Addis Ababa University
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Establishment of Reference Intervals for Selected Clinical Chemistry Parameters among Apparently Healthy Adults in Addis Ababa, Ethiopia

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This is to certify that the thesis prepared by Melkitu Kassaw, entitled: Establishment of reference intervals from selected clinical chemistry parameters among apparently healthy adults in Addis Ababa, Ethiopia, 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.

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Abbreviations

ALB……...Albumin

ALP……...Alkaline phosphatase

ALT……...Alanine aminotransferase

AST……Aspartate aminotransferase

BIL……..Bilirubin

BMI…….Body mass index

BUN……Blood Urea Nitrogen

CI…….Confidence Interval

CK……...Creatinine Kinase

EPHI……Ethiopia Public Health Institute

ETB…….Ethiopian Birr

HBsAg…… Hepatitis B surface antigen

HBV…….hepatitis B virus

HCG…….Human Chorionic Gonadotropin

HCV…… Hepatitis C virus

HGB……Hemoglobin

HIV…….Human Immunodeficiency Virus

IFCC……International Federation for Clinical Chemistry and Laboratory Medicine

NCCLS…….National Committee for Clinical Laboratory Standards

PPS……..Probability Proportional to Size

QC……... Quality Control

SD……Standard Deviation

WHO……World Health Organization

Abstract

Background: Reference interval values are used as medical decision-making tools that are given by clinical laboratory to assist health professionals, medical researchers for clinical trials and for physician to diagnosis, treatment, and follow-up of patients..

Objective: This study aimed to establish reference interval for selected clinical chemistry parameters among apparently healthy adults living in Addis Ababa, Ethiopia.

Method: community based cross sectional study was conducted from March to October 2019 recruiting a total of 448 participants aged from 18-60 years. Medical history, physical examination, anthropometric and socio-demographic data were collected using structured questionnaire. Serum samples were analyzed for selected clinical chemistry parameters using Cobas 6000. Reference intervals were established by calculating 2.5^{th}-97.5^{th} percentile with 90%Cl after excluding outliers using tukey method, Kolmogorov-Sminorv test. The difference between groups was computed using Mann-Whitney test by using SPSS version 23. P-value of <0.05 was taken as statistically significant.

Result: In adults the reference interval of males and females were ALT 1.60-16.89U/l against females 0.47-9.87U/l, AST 8.11-22.50 vs.6.95-18.05 U/l, ALP 44.0-181.65U/l; Albumin 4.40-5.88U/l vs.4.03-5.25U/l, T.protein 6.76-9.57g/l vs. 6.45-8.21g/l, T.bilirubin 0.26-1.15 vs.0.15-0.64mg/dl, D. bilirubin 0.08-0.46mg/dl vs. 0.061-0.25mg/dl, Glucose 62.93-99.39mg/dl vs.59.14-90.72mg/dl, Urea 13.21-33.51mg/dl vs.7.30-12.00mg/dl, Creatinine 0.56-1.02mg/dl vs.0.40-0.80mg/dl and Uric acid 3.41-7.40mg/dl, vs.1.88-5.60mg/dl, respectively. Statistically significant differences were observed between males and females except Albumin and Total proteins.

Conclusion: the reference interval for selected clinical chemistry parameters of adults was lower than the values given by manufacturers and Africans studies. Hence, it is more useful using locally established reference interval than derived from other countries.

Key words: Reference Interval, clinical chemistry,

1. Introduction

1.1 Background

Clinical chemistry and molecular diagnostics are both disciplines of laboratory medicine that study molecules to assist clinical diagnoses. Clinical chemistry is fundamental to diagnostics with millions of tests performed daily to guide patient therapy. Parameters in clinical chemistry indicate how well organ systems are functioning. Enzymes are proteins with catalytic properties, help to accelerate chemical reactions in the body. They are essential for respiration, food digestion, muscle and nerve function among thousands of other functions. Most enzymes work in human body at 37^0c [1]. Clinical enzymology is the application of the science of enzymes to the diagnosis and treatment of disease. Selected Serum enzymes are measured in medical diagnosis to detect injury to a tissue that makes up the measured enzyme. Clinical applications have concentrated mostly on enzymes such as transferases, phosphatase, creatinekinase, γ-glutamyltransferase, lactate dehydrogenase, lipase, and amylase. Liver disease is the most important cause of increased transaminases activity in serum. Enzymes in this category include Alanine transaminases, Aspartate transaminases and alkaline phosphatase [1-3].

Proteins are large and complex molecules made up of hundreds or thousands of smaller units of amino acids that play many vital roles in the body. They do most of the work in cells and are required for catalysis of biochemical reactions, mechanical support and immune protection, movement, transport of ligand, transmits nerve impulses, and control growth and differentiation. Low levels can mean damage or indicate disease condition [4].Bilirubin is made when red blood cells are break down. Usually, the liver cleans out bilirubin from our body. If high level of bilirubin is found in blood it indicates, a problem called jaundice which may cause liver damage [1, 2]. All the blood passes through kidneys' filtered to remove wastes, and control the body's fluid balance and to keep the right levels of electrolytes. Excreted waste products produced from metabolic activity like uric acid from the nucleic acid metabolism, urea from protein and creatinine and the level of those waste products help to assess the well-functioning of the kidneys [5]. The reference interval established for selected clinical chemistry parameters based on local population is valuable for early diagnosis and monitoring of disease [6].

In the area of reference intervals, as an earlier study was reported by Grasbeck and Fellman who published a paper entitled ‘Normal Values and Statistics’ [7].

After presentation of‘ Establishment and Use of Normal Values’ by Grasbeck and Saris, it was realized that the terminology of ‘normal values’ was not adequate and even partially incorrect, so the term ‘reference values’ came into use [8]. Clinical laboratories have for many years reported results Relative to ‘normal’ values However, as pointed out by the philosopher Edmund Murphy [9]. The word ‘normal ’can be interpreted from many frames of reference. The use of the term ‘normal’ has, however, been discouraged, because it can assume different meanings. According to Murphy listed seven of them are statistical – Gaussian distribution – most representative of a class, most commonly present in a class, most suited for survival, that does not harm – in medicine – conventional, ideal [9].

Reference intervals are used both in clinical and research environment. Medical laboratory Reference intervals are primarily utilized for clinical purposes [10] and used as an indicator of good health. On the other hand, reference intervals or limits can be used to screening physiological or pathological conditions. Therefore, it is important in routine health assessment, mainly for screening of diseases [10]. The RI is the most widely used medical decision-making tool[11] and also used for accurate interpretation of laboratory data so as to provide assistance to the clinicians in creating a more comprehensive clinical perspective for diagnosis and management of patients [12]. About 80% medical decisions might be depending upon the laboratory test results to diagnose the patient [13]. The major challenge to generate reference intervals (RIs) from the general population is selection of the appropriate reference groups. Recruiting individuals who represent related demographic groups have to meet the inclusion criteria. In addition, the collection process, analysis of the sample, calculating the reference values with possible stratification of the data into subgroups are among the challenges [14].

The National Committee for Clinical Laboratory Standards (NCCLS) now called Clinical and Laboratory Standards Institute (CLSI) has described a design for the determination of RI. The C28-A3 guideline constituted the most significant step in the development of RIs and is still in recent use. It is recommended that “each laboratory should have its own reference values” and estimate the corresponding RIs according to defined procedures [15]. The guideline provides necessary steps how to select the reference individuals, pre-analytical and analytical considerations, and analysis of reference values for RI establishment study [15] which is an important mission for a clinical laboratory.

Reference individuals selected for the determination of reference intervals should closely resemble the patient population undergoing medical examination and should be of similar age to be clinically significant [16]. The CLSI recommended a minimum of 120 individuals to allow 90% confidence limits to be non-parametrically calculated for the reference limits. The non-parametric methods are used to analyze values taken from the sample group to establish the 2.5 and 97.5 percentiles to form the 95% of reference interval. The direct method which is recommended by CLSI needs healthy individuals as reference. [17] In laboratory, reference interval is the interval between, and including, two reference limits. It gives the boundaries between which a typical measurement is expected to fall high or low. When a measurement occurs that is outside these reference interval boundaries, there is cause for concern. The reference interval is often presented as percentiles of a reference population [18].

Stratification by age and gender are usually the minimum prerequisite. It is necessary to subdivide reference ranges into partition groups. Each partition group should contain 120 samples [17, 18]. Clinical chemistry parameters vary considerably in terms of age, lifestyle, ethnicity, gender, nutrition and other environmental factors [19]. Analytes predictably demonstrate gender specific differences that are great enough to justify maintaining separate gender intervals. Examples of this is creatinine for which the difference in muscle mass between males and females results in both statistically and clinically significant differences [20].Despite several factors affecting reference intervals, the intervals provided on manufacturers' inserted sheets are used in most clinical chemistry laboratories in resource limited settings including Ethiopia. Studies have also demonstrated differences between urban and rural populations due to diverse reasons like, feeding habit, way of life, geographical locations, gender, climate and heredity [21].These suggest that the establishment of selected reference intervals specific for apparently healthy adults living in Addis Ababa is critical for interpretation of laboratory test results and prerequisite of quality services in the Health care delivery.

1.2 Statement of the problem

Several factors including age, gender, lifestyle, ethnicity, nutrition and other environmental factors as well as methods used for testing analytes affect clinical chemistry parameters considerably [16]. The clinical chemistry reference values which are currently in use in Addis Ababa are based on kit inserts provided by companies, which refer mainly to the western population. Adopting non-locally driven reference values used to interpret patient results might lead to failure to detect the underlying disease. It could also unnecessarily categorize patients abnormal for a certain analyte as well as affect participants' selection in clinical trials [17].

In most African studies, a large number of study participants would have been excluded from the study by using instrument derived kit values .A study in Uganda 31% of volunteers were exclude based on western driven references .when they had used locally established reference intervals the exclude rate was only 17% .This incorrect screening of healthy volunteers would have main suggestion on study costs, work load and time [21]. In Kenya more than 58% of the participants were excluded from the trial by applying US generated reference intervals to screening study participants. Similarly, by applying DAIDS toxicity tables which derived from a Caucasian population up to 40%the participant were considering to have adverse event [10].

Another study in Mozambique found that a high amount of participants value were outside of Us driven reference intervals glucose 41.1 % and T.bilirubin also 15.0% from a total of 199 and 253 participants. Additionally, 62.8% of the healthy volunteers' participants have been excluded from the study evaluated for a clinical trial based on US criteria [27].

Locally driven RIs for diagnosis, treatment, and follow-up of patients based on correct interpretation of the laboratory results are therefore compulsory. Most of reference intervals for laboratory tests are based on outdated methods and are replaced by advanced technology and machineries. The life style and nutritional status of people undergoes a remarkable change as well [22].

Those changes recognized by the knowledge of reference intervals that have been established at local setting than using values from western countries or even within the same country using values generated from areas which differ by climate, socioeconomic status, living style and genetic makeup. This has been supported by literature from different countries which reported variations in reference interval for selected clinical chemistry parameters [17, 19].

The study in India highlights that, the importance of region and ethnic specific reference interval for laboratory reporting system [24].

Most studies have noted variables like the patient population and the method used by the laboratory affecting RIs. The reference range used by one laboratory may not be appropriate for others even may not be optimal in some situations [24-29]. A study in Amhara region Gojjam zone showed that most of clinical chemistry reference intervals were higher than the reference intervals derived from western countries. The study indicated that even with in the same country reference intervals vary based on climate, gender, geographic location and monthly income. [31]

In Ethiopia, efforts to establish reference intervals for selected clinical chemistry parameters by studies from the northern part of the country have confirmed the need for establishing locally derived RIs [30, 31]. As far as my literature search goes there is no published study for adult population of Addis Ababa. The current study was, therefore, carried out to establish Clinical chemistry reference intervals in healthy adults living in Addis Ababa, Ethiopia following CLSI and IFCC guidelines [18].

1.3 Significance of the study

Appropriately established reference interval is the vital part of modern laboratory practice. The Reference intervals established in this study will help Clinicians to make medical decisions. It helps them to screen pathological conditions of patients. to the intervals also assist the Health care providers and other health professionals to monitor progression of diseases, and follow up of drug response. The data obtained from this study is used as a baseline data for further study. For policy makers help to revise guideline related to health like treatment guidelines. Specifically, the data generated from this study is mainly used to treat patients and assess the health status of adults living in Addis Ababa.

2. Literature review

Considering the influence of several factors including population and method differences affecting RIs, several studies have tried to establish locally appropriate RIs. According to a study conducted to establish Reference Intervals for common clinical chemistry analytes for Adults living in Hong Kong, by Lo YC.et, al. 2012 the following results were obtained. The respective gender specific RIs for ALT, ALP, AST, creatinine, T-Bili and Uric acid were 8-57U/l, 47-168U/l,12-47U/l, 69-110µmol/, .4.5-22.8µmol/l and 0.22-0.55mmol/l for males and 7-39U/l, 36-105U/l, 11-26U/l, 55-83µmol/l, 3.7-16.3 µmol/l and 0.16-o.37mmol/l, respectively for females. The study found that separately established RIs were necessary between males and females. Particularly, the analyte observed to have both statistically and clinically significant differences in gender was creatinine [23].

A study by Shrilekha et. al, 2013 aimed to establish RIs of Biochemical and Hematological parameters from apparently Healthy Indian population. The findings of this multicenter cross sectional study of biochemical analytes were fasting glucose for females were 76–108mgldl, 2hrs post glucose71–136mg/dl and for males fasting glucose was 78–110 mg/dl and 2hrs post glucose was 68–136mg/dl. Urea for females was 11–31mg/dl versus 13–35mg/dl for males, serum creatinine for females was 0.6–0.9mg/dl and 0.7–1.2mg/dl for males, serum Uric acid for females was 7–6.5 mg/dl and for males 3.5–8.2mg/dl, total bilirubin for females were 0.3–1.0mg/dl and for males 0.3–1.2 mg/dl, total protein for females was 6.7–8g/dl and 6.8–8.5 g /dl for males. Albumin for females was 3.7–4.9g/dl and 3.9–5.1g/dl for males. AST for females was 12–37U/L and14–42U/l for males. The study observed that gender based partitioning is required for most biochemical analytes [24].

A comparative biochemical RIs study by Bakan E. et al. (2016) conducted from adults living in Northeast Turkey (Erzurum). Participants aged from 19-65 years were recruited and their blood analyzed for 34 analytes using direct and indirect method. The study observed gender related significance differences for most analyses of ALP, AST, and ALT. The respective values were 40-127, 9-28 and10-55 U/L for male and 40-116, 9-27 and 6-30 U/L for female. The RIs for TBIL, DBIL CREA and UA were 3.8-23, 1.2-5.3, 59-107 and 202-489 µmol/ for male and 3.1–20.7, 1.1–4.6, 45–83 and 148–369 µmol/for females, respectively. Glucose, TP and alb were 3.7-6.1mmol/L, 62-79 and 42-50g/L respectively for females.

In addition to this, the upper limits of RIs determined by the direct method in males were higher than in females for ALT, AST, TBIL, DBIL, CREA, and UA. The lower limits of RIs in males were higher than females for ALT, CREA, UA, and TBIL according to Regional reference interval study in Turkey, gender related significance differences were observed [25].

A cross-sectional study in Maputo, by Tembe N. et.al. (2014) including 257 young adults between 18 and 24 years performed hematological and biochemical analyses. The RIs determined for Enzyme tests ALT, AST and ALP were 6.5-53.2, 16.8-45.5 and 97.7-266.1U/l for males and 4.8-38.5, 13.5-37.0 and 91.4-240.6U/l for females, respectively. The respective values for T.Bil. Creatinine and Urea were 5.8-36, 58.2-109.0 µm and1.8-5.8mmol/L for males and 4.0-22.4, 45-.0-86.0 and 1.3-5.1mmol/L for females. RIs for Glucose were 3.1-5.7 and 3.2-5.3mmol/L for males and females, respectively while for Albumin the respective values were 43.4-55.2 and 40.1-52.0g/L for males and females. The study concluded Region, age and gender specific reference interval establishment inevitability observed [26].

The Comprehensive Reference Ranges for Hematology and Clinical Chemistry Laboratory parameters Derived from Normal Nigerian Adults by Miri-Dashe T.et.al (2014) who established RIs for males and non-pregnant females. Biochemical analyses revealed for Males and females Creatinine, and Urea values of 76.3-111.1µmol/L, 2.2-4.8Mmol/L and 63.0-117.8µmol/L, 2.5-5.8Mmol/L, respectively. Glucose RI was 3.7-7.97.9Mmol/L for male while 4.2-9.6Mmol/L for females. The RIs for liver function tests like AST, ALT, Bilirubin for male were, 26.0-49.4µ /L,17.3-48.4 µ/L and 3.42-17.1 µmol/L for male while 22.0-58.4 µ/L,19.0-38.0 µ/L and 0.3-10.6 µmol/L, respectively for females. The observation of this study indicated the requirement of gender specific RIs establishment [27].

Population based reference value study by Zeh C. et al.2011 which was conducted on Adolescents and Young Adults aged 18-34 years in Rural Population Western Kenya, revealed RIs of ALT, 12.0-80.6 and 10.7-61.3 U/L, AST 12.5-69.3 and 13.5-48.5 U/L, T-bil 5.3-50.7 and 5.8-36.1µmol/L, Creatinine 54.2-137.8 and 52.4-96.8µmol/L, Glucose 2.1-9.0 and 2.1-6.0mmol/L, BUN 1.8-5.3 and 1.4-4.5mmol/L for males and females, respectively. The study showed that for adolescent and Young adults Age and gender specific separate reference interval is required [10].

A multicenter cross-sectional Reference Interval study by Abebe M, et al (2018) which analyzed routine clinical chemistry parameters was conducted among apparently healthy young adults in Amhara National Regional State, Ethiopia. The study found that, RIs established include: ALT 5.13-42.88 U/L for males and 4.3-37 U/L for females; AST 12.13-46.88 for males and 10-43.8 U/L for females; ALP 77.2-475.8 U/L for males and 89- 381 U/L for females; total bilirubin 0.11-1.18 mg/dl for males and 0.08-0.91 mg/dl for females; creatinine 0.48-1.13 mg/dl for males and 0.47-1.09 mg/dl for females; urea 12-43 mg/dl for males and 10-38.7 mg/dl for females; and uric acid 2.7-6.9 mg/dl for males and 2.1-5.9 mg/dl for females. The mean and standard deviation values for total protein were 5.7±9.7 g/dl for males and 5.6±9.47 for females. Statistically significant differences were observed in clinical chemistry parameter between males and females. Except total cholesterol, all values of males were higher than females [28].

Another study conducted by Mekonnen Z et al (2017) to determine Clinical chemistry parameters reference intervals of healthy adults in Ethiopia showed significant differences between adult males and females. The RIs for the Analytes ALT 6-44.6, AST 10.5-39.0 and ALP 55.3-237.2U/L were for males while 3.0-30, 6.0-32.1 and 49.0-236.0U/L respectively for females. And for T. protein it was 53.0-86.7, 53.2-86g/L for males and females, respectively. T-bill, D .bill. and creatinine RIs were 4.7-37.6, 0 .4-14.3, 17.4-114.0 and 3.6-37.6,0.2-12.2µmol/L for males and females, respectively. The investigators concluded that males had higher mean value and percentile range than females and hence separate RIs are required [29].

3. Objective

3.1 General objective

This study aimed to establish reference interval for selected clinical chemistry parameters among apparently healthy adults from April to October, 2019 in Addis Ababa, Ethiopia.

3.2 Specific objective

- To establish reference intervals for selected clinical chemistry parameters of apparently healthy adults in Addis Ababa, Ethiopia.
- To investigate the differences between sex specific reference intervals of selected clinical chemistry parameters.

4. Materials and methods

4.1 study area

Addis Ababa is the capital city of Ethiopia, at an altitude of 2355 meters above sea level. The city founded by Menelik II in1886 and given the name new flower. It is the largest city in the country by population. The current metro area population of Addis Ababa in 2020 is 4,794,000, a 4.4% increase from 2019. The city is the place for the African Union and Addis Ababa has the central station of the United Nations Economic Commission for Africa and different other global associations, making it one of the most important cities in the entire continent. The city's government is headed by the mayor and the city council. The city has three layers of Government: City Government at the top, 10 Sub City Administrations in the Middle, and 99 Kebele Administrations at the bottom [33]. This study was carried out in four of the 10 sub-cities selected based on Probability Proportional to Size (PPS).

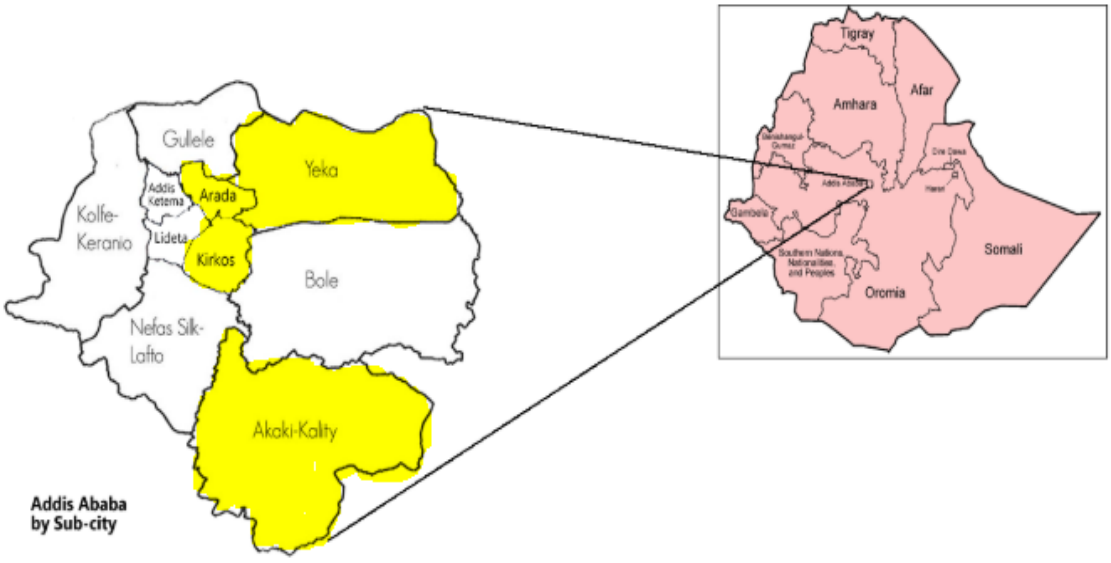

Figure: 1 map of Addis Ababa

4.2 Study design and period

A Community based cross-sectional study was conducted from April to October, 2019 to established reference interval for selected clinical chemistry parameters among apparently healthy adults living in Addis Ababa, Ethiopia.

4.3 Population

4.3.1 Source of population

Source of population were apparently healthy adults who live in Addis Ababa

4.3.2 Study population

The study population were people with age of 18 years and above that live in the selected sub cities of Addis Ababa who fulfill the eligibility criteria.

4.4 Inclusion and Exclusion criteria

4.4.1 Inclusion criteria

- An individual who has no sign and symptoms of any disease
- Negative result for the screening tests
- Adults age between 18–60 years and live in Addis Ababa
- normal body mass index (17.5–25 kg/m^2) were candidates of this study
- volunteers who participate in the study

4.4.2 Exclusion criteria

- chronic illnesses like diabetes mellitus, chronic renal failure, hypertension, ischemic heart disease, liver diseases
- adults with intestinal infections, positive for hemoparasites
- observable mental illness, smokers, chronic alcohol drunker
- BMI less than 17.5kg/m2, hospitalized persons, chronic diseases,
- Pregnant woman

4.5 Study variables

4.5.1 Dependent variables

Selected Clinical chemistry parameters including AST, ALT, Alkaline phosphatase, Glucose, albumin, total protein, direct and total bilirubin, creatinine, urea/BUN and uric acid levels are dependent variables.

4.5.2 Independent variables

- Sex
- Age

4.6 Sample size determination and sampling Technique

4.6.1 Sample size determination

According to a research conducted in African countries, in such large scale studies about 30% of apparently healthy population (Steven *et al*., 2008) do not qualify for reference interval determination for various reasons by common infectious diseases [34]. Therefore, to reach the CLSI sample size recommendation a minimum of 120 samples are needed for reference interval determination. Participants were partitioning by sex and pregnancy. Considering 30% exclusion rate and 10% outliers a total of (120*2) 240 individuals enrolled. (i.e, 30 % x120) excluded during data analysis. Therefore, 30% of 120 individuals added to yield 156 participant thus, 10% of 156 subjects were added to give a total of 172 study subjects to be included in each partition group. Totally, a minimum of 344 (172x2) participants were needed; the study included 344 participants.

The study participants were selected using systematic random sampling by considering woreda as a sampling frame for each sub cities and then households the final selection units. All individuals in the household fulfilling the eligibility criteria and willing to participate were included.

4.6.2 Sampling technique

Probability Proportional to Size (PPS) sampling method was employed, where the size depends on the number of households of Weredas in a sub city. Accordingly, all the woredas in the sub city are considered/selected to be the participants of the study. Since Addis Ababa is very large city, four sub-cities were selected based on PPS, namely Arada, Kirkos, Akaki and Yeka sub-cities. Central Statistical Agency (CSA) assisted with study site selection after obtaining a letter of support from the Federal Ministry of Health. Thus all weredas under the selected sub cities were included.

To recruit 624 participants, the number of households is determined by dividing the total household in the selected city (sub-cities for A.A) by the estimated number of individuals per household which is 4 for urban. Individuals in every household were approached at their households through health extension workers. Given the average number of individuals in each household of 4, the next households were used to recruit the remaining age groups that are not found in the selected household. Once volunteering participants fulfilling the eligibility criteria are identified by the health extension workers, they were invited to go to nearby health facilities for interview using structured questionnaire and to facilitate biological sample collection. Letters has been written to the respective Health facilities (Annexed).

Table 1. Selected sites with household information

Selected Sites	No. Households	Individuals per household	No of population
Akaki	47021	3.8	178679
Kirkos	54398	4.0	217592
Arada	49564	4.1	203212
Yeka	90195	3.8	342741

Note: Source for total population and number of households is from CSA 2007;

Proportional allocation:

$n_i = n/N*N_i$

Where n=total sample size

n_i= number of sample size at each sub city

N=total population of selected sub cities

N_i =total population at that stratum

Samples allocated proportional to population size

Akaki kality=118, Kirkos= 144, Arada=135, Yeka=227

4.7 Measurement and data collection

4.7.1 Data collection

After explanation of the purpose, benefit, minimal risk and the right to withdraw from the study, Socio demographic, clinical, nutritional habit and behavioral information data were collected using well-structured questionnaire. Physical examination as well as disease status of the study participants was obtained during interview and measurements like weight, height and blood pressure were carried out at the site. Blood samples were collected by experienced phlebotomist. Approximately 5 ml blood was collected for clinical chemistry parameters. Stool microscopic examination took place at the site for ova and parasites and further analysis was dome at the department of Medical Laboratory sciences using the concentration technique. Urine samples were collected in leak proof clean containers for chemical analysis and for females to check pregnancy test. Blood, stool and urine samples were collected and taken to the Department of Medical Laboratory Science Laboratories for further processing.

The SOP for Blood, stool and urine sample collection and processing procedure annexed (see annex 1).

4.7.2 Methods for laboratory analysis

The serum samples were collected after the blood was clotted and centrifuged at3000rpm and serum were stored at -80 °C until analysis.

At a pH value of 4.1, albumin displays a sufficiently cationic character to be able to bind with bromcresol green (BCG), an anionic dye, to form a blue-green complex. The color intensity of the blue-green color is directly proportional to the albumin concentration in the sample and is measured photometrically.

ALP: Colorimetric assay in accordance with a standardized method. In the presence of magnesium and zinc ions, p-nitrophenyl phosphate is cleaved by phosphatases into phosphate and p-nitrophenol the p-nitrophenol released is directly proportional to the catalytic ALP activity. It is determined by measuring the increase in absorbance.

ALT catalyzes the reaction between L-alanine and 2oxoglutarate. The pyruvate formed is reduced by NADH in a reaction catalyzed by lactate dehydrogenase (LDH) to form L-lactate and NAD+. The rate of the NADH oxidation is directly proportional to the catalytic ALT activity. It is determined by measuring the decrease in absorbance.

AST in the sample catalyzes the transfer of an amino group between L-aspartate and 2-oxoglutarate to form oxaloacetate and L-glutamate. The oxaloacetate then reacts with NADH, in the presence of malate dehydrogenase (MDH), to form NAD+ the rate of the NADH oxidation is directly proportional to the catalytic AST activity. It is determined by measuring the decrease in absorbance.

Conjugated bilirubin and (direct bilirubin) react directly with 3,5 Dichlorophenyl diazonium salt in acid buffer to form the red-colored azobilirubin. The color intensity of the red azo dye formed is directly proportional to the direct (conjugated) bilirubin concentration and can be determined photometrically.

Total bilirubin, in the presence of a suitable solubilizing agent, is coupled with 3,5-dichlorophenyl diazonium in a strongly acidic medium. The color intensity of the red azo dye formed is directly proportional to the total bilirubin and can be determined photometrically.

This kinetic colorimetric assay is based on the Jaffé method. In alkaline solution, creatinine forms a yellow-orange complex with picrate. The rate of dye formation is proportional to the creatinine concentration in the specimen. The assay uses “rate-blanking” to minimize interference by bilirubin.

Glucose: Enzymatic reference method with hexokinase4, 5 Hexokinase catalyzes the phosphorylation of glucose toglucose6-phosphate by ATP. Glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate in the presence of NADP to gluconate-6-phosphate. No other carbohydrate is oxidized. The rate of NADPH formation during the reaction is directly proportional to the glucose concentration and is measured photometrically. Total Protein: Divalent copper reacts in alkaline solution with protein peptide bonds to form the characteristic purple-colored biuret complex. Sodium potassium tartrate prevents the precipitation of copper hydroxide and potassium iodide prevents auto reduction of copper. The color intensity is directly proportional to the protein concentration which can be determined photometrically.

Uric Acid: Enzymatic colorimetric test, Uricase cleaves uric acid to form allantoin and hydrogen peroxide. In the presence of peroxidase, 4-aminophenazone is oxidized by hydrogen peroxide to a quinone-diimine dye. The color intensity of the quinone-diimine formed is directly proportional to the uric acid concentration and is determined by measuring the increase in absorbance.

Urea: Kinetic test with urease and glutamate dehydrogenase. Urea is hydrolyzed by urease to form ammonium and carbonate. In the second reaction 2-oxoglutarate reacts with ammonium in the presence of glutamate dehydrogenase (GLDH) and the coenzyme NADH to produce L-glutamate. In this reaction two moles of NADH are oxidized to NAD+ for each mole of urea hydrolyzed. The rate of decrease in the NADH concentration is directly proportional to the urea concentration in the specimen and is measured photometrically.

4.8 Data Quality assurance and Quality control

To ensure the precision and accuracy of the test results, all pre-analytical, analytical and post analytical precautions were taken into consideration. The Cobas 6000 analyzer and the protocols used are under the regular control of quality officer and the laboratory team coordinator in the clinical chemistry laboratory at Ethiopian Public Health Institute.

All laboratory staffs received equipment procedure and protocol training by trained personnel working under the supervision of laboratory capacity building in EPHI and ENAO. The laboratory results are validated and verified by the laboratory team coordinator before release. Additionally, to maintain internal quality control, known standards were run and the equipment calibrated before any analysis. As internal quality control, also two levels of controls, pathological and normal, were run. Two levels of controls results have to be within acceptable range before analyzing the sample.

4.8.1 Pre-analytical

Preanalytical factors were also controlled in order to insure that accurate reference ranges are derived. Blood samples were collected by experienced phlebotomist and labelled with code and initial name of the study participant. Stool and urine samples were collected and labelled with the same code and initial similar to the blood sample.

4.7.3 Analytical

Reagents and kits were checked and assessed by known controls. Based on standard operating procedure, the laboratory personnel analyzed the sample. The quality of the result were check by the laboratory team coordinator before release.

4.8.3 Post analytical

The result from the laboratory were arranged and well documented according to the code labeled with the initial name of the study participant.

4.9 Data analysis and interpretation

Data was entered, cleaned and analyzed using SPSS version 23.0 (SPSS Inc.Chicago, USA) to calculate RIs. The reference intervals were calculated non-parametrically based on CLSI recommendation. Nonparametric methods encompass the central 95^{th} percentile of reference values and use the 2.5^{th} and 97.5th percentile as the lower and upper reference limit, respectively.

Descriptive statistics was used to determine the mean, median and 95% range of each parameters. Outliers were identified based on Tukeys method by calculating the Interquartile range. Q1: lower quartile, Q3: upper quartile. The first quartile Q1 is the value ≥ 1/4 of the data, the second quartile Q2 (the median), and the third Q3 is the value ≥ 3/4 of the data. The interquartile range, IQR, is Q3 − Q1(the third quartile-the first quartile).

According to Tukey's rule, the outliers are values more than 1.5 times the interquartile range from the quartiles, either below Q1 − 1.5IQR, or above Q3 + 1.5IQR. Mann Whitney U test was used to assess differences by gender and pregnancy status. The mean, median, and 2.5^{th} and 97.5^{th} percentiles with 90% CIs were calculated and reference intervals established.

4.10 Ethical considerations

The study was conducted after ethical approval was obtained from Research and Ethics review board of the Department of Medical Laboratory Sciences and College of Health Sciences of Addis Ababa University. Support Letters were secured from Ministry of Health, Addis Ababa Health Bureau and sub cities. Informed written consent was also obtained from each study participant before the actual data collection. Individuals who were positive for screening test and other disease conditions at the site were linked to nearby government health centers for further diagnosis and treatment accordingly. Any information from participants was kept confidential.

4.11 Dissemination of Result

The finding of this study will be submitted and presented to the Department of Medical Laboratory Science of Addis Ababa University, and also presented on scientific conferences and published on peer reviewed scientific journals.

4.12 Operational definition

Reference intervals: - The range between, and including two reference values defined by a specific percentage (usually 95%) for common clinical chemistry parameters of healthy individuals.

Healthy adults: - the absence of disease or disabilities based on medical history, physical diagnosis, clinical sign and symptom plus laboratory investigations.

95th percentile ranges: - ranges between, and including the 2.5th percentile and the 97.5th percentile.

Reference individuals; - those individuals represent the reference population from which a reference sample group is selected.

5. RESULT

5.1 Demographic characteristics

A total of 446 apparently healthy adults consented for this study. Complete data from 446 (Non-pregnant females 272 (60.99%), Males 174 (39.01%) were included for analysis. This study attempted to enroll a minimum of 120 adults into each partitions, as recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines for the establishment of reference intervals. The majority are in the age group less than 40 years old (83.5%). The demographic characteristics of the study participants are shown below in Table 2.

Table 2: Socio-demographic characteristics of the study participants

In Addis Ababa, Ethiopia, 2020

Variables		Frequency	Percent
Sex	Male	174	39.01
	Female	272	60.99
Age	18-27	131	29.37
	28-33	123	27.50
	34-39	76	17.04
	40-45	53	11.88
	46-51	39	8.74
	52-60	27	6.05

5.2 Reference Interval for selected clinical chemistry parameters of adults.

Based on the CLSI guideline those who do not fulfill the requirement were excluded from the final analysis. As a result a total of 209 were excluded; of which 46 were excluded based on CRP, 11 urinalysis result, 128 BMI, and 24 with various reason including missing data and outliers. Hence, data from 126 males, 134 non-pregnant females and 153 pregnant women were included in the final analysis of RI determination. Table 3 summarizes the mean (with 95% CI), median and 95% RI for apparently healthy adult males and non-pregnant females in Addis Ababa. In the current study pregnant and non-pregnant woman participate in the study and their RI is separately presented.

The study observed statistically significant differences between males and females in clinical chemistry parameter RIs by using the non-parametric and Mann Whitney U test ($P<0.05$). All clinical chemistry parameter values were higher in males than females except Albumin and Protein (Table3). The overall mean value of males and females were ALT (7.0 vs.4.54), AST (14.5 vs.12.6), ALP (79.94 vs.59.99), Albumin (4.83 vs.4.62) Total protein (7.48 vs.7.28), total bilirubin (0.57 vs.0.37), direct bilirubin (0.21 vs.0.15), glucose (78.20 vs.76.20), Urea (21.6vs.18.3), creatinine (0.81 vs.0.60) and uric acid (5.40 vs.3.78), respectively.

The reference interval of males and females had ALT 1.60-16.89U/l against females 0.47-9.87U/l, AST 8.11-22.50 vs. 6.95-18.05 U/l, ALP 44.0-181.65U/l vs. 32.58-100.43, albumin 4.40-5.88U/l vs.4.03-5.25U/l, T.protein 6.76-9.57g/l vs. 6.45-8.21g/l, T .bilirubin 0.26-1.15 vs.0.15-0.64mg/dl, D. bilirubin 0.08-0.46mg/dl vs. 0.061-0.25mg/dl, Glucose 62.93-99.39mg/dl vs.59.14-90.72mg/dl, Urea 13.21-33.51mg/dl vs.7.30-12.00mg/dl, Creatinine 0.56-1.02mg/dl vs.0.40-0.80mg/dl and Uric acid 3.41-7.40mg/dl, vs.1.88-5.60mg/dl, respectively. As can be seen in Table 3, in the majority of clinical chemistry parameters statistically significance difference was observed between males and females. Males had significantly higher values of liver and renal all function parameters.

Table 3: Mean, Median and 2.5th-95th percentile RI of common clinical chemistry parameters in male and non-pregnant female healthy adults in Addis Ababa Ethiopia, 2020

Analytes	Male (n=126)			Female (n=134)			
	Mean	**Median**	**2.5-97.5 percentile (RI)**	**Mean**	**Median**	**2.5th-97.5 percentile (RI)**	**P-value**
ALT U/l	**7.0**	**6.60**	**1.60-16.89**	**4.54**	**4.20**	**0.47-9.87**	**0.001**
AST U/l	**14.55**	**14.50**	**8.11-22.50**	**12.58**	**12.40**	**6.95-18.05**	**0,001**
ALP U/l	**79.94**	**65.50**	**44.00-181.65**	**59.99**	**57.00**	**32.58-100.43**	**0.001**
Albumin g/l	**4.83**	**4.78**	**4.40-5.88**	**4.62**	**4.62**	**4.03-5.25**	**0.001**
Protein T. g/l	**7.48**	**7.33**	**6.76-9.57**	**7.28**	**7.25**	**6.45-8.21**	**0.149**
Bilirubin T. mg/dl	**0.57**	**0.52**	**0.26-1.15**	**0.37**	**0.36**	**0.15-0.64**	**0.001**
Bilirubin D. mg/dl	**0.21**	**0.21**	**0.08-0.46**	**0.15**	**0.143**	**0.061-0.25**	**0.001**
Glucose mg/dl	**78.20**	**78.10**	**62.93-99.39**	**76.2**	**76.40**	**59.14-90.72**	**0.672**
Urea mg/dl	**21.58**	**20.80**	**13.21-33.51**	**18.3**	**17.30**	**7.30-12.00**	**0.001**
Creatinine mg/dl	**0.81**	**0.80**	**0.56-1.02**	**0.60**	**0.60**	**0.40-0.80**	**0.00`1**

ALT: Alanine Aminotransferase; **AST**: Aspartate Aminotransferase; **ALP**: Alkaline Phosphatase **CI**: confidence interval; **dl**: Deciliter; **g**: Gram; **L.** liter; **mg**: Milligram; **RI**: Reference interval; **U**: Unit.

The Range, 2.5th and 97.5th percentile and the 90% confidence intervals of the lower 2.5th and upper 97.5th RI limits for apparently healthy adult males and non-pregnant females with combined values are summarized in Table 4. CLSI recommends providing RIs with their 90% confidence interval.

Table 4:The upper and lower reference limits with 90% CI of common clinical chemistry parameters of apparently healthy male and Non-pregnant female adults in Addis Ababa, Ethiopia, 2020.(n=413, Male=126, Non-pregnant= 134)

Analytes Unit	**Sex**	**Range**	**2.5th-97.5th percentile RI**	**Lower reference Limit90%CI**	**Upper reference Limits90%CI**
Albumin mg/dl	Female	3.90-5.36	4.03-5.25	3.90-4.23	5.12-5.36
	Male	4.37-5.92	4.40-5.88	4.37-4.45	5.25-5.92
T.protein mg/dl	Female	6.33-8.25	6.45-8.21	6.33-6.53	8.10-8.25
	Male	6.61-9.79	6.76-9.57	6.61-6.83	9.08-9.79
AST(SGOT) U/l	Female	6.30-18.60	6.95-18.08	6.30-8.70	17.05-18.60
	Male	7.80-24.80	8.11-22.50	7.80-8.90	20.90-24.80
ALT(SGPT) U/l	Female	0.10-10.40	0.47-9.87	0.100-1.30	8.90-10.40
	Male	1.40-17.70	1.60-16.89	1.40-1.80	13.10-17.70
ALP U/l	Female	32.58-104.00	32.58-100.43	32.00-36.00	93.00-104.00
	Male	43-.00-222	44.0-181.65	43.00-45.00	128-222.00
Bilirubin(Direct) mg/dl	Female	0.03-0.27	0.061-0.25	0.03-0.08	0.23-0.27
	Male	0.07-0.48	0.08-0.46	0.07-0.10	0.36-0.48
Bilirubin(Total) mg/dl	Female	0.11-0.66	0.15-0.64	0.11-0.18	0.60-0.66
	Male	0.177-1.261	0.26-1.15	0.18-0.28	1.05-1.26
Urea mg/dl	Female	7.30-31.70	11.13-29.23	7.30-12.00	27.50-31.70
	Male	12.30-34.70	13.21-33.51	12.30-14.40	30.70-34.70
Creatinine mg/dl	Female	0.38-0.83	0.40-0.80	0.38-0.44	0.77-0.83
	Male	0.46-1.03	0.56-1.02	0.46-0.65	1.00-1.03
Glucose Mg/dl	Female	56.70-96.30	59.14-90.72	56.70-62.40	86.70-96.30
	Male	58.30-100-30	62.93-99.39	58.30-64.20	94.0-100.36
Uric acid mg\dl	Female	1.60-6.10	1.88-5.60	1.60-2.20	5.40-6.10
	Male	3.20-7.80	3.41-7.40	3.20-3.70	7.10-7.80

The current Reference Intervals of selected clinical chemistry values of adult males and non-pregnant females were compared with the currently used Manufacturers value and the studies conducted in selected studies from other parts of Ethiopia, Tanzania, Nigeria, Botswana, Middle belt Ghana, USA. Although significant differences were noted between male and female for the selected clinical chemistry parameters in this study, the company provided sex specific RI for ALT, AST, ALP and Creatinine only. Though inconsistency seen among the various studies, the RI limits of the current study lie below the upper limit provided by the company for ALT, AST and ALP. For these parameters the company provides upper cutoff only which the current study values are within the accepted limit. Except the study from Northwest Ethiopia and USA which are relatively closer, the upper RI limits given by all the other studies exceed the cutoff given by the company (Table 5).

Table 5: Comparison of clinical chemistry parameter RIs of this study against manufacturer Ranges and other similar studies.

Analyte	Sex	Current Study	Manufacturer	Tanzania 30	Amhara R.egion 28	Ethiopia N.west 29	Botswana 19	Middle belt Ghana 31	Nigeria 27	USA 32
Albumin g/dl	Combined	NA	3.5-5.2	3.56-5.04	3.7-6.2	NA	NA	3.30-5.00	NA	3.5–5.5
	Female	4.03-5.25	NA	3.56-4.93	3.6-6.1	NA	NA	3.35-5.04	NA	NA
	Male	4.40-5.88	NA	3.71-5.07	3.7-6.2	NA	NA	3.27-4.98	NA	NA
ALT U/l	Combined	NA	NA	7.7-48.3	5.0-39.0	6.0-43.0	7.0-46.0	7-51	NA	0-35
	Female	0.47-9.87	Upto33	6.7-44.9	4.3-37.0	3.0-30	7.0-33.0	6-51U/L	19-38	NA
	Male	1.60-16.89	Upto41	9.1-55.3	5.13-42.88	6.0-44.6	8.0-53.0	8-54	17.3-48.4	NA
AST U/l	Combined	NA	NA	14.3-48.1	11.0-46.0	9.0-38	13.0-42.0	14-51	NA	0-35
	Female	6.95-18.05	Upto32	13.5-35.2	10.0-43.8	6.0-32.1	12.0-31.0	13-48	22-58.4	NA
	Male	8.11-22.50	Upto40	15.2-53.4	12.13-46.88	10.5-39.0	14.0-48.0	17-60	26.0-49.4	NA
ALP U/l	Combined	NA	NA	45.6-158.4	87.0-451.28	52.4-237.0	NA	85-241	NA	30-120
	Female	32.58-100.43	35-104	45.3-15.5	89.0-381.0	49.0-236	NA	82-293	NA	NA
	Male	44.00-	40-129	45.4-170.4	77.2-	55.3-	NA	101-353	NA	NA

		181.65			475.8	237.2				
T.protein g/dl	Combined	NA	6.6-8.7	6.63-8.51	5.7-9.6	53.0-86.1	NA	5.06-8.6	NA	5.5–8.0
	Female	6.45-8.21	NA	6.58-8.55	5.6-9.47	5.32-8.60	NA	5.52-8.69	NA	NA
	Male	6.76-9.57	NA	6.72-8.52	5.7-9.7	5.30-8.67	NA	4.67-8.67	NA	NA
Bilirubin (Direct) Mg/dl	Combined	NA	≤0.030m	0.042-0.48	0.02-0.61	0.01-0.80	0.1-0.4	0.047-0.24	NA	0.1–0.3
	Female	0.061-0.25	NA	0.04-0.34	0.01-0.49	0.01-0.71	0.0-0.3	0.041-0.23	NA	NA
	Male	0.08-0.48	NA	0.05-0.49	0.04-0.68	00.00.-0.05	0.1-0.5	0.053-0.245	NA	NA
Bilirubin (Total) g/dl	Combined	NA	Upto1.2	0.30-2.39	0.1-1.1	0.26-2.20	0.2-1.8	0.17-1.51	NA	0.3–1.0
	Female	0.15-0.64	NA	0.26-1.83	0.08-0.91	0.21-2.20	0.2-1.3	0.16-1.56	0.02-0.12	NA
	Male	0.26-1.15	NA	0.35-2.45	0.11-1.18	0.27-2.20	0.3-2.1	0.22-1.88	0.20—1.0	NA
Ureag/dl	Female	11.13-29.23	NA	8.83-27.7	10.0-38.7	NA	5.0-21.0	54.41-0.32l	15.01-34.8	NA
	Male	13.21-33.51	NA	9.43-30.09	12.0-43.0	NA	5.0-22.0	5.41-32.29	13.2-28.8	NA
Creatinine g/dl	Combined	NA	NA	0.4-7-1.01	0.47-1.12	0.23-1.22	0.5-1.1	0.55-1.33	NA	<1.5

	Female	0.40-0.80	0.5-0.95	0.45-0.91	0.47-1.09	0.02-1.08	0.5-0.8	0.25-1.24	0.7-1.33	NA
	Male	0.56-1.02	0.67-1.17	0.54-1.08	0.48-1.13	0.11-1.29	0.6-1.1	0.53-1.34	0.86-1.2	NA
Glucose g/dl	Combined	NA	74-109	54.1-94.2	NA	NA	NA	64.87-115.31	NA	75–115
	Female	59.14-90.72	NA	59.5-91.2	NA	NA	NA	66.67-119.65	79.3-173.1	NA
	Male	62.93-99.39	NA	51.9-95.5	NA	NA	NA	63.03-113.53	66.7-142.4	NA
Uric acid mg/dl	Combined	NA	3.4-7.0	**NA**	2.34-6.60	NA	**NA**	0.65-6.71	NA	NA
	Female	1.88-5.60	NA	**NA**	2.1-5.9	NA	**NA**	1.39-6.40	NA	1.5–6.0
	Male	3.41-7.40	NA	**NA**	2.7-6.9	NA	**NA**	2.12-7.03	NA	2.5–8.0

ALT: Alanine aminotransferase; **ALP**: Alkaline phosphatase; **AST**: Aspartate aminotransferase; **CI**: confidence interval; **dl**: Deciliter; **g**: Gram; **L**: liter; **mg**: Milligram; **NA**: Not available; **USA**: United States of America; **U**: Unit.

6. **DISCUSSION**

To establish reference intervals by using the direct method is challenging, as the method depends on healthy individuals. Thus, most laboratories depend on western countries reference intervals [17, 19, 23, 24, 30, and 31]. Therefore, this study aimed to establish reference intervals for selected clinical chemistry parameters from apparently healthy adult males and females from Addis Ababa.

The current study revealed that reference intervals of ALT and AST in males were higher than females. This is in agreement with the report from Tanzania [30], Amhara [28], North west Ethiopia [29], Botswana [17], and Middle belt Ghana [31]. The current study also observed higher values of ALP in males which are consistent with findings from Tanzania [30], Amhara [28], N. W. Ethiopia [29], M .B. Ghana [31], and with manufacturers' value. The reference values of Albumin and Total protein are not significantly different in current study and this finding agrees with studies from Tanzania, M.B.Ghana, Amhara, and a wider study from N.W. Ethiopia [28-31].

The significantly higher reference interval of Direct and Total Bilirubin in this study in males compared to females is consistent with reports from Tanzania [30], Amhara Region [28], Botswana [17], and Nigeria [27]. In this study, like in studies from Tanzania [30], Amhara [28] and Botswana [17], M.B. Ghana [31], the reference intervals of urea were higher in males than females. The finding for creatinine agrees with studies in Botswana [17], M.B. Ghana [31], and the reference intervals given by the manufacturers, which is higher in males. The level of glucose is also higher in males than females this in consistent with the report from Tanzania [30] while the finding for uric acid is in agreement with the report from Amhara [28], M.B.Ghana [31], and US [32]. The findings of differences between males and females in the current as well as other studies from the northern part of Ethiopia, other African countries and the US underscore the need for sex specific RIs.

On the other hand, the overall reference intervals of selected clinical chemistry values in this study are lower than the reported values from other African countries and the US [17, 28-32]. In addition, the reference values of ALT and AST are lower in the current study as compared to the

intervals provided by the manufacturers. In this study the reference intervals of Albumin and Total Protein is not remarkably different from report from other African countries and US.

The Direct bilirubin reference intervals in current study is lower from Tanzania [17], Amhara Region [30] (24), as well as N.W.Ethiopia [31], but relatively higher than M.B.Ghana [29] and US [37] as well as the reference limit given by the manufactures. Whereas, the reference intervals of total bilirubin is lower in this study as compared to the values given by the manufacturers as well as reports from Tanzania, M.B.Ghana and N.W. Ethiopia [28, 29, 31] but consistent with the finding in Amhara Region [30].

In this study the reference value of urea is lower than those from Amhara Region [30], and higher than a report from Tanzania [28] and Botswana [17]. The reference value of creatinine in current study is in agreement with report from African and US. In current study the reference value of glucose is lower from M.B.Ghana [29], US [37] and manufacturers' limits. The reference interval of uric acid in current study is in line with the findings from Amhara Region [30], M.B.Ghana [29] and the values given by the manufacturer. But the lower limit is higher than M.B.Ghana [29]. In general, there is no consistent pattern in the differences between the RIs in the different countries as well as the values given by the manufacturers. This inconsistency is also noted between the Ethiopian studies and hence requiring each laboratory to establish its own RIs. Several factors including population differences, methodological and selection criteria of the study population could result in the variation between the various studies. For example the study in the Amhara region recruits blood donors while the present study is community based.

In summary, the reference intervals established by the current study indicate the inevitability of separate reference intervals for pregnant woman

7. STRENGTH AND LIMITATION OF THE STUDUY

7.1 Strength of the study

In the current study we used the direct approach which is the first choice for the determination of reference interval. That follows the Clinical and Laboratory Standards Institute/International Federation of Clinical Chemistry and Laboratory Medicine (CLSI/IFCC) recommendation.

Without considering the difficulties to obtaining sufficient numbers of reference individuals from a representative population, separate clinical chemistry reference interval are established for males and Non-pregnant woman who live in Addis Ababa. The samples were analyzed in the National Reference Laboratory which is accredited for common clinical chemistry parameters measured in the current study.

7.2 Limitation of the study

The first limitation in current study includes only four sub cities from the total of 10 in Addis Ababa. Secondly, Adult pregnant females also not included. However, it shades some light as the values given by the manufacturers and other studies for selected clinical chemistry parameters are not disaggregated and hence comparison was possible.

8. CONCLUSION AND RECOMMONDATION

8.1 Conclusion

The current study established Reference intervals for selected clinical chemistry parameters for adults in Addis Ababa. The overall reference intervals of the current study are lower than other studies in Africa, Ethiopia and western countries. The significant differences between males and females in clinical chemistry reference intervals showed by this study and emphasize the need for such inclusive establishment of reference intervals for different populations.

8.2 Recommendations

The established RIs of clinical chemistry parameters are potentially useful in the diagnosis, management and monitoring of disease progression in the study setting. There are significant RIs variations between the current and other Ethiopian studies for most of the common clinical chemistry parameters. Thus, it is recommended to use the established RIs for the populations of Addis Ababa. It is also recommended to establish RI for other populations as well as to determine specific RI for pregnant women.

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Annexes

Annex 1: Lab. sops

Section A: SOP for Blood sample collection

Venipuncture is the collection of blood from a vein which is usually done for laboratory testing. The blood is normally drawn from a vein on the top of the hand or from the inside of the elbow. Venipuncture requires good skills in order to perform the procedure not only correctly, but also painlessly. There are some slight risks associated with venipuncture which may include excessive bleeding, feeling light-headed, fainting, nerve damage, hematoma (accumulation of blood under the skin), and infection. The area where the blood is to be drawn from is first cleaned with a germ-killing solution.

Materials

The equipment used during the venipuncture test can vary, but the following are most commonly used for routine venipuncture:

1. Collection tubes
2. Needles
3. Tourniquet
4. Wipes/Swabs
5. Gauze
6. Bandages
7. Gloves
8. Disposal unit

Procedure

1. The medical technician or phlebotomist will wrap a flexible band on the upper part of the arm to apply some pressure to that area making the vein enlarge with blood.
2. A needle is then gently inserted into the vein.
3. Blood is collected into an attached vial or tube that is airtight and the flexible band is then removed from the arm.

4. If multiple blood samples are to be taken, the phlebotomist must be careful to follow the proper order of draw.

5. Finally, when the necessary blood is collected, the needle will be removed and properly disposed of in a Sharps container and the puncture site will be covered to stop the bleeding.

Section B. stool specimen

Examination of fresh specimens permits the observation of motile trophozoites, but this must be carried out without delay. Liquid (diarrheic) specimens (which are more likely to contain trophozoites) should be examined within 30 minutes of passage (not within 30 minutes of arrival in the laboratory!), and soft specimens (which may contain both trophozoites and cysts) should be examined within one hour of passage. If delays cannot be avoided, the specimen should be preserved within 10% formalin to avoid disintegration of the trophozoites. Formed specimens (less likely to contain trophozoites) can be kept for up to one day, with overnight refrigeration if needed, prior to examination. All specimen containers must be labeled with both the patient's first and last names as well as a second identifier such as the patient's medical record number or date of birth. Containers without two identifiers will be rejected.

Materials Required

- Clean container with spatula or wooden applicator sticks
- 2. Object glass
- Cover-slip
- Pen/ permanent marker for labeling
- Normal saline solution (0.85%; 8.5g/l)
- Lugol's iodine (1% solution)
- Pipettes
- Microscopic slide
- Light microscope

Procedure

1. Place a drop of saline on the centre of the left half of the slide and a drop of Lugol's solution on the center of the right half of the slide on a microscopic slide, which has been labeled with patient ID

2. With a wooden applicator stick or plastic spatulla, pick up a small portion of the stool specimen (size of match head)

3. Mix with a drop of saline to form suspension
4. Similarly, pick up a small portion of the stool specimen and mix with Lugol's solution to form suspension.

5. Cover the drop with a cover slip by holding the cover slip at an angle,

6. Touch the edge of the drop and gently lower the cover slip onto the slide to reduce the possibility of air bubbles in the smear
7. Put the slide under a light microscope with 10x objective.

8. Examine the entire cover slip area by moving the slide systematically backwards and forwards, or up and down.
9. Switch to 40x objective lenses when suspected parasites are seen

10. Results should be recorded directly in the Lab Register.
11. Record if the result is POSITIVE or NEGATIVE.
12. If POSITIVE, record all detected species and its trophozoites/cysts number (for intestinal protozoa) or eggs for helminth.

13. Dispose remaining stool samples and slides with fecal thick smears without contaminating the local environment.

If number of organisms in stool specimen is low, examination of a direct wet mount may not detect parasites. Thus, whenever possible, the stool should be concentrated.worm eggs, larvea, and protozoan cysts may be recovered by concentration but protozoan trophozoites will not be seen as they are usually destroyed during the concentration procedures. This makes direct wet mount examination necessary as the initial phase of microscopic examination. Worm eggs, larvae, and protozoan cysts may be recovered. But the method destroys trophozoite stages. The purpose of concentrating feces is to increase possibility to finding ova, cyst, or larvae in samples that not be able to seen by direct microscopy.

Materials

1. Applicator stick
2. Glass centrifugal tubes
3. Beaker
4. Wire sieve or gauze
5. Vortex
6. Centrifuge.

Reagent:

Reagent I: 10% formalin solution in distilled water.

Reagent II: diethyl ether or ethyl acetate.

Caution;Ether is a highly flammable compound and will ignite and explode quickly if there is a flame or spark nearby.

Procedures

1. Emulsify 1 gm. of feces in 7 ml of 10% formalin in a centrifuge tube.

2. Strain the suspension through a brass wire sieve, and collect in beaker.

3. Pour the filtrate into a 15 ml boiling tube and add 3 ml of ether

4. Mix well 15 sec on vortex or 1 min by hand.

5. Transfer the ether- formalin suspension back into the washed centrifuge tube, and centrifuge at 3,000 rpm for 1 min.

6. Invert the tube quickly to discard the supernatant,On righting the tube, a few drops only should remain with the sediment

7. Mix the sediment well and transfer one drops onto a glass slide and cover it with coverslip.

8. Scan the whole coverslip using 10x objective, turning into 40x for confirmation of identification of parasites.

Formalin- Ether or Formalin- Ethyl acetate method is the recommended concentration procedures.Most types of worm eggs (round worms, tapeworms, schistosomes, and other fluke eggs), larvae, and protozoan cysts may be recovered by this method.

Section C. SOP for Urine analysis

Analysis of urine specimens is useful in monitoring the effectiveness of treatment of chronic problems, and in screening for asymptomatic conditions. Proper collection and transport of specimens is critical to the quality of results produced by the laboratory. The validity of all diagnostic information produced in the lab is contingent on the quality of the specimen received. Consequences of poorly collected and /or poorly transported specimens include failure to isolate the causative organism, and recovery of contaminants or normal flora, which could lead to improper treatment of the patient.

Randomly collected specimens are suitable for urinalysis in the clinical chemistry laboratory and for microscopic analysis. However, they are not regarded as specimens of choice because of the potential for dilution of the specimen when collection occurs soon after the patient has consumed fluids. In this situation, analyte values may be artificially low. All specimen containers must be labeled with both the patient's first and last names as well as a second identifier such as the patient's medical record number or date of birth. Containers without two identifiers will be rejected.

Materials

1. Urine specimen container should be clean, leak proof, particle-free, and preferably made of a clear, disposable material that is inert about urinary constituents.
2. The container and closure should be free of interfering substances (e.g. detergents or trace elements).
3. Urine specimens for routine urinalysis (UA) are collected in clear, dry, chemically-clean containers with tight-fitting lids.
4. The specimen containers should accept a label that will adhere during refrigeration or freezing.
5. Dipstick

Procedure

1. Wash hands with soap and water.
2. Wipe the genital area with tissue paper

Annex II. Information sheet

Project Title: **Establishment of Selected Clinical chemistry parameters Reference Intervals among apparently healthy adults in Addis Ababa ,Ethiopia .**

Project PI: Melkitu Kassaw (BSC, Medical Laboratory Sciences)
Organization: Addis Ababa University

Sponsor: Addis Ababa University and Ministry of Innovation and Technology (MiNT), Ethiopia

Introduction:

Hello! My name is _____ and I am working with researchers from Addis Ababa University. We are conducting a study to Establish Immuno-Hematological and Clinical Chemistry Reference Intervals For adult Ethiopians in Addis Ababa.

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). Especially there is lack of local reference interval for indigenous population. Therefore, the purpose of this proposed study is to Establish Immuno-Hematological and Clinical Chemistry Reference Intervals for adults in Addis Ababa.

You have been chosen for this study. Therefore, we invite you to take part in this study and contribute to the establishment of indigenous reference values which are needed for providing quality laboratory service. Thus, result from this study is anticipated to improve the health status of adults 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 10 ml venous blood (about 1 table spoon) from you by sterile-disposable vacutainer tube and needle (7ml in plane tube and 3 ml in tube containing EDTA). We will conduct laboratory examination to determine different hematological, serological, parasitological and clinical chemistry parameters.

Confidentiality:

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

Risks and Discomfort:

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

Safety:

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

Benefits:

By participating in the study, you will directly benefit by being investigated for any pathogenic organisms and other clinical and hematological abnormalities. Establishing the reference interval and developing the in-house quality control materials will be used in the future to improve the general health status of Ethiopians.

Incentives:

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

Right to refuse or withdraw:

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

Whom to contact:

If you have any questions, you may ask the person whom you are giving your urine, stool and blood.

Melkitu Kassaw 0911 093746

Dr Aster Tsegaye 0911696085

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 IV: 18 ዓመትናከዚያበላይለሆኑአዋቂዎችመረጃ.

የፕሮጀክቱርዕስ፡‘‘እድሜአቸውአምስትዓመትናከዚያበላይለሆኑኢትዬጵያውያንየጤናማሰውደምውስጥየሚገኙየክሊኒካልላቦራቶሪምርመራዎችመጠንሪፈረንስኢንተርቫልእናበላቦራቶሪውስጥየጥራትመመርመሪያንጥረነገርመስራት‘‘፡በበርካታማዕከላትየሚሰራጥናት‘‘

የፕሮጀክቱዋናተመራማሪ:

ተቋማት፡የኢትዮጵያህክምናላቦራቶሪማህበር፣ዩኒቨርስቲዎች፣ሪጅናልላቦራቶሪዎች፣እናብሄራዊየደምባንክአገልግሎትየኢትዮጵያህክምናላቦራቶሪማህበር፣

ስፖንሰር (ወጪውንየሸፈነው)፡የፌደራልሳይንስናቴክኖሎጂሚኒስቴርእናጤናሚኒስቴር

መግቢያ: ጤናይስጥልኝ! ስሜ _____
ነው፡፡የህክምናላቦራቶሪሳይንስትምህርትከሚያስተምሩዩኒቨርስቲዎች፣ሪጅናልላቦራቶሪዎች፣ብሄራዊየደምባንክአገልግሎትእናየኢትዮጵያህክምናላቦራቶሪማህበርጋርእየሰራሁነው፡፡በላቦራቶሪውስጥየጥራትመመርመሪያንጥረነገርእናየጤናማሰውደምውስጥየሚገኙየሄማቶሎጂናየክሊኒካልኬሚስትሪምርመራዎችመጠንሪፈረንስኢንተርቫልእድሜአቸውአምስትዓመትናከዚያበላይለሆኑኢትዮጵያውያንለመስራትበአገራችንየተለያዩክልሎችጥናትእያካሄድንነው፡፡

የምርምርጥናቱአላማ:

የህክምናላቦራቶሪበጤናውአገልግሎትውስጥከፍተኛሚናይጫወታል፡፡ምርመራንለማረጋገጥ፣ህሙማንለመድሃኒቶችምላሽመስጠታቸውንክትትልለማድረግ፣የበሽታዎችንስርጭትለማጥናት፣በሽታለመከላከልእናስለበሽታዎችምንጭምርምርለማድረግአስተዋፅዎያደርጋል፡፡በተለይምበአገራችንየጤናማሰውየላቦራቶሪውጤትማመዳደሪያሪፈረንስኢንተርቫልእናበአገርውስጥየሚመረትየጥራትመመርመሪያየለም፡፡ስለሆነምየዚህጥናትዓላማበአገርውስጥበላቦራቶሪውስጥየሚመረትየጥራትመመርመሪያእናየጤናማሰውየሄማቶሎጂናየክሊኒካልኬሚስትሪውጤትማወዳደሪያሪፈረንስኢንተርቫልእድሜአቸውአምስትናከዚያበላይለሆኑበተለያየክልልለሚኖሩኢትዮጵያውያንመሥራትነው፡፡

እርስዎምለዚህጥናትተመርጠኃል፡፡ስለዚህበዚህጥናትእንዲሳተፉናበአገራችንበላቦራቶሪውስጥየሚመረትየጥራትመመርመሪያእናየጤናማሰውየሄማቶሎጂናየክሊኒካልኬሚስትሪውጤትማመዳደሪያሪፈረንስኢንተርቫልለመስራትአስተዋፅዎእንዲያደርጉተጋብዘኃል፡፡ሁለቱምጥራትያለውየላቦራቶሪአገልግሎትለመስጠትአስፈላጊናቸው፡፡ስለዚህየዚህጥናትውጤትኢትዮጵያውስጥየአዋቂሰዎችጤናንለማሻሻልይረዳል፡፡

የጥናቱአካሄድ:

በጥናቱለመሳተፍከተስማሙየጥናቱአባል/አባላት 15
ደቂቃየሚወስድጥያቄይጠይቁዎታል፡፡ከብደት፣ቁመት፣የክንድእናየደምግፊትልኬትይወሰዳል፡፡ሽንትናአይነምድርበምንሰጠውእ

ቃእንድትሰጡንእንጠይቃለን፡፡፣በተጨማሪም 13 ሚሊሊትር (አንድየሾርባማንኪያየሚሆን)
በንፁህቫኩቴይነርብልቃጥእናመርፌእንቀዳለን (9 ሚሊሊትርበባዶቲዩብ፣ 4
ሚሊሊትርደምእንዳይረጋየሚያደርግንጥረነገር፣ኢዲቲኤ፣ባለበትቲዩብ)፡፡የሄማቶሎጂ፣ሴሮሎጂ፣ፓራሲቶሎጂእናየክሊኒካልኬ
ሚስትሪምርመራዎችንእናካሂዳለን፡፡

ሚስጥርስለመጠበቅ:

በዚህጥናትየሚሰበሰብመረጃበሙሉበሚስጥርይጠበቃል፡፡መረጃበዚህየስምምነትቅፅከተፈቀደውውጪለሶስተኛወገንተላልፎአ
ይሰጥም፡፡የዚህጥናትውጤትሊታተምይችላልነገርግንየጥናቱተሳታፊዎችስምናማንኛውምመለያአይገለፅም፡፡ሚስጥራዊነቱንለመ
ጠበቅየዚህጥናትአባላትመረጃዎችንበተቆለፈክፍልበተቆለፈካቢኔትውስጥያስቀምጣሉ፣የፈቃደኛተሳታፊዎችንማንነትንላለማሳ
ወቅውጤቶችምበኮድይቀመጣሉ፡፡በኮምፒዩተርውስጥለተቀመጡፋይሎችለጥናቱተመራማሪዎችብቻየሚፈቀዱናበሚስጥርቁ
ልፍየሚጠበቁይሆናል፡፡የተሳታፊውጤትለህክምናባለሞያሊተላለፍየሚችለውበተሳታፊውፈቃድብቻነው፡፡የተሰበሰበውሽንት፣
ዓይነምድርናደምለሌላአገልግሎትአይውልም፡፡የሚተርፉትናሙናዎችበአዲስአበባዩኒቨርስቲህክምናላቦራቶሪትምህርትክፍልደህ
ናቦታተቀምጠውለተጨማሪምርመራዎችእንደአስፈላጊታቸውጥቅምላይይውላሉ፡፡በመጨረሻምተሰርቶባቸውየተራረፉየሚደፉ
ናሙናዎችአካባቢንበማይበክልመልኩበጥንቃቄይወገዳሉ፡፡

ጥናቱየሚያስከትላቸውየጤናችግሮችናአለመመቸት:

ሽንትናዓይነምድርበመስጠትየሚደርስመጠነኛአለመመቸትሊኖርይችላል፡፡ሆኖምደምበሚቀዳበትጊዜመጠነኛመጎዳትናየተወሰነ
አለመመቸትሊኖርይችላል፡፡ይሁንእንጂበተቻለመጠንልምድያለውየላቦራቶሪባለሞያበመጠቀምአለመመቸቱንለመቀነስእንሞክ
ራለን፡፡

ደህንነት:

የደምናሙናበሚወሰድበትጊዜበንፁህየደምመቅጃበመጠቀምየሚቀዳውንቦታበ70%
አልኮልበማፅዳትልምድባለውባለሞያይከናወናል፡፡በተጨማሪምጥቅምላይከዋሉበኋላለማስቀመጥየማይሆኑየሚደፉየዓይነምድ
ር፣ሽንትእናደምትራፊዎችየላቦራቶሪደህንነትመመሪያበመከተልይወገዳሉ፡፡

ጥቅማጥቅሞች፡

በዚህጥናትበመሳተፍለበሽታአምጪተህዋስያን፣ደምናሽንትምርመራበማድረግየጤንነትሁኔታማወቅይቻላል፡፡በአገርውስጥበላቦ
ራቶሪውስጥየሚመረትየጥራትመመርመሪያእናየጤናማሰውየሄማቶሎጂናየክሊኒካልኬሚስትሪውጤትማመዳደሪያሪፈረንስኢን
ተርቫልእድሜአቸውአምስትናከዚያበላይለሆኑበተለያየክልልለሚኖሩኢትዮጵያውያንመሰራቱየኢትዮጵያውያንንየጤናሁኔታለማ
ሻሻልይረዳል፡፡

በጥናቱለመሳተፍማትጊያ:

ከዓይነምድር፣ሽንትእናደምምርመራጤናማያልሆነውጤትከተገኘበአቅራቢውወደሚገኝጤናተቋምይላካሉ፣የላቦራቶሪውጤቶቹ
ንበነፃያገኛሉ፡፡ይሁንእንጂበዚህጥናትለመሳተፍምሆነለመድሃኒትክፍያአይሰጥም፡፡ስለተሳትፎዎግንእናመሰግናለን፡፡

ያለመሳተፍመብት:

በዚህጥናትከተሳተፉየቻልነውንሁሉእንክብካቤእናደርጋለን፡፡በማኛውምሰዓትከጥናቱመውጣትእንደሚቻልናይህምበሚያገኙት አገልግሎትላይ (ለምሳሌየጤናአገልግሎት) ምንምአይነትልዩነትአይደረግም፡፡

ጥያቄካለለማነጋገር:

ምንምዓይነትጥያቄካለየዓይነምድር፣ሽንትእናየደምናሙናየሰጡትንሰውመጠየቅይቻላል፡፡

Melkitu Kassa 0911 093746

Dr Aster Tsegaye 0911696085

Annex V: 18 ዓመትእናከዚያበላይለሆኑአዋቂዎችየስምምነትቅፅ

ኮድ._____

ከላይየተገለፀውንመረጃአንብቤአለሁ /ወይምተነቦልኛል፡፡ጥያቄለመጠየቅዕድልተሰጥቶኝጠይቄበሚያረካመልኩተመልሶልኛል፡፡በዚህጥናትለመሳተፍበፈቃደኝነትተስማምቻለሁ፡፡

የዓይነምድርናሙናለመስጠት☐

የሽንትናሙናለመስጠት☐

ደምለመቀዳት ☐

እናበዚህጥናትተሳታፊለመሆን፣በማንኛውምሰዓትከጥናቱለመውጣትመብትእንዳለኝምተረድቻለሁ☐.

የተሳታፊስም፣ቀንእናፊርማ (ወይምአሻራ) ከዚህበታችይፃፉ

_____ _____ /_____ /_____ (ቀን/ወር/ዓመተምህረት)

ያልተማሩከሆኑ;

የተማሩገለልተኛእማኝሰውስም፣ቀንናፊርማ (ከተቻለይህሰውበተሳታፊውቢመረጥናከተመራማሪአባላትግኙነትየሌለውቢሆን)

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

ስልክቁጥር_____

የተመራማሪውስም፣ቀንናፊርማ

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

Annex VI. 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
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 month?	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

	Height (in cm)	_____
45.	Weight (in kg)	_____
46.	MUAC	_____in cm (will be interpreted later)
47.	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/ክፍለ ከተማ _____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	
12.	የትምህርት ደረጃ	6. ያልተማሩ 7. ማበብና መፃፍ 8. አንደኛ ደረጃ (1-8) 9. ሁለተኛ ደረጃ (9-12) 10. ኮሌጅ ዲፕሎማ/ዲግሪ እና ከዚያ በላይ
13.	ሥራ	7. ተማሪ 8. የቤት እመቤት 9. የመንግስት ሠራተኛ 10. የግል ተቀጣሪ 11. ገበሬ 12. ሌላ ካለ ይግለፁ _____
14.	የጋብቻ ሁኔታ	6. ያላገቡ 7. ያገቡ 8. የተፋቱ 9. ባል/ሚስት የሞተባቸው 10. አይመለከታቸውም (ህፃናት)
15.	ሃይማኖት	6. ኦርቶዶክስ ክርስቲያን 7. ሙስሊም 8. ፕሮቴስታንት 9. ካቶሊክ 10. ሌላ ካለ ይግለፁ _____
16.	ብሄረሰብ	_____ድብልቅ ከሆኑ ይግለፁ
17.	መኖሪያ ቦታ	2. ገጠር 2. ከተማ
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 ዓይነት መድሃኒት ወስደኋል?	2. አዎን 2. የለም
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

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

❖ ስለትብብርዎ እናመሰግናለን!

ቃለ መጠይቅ የተደረገበት ቀን:_____

ቃለ መጠይቁን ያካሄደው ስም_____ ፊርማ_____

Declaration

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

Signature:_____

Date of submission:_____

This proposal has been submitted with our approval as advisors.

Advisor:Aster Tsegaye (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Samuel Kindie (MSc, PhD candidate)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia