the Trinder reaction. This method is a single reagent, endpoint reaction that is specific for cholesterol. The reference interval given in Laboratory procedure manual of the manufacturer was 0-200 mg/dl and also minimum and maximum CV% for this analyte was 1.7% and 0.9%. The reaction sequence was as follows:

Cholesteryl ester hydrolase

Cholesteryl ester + H2O ----------> cholesterol + fatty acid

Cholesterol oxidase

Cholesterol + O2 ----------> cholest-4-en-3-one + H2O2

Peroxidase

2H2O2 + 4-aminophenazone + phenol ----------> Quinoneime + $4H_2O$

Triglyceride was measured enzymatically in serum or plasma using TRIGL reagent kit in a series of coupled reactions thus triglycerides are hydrolyzed to produce glycerol. Glycerol is then oxidized using glycerol oxidase, and H2O2, one of the reaction products, was measured as described above for cholesterol. Absorbance is measured at 500 nm. The reference interval given in Laboratory procedure manual of the manufacturer was 0-150 mg/dl also minimum and maximum CV% for this analyte was 1.8% and 0.6%. The reaction sequence was as follows:

Lipase

Triglycerides + $3H_2O$ ----------> glycerol + fatty acids

glycerokinase

Glycerol + ATP ----------> glycerol-3-phosphate + ADP

glycerophosphate oxidase

Glycerol-3-phosphate + O_2 ----------> dihydroxyacetone phosphate + H_2O_2

Peroxidase

H_2O_2 + 4-aminophenazone + 4-chlorophenol ----------> 4-(p-benzoquinone-monoimino) - phenazone + $2H_2O$ + HCl

High-density lipoprotein (HDL) cholesterol: HDL-C was measured using HDLC reagent kit by direct assay in a homogeneous method for direct HDL-C levels in serum. In this method, a magnesium/dextran sulfate solution is first added to the specimen to form water-soluble complexes with non-HDL cholesterol fractions. These complexes are not reactive with the measuring reagents added in the second step. With addition of reagent 2, HDL-cholesterol esters are converted to HDL cholesterol by PEG-cholesterol esterase. The HDL-cholesterol is acted upon by PEG-cholesterol oxidase, and the hydrogen peroxide produced from this reaction combines with 4-amino-antipyrine and HSDA under the action of peroxidase to form a purple/blue pigment that is measured photometrically at 600 nm (secondary wavelength = 700 nm). When the cholesterol measuring enzymes are modified with PEG, they are preferentially more reactive with HDL-cholesterol than the other cholesterol fractions. The reference interval given in Laboratory procedure manual of the manufacturer was >40mg/dl for male and >50mg/dl for female also minimum and maximum CV% for this analyte was 1.8% and 0.6% respectively. The reaction sequence was as follows:

PEG cholesterol esterase

HDLcholesterol esters + H_2O----------->HDLcholesterol + RCOOH

PEGcholesterol oxidase

HDLcholesterol + O_2----------->Δ4cholestenone + H_2O_2

Peroxidase

H_2O_2 +4aminoantipyrine and HSDA ----------->purpleblue dye

Low-density lipoprotein (LDL): LDL was measured by Homogeneous enzymatic colorimetric assay. Cholesterol esters and free cholesterol in LDL are measured on the basis of a cholesterol

enzymatic method using cholesterol esterase and cholesterol oxidase in the presence of surfactants, which selectively solubilize only LDL. Surfactants and a sugar compound inhibit the enzyme reactions to the lipoproteins other than LDL. Cholesterol in HDL, VLDL and chylomicron is not determined. The reference interval given in Laboratory procedure manual of the manufacturer was <100mg/dl also minimum and maximum CV% for this analyte was 2.7% and 1.9% respectively. The reaction sequence was as follow;

Cholesterol esterase / Detergent

LDLcholesterol esters + H2---------->cholesterol + free fatty acids

Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase cholesterol oxidase.

LDLcholesterol + O2---------->Δ 4 cholestenone + H2O2

(In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ 4 cholestenone and hydrogen peroxide)

Peroxidase

2 H2O2 + 4aminoantipyrine + EMSEa) + H_2O + H^+ ---------->red purple pigment + 5 H2O

Factors that influence analysis of lipids

Fasting. Recent food intake exerts little effect on plasma total cholesterol concentration. Plasma triglycerides, however, increase in postprandial plasma to an extent that is related to the fasting triglyceride levels and the amount of fat intake. This is due to the appearance of chylomicrons in the circulation after a fat-containing meal. Transient decreases in HDL and LDL also occur, the magnitude of which depends on the fat content of the meal. In apparently healthy controls, differences between fasting and non-fasting concentrations were small and clinically insignificant for TC, HDL and LDL while it is significant for triglycerides.

Type of Sample- In general, anticoagulants exert osmotic effects in which water leaves the cells and enters the plasma, thus diluting the plasma and lowering the concentrations of non-diffusible components. The magnitude of this effect depends on the anticoagulant used and its concentration. Serum cholesterol and triglyceride concentrations are about 3-5% higher in serum

than in plasma, although no significant serum-plasma difference was observed for HDL. Thus, the serum concentrations of lipids and lipoproteins probably reflect more accurately the subjects physiological state at the time of vein puncture.

Hemolysis can interfere with absorbance readings. Lipemia can affect the triglyceride measurements by interfering with absorbance measurement. Grossly turbid samples are diluted before analysis. An aliquot of the specimen is diluted with normal saline to an extent sufficient so that the value measured in the diluted specimen remains within the range 1 – 1,000 mg/dL

Sample volumes-The sample volumes required are as follows: total cholesterol and/or triglyceride, 0.5 ml; HDL measured with the direct method, along with total cholesterol, 0.2 ml. Any sample remaining after analyses are complete are returned to -80 o C

Storage and sample stability -Serum can be stored at -20°C in a non-self-defrosting freezer for up to 4 weeks. For longer storage (> 4 weeks), they should be maintained at -80°C or lower. Total cholesterol, triglyceride and HDL-cholesterol are stable for at least one year at -80 o C or lower.

Electrolytes

Sodium, potassium and chloride: these three electrolytes were measured by using ISE reagent Kit. The method was ion-selective electrode (ISE) which is an indirect specimen diluted by the instrument prior to analysis. Thus method makes use of the unique properties of certain membrane materials to develop an electrical potential (electromotive force, EMF) for the measurements of ions in solution. The electrode has a selective membrane in contact with both the test solution and an internal filling solution. The internal filling solution contains the test ion at a fixed concentration. Because of the particular nature of the membrane, the test ions will be closely associated with the membrane on each side. The membrane EMF is determined by the difference in concentration of the test ion in the test solution and the internal filling solution. The complete measurement system for a particular ion includes the ISE, a reference electrode and electronic circuits to measure and process the EMF to give the test ion concentration. The sodium and potassium electrodes are based on neutral carriers and the chloride electrode is based

on an ion exchange. The reference interval given in laboratory procedure manual of the manufacturer was 133-145 mmol/L, 3.3-5.1 mmol/L and 96-106 mmol/L for Na ,K and Cl respectively. Also minimum and maximum CV% was 0.5 and1.1% for Na, 1.2 and 3.5% for K, 0.8 and 1.3 %Cl.

Factors that influence the analysis of electrolyte

Sample - Grossly lipemic blood is a source of analytical error with some methods. Thus for lipemic samples, ultracentrifugation of serum or plasma is required before analysis. Hemolysis causes erroneously high K results. In addition, unhemolyzed specimens that are not promptly processed may have increased K concentrations. Erythrocytes contain only one tenth of the Na present in plasma, so hemolysis does not cause significant errors in serum or plasma Na value. A falsely decreased K value is initially observed if an unseparated sample is stored at 37 "C because glycolysis occurs and K shifts intracellular. Even gross hemolysis does not signifcantly alter Cl concentration.

Sample collection and storage -The sample should be collected by standard vein puncture technique. Only lithium heparin plasma may be used. Serum sample should not to remain on the cells after centrifugation. Result in Potassium from the red cells will diffuse into the serum, giving falsely elevated value. Frozen samples are stored at -70°C. When separated from erythrocytes and stored tightly stoppered at 2-8°C, chloride content is stable for several days. Sodium and potassium are stable for 2 weeks at 15- 25°C or 2-8°C.

Temperature and Time for measurement - before measurement of electrolyte done the sample should be at ambient temperature 20-25°C and Because of possible evaporation effects, all samples, calibrators, and QC on the analyzer should be measured within 2 hours.

4.8. Quality control

The collected data was checked for completeness and correctness on a daily bases. Every morning before starting the daily activity the availability of necessary materials was ensured. Additionally, before physical examination and anthropometric measurement was done the instrument like weight scale, Stadiometer and stethoscope were checked for its functionality. In addition, the blood samples were collected following confirmation of overnight fasting. Both levels of (normal and pathological) quality control samples were analyzed at the start of the day

in duplicate. Thus, pool of normal human serum which were stored at -80°C and lyophilized commercial product stored at 2-8°C were analyzed before performing participants sample. Quality control was also analyzed at the end of the shift, with change in reagent and when needed for troubleshooting

4.9 Statistical analysis

All calculations for determining reference ranges were based on the guidelines found in the Clinical and Laboratory Standards Institute (CLSI). After obtaining the result of the laboratory, the data was entered to SPSS software version 23. After cleaning the data important classification of the data such as male, female and pregnant women with their appropriate trimester was done to simplify the process. The data was not normally distributed. So to compensate this bootstrapping method was used and to pull out the outlier's box plot was used. Box-whisker plot was used to reveal gender specific relationship and difference of the analyte (lipid profile and selected electrolytes). Finally, by using Med Calc ver 20.008 software reference intervals of lipid profile and electrolyte was established for adult male, female and pregnant women. The mean, median, minimum, maximum and reference intervals were established at 2.5^{th} and 97.5^{th} percentiles with 90% CI. Probability levels of ≤ 0.05 between the two sexes, adult female and pregnant women were considered as statistically significant**.**

4.10. Operational definition

Reference interval- interval of values between 2.5^{th} and 97.5^{th} percentiles of analytes from apparently healthy individuals

Lipid profile-in this study refers to Total cholesterol, Triglycerides, HDL and LDL

Electrolytes- in this study refers to Sodium (Na), Potassium (K) and Chloride (Cl)

Trimester - duration of pregnancy this is First trimester – conception to 12 weeks, Second trimester – 13 to 26 weeks and Third trimester – 27 to 40 weeks.

Confidence interval- The probability that a population parameter will fall between a set of values

Adult - person between the ages of 18 to 60 years.

4.11. Ethical considerations

Prior to data collection support letter and ethical clearance were obtained from the Department of Medical Laboratory Sciences, College of Health Science of Addis Ababa University and permission to conduct the research was obtained from Addis Ababa Health Bureau and the respective Sub-cities and Woredas. All study participants were informed about the purpose of the study and their participation was on voluntary basis. The participants were directly benefited by being investigated for any pathogenic organisms and other clinical abnormalities. The information obtained during the study was to remain confidential and disclosure of any of the data to third parties other than those allowed in the Informed Consent form not permitted. Also all data was put in locked cabinets in a locked room at the Department of Medical Laboratory Sciences office and the results of the tests were coded to prevent identification of the volunteers. Access to data entered into computerized files was permitted only for authorized personnel directly involved with the study and password protected. Urine, stool and blood collected were not used for other purposes. The leftover samples were 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 analyses, were safely disposed of in an environmentally friendly manner.

4.12. Dissemination of the result

The result of this study will be submitted to Addis Ababa University Department of Medical Laboratory Science then presented for examiner and stakeholders. In the long run, it will be published in different scientific journals**.**

5. Results

5.1 Demographic characteristic of the study population

A total of 630 apparently healthy adults and pregnant women with equal number of male, female and pregnant women (210) for each were recruited to establish a reference interval for lipid profile and selected electrolytes. The age of reference individuals (male and non-pregnant female) ranged from 18 to 60 years .While for pregnant women, the age was 15 to 49. Also the average age for the participant men and non-pregnant women were 30.5 ± 9.2 years. As shown in Figure 1, most of the study participants were in 25 to 29 years age group (110 which is around 26.5 %). Out of 210 pregnant women 54 was at first, 68 at second and 88 third trimesters whereas the average age was 26.6 ± 4.4.

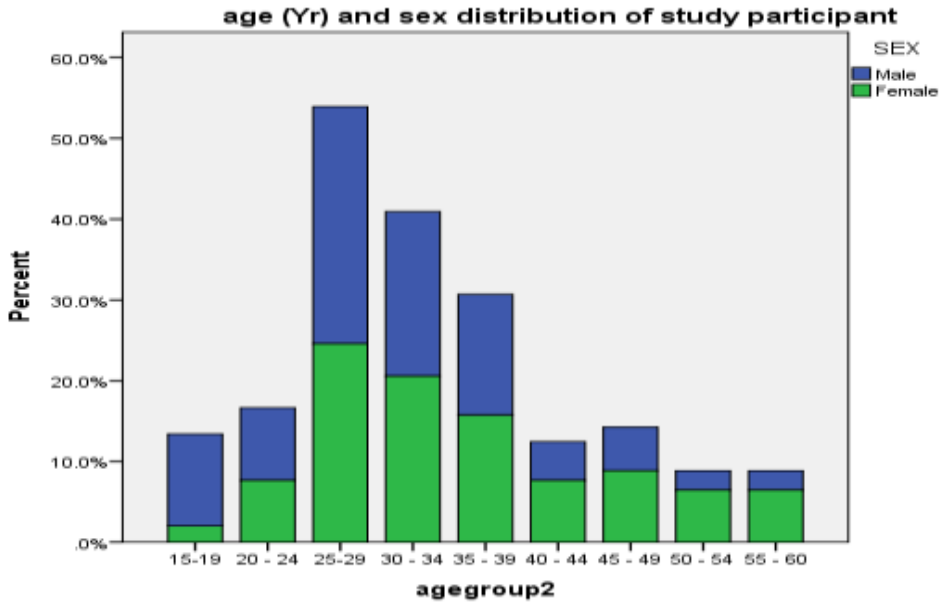


Fig 1: Age and sex distribution of study participants, Addis Ababa, Ethiopia in 2020 .

Regarding gestational week of pregnant women 26 %, 33 % and 41 % were at their first, second and third trimester, respectively. Most of the study participants were in the third trimester .

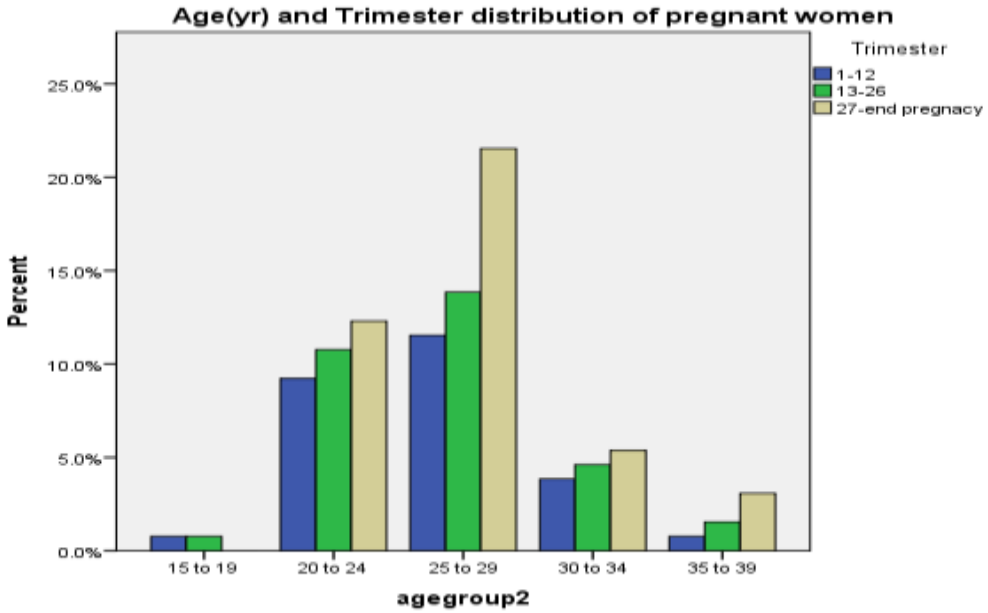


Fig 2: Age and Trimester distribution of pregnant women lived in Addis Ababa, Ethiopia.

As table two indicate greater number of adult female had fasting habit and higher number of adult male had Exercise habit. Most of pregnant women take only folate rather than taking iron and folate at once. Almost all participants had normal BMI (i.e. 18-24.9) and had no habit of chewing "khat", drinking alcohol and smoking cigarettes A study participant who had negative urine and stool test were excluded from the study.

Table 2. Profile of the study participants in 2020.

Participants	**Fasting. habit**	**Exercise. habit**	**Iron**	**Folate**	**Iron and Folate**	**Positive urine test**	**Positive stool test**
Male	74	61	NA	NA	NA	8	20
Female	86	15	NA	NA	NA	58	14
Preg.women	72	12	33	82	29	33	18

Almost all participants had normal BMI (i.e. 18-24.9) and had no habit of chewing “khat”, drinking alcohol and smoking cigarettes A study participant who had negative urine and stool test were excluded from the study.

5.2 Reference Interval of the study participants

Lipid profiles and Electrolytes Reference intervals of the study population are displayed in Table 3. The current established RIs were differing from the manufacturer's provided values for most of lipids (TC, TG, and HDL and LDL). In contrast the RI of electrolyte shows similar value with the manufacturer i.e., upper limit of K for pregnant women was equal to the current one. Also the table shows the main host factors that affect the concentration of lipids and electrolytes.

Table 3. Reference Interval for lipid profile and electrolytes and Correlated Host factors among adults and pregnant women in Addis Ababa, Ethiopia in 2020.

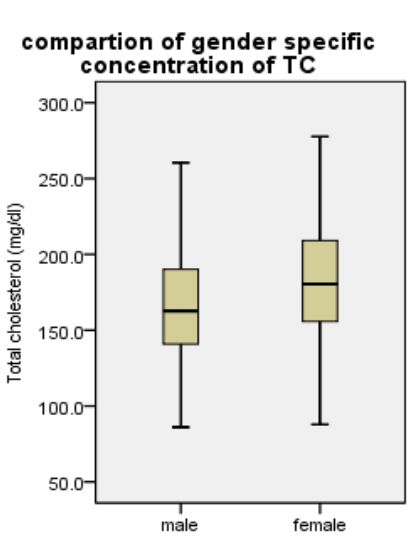
Test Parameters*	95% Reference value of the present study			Correlated Host factors	Reference value of the Manufacture		
	Male	Female			Female		Male
		Non-preg.	Preg.		Non-preg.	Preg.	
TC (mg/dl)	100.1-243	120.4-272	118 –275	Age ,Sex and BMI	0-200	0-200	0-200
TG (mg/dl)	46.5-170	51 -164	64 -304	Age, BMI and sex	0-150	0-150	0-150
HDL (mg/dl)	16 -52 .7	25-57.0	22-62.5	Age , [BMI] male only	>65	>65	>55
LDL (mg/dl)	35-162	51-170	47.8-167	Age, BMI, sex	<100	<100	<100
Na (mmol/L)	138-150	138-149	136-143	[Age, BMI] male only	133-145	133-145	133-145
K (mmol/L)	3.9 -5.5	3.95 -.6.0	3.65-5.1	Age ,BMI	3.3-5.1	3.3-5.1	3.3-5.1
Cl (mmol/L)	99.14- 108	101 -112	100.5-108	Age ,BMI	96-106	96-106	96-106

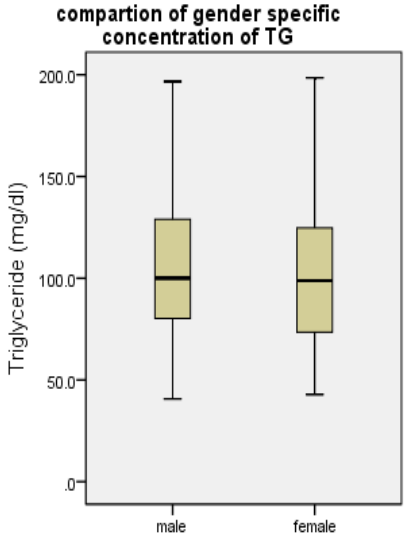
** TC=Total Cholesterol, TG =Triglycerides, HDL= High-Density Lipoprotein, LDL= Low-Density Lipoprotein, Na=Sodium, K= Potassium*

5.3 Sex and Trimester based distribution of Lipid profile and Selected electrolytes

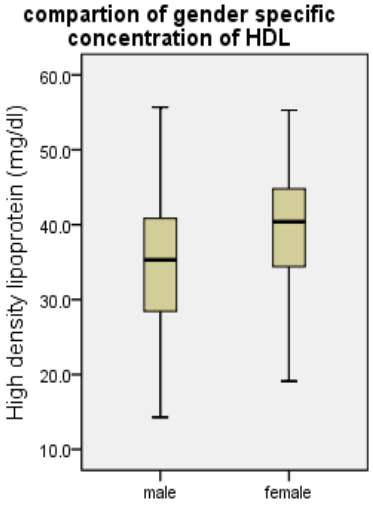
Statistically significant difference ($p<0.05$) between Female (non pregnant women) and male participants were seen for all lipids. Thus female participant had higher values of Total cholesterol, and lower Triglycerides value as compared to male subjects. In overall, the distribution of the value for all lipids between female and male was nearly equal (Figure 3, A-D).

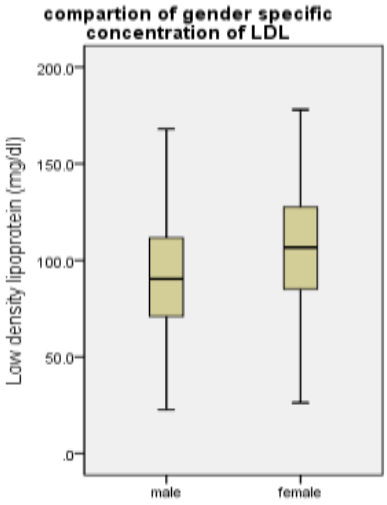
For pregnant women (Figure 3, E-H) median, interquartile range and the maximum value of TC were increased in line with trimester. A low minimum value was seen at the second trimester and values at the third trimester were more dispersed as compared to the other trimester. For TG the overall and interquartile range of the third trimester was much greater than the remainder while at the second trimester it was vice versa. Similar to TC, the median of TG increases as the trimester increases. At the second trimester, a high level of agreement between the values was seen for this analyte. At HDL and LDL side, the values for the second and third trimester were highly distributed than the first trimester. Maximum values were seen at the second trimester for the two analytes in addition the median, interquartile range and minimum HDL value of the third trimester were less than the remainder.


compartion of gender specific concentration of TC
Total cholesterol (mg/dl)
300.0
250.0
200.0
150.0
100.0
50.0
male
female

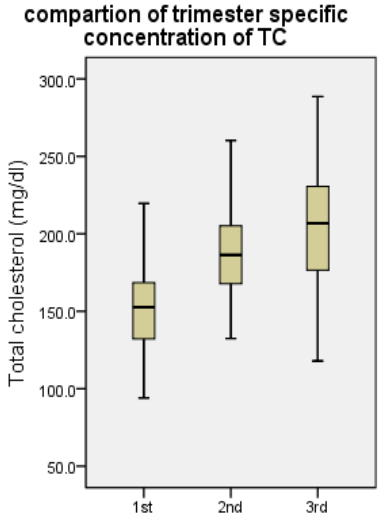

compartion of gender specific concentration of TG
Triglyceride (mg/dl)
200.0
150.0
100.0
50.0
.0
male
female

A B

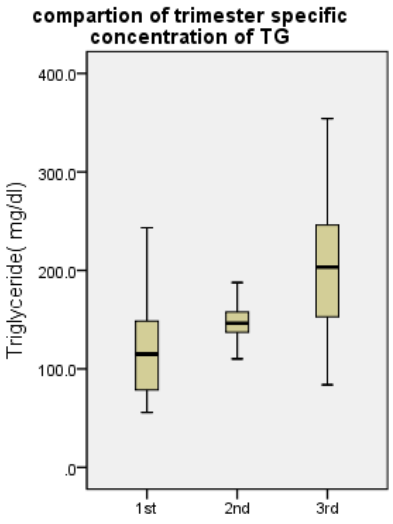

compartion of gender specific concentration of HDL
High density lipoprotein (mg/dl)
60.0
50.0
40.0
30.0
20.0
10.0
male
female


compartion of gender specific concentration of LDL
Low density lipoprotein (mg/dl)
200.0
150.0
100.0
50.0
.0
male
female

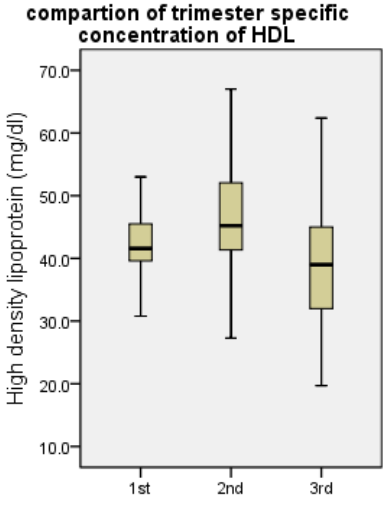
C D



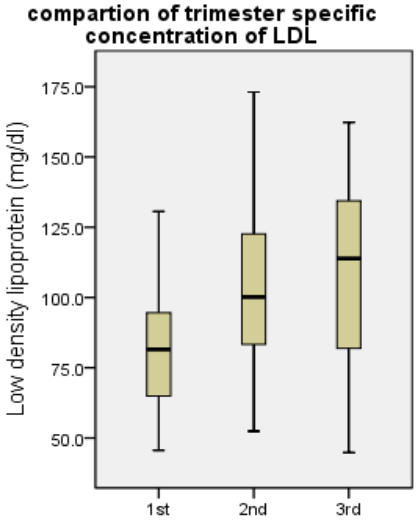
E



F



G

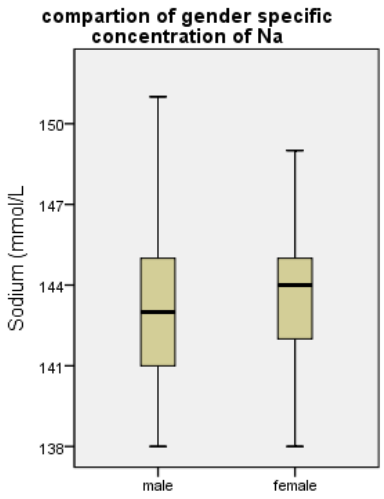


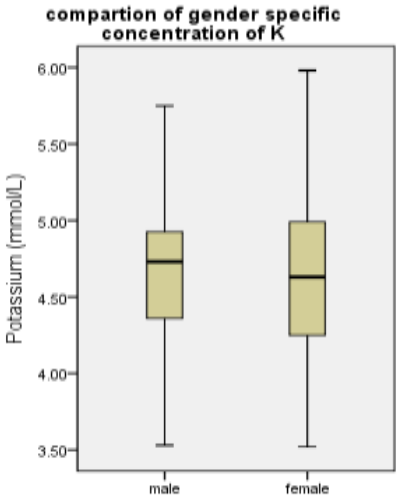
H

Figure 3: Comparison of gender and trimester specific concentration of lipid profile for adults and pregnant lived in Addis Ababa, Ethiopia in 2020.

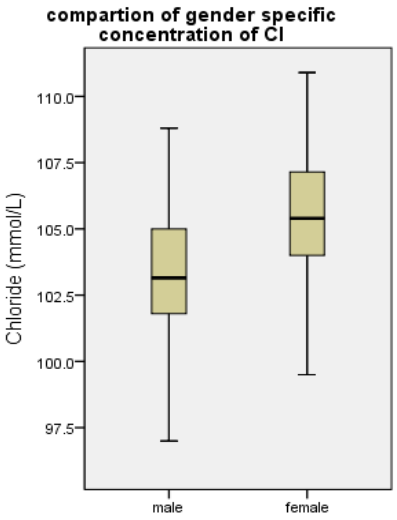
As to the overall distribution of electrolytes, the values of Na^+ and K^+ among male was significantly ($p<0.001$) higher and lower, respectively, than non- pregnant Female study participants (Figure 4, A-C). The overall range and interquartile range of Cl^- and K^+ for female participants were greater than male.

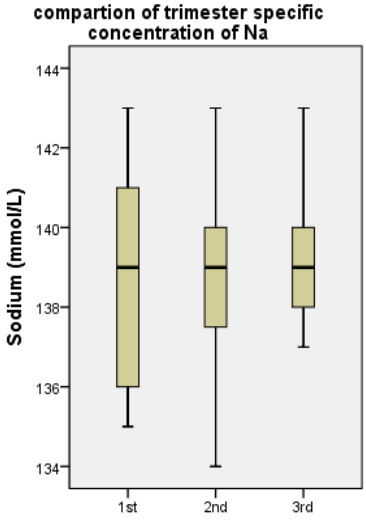
For pregnant women (Figure 4, D-F) the box plot reveal that Na value at the first trimester was more distributed than the other trimester and much greater minimum value was seen for the third trimester. Also similar median and maximum values were seen for all trimesters for this electrolyte. At the K^+ side median and maximum value of the first two consecutive trimesters was similar. Likewise, the median of Cl^- for the first and third trimester were similar. In contrast to the median of TG, the minimum value of K^+ decreased as the trimester increased.



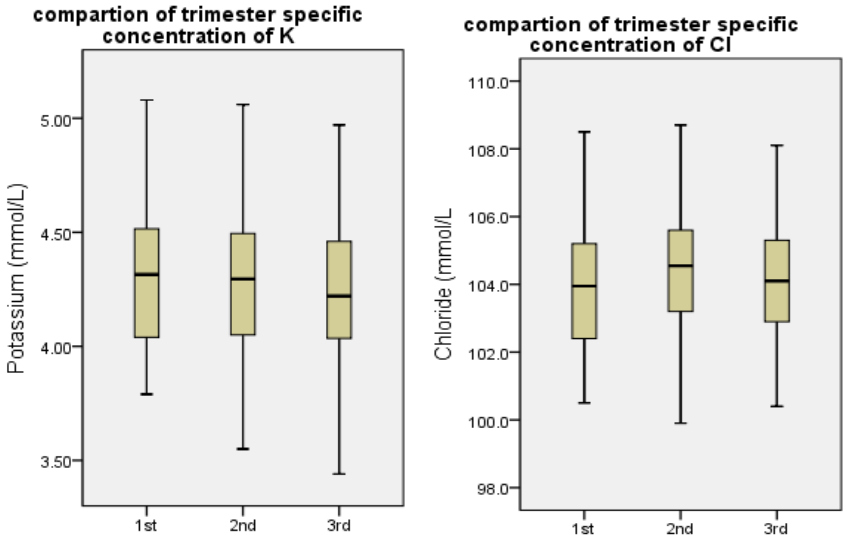


A B





C D



E F

Fig 4: Ccomparison of gender and trimester specific concentration of electrolytes for adults and pregnant women living in Addis Ababa, Ethiopia in 2020.

5.4 Reference intervals of serum lipid profile and selected electrolytes among adult females, males and pregnant women living in Addis Ababa, Ethiopia.

The current study found that females had higher values for serum TC, HDL-c and LDL-c at lower and upper limits than male counter parts. While the upper limit of male of TG was higher than females. (P-value < 0.05) were found for all serum lipid profile. Even though the upper limit of male electrolytes was greater than female, both sexes took similar lower limits for Na^+ and K^+. On the other hand, pregnant women had higher upper limits than non-pregnant for TC, TG and HDL-c but lower limits were lower. Exactly similar LDL-c mean was seen for pregnant and non-pregnant women. Also the gap between lower and upper limits of TG for pregnant women was huge. On the Electrolyte side the upper and lower limit of K^+ and Na^+ were almost equal for both groups thus the difference in this electrolyte between the two sex was only one. There was a notable difference seen between the two sexes for Cl. Serum electrolytes of pregnant women was lower at upper and lower limit than non-pregnant women.

As depicted in Table four reference interval developed for lipids and major electrolyte for adult female and male were, TC [(120.4-272) and (100.1-243) mg/dl], TG [(51-164) and (46.5-170)mg/dl]; HDL-c [(25-57) and (16-52.7)mg/dl], LDL-[(51-170) and (35-162)mg/dl]; Na

[(138-149)and (138-150)mmo/l]; K[(3.9-5.6)and(3.9-5.5) mmol/L] and Cl [(101-112)and (99.14-108) mmol/l].

Table 4: Reference interval of lipid profile and major Electrolytes for male and female adults , Addis Ababa, Ethiopia in 2020.

Analyte	Sex	N	Mean	Median	SD	Min	Max	25 and75%	2.5th -97.5th percentile
TC (mg/dl)	F	138	176	169	39	88	275	149,200	120.4-272
	M	121	163	160	38.13	92.3	260.4	135 ,189	100.1-243
	C	258	166	164	34.3	87.8	247	142 ,187	103.2- 239.5
TG (mg/dl)	F	130	90.6	87.3	28.4	42.9	164	68,109	51 -164
	M	121	103.6	98.9	32.5	32.7	176.6	79.9 ,123	46.5-170
	C	255	99	96	33.1	32.7	188	73.1,119.6	49.7 -171.6
HDL (mg/dl)	F	126	40.8	41.8	7.8	22.5	59	35.6,45.3	25-57
	M	132	34.8	35.35	8.9	14.3	56.3	28.1,41.1	16 -52 .7
	C	263	38	38.3	9.3	14.3	61.8	31.5,44	18.4-56.8
LDL (mg/dl)	F	135	101	94	30	26.3	176	81,119	51 -170
	M	131	92.1	89.9	31.2	22.7	168	70,111	35-162
	C	263	96.2	91.8	30	22.7	168	77.4 ,115	41.2-161
Na (mmol/L)	F	130	143	143	2.5	138	149	141,145	138-149
	M	127	143	143	2.8	138	151	141,145	138-150
	C	259	143	143	2.7	138	151	141,145	138 -149.5
K mmol/L)	F	139	4.5	4.5	0.43	3.7	5.8	4.2,4.8	3.95 -.6.0
	M	130	4.6	4.7	0.4	3.7	5.6	4.3,4.9	3.9 -5.5
	C	270	4.6	4.65	0.43	3.7	5.8	4.3,4.9	3.95 -5.6
Cl (mmol/L)	F	134	105.5	105.5	2.3	99.5	111.1	104,107	101 -112
	M	127	103	103	2.4	98.2	109.9	101,105	99.14- 108
	C	266	105	105	2.9	98.2	112.6	102.6,106.6	99.4-111.6

**N-number of participants, C-combined, M-male, F-female, SD-standard deviation, Min-minimum Max-maximum, CI-confidence interval,*

The calculated 90% confidence intervals for the lower 2.5th and upper 97.5th RIs are shown in Table 5. Table 6 summarizes the reference intervals for pregnant women.

Table 5: The 90 % CI interval of lower and upper reference limit of Lipid profile and Electrolytes for male and female adults living in Addis Ababa, Ethiopia 2020.

Analyte	Sex	N	2.5th-97.5th percentile (RI)	90 %CI UL	90% CI LL
TC (mg/dl)	F	138	120.4-272	242 ,275	87,123.5
	M	121	100.1-243	230,260	92.3,104.5
	C	258	103.2 -239.5	229,246	96,110
TG (mg/dl)	F	130	51 -164	153,167	43,54
	M	121	46.5-170	164.3,176.6	32.7 ,54
	C	255	49.7 -171.6	164.7,177	42.954
HDL (mg/dl)	F	126	25-57	55.1 ,59	22.5,27
	M	132	16 -52 .7	48.8 ,56.3	14.3 -20.5
	C	263	18.4-56.8	55.2,61	16,22.3
LDL (mg/dl)	F	135	51-170	159,176	26.3,58
	M	131	35-162	156.1,168	22.7,43.3
	C	263	41.2-161	156.6 -166.5	30.9,50
Na (mmol/L)	F	130	138-149	148,149	138,139
	M	127	138-150	149,151	138,139
	C	259	138 -149.5	149,150	138,139
K (mmol/L)	F	139	3.95 -.6	5.3,5.8	3.7,4
	M	130	3.9 -5.5	5.3,5.6	3.7,4
	C	270	3.95 -5.6	5.5,5.7	3.8,3.99
Cl (mmol/L)	F	134	101 -112	110.5-112.6	99.5 -101.7
	M	127	99.14- 108	107.7-109.9	98.2,99.5
	C	266	99.4-111.6	110-112	99,99.8

**N-number of participants, UL-upper limit, LL-lower limit, CL-confidence interval,RI reference interval*

As illustrated in the Table six for pregnant women established RI of lipids and major electrolyte were TC(118-275) mg/dl, TG (64 -304) mg/dl, HDL (22-62.5)mg/dl, LDL(47.8-167)mg/dl, Na(136-143) mmo/l, K(3.65-5.1) mmol/L,Cl (100.5-108) mmol/l.

Table 6: Reference interval and 90% CI interval of lower and upper reference limit of Lipids and Electrolytes for pregnant women in Addis Ababa, Ethiopia 2020.

Analyte	N	Mean	median	SD	Min	max	25 % and 75%	2.5^{th} - 97.5^{th}	90%CI UL	90%CI LL
TC (mg/dl)	146	189	186	40	94	288	158, 215	118 –275	260.6 ,288.6	94,121.4
TG (mg/dl)	149	171	158	65	56	354	122, 226	64 -304	287,354	56,75
HDL (mg/dl)	144	42	41.5	9.9	19.7	67	36, 49	22-62.5	59,67	19.7,26
LDL (mg/dl)	146	101	97.5	32	45	191	78, 125	47.8-167	154,191	45,53
Na (mmol/L)	134	139	139	1.8	135	143	138, 140	136-143	142,143	135 ,136
K (mmol/L)	142	4.3	4.3	0.35	3.4	5	4.05, 4.5	3.65-5.1	4.9,5.1	3.4,3.8
Cl (mmol/L)	141	104	104.2	1.9	99.9	108 .7	103, 105.4	100.5-108	107.4,108.7	99.9,101

**N-number of participants, C-combined, M-male, F-female, SD-standard deviation, Min-minimum Max-maximum, CI-confidence interval*

Table seven focuses on comparison between sex using the non-parametric Mann Whitney U test revealed that statistically significant differences (P-value <0.05) between female and male were seen for TC, TG, HDL-c, LDL-c and Cl^-. Also the significant differences between pregnant and non pregnant women were seen for TC, TG, and Na. No Significant differences were seen between the trimester (1st and 2nd, 1st and 3rd and 2nd and 3rd) for all electrolytes and from lipid profile, only HDL and LDL for 1st and 3rdand 3rd and 2nd , respectively.

