ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Establishment of Community based Reference Interval for Common Clinical Chemistry Parameters among Adolescents and Children in Addis Ababa, Ethiopia

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This is to certify that the thesis prepared by Letebrhan G/egzeabher, entitled: **Establishment of Community based Reference Interval for Common Clinical Chemistry Parameters among Adolescents and Children in Addis Ababa, Ethiopia** and submitted in partial fulfillment of the requirements for the Master of Science degree in clinical Laboratory sciences (Clinical Chemistry Track) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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LIST OF ACRONYMS

AAU	Addis Ababa University
ALB	Albumin
ALP	Alkaline phosphatase
ALT	Alanine amino transferase
AST	Aspartate amino transferase
BIL	Bilirubin
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
CD	Clinical Decision Limits
CHS	College of Health Science
CI	Confidence Interval
CLSI	Clinical Laboratory Standard Institute
CREA	Creatinine
C-RIDL	Committee for Reference Intervals and Decision Limits
CRP	C-reactive protein
DMLS	Department of Medical Laboratory Science
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immune Deficiency Virus
IFCC	International Federation of Clinical Chemistry
ISO	International Organization for Standardization
IU	International Unit
KG	Kilo Gram

L	Liter
LFT	Liver Function Test
M	Meter
MG	Milligram
NCCLS	National Committee for Clinical Laboratory Standards
OOR	Out of Range
RFT	Renal Function Test
RI	Reference Interval
RPR	Rapid Plasma Reagent
SD	Standard Deviation
SPSS	Statistical Package for Social Sciences
T.P	Total Protein
U.A	Uric Acid
VCT	Voluntary Counseling Testing
VDRL	Venereal Disease Research Laboratory
WHO	World Health Organization

Abstract

Back ground: Establishment of Reference Intervals (RI) is one of the expected activities of medical laboratories which enable interpretation of the laboratory results according to the existing local setups. Company derived values are being used to interpret results in health facilities of Addis Ababa by the absence of locally RI.

Objective: To establish RI for common clinical chemistry parameters among apparently healthy adolescents and children in Addis Ababa, Ethiopia from April to October, 2019

Methods: A community based cross sectional study was conducted on a total of 516 apparently healthy children (5-11 years) and adolescents (12-17 years) randomly selected from Addis Ababa, Ethiopia from April to October, 2019.After interviewing the health status of participants, socio demographic, nutritional status and life style data were collected using structured questionnaire. Blood (5 ml), urine (5ml) and stool (about 5 grams) samples was taken and examined. Serum levels of selected clinical chemistry parameters were determined using Cobas C501. Data were entered, cleaned and analyzed by SPSS version 21. After the exclusion of outliers using turkey method, Kolmogorov-Sminorv test was used to check its normality. The 95% RI with 90% CI was determined using non-parametric method ($2.5^{th}$and 97.5^{th} percentile).The difference between children and Adolescent values was evaluated using Mann-Whitney test and P-value of <0.05 claimed the presences of stastically significant difference.

Results: There was stastically significant variation between children and Adolescents in both sex and age for level of AST, ALP, TP, TBIL, DBIL, Cr, and UA, but ALT and ALB by age and Urea by sex. The established RI includes: Gluc 61-91 and 57-97 mg/dl, ALT 1.3-13.0 and 1.6-14.0 U/L, AST 12.3-26.7 and 8.0-27.0U/L, ALP 143-375 and 47-452U/L, DBIL 0.01-0.20 and 0.01-0.27mg/dl, TBIL 0.09-0.46 and 0.16-0.71mg/dl, TP6.4-7.8 and 6.4-8.3g/dl, ALB 4.1-5.1 and 4.1-5.2g/dl, UA1.4-4.9 and 1.5-6.8mg/dl, Cr 0.29-0.58 and 0.04-0.64mg/dl and Urea 9-30 and 8-31mg/dl for children and Adolescents, respectively for each test.

Conclusion: The study identified variations with the currently utilized RIs; therefore, establishment and use of local reference ranges should be encouraged because it enhances patient care and health research.

Key words: References interval, Clinical chemistry, Biochemistry analytes, Apparently Healthy, Adolescent, children

1. Introduction

1.1. Background

Reference interval is one of the most important tools to help result interpretation and making decision about patient's condition. Values typically representing the central 95% of healthy individuals, have been superseded by decision limits(1).The concept of reference values was designed in the 1970s by a Scandinavian group, then, it was developed and completed by numerous works of national societies (French and Spanish) as well as at the international level, particularly within the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and National Committee for Clinical Laboratory Standards (NCCLS) (2).

Establishment of Reference intervals in adolescents and children at different age group also used to follow the different phases of physiological development from birth to childhood and adolescence. However, appropriate age- and gender-specific adolescent reference intervals are often lacking. Gaps may be due to study recruitment being performed in school, and missing preschool ages, or that collecting samples is difficult from healthy adolescent. Recent studies and projects have achieved major advancements (3).

According to IFCC, it is necessary for any laboratory to have its own set of reference range. Reference intervals are established by testing the large number of healthy population and helping out what appears to be "Normal" for them (2) and also Reference intervals for common laboratory tests have been derived traditionally from samples of adults living in industrialized countries. However, these intervals can differ substantially from those in children and adolescent from developing countries, such as those in Africa. Optimal cost-effective detection and management of common conditions depends on the availability of appropriate local age-specific reference ranges (4, 5).

Routine capacity for clinical laboratory testing is also increasing in Africa. Clinical trials and clinical care in sub-Saharan Africa requireaccurate laboratory reference range for appropriate assessment of children and adolescent, monitoring disease progression, and reporting of possible toxicity and adverse events.(6).Proper evaluation of result interpretation is importance for children and adolescent depending on the test parameter. Such interpretation is requiring relevant reference interval, ideally established in the local setting. Locally validated reference ranges are also important for clinical trials participation as the use of inappropriate clinical laboratory reference ranges may result in an unnecessary exclusion of patients from participation in important trials or an inability to reliably assess drug-related

toxicities and adverse events. (7).

For developing countries to establish reference interval for a laboratory, a minimum of 120 samples needed from apparently healthy individuals for analysis, by a non-parametric means for each partition and this should be done after a specific time period because reference ranges may change with time and also the methods (4). Statistically, reference intervals are defined as the limiting values denoting a specified percentage (typically central 95%) of values from an apparently healthy reference population with 90% confidence. In the central 95% distribution model, the reference limits are determined by calculating the 2.5th and 97.5th percentiles of test results and exclusion of 5% (8) individuals with liver disease maintain normal function despite extensive liver damage. In such cases, liver disease may only be recognized by using tests that detect liver injury. Most commonly, this is accomplished by measurement of plasma activities of enzymes found within liver cells released in somewhat specific patterns with different forms of injury. Chronic liver injury often involves fibrosis in the liver. Consequently, detection of markers of the fibrotic process might be indicators of degree of liver injury (9).

Liver disease usually classified as hepatocellular, cholestatic (obstructive),or mixed. In hepatocellular diseases (such as viral hepatitis or alcoholic liver disease), features of liver injury, inflammation, and necrosis predominate. In cholestatic diseases (such as gall stone or malignant obstruction, primary biliary cirrhosis, some drug-induced liver diseases), features of inhibition of bile flow predominate. In a mixed pattern, features of both hepatocellular and cholestatic injury are present (such as in cholestatic forms of viral hepatitis and many drug-induced liver diseases) (10).ALP may be elevated in conditions involved in bone formation or increased bone turnover. In children, serum ALP concentrations are considerably higher than in adults and those concentrations correlate with the rate of bone growth. ALP levels are mildly elevated relative to adult levels during the first 3 months of life, increase 2- to 3-fold at puberty, and remain above the adult level for 1 or 2 years (11).

One of the most important tools in confirming a healthy children and adolescent is the growth chart (8). Sexual characteristic changes across puberty are profound; the earlier changes during the growth of adolescent, from birth to puberty are also significant (9).The major functions of the kidney are to excrete metabolic west products as well to maintain water, PH, electrolyte balance, production of calcitriol and erythropoietin. A decrease in kidney function is due to reduction in the performances of nephrons (9).the renal markers serum creatinine, urea, and uric acid all increased with age. The amount of creatinine

produced daily from break down of creatine in muscles correlates with increased muscle mass. Similarly, urea and uric acid are related to protein degradation and would also be expected to increase with growth and development for children and adolescents (12).

The significances of RI of clinical chemistry parameters among different countries and even population groups of the same country attributes the risk of unessential further investigation or default in the detection of the underlying disease or leads to wrong management of patients (13). The aim of this study is, therefore, to determine the local reference ranges for selected clinical chemistry values among a healthy adolescent and children population living in the Addis Ababa, Ethiopia. Values based on age and gender was determined, and local results were compared to levels reported in adolescent and children with other published papers.

1.2. Statement of the Problem

Nearly 80% of physicians' medical decisions are based on laboratory report (4). Most laboratories for example in Pakistan, Kenya, Austrian and Ethiopia rely on the references provided by the manufacturer of the kit and /or developed by another reference laboratory (14-17).Identifying potential interference by a mismatch between clinical and biochemical data is important in planning the subsequent investigation and treatment of patients. Most laboratory tests that do not have disease-specific cutoff values have a "normal" reference interval, defined as the central 95% interpercentile range in a normal distribution for a group of healthy volunteers, False-positive test results are harmful psychologically and financially to the patients (13).

Iranian children and adolescents showed ethnic differences in comparison with the CALIPER study and the other studies in pediatrics in the United States in terms of the number of age groups (12).From a study conducted in different African countries on common biochemical tests like alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bilirubin (direct and total) which are usually used during screening/enrollment and safety monitoring of clinical and vaccine trial participants showed out of range (OOR) values of up to 32% in Kintampo(Ghana) which favors the degrees of ruining for enrolment in to clinical trials and misinterpretations of advert effects using the western RI values. Children are constantly changing and developing and therefore, single reference values may not be appropriate for children of all ages (18).In Kenyan studies involving the age of 1-17 range, the limits of the enzyme like AST and ALT observed to be as high as ten times more as the manufacture's values (15).

Therefore, the aim of this study was to determine the local reference ranges for selected clinical chemistry values among apparently healthy adolescents and children population living in the Addis Ababa, Ethiopia. Values based on age and genders were determined, and local results were compared to levels reported in adolescents and children by other studies.

1.3.Significance of the study

The clinical chemistry reference interval is one of the most important decision-making tools used to differentiate between healthy and diseased individuals. Therefore, in order to accurately differentiate what is healthy and normal locally established RIs are necessary for each laboratory parameters in the population. Many adolescents and children's physiological changes that occur during physical development of the body affect the clinical laboratory parameters. This study is, conducted to determine the biochemical values for population in community settings. Patients will benefit from appropriate interpretation of their laboratory findings based on local standards. It helps unnecessary repeats when patient results become out of range when judged by company derived values. This can be used as reference values in the future evidence-based practices. Moreover, this study would serve as baseline information for further studies at national level.

2. Literature Review

The RIs are descriptive of a specific population and are derived from a reference distribution (usually 95% interval).Whereas clinical decision limits (CDLs) are thresholds above or below which a specific medical decision is recommended and are derived from Receiver Operating Characteristic (ROC) curves and predictive values. CDLs are based on the diagnostic question and are obtained from specific clinical studies to define the probability of the presence of a certain disease or a different outcome (19).

Adeli et al, established biochemical marker reference values across age groups including pediatric and adolescent age groups that are representative of the Canadian population in age category 6-15(children and adolescent). The values were as follows for selected parameters: Albumin (4.1-5.1g/l) and total bilirubin (0.1-0.9mg/dl). The study demonstrated that there are no required additional sex and age partitions. ALKP (153-367IU/L) decreased at an earlier age of 11 years. AST (23-36IU/L) declined steadily until 18 years of age. Males had increased serum creatinine concentrations (0.5-0.9mg/dl) after 12 years of age, respectively, compared to females (0.5-0.8mg/dl). Males also had higher concentrations of urea and uric acid (3.7-7.7mg/dl) between 8–59 and13–79 years of age, respectively than female (2.6-6.2mg/dl).ALT (16-39 IU/L) and glucose (73-91mg/dl) all required 5–15 age partitions, with lower limits that remained relatively un-changed throughout the age range but increasing upper limits in adult and geriatric ages(12).

Houman T et al from the University of Toronto demonstrated that serum concentration of several biomarkers remained relatively constant across the pediatric age range and similar between sexes. But alkaline phosphatase in males aged10-17 years was (86-515IU/L), higher than females (52-432IU/L) while creatinine for the age group of 5-19 males was (27-90µmol/l), and females (35-68µmol/l). Thus, the finding showed both age and sex difference (20).

In a study from Korea, most analytes except albumin (4.0-4.9g/dl) required partitioning either by sex or age. Age-specific partitioned reference intervals for alkaline phosphatase for male age of 6-14 years were (118-341IU/L) and for female age of 6-12 years the value was (123-330 IU/L). Creatinine for male age of 6-9 years (0.32-0.64mg/dl), 10-12years (0.39-0.80mg/dl),13-16years (0.45-0.80mg/dl) and for female age of 6-8 rears (0.32-0.64mg/dl) and 9-14y years (0.37-0.72mg/dl), and total bilirubin for male age of 2-8 years (0.2-0.8mg/dl) and 9-16years (0.2-1.0mg/dl) and for female age of 2-9 years (0.2-0.8mg/dl) and 10-14years (0.2-1.0mg/dl) were established for both males and females after being partitioned by sex.

Additional age-specific partitioning of aspartic amino-transferase (7-31IU/L) in females (2-14y) and total protein (6.3-7.7g/dl)in age of 2-14 years and uric acid in males in age of 2-10 years (2.6-5.9mg/dl) and 11-16years (3.0-7.6mg/dl) was also required. Alanine amino transferase male age of 2-16 years(8-39IU/L) and age of 2-14 years for female(7-31 IU/L), blood urea nitrogen for male age of 2-16 years(8-19mg/dl) and for female age 0f 2-14years (7-17mg/dl), and glucose for male age of 2-16 years (75-117mg/dl) and for female age of 2-14 years (74-121mg/dl)were partitioned only by sex(21).

Iranian children and adolescent showed differences between reference intervals reported in boys and girls. Ages of 7-9 years AST (18-45IU/L), age of 10-14 years (6.3-45.0 IU/L) and in the age of 15-19 years (8.1-42.0 IU/L); there was no difference by gender but the difference is in age category. Similarly, ALT differ in age; the respective values were age of 7-9 years (7-44 IU/L), age of 10-17years (5.2-36.0 IU/L) and FBS also age of 7-10years (58-123mg/dl) and age of 11-19 years (55-123mg/dl)(22).

In the Austrian adolescent study cohort by Barbara B et.al, most biochemical markers reveal similar range to values published in other studies. The respective values were Albumin(37-56g/l), creatinine(0.6-0.9mg/dl), urea(12.40-30.30 mg/dl), uric acid(3.0-5.9mg/dl), AST(<32 IU/L) and ALT(5-20 IU/L)(16).

In Rawalpindi-Islamabad area the values for children and adolescents of plasma glucose (3.1-6.0mmol/L)and total protein (55-83g/l) were higher; urea (2.5-6.2mmol/L), creatinine(31-128umol/l), ALT (14-52 IU/L), ALP (185-706 IU/L) were lower and uric acid (120-410umol/L) and total bilirubin (4-18umol/L) were comparable with those of Karachi population. The authors attributed the variations may be due to selection criteria of population, geographical or analytical factors. The values of plasma glucose, serum urea, creatinine, uric acid, total bilirubin were lower and total protein ALT and ALP were higher than American population. Similarly there were mild to significant differences, in various analyses when compared with different age and sex groups of the Chinese (14).

Comprehensive biochemical reference values that would serve as standards for the interpretation of laboratory results for children and adolescent in routine healthcare practice and screening/ follow-up during clinical trials in the Kintampo area of Ghana was established. Albumin (35-50g/l) decreased progressively in the children; they later increased in the adolescents. Creatinine and urea increased progressively with age throughout the study. Levels of ALT, total protein, and total bilirubin remained almost invariable throughout

the study. Levels of uric acid showed great variation, increasing in the 5–12 year subgroup and decreasing in adolescents. Adolescent males had significantly higher levels of ALT (10–61 IU/L versus 7–41 IU/L), AST (18–67 IU/L versus 11–49 IU/L), direct bilirubin (1.2–4.0 mmol/L versus 0.8–3.9 mmol/L), urea (1.0–5.5 mmol/L versus 1.0–3.6 mmol/L), creatinine (42–79 mmol/L versus 33–78 mmol/L), uric acid (79– 334 mmol/L versus 76–285 mmol/L). However, adolescent females had significantly higher levels of albumin (34.3–48.9 g/L versus 37.8– 50.5 g/L) than males (18).

In university of Zimbabwe, studies on the biochemical parameters in the age of 12-18 showed significant different between males and females were ALT male (5.8-38.8IU/L), female(5.0-43.5 IU/L), AST male(14.4-45.7IU/L), female(12.7-38.8IU/L), and ALP male(73.8-572 IU/L), female(66.1-552 IU/L)(23).

In Kenya a study conducted on children aged 1-17years also revealed significant difference between sex for same parameters those are ALT male(11.18-57.20 IU/L), female(10.24056.84 IU/L), DB male(0.43-3.54umol/l), female(0.21-4.19umol/l), and TP male(30.78g/l),female(29.95-54.57g/l).the lower reference range limits of the enzyme ALT(10.75-57.80 IU/L) vs.(0-50 IU/L) and AST(9.92-54.60 IU/L) vs. (0-50 IU/L) were observed to be as high as ten times more than the manufacture values. The enzyme ALP (61.63-114.31 IU/L) vs. (47-406 IU/L) showed a significant higher lower limit and a significant lower upper limit. Those references interval of ALP was significantly shorter compared to that of the manufacture's value (15).

In Ethiopian (Tigray region) a study conducted in the age of 12-17 yrs. lower and upper limit results were relatively higher than the manufacture's reference value for glucose (65-110mg/dl), alkaline phosphatase (66-456IU/L), albumin, Total bilirubin (0.10-0.81mg/dl) and direct bilirubin (0.03-0.53mg/dl). On the other hand, the upper limit reference value of this study was lower when compared with the manufacturer's upper limit values for AST (14.20-34.90IU/L), ALT (5-23IU/L), Urea (8.14-24.25mg/dl), creatinine (0.37-0.91mg/dl) and Total protein (6.09-7.85g/dl)(24).Generating more data from different parts of Ethiopia is needed to establish our own reference interval, which this study has trid to accomplish for Addis Ababa children and adolescent..

3. Objectives

3.1. General objective

To establish RI for common clinical chemistry parameters for apparently healthy adolescents and children in Addis Ababa, Ethiopia from April to October, 2019

3.2. Specific objectives

- To determines RI for FBS , liver and liver function tests for apparently healthy children in Addis Ababa
- To determines sex specific RI for FBS, and renal liver function tests for apparently healthy adolescents in Addis Ababa

4. Hypothesis

Ho:-There is no statistically difference between reference values for commonly performed LFT and RFT between children and adolescent of Addis Ababa, Ethiopia

5. Materials and methods

5.1. Study design

A community based cross-sectional study was conducted to establish reference intervals for selected clinical chemistry parameters among adolescents and children in Addis Ababa, from April to October 2019

5.2. Study area

Addis Ababa is a capital city of Ethiopia with an altitude of 2300 m above sea level. Commonly known as capital city of Africa as a result of many headquarters of different international and regional organizations such as Africa Union and UN-Economic Commission for Africa are found in the city. Based on the 2007 Census conducted by the Central Statistical Agency of Ethiopia (CSA), Addis Ababa had a total population of2, 739,551 of whom 1,305,387 were males and 1,434,164 females. The estimated population of Addis Ababa in 2018 is 7.178 Million. The city is divided into 10 sub cities and 99 woredas .From those sub cites: Arada, Kirkos, Akaki and Yeka was selected for this study. Randomly selected Weredas within these sub cities were then included in the study.

5.3. Study period

The study was conduct from April to October, 2019 G.C

5.4. Population

5.4.1. Source population

The source populations were all apparently healthy adolescents and children in Addis Ababa, Ethiopia

5.4.2. Study Population

The study population were those adolescent and children who were selected systematically from the randomly sample sub cites of Addis Ababa and who fulfill the eligibility criteria were the study population.

5.5. Inclusion and exclusion criteria

5.5.1. Inclusion criteria

The same inclusion criteria described in the C-RIDL protocol [25] were applied with some modification to cope with the Ethiopian population. The participants was subjectively feeling well, between the ages of 5 and 17 and ideally they were not taking any medication or

supplements but if they were under any of medication names, doses and frequency was recorded.

5.5.2. Exclusion criteria

The participants were excluded if any of the following were identified:

- age less than 5 and greater than 17 year old
- if he or she was diabetic
- on oral therapy or insulin,
- History of chronic liver or kidney disease,
- Had results from their blood samples that clearly point to a severe disease,
- Had been a hospital in-patient or been subjectively seriously ill during the previous 4 weeks of participation,
- He or she was known carrier state of HBV, HCV and HIV
- if he/she CRP result has> 3 mg/dl

5.6. Study variables

5.6.1. Dependent variables

- specific reference intervals for Clinical chemistry parameters (including AST, ALT, Alkaline phosphatase, Glucose, albumin, total protein, direct and total bilirubin, creatinine , urea/BUN and uric acid)

5.6.2. Independent variables

- Sex
- Age

5.7. Measurement and Data collection

5.7.1. Sample size calculation and sampling method

To establish local reference interval, 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, age range) with a power of 90% [CLSI 2008] (27). In the current proposed study, the maximum partition needed were

age (5-11) years; (12-17 years) and sex partition for the older age group, hence a total of three partitions with minimum sample size of 360 participants. .

According to previous studies in other African countries, in such large scale studies about 30% of apparently healthy population [Steven *et al*., 2008] (28) 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 360 for the reference interval determination, a total of 516 individuals were enrolled (i.e, 30 % x 516=155 to be excluded during data analysis; 516-155=361).

Thus, 516 participants were recruited from Addis Ababa. The study participants were selected using convenient sampling method by considering woreda as a sampling frame for each sub cites and then households, schools, youth centers, and religion places the final selection units. All individuals in the household and other places fulfilling the eligibility criteria and willing to participate were included.

5.7.2. Sampling technique

Probability Proportional to Size (PPS) sampling method was employed, where the size depends on the number of households and schools of Woredas (former Kebeles) in a city/town. Accordingly, all the woredas in the town are considered/selected to be the participants of the study. Since Addis Ababa is very large city, four sub-cities were selected based on PPS, namely Arada, Kirkos, Akaki and Yeka sub-cities; thus all woredas under the selected sub cities were included. To recruit 516 participants, community sensitization in the weredas was conducted by experienced health extension workers prior to commencement of the study. Once volunteering participants fulfilling the eligibility criteria are identified by the health extension workers, they were invited to go to nearby health facilities for interview using structured questionnaire and to facilitate biologiocal sample collection. Letter has been written to the Health Bureau by the Federal Ministry of Health. The Bureau wrote support letter to the respective Weredas (Annexed).

*total number of sampled is computed from the total sample size needed (i.e. 516) by the estimated individuals per each study site

Proportional allocation=$Ni=_{n/N*Ni}$

n = total sample size

n_i = sample size at that stratum

N = total population

N_i = total population at that stratum

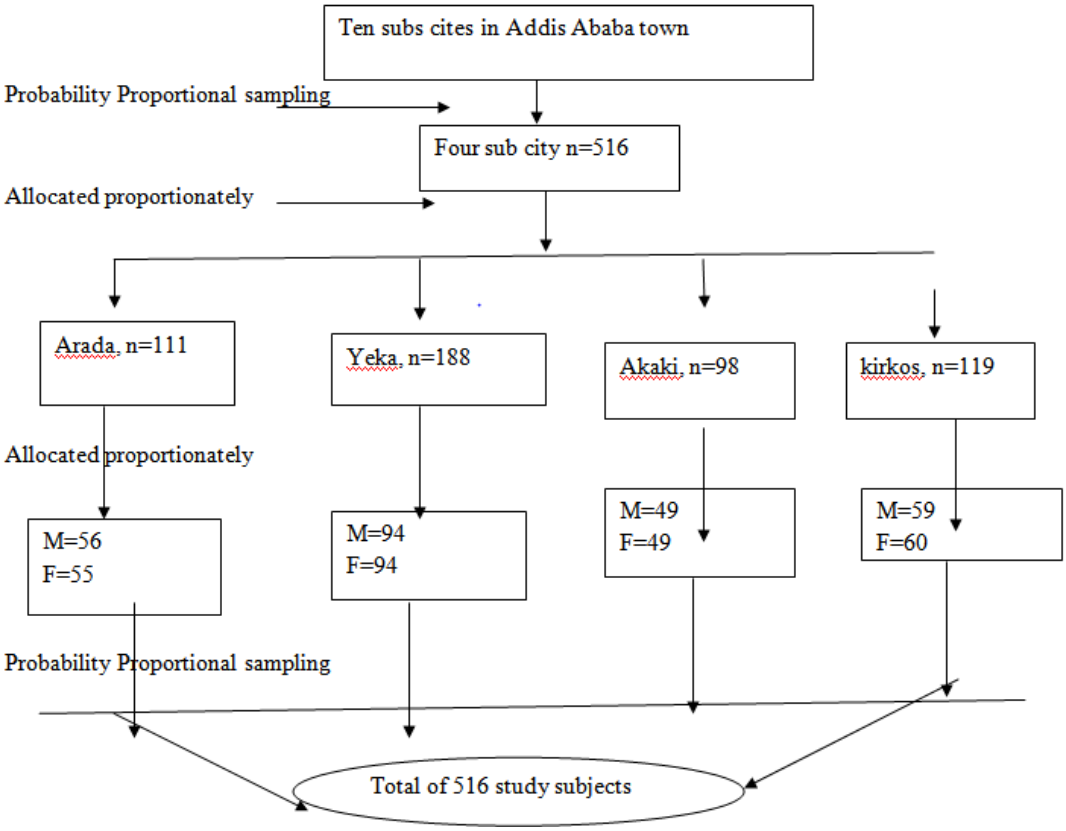

Figure 1.**Schematic presentation of sampling procedure**

5.7.3. Measurement and Data collection

The study aim, risks, benefits of study participation and right to withdraw from the study at any time were explained by the study team to (also for parents/guardians). Data was collected from children who were in good health according to the questionnaire responses, interview and physical examination at the time of blood sampling. Blood specimen was collected for clinical chemistry parameters, and CRP testing and hemo-parasites. . Laboratory results were given to participants upon their requests according to the local Ministry of Health guidelines.

5.7.4. Demographic and clinical data

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

measurement (detail is annexed). There were one supervisor, 4 data collectors per site plus one overall coordinator centrally

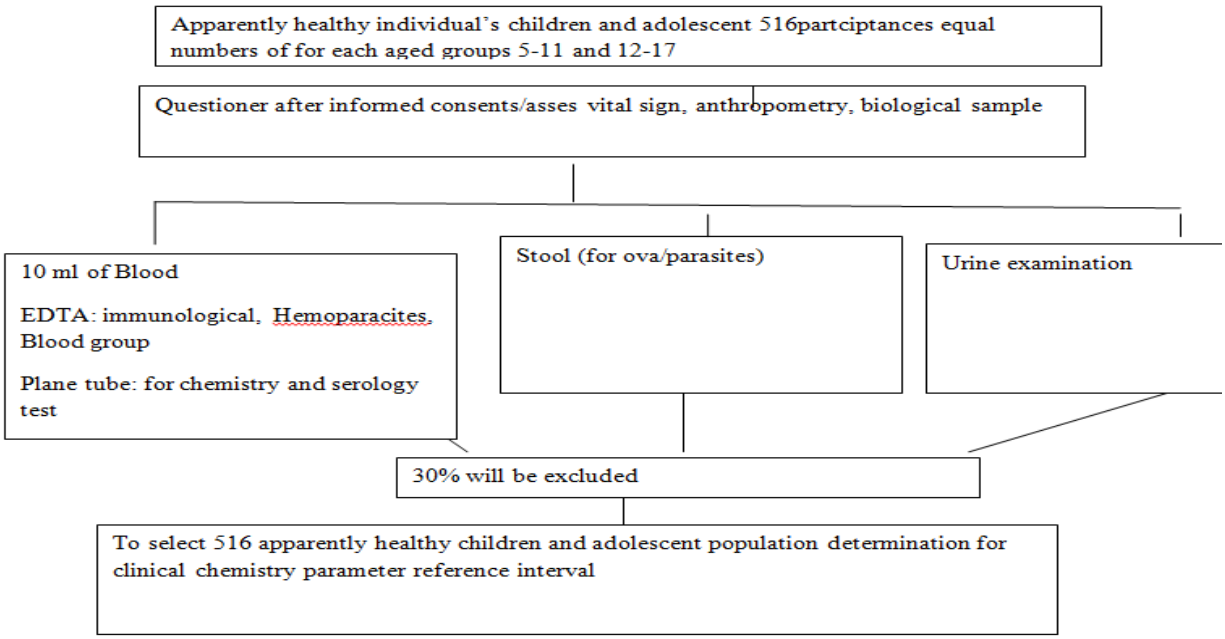

Figure 2.Data collection procedure

5.7.5. Sample collection for laboratory analysis

All samples were collected by experienced laboratory technologist using standard phlebotomy procedures. Blood was collected as follows: 5 ml in a plain tube for chemistries,. The indicated volume was within allowable blood volume that can be collected from children 5-17 years. To minimize diurnal variation of some analytes, blood samples were collected before 11:00 am. The blood was drawn after the participant had sat quietly for approximately 30 min to avoid variations due to postural influence and physical stress. The time period was used for checking the questionnaire.

Whole bloods were used for hemoparasites identification while serum samples for clinical chemistry profiles. Stool sample was collected for parasitological analysis and urine for Urinalysis. Leak proof clean containers were used to collect urine and stool samples.

All samples were labeled with unique identification number (Site name plus 001 to 516).All samples were delivered to the ISO 15189 certified EPHI Central Laboratory on the day of collection. Processing was completed after specimen verification at the Central Laboratory's specimen management section. Serum and left over plasma were collected and stored at -2-8^{O}C and transported in cold chain using cold boxes to EPHI for batch analysis of the clinical chemistry parameters. Urine chemical test, kato technique, concentration and direct stool analysis were performed on site in the respective health facilities where participants are

invited to come to. Then leftover samples were stored at -80^{0}C in Addis Ababa University, Department of Medical Laboratory Sciences for future additional analyses, and each time going through a new ethical clearance process. All results entered into SPSS and hard copies for cross checking were sent to the PI at Department of Medical Laboratory Sciences, AAU.

5.7.6. Laboratory testing and analysis

Using the blood sample collected in the plain tubes for, biochemical analytes, the following parameters were assayed: creatinine, urea, uric acid, aspartate amino transferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), albumin, total protein, total bilirubin, direct bilirubin, and glucose. All tests determined using fully automated Chemistry Analyzer according to manufacturer specifications. Methods for all analysis were traceable to International Federation of Clinical Chemistry (IFCC) standards.

Table 1-Methods used for analytes of clinical chemistry to determine the reference interval of apparently healthy Adolescents and children of Addis Ababa city, Ethiopia 2019

Analytes	Method
FBS	Hexokinase
AST	IFCC Modified without pyridoxal phosphate
ALT	IFCC Modified without pyridoxal phosphate
ALP	p-Nitrophenyl phosphate Diethanolamine
TBIL	Diazotized sulfanilic
DBIL	Diazotized sulfanilic
Urea	Enzymatic –UV kinetic
Cr	Jaffer–Kinetic
U/A	Enzymatic colorimetric test.
TP	Biuret/end point
ALB	Bromocresol green succinate Buffer

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; Cr: Creatinine; TP:Total protein; ALB: Albumin; BIL. D: Bilirubin Direct; BIL. T: Bilirubin Total; FBS: Fasting Blood Sugar

5.8. Operational definitions

Apparently healthy: An individual who has no sign and symptoms and history for any disease and negative result for the screening tests.

RI: The 95 percentile interval between the 97.5th percentile and 2.5th percentile which form the upper and lower reference limit.

Renal function test: - A test in which blood or urine samples are checked for the amounts of certain substances released by the kidneys. A higher- or lower-than-normal amount of a substance can be a sign that the kidneys are not working the way they should.

Liver Function Test: -A group of blood tests used to detect, evaluate, and monitor liver disease or damage. A liver function test measures enzymes, proteins, and other substances that are produced or excreted by the liver, such as alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), bilirubin, and albumin.

Adolescent: young children who are in a markedly pubertal change aged 0f 12–17 Years

5.9. Data Quality Assurance

Each activity including blood sample collection, transportation, storage and analysis was based on good laboratory practices (GLP) using standard operating procedures (SOPs) to ensure data quality. The analysis was done in EPHI. The laboratory had been participating in external quality assessment programs like an onsite evaluation by international digital proficiency testing by One World Accuracy three times per year. All pre-analytical, analytical and post –analytical phases of the quality assurance cycle were maintained

Pre analytical

Standard operation procedures (SOPs) were followed for sample collection processing storage and handling of the sample. Appropriate examination, screening of the subject for acute and chronic diseases was performed .proper orientation was given clearly for the candidates before sample collection and to avoid some factors such as strenuous exercise, eating, drinking and medication. Re assuring the study subjects immediately prior to sample collection was addressed and strict adherence to SOP was made during sample transportation and sample preparation to evade from sample integrity and hemolysis

Analytical

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. Internal quality control was done for each parameter by using two quality control levels. the laboratory strived to comply with the principal of good clinical laboratory practice protocol(GLP) between run precision for the analytes for the analytes was done using 20 measurement made on both the same and separate days using normal control samples. 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

Post analytical

The results obtained from the laboratory were verified by trained personnel before releasing it. The result was entered in to computer carefully and proof read to reduce the corresponding transcriptional error. At the end all leftovers samples were stored at -80^{0}C

5.9. Data analysis and interpretation

All the data was coded and checked for completeness then entered and analyzed using SPSS version 21 statistical software for windows. The data was tasted for normality of its distribution by Kolmogorov–Smirnov; most of the RI parameters were not normally distributed. Therefore, the nonparametric methods for determination of RI were used as recommended by CLSI.5 Median, central 95 percentile, and 90% confidence interval (CI) was calculated. The 97.5th percentile and 2.5th percentile were the upper and lower reference limit for the population. The tukey outlier range statistic had been used to identify and determine outliers. The significant difference between sex among age groups was determined using Wilcoxon rank-sum test (Mann–Whitney U test) and significance difference among age groups between sex is determined using independent Kruskal–Wallis test. P value < 0.05 was considered as statistically significant.

5.9.1 Data quality management

To ensure the data quality, one day training on ethical issue how to approach the respondent and how to administer the questionnaire was given to data collectors and supervisors. A sample (5%) of the Amharic version of the questionnaire was pre-tested for clarity, acceptability, and flow among the non-study subjects. Data collection process was checked by supervisors in a daily basis. The principal investigator was conducting meeting with data collectors and supervision ever day after completion of the data to check data inconsistency and completeness. Systemic observation was carried out to control stool and urine sample sharing among school children. The principal investigator controlled the overall activities.

5.10. Ethical considerations

Ethical approval was obtained from Department of Medical Laboratory Sciences, Addis Ababa University prior to data collection. A written permission from Addis Ababa Health office, sub city health office and wereda has been obtained. Study participants were asked for their permission using assent form and parents/guardians for their consent. All the study participants were informed about the purpose and benefits of the study, about the

confidentiality of their responses and the importance of providing the right information in increasing the validity of the study.

5.11. Dissemination of the result

Finding of the study will be disseminating to the Department of Medical Laboratory Sciences, Health office, study sub cites and other concerned sectors. Hard and soft copy will be made available through the library of AAU for graduate students and the public. Findings will be communicated to the medical community during annual conferences and through publications on peer-reviewed journals.

6. Results

6.1 screening result

From the total of 516 study participants recruited, 45 (8.7%) of participants had CRP result above the normal range, and so excluded from the analysis. Thus, reference interval for selected biochemical tests (Table 3.and 4) was determined for the remaining 471 study participants. Accordingly, for each analyte (FBS, AST, ALT, ALP, TBIL, DBIL, Urea, Cr, U/A, TP and ALB), RI was calculated using the 2.5^{th} and 97.5^{th} percentiles as lower and upper limits at 90% confidence interval in accordance with the CLSI guidelines

6.2 Socio demographic characteristics of the study

A total of 471study participants (children aged 5-11 years and adolescents aged 12-17 years) involved on the research; where the proportion of males and females was 234 (47.7%) and 237 (50.3%), respectively. The mean age of adolescent and children were 14.43 and 9.29years, respectively. The median BMI was 15.25 and 18.33 in children and adolescents, respectively and Std. Deviation of BMI was 131.1 and 2.99 respectively.

6.3 .Reference interval for commonly liver and renal function clinical chemistry tests

The overall mean values for children and Adolescent for glucose (8.4vs 9mg/dl), AST(11.3vs 9.9U/L), ALT(3.6 vs 4.6U/L) ,ALP(67vs 96U/L), Total Bilirubin(0.14 vs 0.18mg/dl), Direct bilirubin (0.5 vs 0.06mg/dl), Total Protein(0.53 vs 0.71g/dl), Albumin(0.30 vs 0.40g/dl), urea(5.3 vs 6.0mg/dl), Creatinine (0.09 vs 0.12mg/dl) and Uric acid(1.0 vs 1.1mg/dl,).The respective RIs for children and adolescents are summarized in Table 2.

The study showed respectively statistically significant differences ($P<0.05$) between children and Adolescent in majority of commonly performed liver and renal function clinical chemistry parameters. Among the analytes urea and ALT required partition by age whereas Glu and ALB needed partition by sex. Children had significantly lower value than Adolescents in most analytes; they had significantly ($P<0.05$) lower value of Direct and total bilirubin, ALT, and UA. Whereas significantly ($P<0.05$) higher value of ALP was seen than Adolescents. The calculated mean, median, 95% CI for mean and 2.5^{th} and 97.5^{th} percentile range (RI) of common liver and renal function clinical chemistry test for apparently healthy children and Adolescents in Addis Ababa city, central Ethiopia is shown in table 2 and the significance of tests by sex shows for Adolescents in table 3.

Table 2:-mean, median and 2.5th and 95th percentile RI of common liver and renal function tests in relation to children and adolescent status of healthy individuals in Addis Ababa, Ethiopia, 2019(N=471, children=169, and adolescent=302)

Analytet	Unit	RI children			RI adolescent				RI Combined		
		Median	Mean (SD)	RI Range	Median	Mean (SD)	RI Range	p. value to age	Median	Mean (SD)	RI Range
Glucose	Mg/dl	76	8.4	61-91	78	12	57-97	0.047	78	9	57-97
ALT	U/L	6.7	3.60	1.3-13.0	6.7	3.7	1.6-14	**0.120**	6.7	4.66	1.6-14
AST	U/L	19	11.31	12.3-26.7	15	9.0	8-26	0.0001	17	9.9	8-27
ALP	U/L	225	67	143-375	183	105	47-452	**0.0001**	208	96	47-452
Direct bilirubin	Mg/dl	0.11	0.5	0.01-0.20	0.14	7.3	0.04-0.27	**0.002**	0.13	0.06	0.01-0.27
Total bilirubin m	Mg/dl	0.28	0.14	0.12-0.48	0.35	0.19	0.16-0.71	**0.0001**	0.32	0.18	0.12-0.71
Albumin	g/dl	4.6	0.30	4.1-5.1	4.7	0.99	4.1-5.2	**0.0001**	4.7	0.40	4.1-5.2
Total protein	g/dl	7.14	0.53	6.4-7.81	7.2	0.77	6.4-8.3	0.0001	7.2	0.71	6.4-8.3
Uric acid	Mg/dl	3.4	1.0	1.4-4.9	4.1	18	1.8-6.8	**0.0001**	3.8	1.1	1.5-6.8
Creatinine	Mg/dl	0.42	0.09	0.29-0.58	0.54	0.35	0.41-0.87	**0.0001**	0.49	0.12	0.29-0.87
Urea	Mg/dl	18	5.3	9-30	18	6.4	8-31	0.154	18	6.0	8-31

*parameters with stasticaly significant difference based on age

ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; AST: Aspartate aminotransferase; L: liter; mg: Milligram; RI: Referenceinterval; IU: International Unit; P. value<0.05 considered as statistically significant

6.4 Sex specific Reference interval for common liver and renal function tests among adolescents

Reference interval for AST, ALP, decreased in female Adolescent. Although DBIL (0.04-0.27mg/dl versus 0.16-0.57mg/dl; P < 0.01), the upper limit was lower than in male Adolescent. Adolescent males had significantly higher levels of ALT(2.0-13.4 U/L versus 1.6-14.0 U/L; P < 0.01), AST (11–26 U/L versus 8–20 U/L; P < 0.01), ALP (48–552 U/L versus 47–392 U/L; (P <0.01), Total bilirubin (0.18-0.71mg/dl versus 0.16-0.57mg/dl; P <0.01), urea(8-31mg/dl versus 9-30; P < 0.01), creatinine(0.41-0.87 versus 0.36-0.68mg/dl; P = 0.01), uric acid (2.5-6.8 versus 1.8-5.6mg/dl; P < 0.01), than female counterparts, as shown in table 3.

Table 3:- Sex specific RIs with 90% CI for lower and upper reference limits of common liver and renal function tests in adolescent in Addis Ababa, Ethiopia, 2019(N=302)

Analyte		Male				Female				P. value to sex
	Unit	N	RI percentile range	Lower reference limit 90% CI	Upper reference limit 90%CI	N	RI percentile Range	Lower reference limit 90% CI	Upper reference limit 90% CI	.
AST(SGOT)	IU/L	137	11-26	10-11	23-27	143	8-20	7.1-10.0	19.0-21.4	0.0001
ALT(SGPT)	IU/L	137	2.0-13.4	1.0-3.5	12.2-14.5	143	1.6-14.0	0.2-2.7	10.6-14.0	0.003
ALP	IU/L	142	48-452	0.41-82	419-478	148	47-392	0.0-58	291-392	0.0001
D. Bilirubin	Mg/dl	134	0.04-0.27	0.03-0.07	0.24-0.28	144	0.04-0.24	0.03-0.05	0.23-0.27	0.002
T. Bilirubin	Mg/dl	136	0.18-0.71	0.12-0.21	0.64-0.78	138	0.16-0.57	0.12-0.18	0.52-0.63	0.0001
Urea	Mg/dl	137	8-31	6.5-11.1	30-33	148	9-30	0.0-9.7	26-30	0.0001
Creatinine	Mg/dl	137	0.41-0.87	0.36-0.43	0.80-0.87	150	0.36-0.68	0.31-0.37	0.65-0.73	0.0001
Total Protein	g/dl	132	6.4-8.0	6.23-6.62	7.91-8.26	149	6.5-8.3	6.42-6.61	8.23-8.71	0.016
Albumin	g/dl	131	4.1-5.2	4.16-4.32	5.15-5.33	146	4.2-5.1	4.21-4.29	5.08-5.20	0.318
Glucose	Mg/dl	134	64-97	63-68	93-99	148	57-94	55-60	81-97	0.813
Uric acid	Mg/dl	140	2.5-6.8	2.1-2.8	6.7-7.2	151	1.8-5.6	1.5-2.1	5.4-5.9	0.0001

*parameters with stasticaly significant difference based on sex

ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; AST: Aspartate aminotransferase; CI: confidence interval: L: liter; mg: Milligram; dl: deciliter; RI: Reference Interval; IU: International Unit; BIL. D: Direct Bilirubin; BIL. T: Total Bilirubin

6.5-Comparability of established RI with other studies in Ethiopia, Africa and with kit insert claiming RIs

In practice, reference values which are used for interpreting test results of adults are also used for both children and adolescents. In the present study, company provided ALT, AST and ALP RIs are almost similar to the newly established RIs of this study; however, these values were found to be different from those established value in literatures.

The study assessed the comparability of the current RI with studies other part of Ethiopia, Africa, Canada and Australia as shown in table 4. No consistent pattern was seen among the various studies. All the parameters studied except ALB portray difference in the reference range values obtained.

Table 4:-Comparison of clinical chemistry parameters RI of current study against manufacturer ranges and other similar studies for the age group of 12-17

Analyte	Sex	Current Study	Manufacturer	Northern Ethiopia[24]	South west Ethiopia[26]	Ghana[18]	Tanzania[7]	Zimbabwe[23]	Kenya[15]	Austria[16]	Canada[12]
FBS mg/dl	C	57-97	74-109	65-110	NA	64.8-120.6	NA	NA	NA	NA	NA
	M	64-97	NA	64.15-108	66.80-125.80	NA	54-93.6	63-111.42	NA	NA	NA
	F	57-94	NA	65.33-111.35	59.70-117.70	NA	50.4-91.6	57.42-120.24	NA	NA	NA
ALT u/l	C	1.6-14	NA	5-23	NA	8-55	NA	NA	10.75-56	10.0-35.80	NA
	M	2.0-13.4	Up to 42	4.54-23.69	14.40-60.70	NA	10-36	5.8-38.8	NA	NA	17-50
	F	1.6-14.0	Up to 33	5.10-20.0	11.0-70.50	NA	7-33	5.0-34.5	NA	NA	14-41
AST u/l	C	8-26	NA	14.20-34.90	NA	14-62	NA	NA	9.92-54.60	15.0-44.60	NA
	M	11-26	Up to 40	15.70-39.10	12.4-58.0	NA	19-42	14.4-45.7	NA	NA	18-36
	F	8-20	Up to 32	13.30-28.50	11.0-72.70	NA	21-52	12.7-38.8	NA	NA	15-34
ALP u/l	C	47-452	116-468	66-456	NA	NA	NA	NA	61.63-114.32	NA	NA
	M	48-452	NA	79-492	21.20-656.40	NA	124-537	73.8-572	NA	NA	113-438
	F	47-392	NA	63.56-253	91.80-440.60	NA	68-498	66.1-552	NA	NA	64-354
DBIL mg/dl	C	0.04-0.27	≤0.30	0.03-0.49	NA	0.05-0.23	NA	NA	0.02-0.22	NA	NA
	M	0.04-0.27	NA	0.03-0.04	NA	NA	NA	0.0-0.25	NA	NA	NA
	F	0.04-0.24	NA	0.02-0.53	NA	NA	NA	0.09-0.35	NA	NA	NA
TBIL mg/dl	C	0.16-0.71	NA	0.10-0.81	NA	0.19-1.26	NA	NA	0.73-4.24	NA	NA
	M	0.18-0.71	1.4	0.09-0.86	NA	NA	0.11-0.87	0.2-1.09	NA	NA	0.1-0.9
	F	0.16-0.57	0.9	0.10-0.72	NA	NA	0.17-1.28	0.17-0.88	NA	NA	0.1-0.9
URE Amg/dl	C	8-31	6-20	8.14-24.25	NA	NA	NA	NA	NA	NA	NA
	M	8-31	NA	9.33-24.99	4.60-27.20	NA	NA	NA	NA	NA	8-20
	F	9-30	NA	7.40-23.00	3.90-48.50	NA	NA	NA	NA	NA	8-19
CRE Amg/dl	C	0.36-0.87	0.53-1.2	0.37-0.91	NA	0.88-0.89	NA	NA	NA	0.58-1.0	NA
	M	0.41-0.87	NA	0.39-0.96	0.30-1.90	NA	0.92-6.83	NA	NA	NA	0.05-0.-
	F	0.36-0.68	NA	0.30-0.85	0.30-1.30	NA	0.91-2.69	NA	NA	NA	0.5-0.8
UAmg/dl	C	1.8-6.8	NA	NA	NA	1.31-5.41	NA	NA	NA	3.22-7.94	?
	M	2.5-6.8	3.4-7.0	NA	NA	NA	NA	NA	NA	NA	?
	F	1.8-5.6	2.4-5.7	NA	NA	NA	NA	NA	NA	NA	?

TPg/dl	C	6.4-8.3	6.6-8.7	6.09-7.85	NA	46.4-86.5	NA	NA	30.38-55.18	NA	NA
	M	6.4-8.0	NA	5.97-7.83	NA	NA	68-84	68.0-90.0	NA	NA	NA
	F	6.5-8.3	NA	6.10-7.90	NA	NA	67-84	43.0-55.0	NA	NA	NA
ALBg/dl	C	4.1-5.2	3.2-4.5	4.42-5.46	NA	34.4-49.39	NA	NA	28.30-48.72	42.32-57.98	NA
	M	4.1+5.2	NA	4.32-5.49	NA	NA	41-51	71.0-90.6	NA	NA	4.1-5.1
	F	4.2-5.1	NA	4.42-5.46	NA	NA	40-49	43.7-57.0	NA	NA	4.1-5.1

ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; AST: Aspartate aminotransferase; CI: confidence interval: L: liter; mg: Milligram; dl: deciliter; RI: Reference Interval; IU: International Unit; BIL. D: Direct Bilirubin; BIL. T: Total Bilirubin M: Male, F: Female, C: Combined

6.6: Out range value

5.6.1-OOR (N) and OOR (%) of current RI as compared to Manufacturer used RI (adopted from leaflet)

The proportion of out range values (OOR %) segregated by Children and Adolescent is displayed based on the company based RIs which is being utilized by EPHI (Ethiopian public health institution) according. Large values were detected for both children and Adolescent especially for total bilirubin (100%) and urea (33.1% and 30%, respectively) and in Adolescent high OOR vales are detected for ALT, ALP, Cr, ALB, UA and AST.

Table 5:-OOR (N) and OOR (%) of current RI study as compared to Manufacturer's provided RI (adopted from leaflet)

analytes	Unit	Children				Adolescent			
		Manufacture r	Current study	OOR (N)	OOR (%)	Manufactur er	Current study	OOR(N)	OOR (%)
Glucose	Mg/dl	74-109	61-91	94	55.6%	74-109	57-97	20	6.2%
ALT	IU/L	0-41	1.3-13.0	03	1.7%	≤ 40	1.6-14	59	19%
AST	IU/L	0-40	12-26	05	2.9%	≤ 40	8-26	43	14%
ALP	IU/L	129-417	143-375	04	2.3%	116-468	47-452	60	19.8%
TBIL	Mg/dl	0.9-1.4	0.09-0.46	141	100%	0.9-1.4	0.16-0.71	261	100%
DBIL	Mg/dl	<0.30	0.02-0.20	14	8.2%	≤ 0.30	0.04-0.27	15	4.9%
UREA	Mg/dl	6-20	9-30	56	33.1%	6-20	8-31	96	30%
CREA	Mg/dl	0.32-0.73	0.29-0.58	06	3.5%	0.53-1.2	0.41-0.87	136	45%
UA	Mg/dl	2.4-7.0	1.4-4.9	28	16.5%	2.4-7.0	1.8-6.8	59	19%
TP	g/l	6.6-8.7	6.4-7.8	04	2.3%	6.09-7.85	6.4-8.3	36	11.9%
ALB	g/l	3.8-5.4	4.1-5.1	0	0%	3.2-4.5	4.1-5.2	135	44.7%

OOR: out of range; N: number; % Percentage, ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; AST: Aspartate aminotransferase; CI: confidence interval: L: liter; mg: Milligram; dl: deciliter; RI: ReferenceInterval; IU: International Unit; BIL. D: Direct Bilirubin; BIL. T: Total Bilirubin

7. Discussion

The established comprehensive biochemical reference values would serve as standards for the interpretation of laboratory results for adolescent and children in routine healthcare practice and screening/follow-up during clinical trials in Addis Ababa, Ethiopia, as well as in populations with similar profiles. One of the difficulties when establishing reference values for children is how the population should be subdivided (18).Even so nearly 80% of physicians' medical decisions laboratory result is based on laboratory report However, there is paucity of reference interval studies done in Ethiopia for common clinical chemistry parameters especially for children and Adolescents.

Grouping children into the 5–11 years, and 12–17 years of age groups (male and female) was performed based on the subgroups used for similar studies in Canada (12) and Northern Ethiopia (24) and also based on recommendations to establish sex-specific reference values during the pubertal stage. Determination of the values by using combined sex for children< 12 years of age was based on the general absence of sex differences for the parameters in these age groups. There is a shortage of comprehensive age- and sex-specific biochemical reference values for children in Ethiopia (26)

The result obtained in this study revealed that though the out of range proportion vary, most analytes reference interval varied from the values provided as reference in the insert package to be used for clinical management in the study area both for children and Adolescents. This anticipated difference for population in different placement confirmed the recommendation of the manufacturers and NCCLSI for each laboratory to establish their own reference interval using their own local population (5).

Besides the presences of analogy among this study, for the respective RIs, and other studies in Africa and across the world, there were also few observable clinical chemistry parameter RIs difference inconsistently in each country .However, relatively higher and wider RI for ALT and AST was observed in southern Ethiopia (24), Northern Ethiopia (26), Ghana (18), Zimbabwe (4), Canada (12) and manufacturer than the current study in Adolescent and children.

Reference range of albumin and protein: children and adolescent in current study was almost comparable with study of Tanzania (7), North Ethiopia (26) and Canada (12)

The lower reference limit of bilirubin (direct) of Adolescent and children in this study was comparable with similar bilirubin RIs study conducted in Kenya (20), and Ghana(18) were as the total BIL not similar with other study and manufacturer clamed. Lower reference range of

current study for ALP was slightly lower than Northern Ethiopia (24), Kenya (15), Tanzania (7), Zimbabwe (23), Canada (12) and Manufacturer but higher than southern Ethiopia (26)

Moreover the lower reference limit of this study for AST, ALT and ALP was slightly higher than the kit manufacturer's declared limit whereas the upper limit of ALT and AST were lower than the values in the leaflets. Although the lower limit of urea in this study was similar to the manufacturer's values and that from northern Ethiopia (24) but the upper limit is higher compared to both. In spite of a matching Upper Reference range of creatinine in current study was slightly lower than Northern and Southern Ethiopia (24, 26), Tanzania (7), Australia (16) and Manufacturer. Factors like demographic variation, ethnic and genetic differences, life style and seasonal differences might be entities which contributed for such relatively inconsistent value of clinical laboratory (20). In the present study, eleven routine chemistry test markers were assessed; many of them required either by age or by sex partitions, but Glucose and Albumin activities did not vary by sex and ALT and Urea by age across the pediatric.

Similarly, the two hepatic enzyme markers were studied: ALP, AST, and all of them varied by age and sex. ALT activities decreased in children, where sex differences were observed with higher upper and lower reference limits in males.AST activities persistently decreased in children; ALP activities demonstrated both increases and decreases with age. Although ALP activities were increased in children, its activities increased prior to and the lower limit decreased during adolescence. Similar to the other hepatic enzymes, ALP and AST activities were generally higher in males than females. Overall, hepatic enzymes required relatively complex age and sex partitioning. Four non enzymatic hepatic markers were also tested: Total protein, albumin, direct bilirubin, and total bilirubin and three renal markers were also included in the present study: urea, creatinine, and uric acid. Uric acid concentrations increased and deviated by sex during adolescence. On the other hand, urea concentrations were stable in children, and in adolescence, and also are showing sex differences. Creatinine concentrations required sex and age partitions. Overall, renal marker concentrations changed in children and adolescence and showed sex differences.

Enzyme activity levels initially increased slightly in children, with the highest levels during the main growth period. After cessation of growth following puberty, a total of 2 age partitions were required for ALP, between5 and 17 years of age, reflecting the period of most profound bone changes. Sex differences were also observed for all partitions between 11 and

17 years, which is suggestive of unique sex-specific bone changes that occur throughout most of life (11).

Albumin and total protein rising concentrations range in both age groups and total protein observed Sex differences in Adolescent. The lower limits of the liver function markers ALT slightly increased with age. These trends are consistent with previous CALIPER reports for the pediatric population (20). the lower limit of AST in children higher than adolescent, AST is expressed in other tissues such as the heart, red blood cells, and kidney and this differential expression pattern may explain why AST activities are higher in children as this trend may parallel the growth and development of organ systems other than the liver at an early age. Sex differences appeared around adolescence for all liver markers and persisted over the subsequent age partitions. Females generally showed lower values than males, in agreement with previous reports for AST (26, 24) but not for ALT.

Total bilirubin increased with age, with the highest concentrations occurring during the transition period from child to adolescent. As expected, concentrations of the renal markers serum creatinine, urea, and uric acid all increased with age. The amount of creatinine produced daily from breakdown of creatine in muscles correlates with increased muscle mass (12). Similarly, urea and uric acid are related to protein degradation and would also be expected to increase with growth and development. This was particularly evident in children and adolescence, in which 2 age partitions were required for creatinine, 3 for urea, and 4 for uric acid consistent with previous CALIPER trends (20).

In summary, the nationally representative data, extensive sampling procedure, and rigorous statistical analysis for the current study have resulted in reference values that are representative of the Addis Ababa population from adolescent and children. Although decision limits may be more useful than reference values for selected analytes in interpretation of test results in a clinical setting, the trends observed here have important implications when using these biochemical markers for disease monitoring and will be useful in a broad public health research context, as well as to help initiate future studies on harmonization of reference intervals

8. Strengths and limitations

Strengths

As the study was community based on children and adolescents, who were thoroughly checked by clinician diagnosing a wide range of condition together with the laboratory result. Moreover, samples were analyzed in a laboratory that has been participating in external quality assessment program like on site evaluation and External Quality Assessment (EQA), proficiency testing (PT) schemes by Randox international Quality Assessment scheme (RIQAS) and also ISO 15189 certified Laboratory

Limitation

The study was limited to determination of RI for Glucose, ALT, AST, ALP, TBIL, DBIL, TP, ALB, UREA, CREA and UA only and young children less than 5 years old were not included.

9. Conclusion and recommendation

9.1. Conclusion

In the current study majority of the common liver and renal function test reference interval of healthy children and adolescent no uniform pattern among different studies: need one's own RI and there was significant difference in the majority of common liver and renal function parameters between children and adolescent. Reference intervals established from samples of adult (manufacturer clamed) are not necessarily applicable for passing medical decision for children and adolescent. Since the awareness of such variation are important in the interpretation of RFT and LFT is results, therefore establishment and use of local reference ranges should be encouraged because it enhances patient care and health research. Overall, adolescent and children reference intervals reported in the present study will allow for more accurate laboratory assessment of pediatric patients tested using biochemical assays on commonly used in clinics and hospitals around the world.

9.2. Recommendations

This reference interval can be used for children and adolescents in Addis Ababa using the same instrument platform. Significant differences in reference intervals are taken into consideration; thus, it is recommended that these reference values be verified by each laboratory using local pediatric blood samples, based on CLSI guidelines, before clinical implementation.

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Annex I: Laboratory Procedures

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 1500-3000 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

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 Formal Ether Sedimentation Technique

1. Wear gloves when handling stool specimens.
2. In a suitable container, thoroughly mix a portion of stool specimen about the size of a walnut into 10mL of saline solution. Mix thoroughly.
3. Filter the emulsion through fine mesh gauze into a conical centrifuge tube.
4. Centrifuge the suspension at relative centrifugal force (RCF) of 600 g (about 2000 rpm) for no less than 10 minutes. The suspension should yield about 0.75ml of sediment for fresh specimens and 0.5 ml for formalin zed feces.
5. Decant the supernatant and wash the sediment with 10 ml of saline solution. Centrifuge again and repeat washing until supernatant is clear.
6. After the last wash, decant the supernatant and add 10 ml of 10% formalin to the sediment. Mix and let stand for 5 minutes to effect fixation.
7. Add 1 to 2 ml of ethyl acetate, Stopper the tube and shake vigorously.
8. Centrifuge at 450 g RCF (about 1500 rpm) for 10 minutes. Four layers should result as follows
    1. a top layer of ethyl acetate;
    2. plug of debris;
    3. layer of formalin; and
    4. sediment
9. Free the plug of debris from the side of the tube by ringing with an applicator stick. Carefully decant the top three layers.

With a pipette, mix the remaining sediment with the small amount or remaining fluid and transfer one drop each to a drop of saline and iodine on a glass slide. Cover with a coverslip and examine microscopically for the presence of parasitic forms

Annex II: Information Sheet (English version)

Addis Ababa University, Collage of health science,

Department of medical laboratory science

E-mail: SMLT@ethionet.et

Tel. +251 112-75-51-70

Participant Information sheet

Principal Investigator: Letebirhan G/Egzeabher(BSc.)

Advisors: Dr. Aster Tsegaye (MSc, PhD)

Dr. Mistre Wolde(MSc, PhD)

Sponsor:-Ministry of science and Technology (MoST) and Addis Ababa University

Study Title: Establishment of Reference Intervals for Clinical chemistry parameters among Adolescent and children in Addis Ababa population, Ethiopia.

Introduction:

Hello!

My name is ----------and I am a second year MSc student of Clinical Laboratory Science in clinical chemistry specialty at Addis Ababa University, College of Health Science, and Department of Medical Laboratory Science. I am conducting a study to establish clinical chemistry parameters Reference Intervals for Addis Ababa population aged 5to17years of age.

.**Purpose of the research:**

The health laboratory plays an indispensable role in the health care system. It supports diagnosis (to rule in or rule out a diagnosis), monitoring of response to treatment, epidemiological surveillance, prevention as well as Research (to understand the patho physiology of a particular disease process). Especially there is lack of local reference interval for indigenous population. Therefore, the purpose of this proposed study is to establish clinical chemistry parameters Reference Intervals for Addis Ababa population aged 5to17years of age.

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

Procedures:

After agreeing that you can take part, the Health Extension workers will ask you some questions which will take up to 15 minutes. Your weight, height and vital signs will be measured. You will be asked to provide urine and fresh stool on a particular container we provide. We will collect 5ml venous blood from you by sterile-disposable vacutainer tube and needle. We will conduct laboratory examination to determine different clinical chemistry parameters and serological tests. You will be asked to provide urine (for chemical tests)

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. Blood and Urine collected will not be used for other purposes. The leftover samples will be stored at the Addis Ababa University Department of Laboratory for additional tests as needed. Finally, all the biological wastes, after analysis will be safely disposed in an environmentally friendly manner.

Risks and Discomfort:

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 clinical nurse working as Health Extension Worker.

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 blood sample (that is not stored) will be discarded following the guideline of bio-safety.

Benefits:

By participating in the study, you will directly benefit by being investigated for any pathogenic organisms and other clinical chemistry abnormalities. Establishing the reference interval will be used in the future to improve the general health status of Addis Ababa population.

Incentives:

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

Right to refuse or withdraw:

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

Whom to contact:

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

Letebrhan G/Egzeabher----------0912446576

Code No._____

Annex III. Consent form for parents/guardians

I have read the information above, or it has been read to me. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. I voluntarily consent that my child participates in this study (provided he/she gives assent for children 12-17 years).

To give his/her stool☐

To give his/her urine☐

To collect her/his blood ☐ and

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

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

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

_____

If illiterate;

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

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

_____

Print name of researcher, date and signature of researcher

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

_____

Code No._____

Annex IV. Assent form for children aged 12-17 years

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

To give my stool ☐

To give my urine ☐

To collect my blood ☐ and

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

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

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

_____

If illiterate;

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

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

_____

Phone number (parents/guardians)_____

_____

Print name of researcher, date and signature of researcher

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

_____

Annex V. Questionnaire

Questionnaires to be filled by health professionals

Part I. General information

Code Number_____ Region _____ Zone _____Woreda_____/city /_sub city _____ Kebele _____

Part II. Personal information

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

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

	Questions 12-17 are additional questions to Students	
12.	Father's Age	_____
13.	Mother's Age	_____
14.	Father's Educational Level	1. Illiterate 2. Read and write 3. Primary (1-8) 4. Secondary (9-12) 5. College diploma/degree and above
15.	Mother's Educational Level	_____
16.	Father's Occupation	
17.	Mother's Occupation	
18.	Monthly income (in birr collected from salary, rent, and other income)	_____Birr
19.	Family Size (Number of People)	_____
20.	Source of water	1. Pipe 2. Spring water 3. Well water 4. River 5. Other sources (specify)
21.	Type of house	1. Mud 2. Cement 3. Wood 4. Bricks 5. others/specify_____
22.	Presence of or contact with Pet animals (e.g. Cat, Dog)	1. Yes 2. No
23.	Presence of domestic animals	1. Yes 2. No
	Part IV. Clinical information	
24.	Did you take any type of drug for any illness for the last three month?	1. Yes 2. No
25.	If yes to Q24, 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	
26.	History of diabetes	1. Yes 2. No

27.	History of Hypertension	1. Yes 2. No
28.	History of Blood transfusion for the last 1 year	1. Yes 2. No
29.	Any history of blood transfusion	1. Yes 2. No
30.	History of Hospital Admission for the last 1 year	1. Yes 2. No
31.	History of Surgical procedure for the last three years?	1. Yes 2. No
32.	History of chronic gastritis	1. Yes 2. No
33.	History of Malaria for the last 6 month	1. Yes 2. No
34.	History of TB for the last two years	1. Yes 2. No
35.	History of Cancer	1. Yes 2. No
36.	History of Cardiac illness	1. Yes 2. No
37.	History of Bleeding disorders	1. Yes 2. No
38.	History of allergy	1. Yes 2. No
39.	History of Wheezing	1. Yes 2. No

	Part V. Anthropometric measurement	
43	Height (in cm)	_____
44	Weight (in kg)	_____
45	MUAC	_____in cm (will be interpreted later)
46	Blood pressure (mm Hg)	_____

❖ We thank you for your cooperation!

Interview Date:_____

Interviewer's Name_____ Signature_____

Annex VI: Information sheet (Amharic Version)

የ ፕሮጀክቱ ርዕስ፡ “እድሜ ቸውከአስራ ሰባት ዓመት በታች ለሆኑ የ አዲስ አበባ ጤፍ ማሰ ውደምሪፈረንስ ኢንተርቫል ማከራት።”

የ ፕሮጀክቱ ዋና ተሚራሚሪ: ለተብርሀን ገ/እግዚአባሄር (ቢ.ኤስ. ሲ)

ስፖንሰር (ወጪውን የ ሸፈነ ው)፡ የ ፌደራል ሳይንስና ቴክኖሎጂ እና ጤፍ ሚኒ ስቴር

መግቢያ:

ጤፍ ይስጥልኝ! ስሜ----------

ስሆን፣ በአዲስአበባዩኒ ቨርስቲየ ህክምና ላቦራቶሪ ሳይንስትምህርትየ ሁለተኛዲግሪት ምህርትእየ ተከታተልኩኝበ ሚሆኑ እድሜ ቸውከሰ ምክትእስከከአስራሰባትዓመትለሆኑ የ አዲስአበባከጤፍ ማሰ ውሪፈረንስኢንተርቫልለ ማውጣትጥና ትእያካሄድኩኝነ ው።

የ ምርምር ጥና ቱ አላማ

የ ህክምና ላቦራቶሪበ ጤፍ ውአገ ልግሎትውስ ጥከፍተኛ ሜኛ ይጫወታል፡ ፡ ምር ሚራንለ ሚረ ጋ ገ ጥ፣ ህ መማንለ ሜድሃኒ ቶችምላ ሽማከጤታቸውን ክትትልለ ሜድረ ግ፣ የ በሽ ታዎችን ስር ጭት ለ ማጥና ት፣ በ ሽ ታለ ማከላክልእና ስለበ ሽ ታዎችምን ጭምር ምር ለ ሜድረ ግአስተዋፅ ዎያደር ጋል፡ ፡ በተለይማከአገ ራችን የ ጤፍ ማሰ ውየ ላቦራቶሪ ውጤት ማወዳደሪያ ሪፈረንስኢንተር ቫልስለሆነ ምሞ ዚህ ጥና ትዓላማ ሞ አዲስአበባ ጤፍ ማሰ ውየ ክሊኒ ካልኬምክትሪ ውጤት ማወዳ ደሪያሪፈረንስኢንተርቫልእድሜ ቸውከአ ምክትእስከከአስራሰባትዓመትለሆኑ ማሠራት ነ ው ፡

እርስዎምላዚህ ጥና ትተሚ ጠዋል፡ ፡ ስለዚህበዚህ ጥና ትእን ዲሳተፉና የ ጤፍ ማሰ ውየ ክሊ ኒ ካልኬምክትሪ ውጤት ማመዳደሪያሪፈረንስኢንተርቫልለ ማከራትአስተዋፅ ዎእን ዲያደር ጉተጋብዘ ዋል፡ ፡ ስለዚህየ ዚህ ጥና ትውጤትኢትዮጵያውስ ጥየ ልጆችን ጤፍ ንለ ማሻ ሻልይረ ዳል፡ ፡

የ ጥና ቱአ ካሄድ:

በ ጥና ቱለ ማሳ ተፍከ ተስ ማሞቁ ጥና ቱአ ባል/አ ባላ ት

ደቂ ቃየ ሚወስ ድጥያ ቄይጠይቁ ዎታል፡ ፡ ክብደ ት፣ ቁ ሞት፣ የ ክን ድእና የ ደ ምግፊትልኬትይ ወሰ ዳል፡ ፡ ሽን ትበ ምን ሰ ጠውእ ቃእን ድትሰ ጡን እን ጠይቃለን፡ ፡ በን ፁህ ቫኩቴይነ ር ብል ቃጥእና ሚ ፈእን ቀዳለን

(5ሚሊሊትር በ ባዶቲዩ ብ)የ ኬሚስ ትሪ፣ ሴሮሎጂ፣ ምር ሚራዎችን እና ካሂዳለን፡ ፡

ሚስጥር ስለማክበቅ:

በዚህጥናትየሚሰበሰብሚጃበማሉበሚስጥርይጠበቃል፡ ፡ ሚጃበዚህየስምማ ትቅፅከ ተፈቀደውጩሊሶስተኛወገንተላልፎአይሰጥም ፡ የዚህጥናትውጤትሊታተምይችላልነገ ርግንየጥናቱተሳታፊዎችስምና ማንኛውምመላያአይገለፅም ፡ ሚስጥራዊነቱንለማክበቅ የዚህጥናትአባልሚጃዎችንበተቆለፈክፍልበተቆለፈካቢኔ ትውስጥያስቀምጣል፣ የፈቃ ደኛተሳታፊዎችንማንነ ትንላለማሳወቅውጤቶችምክኮድይቀጣሉ፡ ፡ በኮምፒዩተርውስጥ ለተቀማጩፋይሎችለጥናቱተሚራሚብቻየ ሚፈቀዱና በሚስጥርቁልፍየ ሚከበቁይሆናል፡ ፡ የተሳታፊውጤትለህክምና ባለሞያሊተላለፍየ ሚቻለውብተሳታፊውፈቃድብቻነ ው፡ ፡ የተሰ በሰበውደምና ሽንትለሌላአገልግሎትአይውልም ፡ የሚኮርፉትናመዋቾበአዲስአበባዩ ኒቨርስቲላቦራቶሪክፍልደህናቦታተቀምጠውለተጨማሪ ምርሙራዎችእንደአስፈላጊታቸው ጥቅምላይይውላሉ፡ ፡ በሜጨፈሻምቱሰርቶባቸውየ ተራረፉየ ሚፎናመዋቾአካባቢንበማ ይበክልሜልኩበጥንቃቄይወገዳሉ፡ ፡

ጥናቱየሚያስከትላቸውየጠፋ ችግሮችና አለመመቾት:

ደምበሚቆዳበትጊዜ ማከነኛ መሳዳትና የተወሰነ አለመመቾት ሊኖርይችላል፡ ፡ ይሁን እንጂ በተቻለማከን ልምድ ያለውየ ጠፋ ባለሞያዎች በማከቀምአለመመቾቱን ለመቀነስ እንሞክራለን፡ ፡ ሆኖምሽንት (ሴትከሆኑ) በማጡት የሚደርስ ማከነኛ አለመመቾት ሊኖር ይችላል፡ ፡

ደህንነት:

የደምናመዋ በሚወሰድበትጊዜ በንፁህ የደምመቅጃ በማከቀምየ ሚቆዳውን ቦታ በ70% አልኮል በመፅዳት ልምድ ባለውየ ጠፋ ባለሞያዎች ይከናወናል፡ ፡ በተጨማሪ ምጥቅም ላይ ከዋሉ በኋላ ለማስቀጭጥየ ሚይሆኑ የ ሚደፉ ደምና ሽንት (ሴትከሆኑ) ትራፊዎች የላቦራቶሪ ደህንነት መመሪያ በማተል ይወገዳሉ፡ ፡

ጥቅማጥቅሞች፡

በዚህጥናትበማተፍየ ደምና ምርሙራበ ሚድረግየ ጤንነ ትሁኔ ታማወቅይቻላል፡ ፡ የ ጠፋ ማ ሰውየ ኬሚስትሪውጤትማወዳደሪያሪፈረንስኢንተርቫልእድሜ ቾውክአምስትእስከከአስ ራሰባትዓመትለሆኑየአዲስአበባጠፋ ማሳውደምማሳራቱየ ጠፋሁኔ ታለማሻሻልይረዳል፡ ፡

በጥናቱለማሳተፍ ማትጊያ:

በደምምርሙራጠፋ ሚልሆነ ውጤትከተገኝበአቅራቢውወደ ሚ ኝጠፋ ተቋምይላካሉ፣ የላቦ ራቶሪውጤቶቹንበነፃያገኛሉ፡ ፡ ይሁንእንጂበዚህጥናትለማሳተፍምሆነ ለመድሃኒትክፍ ያአይሰጥም ፡ ስለተሳትፎዎግንእናማሳግናለን፡ ፡

ያለማኅተፍ ማበት:

በዚህ ጥና ትከተሳተፉየ ቻልነ ውን ሁሉእን ክብካቤእና ደርጋለን፡ ፡ በማቹ ውምሳዓትከጥና ቱማውጣትእንደሜቻልና ይህ ምክ ማያገኙትአገልግሎትላይ (ለምሳሌየ ጡፍ አገልግሎት) ምንምአይነ ትልዩነ ትአይደረግም

ጥያቄ ካለ ለማ ጋገር:

ምንምዓይነ ት ጥያቄ ካለ የደምና ማኖ የሰጡትን ሰውማከየ ቅ ይቻላል በሚከተለው አድራሻ ማከየ ቅ ይቻላል፡ ፡

ለተብርሀን ገ/እግዚአ ባሄር

ሞባይል፡ +251 912446576

ኢሜይል: Ltsgbr@yahoo.com

Annex VII. Consent form for parents/guardians (ለወላጆች/አሳዳጊዎች የስምምነት ቅፅ)

ከላይየተገለፀውን ሚጃአንብቤአለሁ /ወይምተነቦልኛል፡፡
ጥያቄለማከየቅዕድልተሰጥቶኝጨይቄበሚያረካሜልኩተሜልሰልኛል፡፡
ልጄእንዲሳተፍ/እንድትሳተፍተስማምቻለሁ፡፡

አምስት ዓመትና እስከ አስራ ሰባት ዓመት በታች ለሆኑ ልጄ ከተስማማ/ማቹ በዚህ ጥናት እንድትሳተፍ/እንዲሳተፍፈቃደኝነቴን ገልጫለሁ፡፡

የ ዓይነ ምድርናማ ለማነጠት☐
የ ሽንትና ማ ለማነጠት ☐
ደምለመቀዳት ☐
እናበዚህጥናትተሳታፊለሚሆን፣
በማንኛውምሳዓትልጄን ከጥናቱለማነወጣትመብትእንዳለኝምተረድቻለሁ☐.

የተሳታፊስም ቀንእናፊርማ(ወይምአሻራ) ከዚህበታቸይፃፉ

_____ ____ /____ /____

(ቀን/ወር/ዓመተምህረት)

ያልተሙከሆኑ;

የተሙገለልተኛእማኝሰውስም፡

ቀንናፊርማ(ከተቻለይህሰውበተሳታፊውኪሚጥናከተሚሚሚአባላትግኑኝነ ትየሌለውኪ ሆን)

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

_____

ስልክቁጥር _____

የተሚሚሚውስም፡ ቀንናፊርማ

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

_____

ኮድ._____

Annex VIII. Consent form for children (ከ 12—17 ዓመት ለሆኑ ህፃናት የስምምነት ቅፅ)

ከላይ የተገለፀውን ሚጃ አንብቤአለሁ/ወይምተነቦልኛል፡፡ ጥያቄ ለማከየቅ ዕድል ተሰጥቶኝ ጠይቄ በሚያረካ ሜልኩ ተሜልሶልኛል፡፡ ወላጆቼ እስከ ፈቀዱ ድረስ በዚህ ጥናት ለማሳተፍ ተስማምቻለሁ፡፡

የ ዓይነ ምድር ናመኛ ለማስጠት☐

የ ሽንት ናመኛ ለማስጠት☐

ደምላ መቀዳት ☐እና በዚህ ጥናት ተሳታፊ ለሚሆን፣ በማኛውምሰዓት ከጥናቱ ለመውጣት መብት እንዳለኝምተረድቻለሁ☐.

የተሳታፊስም ቀንእናፊርማ(ወይምአሻራ) ከዚህበታችይፃፉ

_____ ____ /____ /____

(ቀን/ወር/ዓመተምህረት)

ያልተሜ ከሆኑ;

የተሜ ገለልተኛ እማኝ ሰው ስም፡ ቀንና ፊርማ(ከተቻለ ይህ ሰው በተሳታፊው ቢሚጥና ከተሜራሜሪ አባላት ግኑኝነት የሌለውቢሆን)

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

_____

ስልክቁጥር (የ ወላጅወይምአሳዳጊ)_____

_____

የ ተሜራሜሪ ውስ ም፡ ቀንና ፊርማ

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

_____

Annex IX: Questionnaire Amharic version (ቃለ ማኪይቅ)

በ ጡፍ ባ ለ ሞያ ዎች የ ሚሞላ ቃለ ማኪይቅ

መመሪ ያ ፡

በ ቅድሚያ ይህ ን ን ቃለ ማኪይቅ ለ መሞላ ትለ ሰ ጡን ጊ ዜና ትብብር አ ድና ቆ ቴ ን እ ገ ል ፃ ለ ሁ ፡ ፡ የ ዚህ ቃለ ማኪይቅአ ላ ማ'በ ላ ቦ ራቶ ሪ ውስ ጥየ ጥራት መሞር ሚ ያ ን ጥረ ነ ገ ር እ ና የ ጡፍ ማሳ ውደ ምውስ ጥየ ማ ኙየ ክ ሊ ኒ ካ ል ኬ ሚሳ ትሪ ምር ሚራዎች ማከን ሪ ፈ ረ ን ስ ኢ ን ተ ር ቫ ል እ ድሜ ል ቸውአ ምክ ት ዓ ሞት ና እ ስ ከ አ ስ ራ ሰ ባ ት ለ ሆኑ ኢ ት ዬ ጵ ያ ውያ ን ለ ማክ ራ ት "ሚ ጃ ለ ማሳ ብ ሰ ብ ነ ው ፡ የ ዚ ህ ጥ ና ት ሃ ሳ ቡ ን ያ ማከ ት የ ጥ ና ቱ ዋ ና ተ ሚ ራ ሚ ሪ በ አ ዲ ስ አ በ ባ ዩ ኒ ቨ ር ስ ቲ የ ህ ክ ምና ላ ቦ ራቶ ሪ ት ምህ ር ት ክ ፍ ል ተ ባ ባ ሪ ፕ ሮ ፌ ሰ ር የ ሆኑ ት ዶ/ር አ ስ ቴ ር ፀ ጋ ዬ ሲ ሆኑ የ ኢ ት ዮ ጵ ያ ህ ክ ምና ላ ቦ ራቶ ሪ ማህ በ ር ያ ስ ተ ዳ ድ ረ ዋ ል ፡ ፡

የ ጥ ና ቱ ን ወ ጪ የ ሸ ፈ ነ ውየ ፌ ደ ራ ል ሳ ይ ን ስ ና ቴ ክ ኖ ሎ ጂ ሚ ኒ ስ ቴ ር ነ ው ፡ ስ ለ ሆነ ምየ እ ር ስ ዎ ቅ ን ት ክ ክ ለ ኛ ሜ ል ስ በ ሰ ዓ ቱ ማክ ጡት የ ዚ ህ ን ጥ ና ት ስ ኬ ት ይ ወ ስ ና ል ፡ ፡ አ ስ ራ አ ምክ ት የ ሚ ሆኑ ተ ቋ ማት ማ ላ ት ም ቁ ኒ ቨ ር ስ ቲ ዎች ፣ ሪ ጅ ና ል ላ ቦ ራቶ ሪ ዎች ፣ እ ና ብሄ ራ ዊ የ ደ ም ባ ን ክ አ ገ ል ግ ሎ ት ጥ ና ቱ ን ለ ሚ ደ ገ ፍ ዝ ግ ጁ ነ ታ ቸ ው ን ገ ል ፀ ዋ ል ፡ ፡ ስ ለ ሆነ ምይ ህ ን ን ቃለ ማኪይቅ ሃ ቀ ኝ ነ ት ና ሃ ላ ፊ ነ ት በ ተ ሞላ ውመ ን ገ ድእ ን ዲ ሞ ሉ በ ት ህ ት ና እ ጠ ይ ቃ ለ ሁ ፡ ፡

አ መሰ ግ ና ለ ሁ!!!

ክ ፍ ል 1.አ ጠ ቃ ላ ይ ሚ ጃ

ኮ ድ_____ ክ ል ል _____ ዞ ን _____

ወ ረ ዳ_____ ከ ተ ማ/ክ ፍ ለ ከ ተ ማ _____ቀ በ ሌ _____

ክ ፍ ል 2.የ ግ ል ሚ ጃ

1. እ ድ ሜ_____
2. ጾ ታ_____
3. የ ት ውል ድ ቦ ታ_____
4. በ ት ውል ድ ቦ ታ ዎ ለ ምን ያ ህ ል ጊ ዜ ኖ ረ ዋ ል ?_____
5. አ ሁ ን ያ ሉ በ ት ቦ ታ ለ ምን ያ ህ ል ጊ ዜ ኖ ረ ዋ ል ?(ከ ት ውል ድ ቦ ታ ዎ የ ተ ለ የ ከ ሆነ)_____ዓ ሞት

ቁጥር.	ጥያቄ	ምላሽ
	ክፍል 3. ሚህበራዊና ኢኮኖሚያዊሚዛ	
6	የትምህርትደረጃ	6. ያልተማሩ 7. ማንብናመፃፍ 8. አንደኛደረጃ (1-8) 9. ሁለተኛደረጃ (9-12) 10. ኮሌጅዲፕሎማዲግሪእናከዚያበላይ
7	ሥራ	7. ተማሪ 8. የቤትእማቴት 9. የመንግስትሠራተኛ 10. የግልተቀጣሪ 11. ገበሬ 12. ሌላካለይግለፁ _____
8	የጋብቻሁኔታ	6. ያላገቡ 7. ያገቡ 8. የተፋቱ 9. ባል/ሚስትየሞተባቸው 10. አይመላከታቸውም(ህፃናት)
9	ሃይማኖት	6. ኦርቶዶክስክርስቲያን 7. ሙስሊም 8. ፕሮቴስታንት 9. ካቶሊክ 10. ሌላካለይግለፁ _____
10	ብሄረሰብ	_____ድብልቅከሆኑይግለፁ
11	ሞሪያቦታ	2. ገጠር 2. ከተማ
	ጥያቄ 7-12 ለተሚዎችተጨሚጥያቄዎች	
12	የአባትእድሜ	_____
13	የእናትእድሜ	_____
14	የአባትየትምህርትደረጃ	6. ያልተማሩ 7. ማንበብናመፃፍ 8. አንደኛደረጃ (1-8) 9. ሁለተኛደረጃ (9-12) 10. ኮሌጅዲፕሎማዲግሪእናከዚያበላይ
15	የእናትየትምህርትደረጃ (ከተ/ቁ 14 ይምረጡ)	_____
16	የአባትሥራ	1. ተማሪ 2. የቤትእማቴት 3. የመንግስትሠራተኛ 4. የግልተቀጣሪ 5. ገበሬ 6. ሌላካለይግለፁ _____
17	የእናትሥራ (ከተ/ቁ 16 ይምረጡ)	_____

18	ውሃ ዊገ ቢ (በ ብር ከ ደ ሞዝ፣ ኪራይ፣ እ ና ሌሎች ገ ቢ ዎች)	_____ብር
19	የ ቤተሰ ብ ብዛ ት	_____
20	የ ውሃ ምን ጭ	6. ቧ ን ቧ 7. የ ምን ጭ 8. የ ጉ ድጓ ድ 9. የ ወን ዝ 10. ሌላ ካ ለ ይግ ለ ፁ _____
21	የ ቤት አ ይነ ት	2. ጭቃ 2. ሲሚን ቶ 3. እ ን ጨ ት 4. ጡብ/ሸ ክ ላ 5. ሌላ ካ ለ ይግ ለ ፁ _____
22	የ ቤት ውስ ጥ ለ ሚዳ እ ን ስ ሳ ሞ ር ወይም ን ክ ኪ (ለ ምሳ ሌ ድ ሞ ት፣ ውሻ)	2. አ ለ 2. የ ለ ም
23	የ ቤት እ ን ስ ሳ ት ሞ ር	2. አ ለ 2. የ ለ ም
	ክ ፍ ል 4. የ ጠ ፍ ሚ ጃ	
24	ባ ፉ ት ሶ ስ ት ወራ ለ ማ ን ኛ ው ም ሣ ይ ነ ት ህ ሙ ማ ን ኛ ው ን ም ሣ ይ ነ ት ሞ ድ ሃ ኒ ት ወ ስ ደ ኋ ል ?	2. አ ዎ ን 2. የ ለ ም
25	ለ ተ ራ ቁ ጥ ር 24 ሜ ል ስ ዎ ወ ስ ጃ ለ ሁ ከ ሆ ነ የ ት ኛ ው ን ዓ ይ ነ ት ሞ ድ ሃ ኒ ት ነ ው ወ ሰ ዱ ት ? (ከ አ ን ድ በ ላ ይ ሜ ል ስ ይ ቻ ላ ል)	8. ፀ ረ -ፕ ሮ ቶ ዞ አ 9. ፀ ረ -ሄ ል ሚ ን ት ስ 10. ፀ ረ -አ ለ ር ጂ 11. የ ወ ሊ ድ ሞ ክ ላ ከ ያ ኪ ኒ ን 12. ፀ ረ -ባ ክ ቴ ሪ ያ 13. ፀ ረ -ቲ ቢ 14. ሌላ ካ ለ ይግ ለ ፁ_____
	የ ሚ ከ ተ ሉ ት የ ህ ሙ ማ ይ ነ ቶ ች አ ሞ ት ያ ው ቃ ል ?	
26	የ ስ ኪ ር ህ ሙ ም ?	2. አ ዎ ን 2. የ ለ ም
27	የ ደ ም ፊ ት ከ ፍ ማ ለ ት ?	1. አ ዎ ን 2. የ ለ ም
28	ባ ለ ፈ ው 1 ዓ ሞ ት ደ ም ት ሰ ጥ ቶ ዎ ያ ው ቃ ል ?	1. አ ዎ ን 2. የ ለ ም
29	ማ ን ኛ ው ም ጊ ዜ ደ ም ት ሰ ጥ ቶ ዎ ያ ው ቃ ል ?	1. አ ዎ ን 2. የ ለ ም
30	ባ ለ ፈ ው 1 ዓ ሞ ት ሆ ስ ፒ ታ ል ተ ኝ ተ ው ያ ው ቃ ሉ ?	1. አ ዎ ን 2. የ ለ ም
31	ባ ለ ፉ ት 3 ዓ ሞ ታ ት የ ቀ ዶ ህ ክ ም ና ተ ደ ር ጎ ል ዎ ያ ው ቃ ል ?	1. አ ዎ ን 2. የ ለ ም
32	የ ቆ የ የ ጨ ጨ ራ ህ ሙ ም አ ለ ብ ዎ ት ?	1. አ ዎ ን 2. የ ለ ም
33	ባ ፉ ት 6 ወ ራ ት የ ወ ባ ህ ሙ ም አ ጋ ጥ ሞ ዎ ት ያ ው ቃ ል ?	1. አ ዎ ን 2. የ ለ ም
34	ባ ለ ፉ ት 2 ዓ ሞ ታ ት የ ቲ ቢ ህ ሙ ም ሮ ዎ ት ያ ው ቃ ል ?	1. አ ዎ ን 2. የ ለ ም
35	ካ ን ሰ ር ህ ሙ ም	1. አ ዎ ን 2. የ ለ ም
36	የ ልብ ህ ሙ ም	1. አ ዎ ን 2. የ ለ ም
37	የ ሞ ድ ሞ ት ች ግ ር /ህ ሙ ም	1. አ ዎ ን 2. የ ለ ም
38	አ ለ ር ጂ (የ ሰ ውነ ት ሞ ቆ ጣ ት)	1. አ ዎ ን 2. የ ለ ም

39	የ ሞተ ንፈስ ችግር (ሲተነ ፍሱሲር ሲር የ ሜኣ ድምፅ)	1. አ ዎን 2. የ ለ ም
	ክፍል 5. ክብደት፣ ቁመት፣ የ ክ ንድና የ ደ ምግፊት ልኬት	
59	ቁ መት	_____ሴ ን ቲ ሜ ት ር
60	ክ ብደ ት	_____ኪ ሎ ግ ራ ም
61	የ ክ ን ድ ማ ለ ኛ ው ክ ፍ ል ዙ ሪ ያ ው (MUAC)	_____ሴ ን ቲ ሜ ት ር
62	የ ደ ም ግ ፊ ት (በ ሚ ሊ ሜ ት ር ሜ ር ኩ ሪ)	_____(mm Hg)

❖ ስ ለ ት ብ ብ ር ዎ እ ና ማ ስ ግ ና ለ ን !

ቃ ለ ማ ኪ ይ ቅ የ ተ ደ ረ ገ በ ት ቀ ን :_____

ቃ ለ ማ ኪ ይ ቁ ን ያ ካ ሄ ደ ው ስ ም_____ ፊ ር ማ_____

Declaration

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

M.Sc. candidate: **Letebrhan G/Egzeabher (B.Sc.)**

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: MistireWolde**(MSc, PhD)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

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

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.