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Determination of community-based reference intervals for selected clinical chemistry parameters among apparently healthy adult individuals in Mekelle city, Tigrai, Northern Ethiopia from December 2018 to May 2019: a cross sectional study.

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This is to certify that the thesis prepared by Gebreslassie Gebremariam Berhe, entitled: **Determination of community-based reference intervals for selected clinical chemistry parameters among apparently healthy adult individuals in Mekelle city, Tigrai, Northern Ethiopia from December 2018 to May 2019: a cross sectional study** 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.

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Abbreviations

ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BilD	Direct Bilirubin
BilT	Total Bilirubin
BUN	Blood Urea Nitrogen
CI	Confidence Interval
CLSI	Clinical laboratory and standard institute
Cr	Creatinine
FBS	Fasting blood sugar
GLU	Glucose
IFCC	International Federation of Clinical Chemistry
IQR	Interquartile range
LJ	Levy Jennings
MGH	Massachusetts General Hospital
PI	Principal Investigator
QC	Quality Controls
RI	Reference Interval
RPM	Revolution per Minute
SD	Standard Deviation
SOP	Standard Operating Procedure
SPSS	Statistical Package for Social Sciences
TB	Tuberculosis
THRI	Tigrai Health Research Institute
TP	Total protein
US	United States
WHO	World Health Organization

Abstract

Background: Reference Intervals (RIs) are important tools to differentiate health and disease individuals. Establishment of RIs specific for a given area is highly recommended, since RI vary from place to place based on genetics, lifestyle, environmental and nutritional factors. Although few studies have been conducted in Ethiopia to determine RI for clinical chemistry in the Amhara region and Gilgel gibe, they are limited to the specific population and there is no such study conducted in Tigrai. Therefore; this study aimed to determine RIs for selected clinical chemistry parameters among apparently healthy adult individuals in Mekelle city, Tigrai, Northern Ethiopia.

Objective: To determine community-based reference intervals for selected clinical chemistry parameters among apparently healthy adult individuals in Mekelle city, Tigrai, Northern Ethiopia from December 2018 to May 2019.

Method: A cross-sectional study was conducted to establish RI for selected clinical chemistry test among apparently healthy adult individuals in Mekelle city, Tigrai, Northern Ethiopia from December 2018 to May 2019. A total of 344 apparently healthy Study participants were selected based on structured questionnaire, physical examinations, parasitological, and urinalysis. SPSS version 23 was used to analyze the data. RI was determined using 2.5^{th} and 97.5^{th} percentiles and P value < 0.05 was considered as statistically significant.

Result: A RI was established for ten clinical chemistry tests. Among them, three parameters had no sex difference [total protein (5.9-8.25 g/dl), albumin (4.3-5.5 g/dl) and fasting blood sugar (73.7-115mg/dl)]; whereas the other seven had a statistically significant between the sexes: alkaline phosphatase (U/L) [49.3-152 and 58.3-178.6], alanine aminotransferase (U/L) [4.2-23.6 and 5.2-33], aspartate aminotransferase, (U/L) [12.3-34.1 and 15.2-36.74], bilirubin direct (mg/dl) [0.0114-0.553 and 0.019-0.606], bilirubin total (mg/dl) [0.075-0.84 and 0.15-1.08], Urea (mg/dl) [8.05-22.85 and 8.7-26.075], and creatinine (mg/dl) [0.46-0.96 and 0.42-1.153] for female and male respectively.

Conclusion: There was a significant difference for ALP, ALT, AST, BilD, BilT, Urea and creatinine between sexes. This finding is important to improve the health system by providing accurate RI.

Keywords: Clinical chemistry tests, Clinical tests, Ethiopia, Reference interval, and Reference value

1. Introduction

1.1.Background

Evidence-based laboratory medicine is an important part of modern laboratory medicine practices (1). It is expected that clinical laboratory data influence 70% of clinical decisions; often providing significant information for healthcare providers in the prevention, diagnosis, treatment, and management of disease (2).

The concept of reference values was introduced in 1969 by Grasbeck and Saris to describe fluctuations of blood analyte concentrations in well characterized groups of individuals. It is first introduced as a philosophy, has gained universal acceptance as one of the most powerful tools in laboratory medicine to aid in the clinical decision-making process (3).

Health and disease can be differentiated by accurate and reliable reference intervals (RIs) of clinical laboratory testing (4). A correct interpretation of laboratory test results with appropriate diagnostic accuracy requires reference values from the appropriate population. Also, the ability to enroll in clinical studies and the interpretation of results from those studies all hinge on using the appropriate reference (5).

Reference intervals are typically established by assaying specimens from a sample group of people who meet carefully defined criteria, which must be spelled out and made available or use by others (6). Standard methods for determining the reference interval are to define and obtain a healthy population of at least 120 individuals and use nonparametric estimates of the 95% reference interval (4).

Clinical biochemistry reference interval defined as the 2.5^{th} and 97.5^{th} percentiles of a healthy population's distribution (7) and used both in the clinical and research environment which is important for accurate interpretation of laboratory data (8). It is hard to underestimate the importance of clinical laboratory test results. A test result by itself is of little value unless it is reported with the appropriate information for its interpretation. (9).

Clinical laboratory reference intervals are an important tool for identifying abnormal laboratory results and providing assistance to clinicians in creating a more comprehensive clinical perspective for diagnosis and ultimately guiding patient management decisions (8). This means that there is no universal definition of what should be regarded as normal and that it is important to define RIs that are suited to the respective population (10).

Laboratory reference intervals for healthy populations have not been established in most African countries. A common practice in these countries, including Ethiopia, is to use reference intervals derived from peoples living in Europe or the United States (US). studies have shown the difference between clinical reference intervals in the African population as compared to those established in western countries (11-13) several studies have also reported that laboratory parameters vary geographically by ethnic origin, genetics, gender, altitude and environmental factors (11, 12, 14-16). Some Studies on reference interval in Kenyan adults, Tanzanian populations, and healthy adult population of Ethiopian showed a notable difference when related to reference values from European countries (17-19).

It is important that reference values are established from appropriate and pertinent populations (20). To establish appropriate RI, the International Federation of Clinical Chemistry (IFCC) and Clinical and Laboratory Standards Institute (CLSI) recommend that RI should be derived locally (10).

1.2.Statement of the problem

Establishing reference intervals has always been a challenge due to significant differences that may exist in disease frequencies, among ethnic groups, genders, and ages. Lifestyle, environment, and genetics; specimen collection techniques, test performance, and test interpretation may also contribute to the diference (9).Producing reference intervals for a general population is a major challenge, as it requires selecting the appropriate reference population and recruiting individuals who represent relevant demographic groups that meet the inclusion criteria (21-23).

Laboratory reference values for populations from western countries are commonly available in the scientific literature textbooks and on the World Wide Web. However, there is lack of published data about laboratory parameters for populations living in tropical sub Saharan Africa (18), and non locally derived RIs are usually used for diagnosis and research purpose (24).

International guidelines recommend that reference intervals are needed for all tests in the clinical laboratory. However, the majority of clinical laboratories in the world adopt RIs established by manufacturers, rather than developing their own (25). International organizations also recommend that population-specific clinical laboratory RIs should be established because gender, age, ethnicity, race, diet, geographical location, and other factors could affect the physiological value of biochemical parameters (26).

There was obsrved difference in the reference intervals of clinical chemistry parameters among different African countries, Caucasian and ethinic group in one country. This may lead to misdiagnosis, inappropriate patient management, and unnecessary use of resources (10, 27-29).

Physicians depend on the availability of appropriate and reliable reference intervals to accurately interpret laboratory test results combined with medical interview and clinical examination. Although health professionals recognize the importance of reference intervals, many laboratories still do not have reference ranges that are specific for their typical patent populations. There is significant gaps in the available reference intervals due to intervals cited in the literature were obtained using older methodologies and instrumentation and cover a specific range of age groups or a relatively small number of samples (9).

Although few studies have been conducted in Ethiopia to determine reference Interval for clinical chemistry in the Amhara region and Gilgel gibe (19, 30, 31), they are limited to the specific population and there is no such study conducted in Tigrai regional state. Besides, the Studies conducted in Ethiopia reported a significant difference in laboratory reference ranges compared with those of other African countries and industrialized [(19, 30). Therefore; This study aimed to determine reference intervals for clinical chemistry parameters among apparently healthy adult individuals in Mekelle city, Tigrai, Northern Ethiopia.

1.3.Significance of the study

This study will benefit health professionals and other stakeholders since, it provides local RI for proper diagnosis, management, treatment and follow up of their customers. It also used to avoide the risk of either unnecessary investigations or failure to detect disease.

The population of this study area will not waste unnecessary money and time due to misdiagnosis. Inaddtion to that the chance of affecting by side effect of druges due to miss treatment will be decreased.

This study can serve as a baseline for further study in the region and also in the country.

2. Literature review

A major need for laboratory medicine and clinical chemistry personnel, in particular, is to provide the clinicians updated & appropriate information in Reference Values. The introduction of the concept of Reference Values and Reference population simplifies the task for laboratories (32). Some of the literature conducted on reference intervals in different countries, including Ethiopia are indicated below.

A combined reference values for adults determined at the Massachusetts General Hospital (MGH) for alkaline phosphatase (ALP) [30-120 U/L], alanine aminotransferase (ALT) [0-35 U/L], aspartate aminotransferase (AST) [0-35U/L], Bilirubin direct (BilD) [0.1-0.3 mg/dl],bilirubin total (BilT) [0.3-1 mg/dl],Total protein(TP) [5.5-8 g/dl] ,Albumin(ALB) [3.5-5.5 g/dl], creatinine (Cr) [0-1.5 mg/dl], Blood urea nitrogen (BUN) [10-20 mg/dl],and Glucose (GLU) [4.2-6.4mmol/l] (33).

A study carried out for Development of reference intervals for serum alkaline phosphatase among adults in Southern China traced to the new IFCC reference measurement procedure, a Serum ALP concentration was obtained from the cohort of eligible reference individuals (n=658). The RI for serum ALP in males age 18–79 years was 48–131 U/L. Females were partitioned into two age groups based on statistical analysis, 18–49 years and 50–79 years, and the RIs derived were 40–106 U/L and 57–159 U/L, respectively. The results demonstrated that overall the values obtained were comparable to reported values except in the case of women in the age range of 50–79 years where values were generally higher. Thus, serum ALP levels were found to be associated with age and gender. Besides, the reference limits established in this study were slightly higher for males than females at age 18–49 years, but slightly lower for males than females at age 50–79 years. These results may be due primarily to physiological changes associated with female climacteric when changes in hormone secretion critically affect bone (calcium and phosphate) metabolism (34).

A study carried out in China for the determination of Reference intervals for serum creatinine levels in the healthy adult population from the age of 18 to 59, the selected participants were divided into different groups by every ten years for an age group. Cr values did not show a statistically significant difference ($P > 0.05$) among the four groups (18–29, 30– 39, 40–49 and 50–59 years) in both genders in our study. Therefore, these four groups were combined into 1 group (18–59 years). The 2.5^{th} and 97.5^{th} percentiles of the Cr levels for adult (18-59 years) was 59 µmol/l- 91 µmol/l for male and 40.9 µmol/l- 65.8 µmol/l for female (35).

A study performed in Kenya for adult population, the result of the reference ranges for ALT, AST, ALP, ALB, TP, Cr, GLU, and BUN, were [0-39 U/L and 0-34 U/L], [(6-40) U/L, and (3-37) U/L], [(13-201) U/L and (5- 227) U/L], [(29-52) g/L, and (28-50) g/L], [(57-89) g/L and (56-88) g/L], [(59-127) µmol/L, and (54-122) µmol/L]; [(2.8-6.8) mmol/L and (2.6-7) mmol/L], [(1.5-5.9) mmol/L and (1.2-6.0) mmol/L] for male versus female respectively. Age differences in the established reference ranges were observed in ALT, ALB, ALP, and Cr in males and ALT, ALB, and Cr in females. Gender differences were observed in ALT, AST, ALB, and Cr in the 18-28-year-old; ALT, AST, and ALB in 29-39-year-old, and AST and ALB, in 40-50-year-old (36).

A study performed in the northern rift valley of Kenya, the established reference value for adult male and female was as follows: BUN (1.8–5.8 mmol/L and 1.9–6.1 mmol/L), and Cr (48–85 µmol/L and 56–99 µmol/L), for female and male respectively. The result of BUN shows no significant sex differences while Cr showed significant sex differences ($P<0.05$). The observed variation in renal function test reference values developed in this study compared to reference range values for the same parameters from other locations suggest variations in analytical methods in addition to ethnic composition and ecological parameters. The higher reference range value for Cr compared to those of other locations could be due to genetic factors and environmental factors. The different lifestyles and genetic composition of the populations could also explain the differences (37).

A study entitled by population-based reference intervals for common blood Haematological and biochemical parameters in the Akuapem north district, Ghana, the concentrations of the liver enzymes, ALT, AST and ALP, and serum Cr were significantly higher in males than females. The RI of ALT (U/L) [9.5 – 39.2 and 11.6 – 53.1], AST (U/L) [15.5 – 46.5 and 18.7 – 65], ALP (U/L) [124 – 479 and 98 – 316], and Cr (mmol/L) [70 – 121 and 81 – 141] for females and males respectively. The distribution of Bilirubin, Albumin and Urea did not differ significantly between males and females. The RI of BilD (mg/dL） [0-0.6), BilT (mg/dL) [0.1–1.4], ALB (g/Ll)[4.6 – 6.8], and Urea (mmol/L) [1.7 – 7.2]. Occult hepatic insults from subclinical viral infections or the usage of herbal preparations may contribute to the differences seen between the values reported here and those in standard clinical texts. Diet, physical environment and socio-economic conditions all affect the physiology of a population, and hence measures of 'normal' physiological functions are expected to differ from population to population (38).

A study conducted in Maputo, Mozambique, on reference value for clinical parameters in young adults, the chemistry values were comparable to US values, with few exceptions. The upper limits for ALT, AST, and BilT, were higher than those from the US. There was a statistically significant difference between genders in all clinical chemistry analytes. Males had significantly higher levels of BilT, GLU, AST, ALT, ALB, Urea, Cr, and ALP than females. The Maputo values were lower compared to western Kenya in the same age group (39).

Table 1: Reference interval (2.5^{th} -97.5^{th} percentiles) for the young adult in Maputo city, Mozambique, May 2004

Analyte	Male	Female
Bilirubin total(µmol/L)	5.8- 36	4- 22.5
Glucose(mmol/L)	3.1- 5.7	3.2- 5.3
ALT(U/L)	6.5- 53.2	4.8- 38.5
AST (U/L)	16.8- 45.5	13.5- 37
ALP (U/L)	97.7- 266.1	91.4- 240.6
Albumin (g/L)	43.4- 55.2	40.1- 52.6
Creatinine (µmol/L)	58.2- 109	45- 86.6
Urea (mmol/L)	1.8-5.8	1.3- 5.1

A study conducted in Middle Belt of Ghana for determination Haematological and Biochemical Reference Values for Healthy Adults, males had significantly higher ALT of 8–54 against 6–51 U/L ($p<0.0001$), AST of 17–60 against 13–48 U/L ($p<0.0001$), ALP of 101–353 against 82–293 U/L ($p<0.0001$), and Cr of 56–119 against 47–110 mmol/L ($p<0.0001$) compared to the females. Although the definite cause of higher liver enzymes in their population is unknown, there is the possibility of this being due to subclinical viral infections or the levels of usage of herbal preparations. On the use of herbal preparations, it has been estimated that the first line treatment for 60% of children with fever resulting from malaria in Ghana, Mali, Nigeria, and Zambia is the use of herbal medicine at home. Screening for Hepatitis viruses was also not performed in this study (40).

A study entitles with Adult Hematology and Clinical Chemistry Laboratory Reference Ranges in a Zimbabwean Population, from a total of 769 adults (54% males) aged 18 to 55 years were included in the analysis. The median age was 28 years and they established clinical chemistry RI for 24 analytes. ALB (4.1- 5.5 g /L and 4.5- 5.9 g/L), ALP (39- 131 IU/L and 49-149 IU/L), ALT (5- 35 IU/L and 9-58.8 IU/L), AST (12-40 IU/L and 17-57 IU/L), BilD (0-0.3 mg/dl and 0-0.4 mg/dl), BilT (0.2-1.1 mg/dl and 0.3-1.6 mg/dl), Cr (0.5-1.1 mg/dl and 0.7-1.3 mg/dl), TP (6.8-9.4 g/l and 71-93 g/l), Urea (3.9-15.4 mg/dl and 4.8-15.4 mg/dl), and random GLU (63.5-99 mg/dl and 61-103 mg/dl). Males had higher levels of urea, Cr, TP, ALB, and liver enzyme levels compared to females ($p<0.001$). BilD from this study was higher compared to the existing reference ranges. High creatinine in males compared to females is expected and explained by a greater skeletal, muscle and bone mass in males. In this study infectious diseases are not screened, such as malaria and helminths. Glucose testing was not fasting (41).

A study carried out in Rwanda for estimation of reference values for serum protein and electrolytes using laboratory-based cross-sectional study, the result of mean and RI (2.5^{th} - 97.5^{th}) is obtained as follows: TP(g/dl) [7.3 (6.3-8.4) and 7.3 (6.3-8.5)]; ALB(g/dl) [4.3 (33.1-5.2) and 4.1 (3.2-5)]; globulins(g/dl) [3.1 (2-4.2) and 3.2 (2.1-4.2)] for male and female respectively. The value of total protein and albumin is comparable to the classical level reference range. This may be due to well-nourished study participants, and deviations from classical ranges with a higher proportion of globulins would be expected in rural undernourished populations with a higher prevalence of infectious diseases. Our RI particularly total protein level is wider than the other published RIs.

This is probably due to a difference in the precision of analytical methods and sample size. With a greater sample size, the standard deviation (SD) decreases leading to narrower RI (42).

A study conducted in Uganda a Chemistry reference intervals were established for analytes including liver function tests, and renal function tests. Those are : ALB (3.7- 5.2 g/dl vs 3.9-5.4g/dl), ALP (47- 160 U/L vs 42-159 U/L), ALT (5.3- 39.9 U/L vs 7.2-43.3 U/L), AST (11.4-28.8U/L vs 13.2-35.9 U/L), BilD (0-0.4 mg/dl vs 0.1-0.5 mg/dl), BilT(0.3-1.9 mg/dl vs 0.4-2.6 mg/dl), Cr (0.5-0.9 mg/dl vs 0.6-1.2 mg/dl), TP (6.8-9 g/dl vs 6.5-8.9 g/dl), and BUN (4.4-14.1mg/dl vs 4.7-15.8 mg/dl) for female versus male respectively. There were statistically significant ($p<0.05$) differences between men and women. Most tests were in agreement with reference intervals published in the US with few exceptions. History of tobacco use, diet, alcohol consumption, fasting status, exercise history, genetic or environmental factors, occupation and socio-economic status could not be obtained and utilized in this study (43).

A study performed in 2014 on normal adult Nigerians a comprehensive reference range for hematology and clinical chemistry laboratory parameters, a reference interval was established as follows: BUN (mmol/L) [2.2– 4.8 and 2.5 – 5.8], Cr (µmol/L) (76.3–111.1 and 63–117.8], GLU (mmol/L) [3.7–7.9 and 4.2–9.6), AST (U/L) [26.0–49.4 and 22.0–58.4), ALT(U/L) [17.3_48.4 and 19.0–38.0], BilT (µmol/L) [3.42–17.1 and 0.3–10.6] respectively for male and female. There was a significant difference by gender for Cr, and BUN ($p<0.05$). Liver enzymes in this study show no significant variation except BilT which is high in males than females ($p = 0.000$), although values for females are slightly higher. The significant differences between males and females, and across countries in clinical chemistry reference ranges illustrated by this study emphasizes the need for such comprehensive establishment of reference values for different populations (44).

A study entitled by biochemical profile at Gilgel Gibe field research center, southwest Ethiopia shows the following result. The 95-percentile range of total serum protein was 4-11.4 mg/dl for men and 4.6-11.7 mg/dl for women. The 95% percentile range for FBS were 66-133 and 68-129 mg/dl for men and women respectively. The mean ALT and AST level of the study population were 27.9 U/L and 31.0 U/L for men and 26.6 U/L and 30.2 U/L for women, respectively. In most age strata, the mean ALT value was higher for men than women. The mean value of ALP was 187.4 U/L for men and 199.5 U/L for women. The highest values of ALP, 227.9 U/L for men and

214.9 U/L for women were observed in the age group of 15-24 years. Distribution of FBS by age showed an increasing pattern which might be explained by the expected relative reduced insulin sensitivity with increasing age. The difference between rhis study and the other studies may be due to the variability between subjects by age, sex, geographic, environment and genetic variation besides the design and sample effect (31).

A study conducted in Gojjam zone northwest of Ethiopia, after careful screening of a total of 799 apparently healthy adults who were consented for this study, complete data from 446 (224 females) were included for the analysis and the established reference interval for ALP (U/L), ALT (U/L), AST (U/L), BilD (mg/dl), BilT (mg/dl), Cr (mg/dl) and TP (g/dl) was 49–236 versus 55.3–237.2; 3– 30 versus 6– 44.6; 6– 32.1 vesus 10.5– 39; 0.012–0.714 vesus 0.0234–0.84; 0.21–2.2 versus 0.275–2.2; 0.245–1.083 versus 0.197–1.29; 5.32–8.6 versus 5.32–8.6 respectively for female and male. Males had high ($P<0.05$) mean and 2.5^{th} - 97.5^{th} percentile ranges of ALT, AST, ALP, Cr, and BilD. The reference intervals of TP and BilT were not significantly different between the two sex groups. Significant ($P<0.05$) higher values of the ALT, ALP, and TP were observed in people living in high land compared to low land residences. The possible reasons for the variation may be due to lifestyle, nutritional, geographical, and environmental factors. The possible reason for the result of this study from western is climate, gender, a month of study and geographical location (19).

A study conducted in Amhara national state, Ethiopia on blood donors, a RIs were established for ALT(U/L) [5.13–42.88 and 4.3–37]; AST(U/L) [12.13–46.88 and 10–43.8]; ALP(U/L) [77.2–475.8 and 89– 381]; BilT(mg/dl) [0.11–1.18 and 0.08–0.91]; Cr(mg/dl) [0.48–1.13 and 0.47–1.09]; TP(g/dl) [5.7–9.7 g/dl and 5.6–9.47]; and Urea(mg/dl)[12–43 and 10–38.7] for male and female respectively. There were statistically significant higher RI in males as compared to females in clinical chemistry parameters. Except for ALT, there was a variation in the reference interval between this study and the other study conducted in the region in the Gojjam zone. The variation may be due to geographical location, demographic, and this study was conducted mainly in young adults. Besides, it may be due to variations in the analytical method, equipment, and reagents used. Some analyses of the upper limit of this study (ALP, albumin, and direct bilirubin) are higher than Uganda, Tanzania, Ghana, and the US. The other factors for the variation for those may be ethnicity, lifestyle, nutrition, culture, seasonal variation and the prevalence of disease (30).

3. Objective

3.1.General Objectives

To determine community-based reference intervals for selected clinical chemistry parameters among apparently healthy adult individuals in Mekelle city, Tigrai, Northern Ethiopia from December 2018 to May 2019.

Specific Objective

- ✓ To determine sex specific reference interval of clinical chemistry tests among apparently healthy adult individuals
- ✓ To compare the reference interval between females and males

4. Materials and Methods

4.1. Study area

This study was carried out at Mekelle City from December 2018 to May 2019. Mekelle is the Capital City of Tigrai Regional State and is located in the Northern part of Ethiopia, at 783 km from the Capital City of Ethiopia, Addis Ababa, with an elevation of 2,254 meters above sea level. Mekelle City is administratively divided into seven sub-cities as follow: Semien, Kedamay Weyane, Hawelti, Ayder, Hadnet, Adi Haqi, and Quiha. According to the projected Central Statistical Agency of Ethiopia (CSA), the town of Mekelle City has a total of population of 310,436 people. Our study was conducted in three sub-cities (Ayder, Hawelti, and Semien) and the sample was collected from the eligible apparently healthy individuals and was transported to Tigrai Health Research Institute for analysis which is found in Hawelti Sub-city (45).

4.2.Study design and period

A cross-sectional study was employed to determined the parameters of clinical chemistry reference intervals in Mekelle city, Tigrai,Northern Ethiopia from December 2018 to May 2019.

4.3.Population

4.3.1. Source population

The source populations for this study were all adult individuals who live in Mekelle city, Tigrai, Norhern Ethiopia.

4.3.2. Study population

The study populations were all adult individuals aged from 18 to 60 years who live in Mekelle city, Tigrai, Norhern Ethiopia those fulfilling eligibility criteria.

4.4.Inclusion and exclusion criteria

4.4.1. Inclusion criteria

Apparently healthy individuals aged from 18 to 60 years, both sexes who lived at least 5 years in the study area were included in the study.

4.4.2. Exclusion criteria

- Individuals who have a history of any acute and chronic illnesses like diabetes mellitus, chronic renal insufficiency, hypertension, ischemic heart disease, anemia, thyroid, liver diseases, cancer of any type
- Individuals taking pharmacologically active substances: all prescription drugs, and have a habit of smoking and alcohol consumption
- Individuals who donated blood within the previous 3 months
- Individuals who received blood transfusion within the previous 1 year
- Individuals who had Hemoparasite and intestinal parasite
- Pregnant women

4.5.Study variable

4.5.1. Dependent variable

- Clinical chemistry parameters (AST, ALT, ALP, FBS, ALB, TP, BilD, BilT, Cr, and urea)

4.5.2. Independent variable

- Sex

4.6.Sample size determination and sampling technique

4.6.1. Sample size determination

The Clinical Laboratory Standards Institute (CLSI) guideline for the global application which was developed through the Clinical and Laboratory Standards Institute consensus process was employed.

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) (46). The dominant form of partitioning applied in clinical laboratory medicine is by social consensus, usually adult age individuals begin from 18 age to the age of retirement which is about 60 (47). According to previous studies in other African countries, in such studies about 30% of the apparently healthy population (16) do 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 a total sample size of 240 for the reference interval determination, a total of 344 individuals was

enrolled (i.e., 30 % x 344=104 to be excluded during data analysis; 344-104=240); thus, giving a total minimum sample size of 344.

Thus, 344 participants were recruited from Mekelle city. The study participants were selected using a multistage sampling technique by considering the sub-city as a primary sampling unit and then households the final selection units. One individual in the household fulfilling the eligibility criteria and willing to participate was included.

4.6.2. Sampling technique

Three sub cities from the total seven sub cities in Mekelle city (Ayder, Hawelti, and Semen) through a simple random sampling method and then the total sample (344) was categorized based on the relative household size in each sub city. The total household numbers of the 3 sub cities were 68,477 (18266, 33319 and 16892 in Semen, Hawelti and Ayder respectively). The total sample size was distributed to each sub city by probability proportional to size(PPS) method based on household. The allocated sample size(household) to Semen, Hawelti, and Ayder were 92, 167 and 85 respectively. In each sub city, there were 5 kebeles, and then we also allocte the sample to each kebele based on the size of the household. After that, data and specimens were collected from each kebele based on systematic sampling technique (K^{th}). Individuals in every K^{th} household were approached at their households through health extension workers. If more than one participants were present in one household, simple random sampling technique was used to select one individual. The table below shows the number of individuals that has been recruited from each kebeles.

Table 2:Total number of houses hold and specimen that was recruited per each Kebele at Mekelle, 2019

subcity	Kebele	Total number of household	Total number of samples recruited	male	female
Semen	Mesfin	4615	24	12	12
	Dedebit	2870	14	7	7
	Yekatit	3216	16	8	8
	Industry	2451	12	6	6
	Meles	5114	26	13	13

	Total	18266	92	46	46
Hawelti	Selam	3397	18	9	9
	Hayelom	4614	24	12	12
	Adi Shimduhun	8793	44	22	22
	Momona	7731	38	19	19
	Hidase	8784	44	22	22
	Total	33319	168	84	84
Ayder	Sertse	3483	16	8	8
	Ginbot 20	3487	18	9	9
	Marta	3637	20	10	10
	Adi ha	5046	24	12	12
	Maryam Dihan	1239	6	3	3
	Total	16892	84	42	42

Note: Source for number of households is from municipal of Mekelle city, 2018

4.7. Measurement and data collection

The study aims, risks, benefits of study participation and right to withdraw from the study at any time were explained by the study team. From those consenting participants, demographic information and a brief medical history were collected with counciling of a physician. Then physical examination was performed by health professionals. A blood specimen was collected for clinical chemistry parameters, and malaria. Stool and urine analyses were also done to screen the participnats. Laboratory results were given to participants upon their requests upon their requests and individuals with positive findings were linked to health facilities.

4.7.1. Demographic and clinical data

Socio-demographic and clinical data were collected using a structured questionnaire by trained data collectors and physical examination and anthropometric measurements were carried out by health professionals.

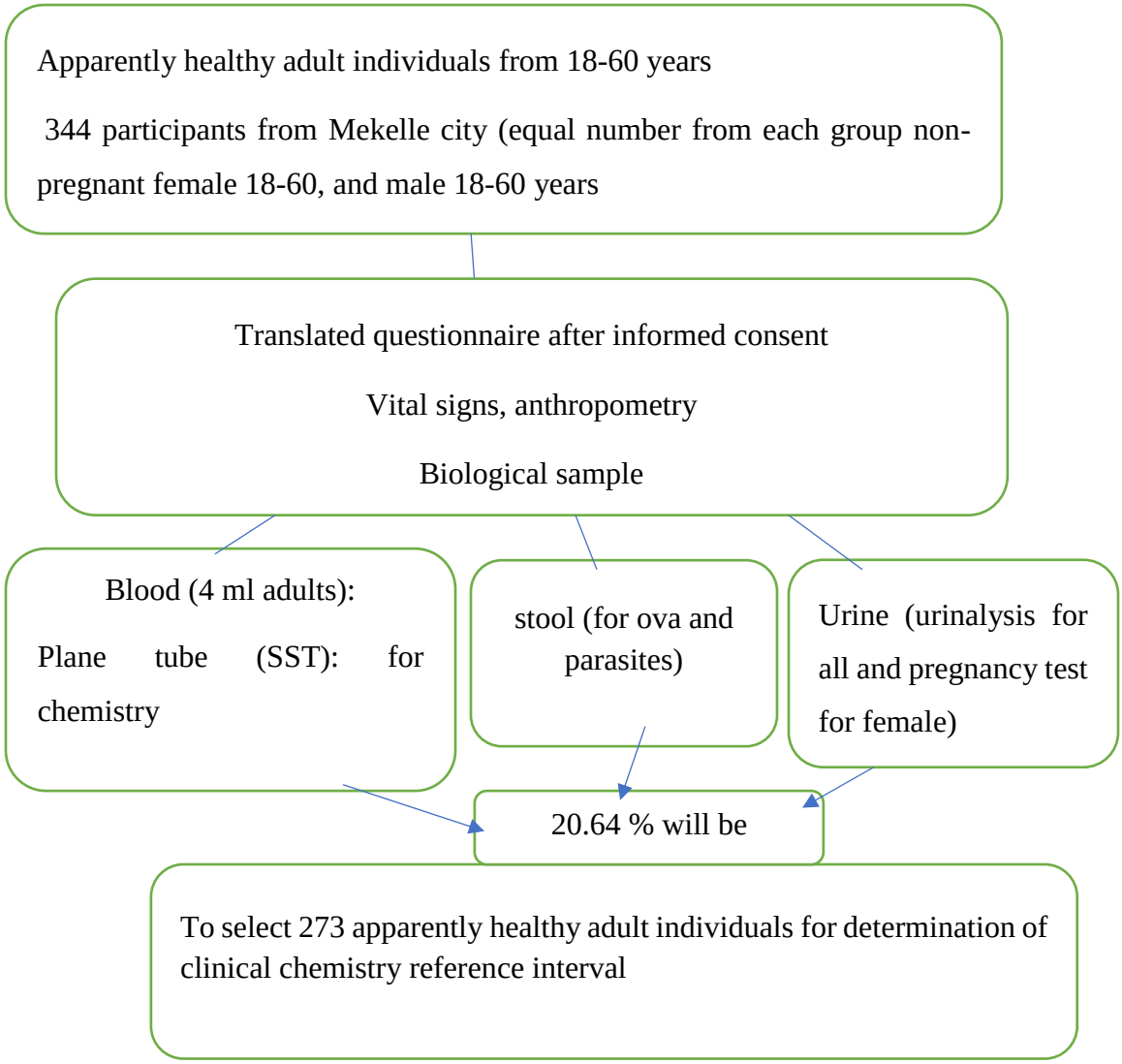

Figure 1: Data collection procedure

4.7.2. Sample collection for laboratory analysis

Blood samples of about 4 ml from adults were collected by a serum separator test tube using a multi-sample needle. To minimize the diurnal variation of some analytes blood samples was collected from 8: 00 am to 11:00 am. Whole blood was used for glucose determination and hemoparasites identification while serum samples for clinical chemistry profiles. A stool sample was collected for parasitological analysis and urine for Urinalysis and determining pregnancy status. Leak-proof clean containers were used to collect urine and stool samples.

All samples were labeled with a unique identification number and site of blood collection. Direct and concentration stool analysis was performed on site in the respective health facilities where participants are invited to health centers. Serum and left-over plasma were collected and stored at $-37^{O}C$ in the Regional laboratory until tested. All results entered into SPSS version 23.

4.7.3. Laboratory testing and analysis

Clinical Chemistry parameters were determined using a fully automated Biosystem A-25 Chemistry Analyzer by spectrophotmetery. The reference interval of each test was determined by the methods/techniques of the following (table 3).

Table 3: Methods used for analytes of clinical chemistry to determine the reference interval of apparently healthy individual of the Mekelle city, Tigrai, Ethiopia 2019

Analyte	Method/technique
ALT	kinetic (IFCC without pyridoxal phosphate activation)
AST	kinetic (IFCC without pyridoxal phosphate activation)
ALP	2-amino 2-methyl 1- propanol (AMP)
Cr	Jaffe compensated
Urea	kinetic urease/GLDH (Glutamate dehydrogenase)
TP	Biuret
ALB	Bromocresol green
BilD and BilT	Diazotized Sulfanilic
FBS	Glucose oxidase

ALP: alkaline phosphatase, ALT: alanine aminotransferase, AST: aspartate aminotransferase, BilD: Bilirubin direct, BilT: Bilirubin total, Cr: creatinine, TP: Total protein, ALB: Albumin, and FBS: fasting blood sugar

All laboratory assays were carried out following standard operating procedures by experienced Medical laboratory technologists. Stool samples were collected and analyzed using direct, Kato Katz and concentration methods.

4.8.Data quality assurance

The questionnaire was pre-tested with few individuals, other than study subjects (5%). This pre-testing of a research instrument was entailed a critical examination of each question as to its clarity, understanding, wording, and meaning as understood by potential respondents to remove possible problems with the question. Besides, adequate training was given to the data collectors before the collection period. Participants were also be adequately oriented on how to collect specimens. The quality of laboratory analysis was maintained by following standard operating procedures of the pre-analytical, analytical, and post-analytical stages, which involves the following steps.

4.8.1. Pre-analytic phase

- The blood was collected from the participants using a standard operating procedure.
- The blood sample container was labeled with the participant's unique code to minimize errors.
- The quality of the collected samples was checked like hemolysis, clot, correct volume, etc.
- The sample was transported using cool box to protect from heat and sunlight to analysis area
- The quality of the centrifuge was checked by tacho method and the serum dispensed after centrifugation immediately to Nunc tube in order to minimize interference
- The quality of the instrument, reagent expiration, reagent volume, and the room temperature were checked before analysis.
- The manufacturer procedure and SOP of the laboratory were strictly followed.
- Qualities of the information's that were collected by the questionnaire from the participants were filled by the principal investigator.

4.8.2. Analytic phase

- The reliability of the study finding especially the analytical part was guaranteed by implementing Quality Controls (QC) sample for the clinical chemistry tests through the whole processes of laboratory works.
- Both normal and pathological quality controls were analyzed before the sample analysis to ensure the proper function, validity, and reliability of the instrument.
- The manufacturer and laboratory SOP for the analytical phase was followed.

4.8.3. Post-analytic phase

- The results of the clinical chemistry tests were registered with correct values and units.
- Data was entered using a double entry method to trace data entry errors which has a strong negative effect on study results and conclusions.

4.9.Data analysis and interpretation

The data was cleaned, entered, processed and analyzed by SPSS Version 23 software. The distribution of the data was checked by Kolmogorov-Smirnov and Shapiro Wilks test. Data that was observed to be lower than 1.5 ×inter quartile range (IQR) of the first quartile, or higher than 1.5 × IQR of third quartile (Using whisker and blot method) was considered as outliers and the outliers was manually deleted. The analyzed data was presented by tables and through other statistical tools.

Mann Whitney U test was used for median comparison between male and female. Reference interval was estimated using the 2.5^{th} percentile for the lower reference limit and 97.5^{th} percentile for the upper reference limit. P-value 0.05 with 95 % confidence interval was considered statistically significance

4.10. Ethical considerations

Before starting the study, ethical clearance was obtained from the ethical review committee of the department of Medical Laboratory Science Addis Ababa University. Permission was obtained from Tigrai Regional Health Bureau and administration of each sub city. Written consent form was obtained from each study participant before data collection. Confidentiality of information (results) was kept between the study participant, investigator and authorized body.

4.11. Dissemination of the result

Findings of this study will be presented to the scientific community in the Addis Ababa University, deparment of Medical laboratory science. The result will be communicated to Tigrai regional health bureau, and other concerned institutions. Finally it will be submitted appropriate journals for publication.

4.12. Operational Definition

Selected clinical chemistry tests: Total protein, albumin, fasting blood sugar, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, bilirubin direct, bilirubin total, Urea, and creatinine.

Refference interval: The interval between 2.5^{th} and 97.5^{th} percentiles and including both percentiles

Addult: Iduviduals who are aged from 18 to 60 years

Renal function test: Tests that are uesd to asses the function of kidney

Liver function test: Tests that are used to asses the function of liver

5. Work flow

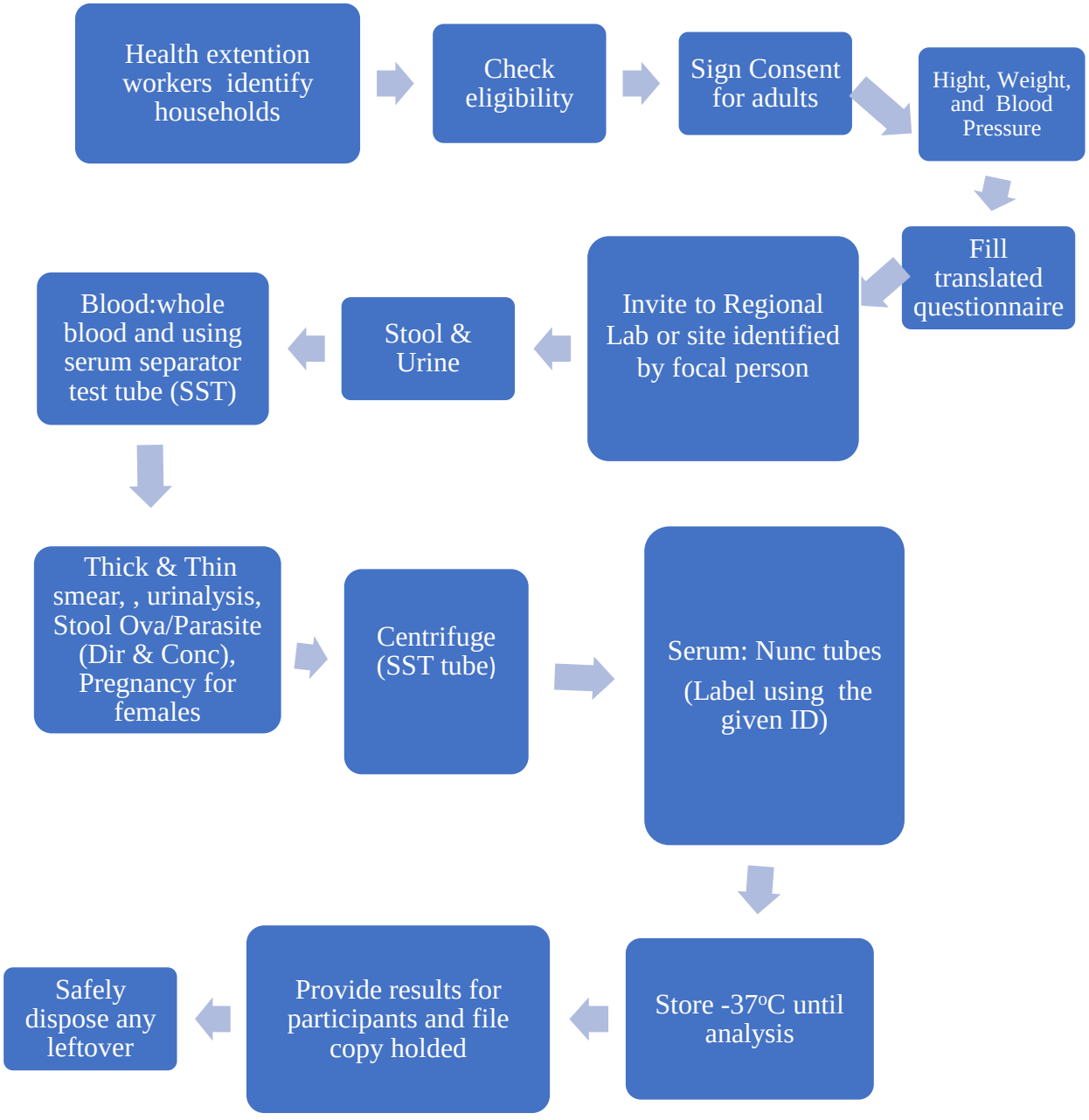

Figure 2: work flow of the study

6. Result

6.1.Sociodemographic characteristics of the participants

About 344 apparently healthy individuals volunteers aged from 18 to 60 years have enrolled in this study. Of those 172(50%) were females. Around 71(20.64%) of the total are rejected due to parasitological infection 37(10.76%),urinary tract infection 5(1.45%), hemolyzed and lipemic sample 5(1.45%),insufficient sample for test 2(0.58%),pregnancy 2(0.58%) and insufficient reagent 20(5.8%). Finally, about 273(79.36%) participants were involved in the clinical chemistry test analysis. Median (IQR) age of the participants were 31 (19-47). Generally, the sociodemographic characteristics of the participants are expressed in table 4 below.

Table 4: Sociodemographic characteristics of the participants of the Mekelle city, Tigrai, Northern Ethiopia, from December 2018 to May 2019 (n=273)

		Frequency	Percent (%)
Sex	Male	136	49.8
	Female	137	50.2
Religion	Ortodox	260	95.2
	Muslim	10	3.5
	Protestant	3	1.1
Marital status	Single	132	48.35
	Married	116	42.5
	Divorced	16	5.9
	Widowed	9	3.3
Level of Education	Illiterate	4	1.5
	Read and write	1	0.4
	Primary (1-8)	17	6.2
	Secondary (9-12)	176	64.5
	College diploma/degree and above	75	27.5
Occupation	Student	123	45.1
	House Wife	5	1.8
	Government employee	63	23.1
	Private employee	82	30.1

6.2.Established Reference interval for parameters of clinical chemistry

About 273 apparently healthy adult individuals were involved in the clinical chemistry test analysis for the establishment of the reference interval for seven liver function tests (alkaline phosphatase, alanininеaminotransferase, aspartate aminotransferase, albumin, total protein, direct bilirubin, and total bilirubin), two renal function tests (creatinine and urea) and fasting blood sugar. Outliers were excluded for each of the tests by whisker and plot method. After excluding the outliers,the remaining participants for each test was indicated in tables 5 and 6. For each of the ten analytes of the clinical chemistry tests mean, median, 95 % CI for the mean, 2.5^{th}-97.5^{th} percentile, 90 % CI for lower and upper limit was determined (shown in table below 5 and 6). Renal function tests both Creatinine (p value = 0.0001) and urea (p value= 0.048) were statistically significant between male and female, but glucose was not statically significant.

Table 5: The established RI of Renal function tests and Glucose for the participants of the Mekelle city, Tigrai, Northern Ethiopia, from December 2018 to May 2019 (n=273).

Analyte (mg/dl)	P	n	Median	Mean	95% CI for mean	2.5^{th}- 97.5^{th} percentile	90% CI for LL	90%CI for UL	P-Value
Cr	C	268	0.77	0.77	0.75-0.8	0.46-1.133	0.42-0.47	1.1-1.16	0.0001
	F	134	0.7	0.705	0.68-0.73	0.46-0.96	0.44-0.47	0.89-1.02	
	M	134	0.865	0.84	0.8-0.87	0.42-1.153	0.4-0.48	1.13-1.17	
Urea	C	268	15.65	15.81	15.31-16.3	8.72-24.35	7.1-9.6	23.3-26.2	0.048
	F	134	15.35	15.21	14.6-15.8	8.05-22.85	5.3-0.1	22.1-23.76	
	M	134	16.05	16.40	15.62-17.2	8.7-26.1	6.9-9.6	24.1 -27.73	
GLU	C	266	90	91.65	90.32-93	73.7-115	70.2-75	112 -116.8	0.198
	F	135	90	90.7	88.9-92.5	71.8-115	67.45-76	109-120.4	
	M	131	92	92.62	90.6-94.6	74-115	70.2-75	112-117	

C: combined,Female: Male, n: number of participants, CI:confidence interval, Cr: Creatinine, and GLU: Glucose, LL: lower limit, UP:upper limit, and P: participants.

For the liver function tests, there was stastistically significance of the between gender. ALP (P value=0.0001), ALT (p value=0.001), AST (p value=0.001), BilD (P value=0.005) and BilT (p value=0.014) were stastistically significance between female and male, but total protien (p value=0.156), and albumin (p value=0.603) were not stastistically significance between sexes (showen in table 6).

Table 6: Established reference interval for Liver function tests among study participants in Mekelle city, Tigrai, Northern Ethiopia from December 2018 to May 2019 (n=273)

Analyte	P	n	Median	Mean	95% CI for mean	2.5^{th}– 97.5^{th} percentile	90% CI for LL	90%CI for UL	P-Value
ALP(U/L)	C	258	92.7	99.45	95-103.8	55.4-170.15	47.7-57.7	157.6-179.1	0.0001
	F	130	88.2	90.5	86.34-94.8	49.3-152	28.5-56.4	132.5-153.5	
	M	128	97.15	105.9	100-111.6	58.3-178.6	51.7- 65.3	166.7-193.5	
ALT(U/L)	C	266	12.95	14.2	13.4-15	4.84-31.06	3.52 -5.26	27.9 - 33	0.001
	F	133	11.3	12.12	11.24-13	4.2-23.6	3.0-5.2	22.6-25.1	
	M	133	15.9	16.3	15-17.5	5.175-33	3.12 -5.8	29.9- 34.7	
AST(U/L)	C	266	22.2	22.7	22-23.3	14.04-34.9	12-15	34 - 36.85	0.001
	F	135	20.7	21.54	20.7-22.4	12.3-34.1	11-14.3	31.5- 35.2	
	M	131	23.3	23.84	22.9-24.8	15.2-36.74	14.3-15.9	34.5- 41	
BilD(mg/dl)	C	269	0.27	0.28	0.26-0.29	0.016-0.58	0.01-0.042	0.55 - 0.61	0.005
	F	136	0.243	0.25	0.23-0.28	0.0114-0.553	0.009-0.04	0.52 - 0.58	
	M	133	0.298	0.302	0.28-0.33	0.019-0.606	0.012-0.106	0 .56 - 0.64	
BilT(mg/dl)	C	262	0.402	0.443	0.42-0.47	0.094-1	0.06-0.12	0.86 - 1.1	0.014
	F	134	0.375	0.407	0.37-0.44	0.075-0.84	0.04-0.115	0 .77 - 0.94	
	M	128	0.416	0.48	0.44-0.52	0.148-1.08	0.023-0.19	0.92- 1.1	
TP(g/dl)	C	267	7.06	7.06	7-7.124	5.9-8.25	5.7 - 6.2	8.12 - 8.4	0.156
	F	133	7.13	7.095	6.9-7.2	5.7-8.4	5.3 - 6.2	8.23- 8.6	
	M	134	7	7.02	6.934-7.1	6.13-8.14	5.8- 6.3	7.9- 8.3	
Alb(g/dl)	C	264	4.9	4.9	4.87-4.9	4.3-5.5	4.2- 4.4	5.42-5.5	0.603
	F	131	4.9	4.9	4.85-5	4.25-5.52	4.2- 4.4	5.5- 5.9	
	M	133	4.9	4.9	4.85-4.9	4.4-5.4	3.8- 4.5	5.3- 5.5	

C: combined, F: Female, M: male, n: number of participants, CI:confidence interval, ALP: alkaline phosphatase, ALT: alanine aminotransferase, AST: aspartate aminotransferase, BilD: Bilirubin direct, BilT: Bilirubin total, TP: Total protein, and ALB: Albumin, LL: lower limit, UP: upper limit, and P: participants

About 27% of female and 36% of male participants were their ALP result were missclassified as abnormally high result by the biosystem(leafleat) RI, but by the established reference interval in the present study they were catagorized as helathy individuals.

Around 2.26% versus 0.75%, 2.22% versus 1.53%, 2.21% versus 2.26%, and 2.24% versus 2.34% of the female and male participants respectively,their ALT,AST,BilD, and BilT results were missclassified as helathy individuals by the A 25 biosystem machine RI, but they were abnormally high as compared to the established reference interval in this study.

About 4.48% of the female and 13.43% and male participants missclassified as abnormaly low their creatinine result by the A 25 biosystem machine RI and 3.73% of the female participants,their creatinine value was considered as abnormally high,but they were helathy individuals by the prsent study. About 2.24% of the male participant, their Creatinine result was cosidered helathy individuals by the A 25 biosystem machine RI but they were abnormally high in the present study.

About 44.78% of the female and 37.1% of the male participants,their urea value was cosidered as abnormally low result while 2.24% of female and 2.24% of male participants considered as helathy individuals by the A 25 biosystem machine RI, but in the presnt study they were classified as helathy individuals and abnormally high respectively.

From the total participants about 22.1% and 0.37% of them, their total protein value were missclassified as abnormally low and helathy individuals by the A 25 biosystem machine RI, but they were healthy individuals and abnormally high in the prsent study respectivelly.

Of the total participants 1.5% of them, their albumin were considered as helathy by the A 25 biosystem machine,but in this study they were considered as low value and 28.41% of the total participants thier albumin were missclassified as abnormally high result as compared to the prsent study.

From total participants 2.26% of them, their glucose value was considerd as low by the manufacturer rather than in the prent study and 19.92% of the total participants were missclassified as abnormally high result but in this study catagorized as healthy.

Table 7:The Miss classified study participants by the biosystem machine RI in Mekelle,Tigrai, Northern Ethiopia from December 2018 to May 2019

	Sex	Mekelle	Manfuturer	Lower limit		Upper limit	
		RI	RI	frequency	Percent(%)	Frequency	Percent(%)
ALP	F	49.3-152	0-105	3	2.3	35	27
	M	58.3-178.6	0-115	3	2.34	46	36
ALT	F	4.2-23.6	0-41*	3	2.26	3	2.26
	M	5.2-33	0-41*	1	0.75	1	0.75
AST	F	12.3-34.1	0-40*	3	2.22	3	2.22
	M	15.2-36.74	0-40*	3	1.53	2	1.53
BILD	F	0.0114-0.553	0-0.2*	3	2.21	3	2.21
	M	0.019-0.606	0-0.2*	3	2.26	3	2.26
bilT	F	0.075-0.84	0-2*	3	2.24	3	2.24
	M	0.148-1.08	0-2*	3	2.34	3	2.34
Cr	F	0.46-0.96	0.5-0.9	6	4.48	5	3.73
	M	0.42-1.153	0.7-1.2	18	13.43	3	2.24
urea	F	8.05-22.85	15-39*	60	44.78	3	2.24
	M	8.7-26.1	15-39*	50	37.31	3	2.24
TP	C	5.9-8.25	6.6-8.3*	59	22.1	1	0.37
ALB	C	4.3-5.5	3.5-5*	4	1.5	75	28.41
GLU	C	73.7-115	70-100*	6	2.26	53	19.92

*F: female,M: male,C: combined, RI: reference interval, ALP: alkaline phosphatase, ALT: alanine aminotransferase, AST: aspartate aminotransferase, BilD: Bilirubin direct, BilT: Bilirubin total, Cr: creatinine, TP: Total protein, ALB: Albumin, GLU: Glucose, and *: combined refernce interval.*

Finally, the study tried to compare the current reference intervals established for Mekelle city apparently healthy adults with other studies in Ethiopia and elsewhere (showen in table 8).

Table 8: Comparison of reference interval (2.5^{th}-97.5^{th} percentile) of clinical chemistry parameters of the Mekelle city with other studies in African and western countries, Tigrai, Northern Ethiopia, 2019(n=273)

Parameters	P	Mekelle	Manufacturer (48)	Gojjam **(19)**	Amhara **(30)**	Mozambique (39)	Uganda (43)	Ghana (40)	Nigeria (44)	Kenya (36)	MGH (33)
ALP(U/L)	C	55.4-170.15	NA	52.4–237	87-451.28	91.1-258.9	NA	85-241	NA	NA	30-120
	F	49.31-152.08	0-105	49-236	89-381	91.4-240.6	47-160	82-293	NA	5-227	NA
	M	58.31-178.57	0-115	55.3-237.2	77.2-475.8	97.7-266.1	42-159	101-353	NA	13-201	NA
ALT(U/L)	C	4.84-31.06	0-41	6-43	5-39	5-48.2	NA	7-51	NA	NA	0-35
	F	4.21-23.56	NA	3-30	4.3-37	4.8-38.5	5.3-39.9	6-51	19- 38	0-34	NA
	M	5.175-33	NA	6-44.6	5.13-42.9	6.5-53.2	7.2-43.3	8-54	17.3-48.4	0-39	NA
AST(U/L)	C	14.04-34.89	0-40	9-38	11-46	13.7-42.8	NA	14-51	NA	NA	0-35
	F	12.32-34.1	NA	6-32.1	10-43.8	13.5-37	11.4-29	13-48	22-58.4	3-37	NA
	M	15.19-36.74	NA	10.5-39	12.13-46.9	16.8-45.5	13.2-36	17-60	26-49.4	6-40	NA
BilD(mg/dl)	C	0.016-0.5825	0-0.2	0.012-0.8	0.02-0.61	NA	NA	0.047-0.234	NA	NA	0.1-0.3
	F	0.01143-0.553	NA	0.012- 0.714	0.01-0.49	NA	0-0.4	0.041-0.222	NA	NA	NA
	M	0.0191-0.606	NA	0.0234-0.84	0.04-0.68	NA	0.1-0.5	0.053-0.24	NA	NA	NA
BilT(mg/d)	C	0.094-1	0-2	0.26-2.2	0.1-1.1	0.26–1.63	NA	0.17-1.51	NA	NA	0.3-1
	F	0.075-0.84	NA	0.21–2.2	0.08-0.91	0.234-1.32	0.3-1.9	0.158–1.56	0.0175-0.62	NA	NA
	M	0.1484-1.083	NA	0.275-2.2	0.11-1.18	0.34-2.11	0.4-2.6	0.22-1.87	0.2-1	NA	NA

Table 8: Comparison of reference interval (2.5^{th}-97.5^{th} percentile) of clinical chemistry parameters of the present study with other studies in African and western countries, Tigrai, North Ethiopia, 2019(n=273) (continue...)**.**

Parameters	P	Mekelle	M**a**nufacturer (48)	Gojjam **(19)**	Amhara **(30)**	Mozambique (39)	Uganda (43)	Ghana (40)	Nigeria (44)	Kenya (36)	MGH (33)
U(mg/dl)	C	8.72-24.35	15-39	NA	11-41	7.8–30.6	NA	5.405-34.2	NA	NA	21.4-42.8
	F	8.05-22.85	NA	NA	10-38.7	7.8-30.6	9.42-30.2	5.405-32.4	15.02- 34.84	7.2-36.03	NA
	M	8.7-26.075	NA	NA	12-43	10.8-34.8	10.1-33.8	5.405-37.2	13.21-28.83	9.01-35.4	NA
Cr(mg/dl)	C	0.46-1.133	NA	0.231-1.22	0.47-1.12	0.533-1.17	NA	0.554-1.335	NA	NA	0-1.5
	F	0.46-0.96	0.5-0.9	0.245-1.08	0.47-1.09	0.509-0.98	0.5-0.9	0.532-1.244	0.713-1.333	0.611-1.38	NA
	M	0.42-1.153	0.7-1.2	0.197-1.29	0.48-1.13	0.66-1.233	0.6-1.2	0.633-1.346	0.863-1.257	0.67-1.437	
TP(g/dl)	C	5.9-8.249	6.6-8.3	5.3-8.61	5.7-9.6	NA	NA	5.06-8.67	NA	NA	5.5-8
	F	5.732-8.38	NA	5.32-8.6	5.6-9.47	NA	6.8-9	5.52-8.69	NA	5.6-8.8	NA
	M	6.13-8.14	NA	5.3-8.67	5.7-9.7	NA	6.5-8.9	4.67-8.64	NA	5.7-8.9	NA
ALB(g/dl)	C	4.32-5.49	3.5-5	NA	3.7-6.2	4.07-5.41	NA	3.3-4.99	NA	NA	NA
	F	4.254-5.524	NA	NA	3.6-6.1	4.01-5.26	3.7-5.2	3.35-5.04	NA	2.8-5.0	NA
	M	4.4-5.396	NA	NA	3.7-6.2	4.34-5.52	3.9-5.4	3.27-4.98	NA	2.9-5.2	NA
GLU(mg/dl)	C	73.7-115	70-100	NA	NA	55.86-99.1	NA	65-115.3	NA	NA	75-115
	F	71.8-115	NA	NA	NA	57.7-95.5	NA	66.7-119	75.7-173	46.85-126	NA
	M	74-115	NA	NA	NA	55.9-102.7	NA	63.06-113.5	66.7-142.34	50.45-122.5	NA

MGH: Massachusetts General Hospital, F: female,M: male, C: combined, P: participants, NA:not available, ALP: alkaline phosphatase, ALT: alanine aminotransferase, AST: aspartate aminotransferase, BilD: Bilirubin direct, BilT: Bilirubin, total, TP: Total protein, ALB: Albumin, Cr: creatinine,U: urea and GLU: glucose.

7. Discussion

Clinical laboratory reference interval has not previously been established in Mekelle city, Tigrai and in the prsent study a reference interval was established using sample driven from an apparently healthy adult aged from 18 to 60 years old. The full range of clinical chemistry analytes was not run for all subjects, primarily due to insufficient access to reagents required to test some parameters.

The reference interval of ALP, ALT, and AST were significantly higher in males as compared to females (indicated in table 6). This finding was consistent to study conducted in Akuapem north district, Ghana (not shown in the table) (38), Gojjam (19), Mozambique (39), and Ghana (40). In addition to that, the values of ALT and AST were statistically significant higher in males than females were also in agreement with Kenya (36). The difference in ALP may be due to physiological changes associated with female climacteric when changes in hormone secretion critically affect bone metabolism (34). The variation of the reference intervals of the liver enzymes betwen males and females may be due to the difference of body mass index (ALT and AST were directly related with increasing BMI) (49) and biological factors between sexes (the value of AST and and ALT increase towards the early post menopause and AST remain high in late menopause while ALT decrease the menopause)(50). This indicates that those enzymes are sex-dependent.

The reference interval of bilirubin direct and total bilirubin determined in this study was statistically significant between sexes. These findings were consistent with those reported in Amhara (30) and Ghana (40). In contrast, bilirubin total was not significant between males and females in the study conducted the Gojjam zone (19). The varation of bilirubin betwen male and female may be due to hormonal infulence of androgen (51, 52).

The lower limit of male creatinine determined in the present study was slightly lower than females,but the upper limit was greater than female. There was statistically significant difference in the distribution of creatinine and urea between females and males. The statistically significant difference of creatinine between males and females in this study was similar to studies conducted at Amhara (30) and Mozambique (39). High creatinine in males compared to females is expected due to greater skeletal, muscle and bone mass in males (41).

Even if the reference value of total protein and albumin determined in the present study were not statistically significant, there was a variation between gender (shown in table 6). The study performed at the Gojjam zone (19), the reference value of total protein was not statistically significant which was similar to this study.

There was great variation for the established RI of ALP between this study and the study conducted in other parts of Ethiopia and African countries for both sexes.The upper limit of the ALP for both sexes was lower than Amhara (30), Mozambique (39)and Ghana (40). On the other hand, the upper limit of ALP for male was higher than Uganda (43) and MGH (33). The reference interval for ALP determined in the present study for males and females was higher as compared to the Manufacturer and China (34). The difference in the reference interval between the present study and other studies may be due to the difference in nutritional status, geographical area, and differences in the age distribution of the participants.

The upper limit of male and female ALT in this study was lower as compared to Gojjam(19), Amhara (30), Mozambique (39), Kenya (36) and MGH (36). The upper limit of female AST was higher than Gojjam (19) and Uganda (43), but lower than Amhara (30), Ghana (40), Kenya (36), Mozambique (39) and MGH (33). The upper limit of male AST was comparable to Uganda (43) and the upper limit of AST was lower than Amhara (30), Mozambique (39), Ghana (40), Nigeria (44) and MGH (33) for both sexes. The upper limit of the reference interval for ALT and AST established in the present study were somewhat lower than the Manufacturer while the lower limits were higher. The possible reason for the difference may be due to subclinical viral infections or the usage of local herbal preparations (38). the value of liver enzyems increase in Alpha-1 anti trypsin deficiency idividuals. The narrow reference range in the case of liver enzymes could be due to uniformity in the diet pattern of the individuals selected for the study and good analyticlal performance (53). The other cause for the difference may be due to nutration (indivduals who take nutrients with high content of copper and iron lead to increase of liver enzymes), age distribution and sample size varation (the mozambique's age distribution was from 18 to 24 years and the male sample size (102) did not satisfay the CLSI guideline).

The upper limit of male bilirubin direct in the present study was higher than Gojjam (19), Amhara (30), Uganda (43), Ghana (40), and MGH (33). The female RI of bilirubin total was also comparable to Amhara (30). The upper limit of female bilirubin total was lower as compared to Gojjam (19), Mozambique (39), Uganda (43), Ghana (40) and higher than Nigeria (44). The upper limit of male bilirubin total was comparable to Nigeria (44). The lower limit reference range driven for bilirubin direct and bilirubin total and the upper limit of direct bilirubin in the present study were somewhat higher than the manufacturer and the upper limit for total bilirubin was lower than the manufacturer. The comparability of the tests in the present study to the study conducted in Amhara (30) may be due to comparability in their lifestyle, nutritional status and the variability may be due to difference in attitude, analysis of the sample by different machines (Mindry BS-20 versus biosystem A 25) and using different equipment and reagents (biosystem reagent versus humastar). Inaddtion to that the variability between countries may be due to the difference in ethnicity, race, geographical location and Attitude (54, 55)

The lower limit of the female urea from the established reference interval was comparable to Mozambique (39).On the other hand, the lower limit of female urea was lower than Amhara (30), Nigeria (44), Uganda (43), MGH (33) and higher than Ghana (40), and Kenya (36). The upper limit of female urea was lower than Amhara (30), Mozambique (39), Ghana (40), Nigeria (44), Kenya (36), Uganda (43) and MGH (33). The lower limit of male urea was lower as compared to Amhara (30), Mozambique (39), Nigeria (44), Kenya (36), Uganda (43), MGH (33) and higher than of Ghana (40). The upper limit of male urea was lower than Amhara (30), Mozambique (39), Ghana (40), Nigeria (44), Kenya (36), Uganda (43) and MGH (33). The established reference interval for urea in the present study was lower than the manufacturer. The difference in lifestyles and genetic composition of the populations may cause for the varation (37). Iaddtion to that the difference of urea reference interval may be due to the difference in sampling method and sample size (in amhara region the sampling method was convinet sampling method and the sample size was about 1,175), dietary intake, and state of hydration of the participants.

The lower lmit of female creatinine in this study was almost inline with China (35).The lower limit female creatinine was higher as compared to Gojjam (19), MGH (33), and lower than Ghana (40), Nigeria (44), and Kenya (36). The upper limit of females was comparable to Gojjam (19), Amhara (30) and Mozambique (39). The upper limit of the female creatinine was lower than Ghana (40), Nigeria (44), Kenya (36), MGH (33), and higher than Uganda (43) and China (35). Lower limit of male creatinine was higher than Gojjam (19) and MGH (33) and lower than Amhara (30), Mozambique (39), Uganda (43), Ghana (40), Nigeria (44), Kenya (36), and China (35). The upper limit of male creatinine was comparable to Amhara (30) and Uganda (43).The upper limit of male creatinine was lower as compared to Gojjam (19), Ghana (40), Nigeria (44) Kenya (36), and MGH (33) higher than China (35). The established lower and upper reference interval for female creatinine was comparable to the manufacturer,but for the male is lower than the manufacturer. The observed variation in renal function test reference values determined in this study compared to reference range values from other locations suggest variations in analytical methods (compensated in the present study versus non compensated in amhara for creatinine), environmental factors, lifestyles, and genetic composition of the populations (37).

The lower limit of combined total protein in this study was higher as compared to Gojjam (19), Amhara (30), Ghana (40), and MGH (33). The upper limit of the combined total protein was lower as compared to Gojjam (19), Amhara (30), Ghana (40), and higher than MGH (33). The lower limit of the total protein was lower than the manufacturer,but the upper limit was comparable. The differences could come from the variability between participants by age distribution, sex, geographical location, environment, frequency of disease and genetic variation (31).

From the established Reference interval of albumin in the present study, the combined upper limit was comparable to Mozambique (39) and the combined lower limit of albumin was higher than Amhara (30), Mozambique (39), and Ghana (40). The upper limit of combined albumin was lower as compared to Amhara (30) and higher as compared to Ghana (40). The established reference interval in the present study for albumin is somewhat higher than the manufacturer. Diet, physical environment and socio-economic conditions all affect the physiology of a population, and hence measures of ‘normal’ physiological functions are expected to differ from population to population (38). The variation may be also due to the difference in geographical location, ethnicity, lifestyle,

culture, seasonal variation and the prevalence of disease and variations in the analytical method, equipment, and reagents used (30).

The combined lower limit of Glucose was higher as compared to Mozambique(39) and Ghana and the upper limit combined glucose was comparable to Ghana(40) and higher than Mozambique(39). The varation of glucose from one study to other study may be due to varation in analytical performance (precision and accuracy of the instrument), the type of sample(plasma, serum vesus whole blood) and the test method used (50). Generally, Differences in the lower and upper reference limits could be due to differences in the geographical location, methods, and types of equipment used, sample size, posture, race, regional differences in the dietary intakes of foods rich in these analytes, and genetics (36). The possible reason for the result of this study differs from the western might be due to variation climate, gender, the month of study and geographical location (19)

There was a misclassification of the participants among all of the tests performed by the A 25 biosystem machine as compared to the reference interval determined in this study. The missclasification of the participants as normal, abormally high or low may be due to difference in genetic ,cultural, altitude, geographical, nutrational status, sample size and lifestyle.

8. Strength and Limitation of the Study

8.1.Strength of the Study

- ✓ Wet mount, concentration (formol-ether), Kato Katz and modified acid-fast technique were performed to exclude participants who were infected with an intestinal parasite.
- ✓ urinalysis was also done as a screening
- ✓ We have done the blood film for hemoparasite detection
- ✓ The study was the first community based

8.2.Limitation of the Study

- ✓ The reference interval was established for ten tests only

9. Conclusion and Recommendation

9.1.Conclusion

There was a significant difference for ALP, ALT, AST, BilD, BilT, Urea and creatinine between sexes. This finding is important to improve the health system by providing accurate local RI.

9.2.Recommendation

This study recommends to laboratories of the Mekelle city to use this established local RI

Other laboratories also perform transferability study in order to use this established local RI.

This study also recommends for researchers and stakeholders to establish further local and national wide studies for reference interval of clinical chemistry parameters.

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Annex

Annex I: Participants' information sheet

A. English version

Title of the project: Determining reference intervals for clinical chemistry parameters among apparently healthy adult individuals in Mekelle city, Tigrai, Northern Ethiopia from December 2018 to May 2019: a cros sectional study.

Principal investigator: Gebreslassie Gebremariam Berhe (BSc, MSc candidate)

Introduction

Dear study participants you are invited to participate in the study on Determining reference intervals for clinical chemistry parameters among apparently healthy adult individuals in Mekelle city, Tigrai, North Ethiopia. This study is approved by the Addis Ababa University Department of Medical Laboratory Science research ethics committee. You are voluntarily participating in this study and you have a full right to stop participation if you have something uncomfortable.

Purpose of the study: The main objective of the study is to Determining reference intervals for clinical chemistry parameters among apparently healthy adult individuals in Mekelle city, Tigrai, Northern Ethiopia.

Duration: the duration of this study depends on the availability of study subjects and it may take 3-6 months.

The associated risk with the study: during sample collection from your vein, there is minor pain or discomfort. The sample is collected by the experienced laboratory personnel and the risk is minimizing as well.

The procedure of the study

If you are agreed to participate in this study, you will give about 3-5 venous blood for clinical chemistry analysis.

Expected Benefit: Dear participants you will have a benefit from this study because this study assesses your liver function, kidney function, and lipid profile changes. No payment is requested for the clinical chemistry tests and you will know your health status early. If your result shows any abnormalities of clinical chemistry tests, you would be managed and treated early.

Confidentiality: The confidentiality of your information and laboratory results are respected strictly. A unique identification number is given to you and your name will not be written in the form and the result of laboratory tests could only be accessed by the researcher.

Agreement: Dear participant, you have read all the information described above and you will request to put your signature to indicate your agreement to participate in the study.

Participant name _____

Date _____ sign_____

If you have any question, please contact the following address:

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B. Amharic version

ለተሳታፊዎች መረጃ መስጫ ሰነድ

የጥናቱ ርእስ፡ በትግራይ ክልል በመቐለ ከተማ በሚነሩ ጤናማ ጎልማሳ ሕብረተሰብ ውስጥ የክሊኒካል ኬሚስትሪ ምርመራ ሪፈረንስ ኢንተርቫል ማጥናት፡፡

የአጥኚው ስም: ገብረስላሴ ገብረማርያም በርሀ

የተቋሙ ስም: አዲስ አበባ ዩኒቨርሲቲ የህክምና ና ጤና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ትምህርት ክፍል

መግቢያ: የተከበሩ የጥናቱ ተሳታፊ በትግራይ ክልል በመቐለ ከተማ በሚነሩ ጤናማ በሚመስሉ ጎልማሳ ሕብረተሰብ ውስጥ የክሊኒካል ኬሚስትሪ ምርመራ ሪፈረንስ ኢንተርቫል በሚደረገዉ ጥናት ለመሳተፍ ተጋብዘል ።ይህ ጥናት በ አዲስ አበባ ዩኒቨርሲቲ ላቦራቶሪ ትምህርት ክፍል የጥናትና የሰነምግባር ኬሚቴ የጸደቀዉ ጥናት መሆኑን መገለጸ እንወዳለን ። በዚህ ጥናት መሳተፍ ሙሉ በሙሉ በእርስዎ ፍቃደኝነት የተመሰረተ በመሆኑ በማንኛውም ሰአትና ቦታ የማቋረጥ ሙሉ መብትዎን የተጠበቀ ነው።

የጥናቱ ዋና አላማ፡- በትግራይ ክልል በመቐለ ከተማ በሚነሩ ጤናማ ጎልማሳ ሕብረተሰብ ውስጥ የክሊኒካል ኬሚስትሪ ምርመራ ሪፈረንስ ኢንተርቫል ማጥናት፡፡

የጥናቱ ጊዜ፡ የጥናቱ ግዜ ከ 3 እስከ 6 ወር ሊወስድ ይችላል፡፡

ከጥናቱ ጋር ተያይዞ የሚመጣ ጉዳት: የደም ናሙና በሚሰጥበት ወቅት ምንም አይነት የከፋ ችግር አያጋጥምዎትም ። ነገር ግን ደም ሲወስድ መጠነኛ የህመም ስሜት ልያስከትል ይችላል ። ሆኖም ግን ናሙናዉን ለመሰብሰብ ልምድ ባላቸዉ ባለሞያ ስለሚመደብና አስፈላጊውን ጥንቃቄ ና እርምጃ ስለሚወስድ የህመም ስሜት አይኖርም።

ከጥናቱ የሚያገኙት ጥቅም ፡ ያለ ምንም ክፍያ የጉበት ና ኩላልት ምርመራ ያደርጋሉ።ችግር ካለ በፍጥነት ህክምና ይሰጣችዋል።
ከርስዎ የምናገኘዉ መረጃ እና ሚስጥራዊነቱ: የእርሶ ስም በዚህ መጠይቅ ላይ አይጠቀስም፡፡ በተጨማሪም የሚሰጡት መረጃ እና በሰጡትም ደም ላይ የሚደረገው የምርመራ ውጤት ከተባለለት ጉዳይ ውጭ እንደማይውል እና ሚስጥራዊነቱ የተጠበቀ እንደሚሆን አረጋግጣለሁኝ፡፡
በዚህ ጥናት ላይ ያለዎትን ጥያቄ በሚከተሉት ኣድራሻ በማንኛውም መጠቀም ይችላሉ።
የኣጥኝው ስም: ገብረስላሴ ገብረማርያም በርሀ ስልክ: 0967559075 ኢሜል: gebreslassiegebre@yahoo.com
ኣማካሪዎች 1) ዶ/ር ሚስጥረ ወልዴ ስልክ: +251-911699710 ኢሜል: mistire08@gmail.com
2) ዶ/ር አስቴር ፀጋየ ሞባይል፡ +251-911696085 ኢሜል: tsegayeaster@yahoo.com

C. Tigrigna version

ናይ መፅናዓይ ሽም: ገብረስላሴ ገብረማርያም በርሀ

ናይ ትካል ሽም: ኣዲስ ኣበባ ዩኒቨርሲቲ ጥዕናን ሕክምና ሳይንስን ኮሌጅ ናይ ሕክምና ላቦራቶሪ ትምህርቲ ክፍሊ

ናይቲ መፅናዕቲ ርእሲ፡- ኣብ ክልል ትግራይ ኣብ ከተማ መቐለ ኣብ ዝነብሩ ጥዕኖኣም ኣብ ዝተሓለወ ዓበይቲ ሕብረተሰብ ዝካየድ ናይ ክሊኒካል ኬሚስትሪ ምርመራ ሪፈረንስ ኢንተርቫል ምፅናዕ

መእተዊ: ዝተከበሩ(ራ) ናይዚ ጽንዓት ተሳታፊ/ት ኣነ ናይ ኣዲስ ኣበባ ዩኒቨርስቲ ጥዕናን ሕክምናን ሳይንስ ኮሌጅ ናይ ሕክምና ላቦራቶሪ ትምህርቲ ክፍሊ ብማስተርስ ድግሪ ተምሃራይ እየ። ንሶም ወይ ንሰን ኣብ ክልል ትግራይ ኣብ ከተማ መቐለ ኣብ ዝነብሩ ጥዕኖኣም ኣብ ዝተሓለወ ሕብረተሰብ ዝካየድ ናይ ክሊኒካል ኬሚስትሪ ምርመራ ሪፈረንስ ኢንተርቫል ምፅናዕ ኣብ ዝብል ናይ መመረቂ መፅናዕቲ ፅሑፍ ተሳታፊ ንክኾኑ/ና ተዓድሞም/ዲመን አለዉ/ዋ።

ናይቲ መፅናዕቲ ዋና ዓላማ፡- ኣብ ክልል ትግራይ ኣብ ከተማ መቐለ ኣብ ዝነብሩ ጥዕኖኣም ዝተሓለወ ዓበይቲ ሕብረተሰብ ዝካየድናይ ክሊኒካል ኬሚስትሪ ምርመራ ሪፈረንስ ኢንተርቫል ምፅናዕን ምፍላጥን።

እቲ ጽንዓት ዝካየደሉ እዋን ፡ ናይቲ ጽንዓት እዋን ካብ 3 ክሳብ 6 ወርሒ ክወስድ ይክእል እዩ፡፡

ቐደም ስዒብ እቲ ጽንዓት፡ ዝተከበሩ(ራ) ናይዚ ጽንዓት ተሳታፊ/ት እዞም ዝስዕቡ ናይዚ ጽንኣት ምስ ተረድኡ/ኣ እና ፍቃደኛ እንድሕር ኮይኖም/ነን ነዚ ጽንዓት ዝከዉን ካብ 3 ክሳብ 5 ሚ.ሊ ዝከዉን ደም ክንወስደሎም/ለን ኢና።

ምስቲ መፅናዕቲ ተታሒዙ ዝመፅእ ሳዕቤን፡- ንምርመራ ዝከዉን ደም ኣብ ዝህበሉ እዋን ምንም ዓይነት ዝኸፍአ ፀገም ኣየጋጥሞምን። ነገር ግን ደም ኣብ ዝውሰደሉ እዋን ዝተወሰነ ናይ ምሕማም ስሚዕት ክህሉ ይኽእል እዩ። ይኹን ዳኣምበር ደም ንምስብሳብ ልምዲ ብዘለዎም በዓል ሞያታት ስለ ዝምደቡን ኣድላይ ዝኾነ ጥንቃቐን ስለዝውሰድ ናይ ምሕማም ስምዕት ኣይህሉን።

ካብዚ መፅናዕቲ ዝረክብዎ ጥቕሚ፡ እዚ መፅናዕቲ ናይ ማስተርስ ድግሪ መመረቂ ፅሑፍ ከም ምኻኑ መጠን ኣብዚ መፅናዕቲ ብምስታፎም ዝረኽብዎ ናይ ገንዘብ ጥቕሚ ዋላኳ እንተዘይሃለዎ ካብቲ መፅናዕቲ ብዝርከብ ውፅኢት ግን ተጠቃሚ እዮም። ነቲ መፅናዕቲ ካብ ዝተወሰደ ደም ዝርከብ ውፅኢት ብነፃ ይረኽቡ እዮም። ብተወሳኺ ኣብቲ ናይ ደም ውፅኢት ለውጢ እንተሃልይዎ ምስ ሓኻይም ንክራኸቡን ንኽምርመሩን ይግበር እዩ።

ናይ ሕክምና መረዳእታ ብምሽጥር ምሕላዉ ዝምልከት ፡ ኣብዚ ጽንዓት ስለ ናቶም ወይ ናተን ንእክቦ ዝኮነ ዓይነት መረዳእታ ብሚስጥር ከም ንሕዘለኩም ነፍልጥ፡፡ ነዚ መጽናዕቲ ኢልና ዘሎ ናቶም/ተን መንነት ዝገልጽ ኩሉ መረዳእታ ናብ ሚሽጥር ክንቕይሮ ኢና፡፡ብተወሳኺ እቲ ትህቡና ደም ኮነ መረዳእታ ካብቲ ጽንዓት ወጻኢ ኣይንጥቀመሉን፡፡

ካብቲ መፅናዕቲ ስለምቁራፅ: - ኣብቲ መፅናዕቲ ምስታፍ ብናቶም/ተን ፍቓደኝነት ዝተመስረተ ኮይኑ ኣብ ማእከል ምቑራፅን ዘይደለይዎ ሕቶ ዘይምምላስ ይኽእሉ/ላ እዮም/የን፡፡ ኣብዚ መፅናዕቲ ዘለዎም/ወን ሕቶን/ ርኢቶን ኣብ ዝኾነ ይኹን ግዜ ክሓቱ/ታ ይኽእሉ/ላ፡፡

ንተወሳኺ ሓበሬታ ነዞም ዝስዕቡ ኣድራሻ ይጠቀሙ፡፡

ናይ መፅናዓይ ሽም: ገብረስላሴ ገብረማርያም በርሀ ሞባይል፡ 0967559075 ኢሜል gebreslassiegebre@yahoo.com

ኣማካሪዎች 1) ዶ/ር ሚስጥረ ወልዴ ስልክ: +251-911699710 ኢሜል: mistire08@gmail.com

2) ዶ/ር አስቴር ፀጋየ ሞባይል፡ +251-911696085 ኢሜል: tsegayeaster@yahoo.com

Annex II： Consent Form

A. English version

Principal investigator: Gebreslassie Gebremariam Berhe (BSc, MSc candidate)

Research title: Determining reference intervals for clinical chemistry parameters among apparently healthy adult individuals in Mekelle city, Tigrai, Northern Ethiopia from December 2018 to May 2019: a cros sectional study.

I have read, or have had this document read to me in a language that I understand, and I understand the purposes, procedures and risks of this research project as described within it. I understand that at any time I may withdraw from this study without giving a reason. I know that no special payment for being participating in the study. I freely agree to participate in this study, as described. I understand that I was given a signed copy of this document to keep.

Name of participant_____ Age____ Address_____ Signature____ Date_____

Interviewer's name_____Signature_____

Principal investigator Name_____ Signature_____

B. Amharic version

የፍቃደኝነት ማረጋገጫ ቅጽ

የጥናቱ ርእስ፡ በትግራይ ክልል በመቐለ ከተማ በሚነሩ ጤናማ ጎልማሳ ሕብረተሰብ ውስጥ የክሊኒካል ኬሚስትሪ ምርመራ ሪፈረንስ ኢንተርቫል ማጥናት፡፡

የአጥኝው ስም: ገብረስላሴ ገብረማርያም በርሀ

የተቋሙ ስም: አዲስ አበባ ዩኒቨርሲቲ የህክምና ና ጤና ሳይንስ ኮሌጅ የህክምና ላቦራቶሪ ትምህርት ክፍል

እኔ ከዚህ በታች የተገለጸዉ በዚህ ጥናት ተሳታፊ ለመሆን ስወስን የጥናቱ ዓላማዎች ኣሳራሮችና ቐድመ ሁኔታዎች በግልጽ በመረዳትና እንዲሁም ከጥናቱ ተሳታፋይነት ፈቓደኝነቴን በማነኛዉም ግዜ የመውጣት መብቴን በማረጋገጥ ነዉ።
ሰለዚህ በጥናቱ ተሳታፊ መሆኔን በፈርማየ እየራጋገጥኩ ይህንን ስውስን በጥናቱ ሊከሰቱ የሚችሉ ስጋቶችን በሚገባ የተረዳሁና ከጥናቱ በማነኛዉም ግዜ ራሴን ለምግለል ብወስን ተገቢ የሆኑ ህክምናዎችና እገዛዎች ሁሉ እንደማትነፈጉኝ በማመን ነው።
እነዚህ መረጃዎች ሁሉ በሚገባ በምረዳዉ ቋንቋ የተገለጸልኝ መሆኑን በፈርማየ ኣጋግጣለሁ።

የተሳታፊው ስም……ፊርማ…… ቀን ……

የኣጥኚው ስም …… ፊርማ ……ቀን ……

ስለ ትብብርዎ አመሰግናለሁ!

C. Tigrigna version

ናይ መፅናዓይ ሽም: ገብረስላሴ ገብረማርያም በርሀ

ናይ ትካል ሽም: ኣዲስ ኣበባ ዩኒቨርሲቲ ጥዕናን ሕክምና ሳይንስን ኮሌጅ ናይ ሕክምና ላቦራቶሪ ትምህርቲ ክፍሊ

ናይቲ መፅናዕቲ ርእሲ፡- ኣብ ክልል ትግራይ ኣብ ከተማ መቐለ ኣብ ዝነብሩ ጥዕኖኣም ኣብ ዝተሓለወ ዓበይቲ ሕብረተሰብ ዝካየድ ናይ ክሊኒካል ኬሚስትሪ ምርመራ ሪፈረንስ ኢንተርቫል ምፅናዕ:

ኣነ ኣብዚ ተገሊጹ ዝኒሆ መጽናዕቲ ተሳታፋይ ንምኳን እንትዉስን እንተለኩ ናይዚ ጽንዓት ዘድልዩ ነገራት ብምርዳእ እና ካብዚ ጽንዓት እዚ ምስታፍ ኣብ ዝኮነ ይኩን እዋን ከም ዘግልል ብምርግጋጸይ እዩ።ስለዚ ኣብዚ ጸንዓት ተሳታፋይ ምኳነይ በፈረማይ የራጋግጽ።እዞም ኩሎም መረደእታታት ብዝግባእ ብዝሩድኡኒ ቋንቋ ዝተገለጹለይ ምኳኖም በፈረማይ የራጋግጽ።

ናይ ተሳታፊ/ፊት መለለዪ ቐፅሪ ---------- ፊርማ ---------- ዕለት----------

ሓበሬታ ዝኣከበ በዓል ሞያ ሽም ---------- ፊርማ ----------ዕለት----------

ስለ ዝተሓባበሩኒ የቐንየለይ!

Annex III: Questionnaire

A. English version

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? _____

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

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
	Part IV: HEALTH STATUS	
12	Did you take any type of drug for any illness for the last three months?	1.Yes 2. No
13	If yes to Q12, 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	
14	History of diabetes	1.Yes 2. No
15	History of Hypertension	1.Yes 2. No
16	History of Blood transfusion for the last 1 year	1.Yes 2. No
17	History of blood donation for the last 3 monthes	1.Yes 2. No
18	History of Hospital Admission for the last 1 year	1.Yes 2. No
19	History of Surgical procedure for the last three years?	1.Yes 2. No
20	History of chronic gastritis	1.Yes 2. No
21	History of Malaria for the last 6 months	1.Yes 2. No

22	History of TB for the last two years	1.Yes 2. No
23	History of Cancer	1.Yes 2. No
24	History of Cardiac illness	1.Yes 2. No
25	History of Bleeding disorders	1.Yes 2. No
26	History of allergy	1.Yes 2. No
27	History of Wheezing	1.Yes 2. No

How frequently 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
28	Alcohol						
29	Khat						
30	Cigarettes						

	Part VI. Anthropometric measurement	
31	Height (in cm)	_____
32	Weight (in kg)	_____
33	MUAC	_____in cm (will be interpreted later)
34	Blood pressure (mm Hg)	_____

- NB: If question 12,14-27 aswer is yes, takes Cigarettes and khat, BP out of 90-120/60-90 mmHg, and BMI <18 and >25, the study participant will be excluded.
- We thank you for your cooperation!

Interview Date: _____

Interviewer's Name_____ Signature_____

B. Amharic version (ቃለ መጠይቅ)

በጤና ባለሞያዎች የሚሞላ ቃለ መጠይቅ

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. ማህበራዊ ና ኢኮኖሚያዊ መረጃ	
6.	የትምህርት ደረጃ	6. ያልተማሩ 7. ማንበብ ና መፃፍ 8. አንደኛ ደረጃ (1-8) 9. ሁለተኛ ደረጃ (9-12) 10. ኮሌጅ ዲፕሎማ/ዲግሪ እና ከዚያ በላይ
7.	ሥራ	7. ተማሪ 8. የቤት እመቤት 9. የመንግስት ሠራተኛ 10. የግል ተቀጣሪ 11. ገበሬ 12. ሌላ ካለ ይግለፁ _____
8.	የጋብቻ ሁኔታ	6. ያላገቡ 7. ያገቡ 8. የተፋቱ 9. ባል/ሚስት የሞተባቸው 10. አይመለከታቸውም (ህፃናት)
9.	ሃይማኖት	6. ኦርቶዶክስ ክርስቲያን 7. ሙስሊም 8. ፕሮቴስታንት 9. ካቶሊክ 10. ሌላ ካለ ይግለፁ _____
10.	ብሄር	_____ድብልቅ ከሆኑ ይግለፁ
11.	የመኖሪያ ቦታ	1. ገጠር 2. ከተማ

	ክፍል 4. የጤና መረጃ	
12.	ባፉት ሶስት ወራት ለማንኛውም ዓይነት ህመም ማንኛውንም ዓይነት መድሃኒት ወስደኋል?	1. አዎን 2. የለም
13.	ለተራ ቁጥር 12 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 _____
	የሚከተሉትን የህመም ዓይነቶች አሞዎት ያውቃል?	
14.	የስኳር ህመም?	1. አዎን 2. የለም
15.	የደም ግፊት ከፍ ማለት?	1. አዎን 2. የለም
16.	ባለፈው 1 ዓመት ደም ተሰጥቶዎ ያውቃል?	1. አዎን 2. የለም
17.	ባለፈው 3 ወር ደም ሰጥቶ ያውቃሉ?	1. አዎን 2. የለም
18.	ባለፈው 1 ዓመት ሆስፒታል ተኝተው ያውቃሉ?	1. አዎን 2. የለም
19.	ባለፉት 3 ዓመታት የቀዶ ህክምና ተደርጎልዎ ያውቃል?	1. አዎን 2. የለም
20.	የቆየ የጨጓራ ህመም አለብዎት?	1. አዎን 2. የለም
21.	ባፉት 6 ወራት የወባ ህመም አጋጥሞዎት ያውቃል?	1. አዎን 2. የለም
22.	ባለፉት 2 ዓመታት የቲቢ ህመም ኖሮዎት ያውቃል?	1. አዎን 2. የለም
23.	ካንሰር ህመም	1. አዎን 2. የለም
24.	የልብ ህመም	1. አዎን 2. የለም
25.	የመድማት ችግር/ህመም	1. አዎን 2. የለም
26.	አለርጂ (የሰውነት መቆጣት)	1. አዎን 2. የለም
27.	የመተንፈስ ችግር (ሲተነፍሱ ሲር ሲር የሚል ድምፅ)	1. አዎን 2. የለም

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
28.	አልኮል						
29.	ጫት						
30.	ሲጋራ						

	ክፍል 6. ክብደት፣ ቁመት፣ የክንድና የደም ግፊት ልኬት	
31.	ቁመት	_____ሴንቲ ሜትር
32.	ክብደት	_____ኪሎ ግራም
33.	የክንድ መሃለኛው ክፍል ዙሪያው (MUAC)	_____ሴንቲ ሜትር
34.	የደም ግፊት (በሚሊሜትር ሜርኩሪ)	_____(mm Hg)

- NB: ተራ ቁጥር 12 እና ከ 13-27 መልሱዎን ላዎ ከሆነ ፣ሲጋራ እና ጨት የሚጠቀሙ ከሆኑ፣የደም ግፊታቸው ከ (90-120)/(60-90) ውጪ ከሆነ፣ BMI <18 እና >25 ከሆነ፣ ተሳታፊው ጥናቱ ውስጥ ላይካተቱም::
- ስለትብብርዎ እናመሰግናለን!

ቃለ መጠይቅ የተደረገበት ቀን: _____

ቃለ መጠይቁን ያካሄደው ስም_____ ፊርማ_____

C. Tigrigna version (ቃለ -መሕተት)

ክፍሊ 1. ኣጠቓላሊ መረዳእታ

ኮድ_____ ክልል ትግራይ ዞባ መቐለ

ክፍለ ከተማ _____ጣብያ _____

ክፍሊ 2. ናይ ዉልቀ መረዳእታ

1. ዕድመ (ብዓመት) _____
2. ፆታ_____
3. ናይ ትዉልዲ ቦታ_____
4. ኣብ ትዉልዲ ቦቶኣም ንኽንደይ ጊዜ ነቢሮም_____
5. ኣብ ሕዚ ዘለዉዎ ቦታ ንኽንደይ ጊዜ ነቢሮም? (ካብ ትዉልዲ ቦታ ዝተፈለየ እንተኾይኑ) _____ዓመት

ክፍሊ 3. ናይ ማሕበራውን ኢኮኖሚያውን መረዳእታ

6. ናይ ትምህርቲ ደረጃ

   11. ዘይተመሃረ 2. ምንባብን ምፅሓፍን 3. ቀዳማይ ብርኪ (1-8) 4. ካልኣይ ብርኪ (9-12)

   5. ናይ ኮሌጅ ዲፕሎማ/ዲግርን ልዕሊኡን

7. ስራሕ

   13. ተመሃራይ/ት 2. ናይ ገዛ እመቤት 3. ናይ መንግስቲ ሰራሕተኛ 4. ናይ ግሊ ተቐፃሪ

   5 ሓረስታይ 6. ካሊእ እንተሃልዩ ይገለፅ_____

8. ኩነታት ሓዳር

   1. ሓዳር ዘይገበረ/ት 2. ሓዳር ዝገበረ/ት 3. ዝተፋተሐ/ት 4. በዓልቲ ገዝኡ/ኣ ዝሞተቶ/ታ

   5. ኣይምልከቶምን (ህፃዉንቲ)

9. ሃይማኖት

   1. ኦርቶዶክስ 2. ሙስሊም 3. ፕሮቴስታንት 4. ካቶሊክ 5. ካሊእ እንተሃልዩ ይገለጽ _____

10. ብሄር 1. _____ 2. ሕዋስ እንተሃልዩ ይገለፅ_____

11. መንበሪ ኣድራሻ 1. ገጠር 2. ከተማ

ክፍሊ 4. ናይ ጥዕና መረዳእታ

12. ኣብ ዝሓለፉ ሰለስተ ወርሒ ዝኮነ ዓይነት መድሓኒት ንዝኮነ ዓይነት ሕማም ወሲዶም ዶ ይፈልጡ ?

   1. እወ 2. ኣይፋሉን

13. ንታራ ቁፅሪ 12 መልሶም እወ እንተኾይኑ ኣየናይ ዓይነት መድሓኒት እዮም ወሲዶም ? (ካብ ሓደ ንላዕሊ መልሲ ምምላስ ይካኣል እዩ)

ı. ፀረ-ፕሮቶዝዋ 2. ፀረ- ሓሰኻ 3. ፀረ-ኣለርጂ 4. ናይ ወሊድ መከላኸሊ ክኒና

5 ፀረ-ባክቴሪያ 6. ፀረ-ቲቢ 7. ካለእ ተሃልዩ ይገለፅ _____

ናይ ዝስዕቡ ናይ ሕማም ዓይነት ሓሚሞም ይፈልጡ ዶ?

14. ናይ ሽኮር ሕማም? 1. እወ 2. ኣይፋሉን

15. ናይ ደም ድፍኢት ልዕል ምባል? 1.እወ 2. ኣይፋሉን

16. ኣብ ዝሓለፈ 3 ወርሒ ደም ሂቦም ይፈልጡ ዶ? 1.እወ 2. ኣይፋሉን

17. ኣብ ዝኾነ ጊዜ ደም ተዋሂብዎም ይፈልጡ ዶ? 1.እወ 2. ኣይፋሉን

18. ኣብ ዝሓለፈ 1 ዓመት ሆስፒታል ሃሪሶም ይፈልጡ ዶ? 1.እወ 2. ኣይፋሉን

19. ኣብ ዝሓለፈ 3 ዓመታት ናይ መጥባሕቲ ህክምና ተገይርሎም ይፈልጡ ዶ? 1.እወ 2. ኣይፋሉን

20. ዝፀንሐ ናይ ጨነራ ሕማም ኣለዎም ዶ? 1.እወ 2. ኣይፋሉን

21. ኣብ ዝሓለፈ 6 ኣዋርሕ ናይ ሕማም ዓሶ ኣጋጢምዎም ነይሩ ዶ? 1.እወ 2. ኣይፋሉን

22. ኣብ ዝሓለፈ 2 ዓመት ናይ ቲቢ ሕማም ሒዝዎም ይፈልጥ ዶ ? 1.እወ 2. ኣይፋሉን

23. ናይ ካንሰር ሕማም 1. እወ 2. ኣይፋሉን

24. ናይ ልቢ ሕማም 1.እወ 2. ኣይፋሉን

25. ናይ ምድማይ ፀገም /ሕማም ኣጋጢምዎም ነይሩ ዶ? 1.እወ 2. ኣይፋሉን

26. ኣለርጂ (ናይ ሰውነት ቁጠዐ) ኣጋጢምዎም ነይሩ ዶ? 1.እወ 2. ኣይፋሉን

27. ናይ ምትንፋስ ችግር ኣጋጢምዎም ነይሩ ዶ? 1.እወ 2. ኣይፋሉን

ካብዞም ዝስዕቡ ክንደይ ግዘ ይጥቀሙ/ይበልዑ (√ እዚ ምልክት የቕምጡ)							
		ብመዓልቲ ሓደ ግዜ	ብመዓልቲ ካብ ሓደ ግዜ ንላዕሊ	ብሰሙን ካብ 2 ክሳብ 3 ግዜ	ብሰሙን ካብ 1 ንላዕሊ	ሓልሓሊፉ (ንኣብነት በዓል፣ፍሉይ ምደላው ክህሉ ከሎ)	ተጠቒመ ኣይፈልጥን
28.	ኣልኮል						
29.	ጫት						
30.	ሽጋራ						

	ክፍሊ 5. ክብደት፣ ቁመት፣ ጭዋዳን ናይ ደም ድፍኢትን	
31.	ቁመት (ሴንቲ ሜትር)	_____
32.	ክብደት (ኪሎ ግራም)	_____ኪሎ ግራም
33.	ናይ ጭዋዳ ማእኸላይ ክፍሊ ዙሪያ (MUAC)	_____ሴንቲ ሜትር
34.	ናይ ደም ድፍኢት (በሚሊሜትር ሜርኩሪ)	_____

NB: ተራ ቁጽሪ 12 ን ካብ 13-27 መልሶም እወ እንተኾይኑ ፣ሲጋራ ን ጫትን ዝጥቀሙ እንተኾይኖም፣ ናይ ደም ድፊኢቶም ካብ 90-120/60-90 ወጻኢ እንተኾይኑ፣ BMI <18 እና >25 እንተኾይኑ, ተሳታፊ ኣብዚ ጽንዓት ተሳታፊ ኣይከውንን ::

❖ ስለ ምትሕብባሮም ነምስግን!!!

ቃለ መሕተት ዝተገበረሉ መዓልቲ: _____

ቃለ መሕተት ዘካየደ ሽም_____ ፊርማ_____

Annex IV. Laboratory Blood sample collection procedure and processing

1. Assemble all the necessary materials for blood collection
2. Identify and prepare the person for collection
3. Label tubes with the client's name/identification number.
4. Wear the rubber gloves and make the person at a comfortable position
5. Tie the tourniquet around the arm of the person just above the bend in the elbow. The tourniquet should be positioned 7.5cm to 10cm above the puncture site.
6. Using the tip of the index finger examine the phlebotomy site, feel the vein, and decide exactly where to place the puncture
7. Disinfect the phlebotomy site by swabbing the skin in small outward circles with an alcohol swab.
8. Insert the needle directly into the vein and withdraw peripheral blood into the SST test tube
9. Withdraw the needle from the vein and cover the puncture site cotton swab and hold pressure at the puncture site for 3 minutes.
10. Properly discard the used materials in a safe container.
11. wait until the blood collected by SST to be clot and centrifuge at 2500-3500 rpm to 3-5 minute
12. Separate the serum to white sample container cup, if we are not able to analyze the specimen immediately store the specimen at the right temperature for the right time for the appropriate test
13. Turn on the clinical chemistry analyzer machine
14. Check the expiry date of all reagents
15. Check the daily, weekly, monthly, quarterly and yearly controls, standards and calibration results of the analyzer
16. Analyze the specimen based on the leaflet procedure for each clinical chemistry parameter test.

Principle and interpretation of the tests

1. Liver function tests

1.1.Alkaline phosphatase

Principle

Alkaline phosphatase (ALP) catalyzes in alkaline medium the transfer of the phosphate group from 4-nitrophenylphosphate to 2-amino-2-methyl-1-propanol (AMP), liberating 4 nitrophenol. The catalytic concentration is determined from the rate of 4-nitrophenol formation, measured at 405 nm.

4-Nitrophenylphosphate +AMP $\xrightarrow{\text{ALP}}$ AMP-Phosphate + 4-Nitrophenol

Interpretation of result

Serum ALP measurements are of particular interest in the investigation of two groups of conditions: bone disease and hepatobiliary disease. Physiological bone growth elevates ALP in serum of growing children and a transient elevation may be found during healing of bone fractures. The response to the liver to any form of biliary tree obstruction is to synthesize more ALP. Intrahepatic obstruction of the bile flow by invading cancer or drugs raises serum ALP. Any drug that is hepatotoxic or induces cholestasis will greatly increase serum ALP.

1.2. Alanine aminotransferase

Principle

Alanine aminotransferase (ALT/GPT) catalyzes the transfer of the amino group from alanine to oxoglutarate with the formation of glutamate and pyruvate. Latter pyruvate is reduced to lactate by lactate dehydrogenase (LDH) in the presence of reduced nicotinamide adenine dinucleotide (NADH). The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of NADH to NAD+, proportional to the activity of ALT present in the sample.

L-Alanine + 2-Oxoglutarate - $\xrightarrow{\text{ALT}}$ L-Glutamate + Pyruvate

Pyruvate + NADH + H $\xrightarrow{\text{LDH}}$ Lactate + NAD+

Result interpretation

The liver is especially rich in ALT, being the enzyme measurement used primarily as a test for infectious and toxic hepatitis, although high levels of both ALT and AST may also be found in cases of liver cell damage and acute pancreatitis, suggesting that the obstruction of the biliary tree by the edematous pancreas and the presence of associate hepatic disease may contribute to elevated

AST levels in these patients. Slight or moderate elevations of AST and ALT activities may be observed after intake of alcohol and after administration of various drugs, such as salicylates, opiates and ampicillin.

1.3.Aspartate aminotransferase

Principle

Aspartate aminotransferase (AST/GOT) catalyzes the transfer of the amino group from aspartate to oxoglutarate with the formation of glutamate and oxalacetate. The oxalacetate is reduced to malate by malate dehydrogenase (MDH) in the presence of reduced nicotinamide adenine dinucleotide (NADH). The reaction is monitored kinetically at 340 nm by the rate of decrease in absorbance resulting from the oxidation of NADH to NAD+, proportional to the activity of AST present in the sample.

L-Aspartate + 2-Oxoglutarate $\xrightarrow{\text{AST}}$ Glutamate + Oxalacetate

Oxalacetate + NADH + H+ $\xrightarrow{\text{MDH}}$ Malate + NAD+

1.4.Bilirubin direct and total

Principle

Direct bilirubin in the sample reacts with diazotized sulfanilic acid forming a coloured complex that can be measured by spectrophotometry. Both direct and indirect bilirubin couple with diazo in the presence of cetrimide. The difference of two measurements total bilirubin (with accelerator) and direct bilirubin (without accelerator) enables the calculation of indirect bilirubin. The terms “direct” and “total” refer to the reaction characteristics of serum bilirubin in the absence or presence of solubilizing (accelerating) reagents. The “direct and indirect” bilirubin’s are only approximately equivalent to the conjugated and unconjugated fractions.

Result interpretation

Conjugated or unconjugated) in plasma is an indication of a disturbance in bilirubin metabolism. This condition is caused either by an overproduction of bilirubin or by an impairment in the metabolic pathway. The increase in bilirubin production is usually caused by a rapid destruction of erythrocytes, resulting from blood diseases such as hemolytic anemia. In newborns the increase in bilirubin may be caused by Rh, ABO, or other blood group incompatibility, by sepsis, hepatic immaturity, or by a variety of hereditary defects in bilirubin conjugation. Impairment in the bilirubin metabolism is caused either by an enzyme deficiency or by a physical obstruction in bilirubin flow such as biliary (bile duct) obstruction. The hyperbilirubinemia leads to kernicterus

(deposition of unconjugated bilirubin in brain and nerve cells) or jaundice (discoloration of mucus membranes, sclera and skin caused by the deposition of bilirubin pigment).

1.5.Total protein

Principle

Protein in the sample reacts with copper (II) ion in alkaline medium forming a coloured complex that can be measured by spectrophotometry.

Result interpretation

The two general causes of alterations of serum total protein are a change in the volume of plasma water and a change in the concentration of one or more of the serum proteins. Hyperproteinemia can be caused by dehydration (inadequate water intake, severe vomiting, diarrhea, Addison's disease, diabetic acidosis) or as a result of an increase in the concentration of specific proteins (immunoglobulins in chronic infections, multiple myeloma). Hypoproteinemia may be caused by hemodilution (salt retention syndromes, massive intravenous infusions), by an impaired synthesis (severe malnutrition, chronic liver disease, intestinal malabsorptive disease), or by an excessive protein loss due to a chronic kidney disease or severe burns.

1.6.Albumin

Pricnciple

The method is based on the specific binding of bromocresol green (BCG), an anionic dye, and the protein at acid pH with the resulting shift in the absorption wavelength of the complex. The intensity of the color formed is proportional to the concentration of albumin in the sample.

BCG + Albumin $\xrightarrow{\text{PH acidic}}$ BCG-albumin complex

Result interpretation

Hyperalbuminemia is of little diagnostic significance except in dehydration. Hypoalbuminemia is found as a result of several factors: reduced synthesis caused by liver diseases; reduced absorption of amino acids due to malabsorption syndromes or malnutrition; increased catabolism as a result of inflammation or tissue damage; altered distribution between intravascular and extravascular space due to increased capillary permeability, overhydration or ascites; abnormal losses caused by renal disease (nephrotic syndrome, diabetes mellitus, chronic glomerulonephritis, systemic lupus erythematosus), gastrointestinal tract disease (ulcerative colitis, Crohn's disease) or skin damage (exfoliative dermatitis, extensive burns); congenital absence of albumin or an albuminemia (48).

2. Renal Function Test

2.1. Creatinine

Principle

Creatinine in the sample reacts with picrate in alkaline medium forming a coloured complex (jaffe method). Serum and plasma samples contain proteins that react in a nonspecific way; nevertheless, the results can be corrected subtracting a fixed value. The use of this correction is known as the Jaffé method compensated. The formation rate of the complex measured through the increase of absorbance at 500 ± 20nm in a prefixed interval of time is proportional to the concentration of creatinine in the sample.

Creatinine + picric acid $\xrightarrow{\text{alkalinePH}}$ creatinine picrate complex

Result interpretations

Creatinine is synthesized in the body at a fairly constant rate from creatine, which is produced during muscle contractions from creatine phosphate. Creatinine in the blood is then removed by filtration trough the glomeruli of the kidney for excretion in the urine. Since the excretion of creatinine in healthy individuals is independent of diet and thus relatively constant, the creatinine Clearance (CC) test is one of the most sensitive tests to diagnose renal function especially the glomerular filtration rate (GFR) the concentration of creatinine in serum being dependent almost entirely upon its rate of excretion by the kidney. Elevated levels of creatinine in serum are usually associated with renal diseases, especially those related to glomerular filtration rate such as glomerular nephritis (45)

2.2.Urea

Principle

Urea in the sample is hydrolyzed by urease to ammonia and carbon dioxide. The second reaction, catalyzed by glutamate dehydrogenase (GLD) converts ammonia and α-ketoglutarate to glutamate and water with the concurrent oxidation of reduced nicotinamide adenine dinucleotide (NADH) to nicotinamide adenine dinucleotide (NAD). Two moles of NADH are oxidized for each mole of urea present. The rate of decrease in absorbance at 340 nm is measured and proportional to the concentration urea in the sample

Urea + H_2O $\xrightarrow{\text{Urease}}$ 2 NH_3 + CO_2

2NH_4+ 2 α Ketoglutarate +2 NADH $\xrightarrow{\text{GLDH}}$ γ Glutamate + 2 NAD + H_2O

Result interpretations

Urea is the nitrogen-containing end product of protein catabolism. States associated with elevated levels of urea in blood are referred to as hyperuremia or azotemia. Parallel determination of urea and creatinine is performed to differentiate between pre-renal and post-renal azotemia. Pre-renal azotemia, caused by e.g. dehydration, increased protein catabolism, cortisol treatment or decreased renal perfusion, leads to increased urea levels, while creatinine values remain within the reference range. In post-renal azotemia's, caused by the obstruction of the urinary tract, both urea and creatinine levels rise, but creatinine in a smaller extent (48).

3. Glucose

Principle

The OnCall Plus Blood Glucose Test Strips are thin strips with a chemical reagent system using glucose oxidase. They work with the OnCall plus Blood Glucose meter to measure the concentration of glucose in whole blood. Blood is applied to the end tip of the test strip. The blood is then automatically absorbed into the reaction cell. This is where the reaction takes place. A transient electrical current is formed during the reaction and detected by the meter. The blood glucose concentration is then calculated based on the electrical current. The result is shown on the meter display. The meters are calibrated to display plasma equivalent results.

Result interpretation

An abnormal increase in blood glucose level, referred to as hyperglycemia, can be associated with diabetes mellitus and hyperactivity of thyroid, pituitary or adrenal glands. An abnormal decrease beyond the fasting level, referred to as hypoglycemia, is observed in cases of insulin overdose, insulin secreting tumors, myxedema, hypopituitarism, Addison´s disease and conditions interfering with glucose absorption. Glucose measurement in the blood is a key test to evaluate and diagnose any carbohydrate-related disorder.

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.

Name of the student: Gebreslassie Gebremariam Berhe

Date _____ Signature _____

Approval of Advisor:

Mistire wolde (MSc, Ph.D., Associate Professor)

Date: _____ Signature: _____

Aster Tsegaye, MSc, Ph.D., Associated professor

Date _____ Signature _____