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Determination of community based reference interval of selected clinical chemistry parameters among apparently healthy Adolescents in Mekelle City, Tigray North Ethiopia from December 2018 to May 2019, and Community based cross sectional study.

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This is to certify that the thesis prepared by GETACHEW BELAY, entitled: **Determination of community reference interval of common clinical chemistry parameters among Adolescents in Mekelle Zones, Tigray North Ethiopia, 2019** 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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Abbreviation

ALB	Albumin
ALP	Alkaline phosphatase
ALT	Alanine amino transferase
AST	Aspartate amino transferase
BIL.D	Bilirubin Direct
BIL.T	Bilirubin Total
CD	clinical decision limits
CLSI	Clinical Laboratory Standard Institute
C-RIDL	Committee for Reference Intervals and Decision Limits
ELISA	enzyme-linked immune sorbent assay
HBV	hepatitis B Virus
HCV	hepatitis C Virus
HDL	High density lipoprotein
HIV	Human immune deficiency Virus
IFCC	International Federation of Clinical Chemistry
ISO	International Organization for Standardization
LDL	Low Density Lipoprotein
RI	Reference Interval
RPR	rapid plasma reagine
T.P	Total Protein
WHO	World Health Organization

Abstract

Background: Clinical laboratory reference intervals (RIs) are essential for clinical diagnosis, treatment and therapeutic monitoring. Locally established RIs are required to correctly interpret clinical laboratory results. In Ethiopia, particular in Tigray region, clinical laboratory test results are interpreted based on RIs derived from a western population.

Objective: To establish reference interval of clinical chemistry parameters among apparently healthy adolescents aged between 12 and 17 years in Mekelle, Tigray northern part of Ethiopia.

Method: Community based cross sectional study was employed from December 2018 to May 2019 in Mekelle city among 172 males and 172 females based on Multi stage sampling technique. Blood samples were tested for Fasting blood sugar (FBS), alanine aminino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), Creatinine, urea, total protein, albumin (ALB), direct bilirubin (BIL.D) and total bilirubin (BIL.T) clinical chemistry parameters using the 25 bio system clinical chemistry analyzer. Results were analyzed using SPSS version 23 software and based on the Clinical laboratory standard institute (CLSI)/ International Federation of Clinical Chemistry (IFCC) C 28-A3 Guideline which defines the reference interval as the 95% central range of 2.5^{th} and 97.5^{th} percentiles. Mann Whitney U test, descriptive stastics and box and whisker were stastical tools used in analysis of the study.

Result: This study observed statistically significant differences between males and females in ALP, ALT, AST, Urea and Creatinine Reference intervals. The established reference intervals for males and females, respectively, were: ALP (U/L) 79.48-492.12 versus 63.56-253.34, ALT (U/L) 4.54-23.69 versus 5.1-20.03, AST 15.7- 39.1 versus 13.3- 28.5, Urea (mg/dL) 9.33-24.99 versus 7.43-23.11, and Creatinine (mg/dL) 0.393-0.957 versus 0.301-0.846. The combined RIs for Total Protein (g/dL) was 6.083-7.85, ALB (g/dL) 4.42-5.46, FBS(mg/dL) 65-110, BIL.D (mg/dL) 0.033-0.532, and BIL.T (mg/dL) 0.106-0.812.

Conclusion: The result showed marked difference among sex for selected clinical chemistry parameters. It also differs from company derived values. Thus, establishing age and sex specific reference intervals locally and nation wide for clinical chemistry parameters is recommended.

Key words: Reference interval, Adolescent, Clinical Chemistry, Ethiopia

1, INTRODUCTION

1.1background

In the mid 20th century, Groesbeck and Fell man published a paper entitled 'Normal Values and Statistics' as an initial study in the field of reference interval (RI). This was followed by a presentation by Groesbeck and Sais on 'Establishment and Use of Normal Values'. In subsequent years it was realized that the terminology of 'normal values' was not adequate and even partially incorrect, so the term 'reference values' came in to use. From1987 to 1991, the International Federation of Clinical Chemistry (IFCC) published a series of 6 papers, in which it was recommended that each laboratory follow defined procedures to produce its own reference interval. Although there were very important developments and implementations between the 1990s and 2008, the C28A3 guideline, published in 2008 by Clinical Laboratory Standard Institute (CLSI) and IFCC constituted the most significant step in the development of RI and is still in current use IFCC Committee for Reference Intervals and Decision Limits (C-RIDL), noted that "The theory of reference values was developed more than 30 years ago, but its application in most clinical laboratories is still incomplete today [1, 2].

Clinicians order laboratory tests for a variety of reasons: screening for disease, diagnosis of disease, monitoring substances like electrolytes, determining prognosis, confirming a previous abnormal test, and medical legal purposes. When a test is used for disease screening, diagnosis or prognosis, the test result is normally compared with a normal range that is defined as usual value for a healthy population [3]. Most countries are introducing specialized clinics for adolescents, and interest has also grown in research on this previously neglected population. The few studies that have focused on reference intervals for adolescents in Africa reported significant differences between adults and adolescents thus indicating the need to have age specific reference intervals when reporting laboratory results for informed decisions to be made [4]. Technological advancement and economic driving forces have led to major changes in clinical laboratories globally, primarily represented by an increase in testing productivity and efficiency [5].

In the clinical laboratory, reference interval (RI) is the interval between, and including, two reference limits used to separates healthy from diseased individuals. According to international guidelines each clinical laboratories and diagnostic test manufacturers should establish their own RIs for healthy individuals belonging to a group of homogenous population [6]. However, the

majority of clinical laboratories in the world adopt RIs established by manufacturers, rather than developing their own or verifying the applicability of those RIs to their specific population [7].

It is known that, reference intervals provided by manufacturers were established mainly based on American and European populations. Given that laboratory test results could be influenced by differences in dietary, genetic, environmental, and social factors, using reference intervals derived from foreign population may lead to a wrong interpretation, which might influence the outcome. In addition, the clinical interpretation of these results in pediatrics is executed in the context of age and gender-specific dynamics because physiological development leads to changes in many analytes measured, particularly during puberty and in the first years of life [8].

For many African populations, the clinical laboratory RIs have not been established, and non-locally derived RIs are usually being used in diagnostic laboratories and clinical trials to screen, diagnose and monitor disease conditions. In Ethiopia, health facilities are dependent on RIs published either in available textbooks or diagnostic kit inserts. Given the dietary habits, geographical location and ethnic diversity as a source of variability, it is necessary to establish population-specific RIs for Ethiopian population [9].

As far as children are concerned, reference intervals should also reflect the different phases of physiological development from birth to adolescence. However, such data are often lacking, incomplete or derived from obsolete methods. Prospective community-based reference interval studies in healthy children are surprisingly scarce [10]. Therefore, this study aimed to establish reference intervals for clinical chemistry parameters among apparently healthy Adolescents in Mekelle, Tigray National Regional State, Ethiopia.

1.2. Statement of the problem

Reference intervals are essential for clinical laboratory test interpretation and patient care. Methods for estimating them are expensive, difficult to perform, often inaccurate, and non-reproducible. [11]. Establishing reference intervals has always been a challenge as significant differences may exist in disease frequencies; biological variation in analytes among ethnic groups, genders and ages; specimen collection techniques; test performance; test interpretation; and other factors. significant gaps in the available reference intervals as frequently intervals cited in the literature were obtained using older methodologies and instrumentation and cover a limited range of age groups or a relatively small number of samples [12].

Additional issues and challenges come into play when establishing reference intervals for use in a pediatric patient population. These issues and challenges largely underlie the reason for the critical gaps that exist today in accurate and up-to-date reference intervals for laboratory tests performed in children and adolescents. Children should not be viewed as small adults in the context of medical practice. Differences in physical size, organ maturity, body fluid compartments, (rates of) growth and development, immune and hormone responsiveness, nutrition and metabolism, are among the many factors that can influence normal analyte levels in children. Hence, the application of adult reference intervals is often not valid in a pediatric setting. Also, there are diseases that children are susceptible, or more susceptible, in acquiring than adults are. Furthermore, separate reference intervals may be necessary for children of different age groups and/or genders, as well as for neonates, and premature babies [13]. Western population reference interval may not match with the African profile. The laboratory method used to establish the reference values can lead to different results.

While sexual characteristic changes across puberty are profound, the earlier changes during the growth of a child, from birth to puberty are also significant. The dominant form of partitioning applied in clinical laboratory medicine is by social consensus. Partitioning in adolescence should ideally be linked to the pubertal Tanner stages. Childhood definitions are particularly problematic as, for example, thyroid stimulating hormone (TSH) falls to adult levels by age 12 years [14]. Although limited researches are conducted in the area of reference interval in Ethiopia there is no study conducted in Mekelle. The objective of this study was to establish reference interval of clinical chemistry tests among adolescents aged between 12 and 17 years in Mekelle, Tigray northern part of Ethiopia.

1.3 Significance of the study

Lack of appropriate local reference values for biochemical parameters are challenges in interpreting results for management of patients and other decision making. Health professionals usually use textbook reference values to compare the reported values. The results of this study, therefore, will be used as reference values in the future evidence-based practices. Patients will get better service as their result will be interpreted based on the locally established value; physicians will have better tool in their patient management process and medical laboratory professionals will have confidence especially flagged result based on RIs established elsewhere are a common challenge. Researchers especially in clinical and vaccine trials will have better tool to identify eligible participants. Moreover, this study would serve as baseline information for further studies.

2. Literature Review:

Reference interval is crucial for disease screening, diagnosis, monitoring, progression and treatment efficacy. The same reference intervals are sometimes used to interpret test results for both adults and children. However, children are not small adults; children have significant differences in physiology and metabolic state, physical size, organ maturity, bodily fluid compartments, and immune and hormone responsiveness when compared to adults. Most notably, dynamic physiological changes, growth and development profoundly influence biomarker concentrations [15].

A study conducted in Canada from approximately 12000 Canadians aged 3–79 years and measured 24 biochemical markers with the Ortho Vitros 5600 FS analyzer or a manual microplate. Data were collected from 500 females and 500 males in each of the following age groups: 3–5 (cycle 2), 6–11, 12–19, 20–39, 40–59, and 60–79 years. The reference interval of ALT (U/L) aged 12-17 years for male and female are 17-50 and 14-41 respectively; ALB(g/dL) aged 6-15 years 4.1-5.1 for both sex; ALP(U/L) aged 11-15 years 113-438, 64-354 for male and female respectively; AST (U/L)aged 12-17 years 18-36, 15-34 for male and female respectively; Bilirubin total aged 6-25 years 0.1-0.9 for both sex; Creatinine (mg/dL) aged 12-15 years 0.5-0.9, 0.5-0.8 for male and female respectively. Urea (mg/dL) reference interval aged 8-19 years are 8-20 and 8-19 for male and female respectively; Glucose (mg/dL) aged 12-19 years 75-93 for both sex [8].

A study conducted in Taiwan from a 4326 subjects including 2029 kindergarten children, 1624 elementary-school children, 325 junior-high-school children, and 348 teachers were selected to determine serum alanine aminotransferase (ALT), blood urea nitrogen (BUN), creatinine (Cr) and glucose levels using a Beckman Synchron CX5 analyzer. The reference interval (2.5^{th} - 97.5^{th}) of the tests for female are Glucose 60–99 (mg/dL), ALT (IU/L) 7–36 , B/C ratio.9–32.5 BUN (mg/dL) 8.4–17 and Creatinine 0.4–1.1(mg/dL); for males are Glucose (mg/dL) 61- 98, ALT (IU/L) 8–41 , B/C ratio 10–34, BUN (mg/dL) 9–18 and Creatinine (mg/dL) 0.4–1.2. The concentrations of glucose and Cr increased with age. On the contrary, the B/C ratio decreased with age [16].

Pediatric reference intervals of liver and renal function tests from birth to Adolescence were conducted in Chinese among 63,086 apparently healthy children and adolescents (0–15 y).The

reference interval of 2.5th and 97.5th percentile were as follows; ALP (U/L) 85-407, 44-306; ALT (U/L) 7-46,6-40; AST (U/L) 13-38, 12-32; Direct Bilirubin(mg/dL) 0.06-0.43; Total Bilirubin (mg/dL) 0.29-1.46; Urea(mg/dL) 9.6-39.5; creatinine (mg/dL) 0.34-0.86 ; Total proptein (g/dL) 5.94-8.04;albumin (g/dL) 3.5-5.22for male and female respectively. Gender partitions were required for alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and creatinine (Cre) [17].

A study conducted in Taita Taveta County, Kenya consisted of healthy female and male population of ages between 1 and 17 years old citizens residing in Taita Taveta region for more than half a year. The research was done in Voi which is located in sisal farming region approximately 591 meters above sea level in Coastal zone of Kenya. Blood used in the study was sampled from a healthy population of children and infants at day time. Cobas Integra® 400 chemistry auto analyzer was used, that is manufactured in Germany (Roche Diagnostics). The reference interval for total protein (g/dL),ALB(g/dL),ALP (U/L),ALT(U/L),AST(U/L),BIL-D(Mg/dL) and BIL-T(Mg/dL) were 5.98 8.57, 3.56-5.3, 74.7-566.6, 3.1-31.1, 10.0-52.1, 0.51-0.969 and 0.262-1.58, respectively [18].

A study was performed in western Kenya in adolescent and young adults in a rural population. The reference interval of adolescents (age 13-17) and young adults(age 18-34) for female and male were for 13-17 years old; to AST (12.0–43.1 and 17.0–59.2, 13.5–48.5 and 12.5–69.3 µ/L) ,ALT (4.2–65.3 and 4.9–42.4, 10.7–61.3 and 12.0–80.6 µ/L), Total bilirubin (3.7–38.5 and 5.7–62.6, 5.8–36.1 and5.3–50.7 µmol/L) creatinine (48.0–87.6 and 49.6–103.7, 52.4–96.8 and 54.2–137.8 µmol/L) Glucose(2.0–7.0 and 1.7–4.1, 2.1–6.0 and 2.1–9.0 mmol/L) BUN (1.2–4.8 and 1.7–4.1; 1.4–4.5 and 1.8–5.3 mmol/L) respectively. Results indicated gender and age variations between young adults and adolescents. Males had higher values for ALT, AST, T-bil and creatinine than females in both age groups, with those differences being significantly greater for T-bil and creatinine indices in both age-groups and AST among the adolescents [19].

A cross-sectional population-based study conducted for Liver Biochemistry Tests in Children in Meru County, Kenya recruited 740 young population of ages one to seventeen years, comprising 360 females and 380 males that were found to be free from HIV, Hepatitis B and syphilis were recruited. DRI - CHEM NX 500I Clinical Chemistry analyzer (Fujifilm, Europe) was used to analyze eight biochemical parameters. The reference intervals were ALB 27.98-

48.96 ,28.62-48.50 (g/L) ; AST 10.16-54.30 ,5.65-58.93 (U/L) ; ALP 61.63-114.31 ,58.21-114.23 (U/L); ALT 11.18-57.20 ,10.24-56.84 (U/L); D-BIL 0.43-3.53 , 0.21-4.19 (µmol/L); T-BIL 12.98-71.78 ,12.19-73.45 (µmol/L); TP 30.78-55.72 , 29.95-54.57 (g/L); GGT 18.82-128.34 , 8.44-127.76 (U/L) for males and females respectively. Significant sex differences were observed in children reference values for total protein. Other parameters (alkaline phosphatase, gamma glutamyl transferase, direct bilirubin, total bilirubin, albumin, alanine aminotransferase, aspartate aminotransferase) did not show significant sex dependent differences [20].

A study was carried out to provide specific reference intervals for children from Papua New Guinea from 327 healthy Melanesian children living in Madang Province. The established intervals were Creatinine (mmol/L) 18–45, Urea (mmol/L) 1.0–5.1, ALT (IU/L) 5–45, ALP (IU/L) 104–259, Bilirubin (µmol/L) 1.5–7.8, Total Protein (g/L) 60–89 and Albumin (g/L) 32.5–46. The PNG reference intervals for serum bilirubin showed some agreement with children from Western countries, and substantial agreement with those from African children. However, the upper limits were much lower in PNG children (7.8 versus 17 µmol/L and 18.9 µmol/L, respectively) [21].

A community based cross sectional study conducted among a total of 302 Zimbabwae adolescents aged ≥ 12 to < 18 years showed that the 2.5^{th} and 97.5^{th} reference interval were as follows; glucose (mg/dL) 65-111.5, 57.5-121.5; ALP (U/L) 73.8-572, 66.1-522; ALT (U/L) 5.8-38.8, 5.0-34.5; AST (U/L) 14.4-45.7, 12.7-38.8; Direct Bilirubin(mg/dL) 0.00-0.25, 0.007-0.2; Total Bilirubin (mg/dL) 0.2-1.09, 0.17-0.89; Urea(mg/dL) 4.6-17, 5.1-15.6; Total proptein (g/dL) 6.8-9, 7.1-9.06;albumin (g/dL) 4.3-5.5, 4.37-5.7 for male and female, respectively. Results showed significant differences between males and females in ALP, ALT and AST [22].

A population-based cross-sectional study was conducted in Gilgel Gibe Field Research Center (GGFRC) in South West Ethiopia from late September 2008 to end of January 2009. The 95 percentile range reference interval of biochemical tests aged between 15 and 24 years were FBS (mg/dl) 66.8-125.8,59.7-117.7; Urea (mg/dl) 4.6-27.2,3.9-48.5; Creatinine (mg/dL) 0.3-1.9,0.3-1.3; ALT (u/L) 14.4-60.7,11-70.5; AST (u/L) 12.4-58, 11-72.7 and ALP (u/L)21.2-656.4, 91.8-440.6 to male and female, respectively [23].

The literatures reviewed above clearly indicated the need for locally established RIs. However, as far as my literature search goes there is no published study in Adolescents of Mekle city.

3. Objectives:

3.1. General objective

To determine community based reference interval of selected clinical chemistry parameters among apparently healthy adolescents aged 12-17 years in Mekelle city, Tigray North Ethiopia from December 2018 to June 2019.

3.2. Specific objectives

1. To determine age and sex specific reference interval for adolescent in Mekelle city.
2. To compare the reference interval values between sexes for the adolescents in Mekelle city.
3. To compare the current reference intervals with those provided by manufacturers.

5. Materials and methods

5.1. Study area:

Mekelle is the capital city of Tigray National Regional state. It is located around 780 kilometers (480 mi) north of the Ethiopian capital Addis Ababa, with an elevation of 2,254 meters (7,395 ft) above sea level and in a semi-arid area with a mean annual rainfall of 714 millimetres (28.1 in). Except for a moderately dense eucalyptus cover on the hills in eastern edges of Mekelle and some exotic species of trees and shrubs lining the streets, the surrounding landscape is almost treeless. The total population of mekelle city is 310,436 according to 2007 census. Administratively, Mekelle is considered a Special Zone, which is divided into seven sub-cities. The seven sub cities of mekelle are Hawelti, Adi-Haki, Kedamay Weyane, Hadnet, Ayder, Semien and Quiha. Within each local administration there are kebeles or ketenas. Our study was conducted in three sub cities, namely, Hawelti, Semen and Ayder [24].

5.2. Study design and period:

A cross sectional community based study was employed in Mekelle city from December 2018 to May 2019.

5.3. Population:

5.3.1. Source population

All adolescent indivividuals who were living in Mekelle city and whose ages were between 12 and 17 years.

5.3.2. Study Population

Apparently Healthy adolescent populations aged between 12 and 17 years, who fulfill the eligibility criteria and were living in Mekelle city for at least 5 years.

5.4. Inclusion and exclusion criteria:

5.4.1. Inclusion criteria

Young apparently healthy children who lived at least for 5 year in Mekelle city were included in the study.

5.4.2. Exclusion criteria

- Those children and parents who were not willing to participate in the study, Individuals with known chronic illnesses like diabetes mellitus, chronic renal insufficiency, hypertension, ischemic heart disease, anemia, thyroid, liver diseases, cancer of any type.
- Individuals who had known infectious disease (HIV,Hepatities)
- Individuals taking pharmacologically active substances and all prescription drugs.
- Individuals who had Hemo-parasite and intestinal parasite were excluded from the study.
- Individuals who received blood transfusion within the previous 1 year.
- Pregnant females.
- Individuals who haven't full filled the questionnaire criteria from question 6 to question 22

5.5. Study variables

5.5.1. Dependent variables

- Selected clinical chemistry parameters

5.5.2. Independent variables

- sex

5.6. Sample size calculation and Sampling method

5.6.1. Sample size calculation

For establishing reference interval the Clinical Laboratory Standards Institute (CLSI) guideline which was developed through consensus process for the global application 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, age range) with a power of 90% [CLSI 2000]. In the current study, the maximum partition needed was 12-17 years old female and male. This age partition is based on previous studies conducted in Canada for AST and ALT [8],[22] and

serum creatinine level similar value after pubertal change [14]. Thus, two age partition groups were needed (2 x 120=240).

According to previous studies in other African countries, in such studies about 30% of apparently healthy population [25] 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 total sample size of 240 for the reference interval determination, a total of 344 individuals were 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 systematic sampling technique by considering sub-cities as a sampling frame and then households the final selection units. One individual in the household fulfilling the eligibility criteria and willing to participate were included in the study.

5.6.2. Sampling Method

Multi stage sampling technique was used from December 2018 to May 2019.

5.7. Measurement and Data collection

5.7.1. Data collection procedure

Three sub cities were selected from the total of 7 sub cities (Ayder, Hawelti and Semen) through simple random sampling method and then the total sample (344) was categorized based on the relative house hold 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 is distributed to each sub city by probability proportional to size based on house hold numbers. Accordingly we were recruited 92, 167 and 85 from Semen, Hawelti and Ayder respectively. All 5 kebeles in each sub city were included and sample size was allocated based on the size of the house hold. After that data was collected and specimen from each kebele based on systematic sampling technique (K^{th}). Besides, if more than one fullfulliung the criteria are obtained in a house hold it was sampled a single participant by lottery method. Below is a table which shows the number of individuals to be sampled from each kebeles.

Table 1: Total number of house holds and sampled per each Kebele

Sub cities	Kebele	Total number of house hold	Total number of recruited	Age of 12-17 male	Age of 12-17 female
Semen	Mesfin	4615	24	12	12
	Dedebit	2870	14	7	7
	Yekatit	3216	16	8	8
	Endistry	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**	41	41

Before data collection, the significance of the research was well explained to the parents (guardians) of the children and to the participant adolescents. Participants were invited to a nearby health facility. After getting an agreement (Consent from parents and assent from those aged 12-17 years), the data collector asked some questions which take up to 10 minutes. The children weight, height and vital sign was measured and recorded. The children also asked to bring urine and fresh stool on a clean, leak proof container provided to them. Direct microscopy (wet mount) was performed at the site of collection and stool specimen was transported to the central laboratory (Tigrai Health Research Institute) for further analysis like concentration and

kato-katz technique. Chemical analysis of urine through reagent strip and after centrifugation urine microscopy was done on site of collection. About 4-ml of fasting blood was collected using a 23-gauge needle through vacutainer method after sterilizing the area with 70% alcohol. The tubes were labeled with the study number (MK001-MK344), the subject's initial name, and date of collection of the sample. Immediately after collection, random blood sugar was analyzed and appropriately recorded on the provided request. The specimens were stored and transported in Styrofoam cool boxes at 4°C and covered to protect them from heat and sunlight, to the main analytical center.

5.7.2. Laboratory analysis

Data collectors were trained for three days about the objective of the study, study participant rights, confidentiality of patient information, procedure of physical examination, procedure of blood sample collection and measurements, and how to approach and interview participants before the actual data collection. The study participants were contacted when they came to the nearby health institution. Study participants who agreed to give written consent after being informed about the purpose of the study and associated risks were physically examined and interviewed. Socio-demographic data and blood sample were collected from those who fulfilled the criteria set to say apparently healthy. About 5 ml of blood sample was collected from each study participant using plane tube at the morning from 8:00 AM to 11:00 AM. At the site of collection fasting blood sugar was tested and recorded on a result format paper. The collected blood samples were transported by ice bag to Tigray health research institute within 60 minutes for processing. Then sample was centrifuged at 2500 rpm (revolution per minute) for 5 minutes to separate serum. Separated sera were stored at -37 ^{0}C until analysis in a nunc tube. After that, Biosystem 25 A clinical chemistry analyzer fully automated clinical chemistry analyzer was used for the measurement of biochemical analytes. Clinical chemistry parameters were determined by the methods/techniques described as follows in a table form:

Table 2: Methods used for analytes of clinical chemistry to determine the reference interval of apparently healthyAdolescents 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 Componsated
Urea	kinetic urease/GLDH (Glutamate dehydrogenase)
TP	Biuret
ALB	Bromocresol green
BilD and BilT	diazotized sulfanilic
FBS	Glucose oxidase

5.8. Data Quality Assurance:

5.8.1 Data Collection Quality Assurance

The Questionnaires were translated from English to Amharic and Tigrigna version by well experienced linguistic Person. Pre testing of the Questionnaires was done on 5% individuals in Wukro city. Training for data collectors were given by the investigator and the consistency of the data was also checked.

5.8.2 Pre analytical Test

Each activity including blood sample collection, transportation and storage were based on good laboratory practices (GLP) using standard operating procedures (SOPs) to ensure data quality. The participants were well prepared and labeling was done from MK 0001 –MK 344 on the questionnaire, plane test tube, stool cup and urine cup. Specimen collection was performed in morning from 8:00AM-11:00AM in order to minimize some interference in clinical chemistry tests. The fore arm was cleaned by 70% alcohol antiseptic before collection and the blood specimen was dispensed to the plane test tube which is without anticoagulant but with separator jell. After collection, random blood sugar was analyzed and appropriately recorded on the provided request. The specimens were stored and transported in Styrofoam cool boxes at 4°C and covered to protect them from heat and sunlight until arrives at the main analytical center.

The serum was dispensed after centrifugation immediately to nunc tube in order to minimize interference in the biological analyte. The serum specimens were stored in deep freezing until analyzed. The stool and urine cup was wide mouthed and analysis was done within 10 minute after collection in the site of collection.

5.8.3 Analytical Test

The analysis was done in Tigray Health Research Institute (THRI) clinical chemistry laboratory which is closely supervised by Ethiopian Public Health Institute (EPHI). The equipment had been calibrated monthly by type-Autocal. In addition, two levels (normal and pathological) of internal quality control (IQC) samples were run along with the serum sample. The control sample results were interpreted using Westgard multi-rule algorithm. The control sample results have to be within acceptable ranges prior serum sample testing. Samplewere run after well understanding the leaflet for each analyte.

5.8.4 Post Analytical Test

All results of clinical chemistry tests, urinalysis and stool examination will be recorded carefully on the provided space by seeing its labeling and it was attached with its questionnaire.

5.9. Data analysis and interpretation:

Data was cleared, edited, checked for completeness manually and entered to SPSS version 23(IBM,USA) software for analysis. Kolmogorov±Sminorv test was used to check data distribution normality. The first quartile (Q.25), the median (Q.50) in addition to third quartile (Q.75) were determined, then interquartile range was calculated (IQR) from the differences of third and first quartiles (Q.75-Q.25). Data that was observed to be lower than 1.5 × IQR of first quartile, or higher than 1.5 × IQR of third quartile was considered as outliers and was manually deleted using the Box and Whisker stastical tool. These exclusions led to some parameter results missing hence difference in sample sizes for different parameters. RIs were calculated in accordance with CLSI/IFCC guideline using non-parametric methods. Mann Whitney U test non– parametric analysis was used to assess sex difference values. The 95% RI was estimated using reference limits at 2.5 th percentile for the lower reference limit and 97.5th percentile for the upper reference limit.

5.10. Operational definitions:

Reference interval: Reference interval is the interval between, and including, two reference limits (2.5^{th} and 97.5^{th} percentile) for apparently healthy individuals.

Adolescent: A young children who are in a markedly pubertal change aged between 12-17 years old.

Selected clinical chemistry tests: Selected clinical chemistry tests are biochemical analytes which are practiced almost in all tropical laboratories from body fluids (FBS, ALP, ALT, AST, BIL D, BIL T, Urea, Creatinine, Total protein and Albumin).

5.11. Ethical considerations

The study was conducted after ethical approval is obtained from Research and Ethics Institutional Review Board of Addis Ababa University College of Health Science, Department of Medical Laboratory Science. Official permission letter was submitted to Tigray reginal Health office. Informed written consent and assent were also obtained from each study participant and guardians before the actual data collection. Participants were informed of risks and benefits of the study, their right to withdraw anytime, how confidentiality was maintained using codes and their right to get their results for free. Individuals positive for stool and urine tests were linked to nearby government hospitals for further diagnosis and treatment accordingly.

5.12. Dissemination of the result

The result will be submitted to Addis Ababa University, College of Health Science, Department of Medical Laboratory Science and Mekelle Zonal Health Office. The findings will be presented in national and international scientific conferences. The findings will also be sent to reputable journal for publication. Above all the RI determined will be communicated to all health facilities in Mekelle for immediate use in clinical management of patients.

6. Work Flow

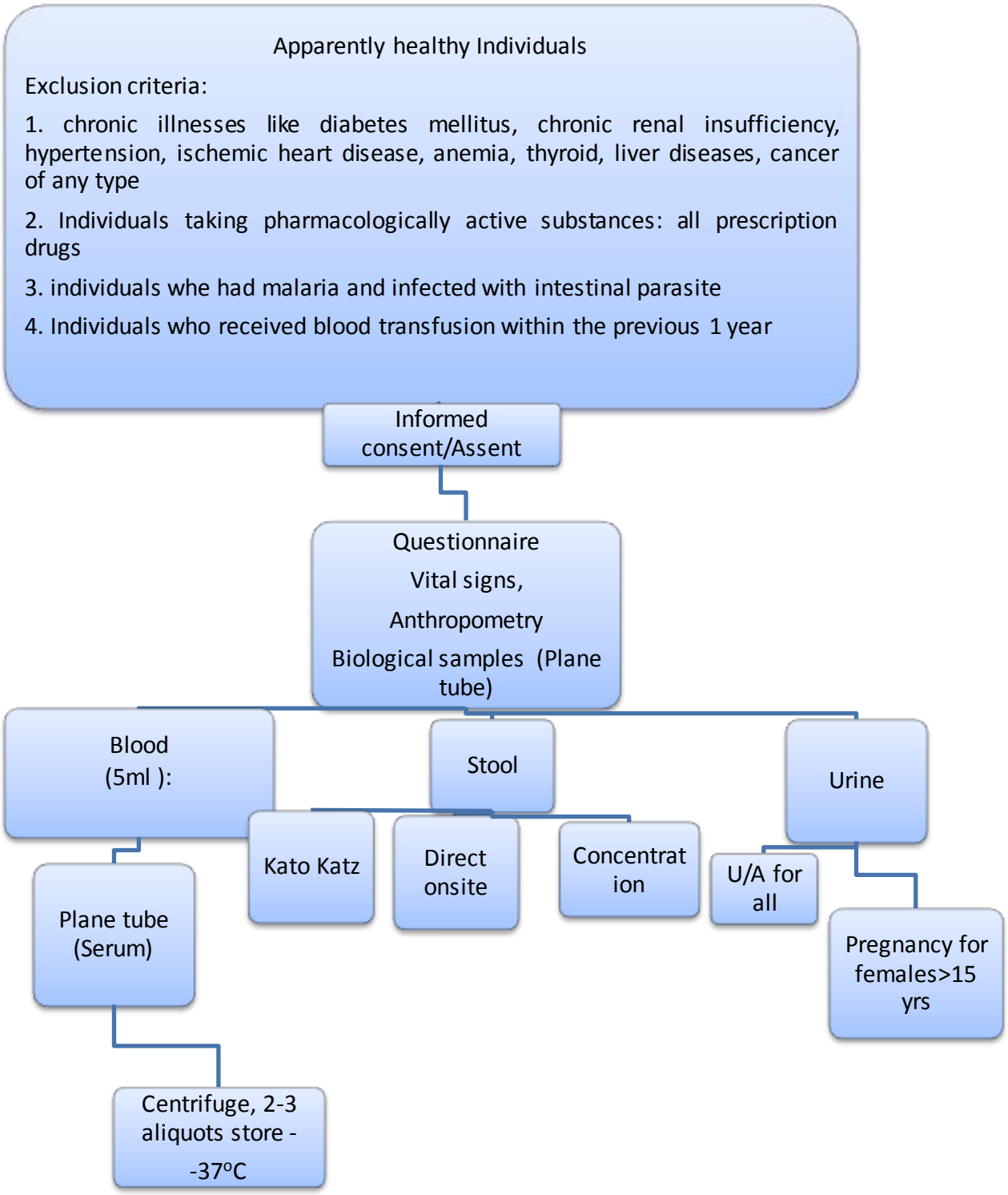

Figure 1: Work flow for sample collection and analysis

7. RESULT

7.1. Socio-demographic Characteristics

A total of 344 apparently healthy adolescents were recruited to establish the RI selected clinical chemistry parameters from Mekelle community Tigray, Ethiopia. Out of all study participants, 172 (50%) were males. The study participants' median age was 15 years. About 18 (5%), 34 (10%) 41 (12%) , 103 (30%), 72 (21%) and 76 (22%) of the study participants were aged 12,13,14,15,16 and 17 years,respectively (Figure 2). By parasitological, urinalysis and hemolysis of specimen, a total of 45 males and 38 females were rejected during clinical chemistry analysis. Of these, 35 males and 25 females had intestinal parasite. In addition, 5 males and 10 females had finding in urinalysis indicating urinary tract infection, respectively; 5 male and 2 female samples were hemolyzed and rejected. Overall rejection rate was about 24% (83/344).

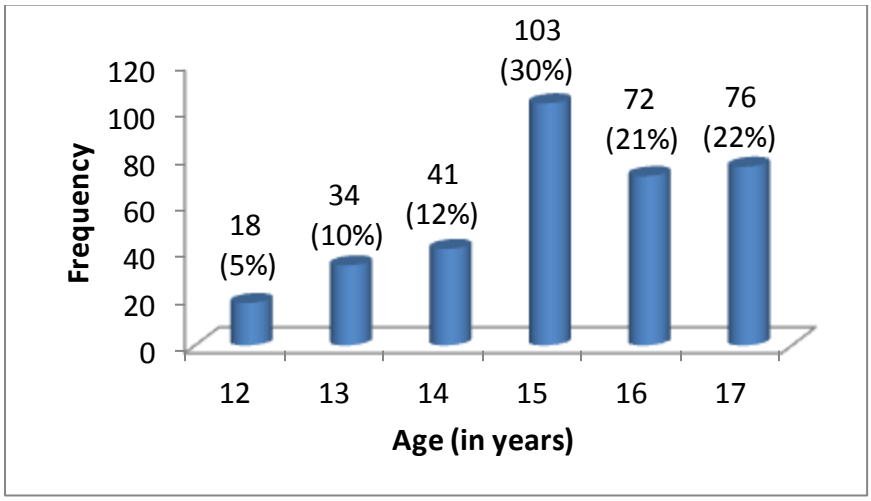

Figure 2: Age distribution of Adolescents in Mekelle Tigray Regional State, Ethiopia, 2019 (N= 344)

7.2. Reference Intervals of Clinical Chemistry Parameters

This study observed statistically significant differences (P-value <0.005) using non-parametric Mann Whitney U test between males and females in ALP, ALT, AST, Urea and Creatinine liver and kidney panel RI. Except for Fasting blood sugar, Bilirubin direct and albumin all tests have higher upper limit in males than females (Table 3).

Table 3: The RIs of clinical chemistry parameters among Adolescents in Mekelle Tigray Regional State, Ethiopia, 2019 (N= 261; Male = 127, and Female = 134).

Analyte	Sex	NO	Mean	Median	95% CI of Mean	Min	Max	25th-75th percentile	2.5th-97.5th percentile, RI	p-value to sex
FBS (Mg/dl)	C	257	87.13	87	85.72, 88.53	60	118	79-100	65-110	0.762
	M	125	86.85	87	84.80, 88.90	62	118	78.50-100.5	64.15-108	
	F	132	87.39	87.5	95.44, 99.35	60	117	79.25-100	65.33-111.35	
ALP (IU/L)	C	256	201	166	187.59, 214.33	13	497	109-271	66-456	<0.001*
	M	123	271.83	268.2	252.20, 291.46	64	553	187-346.1	79.48- 492.12	
	F	126	128.11	114.25	119.93, 136.28	13	285	98.28- 145.23	63.56- 253.34	
ALT (IU/L)	C	252	13	13	12.54, 13.63	3	24	10-16	5-23	<0.001*
	M	120	14.28	13.7	13.46, 15.11	3	25	11.30-17.33	4.54- 23.69	
	F	129	11.74	11.60	11.11, 12.37	4	21	9.15- 13.85	5.1- 20	
AST (IU/L)	C	251	23	22.8	22.38, 23.67	10	40	19.6-26.3	14.2-34.9	<0.001*
	M	123	25.62	25	24.61, 26.63	14	40	21.8- 29.3	15.7- 39.1	
	F	127	20.97	20.8	20.30, 21.64	12	29	18.70- 23.4	13.3- 28.5	
D.BIL (Mg/dl)	C	252	.2639	.243	.2497, .2782	.01	.60	.1835-.3343	.033-.532	0.07
	M	121	.273	.258	.2539, .2915	.01	.54	.206- .346	.034- .496	
	F	129	.251	.234	.2301, .2709	.01	.59	.1610- .3255	.028-.532	
T.BIL (Mg/dl)	C	248	.3921	.3715	.3704, .4138	.02	.95	.2615-.4938	.107-.812	0.059
	M	122	.4183	.3965	.3851, .4515	.02	.93	.2780- .521	.094- .839	
	F	126	.367	.348	.3389, .3950	.02	.81	.2495- .4750	.106- .728	
Urea (Mg/dl)	C	258	15.08	14.65	14.59, 15.57	6	26	12.08-17.73	8.1-24.2	0.006*
	M	125	15.87	15.7	15.14, 16.59	9	26	12.50- 18.55	9.3- 24.9	
	F	132	14.26	14.05	13.62, 14.90	6	24	11.65-16.8	7.4- 23	
Cr (Mg/dl)	C	253	.6607	.670	.6439, .6775	.29	.99	.58-.76	.37-.91	0.001*
	M	122	.697	.690	.6720, .7221	.37	1.05	.5975-.7925	.394- .958	
	F	134	.6248	.640	.6018, .6477	.28	.89	.5400- .7225	.301- .846	
T.P (g/dl)	C	249	6.9	6.84	6.848, 6.960	5.8	8.4	6.6-7.2	6.09-7.85	0.117
	M	122	6.849	6.805	6.771, 6.928	5.8	8	6.510-7.14	5.9- 7.8	
	Female	126	6.964	6.855	6.888, 7.041	6.1	8.1	6.67-7.23	6.1- 7.9	
Alb (g/dl)	C	255	4.883	4.86	4.851, 4.915	4.3	5.6	4.71-5.06	4.4- 5.5	0.094
	M	124	4.848	4.835	4.801, 4.896	4.2	5.6	4.675- 5.028	4.3- 5.5	
	F	132	4.909	4.880	4.865, 4.954	4.3	5.6	4.75- 5.08	4.4- 5.5	

ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; AST: Aspartate aminotransferase; CI: confidence interval :L: litre; mg: Milligram; RI: Reference interval; SGOT: Serum glutamate oxaloacetate transaminase;SGPT: Serum glutamate pyruvate transaminase; U: Unit; T.P: Total protein;ALB: Albumin; Cr: Creatinine; D.Bil: Direct Bilirubin; T.Bil: Total Bilirubin;C:combined; M:male;F:female; P-value ≤ 0.05 cosidered as stasticaly significant.

Based on the newly establshed 95% RI (shown in Table 3), the study tried to analyse the rate of misclassification when using manufacturer's lower and upper limit value for apparently health adolescents in Mekele city. Accordingly, FBS was misclassified as below the lower limit in 10 (3.89%) and above the upper limit in 12 (4.6%) of the study participants when using the RI provided by the company. The upper limit of the manufacturer ALP had a big difference when compared with the current study in both sexes. A total of 110(89.4%) male and 76 (60.3%) female were misclassified by the manufacturer reference interval as an out of range (that is, above the upper limit value). Each of three male and female of the study participants' serum ALT were beyond the upper limit of the current study but within the interval of the manufacturer upper limit value. The manufacturer's AST RI misclassified 6 (4.7%) female and 1 (0.8%) male of the study participant.

A total of 59 (23.4%) study participants' direct bilirubin valuewas misclassified by the manufacturer's RI as being above the upper limt, but they were within thenewly established reference interval. In addition, 215 (4.6%) of the study participant were above the upper limit for total bilirubin campany value. Fifity four (21.7%) of the study participant were below the lower limit of (6.6 g/dL) value of the total protein of the manufacturer's reference interval. Table 4 summarises the rate of misclassifications when using comoany derived RIs as compared to the currently established RI.

The 90% CI for the newly established lower and upper reference intervals is desplaed in Table 5.

Table 4: Misclassification of study participant through lower and upper limit of the current study versus manufacturer RI.

Analyte	sex		Manufacturer	Misclassification			
				Lower limit		Upper limit	
		Current Study		Frequency	Percent (%)	Frequency	Percent (%)
FBS (Mg/dl)	combined	65-110	70-100	10	3.89	12	4.6
ALP (IU/L)	Male	79.48- 492.12	0-115	2	1.6	110	89.4
	Female	63.56- 253.34	0-105	3	2.4	76	60.3
ALT (IU/L)	Male	4.54- 23.69	0-41	3	2.5	3	2.5
	Female	5.1- 20	0-41	3	2.3	3	2.3
AST (IU/L)	Male	15.7- 39.1	0-40	4	3.3	1	.8
	Female	13.3- 28.5	0-40	3	2.4	6	4.7
D.Bil (Mg/dL)	combined	.033-.532	0-0.2	187	74.2	59	23.4
T.Bil (Mg/dL)	combined	.107-.812	0-0.2	6	2.4	215	86.7
Urea (Mg/dl)	Male	9.3- 24.9	15-39	55	44.0	3	2.4
	Female	7.4- 23	15-39	80	60.6	3	2.3
Cr (Mg/dL)	Male	.394- .958	0.7-1.2	61	50.0	3	2.5
	Female	.301- .846	0.5-0.9	23	17.2	3	2.2
T. P (g/dL)	combined	6.09-7.85	6.6-8.3	54	21.7	8	3.2
ALB (g/dL)	combined	4.4- 5.5	3.5-5	6	2.4	74	29.0

ALT: Alanine aminotransferase; **ALP**: Alkaline phosphatase**; AST:** Aspartate aminotransferase; **CI**: confidence interval :**L**: litre; **mg:** Milligram; **RI**: Reference interval; **SGOT**: Serum glutamate oxaloacetate transaminase;**SGPT**: Serum glutamate pyruvate transaminase; **U**: Unit; **T.P**: Total protein;**ALB**: Albumin; **Cr**: Creatinine; **D.Bil**: Direct Bilirubin; **T.Bil**: Total bilirubin

Table 5: The 90% CI of reference limits of clinical chemistry parameters RIs among apparently healthy adolescents in Mekelle, Tigray North Ethiopia, 2019 (N=261; Male=127 and Female=134).

Analyte	unite	NO	Sex	Range	2.5th-97.5th percentile, RI	90% CI (lower reference limit)	90% CI (upper reference limit)
Fasting blood sugar	Mg/dl	257	combined	60-118	65-110	63.1, 67.3	107, 116.6
		125	Male	62-118	64.15-108	62.1, 66.79	107, 118
		132	Female	60-117	65.33-111.35	60.4, 69	106, 116.5
Alkaline phosphatase (ALP)	IU/L	256	combined	13-497	66-456	63.4, 78.3	433, 472
		123	Male	64-553	79.48- 492.12	64.2, 103.7	457.3, 550.9
		126	Female	13-285	63.56- 253.34	15.7, 74.3	221.4 , 283.3
Glutamic pyruvic transaminase(ALT)	IU/L	252	combined	3-24	5-23	4.4, 6.1	21.7, 24.2
		120	Male	3-25	4.54- 23.69	2.7, 7.1	22.5, 25
		129	Female	4-21	5.1- 20	4.4, 6	19, 21.3
Glutamic oxaloacetic transaminase(AST)	Mg/dl	251	combined	10-40	14.2-34.9	12.2, 14.9	33, 35.9
		123	Male	14-40	15.7- 39.1	14.2, 16.9	35.9, 40.2
		127	Female	12-29	13.3- 28.5	12.2, 14.9	27.9, 29.1
Bilirubin Direct	Mg/dl	252	combined	.01-60	.033-.532	.014, .099	.491, .587
		121	Male	.01-.54	.034- .496	.01, .13	.46, .54
		129	Female	.01-.59	.028-.532	.01, .1	.46, .58
Bilirubin Total	Mg/dl	248	combine	.02-.95	.107-.812	.02, .13	.72, .84
		122	Male	.02-.93	.094- .839	.02, .18	.74, .93
		126	Female	.02-.81	.106- .728	.02, .13	.66, .81
Urea	Mg/dl	258	combined	6-26	8.1-24.2	7.4, 8.7	22.7, 25
		125	Male	9-26	9.3- 24.9	8.9, 9.9	22.8, 25.5
		132	Female	6-24	7.4- 23	6.2, 8.3	20.6, 24.3
Creatinine	Mg/dl	253	combined	.29-.99	.37-.91	.32, .4	.89, .93
		122	Male	.37-1.05	.394- .958	.38, .47	.91, 1.05
		134	Female	.28-.89	.301- .846	.28, .37	.81, .89
Total protein	Mg/dl	249	combined	5.8-8.4	6.09-7.85	5.9, 6.2	7.7, 7.9
		122	Male	5.8-8	5.9- 7.8	5.8, 6.2	7.6, 7.9
		126	Female	6.1-8.1	6.1- 7.9	6.1, 6.3	7.8, 8.1
Albumin	Mg/dL	255	combined	4.3-5.6	4.4- 5.5	4.3, 4.5	5.4, 5.5
		124	Male	4.2-5.6	4.3- 5.5	4.2, 4.5	5.3, 5.5
		132	Female	4.3-5.6	4.4- 5.5	4.3, 4.5	5.4, 5.5

ALT: Alanine aminotransferase**; ALP:** Alkaline phosphatase**; AST:** Aspartate aminotransferase**; CI:** confidence interval **:L:** litre**; mg:** Milligram**; RI:** Reference interval**; SGOT:** Serum glutamate oxaloacetate transaminase;**SGPT:** Serum glutamate pyruvate transaminase**; U:** Unit; **T.P:** Total protein**;ALB:** Albumin**; Cr:** Creatinine**; D.Bil:** Direct Bilirubin**; T.Bil:** Total bilirubin

Finally the study tried to compare the current RIs determined for Mekelle city apparently healthy adolescents with other selected studies in Ethiopia and elsewhere. As summarized in Tables 6, variations were noted among the various studies. The ALP upper limit value in males which was much higher than the company derived value was lower than the upper limit reported from zimbabwae and South West Ethiopia (Table 6). Whereas the lower reference limit of ALT for both males and females in the current study is in line with the lower limit reference value of a study conducted in Western Kenya. However, the upper limit reference values in both sexes in the current study is lower than the upper limit reference values of studies conducted in Canada, Taiwan, China, Taita Kenya, West Kenya, Meru Kenya, Zimbabwae and Southern West Ethiopia (Table 6).

Table 6: Comparison of clinical chemistry parameters RI of current study against manufacturer ranges and other similar studies.

Analyte	Sex	Current Study	Manufacturer	Taiwan**[16]**	Canada [8]	China**[17]**	Zimbabwea **[22]**	West Kenya [20]	Meru Kenya **[20]**	Taita Kenya**[18]**	South West Ethiopia **[23]**
FBS (Mg/dl)	C	65-110	70-100	60-99	NA	NA	NA	37.8-118.8	NA	NA	NA
	M	64.15-118	NA	61-98	75-93	NA	65-111.5	NA	NA	NA	66.8-125.8
	F	65.33-121.35	NA	60-99	75-93	NA	57.5-121.5	NA	NA	NA	59.7-117.7
ALP (IU/L)	C	66-456	NA	NA	NA	NA	NA	NA	61.63-114.31	74.7-566.6	NA
	M	79.48- 492.12	0-115	NA	113-438	85-407	73.8-572	NA	61.63-114.31	NA	21.2-656.4
	F	63.56- 253.34	0-105	NA	64-354	44-306	66.1-522	NA	58.21-114.23	NA	91.8-440.6
ALT (IU/L)	C	5-23	0-41	8-38	NA	NA	NA	NA	10.75-57.80	3.1-31.1	NA
	M	4.54- 23.69	NA	8-41	17-50	7-46	5.8-38.8	4.9–42.4	11.18-57.20	NA	14.4-60.7
	F	5.10- 20.03	NA	7-36	14-41	6-40	5.0-34.5	4.2–65.3	10.24-56.84	NA	11-70.5
AST (IU/L)	C	14.23-34.91	0-40	NA	NA	NA	NA	NA	9.92-54.60	10.0-52.1	NA
	M	15.71- 39.08	NA	NA	18-36	13-38	14.4-45.7	17.0–59.2	10.16-54.30	10.0-51.1	12.4-58
	F	13.26- 28.48	NA	NA	15-34	12-32	12.7-38.8	12.0–43.1	5.65-58.93	11.0-54.5	11-72.7
D.Bi (Mg/dL)	C	.033-.532	0-0.2	NA	NA	0.06-0.43	NA	NA	.0269-.226	.51-.969	NA
	M	.034- .49	NA	NA	NA	NA	0.00-0.25	NA	.0251-.206	.13-.94	NA
	F	.028-.532	NA	NA	NA	NA	0.007-0.2	NA	.0123-.245	.182-.97	NA
T.Bil (Mg/dL)	C	.107-.812	0-0.2	NA	NA	0.29-1.46	NA	NA	.737-4.243	.262-1.58	NA
	M	.094- .839	NA	NA	0.1-0.9	NA	0.2-1.09	.333-3.66	.759-4.197	.222-1.548	NA
	F	.106- .728	NA	NA	0.1-0.9	NA	0.17-0.89	.216-2.251	.713-4.295	.28-1.62	NA
Urea (Mg/dL)	C	8.14-24.25	15-39	18.6-38.5	NA	9.6-39.5	NA	3.36-14.29	NA	NA	NA
	M	9.33- 24.99	NA	19.2-38.5	8-20	NA	4.6-17	NA	NA	NA	4.6-27.2
	F	7.43- 23.11	NA	17.9-36.6	8-19	NA	5.1-15.6	NA	NA	NA	3.9-48.5
Cr (Mg/dL)	C	.37-.91	NA	0.4-1.1	NA	NA	NA	NA	NA	NA	NA
	M	.394- .958	0.7-1.2	0.4-1.2	0.5-0.9	0.37-1.05	0.4-1.19	.561-1.173	NA	NA	0.3-1.9
	F	.301- .846	0.5-0.9	0.4-1.1	0.5-0.8	0.34-0.86	0.39-1.19	.543-.99	NA	NA	0.3-1.3
T. P (g/dL)	C	6.083-7.85	6.6-8.3	NA	NA	5.94-8.04	NA	NA	3.038-5.518	5.98-8.57	NA
	M	5.979- 7.829	NA	NA	NA	NA	6.8-9	NA	3.078-5.572	5.88-8.4	NA
	F	6.132- 7.867	NA	NA	NA	NA	7.1-9.06	NA	2.995-5.457	6.0-8.66	NA
ALB (g/dL)	C	4.42- 5.46	3.5-5	NA	NA	3.5-5.22	NA	NA	2.83-4.872	3.56-5.3	NA
	M	4.321- 5.493	NA	NA	4.1-5.1	NA	4.3-5.5	NA	2.798-4.896	3.2-8-5.3	NA
	F	4.42- 5.46	NA	NA	4.1-5.1	NA	4.37-5.7	NA	2.862-4.85	3.74-5.3	NA

ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; AST: Aspartate aminotransferase; L: litre; mg: Milligram; RI: Reference interval; SGOT: Serum glutamate oxaloacetate transaminase; SGPT: Serum glutamate pyruvate transaminase; U: Unit. NA: Not available. T.P: Total protein;ALB: Albumin; Cr: Creatinine; D.Bil: Direct Bilirubin; T.Bil: Total bilirubin;C:Combined;M:male;F:female

8. Discussion

The laboratory reference values currently employed in clinical and research institution in most of African countries including Ethiopia are referred from textbooks or from the manufacturer of the reagent. Clinical laboratory attempts to play a major role in providing valuable information for prevention, diagnosis and management of life threatening diseases to communicable and non-communicable disease [26]. Ethiopian population depends on western derived RIs for disease diagnosis and management because of lack of locally established RIs while a number of studies showed variations between African and western population derived RIs [18-21, 27-30] . Thus, it is required to establish local RIs for adequate medical care and related health issues.

The upper limit of most clinical chemistry parameters among apparently healthy individuals in Africa are higher relative toTaiwan [16], Canada [8] and China [17].This difference may be due to genetic make up, living style and altitude. The manufacturers' reference interval provided in the leaflets of the reagents used in the current study presented a single RI range for both sexes except for ALP and creatinine. However, the majority of African studies including the current study [18-20, 23] reported separate RIs for males and females. In addition, the reference values among apparently healthy individuals of different ages are inconsistent [14]. These discrepancies may cause negative impact in the diagnosis and management of African population.

The current study lower and upper limit results are higher than relative to the manufactures reference value for glucose, alkaline phosphatase, albumin, Total and direct bilirubin. This difference may be due to geographical condition, genetic variation, life style and feeding culture of the study participants. On the other hand, the upper limit reference value of this study is lower when compared with the manufacturer's upper limit values for AST, ALT, Urea, creatinine and Total protein. This inconsistency may be due to feeding style, genetic variation or age difference as the manufacturer's derived limits are mainly based on adult Caucasians and only informed as the values decrease in children.

When detrmining the rates of misclassification, there are a marked discrepancy and misclassification of participants using the manufacturer reference interval as compared the current locally established RI. The difference among the manufacturer and the current study upper and lower limit may be due to genetically difference study participants and geographical

condition. The Fasting blood sugar RI relatively shows consistency between the current study and the manufacturer's RI as well as a RI from Taiwan [16]. However, and the lower limit reported from West Kenya (37.8mg/dl) [19] is remarkable lower compared to the RI established in the current study though the upper limit is in the same range. The current RI for FBS for male and female lower and upper reference limit value is consistent with a study conducted in South West Ethiopia [23].This difference may be due to feeding style.

The current study female's ALP upper limit reference value (253.34 U/L) was lower than the upper limit female reference value detrmined in Canada [8] (354 U/L). On the contrary the current study's lower limit of male ALP (79.48) reference value is less than from a study conducted in Canada (113 U/L) but its upper limit reference value is higher than the upper ALP limit reference value for male in Canada. This difference may be due to environmental condition, diet and analytical method [18]. ALP lower and upper limit in males is higher than females in the current study. This difference may be due to differences in the physiological peak of ALP during puberity between the two sexes. The age onset for the adolescents where peak ALP is detected is earlier in pubertal girls than pubertal boys. The respective age ranges at which ALP peaks are 9 to 13 years for girls and 11 to 16 years for boys and the current study participants age range was 12-17 years old [14].

The lower limit ALT value in the current study for both male and female is in line with the lower limit reference value generated in a study conducted in Western Kenya [19]. However, the upper limit reference values in both sexes in the current study is lower than the upper limit reference values of studies conducted in different countries including a study from Southwest Ethiopia [8, 16-20, 22, 23].This difference may be due to sample size (in china 2683 males and 2292 females were participated), geographical difference and specimen collection time difference (In Taita Kenya collection was performed at day time).

The current AST lower and upper reference limit value is comparable with limits determined in Canada[8] and China[17]. But its upper limit value is lower than the upper limit values provided in different countries including Southwest Ethiopia [16, 18-20, 22, 23]. Such kind of difference may be due to distribution of a disease, geographical difference, sample size and the method we used (use of pyridoxal -5- phosphate elevates concentration of AST. Though all articles, except a study in China [17], did not mention what methods they used, the reagent that was used to

determineAST in the current study was without pyridoxal -5- phosphate, similar to the study in China, in which case reagent difference may not explain the variations noted between the current and the chineese study).

The Total and Direct Bilirubin reference limits in the current study were consistent with reference limit values conducted in Canada, Chaina, Taita Kenya, Zimbabwe [8, 17, 18, 22] except the upper limit value of direct bilirubin in Zimbabwae [22] which is low. In contrast, the current upper limit provided was lower than those reported from west Kenya, Meru Kenya, and Southwest Ethiopia [19, 20, 23].This difference may be due geographical conditions and feeding culture.

The Urea limit values were lower than the limits for Taiwan and China [16, 17] and Females from Southwest Ethiopia [23]. But the male RI value of the current study somewhat agrees with the RI for males from Southwest Ethiopia [23]. In contrast, the the current limit values were higher than those reported from west Kenya and Zimbabwe [19, 22] but in line with RI detrmined in Canada[8]. Creatinine lower and upper limit reference value was comparable to several western and African studies [8, 17-20, 22] except South West Ethiopia [23] where higher upper limit value was reported. In all studies females had less upper limit reference value than males. This difference may be due to geographical condition and diet. High creatinine in males compared to females is expected and in many international settings is explained by a greater skeletal, muscle and bone mass in males. Higher creatinine levels in males than in females may be due to the greater muscle mass in males than females [14].

The albumin reference lower and upper limit value of the current study were comparable with studies conducted in Meru Kenya [20], as well as China, Taita Kenya and Zimbabwe [17, 18, 22]. Regarding Total Protein, the reference limits are consistent with China [17] and Taita Kenya [18]. In the contrary, the current study's Total Protein reference limit values are higher than a study conducted in Meru Kenya[20] but lower than those reported from Zimbabwae[22]. This difference may be due to diet, machine and reagents used.

All in all, there inconsistency between the RIs established in the current study as compared to company derived values or those derived for different populations underscoring the need for locally established appropriate RIs for clinical chemistry parameters.

9. Strength and Limitation of the Study

9.1. Strength of the Study

Since this study is community based, it is more representative than the other studies. Wet mount, concentration (formol ether), Kato Katz and modified acid fast technique are performed in order to exclude participants who were infected with any intestinal parasite. Besides this, blood film and well prepared questionnaire to check individuals' current as well as past health status were employed. Health extension workers were used to screen and recruit participants.

9.2. Limitation of the Study

- Time
- Limited resourse

10. Conclusion and Recommendation

10.1. Conclusion

Reference interval was established for FBS, ALP, ALT, AST, D.BIL, T.BIL, Urea, Creatinine, Total protein and Albumin tests. Using Mann Whitney non-parametric analysis method we appreciated significant statically difference between sexes in Alkaline Phosphatase, Alanine amino transferase, Aspartate amino transferase, Urea and clinical chemistry parameters. In addition, we observed markedly difference in lower and upper limit value among current study, manufacturer and studies done in different countries. The manufacturer reference value had similar for both sexes except to alkaline phosphatase and creatinine. But in our study we observed as it needed to partition besides. So the established reference interval of clinical chemistry parameters will potentially useful in the diagnosis, management and monitoring of disease progression in the study setting.

10.2. Recommendation

We recommend to:

- It will better every regions and also every health facility to set their own reference intervals because they may have difference in geographical, altitudinal and climatic condition difference
- It will better clinicians to use locally established reference intervals than the reference interval from text books and other company values.

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Annexes

Annex I: English version Questionnaire

Questionnaires to be filled by health professionals

Part I. General information

Code Number_____ Region _____ Zone _____

Woreda_____ / city /_sub city _____ Kebele _____

Part II. Personal information

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

No.	Questions	Responses
	Part II: History of common diseases	
6.	Do you consider your self to be healthy?	1. Yes 2. No
7.	Did you take any type of drug for any illness for the last three month?	1. Yes 2. No
8.	If yes to Q6, 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) _____
9.	History of diabetes	1. Yes 2. No
10.	History of Hypertension	1. Yes 2. No
11.	History of Blood transfusion for the last 1 year	1. Yes 2. No
12.	Any history of blood transfusion	1. Yes 2. No
13.	History of Hospital Admission for the last 1 year	1. Yes 2. No

14.	History of Surgical procedure for the last three years?	1. Yes 2. No
15.	History of chronic gastritis	1. Yes 2. No
16.	History of Malaria for the last 6 month	1. Yes 2. No
17.	History of TB for the last two years	1. Yes 2. No
18.	History of Cancer	1. Yes 2. No
19.	History of Cardiac illness	1. Yes 2. No
20.	History of Bleeding disorders	1. Yes 2. No
21.	History of allergy	1. Yes 2. No
22.	History of Wheezing	1. Yes 2. No

	Part IV. Anthropometric measurement	
23.	Height (in cm)	_____
24.	Weight (in kg)	_____
25.	Blood pressure (mm Hg)	_____

NB: If a participant answers **NO** for Q-6 and **yes for one of the** questionnaire from No. 6 to No. 22 and the blood pressure is out of **90-120 systolic** and **60-90 diastolic** except with some preconditions they cannot include in the analysis. In addition, individuals who are lived below 5 year in Mekelle city will be excluded in the study.

We thank you for your cooperation!

Interview Date: _____

Interviewer name_____

Annex II: Questionnaire Amharic version (ቃለ መጠይቅ)

በጤና ባለሞያዎች የሚሞላ ቃለ መጠይቅ

መመሪያ፡

በቅድሚያ ይህንን ቃለ መጠይቅ ለመሙላት ለሰጡን ጊዜና ትብብር አድናቆቴን እገልፃለሁ፡፡ የዚህ ቃለ መጠይቅ አላማ "በላቦራቶሪ ውስጥ የጥራት መመርመሪያ ንጥረ ነገር እና የጤናማ ሰው ደም ውስጥ የሚገኙ የሄማቶሎጂና የክሊኒካል ኬሚስትሪ ምርመራዎች መጠን ሪፈረንስ ኢንተርቫል እድሜአቸው ከ 12-17 ዓመት ለሆኑ የመቀለ ነዋሪዎች ለመስራት መረጃ ለመሰብሰብ ነው፡፡ የዚህ ጥናት ሃሳቡን ያመጡት የጥናቱ ተመራማሪ **ጌታቸው በላይ** በአዲስ አበባ ዩኒቨርስቲ የህክምና ላቦራቶሪ ትምህርት ክፍል የድህረ ምረቃ ተማሪ ሲሆኑ የመመረቂያ ፅሁፋቸው ሊሰሩበት ነው፡፡ ስለሆነም የእርስዎ ቅን ትክክለኛ መልስ በሰዓቱ መስጠት የዚህን ጥናት ስኬት ይወስናል፡፡

አመሰግናለሁ!!!

ክፍል 1. አጠቃላይ መረጃ

ኮድ_____ ክልል _____ ዞን_____

ወረዳ_____ ከተማ/ክፍለከተማ _____ቀበሌ _____

ክፍል 2. የግል መረጃ

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

	ክፍል 3. የጤና መረጃ	
6.	ራስህን ጤነኛ ነኝ ብለህ ታስባለህ?	1. አዎን 2. የለም
7.	ባፉት ሶስት ወራ ለማንኛውም ዓይነት ህመም ማንኛውንም ዓይነት መድሃኒት ወስደኃል?	1. አዎን 2. የለም
8.	ለተራ ቁጥር 6 መልስዎ ወስጃለሁ ከሆነ የትኛውን ዓይነት መድሃኒት ነው ወሰዱት? (ከአንድ በላይ መልስ ይቻላል)	8. ፀረ-ፕሮቶዞአ 9. ፀረ-ሄልሚንትስ 10. ፀረ-አለርጂ 11. የወሊድ መከላከያ ኪኒን 12. ፀረ-ባክቴሪያ

		13. ፀረ-ቲቢ 14. ሌላ ካለ ይግለፁ _____
	የሚከተሉት የህመም ዓይነቶች አሞዎት ያውቃል?	
9.	የስኳር ህመም?	2. አዎን 2. የለም
10.	የደም ግፊት ከፍ ማለት?	1. አዎን 2. የለም
11.	ባለፈው 1 ዓመት ደም ተሰጥቶዎ ያውቃል?	1. አዎን 2. የለም
12.	ማንኛውም ጊዜ ደም ተሰጥቶዎ ያውቃል?	1. አዎን 2. የለም
13.	ባለፈው 1 ዓመት ሆስፒታል ተኝተው ያውቃሉ?	1. አዎን 2. የለም
14.	ባለፉት 3 ዓመታት የቀዶ ህክምና ተደርጎልዎ ያውቃል?	1. አዎን 2. የለም
15.	የቆየ የጨጓራ ህመም አለብዎት?	1. አዎን 2. የለም
16.	ባፉት 6 ወራት የወባ ህመም አጋጥሞዎት ያውቃል?	1. አዎን 2. የለም
17.	ባለፉት 2 ዓመታት የቲቢ ህመም ኖሮዎት ያውቃል?	1. አዎን 2. የለም
18.	ካንሰር ህመም	1. አዎን 2. የለም
19.	የልብ ህመም	1. አዎን 2. የለም
20.	የመድማት ችግር/ህመም	1. አዎን 2. የለም
21.	አለርጂ (የሰውነት መቆጣት)	1. አዎን 2. የለም
22.	የመተንፈስ ችግር (ሲተነፍሱ ሲር ሲር የሚል ድምፅ)	1. አዎን 2. የለም

	ክፍል 4. ክብደት፣ ቁመት፣ የክንድና የደም ግፊት ልኬት	
23.	ቁመት	_____ሴንቲ ሜትር
24.	ክብደት	_____ኪሎ ግራም
25.	የደም ግፊት (በሚሊሜትር ሜርኩሪ)	_____(mm Hg)

ለተ.ቁ 6 የለም ወይም ከ ተ.ቁ 7-21 ለአንድ እና ከዚያ በላይ አዎ ከሆነ መልስዎ ለትናቱ ምርምር ኣይካተቱም::በተጨማሪም ከ 5 ዓመት በታች መቀለ የነሩና የደም ግፊታቸው (በሚሊሜትር ሜርኩሪ) 90-120 systolic እና 60-90 diastolic ውጭ ከሆነ ለጥናቱ ኣይካተቱም ::

❖ ስለትብብርዎ እናመሰግናለን!

ቃለ መጠይቅ የተደረገበት ቀን:_____

ቃለ መጠይቁን ያካሄደው ስም_____ ፊርማ_____

Annex III: Questionnaire Tigrigna version (ቃለ መሕተት)

ብመጀመርታ እዚ ቃለ-መሕተት ዝምላእ ንዝሃብና /ንዝሃባና ንዝሃብክሙና ግዜን ትሕብብርን ኣድናቕተይ ይገልፅ ።።ናይዚ ቃለ-መሕተት ዓላማ ኣብ ላቦራቶሪ ዉሽጢ መመርመሪ ንጥረ ነገር ኣብ ጥዑይ ሰብ ደም ዉሽጢ ዝርከብ ናይ ኪሊኒካል ኬሚስትሪን ምርመራታት መጠን ሪፈረንስ ኢንተርቫል ዕድሚኦም ካብ 12-17 ዓመት ዝኾኑ ናይ መቀለ ነበርቲ ንምስራሕ መረዳእታ ንምእካብ እዩ።።ናይዚ ፅንዓት ዋና ተመራማራይ ጌታቸው በላይ (ኣብ ኣዲስ ኣበባ ዩኒቨርስቲ ናይ ሕክምና ላቦራቶሪ ትምህርቲ ክፍሊ ናይ ካላይ ድግሪ ተምሃራይ) ።። ስለዚ ናቶም ቁኑዕ መልሲ ብግዚኡ ምሃብ ነዚ ፅንዓት ዕዉትነት ይዉስኖ ።።

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 ቦኦኦም ንኽንደይ ጊዜ ነቢሮም_____
5. ኣብ ሕዚ ዘለዉዎ ቦታ ንኽንደይ ጊዜ ነቢሮም? (ካብ ትዉልዲ ቦታ ዝተፈለየ እንተኾይኑ) _____ዓመት

ክፍሊ 3. ናይ ጥዕና መረዳእታ

6. ኣብ ዝሓለፉ ሰለስተ ወርሒ ዝኾነ ዓይነት መድሓኒት ንዝኾነ ዓይነት ሕማም ወሲዶም ዶ ይፈልጡ ?

   1. እወ 2. ኣይፋሉን

7. ንታራ ቁፅሪ 6 መልሶም እወ እንተኾይኑ ኣየናይ ዓይነት መድሓኒት እዮም ወሲዶም ? (ካብ ሓደ ንላዕሊ መልሲ ምምላስ ይካኣል እዩ)

   1. ፀረ-ፕሮቶዝዋ 2. ፀረ- ሓሰኻ 3. ፀረ-ኣለርጂ 4. ናይ ወሊድ መከላኸሊ ክኒና
   5 ፀረ-ባክቴሪያ 6. ፀረ-ቲቢ 7. ካለእ ተሃልዩ ይገለፅ _____

8. ኣብዚ ሕዚ እዋን ዝኾነ ዓይነት ሕማም ይስመዖም ዶ ? 1. እወ 2. ኣይፋሉን

ናይ ዝስዕቡ ናይ ሕማም ዓይነት ሓሚሞም ይፈልጡ ዶ?

9. ናይ ሽኮር ሕማም? 1. እወ 2. ኣይፋሉን
10. ናይ ደም ድፍኢት ልዕል ምባል? 1.እወ 2. ኣይፋሉን
11. ኣብ ዝሓለፈ 1 ዓመት ደም ሂቦም ይፈልጡ ዶ? 1.እወ 2. ኣይፋሉን
12. ኣብ ዝኾነ ጊዜ ደም ተዋሂብዎም ይፈልጡ ዶ? 1.እወ 2. ኣይፋሉን
13. ኣብ ዝሓለፈ 1 ዓመት ሆስፒታል ሂዶም ይፈልጡ ዶ? 1.እወ 2. ኣይፋሉን
14. ኣብ ዝሓለፈ 3 ዓመታት ናይ መጥባሕቲ ህክምና ተገይርሎም ይፈልጡ ዶ? 1.እወ 2. ኣይፋሉን
15. ዝፀንሐ ናይ ጨነራ ሕማም ኣለዎም ዶ? 1.እወ 2. ኣይፋሉን
16. ኣብ ዝሓለፈ 6 ኣዋርሕ ናይ ሕማም ዓሶ ኣጋጢምዎም ነይሩ ዶ? 1.እወ 2. ኣይፋሉን
17. ኣብ ዝሓለፈ 2 ዓመት ናይ ቲቢ ሕማም ሒዝዎም ይፈልጥ ዶ ? 1.እወ 2. ኣይፋሉን
18. ናይ ካንሰር ሕማም 1. እወ 2. ኣይፋሉን
19. ናይ ልቢ ሕማም 1.እወ 2. ኣይፋሉን
20. ናይ ምድማይ ፀገም /ሕማም ኣጋጢምዎም ነይሩ ዶ? 1.እወ 2. ኣይፋሉን

21. አለርጂ (ናይ ሰውነት ቁጠዐ) ኣጋጢምዎም ነይሩ ዶ? 1.እወ 2. ኣይፋሉን

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

	ክፍሊ 4. ክብደት፣ ቁመት፣ ጭዋዳን ናይ ደም ድፍኢትን	
23.	ቁመት (ሴንቲ ሜትር)	_____
24.	ክብደት (ኪሎ ግራም)	_____ኪሎ ግራም
25.	ናይ ደም ድፍኢት (በሚሊሜትር ሜርኩሪ)	_____

ካብ ተቁ 6 እስካብ 22 ንዘለዉ መረዳእታት ንሓደን ካዉኡ ንላዕልን እወ እንተኾይኑ መልሶም ነዚ ፅንዓት ኣይጠቕሙን::ከምኡ ዉን ትሕቲ 5 ዓመት ኣብ መቀለ ዝነበሩን ናይ ደም ድፍኢት (በሚሊሜትር ሜርኩሪ) ካብ 90-120 systolic ወይ 60-90 diastolic ወፃኢ ዝኾኑ ኣብዚ ፅንዓት ኣይካተቱን ::

❖ ስለ ምትሕብባሮም ነምስግን!!!

ቃለ መሕተት ዝተገበረሉ መዓልቲ: _____

ቃለ መሕተት ዘካየደ ሽም_____ ፊርማ_____

Annex IV: Informed Consent

Project Title: Determination of community based reference interval of clinical chemistry parametrs among apparently healthy adolescents in Mekelle city, Tigray North
PI: Getachew Belay

Introduction:

Hello! My name is Getachew Belay and I am Msc student in addis abeba university college of Health science department of Medical Laboratoy Technology. I am doing my final research for graduation on Determination of reference interval of clinical chemistry parametrs among apparently healty adolescents in mekelle city.

Purpose of the research:

The health laboratory plays an indispensable role in the health care system. It supports diagnosis (to rule in or rule out a diagnosis), monitoring of response to treatment, epidemiological surveillance, prevention as well as Research (to understand the pathophysiology of a particular disease process). Especially there is lack of local reference interval for indigenous population and local quality control materials. Therefore, the purpose of this proposed study is to develop reference interval of clinical chemistry parametrs among apparently healthy adolescents aged 12-17 years old.

Your child has been chosen for this study. Therefore, we invite you and your child to take part in this study and contribute to the establishment of indigenous reference values. Both are needed for providing quality laboratory service. Thus, result from this study is anticipated to improve the health status of children at large in Ethiopia.

Procedures:

After agreeing that your child can take part, one or more of our research staff will ask you some questions which will take up to 10 minutes. Your child's weight, height and vital signs will be measured. Your child will be asked to provide urine and fresh stool on a particular container we provide. We will also collect 3 ml venous blood (about half table spoon) from your child by sterile-disposable vacutainer tube and needle.We will conduct laboratory examination to determine different parasitological and clinical chemistry parameters.

Confidentiality:

The information obtained during the study will remain confidential. Disclosure of any of the data to third parties other than those allowed in the Informed Consent form will not be permitted. The

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

Risks and Discomfort:

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

Safety:

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

Benefits:

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

Incentives:

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

Right to refuse or withdraw:

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

Annex V: Information sheet for children 12—17 years (12—17 ዓመት ለሆኑ ህፃናት መረጃ)

የፕሮጀክቱ ርዕስ፡ “እድሜአቸውከ 12-17 ዓመት ለሀኑ የመቀለ ነዋሪዎች የጤናማ ሰው ደም ውስጥ የሚገኙ የክሊኒካል ላቦራቶሪ ኬሚስትሪ41ምርመራዎች መጠን ሪፈረንስ ኢንተርቫል እና በላቦራቶሪ ውስጥ የጥራት መመርመሪያ ንጥረ ነገር መስራት ነው::

የጥናቱ ተመራማሪ: ጌታቸው በላይ

መግቢያ:

ጤና ይስጥልኝ! ስሜ **ጌታቸው በላይ** እባላለው :: የኣዲስ ኣበባ ዩኒቨሪስቲ የድህረ ምረቃ ተማሪ ሲሆን የመመረቂያ ፁሑፌን በ ክሊኒካል ኬሚስትሪ ምርመራዎች መጠን ሪፈረንስ ኢንተርቫል እድሜአቸው ከ 12-17 ዓመት ለሆኑ የ መቀለ ነዋሪዎች እየሰራሁ ነው::

የምርምር ጥናቱ አላማ:

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

አንተም/አንቺም በዚህ ጥናት እንድትሳተፍ/ፊ እየጋበዝን ወላጆችሽ/ወላጆችህ ፈቃዳቸውን ገልፀዋል::: ስለዚህ በዚህ ጥናት በመሳተፍ በአገራችን በላቦራቶሪ ውስጥ የሚመረት የጥራት መመርመሪያ እና የጤናማ ሰው የክሊኒካል ላቦራቶሪ ውጤት ማመዳደሪያ ሪፈረንስ ኢንተርቫል ለመስራት አስተዋፅዎ እንድታደርግ/ጊ ተጋብዘሃል/ሻል:: ሁለቱም ጥራት ያለው የላቦራቶሪ አገልግሎት ለመስጠት አስፈላጊ ናቸው:: ስለዚህ የዚህ ጥናት ውጤት ኢትዮጵያ ውስጥ የአዋቂ ሰዎች ጤናን ለማሻሻል ይረዳል::

የጥናቱ አካሄድ:

በጥናቱ ለመሳተፍ ከተስማማህ/ሽ የጥናቱ አባል/አባላት 10 ደቂቃ የሚወስድ ጥያቄ ይጠይቁሃል/ሻል:: ክብደት፣ ቁመት፣ የክንድ እና የደም ግፊት ልኬት ይወሰዳል:: ሽንትና አይነምድር በምንሰጠው እቃ እንድትሰጠን/ጭን እንጠይቃለን:::፣ በተጨማሪም 3 ሚሊ ሊትር (ግማሽ የሾርባ ማንኪያ የሚሆን) በንፁህ ቫኩቴይነር ብልቃጥ እና መርፌ :: ፓራሲቶሎጂ እና የክሊኒካል ኬሚስትሪ ምርመራዎችን እናካሂዳለን::

ሚስጥር ስለመጠበቅ:

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

ጥናቱ የሚያስከትላቸው የጤና ችግሮችና አለመመቾት:

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

ደህንነት:

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

ጥቅማ ጥቅሞች:

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

በጥናቱ ለመሳተፍ ማትጊያ:

ከዓይነምድር፣ ሽንት እና ደም ምርመራ ጤናማ ያልሆነ ውጤት ከተገኘ በአቅራቢው ወደ ሚገኝ ጤና ተቋም ትላካለህ/ትላኪያለሽ፣ የላቦራቶሪ ውጤቶቹን በነፃ ታገኛለህ/ታገኚያለሽ። ይሁን እንጂ በዚህ ጥናት ለመሳተፍም ሆነ ለመድሃኒት ክፍያ አይሰጥም። ስለተሳትፎህ/ህ ግን እናመሰግናለን።

ያለመሳተፍ መብት:

በዚህ ጥናት ከተሳተፍክ/ሽ የቻልነውን ሁሉ እንክብካቤ እናደርጋለን። በማኛውም ሰዓት ከጥናቱ መውጣት እንደሚቻልና ይህም በምታገኘው/ኚው አገልግሎት ላይ (ለምሳሌ የጤና አገልግሎት) ምንም አይነት ልዩነት አይደረግም።

ጥያቄ ካለ ለማነጋገር:

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

Annex VI: Information sheet

ብመጀመርታ እዚ ቃለ-መሕተት ዝምላእ ንዝሃብና /ንዝሃባና ንዝሃብክሙና ግዜን ትሕብብርን ኣድናቕተይ ይገልፅ ::ናይዚ ቃለ-መሕተት ዓላማ ኣብ ላቦራቶሪ ዉሽጢ መመርመሪ ንጥረ ነገር ኣብ ጥዑይ ሰብ ደም ዉሽጢ ዝርከብ ናይ ክሊኒካል ኬሚስትሪ ምርመራታት መጠን ሪፈረንስ ኢንተርቫል ዕድሚኣም 12-17 ዓመት ዝኾኑ ናይ መቀለ ነበርቲ ንምስራሕ መረዳእታ ንምእካብ እዩ::ናይዚ ፅንዓት ሓሳብ መቕረቢናይዚ ፅንዓት ዋና ተመራማሪን ጌታቸው በላይ(ኣብ ኣዲስ ኣበባ ዩኒቨርስቲ ናይ ሕክምና ላቦራቶሪ ትምህርት ክፍሊ ናይ ካላይ ዲግሪ ተመሃራይ) :: ስለዚ ናቶም ቁኑዕ መልሲ ብግዚኡ ምሃብ ነዚ ፅንዓት ዕዉትነት ይዉስኖ ::

ምስቲ መፅናዕቲ ተታሒዙ ዝመፅእ ሳዕቤን፡- ንምርመራ ዝኽውን ደም ኣብ ዝህብሉ እዋን ምንም ዓይነት ዝኸፍአ ፀገም ኣየጋጥሞምን። ነገር ግን ደም ኣብ ዝውሰደሉ እዋን ዝተወሰነ ናይ ምሕማም ስምዒት ክህሉ ይኽእል እዩ። ይኹን ዳኣምበር ደም ንምእካብ ልምዲ ብዘለዎም በዓል ሞያታት ስለ ዝምደቡን ኣድላይ ዝኾነ ጥንቃቐን ስለዝውሰድ ናይ ምሕማም ስምዐዒት ኣይህሉን።

ካብዚ መፅናዕቲ ዝረክብዎ ጥቕሚ፡ እዚ መፅናዕቲ ናይ ካልኣይ 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 ንከራኽቡን 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:: ስለ ዝኾነ እዚ ቃለ-መሕተት ሓቅነትን ሓላፍነትን ብዝተመልአ መልክዕ ንኽመልኡ ብትሕትና ንሓትት::

ነምስግን!!!

Annex VII. Consent form for parents/guardians

ኣብ ላዕሊ ከም ዝተገለፀ መረዳእታ ኣንቢቤ/ተነቢቡለይ።።ሕቶ ንምሕታት ዕድል ተዋሂብኒ ጠይቐ ብዘርክዕ መለክዑ ተመሊሰለይ።።ቆልዓይ ክሳተፍ/ክትሳተፍ ተስማዕሚዐ ኣለኹ።።ከ 12-17 ዓመት ንታሕቲ ዝኾነ ቆልዓይ እንተተስማዕሚዖም/ን ኣብዚ ፅንዓት ንኽሳተፈ/ፍ ዝፈቐድኩም ምኻነይ ብክታመይ ኣብ ታሕቲ ይገልፅ።።

ናይ ዓይነምድር ናሙና ንምሃብ ☐

ናይ ሽንቲ ናሙና ንምሃብ ☐

ደም ናሙና ንምሃብ ☐ ኣብዚ ፅንዓት ተሳታፊ ንምኻን፣ኣብ ዝኮነ ሰዓት ካብ ፅንዓት ንምዉፃእ መብት ከምዘለኒ

*ናይ ተሳታፊ ሰም፣መዓልትን ፈርማ(ወይም ክታም)*_____ _____ /_____ /_____ (መ/ወር/ዓመተ ምህረት)

ዘይተመሃሩ እንተኾይኖም;

ዝተመሃሩ ገለልተኛ እማኝ ሰብ ስም ፣ መዓልትን ፈርማን (እንተተኻኢሉ እዚ ሰብ ብተሳተፎ እንተምረፅ ካብ ተመራመርቲ ኣባላት ርክብ ዘይብሉ እንተዝኾነ) _____

_____/_____/_____ (መ/ወር/ዓመተ ምህረት) ናይ ተመራመሪ ስምን፣መዓልትን ፈርማን

Annex VIII. Assent form for Adolescents

ኣብ ላዕሊ ከም ዝተገለፀ መረዳእታ ኣንቢቤ/ተነቢቡለይ።።ሕቶ ንምሕታት ዕድል ተዋሂብኒ ጠይቐ ብዘርክዕ መለክዑ ተመሊሰለይ።።ከሳተፍ ተስማዕሚዐ ኣለኹ።። ንኽሳተፈ/ፍ ዝፈቐድኩ ምኻነይ ብክታመይ ኣብ ታሕቲ ይገልፅ።።

ናይ ዓይነምድር ናሙና ንምሃብ ☐

ናይ ሽንቲ ናሙና ንምሃብ ☐

ደም ናሙና ንምሃብ ☐ ኣብዚ ፅንዓት ተሳታፊ ንምኻን፣ኣብ ዝኮነ ሰዓት ካብ ፅንዓት ንምዉፃእ መብት ከምዘለኒ

*ናይ ተሳታፊ ሰም፣መዓልትን ፈርማ(ወይም ክታም)*_____ _____ /_____ /_____ (መ/ወር/ዓመተ ምህረት)

ዘይተመሃሩ እንተኾይኖም;

ዝተመሃሩ ገለልተኛ እማኝ ሰብ ስም ፣ መዓልትን ፈርማን (እንተተኻኢሉ እዚ ሰብ ብተሳተፎ እንተምረፅ ካብ ተመራመርቲ ኣባላት ርክብ ዘይብሉ እንተዝኾነ) _____

_____/_____/_____ (መ/ወር/ዓመተ ምህረት) ስምን፣መዓልትን ፈርማን

Annex IX: Principle of Clinical chemistry tests

ALKALINE PHOSPHATASE (ALP) – AMP:

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 405nm.

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

ALANINE AMINOTRANSFERASE (ALT/GPT) IFCC WITH OUT PYRIDOXAL-5-PHOSPHATE:

PRINCIPLE

Alanine aminotransferase (ALT or GPT) catalyzes the transfer of the amino group from alanine to 2-oxoglutarate, forming pyruvate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm, by means of the lactate dehydrogenase (LDH) coupled reaction.

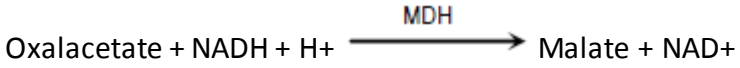
Alanine + 2 – Oxoglutarate $\xrightarrow{\text{ALT}}$ Pyruvate + Glutamate

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

ASPARTATE AMINOTRANSFERASE (AST/GOT) IFCC WITH OUT PYRIDOXAL-5-PHOSPHATE: PRINCIPLE OF THE METHOD

Aspartate aminotransferase (AST or GOT) catalyzes the transfer of the amino group from aspartate to 2-oxoglutarate, forming oxalacetate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm, by means of the malate dehydrogenase (MDH) coupled reaction.

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



BILIRUBIN (DIRECT AND TOTAL): DIAZOTIZED SULFANILIC

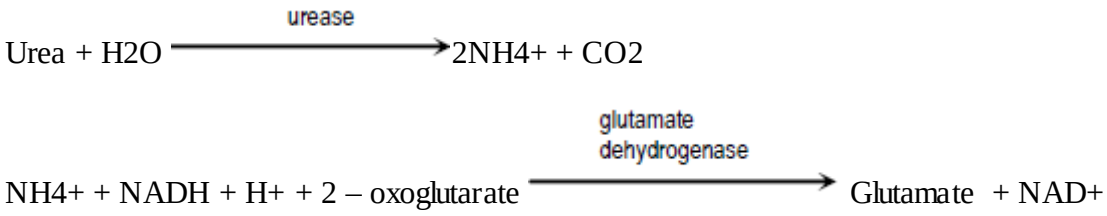
PRINCIPLE

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 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 bilirubins are only approximately equivalent to the conjugated and unconjugated fractions.

UREA/BUN – UV (UREASE / GLUTAMATE DEHYDROGENASE):

PRINCIPLE

Urea in the sample consumes, by means of the coupled reactions described below, NADH that can be measured by spectrophotometry at 340 nm.



CREATININE (JAFFE COMPONSATED):

PRINCIPLE

Creatinine in the sample reacts with picrate in alkaline medium forming a coloured complex (Jaffé method). The complex formation rate is measured in a short period to avoid interferences. Serum and plasma samples contain proteins that react in a non specific way; nevertheless, the results can be corrected subtracting a fixed value. The use of this correction is known as the Jaffé method compensated.

PROTEIN (TOTAL) BIURET

PRINCIPLE

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

ALBUMIN: BROMOCRESOL GREEN:

PRINCIPLE

Albumin in the sample reacts with bromocresol green in acid medium forming a coloured complex that can be measured by spectrophotometry.

Glucose Oxidase Method

PRINCIPLE

The On.Call Plus Blood Glucose Test Strips are thin strips with a chemical reagent system using glucose oxidase. They work with the On.Call 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, mixedema, hypopituitarism, Addison´s disease and conditions interfering with glucose absorbtion. Glucose measurement in the blood is a key test to evaluate and diagnose any carbohydrate-related disorder.

Annex X: Laboratory Procedures

Urine Microscopy procedure

1. Mix the urine specimen
2. Transfer about 10 ml of urine into a labeled centrifuge tube.
3. Centrifuge the specimen at a medium speed (from 1500 – 2000 rpm) for 3-5 minutes
4. Discard the supernatant by quick inversion of the tube
5. Re suspend the sediment that is at the bottom of the tube, by tapping the tube by your fingers
6. Take the sediment by Pasteur pipette from the tube and transfer a drop into the clean and dry slide.
7. Apply cover slide on the urine sediment that is on the slide.
8. Put on the microscope and look under 10x objective of the microscope.
9. Then after looking through the low power objective, change the objective in to 40x objective.
10. Then report what you get under low power and high power objective on the laboratory request form of the patient.

Urine Reagent strip procedure

- Dip the test – strip in the urine specimen. Remove the test-strip immediately and let the excess urine drain off on a paper towel, or tap the edge of the strip.
- Read the color change
- Report the result according to the color chart provided by manufacturer.
- Always read the test strip in good white light and ignore color developing on the test area after the period specified as the reading time of the test.
- Be careful not to wet the reagent strip excessively. So that the acid buffer from the protein area runs into the pH area, causing an orange discoloration.

Procedure for stool examination using wet mount preparation

1. Instruct the participant how to collect the stool in the labeled, clean, dry and leak proof container.
2. Receive the sample and check with participant Id and observe the appearance of the stool and record it.
3. Place a drop of fresh physiological saline on one end of a slide and a drop of iodine on the other end.
4. Using a wire loop or piece of stick, mix a small amount of specimen, about 2 mg, (matchstick head amount) with the saline and a similar amount with the iodine.
5. Make smooth thin preparations.
6. Cover each preparation with a 22x22 cover glass.
7. Examine systematically the entire saline preparation for larvae, ciliates, helminthes eggs, cysts, and oocysts. Use the 10x objective with the condenser iris closed sufficiently to give good contrast.
8. Use the 40x objective to assist in the detectionand identification of eggs, cysts, and oocysts.
9. Always examine several microscope fields with this objective before reporting 'No parasites found'.
10. Use the iodine preparation to assist in the identification of cysts
11. Report the presence of larvae, ciliates, helminthes eggs, cysts, and oocysts

Procedure of formol-ether concentration method

1.Using a rod or stick, emulsify an estimated 1 g(pea-size) of faeces in about 4 ml of 10%formol water contained in a screw-cap bottle or tube.

2.Add a further 3–4 ml of 10% v/v formol water, cap the bottle, and mix well by shaking.

3.Sieve the emulsified faeces, collecting the sieved suspension in a beaker.

4.Transfer the suspension to a conical (centrifuge) tube made of strong glass, copolymer, orpolypropylene.

5. Add 3–4 ml of diethyl ether o rethyl acetate.

6.Stopper the tube and mix for 1 minute.

7.With a tissue or piece of cloth wrapped around the top of the tube, loosen the stopper (considerable pressure will have built up inside the tube).

8.Centrifuge immediately at 750–1,000 g (approx. 3000 rpm) for 1 minute.

9. Using a stick or the stem of a plastic bulb pipette, loosen the layer of faecal debris from the side of the tube
10. Invert the tube to discard the ether, faecal debris, and formol water. The sediment will remain.
11. Return the tube to its upright position and allow the fluid from the side of the tube to drain to the bottom.
12. Tap the bottom of the tube to re suspend and mix the sediment.
13. Transfer the sediment to a slide, and cover with a cover glass.
14. Examine the preparation microscopically using the 10x objective with the condenser iris closed sufficiently to give good contrast. Use the40x objective to examine small cysts and eggs.
15. To assist in the identification of cysts, run a small drop of iodine under the cover glass. Although the motility of Strongyloides larvae will not be seen, the non-motile larvae can be easily recognized.
16. If required, count the number of each species of egg in the entire preparation. This will give the approximate number per gram of faeces.

Formol ether oocyst concentration technique-2

Follow steps 1 to 7 of the above method. Continue as follows:

8. Centrifuge immediately at *low* speed, i.e. RC 300–400 g (about 1 000 rpm) for 1 minute. Using a plastic bulb pipette or Pasteur pipette, carefully remove the entire column of fluid below the faecal debris and ether and transfer this to another centrifuge tube.
7. Add formol water to make the volume up to 10–15 ml. Centrifuge at RCF 750–1 000 g (about 3 000 rpm) for 5–10 minutes.
8. Remove the supernatant. Tap the bottom of the tube to re suspend and mix the sediment.

Transfer the sediment to a slide and examine for oocysts using the 40x objective.

Modified Ziehl-Neelsen (Zn) method for *Cryptosporidium* and *C. cayetanensis*

1. Prepare a smear from the sediment obtained by the formol ether oocyst concentration technique
2. Air-dry the smear.
3. Fix the smear with methanol for 2–3 minutes.
4. Stain with unheated carbol fuchsin for 15 minutes.

5.Wash off the stain with water.

6.Decolorize with 1% acid alcohol for 10–15 seconds.

7.Wash off with water.

8.Counterstain with 0.5% malachite green (or methylene blue) for 30 seconds.

9.Wash off with water and stand the slide in a draining rack for the smear to dry.

10. Examine the smear microscopically for oocysts, using a low power magnification to detect the oocysts and the oil immersion objective to identify them.

Procedure for Preparation of duplicate Kato-Katz smears

1.Place a small amount (at least 2 gram) of stool on the scrap paper or newspaper.

2.Mark the slide as 'Subject ID -- A'.

3. Place the 41.7mg containing template with the hole on the centre of the marked microscope slide.

4. Press a piece of small nylon screen on top of the stool so that part of the stool is sieved through the mesh and accumulates on top.

5.Scrape the sieved stool from the upper surface of the screen using the spatula.

6.Fill the hole of the template on the microscope slide completely with stool from the spatula and remove any excess stool with the spatula.

7.Carefully remove the template by lifting it vertically. Avoid horizontal movements.

8. Place a pre-soaked cellophane clipping on top of the stool aliquot on the microscope slide.

9.Remove excessive glycerol-malachite green solution using tissue paper before placing the cellophane clipping on the aliquot.

10. Take a second, clean, microscope slide and place it on top of the cellophane. Press the top microscope down so the stool aliquot spreads evenly. Avoid lifting, wrinkling or moving the cellophane when spreading the smear.

11. Caution: support the slide from below to avoid cracking/breaking.

12. Place the slide in the microscope slide box.

13. Mark a second microscope slide as 'subject ID - B'.

14. Repeat steps 1-12 to prepare the A and B smear of remaining samples

15. Take a smear, either A or B smear,

16. Examine the whole smear systematically using 10x or 40x objective by two laboratory technologist independently
17. Count the number of egg for each soil-transmitted helminthes species and Schistosoma species separately.
18. Take an average number of eggs from A and B smears and Multiply the number of eggs by 24 and report number of eggs per gram of faces/EPG/

NB: For Hook wormslide should be read within 30–60 minutes.

General clinical chemistry tests procedure

- Collect whole blood of 5ml from for arm and transfer gently to the pale test tube from the syringe
- after clotting the whole blood centrifuge from 1500-300 rpm to 2-3 minute
- 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
- Turn on the clinical chemistry analyzer machine
- Check the expire date of all reagents
- Check the daily, weekly, monthly, quarterly and yearly controls, standards and calibration results of the analyzer
- Analyze the specimen based on the leaflet procedure for each clinical chemistry parameter tests.

Declaration

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

M.Sc. candidate: GETACHEW BELAY (B.Sc.)

Signature: _____

Date of submission:_____

This thesis has been submitted with our approval as advisors.

Advisor:MISTIRE WELDE(MSc, PhD, ASSOCIATED PROFFESSOR)

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Date: _____

Place: Addis Ababa, Ethiopia

Advisor: ASTER TSEGAYE (MSc, PhD, ASSOCIATED PROFFESSOR)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.