ADDIS ABABA UNIVERSITY COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Establishment of serum lipid profile and electrolytes reference intervals for apparently healthy children and adolescents in Addis Ababa, Ethiopia

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This is to certify that the thesis prepared by Ousman Mohammed, entitled: Establishment of serum lipid profile and electrolytes reference intervals for apparently healthy children and adolescents in Addis Ababa, Ethiopia and submitted in partial fulfillment of the requirements for 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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Abbreviations

ATP —Adenosine Triphosphate

CLSI— Clinical and Laboratory Standards Institute

CVD—Cardiovascular Diseases

EMF —Electromotive Force

EPHI— Ethiopian Public Health Institute

HDL—High-Density Lipoprotein

HSDA — 2-hydroxy-3-sulfopropyl-3, 5-dimethoxyaniline

IFCC—International Federation of Clinical Chemistry

ISE — Ion-Selective Electrode

ISO—International Organization for Standardization

LDL—Low-Density Lipoprotein

PI —Principal Investigator

RI- Reference Interval

TC—Total Cholesterol

TG—Triglycerides

VLDL —Very Low Density Lipoproteins

Abstract

Background: Reliable and accurate reference intervals for laboratory analyses are an integral part of correct interpretation of clinical laboratory test results. In Addis Ababa, Ethiopia, there were no reference intervals established on lipid profile and electrolytes which are essential in the assessment of early dyslipidemia and electrolyte abnormalities.

Objective: To establish reference intervals on serum lipid profile and electrolytes for apparently healthy children and adolescents from April to October 2019 in Addis Ababa, Ethiopia.

Methods: Community based cross sectional study was conducted from April to October 2019. A total of 522 participants aged 5-17 years were recruited from communities. After physical examination, socio-demographic, anthropometric data and blood samples were collected. Serum levels of lipid profile and electrolytes were determined using Cobas c501. In accordance with Clinical and Laboratory Standards Institute guidelines reference intervals for four lipid profile and six electrolytes were established by calculating 2.5^{th} and 97.5^{th} limits with 90% confidence interval using SPSS version 23.

Results: In children, the reference intervals for serum potassium, sodium, chloride, calcium, magnesium and phosphate in mmol/L were 4.37-5.2, 137-145.5, 101.9-107.9, 2.34-2.7, 0.74-0.97, and 1.42-1.85 and for total cholesterol, triglycerides, low density lipoprotein and high density lipoprotein the respective values were 100.76-171.7, 44.16-126.36, 60.6-105.6 and 31.6-53.7 in mg/dl, for both sexes. For adolescents 4.03-5.58, 137-146, 98.9-120.9, 2.39-2.7, 0.73-0.96, 0.96-1.8 for serum potassium, sodium, chloride, calcium, magnesium, phosphate in mmol/L and 97.2-189.1, 40.5-143.6, 41.7-120.9 and 21.3-57 in mg/dl for total cholesterol, triglycerides, low density lipoprotein and high density lipoprotein, respectively, for both sexes.

Conclusion: The obtained reference intervals by the current study revealed that both lower and upper limits were in disagreement with the manufacture as well as published literatures. The study also observed significant differences in the reference values between genders for all analytes except sodium, calcium and magnesium at the adolescents. Therefore, it is important to use the current reference intervals.

Key words: reference intervals, lipid profile, electrolyte, children and adolescent

1. Introduction

1.1 Background

Lipids are any organic compounds including fats, oils, and steroids and because of certain components they do not interact with water. They are important as dietary constituents of the high energy value, electrical insulators, in the structure and function of membranes of cells and organelles. Transported in the blood combined with lipoprotein particles (1). Lipid profile tests are used for screening atherosclerotic risk and in the diagnosis and treatment of dyslipidemia. The onset of this disease occurs during the first years of life and manifestation hyperlipidemia in childhood is associated with atherosclerosis at older age (2, 3). The differences in factors like body fat distribution, activity of enzymes involved in lipid hydrolysis, insulin response, and some specific apolipoproteins can partly explain the inter-ethnic differences in serum lipids. It is therefore the evaluation of serum concentrations of these parameters at early ages is most extremely important. Hence, it is important to have valid reference intervals available to identify children at risk as early as possible (4).

Electrolytes are substances that dissociate into negative or positively charged ions when dissolved in water. They are widely distributed in body fluids, maintain physiological functions such as osmotic balance, acid-base balance and intra and extracellular gradients, and play a major role in maintaining metabolic functions. The main extracellular electrolytes are Na, Cl and Ca while K, Mg and PO4 are the major intracellular electrolytes (5, 6). Sodium is the mostly responsible for numbers of important functions, mostly related to fluid and water regulation and potassium for the function of excitable tissues such as skeletal, cardiac muscles and nerves. Monitoring potassium is vital as small changes of its level can largely affect the heart's rhythm and ability to contract (7). Calcium and magnesium are two minerals whose presence is essential for the body, their deficiency causing severe damage to the metabolic functions (8, 9). In addition, phosphorus plays a key role in energy metabolism as part of the ATP structure and phosphorylation and dephosphorylation reactions. Hypophosphatemia affects the production of ATP causing muscle weakness and impairment of hematopoiesis with symptoms of anemia and reduced oxygen delivering (10, 11). Serum electrolyte imbalance resulting from various reasons require urgent

management and in children the problem remain unrecognized resulting in mortality and morbidity. Therefore, it is very essential to recognize the reference intervals adapted to the population which helps in early detection, close monitoring and correction of electrolyte abnormalities in order to make quick and accurate decisions (12).

The idea of population-based reference intervals was first introduced in human medicine in 1969 by Grasbeck. Reference interval is the interval between which 95% of values of a reference population fall into, in such a way that 5% of the values may be outside of the limits. The International Federation of Clinical Chemistry (IFCC) recommends the use of the term "reference" in place of "normal" values. The meaning "normal" has comprises several definitions statistical, epidemiologic, and clinical senses. The use of the term reference intervals avoids the confusion associated with normal and may be used with either health- or disease-associated values (13, 14).

Most reference intervals currently in Africa are derived from foreign populations and may not be reflective of population values elsewhere (15, 16). Interpretation of laboratory results relying on reference intervals derived elsewhere from non-representative reference populations is potentially problematic for both clinical care and clinical trials participation and may lead to mismanagement, an underestimation or overestimation of disease conditions and unnecessary exclusion of patients from participation in important trials. The proper medical evaluation of children is largely dependent on correct interpretation of all laboratory results combined with data collected through physical examinations and medical histories. Such numeric laboratory results interpretation requires relevant reference intervals, ideally established in the local setting (17, 18).

The role of laboratory medicine should not be undermined because about 70-80 % of decisions in diagnosis are based on laboratory test results (19). Appropriate interpretation of these test results has certainly rely on accurate reference intervals determined from a healthy population and partitioned by common subclasses, including age and sex. Reference intervals (RIs) are generally used in the case of medical diagnosis, therapeutic management, following prognosis or any other physiological assessment. In every clinical laboratory, reference intervals for each and every analytes and specimen source are need to be established or verified. Regardless of the major challenges, requiring selection and recruitment of large numbers of healthy individuals representing local community, population-based RIs are widely used in process of making clinical

decision. Even though population-based RI is commonly accepted by all members of group, the best method for their establishment is frequently debated. In addition, only very few manufacturers or laboratories are committed to undertake their own reference interval studies. The rest manufacturers and laboratories depend on RIs from studies done a few decades ago, when both the analytical methods and life style of the population were very different. Therefore, it is a very essential task to derive RIs carefully by the laboratory based on standard protocols (20).

Population-based RIs are derived from a group of well-defined "healthy" reference individuals. Determining how "health" is crucial to the selection of appropriate reference individuals. At least, a clinical history, parasitic screening, and physical examination should be performed. Establishing exclusion criteria is crucial thereby to select reference individuals to achieve a reference group that is most representative of the patient population in health (21). Partitioning or stratification of reference individuals into subclasses (e.g., by age or sex) may be necessary if there are significant differences between the observed data beside this Physiological and clinical significance is considered. Generally, a separate reference interval should be established for a subclass if the difference between the observed means of two subclasses is statistically significant ($P < 0.05$) (22).

The minimum information that should be provided for all reference values includes the constituency of the reference population (e.g. age, sex, and environment), the exclusion criteria for reference individuals, and the method by which the samples were collected, and the analytical and statistical methods used for generating the reference values. The main effects to consider are age and sex to partition for these factors. The pre-analytical and analytical factors should reflect the usual practice in the laboratory. The particular reference interval is thought to be no longer suitable whenever a pre-examination or examination procedure is changed. Thus, the reference interval data should be updated every few years as the reference population change with environmental and nutrition factors (23). Therefore, the current study was intended to address critical gaps in serum lipid profile and electrolytes reference intervals by determining variability with sex and age among children and adolescents in Addis Ababa.

1.2 Statement of the Problem

Although the availability of both accurate laboratory tests and reliable reference intervals are vital for appropriate clinical assessment of children, reference interval establishment can be challenging due to recruitment difficulties, time consumption, resource requirements, and difficulty of obtaining an adequate sample of a reference population. Many of the challenges encountered when establishing children and adolescents reference intervals are related to child development and growth, which can profoundly influence the concentrations of many analytes. Physiological differences like physical size, organ maturity, body fluid compartments, nutrition, and metabolism are more likely affect normal analyte concentrations in these population. These marked fluctuations in Childhood period makes vital the determination of age and gender specific reference intervals and, therefore, improves the clinical laboratory service (24-27).

According to studies discrepancy in RIs for chemistry analytes were much greater than the analytical inaccuracy of tests. For example, the data presented by International Measurement Evaluation Program from Australian and New Zealand participants showed the reference interval variations both upper and lower limit was much higher than that was seen in the measurement result discrepancy (28). IFCC also recommended, for every indigenous population to have their own set of reference limits for each analyte derived from healthy representative individuals (29).

Cardiovascular diseases (CVD) are the leading cause of death in the global adult population (30). Now a day, child and adolescent obesity even in developing countries becomes a great health issue, and which is potentially associated dyslipidemia. Childhood dyslipidemia has become a great health concern due to its potential association with CVD and the metabolic syndrome with consequent occurrence of cardiovascular mortality. It has been recommended to screen dyslipidemia in children ≥2 years old who have a family history of CVDs (31). The normal lipid levels can definitely vary among populations. Coronary heart disease mortality rate among individuals with the same cholesterol level is entirely different in different cultures and each country and every nations is encouraged to have its own reference intervals as clinical decision tool (32).

Reference intervals for clinical laboratory parameters have traditionally been obtained from European and North American populations (33). However, differences have been reported between

these values when compared to healthy African population values (34). Studies conducted in Africa over the last decade have highlighted differences in biochemical parameters including serum lipid and electrolytes, even in children and adolescents, compared to those derived from industrialized populations. For instance, triglycerides, HDL-cholesterol, LDL-cholesterol Calcium, potassium and phosphate differences by Buchanan A et al. in Tanzanian children and sodium, potassium, chloride, calcium and phosphate by Gitimu M et al. in Taita Taveta, Kenya children and adolescents were observed. Additionally, variations in several parameters have also been reported between different African ethnic groups. Despite these differences, few data exist on biochemical reference intervals for healthy children and adolescents in sub-Saharan Africa (35-38). Similarly, in Addis Ababa most of the laboratories follow reference intervals established in the Western population and from reagent manufacturer. The current study was part of a national program aimed at addressing the gaps in children and adolescents reference intervals on serum lipid profile and electrolytes. This study could also provoke the clinician to address the appropriateness of the reference values in use, to consider the source of the reference values in clinical decision making. In addition, this study investigated the influences of age and sex on the concentrations of serum lipid profile and electrolytes which are commonly measured in many laboratories.

1.3 Significance of the study

Reference interval is important to transform a numerical value obtaining from analyte concentration measurements from body fluid samples, into clinically meaningful information. A RI is intended to inform the clinical care provider that laboratory values within the interval indicate a non-diseased condition. Since there is no fixed RIs on lipid profile and serum electrolytes derived from Addis Ababa children and adolescents, this study provides appropriate reference intervals on these analytes for local community. Establishment of RIs benefit patients to get appropriate medical evaluation. In addition, conducting this study could provide the physician with locally derived RIs on serum lipid and electrolytes which is actually helpful for proper diagnosis, treatment and monitoring of abnormal results. It could also be used as transference of this RIs to other comparable community after validation performed. Lastly the study could serves as base line data for the upcoming researchers in this area and also gives information for policy makers.

2. Literature Review

The standard for the production of human population-based RIs was commissioned by the International Federation of Clinical Chemistry (IFCC) in 1970 and resulted in a 6-part series that was adopted by various professional organizations, including the Clinical and Laboratory Standards Institute (CLSI). A revision released by the CLSI in 2008 includes recommendations for transference and validation of RI from other sources and for the use of robust methods for determining RI from small sample sizes (39).

The reference values of lipid parameters released by the National Cholesterol Education Program Expert Panel on Cholesterol Levels in Children and adolescents to define pediatric dyslipidemia were TC ≥ 200 mg/dL, LDL-C ≥ 130 mg/dL, and HDL-C < 40 mg/dL. In addition, the thresholds values of triglyceride recommended by the National Cholesterol Education Program for defining hypertriglyceridemia have been ≥100 mg/dL and ≥ 130 mg/dL in children aged 0–9 and 10–19 years, respectively (40).

The study conducted by Adeli K et al., 2009-2011, in Canada two or more age partitions were required for lipid profile and electrolyte tests. However, sex partitioning was not required for total cholesterol and potassium. Total cholesterol, chloride, LDL cholesterol, triglyceride, potassium and sodium remain almost invariable with sex throughout the child period. While the value of HDL cholesterol is somehow different. K (with 6-79 age group the value was 3.8-4.9 mmol/L for both biological genders. For LDL (with age group of 6-24 yrs) was 46-143 mg/dl for males and females while Na at age group of 6-15 and 16-49 yrs were 136-143 and 137-143 in mmol/L respectively for both sexes. Chloride (6-11 and 12-29yrs) were 101-107, 101-106 for males and 101-107 and 100-107 for females in mmol/L respectively. The value calcium, total at age of 6-15 and 15-19 years were 9.3-10.5 and 6.2-10.4 in mg/dL for both sexes respectively. For 5 to <13 years and 16 to <19 years of the levels of phosphate in mg/dl were 4.1-5.9 and 2.9-5.0 for combined sex while at 13 to <16 years age class the value slightly different 3.5-6.2 mg/dl for males and 3.2-5.5 mg/dl for females. Magnesium level from1 to <19 years was 2.09-2.84 mg/dl for both boys and girls. Total cholesterol (6-15 and 16-19 yrs) were 116-205 and 100-182 in mg/dl for both genders in the given age groups. Evidenced from this study, no sex variations were seen in the reference value of cholesterol total. As for triglyceride (6-29 yrs) was 35-186 in mg/dl for

combined sexes. In contrast to triglyceride the level of HDL became invariable between sexes at age of 6-14 yrs. 35-81 mg/dl. Little change of this value in older age (15-79 yrs) 31-70 and 35-89 in mg/dl for males and females in a relative manner (41).

A community based study done for establishing reference intervals of clinical chemistry analytes in Australia and New Zealand conducted by Tate JR et al., 2013, revealed that 3.6–5.3 mmol/L for age group of 2 yrs to <18yrs for potassium while 133–144 and 97–110 in mmol/L for sodium and chloride at age group of 1 week to <18y for both genders in the given order. The value of calcium for both sexes at age of 2 to <18 years and magnesium 1week to <18years were 2.20–2.65 mmol/L and 0.65–1.10 mmol/L respectively. Finally, the levels of phosphate at different age partitions of 4y to <15y, 15y to <18y and 18y to <20y were 0.90–2.00 mmol/L, 0.80–1.85 mmol/L and 0.75–1.65 mmol/L in a relative manner for combined sexes (42).

In another study conducted on deriving Pediatric reference intervals for general clinical chemistry components in Denmark and Sweden done by Ridefelt P et al., 2017, proposed that total cholesterol (105.3-214.5 mg/dl), triglyceride (32.04-194.9 mg/dl), sodium (136-146 mmol/L for both sexes at the ge range of 0.5-17 years),while for potassium, calcium and magnesium were 3.3-4.6 mmol/L, 2.24-2.60 mmol/L and 0.71-0.94 mmol/L at age class of 5-17years. The study evidenced that no sex or age partitions were required in the establishment of reference intervals for the above given analytes at the childhood period (43).

Hilsted L et al., 2013 collected blood samples from 1429 healthy Danish children, (5-19 years old) for establishing reference intervals and were analyzed on a Roche-Modular-P/ISE-system. The estimated reference limit of females calcium concentration at 5-13 yrs and 14-18 yrs were 2.26-2.58, 1.95-2.58 and for males 2.22-2.58, 2.10-2.58 in mmol/L in a relative manner. Cholesterol and LDL-cholesterol (for 5-18 yrs old combined sexes) 105.3-214.5 mg/dl and 43-132.6 mg/dl respectively; HDL-cholesterol (5-18 yrs old females and 5-13 yrs males) was 39-89.7 mg/dl, for 14-18 yrs old males 31.2-78 mg/dl; magnesium (for 5-13, 14-18 yrs old females and 5-18 yrs old males) 0.73-, 0.65-, 0.71- with the same upper limit (0.93) in mmol/L respectively; phosphate (5-13 and 14-18 yrs females) were 1.09-1.72 mmol/L, 0.72-1.49 mmol/L, for males with the same age ranges were 1.07-1.74, 0.85-1.74 in mmol/L; potassium and sodium (5-18 yrs amd sex both) were 3.26-4.29, 135-147 in mmol/L respectively; triglycerides (5-18 yrs females,

5-10yrs and 11-18 yrs males) were 30.3- as common lower limit and 173.6, 140.6 and 226.1 in mg/dl in a relative manner (44).

Reference values from Texas children's Hospital Clinical Laboratory, USA done by Dean B. Andropoulos, 2011, at age of 5-15 years founds that total cholesterol 135–200 mg/dl, Triglycerides 20–150mg/dL; HDL (6-15 and >16 years) 38–75 mg/dl and 30–64 mg/dl for males while 35–73 mg/dl and 35–80 mg/dl for females respectively. LDL (6–16yrs) 64–130, 60–140 and at 17 yrs. 63–135, 59–141 in mg/dl for males and females respectively. The RIs for Potassium, sodium and chloride at age of (5-17 yr.) were 3.5–5.5, 136–145 and 95–105 in mmol/L for both sexes in respective order. Total calcium at varies age groups of 4–11yrs, 12–13yrs, 14–15yrs and ≥16yrs were 8.8–10.1, 8.8–10.6, 9.2–10.7, and 8.9–10.7 in mg/dl for both girls and boys. 1.6–2.3 mg/dl, 1.6–2.2 mg/dl and 1.5–2.3 mg/dl were the values of magnesium at the given age partitions 6–9yrs, 10–13yrs, and ≥14yrs for combined sexes respectively. The levels serum phosphate 5–7yrs, 8–11yrs, 12–16yrs and ≥17yrs with little differences were 3.1–6.3, 3.0–6.0, 2.5–5.0 and 2.3–4.8 in mg/dl respectively (45).

Zierk J et al. 2015 collected samples by applying an indirect method from 32000 different inpatients and outpatients from a German pediatric tertiary care center and measurements were performed on a Cobas Integra 800 during clinical care over a 6-year period for providing population based reference intervals for aged ≤18 year's children. The continuous reference intervals for plasma electrolytes like sodium, chloride, potassium, calcium, magnesium and phosphate were about 130-145, 100-115, 3.2-5.75, 1.9-2.7, 0.61-1.0 and 1.2-2.6 in mmol/L for combined sexes and all age levels respectively (46).

Dathan Stumpf et al. 2016, collected fasting samples from healthy Germany children and adolescents, presenting 0.5-16 years of age and gender related reference intervals. The study revealed that serum concentration of TC, LDL-C and TG were higher in girls than in boys. While the data of high density lipoproteins (HDL) showed higher concentrations in boys than in girls and no age partitions were needed for all four lipid profile (47).

Population was stratified into only two groups, pediatrics aged between 0-14 in a study done by Molla A et al. in Pakistan. They found that serum total cholesterol level at age group of 0-14 years were 110-219 mg/dl, 113-233 mg/dl; triglyceride at the same age group were 18-150 mg/dl, 22-

157 mg/dl for males and females respectively. For electrolyte at the same age group sodium 135-145 mmol/l, 132-148 mmol/l; potassium 3.4-5.0 mmol/l, 3.7-5.2 mmol/l, calcium 7.9-11 mg/dl, 6.7-11.9 mg/dl, phosphate 3.9-5.5 mg/dl, 3.8-5.7 mg/dl and chloride 101-112 mmol/l, 101-114 mmol/l were established for males and females in a relative manner. As for the age group of (15-60 yrs.) total cholesterol 114-273 mg/dl, 107-272 mg/dl; triglyceride 24-321mg/dl, 17-269 mg/dl; potassium 3.5-4.9 mmol/l, 3.6-4.9 mmol/l; calcium 8.5-10.5 mg/dl, 8.2-10.4 mg/dl, phosphate 2.7-4.6 mg/dl, 3.0-4.6 mg/dl and chloride 98-115 mmol/l, 99-117 mmol/l for males and females respectively while for sodium 134-150 mmol/l both sexes (48).

A reference interval for 1-10 years old apparently healthy children were found for plasma electrolytes like potassium 3.2-4.9 mmol/L; sodium 127.5–141mmol/L; calcium 1.97–2.65 mmol/L, phosphate 1.27–2.29 mmol/L, total cholesterol 85.8–183.3 mg/dl and triglyceride 32.9–240.3 mg/dl as a study done by Manning L et al. in Melanesian, Papua New Guinea. All assays were performed on the Cobas Integra 800 platform (Roche Diagnostics) using reagents supplied by the manufacturer. The study established common reference intervals without need of separations with sex and ages (49).

The recruited children and adolescents have been divided into age cohorts of 5 to <13 years and ≥13 to <18 years by Buchanan AM et al. in a study done in Kilimanjaro region, Tanzania for establishing biochemistry reference values. They obtained reference intervals for chloride in the age range of 5 to <13 years was 98–108 mmol/L; potassium 3.2–5.2 mmol/L; sodium 134–141 mmol/L; calcium 2.2-2.56 mmol/L; magnesium 0.77-0.99 mmol/L and phosphate 1.20-1.90 mmol/L for combined sexes. While for age of ≥13 to <18 years the established RIs were separated for males and females; as for chloride 98–105, 99–106 mmol/ L; potassium 3.6–5.1, 3.6–5.0 mmol/L; calcium 2.18-2.6 mmol/L, 2.2-2.6 mmol/L; magnesium 0.77-0.98 mmol/L, 0.75-0.98 mmol/L and phosphate 0.92-1.79 mmol/L, 0.87-1.83 mmol/L in a relative manner with sex. But for sodium at age of ≥13 to <18 the value was the same for combined sexes 134–140 mmol/L. Similarly for Cholesterol at age of ≥5 to <13 years 82.3-191.5 mg/dl for both genders, at age of ≥13 to <18 years 91.7–177.8 mg/dl and 101-200 mg/dl for males and females respectively; Triglycerides at age range of ≥5 to <13 yrs was 32.9–157.5 mg/dl for both sexes, at age of ≥13 to <18 years 33.8–222.5 and 31.15–174.4 in mg/dl for males and females in the respective order; HDL-cholesterol at age of ≥5 to <13 years 29.3–76.4 md/dl for both sexes, at age of ≥13 to <18

years 25–74.5 mg/dl and 30–73 mg/dl for males and females respectively; LDL-cholesterol at age of ≥5 to <13 years for both sexes was 35.1–123.6 mg/dl, while at age of ≥13 to <18 years 37.4–109.6 and 48–121.7 in mg/dl for males and females respectively (50).

65.74-166.28 mg/dl, 44.29-169.17 mg/dl, 99–114 mmol/L, 1.03–1.84 mmol/L, 3.6–5.6 mmol/L, and 135–151 mmol/L were the derived combined reference intervals by Dosoo DK et al. 2014 for 5-12 years children in the Middle Belt of Ghana for total cholesterol, triglyceride, chloride, phosphate, potassium and sodium respectively. While at the pubertal period (13-17years) the separated reference intervals for total cholesterol 65.74-150.8 mg/dl, 69.61-177.88 mg/dl; triglyceride 35.43-159.43 mg/dl, 35.43-150.57 mg/dl; chloride 95–117 mmol/L, 96–116 mmol/L; phosphate 0.95–1.79 mmol/L, 0.96–1.77 mmol/L; potassium 3.6–5.8 mmol/L, 3.6–5.9 mmol/L and sodium 132–156 mmol/L, 132–152 mmol/L for males and females respectively. According to this study total cholesterol and phosphate revealed significant sex differences but not triglyceride and other serum electrolytes (51).

A community based, cross sectional study using a total of 302 adolescents conducted by Gomani P et al., 2015 for determining reference intervals for Zimbabwean adolescents (aged ≥12 to < 18 years). Sodium 133-155 mmol/L, 135-153 mmol/L; Potassium 3.46-5.34 mmol/L, 3.4-5.3 mmol/L; chloride 96.0-111 mmol/L, 96.0-107 mmol/L; phosphate 0.87-1.81, 0.78-2.0 mmol/L; calcium 1.83-2.73 mmol/L, 1.83-2.85 mmol/L; Triglycerides 29.4-171.8 mg/dl, 26.7-139.7 mg/dl; total cholesterol 91.3-214.1 mg/dl, 75.3-210.6 mg/dl; LDL-C 37.4-138.8 mg/dl, 30.4-129.5 mg/dl; HDL-C 32.8-85.8 mg/dl, 29.6-80.7 mg/dl for girls and boys respectively (52).

A prospective study carried out on children and adolescent's population from Taita Taveta County, in Kenya found that there was significant variations of electrolytes analytes compared to those supplied by the manufacturers together with the reagent kits. According to the study done by Gitimu M et al. only sodium level was statically significant difference (p value =0.038) between male and female study participants (53). However, as far as my literature search goes there is no published reference interval for children and adolescents in Ethiopia. Thus, the present study tried to established reference intervals on serum lipid profile and electrolytes in new study setting in Addis Ababa, Ethiopia.

3. Objectives

3.1. General objective

To establish reference intervals on serum lipid profile and electrolytes in apparently healthy children and adolescents from April to October 2019 in Addis Ababa, Ethiopia

3.2. Specific objectives

- To establish common reference intervals on electrolytes for apparently healthy children and adolescents living in Addis Ababa.
- To establish common reference intervals on lipid profile tests for apparently healthy children and adolescents living in Addis Ababa.
- To explore differences in reference values of serum lipid and electrolytes with sexes and age groups of study participants.

4. Hypothesis

The current reference intervals on serum lipid and electrolytes were different from reference intervals adopted from manufacturer leaflets.

5. Materials and methods

5.1. Study area

The study was conducted in apparently health children and adolescents living in Addis Ababa, Ethiopia. Addis Ababa is the capital city of Ethiopia and the largest city in the country by population with an area of 530.14 square kilometers. The city has through recent years seen a robust annual growth rate, and population counts as of 2017 are growing closer to 4 million with male to female ratio of 0.9. It has three administration levels: City Government which is on the top, 10 sub-city administrations in the middle one, and 116 woreda administrations at the lowest level. Four subcities namely Arada, Yeka, Kirkos and Akaki were included to recruit children and adolescents based on Probability Proportional to Size (PPS) sampling method (54).

5.2. Study design and period

Community based cross sectional study was conducted from April to October 2019.

5.3. Population

5.3.1. Source population

All children and adolescents who were living in Addis Ababa used as source population.

5.3.2. Study Population

All apparently healthy children and adolescents who live in Addis Ababa and fulfill the recruitment criteria were taken as the study subjects.

5.4. Inclusion and exclusion criteria

5.4.1. Inclusion criteria

- Apparently healthy individuals aged between five years to seventeen who were voluntary to participate in the study and lived in the study area at least for five years.

5.4.2. Exclusion criteria

- Those experiencing an acute illness and having chronic diseases
- Individuals taking alcohol

- Individuals having malaria, intestinal parasites and abnormal urine chemical tests and C-reactive protein

5.5. Study variables

5.5.1. Dependent variables

- RIs of serum lipid profile (TC, TG, LDL-C and HDL-C)
- RIs of serum electrolytes (K^+, Na^+, Cl^-, Ca, Mg and Po4)

5.5.2. Independent variables

- Sex
- Age

5.6. Sample size determination and Sampling technique

5.6.1. Sample size determination

CLSI recommends that the best means to establish a reference interval is to collect samples from a sufficient number of reference individuals to yield a minimum of 120 samples for analysis, by using non-parametric means for each partition and direct sampling method with a power of 90%. The Clinical Laboratory Standards Institute/International Federation for Clinical Chemistry (CLSI/IFCC) recommended non-parametric methods of analysis to establish RIs. Separate reference intervals for boys and girls for different age groups may not be justified unless they could be clinically useful and/or are well grounded physiologically (15, 22). In order to estimate a non-parametric reference intervals with 90% confidence interval, a minimum of 120 reference individuals from each sample groups were needed (39).This number does not consider any losses or deletion of observations. Participants were partitioned by ages; 5–12 years and 13–17 years and the last age group was further grouped by sex because of sex-related differences during pubertal period (50-52). Thus, three partitions groups were needed (3 x 120=360). According to previous studies in other African countries, in such large scale studies about 30% of apparently healthy population (Steven et al., 2008) do not qualify for reference interval determination for various reasons. 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 522 individuals were enrolled.

Therefore, participants were recruited from selected sub-cities then woredas after communicating with the statistical agency.

5.6.2. Sampling technique

Since Addis Ababa is very large city, four sub-cities were randomly selected based on lottery method, namely Arada, Kirkos, Akaki and Yeka; thus randomly selected woredas under the selected sub-cities were included. Probability Proportional to Size (PPS) sampling method was employed, where the size depends on the number of populations of woredas in a sub-city. The study participants were selected using convenient sampling method from the randomly selected sub-cities then woredas. The present study recruited children and adolescents from communities through village to village mobilization by the health extension workers to recruit 522 study participants. A households which might have more than one participant's, only one child and adolescent were randomly selected by lottery method in order to ensure representativeness. Once volunteering participants fulfilling the eligibility criteria are identified by the health extension workers and data collectors, they were invited to go to nearby health facilities for interview using structured questionnaire and to facilitate blood, urine and stool sample collection.

Table 1: Selected sites with household information

Selected Sites	**No. Households**	**Individuals per household**	**No. population**
Akaki	47021	3.8	178,680
Kirkos	54398	4.0	217,592
Arada	49564	4.1	203,212
Yeka	90195	3.8	342,741

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

Proportional allocation: $n_i=n/N*N_i$

Where n=total sample size

n_i= sample size at that stratum

N=total population

N_i =total population at that stratum

The number of house changed into number of population

Thus, samples were allocated proportionally based on their population size.

- Akaki Kality=99 Arada=113 Yeka=189 Kirkos=121

5.7. Measurement and Data collection

5.7.1. Data collection procedure

The study aims, risks, benefits of study participation and right to withdraw from the study at any time were explained to participants' parents/guardians of children. Following complete explanation of the protocols and aims of the study, oral assent and written consents were obtained from students and their parents, respectively. An a priori sampling method was used thereby individuals had assessed whether they fulfil the well-defined exclusion/ inclusion criteria before being selected as a referent individual. From those consenting/assenting participant demographic information, anthropometric and a brief medical history were collected. In the data collection process there were an initial in-home interview by health extension worker and along with the researcher to collect general health information on lifestyle habits and medical history followed by a visit to a mobile examination center to obtain samples and physical examination. Prior to blood collection, volunteers were requested to fast overnight for 10–12 hr. The participants and/or parents/guardians were interviewed and participants were asked to be measured their heights, weights and mid-upper arm circumference. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2) and participants with obesity and severe thinness were excluded from the study. Socio-demographic and clinical data were collected using structured questionnaires by trained data collectors and researchers. Physical examination and anthropometric measurements were carried out by health officer. Finally, the sample collection was performed at 8:30 am till 11:00 am. Beside this, volunteers were requested to sit for 15–20 min prior to collection to avoid variation due to postural influence and physical stress. Approximately 10ml of blood samples were collected using serum separator and ethylene diamine-

tetra-acetic acid (EDTA) tubes; then the samples were transported from collection area to lab by packing inside the icebox. In addition, stool sample was collected and direct wet mount test performed immediately and the leftover sample was preserved for concentration test. Midstream urine sample was also collected on site and chemical tests were ran. As a practical matter, samples were collected and handled properly and in a manner consistent with the routine practices for patient specimens. The volunteer's information was kept confidentially by a system of code number identification so that the answers were unknown to the analyst but persons with abnormal results could be recalled.

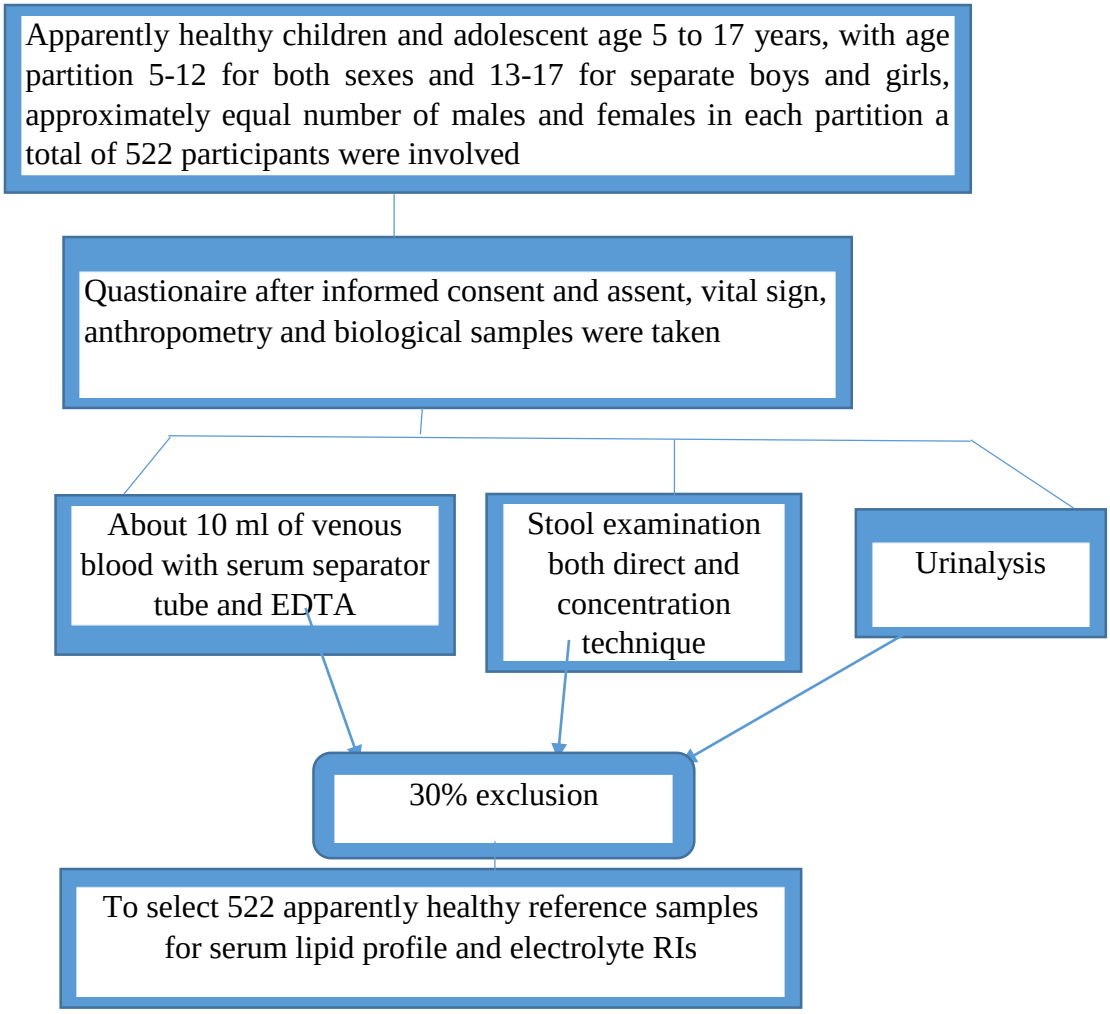


Figure 1: Data collection procedure

5.7.2 Laboratory testing and analysis

Blood specimen collection, handling, storage, transportation and processing: By explaining the blood drawing procedure to the participants, from each individual about 10 ml of blood samples were collected in serum separator and EDTA tubes following standard operational procedures. The serum separator tube blood sample was centrifuged within 1 hr. of collection for 2-3 minutes at 1500-3000 revolutions per minute, then immediately the serum separated and store at -80 ^{o}C. According to Candadian study, at −80 °C storage serum lipid and electrolytes were stable at least up to 13 months period and do not require immediate testing for reference interval determination. During the test procedures all samples were subjected to a single freeze–thaw cycle.

Electrolyte analysis: - Laboratory analysis included 6 commonly performed serum electrolytes (Na, K, Cl, Ca, Mg and PO4) tests. All these tests were performed with Cobas c 501 automatic chemistry analyzer at Ethiopian Public Health Institute which is a nationally accredited laboratory. Analytical methods were regulated in accordance with manufacturer's instructions thereby preventive maintenance, function checks, and quality controls have been done always.

Serum electrolytes (Na^+, K^+ and $Cl^{-)}$ levels were measured by using an Ion-Selective Electrode (ISE) indirect method. It uses certain membrane materials with unique properties to develop an electrical potential (electromotive force, EMF) for the measurements of ions in solution. The electrode provides a selective membrane in contact with both the test solution and an internal filling solution which contains the test ion at a fixed concentration. Because of the particular nature of the membrane, the test ions were closely associated with the membrane on each side. The membrane EMF was determined by the difference in concentration of the test ion in the test solution and the internal filling solution.

Calcium ions react with 5-nitro-5'-methyl-BAPTA 5-nitro-5'-methyl-(1, 2-bis (o-aminophenoxy) ethan-N, N, N', N'-tetra acetic acid (NM-BAPTA) under alkaline conditions to form a complex.

This complex reacts in the second step with EDTA.

Ca^{2+} + NM-BAPTA alkaline pH→ calcium-NM-BAPTA complex

Calcium-NM-BAPTA complex + EDTA→ NM-BAPTA + calcium EDTA complex

The change in absorbance is directly proportional to the calcium concentration and is measured photometrically.

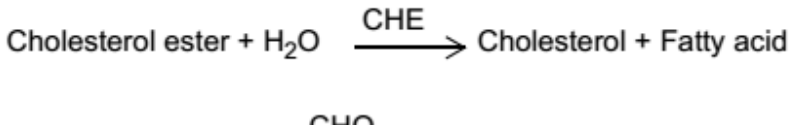
Magnesium can be measured using colorimetric endpoint method by mixing sample with reagent one and addition of reagent two and start of reaction. Finally in alkaline solution, magnesium forms a purple complex with xylidyl blue, diazonium salt then the magnesium concentration is measured photometrically via the decrease in the xylidyl blue absorbance.

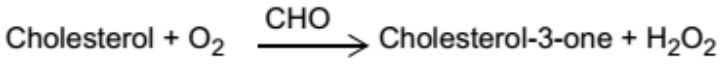
Inorganic phosphate can be measured after forming an ammonium phosphomolybdate complex having the formula (NH4)3[PO4 (MoO3)12] with ammonium molybdate in the presence of sulfuric acid as follow.

Phosphate + ammonium molybdate $\underline{H2SO4}$ → ammonium phosphomolybdate

The concentration of phosphomolybdate formed is directly proportional to the inorganic phosphate concentration and is measured photometrically.

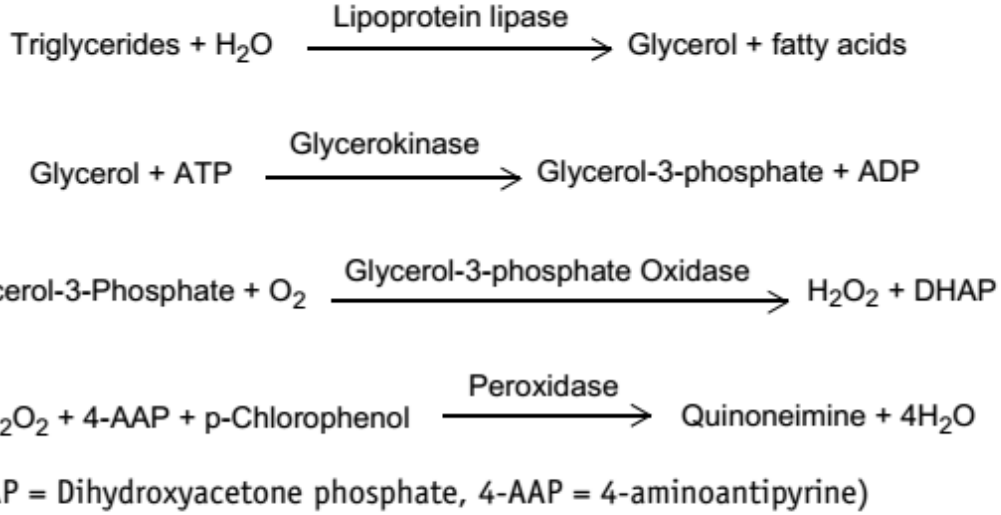
TOTAL CHOLESTEROL: Cholesterol was measured by enzymatic, colorimetric method. Cholesterol esters are splitted by the action of cholesterol esterase to produce free cholesterol and fatty acids. Then cholesterol oxidase catalyzes the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. By the action of peroxidase hydrogen peroxide perform its effect on the oxidative coupling of phenol and 4-aminophenazone to form a red quinone-imine dye. Finally determined by measuring the increase in absorbance. The reaction sequences were as follow:





(CHE = Cholesterol Esterase, CHO = Cholesterol oxydase, POD = Peroxidase)

TRIGLYCERIDES TEST: A series of coupled reactions in the determination of triglycerides which are hydrolyzed to produce glycerol. Increase in absorbance was measured. The reaction sequences were as follow:



The color intensity of the red dyestuff formed was directly proportional to the triglyceride concentration and can be measured photometrically.

HIGH DENSITY LIPOPROTEIN CHOLESTEROL (HDL-C): HDL-C was measured by direct assay in a homogeneous method for directly HDL-C levels in serum in the presence of magnesium ions, dextran sulfate selectively forms water-soluble complexes with LDL, VLDL and chylomicrons are resistant to PEG-modified enzymes action. The reactions were as follow:

HDL-cholesterol esters + H2O PEG-cholesterol esterase HDL-cholesterol + RCOOH

In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to

Δ4-cholestenone and hydrogen peroxide.

HDL-cholesterol + O2 PEG-cholesterol oxidase Δ4-cholestenone + H2O2

The generated hydrogen peroxide reacts with 4-amino-antipyrine and HSDA to form a purple-blue dye by the action of peroxidase. The color intensity of this dye was directly proportional to the cholesterol concentration and measured photometrically.

2 H2O2 + 4-amino-antipyrine + HSDA* + H+ + H2O peroxidase purple-blue pigment + 5 H2O

LOW DENSITY LIPOPROTEIN CHOLESTEROL (LDL-C): Cholesterol esters and free cholesterol in LDL were measured on the basis of a cholesterol enzymatic method using cholesterol esterase and cholesterol oxidase in the presence of surfactants which selectively solubilize only LDL. The enzyme reactions to the lipoproteins other than LDL are inhibited by surfactants and a sugar compound. Cholesterol in HDL, VLDL and chylomicron is not determined.

LDL-cholesterol esters + H2O detergent cholesterol esterase cholesterol + free fatty acids

By the action of cholesterol esterase cholesterol esters are broken down quantitatively into free cholesterol and fatty acids

LDL-cholesterol + O2 cholesterol oxidase Δ4-cholestenone + H2O2

Cholesterol oxidase along oxygen oxidized cholesterol to Δ4-cholestenone and hydrogen peroxide.

2 H2O2 + 4-aminoantipyrine + EMSEa + H2O + H^+ peroxidase red purple pigment + 5 H2O

The color intensity of this dye was directly proportional to the cholesterol concentration and measured photometrically.

5.8. Data Quality Assurance and Quality Control

After completion of each questionnaire, cross checking was done among data collectors and principal investigator to assure the completeness of the information gathered. The label on the test tube and subject's unique identification number on questionnaire was also checked for similarity. Weight was measured without heavy cloths and shoes, while height without shoes in order to get accurate measurement. The collected data was entered into SPSS version 23 daily, so as to increase the accuracy and minimize data entry errors. After checking the expiry date of both the reagents and controls, Cobas c501 clinical chemistry analyzer was checked for delivering correct result by using normal and pathological controls. Normal control which was under normal reference interval and high (pathological) control which is above the normal reference interval could be used. Before any study participant's sample was processed, dual quality controls (normal and pathological) and pooled serum samples were always performed and then study participant's sample was analyzed. Besides calibration also ran as needed but (for Na^+, K^+ and Cl^- every 24 hours) and all samples tested passed through automated interference detectors for hemolysis, lipemia and icterus. These serum indexes had also checked visually. At the end of testing, results were screened for missing results. The analytical performance of all assays were strictly assessed, and participant's sample was analyzed only when all assays were acceptable. All the necessary procedures and steps were followed based on the manufacture instructions. The collected results was checked for completeness on daily basis by the principal investigator. In general to ensure the accuracy and precision of the test results, all pre-analytical, analytical and post-analytical precautions were taken into consideration.

5.8.1 Pre analytical phases

Blood, stool and urine samples were collected from the study subjects and properly labeled with their initial name and code. The blood sample was collected by the trained laboratory personnel and the principal investigator extremely tried to collect good quality sample in a way to analyzed it and produced reliable result. Participants were instructed to collect mid-stream urine and not exposing to sun light. As well to avoid contamination of stool samples with dusty particles. Samples were transported to lab in triple package system with in the icebox. Generally, standard operating procedures were followed for each sample collection, transportation, preparation and storage.

5.8.2 Analytical phases

The tests were done by well-trained laboratory personnel following standard operational procedures of each test methods. The reagents, kits and the methods have been assessed with known physiologic and pathologic controls materials. Finally the results were checked by the principal investigator.

5.8.3 Post analytical phases

The results were documented with participant's identification in order to avoid errors in the test results, repeatedly checked before delivering to the responsible bodies.

5.9. Data analysis and interpretation

The data was analyzed in accordance with Clinical & Laboratory Standards Institute (CLSI) C28-A3 guidelines (39). The serum lipid profile and electrolytes reference intervals of apparently healthy children and adolescents were calculated using non-parametric methods. First, we applied inspection of reference observation distribution via scatterplot followed by identification of the outliers in interquartile ranges (IQR: Q3-Q1; Q1: lower quartile, Q3: upper quartile). At levels of < Q1 - 1.5 IQR and / or > Q3 + 1.5 IQR was considered as outliers and discarded manually following Box and Whisker plot and not included in the final reference estimation. Mann Whitney U test was used to assess sex differences because the data may not be distributed normally. The mean, median, SD, and 90% CIs were calculated and reference intervals were established at 2.5^{th} and 97.5^{th} percentiles.

5.10 Operational definitions

Apparently healthy individuals— a person having no chronic diseases and experience acute illness.

Reference individual —a person selected from reference population for testing on the basis of well-defined criteria.

Reference population — a group encompassing of all the reference individuals.

Reference sample group — an adequate number of individual's recruited from reference population to represent it.

Reference interval —the interval between, and consisting, two reference limits.

Cholesterol —a steroid with hydroxyl group in the C3 position which is synthesized in tissue.

Triglycerides— esters of the trihydric alcohol glycerol with 3 long-chain fatty acids which are partly synthesized in the liver and partly ingested in food.

High density lipoproteins (HDL) —the one responsible for the reverse transport of cholesterol from the peripheral cells to the liver.

Low density lipoproteins (LDLs) —a lipoprotein derived from VLDLs responsible for causing and influencing the progression of atherosclerosis.

Child—a person between five to twelve years.

Adolescent—a person aged between thirteen to seventeen years.

5.11. Ethical considerations

Before starting data collection support letter and ethical clearance letter was obtained from the Department of Medical Laboratory Sciences, College of Health Science of Addis Ababa University and permission to conduct the research was obtained from Addis Ababa Health Bureau and the respective Sub-cities and Woredas. Information sheet was prepared and read to all study participants. All participants were informed about the purpose of the study and their participation was on voluntary basis and verbal assent from participants and written informed consent from parents/guardians were obtained. Name of the participants were coded by number on the questionnaire; to ensure confidentiality. Screening tests results were submitted to the participant's parent and responsible body for intervention through the health extension workers and data collectors.

5.12. Dissemination of the result

The finding of this study will be presented and submitted to AAU, Medical Laboratory Department, and stakeholders. It will also be presented on scientific conferences and published on peer reviewed scientific journals.

6. Results

6.1. Socio demographic characteristics

Five hundred and twenty two apparently healthy individuals participated in the study, with equal number of male: female ratios N=261 (50%) each. Of the total study samples, 54.6% (285/522) were in the adolescent age group (13-17 years) and the rest 45.4% (237/522) were children (5-12 years). The numbers of children in the age groups of 5-12 years were relatively smaller as compared to 13-17 years, since the study have established separate reference intervals for the adolescent group. Participants were divided into age cohorts of ≥5 to <13 years and ≥13 to ≤17 years. The oldest age cohort was further subdivided by gender. This study was attempted to enroll a minimum of 120 children into each cohort, as recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines for the establishment of reference intervals. Regarding the ethnicity of the study participants Amhara 183 (35.1 %), Oromo 77 (14.8 %), South nation nationality people 108 (20.7 %), Tigre 31 (5.9 %), and the rest 123 (23.6 %) were mixed origin. All were students and about of 97% were born in Addis Ababa.

6.2 Age and gender distribution, partitioning and screening results of the reference population

Figure 3 illustrates a bar chart for age and gender distribution of the reference population. The average age of participants with SD was 12.6±3.1 years for boys and 12.7±2.9 years for girls. As a whole, male female ratio was equal (50% Vs 50%). Regarding life style condition of study participants, almost all had normal weight, none were included as overweight and underweight. About 95% of participants had at least twice a week exercise habit and this was almost equal ratio for both genders. None of the participants had the habit of drinking alcohol, chewing chat and smoking. Of 522 participants, 22 with more than 3 mg/L C-reactive protein results were excluded while no participants were excluded due to malaria tests, intestinal parasite, and urine chemical tests. Hence a total, 500 children and adolescents had met eligibility criteria before assessing the lipid and electrolyte results. Analytes were graphed using scatter plots to observe their distribution as shown by Figures 3 & 4, followed by identification of the outliers in each analytes with interquartile range function.

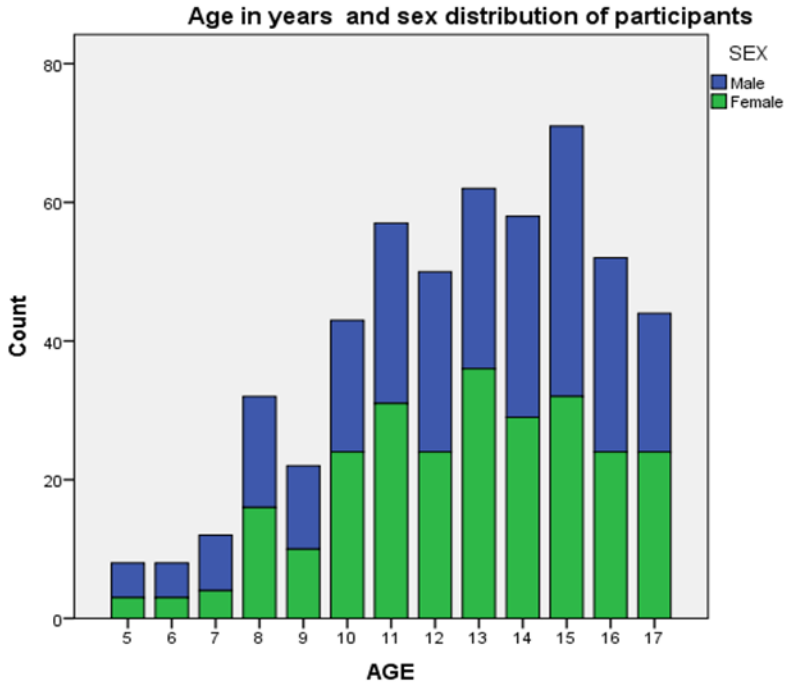
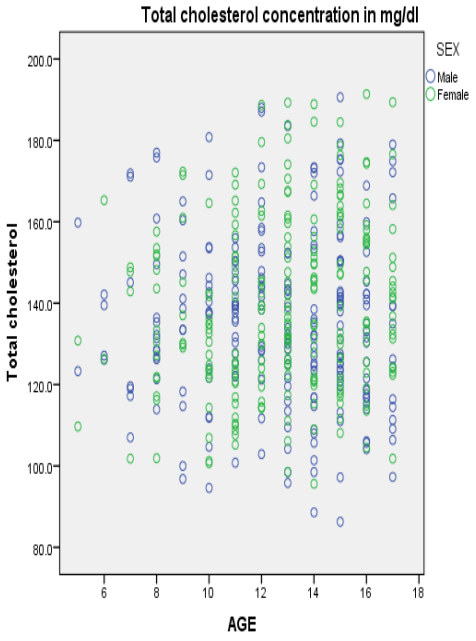
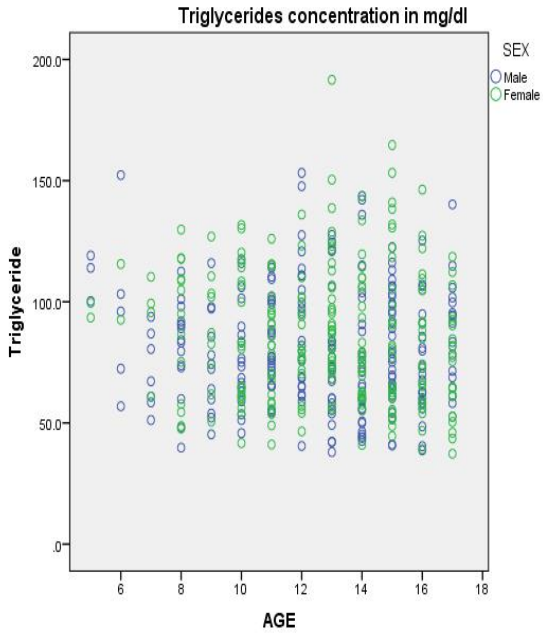


Figure 2: Bar chart showing age and gender distribution of the reference population.

Inspection of the scaterplot, the concentration of total cholestrol were slightly increasing throuhout age until it reached around 13 years then after goes with almost similar pattern. High lower limit values of TC were found at childhood age. In contrast the values of triglycerides almost similar throughout age range and did not show much variability with age. On the other hand HDL-C concentration follow the same pattern with total cholesterol increasing with age of children especially after 8 years. HDL-C was much lower at the age range of 5-7 years and sight increases from 8 to10 years with similar pattern between males and females. And starting from 12 years the values were relatively high throughou adolescences. Observation from the scatterplot serum LDL-C levels had showed some variation at lower age and amost compact after age of 9 years. However, it showed a small decrease in at age 9 years and oldest age. The overall distribution of participants lipid profile were somehow uniform with age after excluding extreme values which may greatly affect the reference intervals (Figure 3A-D).



A



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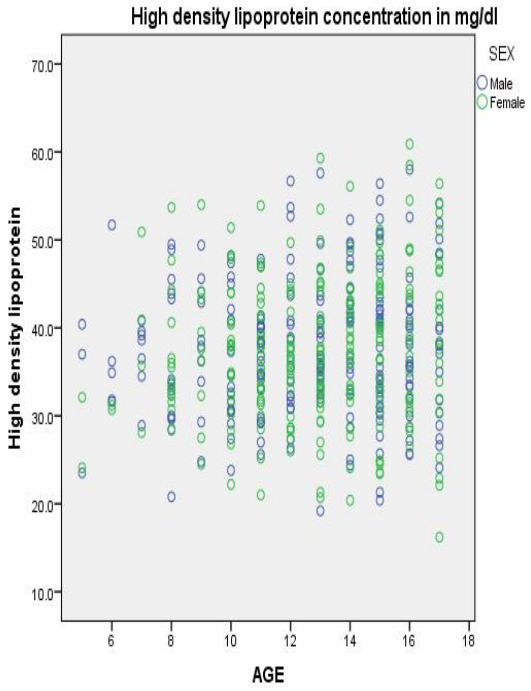

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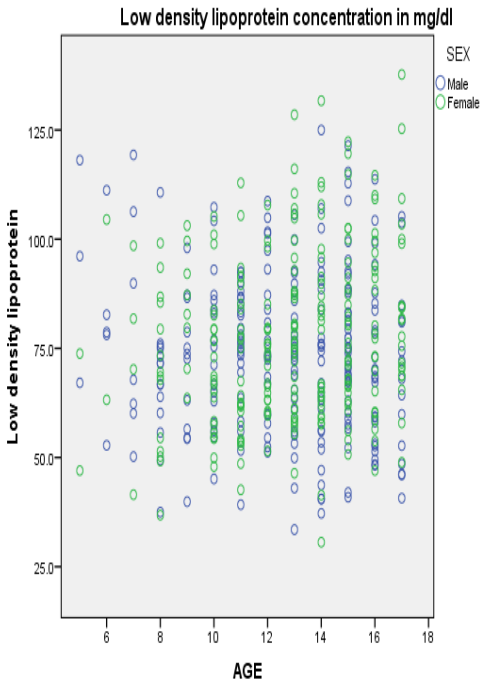


D

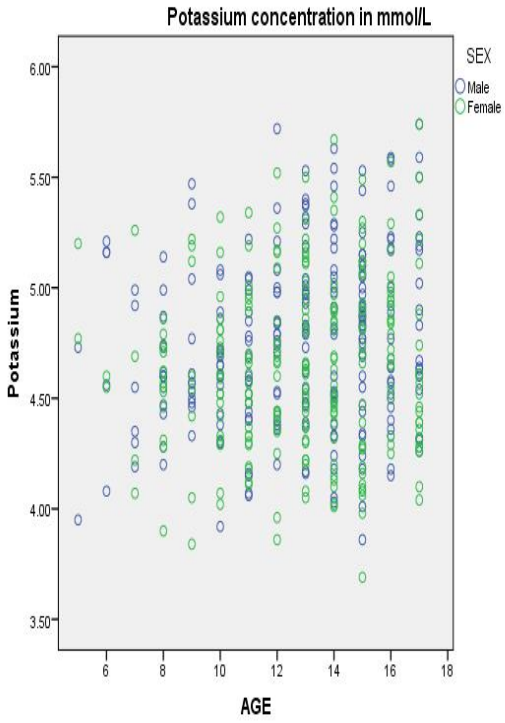
Figure 3A-D: Scatterplot distributions for serum lipid among the 5 to 17 year age range.

Evidence from the scaterplot as shown in Figure 4A to F showed that potassium values were different with sexes, and at lower age partition relatively low. The distribution indicated that at after age of 12 years it was riched pick values and remained relatively stable throughout the age cohort. Unlike others sodium concentration was very scattered but with no differences with age. Regarding values with sex and age, sodium had a uniform distribution. In other ways chloride concentrations were somewhat vary with sex. The decreased chloride level was shown in the last age range.

Magnesium had similar distribution throughout age. It indicated that male's value was a little higher than females did. Concerning the calcium values, it was concentrated in certain point with no differences in age. From the scatterplot, sex differences was not observed by calcium concentration. Unlike other electrolytes the phosphate distribution revealed a constantly high concentration until age of 13 years. The lower limit sharply decreased after 13 years. At the adolescence period phosphate variation with sex was clear. Generally, evidences from inspection

of the scatterplots all lipid profile were increased continuously with age except LDL-C and the same was true for potassium.

A



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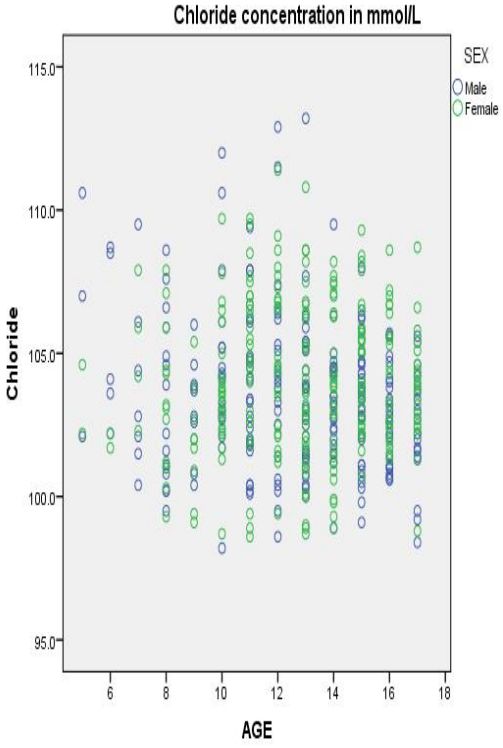

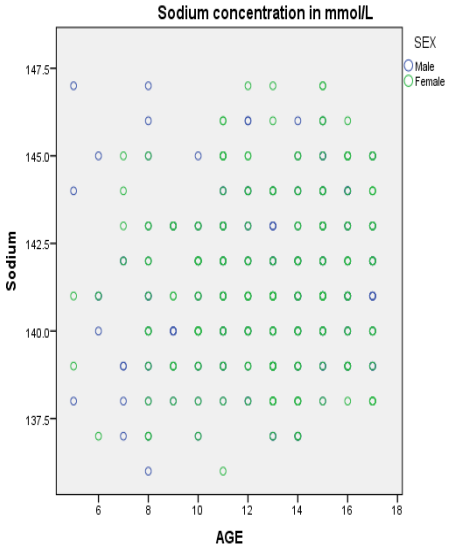

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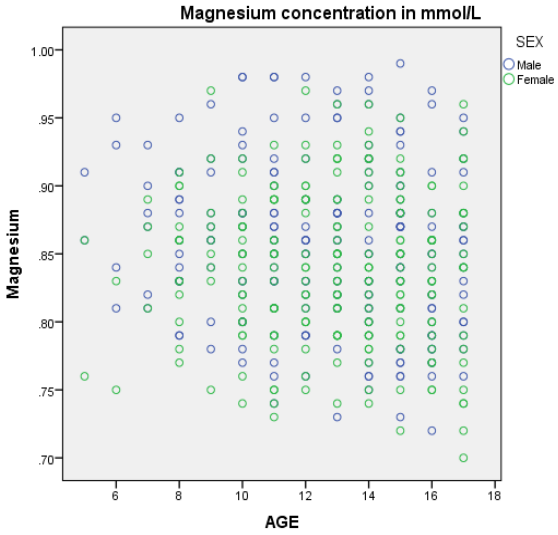

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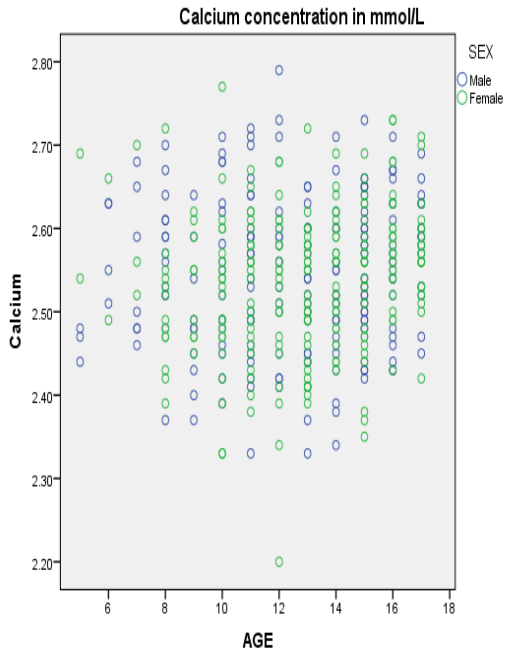

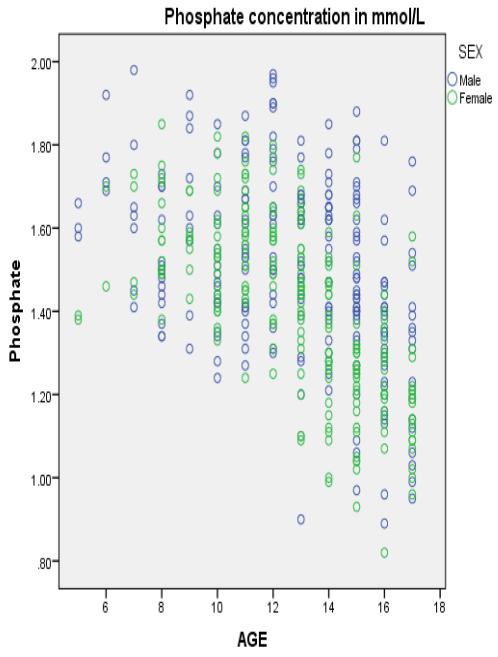

E F

Figure 4A-F: Scatterplot distributions for serum electrolytes over the 5 to 17 year age range.

6.3 Establishment of reference intervals on serum electrolytes and lipid profile for Addis Ababa children and Adolescents

Different number of outliers were excluded from each analyte before determining reference intervals. The reference values for four lipid profile and six serum electrolytes were shown in the below table (Table 2). At the adolescent group sex partitions were performed, since significance differences observed between sexes. Adolescent females had high TC, TG, LDL-C and Ca values in both lower and upper limits. And females also had high HDL-C, K, Cl and Na values in the upper limit than did males. On the other hand adolescent male participants had slightly high value of phosphate in the upper limit. Similarly, males had high Mg and Cl in both limit than did females. The electrolyte values between children and adolescents were very similar except PO4. High concentration of PO4 was observed at the low age cohort than adolescents in both lower and upper limit. As for lipid profile children (5-12years) had relatively high TC, LDL-C, HDL-C and TG in the lower limit than adolescents combined values.

Table 2: Established Reference Intervals of serum lipid profile and electrolytes for apparently healthy children and adolescents in Addis Ababa, Ethiopia, February-October, 2020

Analyte	Age in years	Sex	Lower limit	Upper limit
TC (mg/dl)	5-12	Combined	100.76	171.7
	13-17	Combined	97.25	189.1
		Male	92.7	170.2
		Female	104.2	191.6
TG (mg/dl)	5-12	Combined	44.16	126.36
	13-17	Combined	40.55	143.6
		Male	39.7	132.4
		Female	44.3	150.5
Ca (mmol/L)	5-12	Combined	2.34	2.7
	13-17	Combined	2.39	2.7
		Male	2.36	2.7
		Female	2.39	2.72
Na (mmol/L)	5-12	Combined	137	145.4
	13-17	Combined	137	146
		Male	137	145
		Female	137	147
Mg (mmol/L)	5-12	Combined	0.74	0.97
	13-17	Combined	0.73	0.96
		Male	0.73	0.97
		Female	0.72	0.94
PO4 (mmol/L)	5-12	Combined	1.42	1.85
	13-17	Combined	0.96	1.80
		Male	0.93	1.81
		Female	0.96	1.73
K (mmol/L)	5-12	Combined	4.37	5.20
	13-17	Combined	4.03	5.58
		Male	4.1	5.58
		Female	4.0	5.74
Cl (mmol/L)	5-12	Combined	101.9	107.9
	13-17	Combined	98.95	108.5
		Male	99.5	107
		Female	98.9	108.6
LDL (mg/dl)	5-12	Combined	60.6	105.6
	13-17	Combined	41.7	120.95
		Male	40.6	107.9
		Female	50.5	128.7
HDL (mg/dl)	5-12	Combined	31.6	53.7
	13-17	Combined	21.3	57
		Male	22.9	56.9
		Female	20.9	58.5

The mean, median, SD and reference intervals for children and adolescents were determined by using 2.5th and 97.5th percentiles for each serum lipid and electrolyte parameters. Using the non-parametric Mann Whitney U test, a statistically significant differences (P-value <0.05) between males and females were found for PO4, TC, TG, K, Cl and LDL-C. As shown in Table 3 below, the mean concentrations of and (RI) for TC was 132.67 mg/dL (100.76-171.7), for TG 82.61 mg/dl (44.16-126.36), HDL-C 36.5 mg/dl (31.6-53.7), LDL-C 72.5 mg/dl (60.6-105.6), potassium 4.56 mmol/L (4.37-5.2), sodium 140.9 mmol/L (137-145.4), chloride 103.5 mmol/L (101.9-107.9), calcium 2.52 mmol/L (2.34-2.7), magnesium 0.85 mmol/L (0.74-0.97) and phosphate 1.54 mmol/L (1.42-1.85) for both boys and girls aged between 5-12 years. The mean (RI) values for the analytes in adolescents were TC 136.5 mg/dl (97.2-189.1), TG 81.85 mg/dl (40.55-143.6), HDL-C 38.3 mg/dl (21.3-57), LDL-C 76 mg/dl (41.7-120.95), potassium 4.74 mmol/L (4.03-5.58), sodium 141 mmol/L (137-146), chloride 103.5 mmol/L (98.95-108.5), calcium 2.54 mmol/L (2.39-2.7), magnesium 0.84 mmol/L (0.73-0.96) and phosphate 1.37 mmol/L (0.96-1.8), for combined sexes. There was no statistically significant difference between sexes and age partitions regarding the mean and median concentration of all electrolytes except phosphate.

Table 3: Mean, Median, SD, Minimum, Maximum, Reference interval, and P-values of serum lipid profile and electrolytes for children and adolescents, Addis Ababa, Ethiopia

Analyte	Age in year	Sex	N	Mean	Median	SD	Min	Max	2.5th percentile (90% CI)	97.5th percentile (90% CI)	P-value for sex
TC (mg/dl)	5-12 y	C	200	132.7	130.8	17.3	94.6	173.4	100.76 (96.8-104.3)	171.7 (165-172.7	
	13-17 y	M	128	129	129	18	86.3	175.4	92.7 (86.3-98.5)	170.2 (157.8-175.4)	**0.001**
		F	137	143	141.6	22.9	95.6	200.1	104.2 (95.6-110.8)	191.6 (184.8-200)	
		C	265	136.5	134.4	21.9	86.3	200.1	97.25 (95.6-104)	189.1 (180.5-192.8)	
TG (mg/dl)	5-12 y	C	203	82.61	81.8	21.86	40.5	136	44.16 (40.86-49.4)	126.4 (117.7-132.58)	
	13-17 y	M	129	75.9	70.6	24.15	38	143.7	39.7 (38-42)	132.4 (118.1-143.7)	**0.028**
		F	133	87	81.9	28.4	37.3	164.7	44.3 (37.3-50.1)	150.5 (139.1-164.7)	
		C	262	81.6	77.2	27	73.3	164.7	40.55 (38.35-43.0)	143.6 (135.96-150.4)	
Ca (mmol/L)	5-12 y	C	196	2.52	2.52	0.086	2.33	2.73	2.34 (2.33-2.39)	2.7 (2.68-2.72)	
	13-17 y	M	126	2.54	2.54	0.08	2.33	2.73	2.36 (2.33-2.41)	2.7 (2.67-2.73)	0.565
		F	131	2.54	2.56	0.082	2.37	2.73	2.39 (2.37-2.41)	2.72 (2.68-2.73)	
		C	257	2.54	2.55	0.08	2.33	2.73	2.39 (2.37-2.41)	2.7 (2.68-2.73)	
Na (mmol/L)	5-12 y	C	193	140.9	141	2.26	136	147	137 (136-137)	145.4 (145-146.4)	
	13-17 y	M	131	141.2	141	1.96	137	145	137 (137-138)	145 (144.4-145)	0.815
		F	137	141.3	141	2.56	137	148	137 (137-138)	147 (146-148)	
		C	268	141	141	2.3	137	148	137 (137-138)	146 (145-147)	
Mg (mmol/L)	5-12 y	C	201	0.85	0.85	0.055	0.73	0.98	0.74 (0.74-0.75)	0.97 (0.95-0.98)	
	13-17 y	M	130	0.85	0.84	0.06	0.72	0.97	0.73 (0.72—0.76)	0.97 (0.96-0.97)	0.124
		F	139	0.83	0.83	0.055	0.7	0.96	0.72 (0.7-0.74)	0.94 (0.92-0.96)	
		C	269	0.84	0.84	0.058	0.7	0.97	0.73 (0.72-0.74)	0.96 (0.94-0.97)	
PO4 (mmol/L)	5-12 y	C	207	1.54	1.54	0.15	1.24	1.95	1.42 (1.4-1.45)	1.85 (1.8-1.9)	
	13-17 y	M	133	1.46	1.47	0.22	0.89	1.88	0.93 (0.89-1.02)	1.81 (1.76-1.88)	<**0.001**
		F	139	1.3	1.28	0.19	0.82	1.77	0.96 (0.82-1.01)	1.73 (1.63-1.77)	
		C	272	1.37	1.36	0.217	0.82	1.88	0.96 (0.9-0.99)	1.8 (1.72-1.81)	
K (mmol/L)	5-12 y	C	196	4.56	4.57	0.31	3.84	5.27	4.37 (4.3-4.43)	5.2 (5.16-5.24)	
	13-17 y	M	134	4.81	4.82	0.38	4.01	5.74	4.1 (4.01-4.2)	5.58 (5.45-5.74)	**0.009**
		F	139	4.7	4.65	0.44	3.69	5.83	4.0 (369-4.05)	5.74 (5.5-5.83)	
		C	272	4.74	4.77	0.42	3.69	5.83	4.03 (3.99-4.08)	5.58 (5.49-5.78)	
Cl (mmol/L)	5-12 y	C	203	103.5	103.3	2.38	98.2	108.5	101.9 (101.6-102.2)	107.9 (107.6-108.1)	
	13-17 y	M	130	103.0	103.1	1.8	98.9	108	99.5 (98.9-100)	107 (106.1-108)	**0.006**
		F	135	103.8	103.7	2.53	98.7	109.3	98.9 (98.7-99.8)	108.6 (108.2-109.3)	
		C	265	103.5	103.4	2.26	98.7	109.3	98.95 (98.8-99.8)	108.5 (107.8-108.6)	
LDL (mg/dl)	5-12 y	C	203	72.5	73.1	15.2	39.9	112.9	60.6 (57.8-63.2)	105.6 (99.3-110.2)	
	13-17 y	M	128	70	70.2	16.6	40.5	113.7	40.6 (40.5-42.6)	107.9 (96.6-113.7)	**0.008**
		F	138	81.2	79.3	20.1	41.4	137.7	50.5 (41.4-55.7)	128.7 (116-137.7)	

		C	266	76	74	19.36	40.5	137.7	41.7 (40.7-46.2)	120.95 (113.3-128.5)	
HDL (mg/dl)	5-12 y	C	199	36.5	35.7	7.1	20.8	54	31.6 (30.5-32.9)	53.7 (49.4-53.9)	
	13-17 y	M	131	38.29	38	8.24	19.2	58	22.9 (19.2-25.6)	56.9 (52.4-58)	0.832
		F	137	38.32	37.8	8.7	16.2	60.9	20.9 (16.2-24.4)	58.5 (53.6-60.9)	
		C	268	38.3	38	8.48	16.2	60.9	21.3 (20.25-24.3)	57 (54-58.65)	

**N=number of participants, C=combined, M=male, F=female, SD=standard deviation, Min=minimum Max=maximum, CI=confidence interval, P < 0.05= statistically significant difference between males and females*

In this study participants had very low level of HDL-C in comparison with manufacturer reference intervals. Misclassifications were observed in all analytes for both partitions. The major discrepancy was seen for HDL-c (Table 4).

Table 4: Frequency of misclassified values

Analyte	5-12 years				13-17 years			
	Manufacturer RIs	Current RIs	N	%	Manufacturer RIs	Current RIs	N	%
K (mmol/L)	3.5-5.1	4.34-5.2	50	24.2 %	3.5-5.1	4.03-5.58	53	19.5 %
Na (mmol/L)	136-145	137-145	2	1.04 %	136-145	137-146	6	2.2 %
Cl (mmol/L)	98-107	101.9-107.9	37	18.2 %	98-107	98.95-108.5	19	7.2 %
Ca (mmol/L)	2.2-2.7	2.34-2.7	4	4.0 %	2.1-2.55	2.39-2.7	102	39.7 %
Mg (mmol/L)	0.7-0.86	0.74-0.97	72	36 %	0.70-0.91	0.73-0.97	37	13.8 %
PO4 (mmol/L)	1.05–1.85	1.42-1.85	35	16.9 %	0.95–1.65	0.96-1.8	23	8.4 %
TC (mg/dl)	<200	100.7-171.7	12	6 %	<200	97.25-189.1	8	3 %
TG (md/dl)	<200	44.16-126.36	9	4.4 %	<200	40.55-143.6	6	2.3 %
LDL-c (mg/dl)	<100	60.6-105.6	9	4.4 %	<100	41.7-120.95	29	10.9 %
HDL-c (mg/dl)	>55	31.6-53.7	148	74.4 %	>55	21.3-57	200	74.6 %

Note N=number, RIs =reference intervals

6.4 Comparison of currently established reference intervals on children and adolescents with other published data

Serum electrolytes such as phosphate and potassium showed notable differences compared to the Canadian, Sweden, Denmark and German derived reference intervals. The current study participant's HDL-C, TC, TG and LDL-C concentration were lower than the below shown counterparts (Table 5). As can see from the table both the company derived RI that is currently being used in the country and published reports elsewhere lack sex disaggregated data. As can be seen from the table, no consistency found between studies. For example, the upper limit for potassium varies between 3.75 Germany and 5.5 mmol/L in USA, chloride between 105 and 115 for the two countries, respectively. The current finding for Ethiopians lies between the two. In contrary, the upper limit of triglycerides is the lowest for the current study compared to others listed in the table; 126.36 for Ethiopian versus 194.9 mg/dl for children from Sweden.

Table 5-8: Comparison of currently established reference intervals on children and adolescents with other published data

Table 5: Comparison of commonly used serum lipid profile and electrolytes reference values for children (5-12 years) in Addis Ababa, Ethiopia, with other published studies

Analyte	Sex	Current RI (with Cobas c 501)	Canada (using Cobas) (41)	Australia (using Architect c8000) (42)	Sweden (using Cobas) (43)	Texas USA (45)	German (using Cobas) (46, 47)	Denmark (with Cobas) (44)	Pakistan (using Cobas) (48)
K (mmol/L)	C	4.37-5.2	3.8-4.9	3.6–5.3	3.3-4.6	3.5–5.5	3.2-3.75	3.26-4.29	NA
	M	--	NA	NA	NA	NA	NA	NA	3.4-5.0
	F	--	NA	NA	NA	NA	NA	NA	3.7-5.2
Na (mmol/L)	C	137-145.4	136-143	133–144	136-146	136–145	130-145	135-147	NA
	M	--	NA	NA	NA	NA	NA	NA	135-145
	F	--	NA	NA	NA	NA	NA	NA	132-148
Cl (mmol/L)	C	101.9-107.9	NA	97–110	NA	95–105	100-115	NA	NA
	M	--	101-107	NA	NA	NA	NA	NA	101-112
	F	--	101-107	NA	NA	NA	NA	NA	101-114
Ca (mmol/L)	C	2.34-2.7	2.33-2.63	2.2-2.65	2.24-2.60	2.2-2.52	1.9-2.7	NA	NA
	M	--	NA	NA	NA	NA	NA	2.22-2.58	2.0-2.75
	F	--	NA	NA	NA	NA	NA	2.26-2.58	1.68-2.98
Mg (mmol/L)	C	0.74-0.97	0.86-1.17	0.65-1.10	0.71-0.94	0.66-0.94	0.61-1.0		NA
	M	--	NA	NA	NA	NA	NA	0.71-0.93	NA
	F	--	NA	NA	NA	NA	NA	0.73-0.93	NA
PO4 (mmol/L)	C	1.42-1.85	1.32-1.91	0.9-2.0	NA	0.97-1.94	1.2-2.6		NA
	M	--	NA	NA	NA	NA	NA	1.07-1.74	1.26-1.78
	F	--	NA	NA	NA	NA	NA	1.09-1.72	1.23-1.84
TC (mg/dl)	C	100.76-171.7	116-205	NA	105.3-214.5	135–200	NA	105.3-214.5	NA
	M	--	NA	NA	NA	NA	87.8-199	NA	110-219
	F	--	NA	NA	NA	NA	105-222	NA	113-233
TG (mg/dl)	C	44.16-126.36	35-186	NA	32.04-194.9	20–150	NA	NA	NA
	M	--	NA	NA	NA	NA	38.3-415	30.3- 140.6	18-150
	F	--	NA	NA	NA	NA	41-420	30.3- 173.6	22-157
LDL-C (mg/dl)	C	60.6-105.6	46-143	NA	NA	NA	NA	43-132.6	NA
	M	--	NA	NA	NA	64–130	41-128.7	NA	NA
	F	--	NA	NA	NA	60–140	49-150.2	NA	NA
HDL-C (mg/dl)	C	31.6-53.7	35-81	NA	NA	NA	NA	NA	NA
	M	--	NA	NA	NA	38–75	24.2-68.3	31.2-78	NA
	F	--	NA	NA	NA	35–73	24.6-68.3	39-89.7	NA

**NA=not available, C=combined, M=male, F=female*

Comparison of the present study's potassium RI for children aged 13-17 years with that from Canada, German, Denmark and Pakistan showed that both the upper and lower RI limits are higher than the values from these countries. Sodium also showed higher from these countries but the upper limit was similar with Sweden and Denmark but lower than Pakistan counterparts. The derived RI by the current study for magnesium was lower than the below listed countries. Regarding lipid profile, TC and HDL-C were lower in the present study than others. However, TC was higher than Canadian. There were consistency among different countries for LDL-C RI in lower limits (Table 6).

Table 6: Comparison of commonly used serum lipid profile and electrolytes reference values for adolescents (13-17) in Addis Ababa, Ethiopia, with other studies

Analyte	Sex	Current study	Canada (using Cobas) (41)	Australia (using Architect c8000) (42)	Sweden (using Cobas) (43)	Texas USA (45)	German (using Cobas) (46, 47)	Denmark (with Cobas) (44)	Pakistan (using Cobas) (48)
K (mmol/L)	C	4.03-5.58	3.8-4.9	3.6–5.3	3.26-4.29	3.5–5.5	3.2-3.75	3.26-4.29	NA
	M	4.1-5.58	NA	NA	NA	NA	NA	NA	3.5-4.9
	F	4.0-5.74	NA	NA	NA	NA	NA	NA	3.6-4.9
Na (mmol/L)	C	137-146	137-143	133–144	135-147	136–145	130-145	135-147	134-150
	M	137-145	NA	NA	NA	NA	NA	NA	NA
	F	137-147	NA	NA	NA	NA	NA	NA	NA
Cl (mmol/L)	C	98.95-108.5	NA	97–110	NA	95–105	100-115	NA	NA
	M	99.5-107	101-106	NA	NA	NA	NA	NA	98-115
	F	98.9-108.6	100-107	NA	NA	NA	NA	NA	99-117
Ca (mmol/L)	C	2.39-2.7	1.55-2.6	2.2-2.65	NA	2.22-2.7	1.9-2.7	NA	NA
	M	2.36-2.7	NA	NA	2.10-2.58	NA	NA	2.10-2.58	2.13-2.63
	F	2.39-2.72	NA	NA	1.95-2.58	NA	NA	1.95-2.58	2.13-2.6
Mg (mmol/L)	C	0.73-0.96	0.86-1.16	0.65-1.10	NA	0.62-0.94	0.61-1.0	NA	NA
	M	0.73-0.97	NA	NA	0.71-0.93	NA	NA	0.71-0.93	NA
	F	0.72-0.94	NA	NA	0.65-0.93	NA	NA	0.65-0.93	NA
PO4 (mmol/L)	C	0.96-1.8	NA	0.8–1.85	NA	0.74-1.55	1.2-2.6	NA	NA
	M	0.93-1.81	1.13-2.0	NA	0.85-1.74	NA	NA	0.85-1.74	0.9-1.5
	F	0.96-1.73	1.03-1.8	NA	0.72-1.49	NA	NA	0.72-1.49	0.97-1.50
TC (mg/dl)	C	97.25-189.1	100-182	NA	105.3-214.5	135–200	NA	105.3-214.5	NA
	M	92.7-170.2	NA	NA	NA	NA	87.8-199	NA	114-273

	F	104.2-191.6	NA	NA	NA	NA	105-222	NA	107-272
TG (mg/dl)	C	40.55-143.6	35-186	NA	32.04-194.9	20–150	NA	NA	NA
	M	39.7-132.4	NA	NA	NA	NA	38.3-415	30.3-226.1	24-321
	F	44.3-150.5	NA	NA	NA	NA	41-420	30.3- 173.6	17-269
LDL-C (mg/dl)	C	41.7-120.95	46-143	NA	NA	NA	NA	43-132.6	NA
	M	40.6-107.9	NA	NA	NA	64–130	41-128.7	NA	NA
	F	50.5-128.7	NA	NA	NA	60–140	49-150.2	NA	NA
HDL-C (mg/dl)	C	21.3-57	NA	NA	NA	NA	NA	NA	NA
	M	22.9-56.9	31-70	NA	NA	30–64	24.2-68.3	31.2-78	NA
	F	20.9-58.5	35-89	NA	NA	35–80	24.6-68.3	39-89.7	NA

**NA=not available, C=combined, M=male, F=female*

Although all the assays were performed using the Cobas platform (Roche Diagnostics) which may harmonized the methodology-dependent differences, it was very likely that reference interval differences between the current study and their African counterparts. For instance, some differences were observed in all parameters among African populations. However, no notable differences were observed in TC, LDL-C and TG values among different African countries except Guinean, who had higher upper limit of TG. The current RI on HDL-C were much lower than Tanzanian children. Study conducted in Guinea reported very low Na lower reference limit. In contrast, the current delivered high lower reference limit for potassium.

Table 7: Comparison of established reference intervals on serum lipid profile and electrolytes for Addis Ababa children (5-12 years) with other African studies

Analyte	Sex	Current study (using Cobas)	Manufacturer (Cobas)	Guinea (using Cobas) (49)	Tanzania (using Cobas) (50)	Ghana (using Cobas) (51)	Kenya (using Cobas) (53)
K (mmol/L)	C	4.37-5.2	3.5-5.1	3.2-4.9	3.2–5.2	3.6–5.6	NA
	M	--	NA	NA	NA	NA	3.1-6.6
	F	--	NA	NA	NA	NA	3.4-5.7
Na (mmol/L)	C	137-145.4	136-145	127.5–141	134–141	135–151	NA
	M	--	NA	NA	NA	NA	136.8-148.9
	F	--	NA	NA	NA	NA	144.14-147.1
Cl (mmol/L)	C	101.9-107.9	98-107	NA	98–108	99–114	NA
	M	--	NA	NA	NA	NA	101.4-110.6
	F	--	NA	NA	NA	NA	99.5-111.7
Ca (mmol/L)	C	2.34-2.7	2.2-2.7	1.97–2.65	2.2-2.56	NA	NA
	M	--	NA	NA	NA	NA	2.3-2.7
	F	--	NA	NA	NA	NA	2.37-2.41
Mg (mmol/L)	C	0.74-0.97	0.70-0.86	NA	0.77-0.99	NA	NA
	M	--	NA	NA	NA	NA	NA

	F	--	NA	NA	NA	NA	NA
PO4 (mmol/L)	C	1.42-1.85	NA	1.27–2.29	1.20-1.90	1.03–1.84	NA
	M	--	1.05–1.85	NA	NA	NA	1.2-3.1
	F	--	1.05–1.80	NA	NA	NA	1.3-4.2
TC (mg/dl)	C	100.76-171.7	<200	85.8–183.3	82.3-191.5	65.74-166.28	NA
	M	--	<200	NA	NA	NA	NA
	F	--	<200	NA	NA	NA	NA
TG (mg/dl)	C	44.16-126.36	<200	32.9–240.3	32.9–157.5	44.29-169.17	NA
	M	--	<200	NA	NA	NA	NA
	F	--	<200	NA	NA	NA	NA
LDL-C (mg/dl)	C	60.6-105.6	<100	NA	35.1–123.6	NA	NA
	M	--	<100	NA	NA	NA	NA
	F	--	<100	NA	NA	NA	NA
HDL-C (mg/dl)	C	31.6-53.7	NA	NA	29.3–76.4	NA	NA
	M	--	>55	NA	NA	NA	NA
	F	--	>65	NA	NA	NA	NA

**NA=not available, C=combined, M=male, F=female*

Comparison was also made with similar studies in other African countries for adolescents as shown in Table 8 below. Although RIs for most electrolytes were similar among African countries, some differences were observed. Interestingly, all electrolyte's RI obtained by the current study showed slightly higher in both lower and upper limits than manufacture values. Particularly, Sodium reference limits in the current study were highly inconsistent with reference limit values conducted in Tanzania, Kenya, Ghana and Zimbabwe. This study also provided higher potassium limit than other African studies except Ghanaian counterparts. Concerning the lipid profile tests, the present study found lower HDL-C values in both sexes than Tanzanian, Zimbabwe and Manufacturer RI. However, better consistency were found for TC, TG and LDL-C (Table 8).

Table 8: Comparison of established reference intervals on serum lipid profile and electrolytes for Addis Ababa adolescents (13-17 years) with other African studies

Analyte	Sex	Current RI (using Cobas)	Manufacturer (Cobas)	Tanzania (using Cobas) (50)	Ghana (using Cobas) (51)	Zimbabwe (Hitachi 902) (52)	Kenya (using Cobas) (53)
K (mmol/L)	C	4.03-5.58	3.5-5.1	NA	NA	NA	NA
	M	4.1-5.58	NA	3.6–5.1	3.6–5.8	3.4-5.3	3.8-5.4
	F	4.0-5.74	NA	3.6–5.0	3.6–5.9	3.46-5.34	3.9-5.2
Na (mmol/L)	C	137-146	136-145	134–140	NA	NA	NA
	M	137-145	NA	134–140	132–156	135-153	132.9-146.5
	F	137-147	NA	134–140	132–152	133-155	140.4-142.5
Cl (mmol/L)	C	98.95-108.5	98-107	NA	NA	NA	NA
	M	99.5-107	NA	98–105	95–117	96.0-107	95.1-111.5
	F	98.9-108.6	NA	99–106	96–116	96.0-111	97.2-111.5
Ca (mmol/L)	C	2.39-2.7	2.1-2.55	NA	NA	NA	NA
	M	2.36-2.7		2.18-2.6	NA	1.83-2.85	2.0-2.5
	F	2.39-2.72	NA	2.2-2.6	NA	1.83-2.73	2.0-2.6
Mg (mmol/L)	C	0.73-0.96	0.70-0.91	NA	NA	NA	NA
	M	0.73-0.97	NA	0.77-0.98	NA	NA	NA
	F	0.72-0.94	NA	0.75-0.98	NA	NA	NA
PO4 (mmol/L)	C	0.96-1.8	NA	NA	NA	NA	NA
	M	0.93-1.81	0.95–1.65	0.92-1.79	0.95–1.79	0.78-2.0	1.0-2.56
	F	0.96-1.73	0.90–1.55	0.87-1.83	0.96–1.77	0.87-1.81	0.9-2.7
TC (mg/dl)	C	97.25-189.1	<200	NA	NA	NA	NA
	M	92.7-170.2	<200	91.7–177.8	65.7-150.8	75.3-210.6	NA
	F	104.2-191.6	<200	101-200	69.6-177.9	91.3-214.1	NA
TG (mg/dl)	C	40.55-143.6	<200	NA	NA	NA	NA
	M	39.7-132.4	<200	33.8–222.5	35.43-159.43	26.7-139.7	NA
	F	44.3-150.5	<200	31.15–174.4	35.43-150.57	29.4-171.8	NA
LDL-C (mg/dl)	C	41.7-120.95	<100	NA	NA	NA	NA
	M	40.6-107.9	<100	37.4–109.6	NA	30.4-129.5	NA
	F	50.5-128.7	<100	48–121.7	NA	37.4-138.8	NA
HDL-C (mg/dl)	C	21.3-57	NA	NA	NA	NA	NA
	M	22.9-56.9	>55	25–74.5	NA	29.6-80.7	NA
	F	20.9-58.5	>65	30–73	NA	32.8-85.8	NA

**NA=not available, C=combined, M=male, F=female*

8. Discussion

CLSI and IFCC recommended that RIs should be established for each region and locality because appropriate interpretation of any numeric laboratory test results has certainly rely on accurate reference intervals (39). Establishing reference intervals using direct method is challenging in children, since samples from healthy children is difficult to obtain. Thus, Ethiopian reference intervals are adopted from western countries. Hence, the present study particularly focused on developing the reference intervals using direct method for serum lipid and electrolytes.

There were statistically significant sex differences found in TC, TG, LDL-C, potassium, phosphate and chloride for 13-17 years study participants. Calcium and HDL-C were similar throughout the childhood and adolescence period with no differences in sex.

The increase in potassium concentration which was increased with maturity towards adolescence in the present study likely reflects different stages of physiolocal changes during maturation. Compared to values determined for other population groups, the K values for this study participants were higher than those reported from Canada (41), Sweden (43), Denmark (44), manufacturer's values, Guinea (49) in both upper and lower limits. Potassium RI also higher in lower limit than USA (45), Tanzania (50) and Ghana (51). In line with this study exactly the same figure was obtained in the upper limit of potassium by Tanzanian study (50). The established RIs for Tanzanian and Ghana males and females were lower than the current findings. In consistent with the current finding a study done in Ghana found that females had slightly higher K value in the upper limit. The difference between the current result and other findings may be due to nutrition, genetic variation or method differences.

Regarding sodium, the current study determined an interval which was almost similar for both sexes and age range. Evidences from this and other studies that the level of sodium peak at early age and remain constant. The current study's lower and upper RI for sodium were relatively higher than the manufacture's values, and those established in Canada (41), Germany (46), Australia (42), Tanzania (50) and Guinea (49) in both sexes and age group. However, the current study's Na upper limit was lower than findings from Ghana (51). The level of sodium was more significantly inconsistent among different African countries including the current study. In agreement with the present finding, adolescent females had slightly high upper limit value than males in Zimbabwean

study (52). Similarly the current study's chloride RIs were higher in both limits than stated in the manufacturer's leaflet and those reported by studies from Canada (41), USA (45) but lower in the upper limit from that reported from Australia (42) and Ghana (51). It was observed completely dissimilar RI result between Kenyan and the current study in both limits of Cl (53). According to findings including current work, adolescent females had slightly high Cl concentration in upper limit than males. Sodium and chloride are particularly prone to salt amount of intake; this is the most likely explanation for this major difference in results. This may be also explained by the variations in the aldosterol levels and genetic factors, life style, hot weather, exposure to sun light for long time and sweating rate.

Serum calcium slightly increased through the adolescent's period, with the lowest concentrations observed in the both male and female children aged 5-12 years. The current finding revealed that no significant sex differences among participants. Relatively the higher values of calcium were observed in the present study participant's in both lower and upper limits than studies in Canada (41), Australia (42), Sweden (43), Guinea (49) and Tanzania (50) while there were better agreement with values given in the Manufacturer's leaflet. Overall, differences in calcium concentration may be mainly due to differences in serum vitamin D or parathyroid hormone (PTH). Probably it may also be due to dietary factors or ethnic differences.

In In contrast, children participant's had low magnesium RI values in the upper limit than other studies such as Canada (41), Australia (42), Germen (46) and Tanzania (50). However it was relatively higher than values provided by the manufacturer. As for adolescents, the magnesium values were higher than study from Denmark (44) but lower than Tanzanian study (50). On the other hand, higher concentration of phosphate in lower age cohort was observed. Hence, significant increase in Phosphate in early stages of life in children is attributed to bone growth. In line with this finding others studies revealed that children had high phosphate value (41, 43, 45, 46, 49, 50). Comparing the present phosphate RI, children participant had lower upper limit than reported RIs from Germany (46), Australia (42), Canada (41), Guinea (49) and Tanzania (50). Regarding adolescent participants, their phosphate values were higher in both sexes compared to values from Pakistan (48) and Denmark (44). However, it was lower in both lower and upper limits than Kenyan study (53). The reason for these differences in electrolytes RIs may be due to the differences of analyzer, method of analysis, number of participants, diet, living style and genetics.

In the present study, sex associated differences were observed for all lipid profile tests except HDL-C at the older age cohort. Concentrations of LDL-c, HDL-c, TC and TG increased with age in both biological genders. Similar results were found by Tanzanian (50) and Guinea (49) studies Comparing participating children TC results with Canadian (41), Sweden (43), USA (45), and Denmark (45) studies, the results were lower in both lower and upper limits. While TC RI limit reported in the current study was high compared to Tanzanian (50) and Guinean study only in the upper limit side (49). However, TC RI value was higher than a finding from Ghana in both limits (51). Similarly at the adolescences age the current findings on TC was also lower than reports from USA (45), Germany (47), and Denmark (44). Moreover, only the upper limit of TG in children and adolescents were less than that reported from Canada (41), USA (45), Australia (42), Sweden (43), Guinea (49), Tanzania (50) and Ghana (51) counterparts. This difference may be due to variation in number of participants, exclusion criteria of conditions which likely affect lipid levels, partition, difference in both statistics and lab methods, consumption of saturated and unsaturated fats, or enzymes involved in lipolysis.

Children in the current study had lower LDL-C upper reference limit than Canada (41), Denmark (44), and Tanzania (50). Relatively similar to findings from Tanzanian and Zimbabwe studies, the current work revealed that female adolescents had relatively high LDL-C than did male. But these findings oppose the common believe that the effect of estrogen hormone in decreasing the LDL-C concentration in female adolescents. Low HDL-C levels are considered to be a stronger predictor of occurrence and recurrence of myocardial infraction as well as stroke and are also associated with premature and severe cardiovascular diseases (2). Hence, the current study obtained that both the lower and upper limit of HDL-C among children and adolescents were lower in relative to all other studies (41, 43, 45, 50, 52). This low HDL-C concentration may be explained by the difference of diet, lifestyle, body posture and genetics. Overall, the rising of total cholesterol and LDL-C upper limit concentrations from early childhood to adolescences possibly explained as the increasing demand for steroid hormone synthesis and related with physiologic development. In summary, while comparing the present work and western, it was observed that lower results were recorded for cholesterol, triglyceride, LDL-C and HDL-C.

9. Strength and Limitations of the study

9.1 Strengths

Inspite of the challenges for obtaining sample from children, this study is one of the efforts to establish reference values for fasting lipid profile and serum electrolyte parameters in a representative sample of children and adolescents for Addis Ababa city. The study has recruited more than CLSI recommended minimum number (120) of observations which has the advantage of allowing for the non-parametric computation of 90% confidence limits for each reference limit. The direct aprior method which is the best one was employed, by collecting sufficient sample from apparently healthy children and adolescents. Serum lipid and electrolytes were analyzed at nationally accredited lab with Cobas c501.

9.2 Limitations

The drawback with the present study is that age groups from birth to 5 years of age was lacking.

10. Conclusion and Recommendations

10.1 Conclusions

The present study was part of the national project that addresses critical gap of the area and established common reference intervals on routinely performed lipid profile and serum electrolytes for children and adolescents. This report was the first effort in Addis Ababa to establish the RIs for fasting lipid and serum electrolyte in children and adolescents aged between 5-17 years.The current reference intervals in children and adolescents provide an important update for serum electrolytes and these will aid in the early assessment of lipid and electrolyte abnormality in child and adolescent populations. The study also observed significant differences in the reference values between genders for TC, TG, LDL-C, PO4, K and Cl at the pubertal age and only Mg at lower age cohort. Indeed, major differences were observed in most of these analytes in comparison with published literatures. So, the current work further confirms the need to use locally established reference intervals. The generated reference intervals are primarily applicable to the Addis Ababa children and adolescents to aid the physicians in differentiating between healthy and diseased populations, but could be used by any other laboratory after validation test if not possible to derive RIs for the local population.

10.2 Recommendations

We suggest that these data may be used for interpretation of an individual's result for decision making, for epidemiological and intervention studies and for use by health care providers and health policy makers for definition of abnormal lipid and electrolyte levels in the Addis Ababa children and adolescents. After this physicians or any health care provider need to adhere to this RIs values for the appropriate interpretation of laboratory data and provide the highest quality of care to patients. Further research is needed on under five children and infants and similarly we recommend conducting nationwide study to derive biochemical reference intervals including lipid profile and serum electrolytes for Ethiopian population as a whole.

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12. Annexes

Annex I- Standard operating procedures (SOPs)

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

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

Performing the Chemical Test by Reagent Strip

Fill name and the date on the lab sheet for Chemical Examination of Urine.

Using the urine controls package insert provided by your instructor, accurately record the Expected Range for each test pad for both controls.

Accurately record the name and identification number of each participant's sample.

Carefully mix urine by inverting tube or swirling urine if it is in a cup.

When testing participants samples, observe and record color and clarity.

Carefully remove one strip from container, taking care not to allow reagent pads to touch hands or other surfaces.

Recap container of strips immediately finger tight. Exposure to air will cause deterioration of the chemicals on the pads.

Briefly (no longer than 1 second) dip test strip into the urine making sure that all pads are moistened.

Draw the edge of the strip along rim of specimen container to remove excess urine.

Start the timer when you have removed the moistened strip from the urine or control.

Blot edge of strip on biowipe or paper towel to remove excess urine. Failure to blot may result in chemicals from adjacent pads "bleeding" into each other causing erroneous results.

Read each pad at the time shown on the strip container, starting with the shortest time. Hold the strip close to the color blocks but Do Not Allow the Wet Strip to Touch the Color Chart. Match the colors carefully – THIS IS CRITICAL. Failure to read the reaction at the time indicated may cause erroneous results.

Record results on the report form using appropriate units as necessary. Negative results should be reported out as "Neg". Positive results should be reported in the proper format, using appropriate unites where indicated.

Discard the reagent strip into regular trash when you have finished recording the results

High sensitive C- reactive protein test procedures

The human serum containing CRP mixed with latex particles coated with monoclonal anti-CRP antibodies

After few minutes the presences of CRP leads to precipitation and color development

Then the precipitate is determined turbidimetrically with fully automated Cobas c501 analyzer

Annex II. Information sheet for children aged 5-17 years

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

Principal Investigator: Ousman Mohammed

Organization: Addis Ababa University

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

Introduction:

Hello! My name is _____ and I am working with researchers from Department of Medical Laboratory Science, Addis Ababa University. We are conducting a study to Establish Immuno-Hematological and Clinical Chemistry Reference Intervals for Ethiopians aged ≥ 5years from various localities in the country.

Purpose of the research:

The health laboratory plays an indispensable role in the health care system. It supports diagnosis (to rule in or rule out a diagnosis), monitoring of response to treatment, epidemiological surveillance, prevention as well as Research (to understand the pathophysiology of a particular disease process). Especially there is lack of local reference interval for indigenous population. Therefore, as part of national project the purpose of this proposed study is to establish Llipid profiles and selected Electrolyte Reference Intervals for Addis Ababa population aged ≥ 5years.

You have been chosen for this study as well your guardian/family has been asked and gave consent for your participation in the study. Therefore, we invite you to take part in this study and contribute to the establishment of indigenous reference values which are needed for providing quality laboratory service. Thus, result from this study is anticipated to improve the health status of the Addis Ababa children and adolescent.

Procedures:

After agreeing that you can take part, one or more of researchers will visit your school on a certain day and ask you/your parents some questions which will take up to 15 minutes. Your weight, height and vital signs will be measured. You will be asked to provide urine and fresh stool on a particular container we provide. We will also collect about 10 ml venous blood (about 1 table spoon) from you by sterile-disposable vacutainer tube and needle (7ml in plane tube and 3 ml in tube containing EDTA). We will conduct laboratory examination to determine different hematological, serological, urinalysis, parasitological and selected 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, we will keep records in locked cabinets in a locked room at the office and the results of the tests will be coded to prevent identification of the volunteers. Access to data entered into computerized files will be permitted only for authorized personnel directly involved with the study and will be password protected. Individual-specific information may be provided to responsible local medical personnel only with your permission. Urine, stool and blood collected will not be used for other purposes. The leftover samples will be stored at the Department of Medical Laboratory Sciences of AAU in a secure place for additional tests as needed. Finally, all the biological wastes, after analysis will be safely disposed in an environmentally friendly manner.

Risks and Discomfort:

There will be minimal discomfort in giving urine and stool samples. In addition 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 laboratory professionals after disinfecting the site of picture by 70% ethanol.

Moreover, leftover stool, urine and blood sample (that is not stored) will be discarded following the guideline of bio-safety.

Benefits:

By participating in the study, you will directly benefited by being investigated for any pathogenic organisms and other clinical and hematological abnormalities. The established 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 or we the principal investigator (PI) of the study or the investigators/focal persons using the following addresses:

1. Dr Aster Tsegaye, Addis Ababa University 09 11 69 60 85
2. Dr. Mistire Wolde, Addis Ababa University 09 11 69 97 10

IRB address: Addis Ababa University, College of Health Science +251 -11-896-13 96

Code No._____

Annex III. Assent form for children aged 5-17 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 IV: Information sheet for Parents/guardians

Project Title: Establishment of Serum lipid and electrolytes Parameters Reference Intervals for Addis Ababa children and adolescents.

Project PI: Aster Tsegaye (PhD, Associate Professor at Department of Medical Laboratory Sciences, Addis Ababa University), Ousman Mohammed (BSc)

Organization: Addis Ababa University

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

Introduction:

Hello! My name is _____ and I am working with researchers from the various Medical Laboratory Science teaching Universities, Regional Laboratories, National Blood Bank of Ethiopia and EMLA. We are conducting a study to Establish Immuno-Hematological and Clinical Chemistry Reference Intervals for Ethiopians aged ≥ 5years from various localities in the country.

Purpose of the research:

The health laboratory plays an indispensable role in the health care system. It supports diagnosis (to rule in or rule out a diagnosis), monitoring of response to treatment, epidemiological surveillance, prevention as well as Research (to understand the pathophysiology of a particular disease process). Especially there is lack of local reference interval for indigenous population and local quality control materials. Therefore, as part of national project the purpose of this proposed study is to establish Llipid profiles and selected Electrolyte Reference Intervals for Addis Ababa population aged ≥ 5years. 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 and to develop in-house quality control materials. Both are needed for providing quality laboratory service. Thus, result from this study is anticipated to improve the health status of Addis Ababa children and adolescents.

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 15 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 about 10 ml venous blood (about 1 table spoon) from your child by

sterile-disposable vacutainer tube and needle (7ml in plane tube and 3 ml in tube containing EDTA).

Confidentiality:

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

Risks and Discomfort:

There will be minimal discomfort in giving urine and stool samples. In addition, 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 benefited by being investigated for any pathogenic organisms and other clinical and hematological abnormalities. The established reference interval will be used in the future to improve the general health status of Addis Ababa population.

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.

Whom to contact:

If you have any questions, you may ask the person whom you are giving information or we principal investigator (PI) of the study or the investigators/focal persons using the following addresses:

1. Dr Aster Tsegaye, Addis Ababa University 09 11 69 60 85
2. Dr. Mistire Wolde, Addis Ababa University 09 11 69 97 10

IRB address: Addis Ababa University, College of Health Science +251 -11-896-13 96

Code No._____

Annex V. 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 5-17 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) _____

Annex VI: Information sheet for children 5—17 years (5—17 ዓመት ለሆኑ ህፃናት መረጃ)

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

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

ተባባሪ ተመራማሪዎች የስም ዝርዝር ተያይዟል

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

ስፖንሰር (ወጪውን የሸፈነው)፡ የፌደራል ሳይንስና ቴክኖሎጂ ሚኒስቴር

መግቢያ:

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

የምርምር ጥናቱ አላማ:

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

አንተም/አንቺም በዚህ ጥናት እንድትሳተፍ/ፊ እየጋበዝን ወላጆችሽ/ወላጆችህ ፈቃዳቸውን ገልፀዋል፡፡፡ ስለዚህ በዚህ ጥናት በመሳተፍ በአገራችን በላቦራቶሪ ውስጥ 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 15 ደቂቃ የሚወስድ ጥያቄ ይጠይቁሃል/ሻል፡፡ ክብደት፣ ቁመት፣ የክንድ እና የደም ግፊት ልኬት ይወሰዳል፡፡ ሽንትና አይነምድር በምንሰጠው እቃ እንድትሰጠን/ጭን እንጠይቃለን፡፡፣ በተጨማሪም 10 ሚሊ ሊትር (አንድ የሾርባ ማንኪያ የሚሆን) በንፁህ ቫኩቴይነር ብልቃጥ እና መርፌ እንቀዳለን (7ሚሊ ሊትር በባዶ

ቲዩብ፣ 3 ሚሊ ሊትር ደም እንዳይረጋ የሚያደርግ ንጥረ ነገር ፣ኢዲቲኤ፣ ባለበት ቲዩብ)። የሄማቶሎጂ፣ ሴሮሎጂ፣ ፓራሲቶሎጂ እና የክሊኒካል ኬሚስትሪ ምርመራዎችን እናካሂዳለን።

ሚስጥር ስለመጠበቅ:

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

ጥናቱ የሚያስከትላቸው የጤና ችግሮችና አለመመቸት:

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

ደህንነት:

የደም ናሙና በሚወሰድበት ጊዜ በንፁህ የደም መቅጃ በመጠቀም የሚቀዳውን ቦታ በ70% አልኮል በማፅዳት ልምድ ባለው ባለሞያ ይከናወናል። በተጨማሪም ጥቅም ላይ ከዋሉ በላ 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 የሰጠሽውን/የሰጠሽውን ሰው መጠየቅ ይቻላል ወይም የፕሮጀክቱ ዋና ተመራማሪን ወይም ተባባሪዎችና በየተቋሙ የሚገኙ ተወካዮችን በሚከተለው አድራሻ መጠየቅ ይቻላል፡፡

1. ዶ/ር አስቴር ፀጋዬ፣ መሪ ተመራማሪ፣ አ.አ.ዩ/አ.አ. 09 11 69 60 85

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

ኮድ._____

Annex VII. Consent form for children 5-17 years 5—17 ዓመት ለሆኑ ህፃናት የስምምነት ቅፅ)

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

የ ዓይነምድር ናሙና ለመስጠት ☐

የሽንት ናሙና ለመስጠት ☐

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

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

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

ያልተማሩ ከሆኑ;

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

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

ስልክ ቁጥር (የወላጅ ወይም አሳዳጊ)_____

የተመራማሪው ስም፣ ቀንና ፊርማ

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

Annex VIII: Information sheet for Parents/guardians (ለወላጆች/አሳዳጊዎች መረጃ)

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

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

ተባባሪ ተመራማሪዎች የስም ዝርዝር ተያይዟል

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

ስፖንሰር (ወጪውን የሸፈነው)፡ የፌደራል ሳይንስና ቴክኖሎጂ ሚኒስቴር

መግቢያ:

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

የምርምር ጥናቱ አላማ:

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

ልጅዎ ለዚህ ጥናት ተመርጧል/ጣለች፡፡ ስለዚህ በዚህ ጥናት እንድትሳተፉና በአገራችን በላቦራቶሪ ውስጥ የሚመረት የጥራት መመርመሪያ እና የጤናማ ሰው የሄማቶሎጂና የክሊኒካል ኬሚስትሪ ውጤት ማመዳደሪያ ሪፈረንስ ኢንተርቫል ለመስራት አስተዋፅዎ እንዲያደርጉ ተጋብዘዋል፡፡ ሁለቱም ጥራት ያለው የላቦራቶሪ አገልግሎት ለመስጠት አስፈላጊ ናቸው፡፡ ስለዚህ የዚህ ጥናት ውጤት ኢትዮጵያ ውስጥ የልጆችን ጤና ለማሻሻል ይረዳል፡፡

የጥናቱ አካሄድ:

በጥናቱ ልጅዎ እንዲሳተፍ ከተስማሙ የጥናቱ አባል/አባላት 15 ደቂቃ የሚወስድ ጥያቄ ይጠይቁዎታል፡፡ የልጅዎ ክብደት፣ ቁመት፣ የክንድ እና የደም ግፊት ልኬት ይወሰዳል፡፡ ልጅዎ ሽንትና አይነምድር በምንሰጠው እቃ እንድትሰጠን/እንዲሰጠን እንጠይቃለን፡፡ በተጨማሪም 10 ሚሊ ሊትር (አንድ የሾርባ ማንኪያ የሚሆን) በንፁህ ቫኩቴይነር ብልቃጥ እና መርፌ

እንቀዳለን (7ሚሊ ሊትር በባዶ ቲዩብ፣ 3 ሚሊ ሊትር ደም እንዳይረጋ የሚያደርግ ንጥረ ነገር ፣ኢዲቲኤ፣ ባለበት ቲዩብ)። የሄማቶሎጂ፣ ሴሮሎጂ፣ ፓራሲቶሎጂ እና የክሊኒካል ኬሚስትሪ ምርመራዎችን እናካሂዳለን።

ሚስጥር ስለመጠበቅ:

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

ጥናቱ የሚያስከትላቸው የጤና ችግሮችና አለመመቸት:

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

ደህንነት:

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

ጥቅማ ጥቅሞች፡

በዚህ ጥናት በመሳተፍ ለበሽታ አምጪ ተህዋስያን፣ ደምና ሽንት ምርመራ በማድረግ የልጅዎን ጤንነት 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 ከልጅዎ ከተገኘ በአቅራቢው ወደ ሚገኝ ጤና ተቋም ይላካሉ፣ የልጅዎን ላቦራቶሪ ውጤቶች በነፃ ያገኛሉ። ይሁን እንጂ በዚህ ጥናት ለመሳተፍም ሆነ ለመድሃኒት ክፍያ አይሰጥም። ስለተሳትፎዎ ግን እናመሰግናለን።

ያለመሳተፍ መብት:

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

ጥያቄ ካለ ለማነጋገር:

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

1. ዶ/ር አስቴር ፀጋዬ፣ መሪ ተመራማሪ፣ አ.አ.ዩ/አ.አ. ሪጅናል ላቦራቶሪ 09 11 69 60 85

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

ኮድ._____

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

ከላይ የተገለፀውን መረጃ አንብቤአለሁ /ወይም ተነቦልኛል፡፡ ጥያቄ ለመጠየቅ ዕድል ተሰጥቶኝ ጠይቄ በሚያረካ መልኩ ተመልሶልኛል፡፡ ልጄ እንዲሳተፍ/እንድትሳተፍ ተስማምቻለሁ፡፡ ከ 5-17 ዓመት በታች ለሆኑ ልጄ ከተስማማ/ማች በዚህ ጥናት እንድትሳተፍ/እንዲሳተፍ ፈቃደኝነቴን ገልጫለሁ፡፡

የ ዓይነምድር ናሙና ለመስጠት ☐

የሽንት ናሙና ለመስጠት ☐

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

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

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

ያልተማሩ ከሆኑ;

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

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

ስልክ ቁጥር _____

የተመራማሪው ስም፣ ቀንና ፊርማ

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

Annex X. 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.	Ethnicity	_____If mixed, specify_ _____
	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 Q29, what type of drug? (more than one answer possible)	1. Anti-protozoa 2. Anti-helminthic 3. Anti-allergy 4. Birth control pills 5. Anti-bacterial 6. Anti-TB **7.** Other (specify) _____
	History of common diseases	
26.	History of diabetes	1. Yes 2. No
27.	History of Hospital Admission for the last 1 year	1. Yes 2. No
28.	History of Surgical procedure for the last three years?	1. Yes 2. No
29.	History of chronic gastritis	1. Yes 2. No
30.	History of Malaria for the last 6 month	1. Yes 2. No
31.	History of TB for the last two years	1. Yes 2. No
32.	History of Cancer	1. Yes 2. No

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

❖ We thank you for your cooperation!

Interview Date: _____

Interviewer's Name_____ Signature_____

Annex XI: Questionnaire Amharic version (ቃለ መጠይቅ)

በጤና ባለሞያዎች የሚሞላ ቃለ መጠይቅ

መመሪያ፡

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

አመሰግናለሁ!!!

ክፍል 1. አጠቃላይ መረጃ

ኮድ_____ ክልል _____ ዞን_____

ወረዳ_____ ከተማ/ክፍለከተማ _____ቀበሌ _____

ክፍል 2. የግል መረጃ

6. እድሜ _____
7. ጾታ_____
8. የትውልድ ቦታ_____
9. በትውልድ ቦታዎ ለምን ያህል ጊዜ ኖረዋል?_____
10. አሁን ያሉበት ቦታ ለምን ያህል ጊዜ ኖረዋል? (ከትውልድ ቦታዎ የተለየ ከሆነ) _____ዓመት

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: Ousman Mohammed (B.Sc.)

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: Mistire Wolde (BSc, MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: Aster Tsegaye (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.