Table:7 Comparison of lipid profile and electrolyte reference intervals by sex and pregnancy status and inter Trimester difference of pregnant women in Addis Ababa, Ethiopia 2020.

Analyte	Female and Male	Pregnant and non pregnant women	1st and 2nd Trimester	1 st and 3rd Trimester	2nd and 3rd Trimester
TC (mg/dl)	0.005,	0.012	0.0004	0.0012	0.041
TG (mg/dl)	0.006,	< 0.0001	0.03	0.00001	0.0004
HDL (mg/dl)	<0.001	0.189	0.033	0.141	0.014
LDL (mg/dl)	0.00001	0.78	0.018	0.001	0.203
Na (mmol/L)	0.634	<0.0001	0.764	0.241	0.895
K (mmol/L)	0.16	<0.0001	0.486	0.524	0.227
Cl (mmol/L)	< 0.0001	1	0.53	0.651	0.543

5.5 Comparison of current established reference interval of Lipids and Electrolytes with other published data.

As presented in Table eight, comparison with other selected studies outside Africa and manufacturers RIs revealed that the study participants had higher upper limit of serum lipid and electrolytes than the manufacturer except HDL-c, which had lower value while the value of other countries may be higher or lower than the current values. China had higher lower limit and lower upper limit for TC in both sexes while Russia, Saudi and Turkey had higher value for both limits than the current. For TG China had lower limit for female while upper limit of female and male was higher. In relation to this Russia, Turkey and Saudi Arabia had a lower limit for male and RI for female while upper limit of male was higher than the present TG values. Russia and Saudi

Arabia derived LDL-c RIs were higher than the present one. Notably, lower HDL-c RI was seen for our study than the other. On the other hand, the present study reported higher RIs values for all selected serum electrolytes Table 8.

Table 8: Comparison of established reference intervals of serum lipid profile and selected electrolytes for Addis Ababa, Ethiopian adults with Manufacturer and other previous studies in 2020.

Analyte	Sex	Current study Cobas	Manufacturer Cobas	China Beckman Coulter (17)	Russia Beckman Coulter (16)	Saudi Arabia Architect 1600c (25)	Izmir(Turkey) Abbott (26)
TC (mg/dl)	F	120.4-272	NA	122-222	NA	NA	NA
	M	100-243	NA	125-240	NA	NA	NA
	C	103-239.5	0-200	NA	128-297	136-248	122-272
	N	258	NA	1451	351	393	393
	Med	164		NA	182	NA	NA
TG (mg/dl)	F	51-164	NA	43-203	37-161	34-142	39-200
	M	46.5-170	NA	47-305	40-238	44-338	51-315
	C	49.7-171.6	0-150	NA	NA	NA	NA
	N	255	NA	1450	181	393	393
	Med	96	NA	NA	87	NA	NA
HDL (mg/dl)	F	25-57	>65	36-85	39-87	38-85	36-79
	M	16 -52 .7	>55	31-76	30-69	29-69	31-67
	C	18.4-56.8	NA	NA	NA	NA	NA
	N	263	NA	1450	331	393	393
	Med	38.3		NA	58	NA	NA
LDL (mg/dl)	F	51,170	NA	50-133	77-189	NA	NA
	M	35-162	NA	52-149	86-229	NA	NA
	C	41.2-161	<100	NA	91-206	70-169	NA
	N	263	NA	1450	275	389	195
	Med	91.8		NA	147	NA	NA
Na (mmol/L)	F	138-149	NA	NA	NA	NA	NA
	M	138-150	NA	NA	NA	NA	NA
	C	138 -149.5	133-145	136-144	135-141	135-144	139-147
	N	259	NA	1324	699	393	393
	Med	143		140	138	NA	NA
K (mmol/L)	F	3.9,5.6	NA	NA	NA	NA	3.6-5
	M	3.9,5.5	NA	NA	NA	NA	3.7-5.7
	C	3.9,5.6	3.3-5.1	3.7-4.7	3-5 -4.5	3.7-4.9	NA

	N	270	NA	1591	684	393	393
	Med	4.65		NA	4.0	NA	NA
Cl (mmol/L)	F	101,112	NA	NA	NA	NA	NA
	M	99.14- 108	NA	NA	NA	NA	NA
	C	99.4-111.6	96-106	101-109	99-107	101-111	103-111
	N	266	NA	1323	701	393	195
	Med	105	NA	NA	103	NA	NA

N-number of participants, C-combined, M-male, F-female, Med-median

Tables nine presented the adults RIs and median values of the current study as compared to other studies from the other parts of Africa. The current RI of TC was higher than others. As for TG Zimbabwe RIs were lower as the remaining country had a higher upper limit. There was a huge gap between upper and lower values of Egyptian RIs for both sexes. Egypt derived LDL-c RI was higher, the other had a higher upper limit. There was no notable difference seen for electrolytes reference interval. Although the present study fit the minimum requirement of CLSI to establish reference interval number of study population for all analyte were the less than the population those participate in Ghana (11), Kenya (26) Turkey (25) and Zimbabwe (14) study.. Also median value of TC, TG and K were higher than Ghana (11), Kenya (26) and Zimbabwe (14). In contrary to this present study median value of HDL was less than the value established for this country.

Table 9: Comparison of established reference intervals of serum Lipid and Electrolytes for Addis Ababa, Ethiopian Adult with other African studies RI in 2020.

Analyte	**Sex**	**Current study** Cobas	**Egypt** Cobas (23)	**Ghana** VitalabSelectra E (12)	**Kenya** Cobas (27)	**Zimbabwe** Cobas (15)	**Rwanda** Cobas (13)
TC (mg/dl)	F	120.4-272	NA	82 – 218	101-230	101-240	NA
	M	100-243	NA	70-195	97.5 - 214.5	99-232	NA
	C	103-239.5	135-271	78- 210	101-222	99-237	NA
	N	258	250	622	1508	769	NA
	Med	164	178	125	148	156	NA
TG mg/dl	F	51-164	36-311	35.5-187	36-222.5	34-159.5	NA
	M	46.5-170	44 -359	35.5-196	36-240	37-156	NA
	C	49.7,171.6	NA	35.5-196	36-231	35-155	32-172
	N	255	286	619	1477	769	172
	Med	96	87	80	80	69	82
HDL (mg/dl)	F	25-57	28-78	NA	NA	32-85	NA
	M	16 -52 .7	26-71	NA	NA	31-87	NA
	C	18.4-56.8	NA	NA	NA	31-85	29-86
	N	263	286	NA	NA	769	130
	Med	38.3	50	NA	NA	49	48
LDL (mg/dl)	F	51,170	NA	NA	NA	52-160	NA
	M	35-162	NA	NA	NA	41-159	NA
	C	41.2-161	55-185	NA	NA	47-154	NA
	N	263	373	NA	NA	769	NA
	Med	91.8	103	NA	NA	90	NA
Na (mmol/L)	F	138-149	NA	135-150	140-155	135-148	NA
	M	138-150	NA	135-151	142-152	136-149	NA
	C	138 -149.5	131-148	135-150	141-152.5	136-149	137-147
	N	259	544	541	1541	769	122
	Med	143	140	144	146.5	142	142
K (mmol/L)	F	3.9,5.6	NA	3.4 -5.1	3.8-5.8	3.5-5-2	NA
	M	3.9,5.5	NA	3.6 -5.2	3.9-5.8	3.6-5.3	NA
	C	3.9,5.6	3.4-5.3	3.6-5.2	3.9-5.8	3.6-5.2	3.3-5
	N	270	543	583	1535	769	131

	Med	4.65	4.3	4.4	4.6	4.3	4.1
Cl (mmol/ L)	F	101,112	NA	108-113	101-113	96-107	NA
	M	99.14- 108	NA	101-115	100-111	95-107	NA
	C	99.4-111.6	94-111	102-114	100.5-112	96-107	100-112
	N	266	538	531	1541	769	121
	Med	105	103	107	105.6	101	106

N-number of participants, C-combined, M-male, F-female, Med-median

Table Ten displays the comparison of the RIs and median of pregnant women of the current study versus other countries study. While the current study was trimester independent RI of TC was nearly equal to the second trimester of Nigerian study and far lower than second trimester RI of Denmark pregnant women. For TG the current study found that less UL than Sweden and Denmark second trimester RI. For electrolytes we found that nearly equal UL and LL for Na with Denmark study and for K with Kenyan study. Number of study population of the current study less than Denmark and Kenya population for all lipids and electrolyte also Current lipids Median was higher than other study. While current Na median was equal to Nigerian study and for K median it was equal to Kenyan study.

Table 10: Comparison of established reference intervals of serum lipid profile and selected electrolytes for Addis Ababa Ethiopia pregnant women with other African studies in 2020.

Analyte	**Gestational week**	**Manufacturer** Cobas	**Current study** Cobas	**Kenya** Integra 400 (29)	**Denmark** Cobas (30)	**Sweden** Cobas (19)	**Nigeria** V350 analyzer (22)
TC (mg/dl)	1-12	0-200	118 –275	NA	144-269	NA	156-245
	13-26	NA	NA	NA	160-304	NA	207-277
	27-end	NA	NA	NA	172-343	NA	66-238
	N	NA	146		367	NA	NA
	Med	NA	186		NA		175.5
TG(mg/dl)	1-12	0-150	64-304	NA	68-222	79-384	80-235
	13-26				74-280	96-388	116-276
	27-end				118-420	144-455	80-205
	N	NA	149		367	NA	NA
	Med	NA	158		NA		115.7
HDL (mg/dl)	1-12	>65	22-62.5	NA	55-113	NA	NA
	13-26				55-117	NA	
	27-end				47-113	NA	NA
	N	NA	144	NA	367	NA	NA
	Med	NA	41.5	NA	NA		NA
LDL (mg/dl)	1-12	<100	47.8-167	NA	47-156	NA	NA
	13-26				51-168	NA	
	27-end				62-218	NA	NA
	N	NA	146	NA	367	NA	NA
	Med	NA	97.5	NA	NA		NA
Na(mmol/L)	1-12	133-145	136-143	126.3-140.6	135-142	128-140	134-142
	13-26				135-142	130-140	135-142
	27-end				135-141	127-	135-152
	N	NA	134	296	367	140	NA
	Med	NA	139	132.8	NA		139
K(mmol/L)	1-12	3.3-5.1	3.65-5.1	3.6-5.2	3.2-4.2	3.26-4.6	4.1-5.2
	13-26				3-2-4.2	3.46-4.74	3.9-5.3
	27-end				3.2-4.1	3.32-5.09	3.9-5.7
	N	NA	142	296	359		NA
	Med	NA	4.3	4.3	NA		4.1
Cl(mmol/L)	1-12	96-106	100.5-108	89-105.7	NA	97-107	105-117
	13-26				NA	99-108	103-115
	27-end				NA	97-109	111-127

	N	NA	141	296	NA		NA
	Med	NA	104.2	99.5			112

6. Discussion

Different studies conducted in some African countries reported that there is a significant difference in normal laboratory reference interval compared with those of other African countries and western world (29). Despite this, including Ethiopia, different developing countries interpret the laboratory results with reference intervals provided by manufacturers' inserts sheets. As laboratory results affect around 70% of medical decisions in preventing, diagnosing, treatment and management of patient disease (14) the laboratory test result should be interpreted with reference intervals, which represent the local population. It is challenging to develop direct reference intervals, mainly selection of reference population and method. Thus, for better medical care and decision for our population the current study tries to focus on developing RIs, which focused on serum lipid profile and selected common electrolytes.

The current study showed that there was statistically significant sex difference observed for TC, TG, HDL-c, LDL-c and Cl for study population while Na and K were similar for both sexes. On the other hand, statistically significant differences were observed for TG, TC, Na and K between pregnant and non-pregnant women. HDL-c, LDL-c and Cl were similar for both genders. The manufacturers' reference interval provided in the leaflets of the reagents used in the current study presented a combined reference interval for both sexes except for HDL-c. However, most of the RIs of foreign countries that was used in this study for comparison including the current studies (12-15) reported separate RIs for males and females. This may be valuable to see significant differences between sexes (22). Evidences from the current study, and from some other Africa countries reference intervals were higher than Russia (16), Saudi Arabia (25), ISMIR (Turkey) (26) and China reference interval (17). This may be due to genetic makeup, lifestyle and geographical location of the countries.

According to the work, TC value was lower than Russia (16), Saudi Arabia (25), ISMIR (Turkey) (26), china (only at upper limit for both sexes) (17) and Egypt reference interval (23). While upper and lower limit was higher than the study from Ghana that established RI for both sexes (12). For TG current female upper and lower limits as well as male lower limits were higher than Russia (16) and Saudi Arabia reference intervals (25). On the other hand, higher lower limit and lower upper limit of current study were observed than China (17), Egypt (23),

Zimbabwe (15) and Ghana (12). The upper limit of TG value in the current study for both males and females was in line with the upper limit of reference interval generated for the Rwandan population (13). This difference may be because of lifestyle, genetic makeup, variation in number of participants, difference in statistics and lab methods. Unlike this Current TC reference limit was in line with Kenya (27) and Zimbabwe (15) population. This may be due to similar geographical location of the countries.

Contrary to other study the current work found that extremely lower values of HDL-c at both reference limits. Thus, the present study revealed that HDL-c reference limit of the study population was much lower than manufacturer and other countries reference limit for both sexes (25-29). Although many prospective studies from different racial and ethnic groups worldwide have confirmed that less HDL-c is a strong, consistent, and independent predictor of incident cardiovascular events (myocardial infarction, ischemic stroke) (28). Better agreement was seen on the current lower limit of female LDL-c with China (17) and Zimbabwe (15) reference interval while male upper limit of this country was less than the current one. On the contrary, both the upper and lower limit of current LDL-c value was much less than Russia (16) and Saudi Arabia (25) reference interval of the study population. This disagreement of HDL-c and LDL-c reference limits might be explained by differences in lifestyle, feeding culture and genetic origin of the study population. Studies show that effects of estrogen hormone make serum LDL cholesterol level lower and HDL cholesterol levels higher in women compared with men of the same age (34). In comparison to this our current finding showed that male LDL cholesterol level was lower than female. This may be a predictor for further finding to dig the root cause of this discrepancy, or possibly the study subjects are from Addis Ababa, where the average altitude is 2000 meter above sea level, which is higher altitude than most studies elsewhere.

The current finding supports previous studies which indicated that Electrolyte concentrations did not show significant differences between females and male (35). Current Na value was in line with Ghana (12) and Zimbabwe (15) reference limit developed for study population at upper limit for both sex. While little higher value was seen in present study than china (17), Russia (16), Saudi Arabia (25) and Rwanda (13) reference limits of the study population for both sex. Also the lower limit of Turkey (26) value was higher than the present value except the upper limit of Turkey population which is lower (26). Our current potassium value was higher at both

limit for both sex than Russia (16), Saudi Arabia (25), china (17), Ghana (12), Zimbabwe (15) and Rwanda (13) reference limit. Interestingly exact similar lower limit at both sex was seen with Kenyan population reference interval (27). This may be due to the similar geographical location and weather condition of the two countries. The discrepancy of sodium and potassium reference limit between the present study and the remaining country may be due to weather conditions, lifestyle, level of salt intake, and sweating level of the study population.

Chloride in association with Na plays a great role in maintaining body fluid balance and different vital functions of the body (36). There was better similarity on the current developed reference limit of Cl than other electrolytes to Kenya (27), Rwanda (13), Russia (16), Saudi Arabia (25) and Turkey (26) reference interval. The reference limit of Cl developed for Ghana (12) and Egypt (23) population was higher at both limits than the current while the value of Zimbabwe (15) population was lower. This variance may be due to salt intake, weather condition and level of aldosterone hormone in the body of the study population. At all, better agreement was seen for electrolyte reference intervals than lipids.

Pregnancy is known to change metabolic processes involved in lipid and lipoprotein more than others do. These metabolic alterations are likely evolved to meet the metabolic demands of the growing fetus (37). Most of the changes in serum biochemistry parameters were associated with the rising level of estrogen and progesterone hormone (30). The body may not be able to balance the changes. Also, biochemical profiles can become significantly distorted from the values normally noted during pregnancy (38, 39). There was a significant difference seen between pregnant and non-pregnant women for TC, TG, Na and K while HDL-c and LDL-c were similar. Electrolyte reference limit of pregnant women were slightly lower than non-pregnant women in the current study, this was because of that the plasma volume and glomerular filtration rate increases during pregnancy resulting in a decrease in most serum constituents (30).

Current TC value was higher than the manufacturer and Nigeria (22) reference limit developed for the study population while it was far lower than Denmark (30) value at both reference limits. Like TC, the present TG value was higher than the manufacturer and Nigeria value (22) reference limit. Also the value was far lower than the reference limit developed for Denmark (30) and Sweden (19) population. Although our study was trimester independent, studies showed that HDL-c concentration changes non-significantly in the first trimester, but significantly

increases in the second trimester and slightly decreases in the third trimester (40). Current study was in line with finding which show pregnant women HDL reference limit did not show significant difference to non-pregnant women. While a study revealed that LDL value elevated during pregnancy, current LDL values of pregnant and non-pregnant women were similar (41). The present HDL value was lower than the manufacturer and Denmark (30) reference interval at both limits. The trimester independent LDL value was higher than the manufacturer and similar to the second trimester reference limit of Denmark (30). This lipid inconsistency between our study population and others may be due to genetics, lifestyle, feeding habits, and number of study participants.

In agreement to our current study, Denmark potassium reference values for pregnant women were slightly lower than non-pregnant women. This is in contrast to Palm et al. who observed a minor increase and a much higher upper limit during pregnancy (19). Sodium value of Denmark pregnant women was very close to non-pregnant women but our pregnant women value was significantly lower than non-pregnant women. Present Na value was in agreement with Denmark (30) and Nigeria (22). Whereas it was higher than the intervals from Kenya (29) and Sweden (19) reference limit. At K side a very close relation was seen between the current reference limit and Kenya value (29). However, it was higher than the Denmark (30) and Sweden (19) reference limit. Also it was much lower than the Nigerian (22) reference limits developed for the study population. Trimester independent reference limit of our current Cl value agreed to Sweden (19) at both limits and higher and far lower than Kenya (29) and Nigerian (22) value at both limits respectively. This difference of electrolyte may be due to lifestyle including level of salt intake. The salt intake hypothesis assumes that pregnant women are more sensitive to salt intake than non-pregnant women are (42), weather condition (hot weather) and method of analysis.

As both lipid profiles and electrolytes are laboratory parameters important in the diagnosis of a currently rising non-communicable diseases like CKD, such variations among RIs between populations underscore the need for establishing population specific RI as good medical decisions which are guided by laboratory results interpreted by population based reference intervals are needed.

7. Strength and Limitations

7.1. Strengths of the study

Despite many obstacles during the study period, the current study reports the first community based RIs for serum lipid profile and selected electrolytes for Addis Ababa adults. As Addis Ababa is a populated city of the country, we found an optimum study population to collect samples, which is more than the recommendation of CLSI, which is a minimum of 120 samples.

7.2. Limitation of the study

Notwithstanding its opportunity and significant of our study, we encounter shortage of important material and reagent for sample collection and analysis. While the participant had the right to withdraw from the study some of which refused during sample collection this resulted in wastage of some material. Reference interval, which established for pregnant women, was trimester independent this may result in misinterpretation of the physiological and biochemical change that occurred during each trimester.

8. Conclusion and Recommendations

8.1. Conclusion

Current study develops reference interval for lipid profile and major electrolytes for adults and pregnant women of Addis Ababa. This implied that one-steps forward were seen in Ethiopian healthcare system. Also, the study shows there was difference in reference interval of lipid and major electrolyte for Adults and pregnant women of Addis Ababa to other African and western population. Significant difference seen between sexes for all lipids and Cl and also between pregnant women and adult female for TC, TG, and Na and K. From general perspective, our study revealed that the value of HDL-c was very low.

8.2 Recommendation

Current study and different literature indicate that the use of reference intervals which did not represent the population result in misinterpretation of laboratory results. Thus, we suggest that applying this study aids the physician in the correct medical decision. Also, the application of this study in the disease management and control program, during policy making related to the health of adults, in the interpretation of laboratory results during screening and management of disease and in the maternal health program will create a good environment in the health care system of Addis Ababa, Ethiopia. We also suggest that there should be effort to establish regional specific reference interval for the parameters included and those not included in this study.

9. References

1. Tietz NW. Fundamentals of Clinical Chemistry, 6th ed. Burtis CA, Ashwood ER, eds. WB Saunders Co 2001:970, 1004-1009.
2. Bayram F, Kocer D, Gundogan K, Kaya A, Demir O, Coskun R, *et al.* Prevalence of dyslipidemia and associated risk factors in Turkish adults. J Clin Lipidol. 2014; 8(2):206–16.
3. Laatikainen T, Pietinen P, Valsta L, Sundvall J, Reinivuo Z, J Tuomilehto, *et al*. Sodium in the Finnish diet: 20-year trends in urinary sodium excretion among the adult population. European Journal of Clinical Nutrition. 2006; (60):965–970.
4. Umz M, Rabinowitz L, Wingo C. An Integrated View of Potassium Homeostasis. N Engl J Med. 2015;373(1):60-72.
5. Kassahun H, Belay Z, chala E, Endriyas K, Abebe T, Aklilu H. Serum electrolytes disorder and its associated factors among adults admitted with metabolic syndrome in Jimma Medical Center, South West Ethiopia: Facility-based cross sectional study**.** PLoS One.2020:5.
6. Ferraro S, Braga F, Panteghini M. Laboratory medicine in the new healthcare environment. Clinical Chemistry and Laboratory Medicine (CCLM).2016; 54:523-33.
7. Solberg HE. The IFCC recommendation on estimation of reference intervals. The RefVal program. Clinical Chemistry and Laboratory Medicine (CCLM). 2004; 42(7):710-4.
8. Cerioti F. Prerequisite for use of common reference interval [cited 2021 Sep 13]; Available from: http://www.nobi.nih.gov/pubmed//17909616
9. Clinical and Laboratory Standards Institute. Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guidelines. 3rd ed. Wayne, PA: CLSI; 2008.
10. ISO. Medical laboratories—particular requirements for quality and competence. Geneva: ISO; 2012. ISO 15189.
11. Priya P, Catherine P, Heli T, Alexandre M. Physiological changes in pregnancy.CVJ Africa. 2016; 2-27.
12. David K. D, Kingsley K, Dennis A, Evans K, Josephine O, *et al.* Hematological and Biochemical Reference Values for Healthy Adults in the Middle Belt of Ghana. PLoS One .2012; 7.

13. Jean B. Clinical Chemistry Reference Intervals in a Rwandan Population. British Journal of Medicine & Medical Research.2013;3:532-54.
14. WHO. Prevention of Cardiovascular Disease Guidelines for assessment and management of cardiovascular risk. Geneva, World Health Organization.2007.
15. Wadzanai P., Gibson M, Willard T, Nehemiah N, Nyaradzo M. Adult Hematology and Clinical Chemistry Laboratory Reference Ranges in a Zimbabwean Population. PLoS One.20167.
16. Svetlana E, Kiyoshi I, Anna R, Irina S, Nina V, Shogo K, et al. Establishing reference intervals for major biochemical analytes for the Russian population. Elsevier Inc. 2020;47–58.
17. Liangyu X, Ming C, Min L, Zhihua T, Shijun L, XuzhenQ, *et.al*. Nationwide Multicenter Reference Interval Study for 28 Common Biochemical Analytes in China. Journal of Medicine.2016. 95(9).
18. Fassil S, Mekitew L, Awoke M, Yeweyenhareg F, Terefe G. Non-communicable Diseases in Ethiopia: Disease burden, gaps in health care delivery and strategic directions. Ethiop. J. Health Dev. 2018; 32 (3).
19. Palm M, Larsson A, Hansson LO, Axelsson O. Reference values for clinical chemistry tests during normal pregnancy. Br J Obstet Gynaecol.2008;115:874–8.
20. Blackburn S. Maternal, fetal and neonatal physiology. a clinical perspective. 4th ed. Maryland Heights, MO: Elsevier Saunders; 2013.
21. Koram K, AddaeM,Ocrans J, Amankwah A, Rogers W, krumah F. population based reference intervals for common blood hematological and biochemical parameters in the Akuapem north district. Ghana medical journal .2007; 41(4).
22. Miri-Dashe T, Osawe S, Daniel M, Choji R, Mamman I, *et al*. Comprehensive reference ranges for hematology and clinical chemistry laboratory parameters derived from normal Nigerian adults. PLoS One.2014; 15:9(5).
23. Heba B, Kiyoshi I, May S, Ahmed A, Sarah A, Dalia R, *et al*. Establishment of reference intervals of clinical chemistry analytes for the adult population in Egypt. PLoS One.2021; 16(3).
24. https://worldpopulationreview.com/world-cities/addis-ababa-population.

25. Anwar B, Kiyoshi I, Abdulaziz A, Waleed T, Suhad B, Mustafa N. Establishment of reference intervals of clinical chemistry analytes for the adult population in Saudi Arabia. Journal of Clin Chem Lab Med .2016; 54(5):843–855.
26. Mehmet K, Serap C. Determination of Reference Intervals of Healthy Adults Aged Between 20-50 Years in Izmir. Turk J Biochem.2010; 35 (3).
27. Rukia K, Christian B, Frederick S, Douglas S, Warren B, Nelson L, *et.al*. Reference Ranges for the Clinical Laboratory Derived from a Rural Population in Kericho, Kenya. PLoS One.2008;3.
28. Toth P, Barter J, Rosenson S. High-density lipoproteins: a consensus statement from the National Lipid Association. J Clin Lipidol.2013; 7:484–525.
29. Richard M, Joseph K, Stanley K, Eliud N. Reference Intervals for Serum Biochemistry Analyses for Pregnant Mothers of Taita-Taveta County, Kenya. IJRSI.2020; 7.
30. Anna K , Pal B, Nina P , Malene R , Maja J , Brian B, *et al*. Laboratory reference intervals during pregnancy, delivery and the early postpartum period. Clin Chem Lab Med. 2010 ;48(2):237–248
31. Seako M, Ishmael K. Reference intervals for serum total cholesterol, HDL cholesterol and non-HDL cholesterol in Botswana adults**.** S Afr Med J.2011; 25:101-8. PMID:21920130
32. Alice T, Linda P, Elie K, Issaka S, Jacques S, Jean S. Reference Ranges of Cholesterol SubFractions in Random Healthy Adults in Ouagadougou, Burkina Faso. PLOS one .2015; 10:1.
33. Center, South West Ethiopia: Facility-based cross sectional study**.** PLoS One.2020:5.
34. Guetta V, Ocannon R .Cardiovascular Effects of Estrogen and Lipid-Lowering Therapies in Postmenopause women heart association AHA.1996;93: 1928-1937.
35. Sara R, Lisa A. Stephany M, Akin R, Kaith R, Doug **L, *et* *al.*** Evaluation of sex differences in acid/base and electrolyte concentrations in acute large vessel stroke. Elsevier Inc. 2020;323**.**PMID:31697942
36. Masayuki O, Keiko A, Satoshi S, Keiko S. Twenty-four-hour urinary sodium and potassium excretion and associated factors in Japanese secondary school students.MPG.2016;39:524–529.
37. Kaaja R. Insulin resistance syndrome in preeclampsia. Sem Reprod Endocr.1998;16(1):41-46.

38. Pillay P, Piercy C, Tolppanen H, Mebazaa A. Physiological changes in pregnancy. Cardiovasc J Afr. 2016; 27(2):89–94.
39. Blackburn S. Maternal, fetal and neonatal physiology. a clinical perspective. 4th ed. Maryland Heights, MO: Elsevier Saunders; 2013.
40. Hongliang W, Qinyu D , Haiyan Z, Ning L , Zhiyin L, Dongxu H, *et al* .Associations between maternal serum HDLc concentrations during pregnancy and neonatal birth weight: a population-based cohort study. BMC.2020;19:93. https://doi.org/10.1186/s12944-020-01264-0.
41. Anne B, Keelin O. Cholesterol in pregnancy: a review of knowns and unknowns. Journal of Obstetric Medicine.2011; 9.
42. Honda T, Honda K, Kokubun C, Nishimura T, Hasegawa M, Nishida A, *et al*. Time-course changes of hematology and clinical chemistry values in pregnant rats. J Toxicol Sci .2008; 33:375–80.
43. Yanamandra N, Chandra Haran E.Anatomical and physiological changes in pregnancy and their implications in clinical practice. Cambridge University Press. 2012; 1–7.

10. Annexes

Annex I- Standard operating procedures (SOPs)

Stool specimen collection and handling: Stool samples will be collected in a clean, dry clean stool cup. During the study, a total of 522 fresh stool samples will be collected strictly following standard operational procedures with sterile stool cups. Proper stool specimens will be taken from each participant to reduce the chance of occurrence of false negatives. Then a drop of normal saline was put on the cleaned microscope Slides, a small amount of stool specimen with a wooden stick will be taken and mixed with saline and examined as soon as possible (within 30 minutes of passage). And the leftover stool sample will be preserved with 10% formalin and transported to the side laboratory following a safe transportation manner.

Stool sample processing for Formol ether Concentration: a fresh stool sample will be dispensed into 10 ml of 10% formalin in a round bottom tube and the stool and formalin will be mixed thoroughly and will let the mixture stand for a minimum of 30 min for fixation. Strain a sufficient quantity through wet into a conical 15ml centrifuge tube to give the desired amount of sediment (0.5 to 1 ml), 10% formalin will be added to the top of the tube and centrifuge for 10 min at 500 x g. Supernatant fluid will be discarded and suspend the sediment on the bottom of the tube, ethyl acetate will be added and shake vigorously by holding the tube so the stopper is directed away from our face. Centrifuge for 10 min at 500 x g. The sediment will be examined using 10 X and 40 X microscopic examination.

Performing the urine Chemical Test by Reagent Strip

Fill name and the date on the lab sheet for Chemical Examination of Urine. Using the urine controls package insert provided by your instructor, accurately record the Expected Range for each test pad for both controls. Accurately record the name and identification number of each participant's sample. Carefully mix urine by inverting the tube or swirling urine if it is in a cup. When testing participants' samples, observe and record color and clarity. Carefully remove one strip from the container, taking care not to allow reagent pads to touch hands or other surfaces. Recap container of strips immediately finger tight. Exposure to air will cause deterioration of the chemicals on the pads. Briefly, (no longer than 1 second) dip test strip into the urine making sure that all pads are moistened. Draw the edge of the strip along the rim of the specimen container to remove excess urine. Start the timer when you have removed the moistened strip from the urine or control. Blot edge of strip on bio wipe or paper towel to remove excess urine. Failure to blot may result in chemicals from adjacent pads "bleeding" into each other causing erroneous results. Read each pad at the time shown on the strip container, starting with the shortest time. Hold the strip close to the color blocks but Do Not Allow the Wet Strip to Touch the Color Chart. Match the colors carefully – THIS IS CRITICAL. Failure to read the reaction at the time indicated may cause erroneous results. Record results on the report from using appropriate units as necessary. Negative results should be reported out as "Neg". Positive results should be reported in the proper format, using appropriate units where indicated. Discard the reagent strip into regular trash when you have finished recording the results.

High sensitive C- reactive protein test procedures

The human serum containing CRP mixed with latex particles coated with monoclonal antiCRP antibodies After few minutes the presences of CRP leads to precipitation and color development then the precipitate is determined turbid metrically with fully automated Cobas c501 analyzer

Annex II: Information sheet for adults (≥18 years)

Research Title: Establishment of lipid profile and electrolytes reference intervals for Addis Ababa children and adolescents.

Principal Investigator: FikiriteAboneh

Organization: Addis Ababa University

Sponsor: Ministry of Science and Technology (MoST), Ethiopia

Introduction:

Hello! My name is FikirteAboneh and I am working with researchers from the various Medical Laboratory Science teaching Universities, Regional Laboratories, National Blood Bank of Ethiopia and EMLA. We are conducting a study to develop In-House Quality Control Material and Establish Immuno-Hematological and Clinical Chemistry Reference Intervals For Ethiopians aged ≥18years from various localities in the country.

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 pathophysiology of a particular disease process). Thereis a lack of local reference interval for indigenous population and local quality control materials. Therefore, the purpose of this proposed study is to develop In-House Quality Control Material and Establish Immuno-Hematological and Clinical Chemistry Reference Intervals for Ethiopians aged ≥18years in Addis Ababa Ethiopia.

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 and to develop in-house quality control materials. Both are needed for providing quality laboratory service. Thus, the result from this study is anticipated to improve the health status of the adult population at large in 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 tablespoon) from you by sterile-disposable vacutainer tube and needle (9ml in plane tube and 4 ml in tube containing EDTA). We will conduct laboratory examinations 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 of 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 samples (that are 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 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 against in any form of service like health.

Code No._____

Annex III. 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)

የፕሮጀክቱ ርዕስ፡ "እድሜአቸው አምስት ዓመትና ከዚያ በላይ ለሆኑ ኢትዬጵያውያን የጤናማ ሰው ደም ውስጥ የሚገኙ የክሊኒካል ላቦራቶሪ ምርመራዎች መጠን ሪፈረንስ ኢንተርቫል እና በላቦራቶሪ ውስጥ የጥራት መመርመሪያ ንጥረ ነገር መስራት "፡ በበርካታ ማዕከላት የሚሰራ ጥናት "

የፕሮጀክቱ ዋና ተመራማሪ: አስቴር ፀጋዬ (ፒ. ኤች. ዲ፣ በአዲስ አበባ ዩኒቨርስቲ የህክምና ላቦራቶሪ ትምህርት ክፍል ተባባሪ ፕሮፌሰር)

ተባባሪ ተመራማሪዎች የስም ዝርዝር ተያይዟል

ተቋማት፡ የኢትዮጵያ ህክምና ላቦራቶሪ ማህበር፣ ዩኒቨርስቲዎች፣ ሪጅናል ላቦራቶሪዎች፣ እና ብሄራዊ የደም ባንክ አገልግሎትየኢትዮጵያ ህክምና ላቦራቶሪ ማህበር፣

ስፖንሰር (ወጪውን የሸፈነው)፡ የፌደራል ሳይንስና ቴክኖሎጂ ሚኒስቴር

መግቢያ:

ጤና ይስጥልኝ! ስሜ _____ ነው፡፡ የህክምና ላቦራቶሪ ሳይንስ ትምህርት ከሚያስተምሩ ዩኒቨርስቲዎች፣ ሪጅናል ላቦራቶሪዎች፣ ብሄራዊ የደም ባንክ አገልግሎት እና የኢትዮጵያ ህክምና ላቦራቶሪ ማህበር ጋር እየሰራሁ ነው፡፡ በላቦራቶሪ ውስጥ የጥራት መመርመሪያ ንጥረ ነገር እና የጤናማ ሰው ደም ውስጥ የሚገኙ የሄማቶሎጂና የክሊኒካል ኬሚስትሪ ምርመራዎች መጠን ሪፈረንስ ኢንተርቫል እድሜአቸው አምስት ዓመትና ከዚያ በላይ ለሆኑ ኢትዮጵያውያን ለመስራት በአገራችን የተለያዩ ክልሎች ጥናት እያካሄድን ነው፡፡

የምርምር ጥናቱ አላማ:

የህክምና ላቦራቶሪ በጤናው አገልግሎት ውስጥ ከፍተኛ ሚና ይጫወታል፡፡ ምርመራን ለማረጋገጥ፣ ህሙማን ለመድሃኒቶች ምላሽ መስጠታቸውን ክትትል ለማድረግ፣ የበሽታዎችን ስርጭት ለማጥናት፣ በሽታ ለመከላከል እና ስለበሽታዎች ምንጭ ምርምር ለማድረግ አስተዋፅዎ ያደርጋል፡፡ በተለይም በአገራችን የጤናማ ሰው የላቦራቶሪ ውጤት ማመዳደሪያ ሪፈረንስ ኢንተርቫል እና በአገር ውስጥ የሚመረት የጥራት መመርመሪያ የለም፡፡ ስለሆነም የዚህ ጥናት ዓላማ በአገር ውስጥ በላቦራቶሪ ውስጥ የሚመረት የጥራት መመርመሪያ እና የጤናማ ሰው የሄማቶሎጂና የክሊኒካል ኬሚስትሪ ውጤት ማወዳደሪያ ሪፈረንስ ኢንተርቫል እድሜአቸው አምስትና ከዚያ በላይ ለሆኑ በተለያየ ክልል ለሚኖሩ ኢትዮጵያውያን መሥራት ነው፡፡

እርስዎም ለዚህ ጥናት ተመርጠኃል፡፡ ስለዚህ በዚህ ጥናት እንዲሳተፉና በአገራችን በላቦራቶሪ ውስጥ የሚመረት የጥራት መመርመሪያ እና የጤናማ ሰው የሄማቶሎጂና የክሊኒካል ኬሚስትሪ ውጤት ማመዳደሪያ ሪፈረንስ ኢንተርቫል ለመስራት አስተዋፅዎ እንዲያደርጉ ተጋብዘኃል፡፡ ሁለቱም ጥራት ያለው የላቦራቶሪ አገልግሎት ለመስጠት አስፈላጊ ናቸው፡፡ ስለዚህ የዚህ ጥናት ውጤት ኢትዮጵያ ውስጥ የአዋቂ ሰዎች ጤናን ለማሻሻል ይረዳል፡፡

የጥናቱ አካሄድ:

በጥናቱ ለመሳተፍ ከተስማሙ የጥናቱ አባል/አባላት 15 ደቂቃ የሚወስድ ጥያቄ ይጠይቁዎታል፡፡ ክብደት፣ ቁመት፣ የክንድ እና የደም ግፊት ልኬት ይወሰዳል፡፡ ሽንትና አይነምድር በምንሰጠው እቃ እንድትሰጡን እንጠይቃለን፡፡፣ በተጨማሪም 13 ሚሊ ሊትር (አንድ የሾርባ ማንኪያ የሚሆን) በንፁህ ቫኩቴይነር ብልቃጥ እና መርፌ እንቀዳለን (9ሚሊ ሊትር በባዶ ቲዩብ፣ 4 ሚሊ ሊትር ደም እንዳይረጋ የሚያደርግ ንጥረ ነገር ፣ኢዲቲኤ፣ ባለበት ቲዩብ)፡፡ የሄማቶሎጂ፣ ሴሮሎጂ፣ ፓራሲቶሎጂ እና የክሊኒካል ኬሚስትሪ ምርመራዎችን እናካሂዳለን፡፡

ሚስጥር ስለመጠበቅ:

በዚህ ጥናት የሚሰበሰብ መረጃ በሙሉ በሚስጥር ይጠበቃል፡፡ መረጃ በዚህ የስምምነት ቅፅ ከተፈቀደው ውጪ ለሶስተኛ ወገን ተላልፎ አይሰጥም፡፡ የዚህ ጥናት ውጤት ሊታተም ይችላል ነገር ግን የጥናቱ ተሳታፊዎች ስምና ማንኛውም መለያ አይገለፅም፡፡ ሚስጥራዊነቱን ለመጠበቅ የዚህ ጥናት አባላት መረጃዎችን በተቆለፈ ክፍል በተቆለፈ ካቢኔት ውስጥ ያስቀምጣሉ፣ የፈቃደኛ ተሳታፊዎችን ማንነትን ላለማሳወቅ ውጤቶችም በኮድ ይቀመጣሉ፡፡ በኮምፒዩተር ውስጥ ለተቀመጡ ፋይሎች ለጥናቱ ተመራማሪዎች ብቻ የሚፈቀዱና በሚስጥር ቁልፍ የሚጠበቁ ይሆናል፡፡ የተሳታፊ ውጤት ለህክምና ባለሞያ ሊተላለፍ የሚችለው በተሳታፊው ፈቃድ ብቻ ነው፡፡ የተሰበሰበው ሽንት፣ ዓይነምድርና ደም ለሌላ አገልግሎት አይውልም፡፡ የሚተርፉት ናሙናዎች በአዲስ አበባ ዩኒቨርስቲ ህክምና ላቦራቶሪ ትምህርት ክፍል ደህና ቦታ ተቀምጠው ለተጨማሪ ምርመራዎች እንደ አስፈላጊታቸው ጥቅም ላይ ይውላሉ፡፡ በመጨረሻም ተሰርቶባቸው የተራረፉ የሚደፉ ናሙናዎች አካባቢን በማይበክል መልኩ በጥንቃቄ ይወገዳሉ፡፡

ጥናቱ የሚያስከትላቸው የጤና ችግሮችና አለመመቸት:

ሽንትና ዓይነምድር በመስጠት የሚደርስ መጠነኛ አለመመቸት ሊኖር ይችላል፡፡ ሆኖም ደም በሚቀዳበት ጊዜ መጠነኛ መጎዳትና የተወሰነ አለመመቸት ሊኖር ይችላል፡፡ ይሁን እንጂ በተቻለ መጠን ልምድ ያለው የላቦራቶሪ ባለሞያ በመጠቀም አለመመቸቱን ለመቀነስ እንሞክራለን፡፡

ደህንነት:

የደም ናሙና በሚወሰድበት ጊዜ በንፁህ የደም መቅጃ በመጠቀም የሚቀዳውን ቦታ በ70% አልኮል በማፅዳት ልምድ ባለው ባለሞያ ይከናወናል፡፡ በተጨማሪም ጥቅም ላይ ከዋሉ በኋላ ለማስቀመጥ የማይሆኑ የሚደፉ የዓይነምድር፣ ሽንት እና ደም ትራፊዎች የላቦራቶሪ ደህንነት መመሪያ በመከተል ይወገዳሉ፡፡

ጥቅማ ጥቅሞች፡

በዚህ ጥናት በመሳተፍ ለበሽታ አምጪ ተህዋስያን፣ ደምና ሽንት ምርመራ በማድረግ የጤንነት ሁኔታ ማወቅ ይቻላል፡፡ በአገር ውስጥ በላቦራቶሪ ውስጥ የሚመረት የጥራት መመርመሪያ እና የጤናማ ሰው የሄማቶሎጂና የክሊኒካል ኬሚስትሪ ውጤት ማመዳደሪያ ሪፈረንስ ኢንተርቫል እድሜአቸው አምስትና ከዚያ በላይ ለሆኑ በተለያየ ክልል ለሚኖሩ ኢትዮጵያውያን መሰራቱ የኢትዮጵያውያንን የጤና ሁኔታ ለማሻሻል ይረዳል፡፡

በጥናቱ ለመሳተፍ ማትጊያ:

ከዓይነምድር፣ ሽንት እና ደም ምርመራ ጤናማ ያልሆነ ውጤት ከተገኘ በአቅራቢው ወደ ሚገኝ ጤና ተቋም ይላካሉ፣ የላቦራቶሪ ውጤቶቹን በነፃ ያገኛሉ፡፡ ይሁን እንጂ በዚህ ጥናት ለመሳተፍም ሆነ ለመድሃኒት ክፍያ አይሰጥም፡፡ ስለተሳትፎዎ ግን እናመሰግናለን፡፡

ያለመሳተፍ መብት:

በዚህ ጥናት ከተሳተፉ የቻልነውን ሁሉ እንክብካቤ እናደርጋለን፡፡ በማኛውም ሰዓት ከጥናቱ መውጣት እንደሚቻልና ይህም በሚያገኙት አገልግሎት ላይ (ለምሳሌ የጤና አገልግሎት) ምንም አይነት ልዩነት አይደረግም፡፡

ጥያቄ ካለ ለማነጋገር:

ምንም ዓይነት ጥያቄ ካለ የዓይነምድር፣ ሽንት እና የ ደም ናሙና የሰጡትን ሰው መጠየቅ ይቻላል ወይም የፕሮጀክቱ ዋና ተመራማሪን ወይም ተባባሪዎችና በየተቋሙ የሚገኙ ተወካዮችን በሚከተለው አድራሻ መጠየቅ ይቻላል፡፡

1. ዶ/ር አስቴር ፀጋዬ፣ መሪ ተመራማሪ፣ አ.አ.ዩ/አ.አ. ሪጅናል ላቦራቶሪ 09 11 696085

በአዲስ አበባ ዩኒቨርስቲ የጤና ሳይንስ ኮሌጅ የምርምር ስነምግባር ቢሮ ስልክ፡ +251 -11-896-13 96

ኮድ._____

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

ከላይ የተገለፀውን መረጃ አንብቤአለሁ /ወይም ተነቦልኛል፡፡ ጥያቄ ለመጠየቅ ዕድል ተሰጥቶኝ ጠይቄ በሚያረካ መልኩ ተመልሶልኛል፡፡ በዚህ ጥናት ለመሳተፍ በፈቃደኝነት ተስማምቻለሁ፡፡

የ ዓይነምድር ናሙና ለመስጠት ☐

የሽንት ናሙና ለመስጠት ☐

ደም ለመቀዳት ☐ እና በዚህ ጥናት ተሳታፊ ለመሆን፣ በማንኛውም ሰዓት ከጥናቱ ለመውጣት መብት እንዳለኝም ተረድቻለሁ ☐.

የተሳታፊ ስም፣ ቀን እና ፊርማ (ወይም አሻራ) ከዚህ በታች ይፃፉ

_____ _____ /_____ /_____ (ቀን/ወር/ዓመተ ምህረት)

ያልተማሩ ከሆኑ;

የተማሩ ገለልተኛ እማኝ ሰው ስም፣ ቀንና ፊርማ (ከተቻለ ይህ ሰው በተሳታፊው ቢመረጥና ከተመራማሪ አባላት ግኑኝነት የሌለው ቢሆን)

_____ _____/_____/_____ (dd/mm/yy) _____

ስልክ ቁጥር _____

የተመራማሪው ስም፣ ቀንና ፊርማ

_____ _____/_____/_____ (dd/mm/yy) _____

Annex IV. 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

1. Age (in years) _____
2. Sex _____
3. Place of Birth
4. For how long (years) did you live in the birth place? _____
5. How long do you live in this specific area? (If different from the birth place) _____years

No.	Questions	Responses
	Part III. SOCIO-DEMOGRAPHIC INFORMATION	
6.	Educational status	1. Illiterate 2. Read and write 3. Primary (1-8) 4. Secondary (9-12) 5. College diploma/degree and above
7.	Occupation	1. Student 2. House wife 3. Government employee 4. Private employee 5. Farmer 6. Others (specify) _____
8.	Marital status	1. Single 2. Married 3. Divorced 4. Widowed 5. Not applicable (children)
9.	Religion	1. Orthodox Christian 2. Muslim 3. Protestant 4. Catholic 5. Others (Specify) _____
10.	Ethnicity	_____If mixed, specify _____
11.	Residence	1. Rural 2. Urban
	Questions 7-12 are additional questions to Students	
12.	Father's Age	_____
13.	Mother's Age	_____
14.	Father's Educational Level	1. Illiterate 2. Read and write 3. Primary (1-8) 4. Secondary (9-12) 5. College diploma/degree and above
15.	Mother's Educational Level	_____

16.	Father's Occupation	
17.	Mother's Occupation	
18.	Monthly income (in birr collected from salary, rent, and other income)	_____Birr
19.	Family Size (Number of People)	_____
20.	Source of water	1. Pipe 2. Spring water 3. Well water 4. River 5. Other sources (specify)
21.	Type of house	1. Mud 2. Cement 3. Wood 4. Bricks 5. others/specify_____
22.	Presence of or contact with Pet animals (e.g. Cat, Dog)	1. Yes 2. No
23.	Presence of domestic animals	1. Yes 2. No
	Part IV. Clinical information	
	Questions 24-28 for female participant who are pregnant specify	
24.	Gestation _____(weeks)	
25.	Parity _____	
26.	Iron supplementation:	1. Yes 2. No
27.	Folate supplementation	1. Yes 2. No
28.	Iron and folate combined supplementation	1. Yes 2. No
29.	Did you take any type of drug for any illness for the last three months?	1. Yes 2. No
30.	If yes to Q29, what type of drug? (more than one answer possible)	1. Anti-protozoa 2. Anti-helminthic 3. Anti-allergy 4. Birth control pills 5. Anti-bacterial 6. Anti-TB

		7. Other (specify) _____
	History of common diseases	
31.	History of diabetes	1. Yes 2. No
32.	History of Hypertension	1. Yes 2. No
33.	History of Blood transfusion for the last 1 year	1. Yes 2. No
34.	Any history of blood transfusion	1. Yes 2. No
35.	History of Hospital Admission for the last 1 year	1. Yes 2. No
36.	History of Surgical procedure for the last three years?	1. Yes 2. No
37.	History of chronic gastritis	1. Yes 2. No
38.	History of Malaria for the last 6 month	1. Yes 2. No
39.	History of TB for the last two years	1. Yes 2. No
40.	History of Cancer	1. Yes 2. No
41.	History of Cardiac illness	1. Yes 2. No
42.	History of Bleeding disorders	1. Yes 2. No
43.	History of allergy	1. Yes 2. No
44.	History of Wheezing	1. Yes 2. 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**
45.	Roots and Tuber (Potato, sweet potato, Enset, Cassava)						
46.	Legumes (Beans, peas, chicken pea, etc)						
47.	Cereals (Corn, Teff, Wheat, sorghum, etc)						
48.	Vegetables (Tomato, cabbage, etc)						
49.	Fruits (Orange, banana, etc)						
50.	Meat (including poultry, fish, etc)						
51.	Milk and Milk products						

	(Butter, yoghurt, cheese, etc)						
52.	Egg						
53.	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
54.	Alcohol						
55.	*Khat*						
56.	Cigarettes						

	Part V. Life style/Habit Continued…	
57.	Do you have Fasting habit?	1. Yes 2. No
58.	If Yes, how is your fasting habit?	1. Eating vegetable food only 2. Complete abstinence from food then eating all kinds of food 3. Complete abstinence from food then eating vegetable food only
59.	Did you eat undercooked/raw meat?	1. Yes 2. No
60.	Do you have the habit of physical Exercise?	1. Yes 2. No
61.	If yes, how many times do you do the exercise per week?	
62.	Any sexual contact	1. Yes 2. No 3, Not applicable (children)
63.	If yes to Q45, condom use`	1. Yes 2. No
	Part VI. Anthropometric measurement	
64.	Height (in cm)	_____
65.	Weight (in kg)	_____
66.	MUAC	_____in cm (will be interpreted later)
67.	Blood pressure (mm Hg)	_____

❖ We thank you for your cooperation!

Interview Date: _____

Interviewer's Name_____ Signature_____

Annex XIV: Questionnaire Amharic version (ቃለ መጠይቅ)

በጤና ባለሞያዎች የሚሞላ ቃለ መጠይቅ

መመሪያ:

በቅድሚያ ይህንን ቃለ መጠይቅ ለመሙላት ለሰጡን ጊዜና ትብብር አድናቆቴን እገልፃለሁ፡፡ የዚህ ቃለ መጠይቅ አላማ "በላቦራቶሪ ውስጥ የጥራት መመርመሪያ ንጥረ ነገር እና የጤናማ ሰው ደም ውስጥ የሚገኙ የሄማቶሎጂና የክሊኒካል ኬሚስትሪ ምርመራዎች መጠን ሪፈረንስ ኢንተርቫል እድሜአቸው አምስት ዓመትና ከዚያ በላይ ለሆኑ ኢትዬጵያውያን ለመስራት" መረጃ ለመሰብሰብ ነው፡፡ የዚህ ጥናት ሃሳቡን ያመጡት የጥናቱ ዋና ተመራማሪ በአዲስ አበባ ዩኒቨርስቲ የህክምና ላቦራቶሪ ትምህርት ክፍል ተባባሪ ፕሮፌሰር የሆኑት ዶ/ር አስቴር ፀጋዬ ሲሆኑ የኢትዮጵያ ህክምና ላቦራቶሪ ማህበር ያስተዳድረዋል፡፡ የጥናቱን ወጪ የሸፈነው የፌደራል ሳይንስና ቴክኖሎጂ ሚኒስቴር ነው፡፡ ስለሆነም የእርስዎ ቅን ትክክለኛ መልስ በሰዓቱ መስጠት የዚህን ጥናት ስኬት ይወስናል፡፡ አስራ አምስት የሚሆኑ ተቋማት ማለትም ዩኒቨርስቲዎች፣ ሪጅናል ላቦራቶሪዎች፣ እና ብሄራዊ የደም ባንክ አገልግሎት ጥናቱን ለመደገፍ ዝግጁነታቸውን ገልፀዋል፡፡ ስለሆነም ይህንን ቃለ መጠይቅ ሃቀኝነትና ሃላፊነት በተሞላው መንገድ እንዲሞሉ በትህትና እጠይቃለሁ፡፡

አመሰግናለሁ!!!

ክፍል 1. አጠቃላይ መረጃ

ኮድ_____ ክልል _____ ዞን_____

ወረዳ_____ ከተማ/ክፍለከተማ _____ቀበሌ _____

ክፍል 2. የግል መረጃ

1. እድሜ _____
2. ጾታ_____
3. የትውልድ ቦታ_____
4. በትውልድ ቦታዎ ለምን ያህል ጊዜ ኖረዋል?_____
5. አሁን ያሉበት ቦታ ለምን ያህል ጊዜ ኖረዋል? (ከትውልድ ቦታዎ የተለየ ከሆነ) _____ዓመት

ቁጥር.	ጥያቄ	ምላሽ
	ክፍል 3. ማህበራዊና ኢኮኖሚያዊ መረጃ	
24.	የትምህርት ደረጃ	6. ያልተማሩ 7. ማበብና መፃፍ 8. አንደኛ ደረጃ (1-8) 9. ሁለተኛ ደረጃ (9-12) 10. ኮሌጅ ዲፕሎማ/ዲግሪ እና ከዚያ በላይ
25.	ሥራ	7. ተማሪ 8. የቤት እመቤት 9. የመንግስት ሠራተኛ 10. የግል ተቀጣሪ 11. ገበሬ 12. ሌላ ካለ ይግለፁ _____
26.	የጋብቻ ሁኔታ	6. ያላገቡ 7. ያገቡ 8. የተፋቱ 9. ባል/ሚስት የሞተባቸው 10. አይመለከታቸውም (ህፃናት)
27.	ሃይማኖት	6. ኦርቶዶክስ ክርስቲያን 7. ሙስሊም 8. ፕሮቴስታንት 9. ካቶሊክ 10. ሌላ ካለ ይግለፁ _____
28.	ብሄረሰብ	_____ድብልቅ ከሆኑ ይግለፁ
29.	መኖሪያ ቦታ	2. ገጠር 2. ከተማ
	ጥያቄ 7-12 ለተማሪዎች ተጨማሪ ጥያቄዎች	
30.	የአባት እድሜ	_____
31.	የእናት እድሜ	_____
32.	የአባት የትምህርት ደረጃ	6. ያልተማሩ 7. ማንበብና መፃፍ 8. አንደኛ ደረጃ (1-8) 9. ሁለተኛ ደረጃ (9-12) 10. ኮሌጅ ዲፕሎማ/ዲግሪ እና ከዚያ በላይ

33.	የእናት የትምህርት ደረጃ (ከተ/ቁ 14 ይምረጡ)	_____
34.	የአባት ሥራ	1. ተማሪ 2. የቤት እመቤት 3. የመንግስት ሠራተኛ 4. የግል ተቀጣሪ 5. ገበሬ 6. ሌላ ካለ ይግለፁ _____
35.	የእናት ሥራ (ከተ/ቁ 16 ይምረጡ)	_____
36.	ወሃዊ ገቢ (በብር ከደሞዝ፣ ኪራይ፣ እና ሌሎች ገቢዎች)	_____ብር
37.	የቤተሰብ ብዛት	_____
38.	የውሃ ምንጭ	6. ቧንቧ 7. የምንጭ 8. የጉድጓድ 9. የወንዝ 10. ሌላ ካለ ይግለፁ _____
39.	የቤት አይነት	2. ጭቃ 2. ሲሚንቶ 3. እንጨት 4. ጡብ/ሸክላ 5. ሌላ ካለ ይግለፁ _____
40.	የቤት ውስጥ ለማዳ እንስሳ መኖር ወይም ንክኪ (ለምሳሌ ድመት፣ ውሻ)	2. አለ 2. የለም
41.	የቤት እንስሳት መኖር	2. አለ 2. የለም
	ክፍል 4. የጤና መረጃ	
	ከ 24-28 ያሉት ጥያቄዎች ለነፍሰጡር ሴቶች ብቻ ነው	
45.	ከፀነሱ ስንት ጊዜዎ ነው?	_____(ሳምንት)
46.	ለስንተኛ ጊዜ ነው የፀነሱት?	_____
47.	ተጨማሪ ብረት ንጥረነገር	2. አዎን 2. የለም
48.	ተጨማሪ ፎሌት ንጥረነገር	2. አዎን 2. የለም
49.	ተጨማሪ የብረት ንጥረነገር ና ፎሌት	2. አዎን 2. የለም
50.	ባፉት ሶስት ወራ ለማንኛውም ዓይነት ህመም ማንኛውንም	2. አዎን 2. የለም

	ዓይነት መድሃኒት ወስደኋል?	
51.	ለተራ ቁጥር 29 መልስዎ ወስጃለሁ ከሆነ የትኛውን ዓይነት መድሃኒት ነው ወሰዱት? (ከአንድ በላይ መልስ ይቻላል)	8. ፀረ-ፕሮቶዞአ 9. ፀረ-ሄልሚንትስ 10. ፀረ-አለርጂ 11. የወሊድ መከላከያ ኪኒን 12. ፀረ-ባክቴሪያ 13. ፀረ-ቲቢ 14. ሌላ ካለ ይግለፁ _____
	የሚከተሉት የህመም ዓይነቶች አሞዎት ያውቃል?	
52.	የስኳር ህመም?	2. አዎን 2. የለም
53.	የደም ግፊት ከፍ ማለት?	1. አዎን 2. የለም
54.	ባለፈው 1 ዓመት ደም ተሰጥቶዎ ያውቃል?	1. አዎን 2. የለም
55.	ማንኛውም ጊዜ ደም ተሰጥቶዎ ያውቃል?	1. አዎን 2. የለም
56.	ባለፈው 1 ዓመት ሆስፒታል ተኝተው ያውቃሉ?	1. አዎን 2. የለም
57.	ባለፉት 3 ዓመታት የቀዶ ህክምና ተደርጎልዎ ያውቃል?	1. አዎን 2. የለም
58.	የቆየ የጨጓራ ህመም አለብዎት?	1. አዎን 2. የለም
59.	ባፉት 6 ወራት የወባ ህመም አጋጥሞዎት ያውቃል?	1. አዎን 2. የለም
60.	ባለፉት 2 ዓመታት የቲቢ ህመም ኖሮዎት ያውቃል?	1. አዎን 2. የለም
61.	ካንሰር ህመም	1. አዎን 2. የለም
62.	የልብ ህመም	1. አዎን 2. የለም
63.	የመድማት ችግር/ህመም	1. አዎን 2. የለም
64.	አለርጂ (የሰውነት መቆጣት)	1. አዎን 2. የለም
65.	የመተንፈስ ችግር (ሲተነፍሱ ሲር ሲር የሚል ድምፅ)	1. አዎን 2. የለም

ክፍል 5. የአመጋገብ እና የህይወት ልምድዎ

የሚከተሉትን የምግብ ዓይነቶች ምን ያህል ጊዜ ይመገቧቸዋል ? (“√ “ ይህን ምልክት ያስቀምጡ)							
ተ/ቁ	**የምግብ ዓይነት**	**1** **በቀን አንድ ጊዜ**	**2** **በቀን ከአንድ ጊዜ በላይ**	**3** **በሳምንት ከ 2 እስከ 3 ጊዜ**	**4** **አልፎ አልፎ (ለምሳሌ፣ ለበዓል፣ ልዩ ዝግጅቶች ሲኖሩ)**	**5** **ተጠቅሜ አላውቅም**	**ማብራሪያ**
45.	ሥራ ሥር (ድንች፣ ስኳር ድንች፣ እንሰት፣ ካሳቫ ወዘተ)						
46.	አባዝርት (Legumes፡ ባቄል፣ አተር፣ ሽንብራ ወዘተ)						

47.	ጥራጥሬ (በቆሎ፣ ጤፍ፣ ስንዴ፣ ማሽላ)						
48.	አትክልት (ቲማቲም፣ ጎመን፣ ወዘተ)						
49.	ፍራፍሬ (ብርትኳን፣ ሙዝ፣ ወዘተ)						
50.	ሥጋ (የዶሮ፣ የ አሳን ጨምሮ)						
51.	ወተትና የወተት ተዋፅዎ (እርጎ፣ ቅቤ፣ አይብ፣ ወዘተ)						
52.	እንቁላል						
53.	ሻይ እና/ወይም ቡና						
የሚከተሉትን ምን ያህል ይበላሉ/ይጠቀማሉ (√ ይህን ምልክት ያስቀምጡ)							
		በቀን አንድ ጊዜ (ሁልጊዜ)	በቀን ከ1 ጊዜ በላይ	በሳምንት ከ 2 እስከ 3 ጊዜ	በሳምንት 1 ቀን	አልፎ አልፎ (ለምሳሌ፣ ለበዓል፣ ልዩ ዝግጅቶች ሲኖሩ)	ተጠቅሜ አላውቅም
54.	አልኮል						
55.	ጫት						
56.	ሲጋራ						

	ከ ክፍል 5 የቀጠለ. የህይወት አመራርና ልምዶች	
57.	የመዖም ልምድ አለዎት?	2. አዎን 2. የለም
58.	መልስዎ አዎን ከሆነ፣ የመዖም ልምድዎ እንዴት ነው?	4. አትክልቶችን ብቻ መመገብ 5. በአጠቃላይ ከምግብ መታቀብ ከዚያም ያገኙትን መመገብ 6. በአጠቃላይ ከምግብ መታቀብ ከዚያም አትክልቶችን መመገብ
59.	በደንብ ያልበሰለ ወይም ጥሬ ሥጋ ይመገባሉ?	2. አዎን 2. የለም
60.	የሰውነት እንቅስቃሴ የማድረግ ልምድ አለዎት?	2. አዎን 2. የለም
61.	መልስዎ አለኝ ከሆነ በሳምንት ለምን ያህል ጊዜ ይንቀሳቀሳሉ?	
62.	የግብረ ሥጋ ግኑኝነት አድርገው ያውቃሉ	1. አዎን 2. የለም 3, አይመለከትም (ለህፃናት)
63.	ለ ተ/ቁ 66 መልስዎ አዎን ከሆነ፣ ኮንዶም ይጠቀማሉ?`	2. አዎን 2. የለም
	ክፍል 6. ክብደት፣ ቁመት፣ የክንድና የደም ግፊት ልኬት	

64.	ቁመት	_____ሴንቲ ሜትር
65.	ክብደት	_____ኪሎ ግራም
66.	የክንድ መሃለኛው ክፍል ዙሪያው (MUAC)	_____ሴንቲ ሜትር
67.	የደም ግፊት (በሚሊሜትር ሜርኩሪ)	_____(mm Hg)

❖ ስለትብብርዎ እናመሰግናለን!

ቃለ መጠይቅ የተደረገበት ቀን:_____

ቃለ መጠይቁን ያካሄደው ስም_____ ፊርማ_____

Declaration

I, the undersigned, declare that this MSc thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

M.Sc. candidate: Fikirite Aboneh (BSC)

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: Mr. Samuel Kindie (MSc, PhD candidate)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Aster Tsegaye (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia