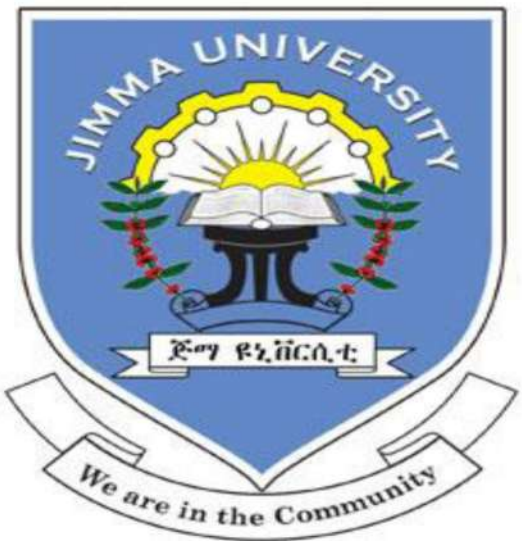
REFERENCE INTERVAL ESTABLISHMENT FOR SELECTED CLINICAL CHEMISTRY PARAMETERS FOR APPARENTLY HEALTH INDIVIDUALS WITH AGE GREATER THAN OR EQUAL TO FIVE YEARS IN SOUTH WEST ETHIOPIA



A RESEARCH THESIS SUBMITTED TO SCHOOL OF MEDICAL LABORATORY SCIENCES; JIMMA UNIVERSITY INSTITUT OF HEALTH IN PARTIAL FULFILLMENT FOR THE REQUIREMENT OF MASTERS DEGREE IN CLINICAL LABORATORY SCIENCE SPECIALITY IN CLINICAL CHEMISTRY

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REFERENCE INTERVAL ESTABLISHMENT FOR SELECTED CLINICAL CHEMISTRY PARAMETERS FOR APPARENTLY HEALTH INDIVIDUALS WITH AGE >=5YEARS IN SOUTH WEST ETHIOPIA

BY

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ABSTRACT

Background:*Reference Intervals are the result of quantitative analysis of a clinical chemistry parameters, obtained from a group of apparently health individual or group of individuals who are selected according to the clearly defined criteria of International Federation Of Clinical Chemistry/Clinical Laboratory Science Institute(IFCC/CLSI)recommend. Selecting reference individuals are an essential but difficult step in the production of RIs throughout the world. As the diversity of the global population increases, a serious need exists to develop meaningful and reliable RI that can be used across regions and population groups.*

Objective: *To determine Reference Interval of selected clinical chemistry parameters for apparently healthy individuals with age ≥5 years and to show sex and age difference in reference intervals in south west, Ethiopia.*

Methods*: A community based cross sectional study was conducted by involving a total of 998 apparently healthy male and female subjects of age 5-71years from the three town with the ratio of their population and the study was conduct from march 2017-May 2017G.C.A serum sample was used for clinical chemistry parameters analysis by using HUMANSTAR-100 analyzer(HUMAN, Germany). Control samples were used for every test batch for all parameters. RIs were constructed using non-parametric methods to estimate 2.5 and 97.5 percentiles of distribution as lower and upper reference limits, respectively. Data were entered using Epi-data and analyzed using SPSS version 20 software. Mann Whitney U test and Kruskal Wallis test were used to show sex and age difference respectively.*

Result*: There were significant differences in relation to gender in all age group RI for aspartate aminotransferase, Alanine aminotransferase, alkaline phosphatase, urea and creatinine. There were also significant age differences between all age group in the RI of both sex for aspartate aminotransferase and Alanine aminotransferase.*

Conclusion:*. The findings of this study provide sex and age specific Reference Intervals for age >=5 years from south west Ethiopian region. Some parameters of this study show sex and age difference and also a difference between other countries and reagent providers RI. Adoption of this RI is recommended in this region and determination of similar RI for other regions in Ethiopia.*

Key words: RI, Clinical Chemistry, healthy individuals, South West Ethiopia

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ABBREVIATION AND ACRONYMS

ALP	Akaline Phosphatase
ALT	Alanine AminoTransferase
AST	Aspartate Aminotransferase
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
CDL	Clinical Decision Limit
CI	Confidence Intervals
CLSI	Clinical and Laboratory Standards Institute
Cr	Creatinine
C-RIDL	Committee on RI and Decision Limits
C-RP	C-reactive protein
CV	Coefficient of Variation
FBS	Fasting Blood Sugar
Glu	Glucose
HBsAg	Hepatits B surface antigen
HCV	Hepatits C virus
HDL-C	High- Density Lipoprotein-Cholesterol
HIV	Human immune deficiency virus
IFCC	International Federation of Clinical Chemistry
JUMC	Jima University Medical Center
LL	Lower Limit

MRA	Multiple Regression Analysis
NCCLS	National Committee for Clinical Laboratory Standards
RI	Reference Interval
SD	Standard Deviation
SDR	Standard Deviations Ratio
SI units	International System of Units
SOP	Standard Operational Procedure
T.chol	Total Cholesterol
TG	Triglycerides
UA	Uric Acid
UL	Upper Limit
WHO	World Health Organization

CHAPTER ONE: INTRODUCTION

1.1 Background

Reference Intervals are one of the most widely used clinical decision making tools. The concept of RIs was first introduced by Gräsbeck and Saris in 1969. Before 1969, no any information was existed on the theory and importance of RIs. This was partly due to the limited number of laboratory tests offered as well as the lack of quality indicators, which resulted in large deviations from values usually found in the general population. This concept first started out as a philosophy but has since become a major discussion point in laboratory medicine and one of the most powerful means to assist in the clinical decision making activity (1).

RIs in clinical chemistry are the results of quantitative analysis of clinical chemistry parameters obtained from an individual or group of individuals that are selected according to clearly defined criteria that was recommended by IFCC/CLSI (3).

To establish a new RI laboratory should collect samples of apparently healthy individuals within its reference population, analyze the samples using its own instrumentation, and then use the appropriate statistical methods to compute the RIs. Current guidelines on establishing RIs provided by the CLSI (4)define RIs as ranges of values within which a specified percentage of measurements of healthy individual should include, and provide clinicians a normal range of comparison, when evaluating and interpreting a patient's laboratory tested results. Establishment of RI is needs the proper measurement of analyte from health individual for the correct interpretation of a patient's results(5,6).

The limits are defined by the range of values that 95% of the reference sample falls between 2.5% and 97.5%, In this case 2.5% of the reference sample will have test values that fall below the lower limit and 2.5% will have values that exceed the upper limit(7,8).

Ceriotti and co-workers reported further that the width of the RI is influenced by three sources of variability: The intra- and inter-individual, biological variability of the selected reference individuals and analytical variability of the measurement system, respectively(8–10).

The major need for laboratory medicine in general and clinical chemistry personnel in particular, is to provide the clinicians updated & appropriate information in RIs. Introduction to the concept of RIs and reference population simplifies the task for laboratories; as long as they define the

reference population, the outcome can always be recognized as RIs. Selecting reference individuals are an essential but difficult step in the production of RIs throughout the world (11).

The Clinical and Laboratory Standards Institute (CLSI) published and updated a guideline for laboratories and manufactures to perform their own RI studies, this is due to the result of measured laboratory parameters are influenced not only by individual factors such as age, sex, and lifestyle, but also by population and ecological factors such as ethnicity, climate, and altitude and also vary between individuals(4).

RIs are supposed to be established or verified for each analytic and specimen source in every clinical laboratory. In reality, however, very few laboratories or manufacturers carry out their own RI studies. Other laboratories and manufacturers refer to RIs from studies done a few decades ago, when both the analytical methods and life style of the population were very different. So it is a very important task to carefully establish RIs by the laboratory based on standard protocols(4,6,12).

The laboratory results from a patient are compared with the health associated RIs of: interpreting clinical laboratory data, supporting correct medical diagnosis and decision on therapeutic management and other physiological assessment. The carefully determined health associated RIs along with patient's clinical data help in determining the clinical status of a patient. Very sound understanding of RIs is therefore vital as diagnosis of disease frequently depends on results of laboratory analyses measured from blood, serum, urine and other biological fluids. The poorly defined RIs due to improper basic and uniform process do not serve the medical needs of patients. The lack of direct comparability of results from different laboratories poses further problems of patients as well as to the treating physician(13).

The recommendation of International Federation of Clinical Chemistry (IFCC) that each laboratory produces its own RIs and estimates the corresponding RIs according to defined procedures are impractical and also did not met the need of patients and physicians. It was also recommended that if due to some reason the laboratory fails to establish the detailed reference studies, they are required to validate RIs published elsewhere using their own methodology for the population served by them(14,15). Because, One of the possible causes of difference in local RIs may be due to racial makeup of the local population which may give difference in concentration of some common analytes; There for the inclusion of these differences in local RIs requires very careful attention(16,17).

There are three principal methods to determine RIs: 1) Conventional method or priory method which conducts a comprehensive RI determination study using the International Federation for Clinical Chemistry (IFCC) recommendations, 2) The posterior method where data is analysed that has been pretested and where sufficient information to determine selection criteria is available, and 3) Indirect determination analysing large amounts of data from laboratory databases and applying statistical calculation to the data set(8,18). In this study the conventional or priory methods i.e recommended by IFCC was followed.

1.2 Statement of the problem

The importance of RIs is not so much to define "normal" and "abnormal" or health or disease, but assist clinicians to make appropriate diagnosis and monitor treatment of patients. Clinicians require decision-making values, rather than "normal values". Although medical decision making is increasingly based on laboratory results, thus locally established laboratory RI is mandatory(19,20).

Establishing RIs has always been a challenge as significant differences may exist on disease frequencies, biological variation in analyses among ethnic groups, genders and ages, specimen collection techniques, test performance, test interpretation, and other factors. However, it has a challenge; health professionals understand the importance of RIs, but many laboratories still do not have comprehensive data onto RIs that are specific to their typical patient populations. There continues to be significant gaps between the available RIs that frequently cited in the literature were obtained using older methodologies and instrumentation and cover a limited range of age groups or a relatively small number of samples size(6,21).

Ferruccio Ceriotti, a former chairman of the IFCC Committee for RIs and Decision Limits (C-RIDL), noticed that "The theory of RIs was developed many years ago, but its application for most clinical laboratories is still incomplete today." and that "This is due to the fact that obtaining a 'good' RI is a very demanding activity, in terms of time, money and knowledge"; Large multicenter studies are needed to make real progress in this field and bridge the large gap now existing between a very nice theory (IFCC and CLSI documents) and a very poor practice"(14,22,23).

Friedberg et al. noticed that College of American Pathologists (CAP) surveys of 500 laboratories in 2001 found that 390 (78%) was adopted manufacturers' values for RIs (24).

As the diversity of the global population increases, a serious need exists to develop meaningful and reliable RIs that can be used across regions and population groups. Critical gaps between reliable RI can potentially contribute to incorrect disease diagnosis and lead to inappropriate treatment. It is critically important that more reliable and comprehensive RIs be established for specific populations (25,26).

Laboratory RIs of populations of western countries are widely published in scientific literature, textbooks and on the worldwide web. However, there is a general lack of published data

regarding laboratory parameters for populations living in tropical sub-Saharan Africa and the few studies that have been undertaken have indicated differences in RIs of African populations compared to those derived from western populations. These differences suggest the need for the development of locally derived RIs(27,28).

1.3 Significant of the study

This study was conducted to establish clinical chemistry parameter RI for apparently healthy individuals in south west Ethiopia. Establishing RI of specific population is important to provide the accurate RI for patient treatment and monitoring. So this study also provides one means for an evidence based practice in patient diagnosis and monitoring in clinical trials and routine work. In addition it contribute its part in the future national level study on RI and use for policy making and research in the field of clinical chemistry.

CHAPTER TWO: LITERATURE REVIEW

Reference intervals need serious reconsideration, for their applicability in the specific setting and for the population that is being served. Because reference ranges that are currently used in many laboratories in Africa, especially in Ethiopia, were established by instrument manufacturers or using text book values, or from reagent suppliers. In recent years several studies have been carried out in Africa using the recommended International federation of clinical chemistry (IFCC) guidelines. Several reference determination studies have been carried out in other parts of Africa, few have been carried out in Ethiopia(17,29,30).

A Study done on pediatric, adolescent and geriatric age group in Canada shows alkaline phosphatase (ALP), and aspartate amino transferase (AST) all decreased in concentration across the pediatric age range. ALP decreased at the 16years of age in boys; in girls, however, ALP decreased at an earlier age of 11 years. ALP concentrations then remained relatively stable throughout adulthood and geriatric ages. Creatinine(Cr),Total cholesterol(T.chol), Triglyceride(TG), Uric Acid(UA), and urea all had increased concentrations in adulthood vs childhood. Alanine amino trasferas (ALT), and glucose all required 5–6 age partitions, with lower limits that remained relatively unchanged throughout the age range but increasing upper limits in adult and geriatric ages (31).

The study which included healthy adults age between 20-50 years in Turkey was revealed the RIs for ALP, ALT, AST and creatinine in males were narrower and uric acid in females were wider RIs than the intervals provided by the manufacturer. serum triglycerides median values were 1.28 and 0.87 mmol/L, for male and female respectively. 10.7% of male had high triglyceride levels associated with low levels of HDL-C. And also the upper limits were higher for T.chol for both sex, TG values of men were higher than women, and Mean serum T.chol, and TG levels were increased with age (32).

Similarly Study done on RI for renal profile in north Indian population reveled that RI for urea, Cr and uric acid were lower in female (16-42mg/dl, 0.6-1.2mg/dl, 2.4-6.8mg/dl) as compared to male (17.00-44.35mg/dl, 0.7-1.5mg/dl, 2.8-7.2mg/dl) with lower cutoff of lower and upper limits(33).

Study done in china show that some analytes were changed significantly with age in both sexes: T.Chol, TG, and Urea. ALP, Glu, and TG increased with age only in females. In males, the level of lipids increased mainly until the age of 40, where as in female, the increase occurred more

prominently after 40 years of age. Also MRA results revealed that test results for analytes (UA, TG, HDL-C, ALT,) in females and analytes (UA, T.Chol, TG, HDL-C, ALT,) in males were related to BMI. Of all the related analytes, only HDL-C decreased with the increase of BMI.(12).

A Population based RIs for common blood hematological and biochemical parameters study in the Akuapem north district Ghana showed the concentrations of the liver enzymes, ALT, AST and ALP and serum Cr were significantly higher in males than in females and based on this analysis, separate RIs for males and females were reported for these analytes. The RIs for ALT were 11.6 – 53.1 U/L median = 24.3 U/L (male) , 9.5 – 39.2 U/L, median = 18.0 U/L (female). The RIs for AST were (male) 18.7 – 65.0U/L median = 31.3U/L and (female) 15.5 – 46.5 U/L, median = 25.7 U/L. The distribution of Urea did not differ significantly between males and females (median =4.4mmol/L, RI 1.7 – 7.2mmol/L)(34).

A similar Study conducted in western Kenya shows a liver and kidney function tests indicated gender and age variations between young adults and adolescents. Males had higher values for ALT, AST, and Cr than females in both age groups, with those differences being significantly greater for Cr indices in both age-groups and AST among the adolescents. There were no gender differences in blood urea nitrogen and Glu levels for all age groups and no significant differences in AST, ALT and Glu between the two age groups for both males and females. However young adult men and women did have higher values for Cr and BUN compared to adolescent males and females, respectively(35).

A study in north rift valley Kenya shows that among the renal function tests, age 18-28 showed significant differences for Cr, and UA with males having greater mean RIs than their female. creat and UA showed a significant sex difference in age 29-39 while in age 40-50, only UA showed a significant sex difference for all the renal function analytes. UA showed significant sex differences in all the age categories with males having higher values than females. Within females, RIs for Cr for age 40-50 were greater than those of age 18-28. But, Other renal function tests did not show any significant difference(28).

Population based study in Rwanda of 187 subjects (age range: 17-54 years) presented as median, with 2.5th-97.5th percentiles (95% RI) in brackets are as follows: For males: ALT: 25 (12-43) U/L; AST: 29 (16-47) U/L; Cr: 0.8 (0.5-1.1) mg/dL; UA: 5 (3-7) mg/dL. For males and females:

ALP: 71 (27-122) U/L; TG: 82 (32-172) mg/dL; HDL-C: 48 (29-86) mg/dL; Glu: 87 (70-114) mg/dL (36).

Another RI study in Botsowana found that differences between the newly established RIs and those currently in use in Botswana, and the current in use RIs had higher upper limits for ALT, BUN, and decreased lower limits for ALT, creatinine, BUN. Also males have higher levels of AST, ALT, Cr, blood urea nitrogen than female (25).

Study in rural north Uganda also observed significant age differences in RIs for ALP , AST , glucose , Cr , BUN and UA (30).

A study conducted in Mozambique shows that there was a statistically significant difference between genders in all clinical chemistry analytes, with the exception of triglycerides. Males had significantly higher levels of Glu, ALP, AST, ALT, UA, urea and Cr than females. Females had significantly higher levels of T.chol and HDL-C than males(37).

Comprehensive RIs study done among Nigerian adult shows that the differences in values exist by gender as well as by country. Glu and liver enzymes were significantly higher in Nigerian men than women while it was the opposite for urea levels. Also AST and ALT values from Nigerians were higher than those reported for Kenyans, Tanzanians, and USA(38).

Study done in Ethiopia Gilgal Gibe research center showed the total mean cholesterol level at\ different age group was higher for women than men. Total serum triglyceride level was slightly higher for men than women. The median values (95%)for FBS were 95.0 mg/dl . The mean (95% CI) values for BUN, Cr and UA were 14.3 mg/dl, 0.9 mg/dl and 4.6 mg/dl for men and 13.8 mg/dl, 0.9 mg/dl and 4.1 mg/dl for women, respectively. The mean (95% CI) ALT and AST level of the study population were 27.9 U/L and 31.0 U/L for men and 26.6 U/L and 30.2 U/L for women, respectively. In most age group, the mean ALT value was higher for men than women. Highest values of ALP, 227.9 U/L for men and 214.9 U/L for women were observed in the age group of 15-24 years(17).

As to our knowledge, there was no comprehensive study conduct to establish any RI in southwest region,Ethiopia. Thus, this study was aimed to establishing RIs for selected clinical chemistry parametes in South west Ethiopia

CHAPTER THREE: OBJECTIVES

3.1 General objective

To determine the RIs for clinical chemistry parameters for apparently healthy individual age ≥5 years in South West, Ethiopia.

3.2 Specific objectives

- To establish RIs for liver enzyme(ALP,AST,ALT) for both sexes and age ≥5 years in south western Ethiopians
- To establish RIs for Kidney function(Cr,Urea,UA) for both sexes and age ≥5 years in South Western Ethiopians
- To determine RIs of blood glucose level for sex male and female individuals age ≥5 years in south western Ethiopians
- To establish RIs for lipid profile(HDL,T.CHol,TG) for sex male and female individuals age ≥5 years in south western Ethiopians

CHAPTER FOUR: MATERIALS AND METHODS

4.1. Study area

This study was conducted in southwest Ethiopia including Jimma, Bonga and Mattu Towns by considering their population diversity and convenience to the study.

Jimma, is the largest town in south-weste Ethiopia. It located in oromia regional state , Jimma Zone. It has an elevation of 1780m above sea level. Based on the 2007 Census conducted by the Central Statistical Agency of Ethiopia (CSA), the town has a total population of 120,960, of which 60,824 are men and 60,136 women. With an area of 50.52 square kilometers, Jimma has a population density of 2,394.30 all are urban inhabitants. A total of 32,191 households were counted in this town, which results in an average of 3.76 persons to a household, and 30,016 housing units. The three largest ethnic groups reported in Jimma were the Oromo (46.71%), the Amhara (17.14%) and the Dawro (10.05%); all other ethnic groups made up 26.1% of the population(39).

Bonga town is located in the Kaffa zonea of the SNNPR, an elevation of 1,714 meters above sea level. Based on the 2007 Census conducted by the CSA, this town has a total population of 20,858, of whom 10,736 are men and 10,122 women(39)

Mettu Town is the capital city of Illu Ababora Zone of the Oromia Region in south-west Ethiopia. It is located around 265Km away from Jimma **city** and its altitude of 1605 meters above sea level. The 2007 national census reported a total population for Metu of 28,782, of whom 14,400 were men and 14,382 were women(39).

4.2. Study design and period

Community based cross sectional study was conducted from March 13,2017-May 30,2017G.C.

4.3 Population

4.3.1 Source population

All population living in south west Ethiopia those age five years and above.

4.3.2 Study population

The study population were school children (primary and secondary school), University students, University staff, volunteers from community and pensioners.

4.4. Inclusion and exclusion criteria

4.4.1. Inclusion criteria

Apparently healthy individuals who have been living in the study areas for six months before the study period and who were greater or equal to 5 years old..

4.4.2. Exclusion criteria

The exclusion criteria was determined based on the protocol provided by IFCC/C-RIDL(6). The height, weight, and blood pressure were measured on site. The exclusion criteria were as follows:

- Pathophysiological States - Renal failure, cardiac diseases, chronic respiratory diseases, liver diseases, malignancies.
- Known carrier state for hepatitis B virus, hepatitis C virus, or human immunodeficiency virus.
- Systemic Diseases – Hypertension and Diabetes mellitus
- The chronic intake of pharmacologically active agents like alcohol, tobacco or oral contraceptives, (for more than six months during the time of the health checkup)
- Replacement or Supplementation Therapy e.g. Thyroxin, Insulin
- Modified Physiological States- Pregnancy, psychological and mental disorders- exercise/physical training /
- Other Factors - Obesity (BMI >30 kg/m^2).

4.5. Sample size and sampling technique

4.5.1. Sample size determination

The sample size was determined according to the CLSI recommendation to use well defined exclusion and portioning criteria for the selection of the reference individuals. So based on the CLSI guideline the minimum sample size required for RI determination is 120 healthy individuals for sex and age partitioning.

According to previous studies in other African countries, in such large scale studies about 30% (40) did not qualify for RI determination for various reasons when tested for the common viral infections and syphilis. We proposed depending on this study to reach a total minimum sample size of 720 , we need 1030 individuals i.e(30% x 1030=309 which is 1030-309 = 721). But due to absence of volunteers, we were collected 998 sample and 12% were excluded with post exclusion criteria.

4.5.2. Sampling technique

Non probability convenience sampling technique was used and all volunteers' were selected from all schools, university students, employees and other volunteers'.

4.6 Study variable

4.6.1 Dependent variable

Clinical chemistry parameters RIs

4.6.2 Independent Variable

- Age
- Sex
- Ethnicity
- Altitude
- BMI

4.7. Data collection technique and instruments

4.7.1 .Questionnaire

Predesigned questionnaire was used to collect socio-demographic, anthropometric and physical examination data. Height and weight was taken using calibrated equipment's and standardized techniques. Physical examination and interview with study participants were done by a trained clinical nurse.

4.7.2 Blood Specimen collection and processing

4.7.2.1 Blood specimen collection and processing

Five milliliters of venous blood was drawn by antecubital venipuncture using vacutainer system within serum separator tube. The collected Whole blood was left for 30 minutes until it clotted, serum samples were obtained by centrifugation at room temperature at 1500 revolution per minutes (rpms) for 10 minutes. The separated serum samples were stored at -20^{o}c refrigerator till laboratory analysis. The time of collection was performed at 8:30am to 11:30am

4.7.3 Laboratory test Methodology

Analysis of samples was done after proper standardization of the instruments with the help of calibrators and controls.

4.7.3.1 Screening test

The qualitative determination of C Reactive Protein (C-RP)(Human,Germany) testes in participant's serum specimens was done for screening the participants for the presence of infection and inflammation. Hepatitis B virus was screened by blue cross biomedical (beijing)Co.Ltd, One test strip which is a rapid test for the qualitative detection of HBsAg in human serum and plasma. Hepatitis C virus (HCV) was screened by Atlas Link(Beijing) Technology Anti-HCV cassette which is a rapid test for the qualitative detection of antibodies to HCV in human serum, plasma or whole blood.

4.7.3.2 Clinical Chemistry parameters test Methodology

All specimens from each individual were assayed in a single batch, using the same lots of reagents to minimize the analytical variation. Blood serum samples were analyzed for serum Glu, lipid profiles (T.chol, HDL-C and TG), liver function tests (ALT, AST, ALP), renal function tests(Urea, Cr, and UA) parameters using IFCC approved method(3) (Table 4.1). All tests were performed on fully auto analyzer Humastar100 chemistry analyzer(Germany) using human reagents.

Table 4. 1.Test Methods used to analysis the parameters for RI in southwest,Ethiopia ,2017

PARAMETERS	METHODS
ALP	Orthophospheric mono ester phosphohydrolase,Alkaline Optimum,Ec3.1.3.1at 405nm
AST	IFCC MOD,Liqui UV test,Ec2.6.1.1at 340nm
ALT	IFCC MOD,Liqui UV test,Ec2.6.1.2 at 340nm
CRE	JAFFE reaction,auto creatin photo metric colorimeteric(490-510)nm
UREA	GLDH Method,enzymatic colorimetric test with lipid clearing factor at 345nm
UA	PAP-METHOD,enzymatic colorimetric test with lipid clearing factorat 520nm
T.chol	CHOD=PAP-method,enzymatic colorimetric test with lipid clearing factor at 500nm
TG	GPO-PAP-METHOD,enzymatic colorimetric test with lipid clearing factor at 593nm
HDL-C	Direct Homogeneous test for the determination of HDL CHOL,ENZYMATIC COLORIMETRIC TEST
GLU	God-pap method enzymetic colorimetric test for glucose at 500nm

4.9. Statistical analysis

All socio-demographic and some clinical data as well as biochemical data were first entered in to Epidata, cleaned and checked for completeness. Then exported and statistical analyzed using SPSS-version 20 statistical software for windows.

In present study the data were non normal distribution, therefore non-parametric methods for determination of RI were adopted as recommended by IFCC and NCCLS (3). Median and central 95% confidence interval (CI) was calculated. The 97.5 percentile and 2.5 percentile were form the upper and lower reference limit to the population. The significance difference between sex among age groups were determined by using mann whiteny u test and significance difference between age groups among sex were determined by using kruskal wallis test. P-value <0.05 was considered as significance difference. The different age groups were categorized as: Category 1 (5- 15 years), Category 2 (16-49 years), and Category 3 (>=50 years).

4.10. Quality assurance

In order to obtain reliable and valid data, training /orientation was given to data collectors prior to data collection to ensure the quality of data. Standard operating procedures (SOP) for sample collection, processing, transportation and storage was followed to have reliable result. To minimize pre-analytical variation, the same phlebotomist was collecting the blood specimens from each volunteers. Commercially available quality controls (Normal and Pathological) were carried out to evaluate instrument performance and reagent stability in every test run.

4.11. Ethical consideration

Ethical clearance was obtained from Jimma University, Institute of Health Ethical Review Committee. Support letter from Health Science Research coordinating office was written to concerned body and the permission was obtained from concerned office. A written informed consent was obtained from the study participants after describing the benefits and risks of the study. The collected data was treated with the highest level of confidentiality. The specimens collected from the participants were analyzed only for the intended purposes. Those study participants who have positive laboratory result during the screening process, were sent to communicate the clinician working in the hospitals and they had gotten the necessary treatment and counseling according to their disease condition.

4.12. Operational Definitions

Apparently healthy: individual who pass all vital sign and screening test in normal result (no sign of illness or disease)..

Reference population; This is a group of persons who meet the defined criteria for a reference individual.

Reference individuals; Individual that apparently healthy and included in the study

Reference sample; An adequate number of reference individuals meeting the selection criteria to be included into the sampling group and who represent the reference population.

Reference Interval ; A RI is the interval between, and including, two reference limits, which are values derived from the distribution of results obtained from a sample of a reference population.

Reference limits; Define the value of the upper limits and the lower limits of the reference distribution, and are estimates of true limits.

Decision limit; Indicates the cut-off point or thresholds between health and disease used by clinicians to make diagnostic decision and medical action.

Parameter; A quantity that defines certain features of a population (mean, SD, CV, mode, average).

Confidence interval (CI); A value that, within a given probability, will contain the value of the unknown population parameter and indicates the imprecision of that estimate

CHAPTER FIVE: RESULTS

5.1 Socio -demographic distribution

From estimated sample size of 1030 only a total of 998 volunteers blood samples were collected from Jimma=626,Bonga=247 and Mattu=125 in the southwest Ethiopia. Post screening for HBsAg, HCV, C-RP were done and 115(12%) were reactive for screening tests, and the remaining 883(430 male and 453 female) were included in the study (table 5.1).

Table 5 1 **Socio demographic distribution of participants in south west Ethiopia, 2017**

Variables	**Frequency**	**Percent**
Male	430	48.7
Female	453	51.3
AGE CATEGORY IN YEARS		
5_15	369	41.8
16-49	254	28.8
>=50	260	29.4
ETHNICITY		
Oromo	356	40.3
Kaffa	150	17
Amhara	187	21.2
Tigrea	26	2.9
Dawuro	76	8.6
Others	88	10
RELIGION		
Muslim	192	21.7
Catholic	56	6.3
Orthodox	458	51.9
Protestant	173	19.6
Others	4	0.4

5.2. RI for liver function, renal function and metabolic parameters

RIs for ten clinical chemistry parameters were determined for males and females with an age range of 5 to 71 years with median age of 18yrs and 22yrs for females and for males, respectively.

Table 5.2 and 5.3 shows age and sex specific RIs for each parameter based on the p-values for the difference between male and female participants ($p<0.05$ indicate significant difference) The tables also indicate the number of age and sex specific participants used for determining the RIs for each parameter which were all above the minimum sample size (N = 120) suggested by CLSI(3).

The significant sex difference RIs were seen : among all(1,2,3) age group for the analytes(ALP, AST, and ALT) , Among age group 1and 2 for the anaytes (Cr and UREA), Among age group 2 and 3 for (UA,HDL-C and T.chol), and among age group 3 only TG.

But(UA, HDL-C,T.chol) among age group 1, TG among age group 2, (Cr and urea) among age group 3 and glucose among all age groups were no significant difference seen b/n sex(Table 5.2 and 5.3).

ALP and AST all decreased in median value with age increases. ALP concentration continued relatively stable throughout middle age(M=214.5(91.7-783),F=189(77.7-533.6)) and geriatric ages(M=164(72.4-369),F=199(99.6-449.8).

Cr, T. chol, TG, UA, and urea all had increased concentrations in age>=16 vs age <16. Serum Cr concentrations increased considerably throughout the age increase.

Male 215(73.8-533.8)mg/dl had higher TG than female 175(62-529)mg/dl in age category >=50years. Median HDL-C(Male=37.7,38,40;Femal=39,44.7,46) concentration increased with age increases; however, male median HDL-C concentrations lower than female throughout all age categories. Lower limits of Glu remained relatively unchanged throughout the age category, but increasing upper limits in 16-49 years and >=50 years ages.

Table 5 2 RI for LFT (ALP,ALT,AST) and RFT(Cr, UREA,UA) of southwest Ethiopia 2017

parameter	Age category	sex	N	Median	Percentiles		Sig. difference b/n sex
					2.5	97.5	
ALP (U/L)	5_15	M	163	650.00	225.30	891.80	*P=.000
		F	206	555.00	154.70	962.30	
	16-49	M	132	214.50	91.65	783.47	*P=0.002
		F	122	189.00	77.72	533.62	
	>=50	M	135	164.00	72.40	369.00	*P=0.000
		F	125	199.00	99.60	449.75	
AST (U/L)	5_15	M	163	31.00	11.00	96.00	*P=0.000
		F	206	25.00	12.00	53.00	
	16-49	M	132	25.00	13.00	64.00	*P=0.000
		F	122	21.00	11.00	56.00	
	>=50	M	135	25.00	11.00	71.00	*P=0.000
		F	125	22.00	11.00	52.00	
ALT (U/L)	5_15	M	163	18.00	6.00	57.00	*P=0.000
		F	206	16.00	5.00	40.00	
	16-49	M	132	19.00	4.33	56.00	*P=0.000
		F	122	15.50	5.00	48.00	
	>=50	M	135	21.00	7.40	66.00	*P=0.000
		F	125	17.00	4.00	47.70	
Cr(mg/dl)	5_15	M	163	0.56	0.24	1.29	*P=0.025
		F	206	0.53	0.28	0.92	
	16-49	M	132	0.79	0.15	1.35	*P=0.000
		F	122	0.62	0.26	1.31	
	>=50	M	135	0.71	0.14	1.25	P=0.072
		F	125	0.62	0.28	1.05	
UA(mg/dl)	5_15	M	163	3.87	1.58	9.15	P=0.174
		F	206	3.85	1.93	6.42	
	16-49	M	132	5.26	2.80	9.08	*P=0.000
		F	122	3.80	1.80	8.90	
	>=50	M	135	5.60	3.02	9.85	*P=0.000
		F	125	4.41	2.72	9.08	
Urea (mg/dl)	5_15	M	163	20.00	9.14	46.39	*P=0.000
		F	206	16.80	5.62	32.16	
	16-49	M	132	20.75	9.09	38.03	*P=0.002
		F	122	17.90	9.56	35.87	
	>=50	M	135	22.00	10.16	42.86	P=0.192
		F	125	20.60	9.52	40.36	

M=male, F=female, p=p- value, ALT=alanine amino trasfarase , AST=aspartate Amino trasfarase, Cr=Creatinine, UA=Uric Acid, *p=significant sex difference

Table 5 3 RI for lipid profile (T.Chol,HDL-C,TG) and RBS of southwest Ethiopia 2017

parameter	Age category	sex	N	Median	Percentiles		Sig. Difference b/n sex
					2.50	97.50	
HDL-C(mg/dl)	5_15	M	163	37.70	9.65	92.50	P=0.838
		F	206	39.00	14.80	90.00	
	16-49	M	132	38.00	19.00	88.00	*P=0.000
		F	122	44.70	21.78	129.90	
	>=50	M	135	40.00	16.70	108.00	*P=0.000
		F	125	46.00	21.75	133.00	
Triglycer (mg/dl)	5_15	M	163	120.00	48.80	306.90	P=0.812
		F	206	119.00	56.00	266.00	
	16-49	M	132	131.50	63.00	382.00	P=0.205
		F	122	119.00	57.00	469.00	
	>=50	M	135	215.00	73.80	533.80	*P=0.027
		F	125	175.00	62.00	529.00	
Cholesterol (mg/dl)	5_15	M	163	158.00	96.50	282.50	P=0.213
		F	206	164.50	88.00	262.00	
	16-49	M	132	161.50	99.97	344.50	*P=0.002
		F	122	187.00	103.75	366.00	
	>=50	M	135	212.00	97.00	337.00	*P=0.043
		F	125	225.00	113.00	345.00	
Glucose (mg/dl)	5_15	M	163	94.70	49.89	96.70	P=0.103
		F	206	92.75	52.00	158.85	
	16-49	M	132	96.00	66.00	189.00	P=0.124
		F	122	91.75	59.00	203.80	
	>=50	M	135	94.70	57.70	213.90	P=0.096
		F	125	103.00	66.80	233.00	

M=male, F=female, p= p-value, HDL-C=high density lipoprotein cholesterol

*p=significant sex difference

5.3 RIs difference b/n age groups among sex for selected clinical chemistry parameters in southwest, Ethiopia.

Multiple Comparisons Kruskal wallis test used for age difference among sex; p-values less than 0.05 were considered statistically significant age difference.

A significant age difference between age category 1 and 2 within males were seen in AST,ALP, Cr , UA and TG, and within females were seen in ALP, AST, Cr , Urea, T.Chol and TG.

A Significant age difference between age category 1 and 3 were observed within males in all anaytes, except HDL-C, Glu and Urea tests, whereas within female were seen in all parameters.

Significant age difference between age category 2 and 3 were seen within male in ALP, Cr,T. Chol and TG ,and within female were seen in T.chol, TG, Glu, UA and Urea (Table 5.3).

There was no significant differences were seen between age category 1 and 2 within male for T.chole, Glu, ALT, HDL-C and urea, and within female for GLU,ALT,TG and UA.

There was no significant age differences were seen between age category 1 and 3 within male for Glu, HDL-C and urea, And between age category 2 and 3 within male for Glu, AST, ALT, HDL-C and Urea, whereas within female for ALP, Cr, AST, ALT and HDL-C (Table 5.3).

Table 5 4 Kruskal Wallis Test to show differences b/n age groups among male and female for each parameters.

Age cat.	Sex		ALP	Chol	Cr	Glucose	GOT	GPT	HDL	Trigly c	Uric Acid	Urea
B/n age group 1 & 2	male	Chi-Square	146.406	3.579	86.058	0.496	20.539	2.912	0.587	5.26	61.207	0.148
		df	1	1	1	1	1	1	1	1	1	1
		Asymp. Sig.	*0.000	0.059	*0.000	0.481	*0.000	0.088	0.443	*0.022	*0.000	0.700
	female	Chi-Square	159.74	24.236	28.71	0.981	16.809	1.425	22.218	2.183	2.75	4.283
		df	1	1	1	1	1	1	1	1	1	1
		Asymp. Sig.	*0.000	*0.000	*0.000	0.322	*0.000	0.233	*0.000	0.14	0.097	*0.038
B/n age group 1 & 3	male	Chi-Square	219.265	59.264	15.345	3.049	20.003	10.529	0.805	66.064	85.98	2.282
		df	1	1	1	1	1	1	1	1	1	1
		Asymp. Sig.	*0.000	*0.000	*0.000	0.081	*0.000	*0.001	0.37	*0.000	*0.000	0.131
	female	Chi-Square	161.094	91.927	35.915	24.088	15.425	3.971	24.386	52.182	41.822	35.598
		df	1	1	1	1	1	1	1	1	1	1
		Asymp. Sig.	*0.000	*0.000	*0.000	*0.000	*0.000	*0.0460	*0.000	*0.000	*0.000	*0.000
B/n age group 2 &3	male	Chi-Square	22.453	33.791	9.888	1.628	0.074	3.375	0.362	32.732	3.713	2.553
		df	1	1	1	1	1	1	1	1	1	1
		Asymp. Sig.	*0.000	*0.000	*0.002	0.202	0.786	0.066	0.547	*0.000	*0.054	0.111
	female	Chi-Square	3.28	21.34	0.821	15.344	0.216	1.261	0.271	27.893	21.115	10.198
		df	1	1	1	1	1	1	1	1	1	1
		Asymp. Sig.	**0.07**	***0.00**	**0.365**	***0.000**	**0.642**	**0.261**	**0.602**	***0.000**	***0.000**	***0.001**

df=degree of freedom, * Significant age difference

*1,2,3(5-15,16-49,>=50)

CHAPTER SIX: DICUSSION

The clinical chemistry parameters RIs determined in this study were; blood Glucose, T.chole, TG, HDL-C, UREA, Cr, UA, ALT, AST and ALP. Among 998 study participants involved, only 883 were selected for the study and 115 study participants excluded from the study because they were reactive for serological or screening tests. Out of 883, 430 males and 453 females were fulfilling the inclusion criteria in the study and each group exceeded the minimum of 120 participants per subgroup for the non-parametric test that required for 95%CI that recommended by CLSI (3) to RI determination.

There is high median value for all parameters in this study in male within all age group except for ALP within age category 3, and for HDL-C and T.chol within all age categories. This was correlated with study done in India(33), Ghana (34) and Kenya (35).

ALP decreases with age increases among male and female .The decrease in serum ALP could be due to reduced bone growth as age advances (41,42).

The significantly higher median values of the RI for AST in male compared to female indicates sex differences in these clinical chemistry parameters. Sex differences in AST have been known to exist due to differences in muscle mass which affects AST. Similar findings have been reported in South India (11),Tanzanian (27), Kercho-Kenya (43) and Uganda (44) populations.

AST is expressed in other tissues such as the heart, red blood cells, and kidney, and this differential expression pattern may explain why AST activities were higher in childhood and declined into adulthood, as this may due to growth and development of organ systems other than the liver at an early age. Sex differences appeared around adolescence for all liver markers and persisted over the subsequent age category(45).

The upper limits of ALT increased with age whereas AST generally decreased (46). The rise in ALT in the adult and geriatric age may reflect the increased development of fatty liver and the metabolic syndrome, which generally occur later in life(31,47).

Sex differences observed for UA could be attributed to the differences due to effects of sex hormone patterns and differences in body mass between genders(27,44). The increase in serum

RI for UA in males with progression of age could be as a result of the increase in weight with advancing age as indicated by study done on Japanese population(48).

Concentrations of serum Cr, Urea, and UA all increased with age. The amount of Cr produced daily from break- down of Creatine in muscles correlates with increased muscle mass. Similarly, Urea and UA are related to protein degradation and would also be expected to increase with growth and development. This was particularly evident in childhood and adolescence, consistent with study in Canada (46).

The rising T. chol concentrations from early childhood to adulthood may indicate the growing demand for hormone synthesis associated with growth and development. Median value of HDL-C almost similar with age in males but increased in females. These results support the role of estrogen in modulating HDL-C, a mechanism to explain the protection against cardiovascular disease in premenopausal women(49).

The age category within childhood shows the regulation of glucose metabolism at an early age. However, The rising upper limit of glucose concentrations later in life may provide insight into underlying insulin resistance that occurs with age (50).

The observed significant increase of some biochemical analytes and decrease of others in one or both sexes as age progresses is an indication that the analytes are age dependent(51). From some research it was noted that there was variation in age and sex in all categories , this could be contributed to diet, environmental factors(52) and analytical methods as indicated by study done in southern Tanzania(27).

Generally, physiological functions have been shown to vary with population due to differences in diet, genetics, physical, environmental and socioeconomic conditions(51–53).

The following table 6.1 shows the comparison of current study with manufactures RI and RI study in our country of other area and other country for age >15years.

The RI of ALP in our study higher in both sex than other Ethiopia (Gilgal gibe),Tanzanian and RI from reagent producers. This may be due to age 16year to 20 years were included in our study as adult age where bone development was going on. T.chol, TG, UA and glucose of our result

are also higher than other RI study, but Cr is lower than others. Urea is lower than reagent manufacture's but higher than Tanzanian's.

The upper limit of AST is higher than other but, ALT is almost similar with Gilgal gibe and Tanzania's but lower than reagent manufacture's as shown in the table below(Table 6.1).

Table 6 1 comparison of this RI with other RI studies for greater than 15years old ,2017

parametrs	Sex								
	Male					Female			
	Unit	Current RI	Gilagal Gibe (Ethio)	Tanzanian (Mbye)	Reagent manufacturer (Human,Germany)	current RI	Gilgal Gibe(Ethio)	Tanzanian (Mbye)	Reagent manu facturer (Human,Germany)
ALP	U/L	89.8-684.6	55.8-362.9	45.9-170.4	80-306	99-492	70.4-384.4	45.3-155	64-306
T.Chol	mg/dl	101-333.5	52.1-252.2	89-218	0-200	110-350	58-286.4	108-213	0-200
Craetinine	Mg/dl	0.14-1.27	0.3-1.4	0.58-1.15	0.6-1.1	0.28-1.22	0.3-1.3	0.48-0.97	0.5-0.9
Glucose	mg/dl	60.85-195.85	66.8-133.0	52-95	75-115	61.64-207.4	68-129.0	59-90.65	75-115
AST	U/L	11-67.5	13-59.5	15.2-53.4	0-35	11-53.8	12-59.9	13.5-35.25	0-31
ALT	U/L	6.0-59.0	11.2-56	9.1-55.3	0-45	5.2-48.6	10.1-54.0	6.7-44.9	0-34
HDL-C	mg/dl	17.79-98.49	N/P	N/p	>40	21.92-129.46	N/p	N/P	>40
Triglycerides	mg/dl	64-476	41.3-275.8	35-268	0-15	60-496.6	41.0-261.2	34-194	0-150
Uric Acid	mg/dl	2.91-9.45	2.5-7.9	3.27-7.66	3.4-7.0	2.17-8.99	2.0-7.2	2.47-6.01	2.4-5.7
Urea	mg/dl	9.65-38.49	9.2-69	8.63-27.55	10.0-50	9.64-38.88	9.0-71.6	8.09-25.3	10.0-50

N/p =Not provided

The limitation of this study

- HIV screen tests were not done
- Most tests are not included in the study due to the scarcity of time and resources.
- Rural population are not incorporated

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CHAPER SEVEN; CONCLUSION AND RECOMMENDATION

7.1 CONCLUSION

This study has established baseline RIs for some clinical chemistry parameters and provide sex and age specific RIs for age >=5 years from south west Ethiopian region in "apparently healthy" population. Some parameters of this study show sex and age difference and also a difference between other countries, even in same country and reagent providers RI.Though our finding showed there might be a variation in RI across the country due to; physical condition, environment and socioeconomic conditions that affect the physiology of a population. It is evident from this study that there is the need to establish RIs that are applicable to specific populations rather than take a set of RI determined for one population and apply it to another population, especially for clinical trials and for those need critical decision limit.

7.2 RECOMMENDATION

- We recommend conducting nationwide study to determine the clinical chemistry RI of the Ethiopian population as a whole or regionally.
- Similar studies should be carried out to establish RIs for remaining clinical chemistry Parameters.
- Future studies should target infants and children less than 5 year in the region.
- Future similar studies should also incorporate for fasting blood samples for glucose and lipid profiles.

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ANNEXS

Annex I. Clinical Chemistry Standard Operating Procedure (SOP)

Total Cholesterol

1.Purpose

The specific cause for high or low level of serum Cholesterol (HDL, and LDL) is unknown. But due to some physiologic changes the plasma cholesterol level deviate from specified normal levels. Usually the level of serum cholesterol reflects the level of lipoproteins or lipids in plasma because HDL and LDL are cholesterol like lipoproteins.

A high level of serum cholesterols which reflects high values of LDL can be due to

- Inherited defect in lipoprotein metabolism
- Disturbance of endocrine physiology
- Liver disease
- Hypothyroidism is associated with hyper –cholestremia which shows increased LDL and decreased HDL
- Early hepatitis associated with increased in serum cholesterol levels, and as the disease progress the level falls probably because of decrease synthesis by the damage or necrosing liver cells.
- In nephrotic syndrome there is elevated VLDL or VLDL and LDL together
- Diabetes mellitus (effective insulin lack) is also associated with elevated values of serum cholesterol and triglyceride
- Emotional stress (associated) results in some individuals with high serum level of cholesterol.
- There is a statistically significant correlation between high serum cholesterol level and the incidence of coronary heart disease
- In pregnancy moderate increase is total cholesterol levels is observed
- In post menopausal women there is moderate increase in plasma cholesterol levels

Low level

- Estrogen therapy which decrease LDL.
- Progressive hepatocyte necrosis due to viral hepatitis and toxic hepatitis
- Cirrhosis of the lever (decrease by synthesis of lipoproteins)
- An inherited defect of either LDL or HDL metabolism
- Impairment of liver functions
- In hyper thyroidism

Lipids are literally define as organic compounds those are fatty, oily and waxy in nature and practically insoluble in water but dissolve freely in non- polar solvents. Lipids may be classifying as Simple lipid, compound lipid and derived lipids. Simple lipids are esters of alcohol commonly found from free fatty acid and glycerol which also include vegetable oil animal fats and waxes of insect. These commonly contain carbon skeleton C_4 to C_{20} e.g. glycerol and fatty acid. Compound lipids contain other groups besides fatty acids and glycerol. These groups are non lipid substance they can be proteins, sugars, phosphates, sugars and others. From these lipid combination with protein like albumin and globulin in complex, form lipoproteins. On the basis of density lipoproteins are classified as VLDL, LDL, HDL. The major components of the VLDL are triglycerides while the major components of LDL and HDL are cholesterol ester and phospholipids.

2. Principle

The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4 aminophenazone in the presence of phenol and peroxidase. In the presence of cholesterol esterase, the cholesterol ester in the sample is hydrolyzed to cholesterol and free fatty acids. Produced cholesterol oxidized by cholesterol oxides to cholesterene and hydrogen peroxide. Hydrogen peroxides are detected by chromogen oxygen accepter phenol -4-aminophenazo in the presence of peroxides, then red quenoid is formed, this is proportional to cholesterol present in the sample.

3.Sample Requirement

Serum, heparanized or EDTA plasma

4. Equipment

- Cholesterol reagent kit insert
- Clinical chemistry analyzer
- Reagent bottle
- Sample cup
- Pipettes
- Tippes
- Reaction cuvette
- Centrifuge

5.Reagent

-phosphate buffer (ph 6.5) 100mmo/l

-4-amino phenazone 0.3mmo/l

-phenol 5mmo/l

-peroxidase >5ku/l

-cholestrolesterase >150u/l

-Cholestroloxidase 0.05%

-cholestrol standard

6.Report

HUMAN STARE100 reads the result automatically.

* The result is reported in **mg/dl**

* Normal range **<220mg/dl**

* Linearity range upto 750 mg/dl Report the result with normal range if control with in the limit range and not flagged, and result in linearity range.

TRIGLYCERIDES

1. Value

Triglycerides and cholesterol level are elevated in plasma when there are risk factor releted to atherosceleotic disease. Also elevated leveles can be due to secondary to disease like, diabetes mellitus, billary obstruction, endocrine disturbance followed by metabolic disorders or inherited trait.

2. Principle

Triglycerides are determined after enzymatic hydrolysis with lipase .indicater is quinoneimine formed from hydrogen perodxide, 4-aminoantipyrin and 4-chlorophenol under the catalytic influence of peroxidase.

$$\text{Triglyceride} \xrightarrow{\text{lipase}} \text{glycerol} + \text{fatty acid}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{GPO}} \text{dihydroxyacetone phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-aminoantipytrin} + \text{4-chlorophenol} \xrightarrow{\text{POD}} \text{quininеimine} + \text{Hcl} + H_2O$$

3. Specimen

- Serum, heparinised plasma or EDTA plasma.
- Stable for 3 days at 2-8^{0}c and 4month at -20^{0}c

4. Equipment

- triglyceride reagent kit insert
- Clinical chemistry analyzer
- Reagent bottle
- Sample cup
- Pipettes
- Tippes

- Reaction cuvette

5. Reagent

Reag.1- PIPS buffer (ph 7) 50mmol/l

-4-chlorophenol 5mmol/l

-4-aminoantipyrin 0.25mmol/l

-Magnesium ion 4.5mmol/l

-ATP 2mmol/l

-Lipase ≥ 1.3u/ml

-Peroxidase ≥0.5 u/ml

-Glycerol kinase ≥ 0.4u/ml

-Glycerol-3-phosphate oxidase ≥1.5u/ml

- Standard 200mg/dl

Reagents are ready for use !

6. Report

- HUMAN STARE100 reads the result automatically.
- The result is reported in mg/dl

Normal range:

Suspect over 150 mg/dl

Increased over 200 mg/dl

Linearity range upto750 mg/dl

HIGH DENSITY LIPOPROTIEN (HDL-C)

1. Principle

Chylomicrons,Low density lipoprotein (LDL) and very low-density lipoprotein (VLDL) precipitate with phosphotungstic acid in the presence of magnesium ions at room temperature, High density lipoprotein (HDL) remains in solution and can be quantitated by measuring the residual cholesterol concentration in the supernatant.

2. Specimen

Plasma or Serum

Stable for at least 6 days at 2-8^{0}C

3. Equipment

- HDL reagent kit insert
- Clinical chemistry analyzer
- Reagent bottle
- Sample cup
- Pipettes
- Tippes
- Reaction cuvette

4. Reagent

A. Phosphotungstic acid ---------- 14 mmol/L

$MGC1_2$----------2mmo1/L

Stable at 2-8^{0}c up to expiry date indicated in the label.

5. Report

HUMAN STARE100 reads the result automatically

GLUCOSE

1.Principle

The glucose is determined after enzymatic oxidation in the presence of glucose oxidase.the formed hydrogen peroxide reacts under catalysis of peroxidase with phenol and 4-aminoantipyrine to a red-violet quinoneimine dye as indicator.

2.Specimen

Serum,plasma. The glucose is stable for 24hrs at 2-8^{0}c

3.Equipment

- glucose reagent kit insert
- Clinical chemistry analyzer
- Reagent bottle
- Sample cup
- Pipettes
- Tippes
- Reaction cuvette

4. Reagent

Enzyme reagent

standerd

Stable at 2-8^{0}c up to expiry date indicated in the label.

5. Report

HUMAN STARE100 reads the result automatically.

The result is reported in mg/dl

Normal range

Serum or plasma fasting75-115 mg

Linearity range upto400 mg/dl

Report the result with normal range

If control with in the limit range

Not flagged, and result in linearity range

Not pathologic.

6 Source of error

- Expired controls
- Expired reagents
- Contaminated, sample cubs, Reaction wells and reagent bottles
- Screeched flow cell.
- Contamination of samples, reagents, cuvetts with glove powder
- Heamolyzed sample

GOT/AST

1. Value

GOT/ AST is present in the cytoplasm and mitochondria of a cell. The concentration of mitochondria is greater than to cytoplasm. This enzyme found in all tissue mainly in heart, liver, skeletal muscle, kidney, pancreas, spleen. Lung and erythrocytes respectively to the concentration present, Therefore this enzyme elevate in different pathological condition

a. acute myocardial infarction
b. In viral hepatitis
c. In infectious mononucleasis
d. Hepatocyte necrosis
e. Intrahepatic cholestasis and cirrhosis
f. In progressive muscular dystrophy and dermatomyositis
g. In pulmonary embolism
h. In acute pancratitis
i. Muscle injury, gangrene and hemolytic disease.
j. Carcinoma of the liver.

2. Principle

GOT (AST) enzyme present in serum catalyzes the transfer of amino group from the amino acid aspartate to the α-keto glutarate. The reaction products are L-glutamate and oxaloacetale . In the presence of an enzyme MDH and Coenzyme NADH Oxaloacetic acid is reduced to L-malate and proportionally NADH is oxidized to NAD^+. The Consumption of NADH is directly proportional to the amount of oxalo acetate released. The released oxaloaecte is directly proportional to the activity of the enzyme present in the sample. Decrease in absorbance due to NADH is read at 340 nm every minute and the average decrease in absorbance per minute is calculated.

3. Specimen

- Serum, heparinised plasma or EDTA plasma.
- Hemolyzed sample should never be used
- 10%loss of activity with in three days at room temperature
- 8% loss of activities at4^oc.

4. Equipment

- AST reagent kit insert
- Clinical chemistry analyzer
- Reagent bottle
- Sample cup
- Pipettes
- Reaction cuvette

5. Reagent

Buffer

- Tris buffer(ph 7.5) 100mmol/l
- L-aspartate 300mmol/l
- LDH ≥0.9ku/l
- MDH ≥0.6ku/l

Substrate

- 2-oxoglutarate 60mmol/l
- NADH 0.9mmol/l
- Saline(0.85 %NaCl), if desired for specimen dilution
- Distilled or deionized water
- Control Material

Reagent storage temperature $2 - 8\ ^{0}c$

7. Result

- The result is reported as u/l
- Normal range <32u/l for male
  <30u/l for female
- Report the result with normal range

If control with in the limit range

SGPT/ALAT

1.Purpose

ALAT/GPT is present in all tissue. Relativity it's concentration in Lever is greater than the rest tissue of our body. So determination of GPT/ALAT is mainly for diagnosis of liver disease since in hepatocytes necrosis this cytoplasmic enzyme is released to plasma resulting in elevated serum ALAT /GPT activities. Even before clinical Symptoms appear. Serum ALT is increase in viral hepatitis, infectiousmononucleasise, cirrhosis, intra hepatic cholestasis.

Also it uses to resolve ambiguous increase in serum GOT/ AST in case of myocardial infarction. Where there is no CPK is enzyme test. If both serum ALT and AST are elevated AST source is liver where as only AST increase may be due to myocardial infarction.

2.Principle

GPT present in the serum samples catalyzes the transfer of amino group from L-alanin to α -ketoglutarate forming pyruvate and glutamate. pyruvate is reduced by NADH in the presence of LDH enzyme.
The amount of NADH oxidized is directly proportional to the amount of pyruvate released and the amount of pyruvate released is directly proportional to the enzyme activity present in the sample. Decrease in absorbance due to NADH consumption is read at 340.

3.Sample Requirement

- Serum, heparnised plasma or EDTA plasma.

4.Equipment

- ALT reagent kit insert
- Clinical chemistry analyzer
- Reagent bottle
- Pipettes

5.Reagent

Buffer

- Tris buffer 150mmol/l

- L- alanin 750mmol/l

- LDH ≥1.2ku/l

Substrate

- 2-oxoglutarat 90mmol/l

- NADH 0.9mmol/l

- Saline(0.85 %NaCl), if desired for specimen dilution
- Distilled or deionized water
- Control Material

6.Result

The result is reported as U/L.

Normal range <40u/l for male

<38u/l for female

Report the result with normal range and if control with in the limit range.

If the graph is linear,

Not flagged, and

Not pathologic.

ALKALINE PHOSPHATASE (ALP)

1.Value

Determination of serum alkaline phosphates activities are of clinical interest in the diagnosis of liver disease and bone disease even though high values of serum alkaline phosphate be due to physiological or pathological factors

a. Physiological factors
    1. In growing children
    2. In pregnant women at 3rd trimester which is placental origin.
    3. Healing of bone fracture
b. Pathologic factors elevated serum ALP in
    1. Hepatobillay disease which can be intrahepatic or extrahepatic obstruction or lepatocytes necrosis (by drugs, alcohol, viral)
    2. Bone diseases like paget's disease, osteomalacia, osteoporesis, rickets Fan conic syndrome and osteoblastic tumors.
    3. Hyper-parathyroidism
    4. In chromic renal disease
    5. In conjustive heart failure.

Also low results are observed in genetic inborn absence of alkaline phosphates, hyphoparathyrodism and in pernicious anemia.

2. Principle

Alkaline phosphates presents in the sample hydrolyze paranitrophenyl phosphate at Ph 9.8 to p-nitrophenol and in organic phosphate. At this P P- nitrophenol is converted to a colored quinoid derivative. the rate at with p-nitophenol produced can be measured every minute by measuring the increasing in absorbance at 405 nm (410nm) and the increase in absorbance is directly proportional to alkaline phosphalase activity present in the sample.

3. Specimen

- Serum or heparinised plasma.
- 0% loss at 40c for seven days
- 10%loss of activities at room temperature for seven days

4. Equipment

- ALP reagent kit insert
- Clinical chemistry analyzer
- Reagent bottle
- Sample cup
- Pipettes
- Reaction cuvette

5. Reagent

-Buffer contain – diethanolamine buffer(Ph9.8)

Magnesium chloride

- Substrate contain – P-Nitropheny/Phisphate

- Saline (0.85 %NaCl), if desired for specimen dilution

- Distilled or deionized water

- Control Materialc

Reagent storage temperature $2 – 8^{0}$ c

7. Report

- The result is reported as U/1

Normal range 35-104 u/l for female

40-129 u/l for male

Report the result with normal range

If control with in the limit range

If the graph is linear,

Not flagged, and

UREA/BUN

1.Purpose

The measurement of serum / plasma Urea/BUN is indicative of renal damage.

Markedly and prolonged increase serum /plasma urea is observed in renal failure. Also there are slight increases in case of

- Dehydration
- Diuretic therapy
- Gastrointestinal blood loss
- Any condition associate with

Increase protein breaks down, such as pneumonia, malaria, Meningitis, May in trauma, and surgical operation.

Low serum /plasma urea level may be seen.

- Pregnancy
- Malnutrition & AIDS
- Sever liver disease
- Water over load.

2.Principle

Urea present in the sample hydrolyzed to $NH4^+$ and CO_2 by catalytic activity jof urea's enzyme al 37^oc the released NH^+_4 is coupled will 2- Keto glutaiate, is the presence of co- enzyme NADH, which is catalyzed by an enzyme glutamate dehydrogenises (GLDH). The reaction product is glutamate, NAD^+, and H_2O.

In the reaction NADH is converted to NAD^+, and the amount of NADH consumed is directly proportional to the amount or urea present in the simple decrease in absorb me due to NADH is converted to Nad+, and the amount of NADH consumed is directly proportional to the amount of urea present in the sample decrease in absorb me due to NAPH is read at 340nm, every minute**.**

3.Sample requirement

Serum or EDTA plasma

4.Equipment

- Urea kit insert
- Clinical chemistry analyzer
- Reagent bottle
- Sample cup

5.Reagent

Enzyme

tris buffer (ph7.8)	120mmol/l
ADP	750mmo/l
Urease	≥ 40 ku
GLDH	

Substrate ≥ 0.4ku

2-oxoglutarate	25mmol/l
NADH	1.2mmo/l

Prepare 4 part enzyme + 1part substrate

7.Report

- The result is reported in mg/dl
- Report the result with normal range i.e. 15-45mg/dl for male & female

If control with in the limit range

If the graph is linear,

Not flagged, and

Not pathologic

CREATNINE

1.Value

Creatinine is all waste products by the kidneys mainly by glomeruler filtration. The concentration of creatinin in plasma of a healthy individual is fairly constant, independent from water in take, exercise and rate of urine concentration. Therefore increased plasma creatinine values always indicate decreased excretion i.e. impaired kidney function. The creatinine clearance enables a quite good estimation of the glomerular filtration rate which allows better detection of kidney disease and monitoring of renal function. For this purpose creatinine is measured simultaneously in serum and urine collected over a defined time period.

2.Principle

Creatinine forms a colored orange-red complex in an alkaline picrate solution. The difference in absorbance at fixed times during conversion is proportional to the concentration of creatinine in the sample.

Creatinine + picric acide ⟶ Creatinine picrate complex.

3.Specimens

Serum, heparinized or EDTA plasma,

4.Equipment

- Creatinin kit insert
- Clinical chemistry analyzer
- Reagent bottle
- Sample cup
- Pipettes
- Reaction cuvette

5.Reagent

Picric acid	13.9mmol/l
Sodium hydroxide	160mm

Creatinine standard. 2mg/dl

Dist. water

Nacl 0.85%

6.Report

- The result is reported in mg/dl

- Report the result with normal range

*If control with in the limit range

*If the graph is linear,

Not flagged, and

Not pathologic

7.Source of error

- using expired reagents and controls
- Reagent contamination.

8.Safety

- Use protective – gloves, guans and face shield

Annex-II QUESTIONNAIRE

JUMMA UNIVERSITY

INSTITUTE OF HEALTH

SCHOOL OF MEDICAL LABORATORY SCIENCE

QUESTIONNAIRE FOR SCHOOL CHILDREN

1. Anthropometric measurement

1.1 weight in the nearest 0.1 Kg _____

1.2 Height in the nearest 0.1 cm _____

2. Identification

2.1 Code _____

2.2 Address _____

4 Socio-demographic information

4.1 Age in years _____

4.2 Sex

1. Male 2. Female

4.3 For how money years have you been here _____

4.4 Mother's Occupational status

1. Merchant 3. Former 5. Student
2. Employee 4. Daily laborer 6. Others -------

4.5 Father's Occupational status

1. Merchant 3. Former 5. Student
2. Employee 4. Daily laborer 6. Others---------

4.6 Mother's Educational status

1. Illiterate
2. Read and write
3. Grade 1-4
4. Grade 5-8
5. Grade 9-12
6. College/university

4.7 Father's Educational Status

1. Illiterate
2. Read and write
3. Grade 1-4
4. Grade 5-8
5. Grade 9-12
6. College/university

4.8 Educational of the child

1. Illiterate
2. Read and write
3. Grade 1-4
4. Grade 5-8
5. Grade 9-12

4.9 Religion

1. Muslim
2. Catholic
3. Orthodox
4. Protestant
5. Others_____

4.10 Ethnicity

1. Oromo
2. Kaffa
3. Amhara
4. Tigrea
5. Dawuro
6. Others_____

4.11 Family size _____

5 Life style factors and nutritional habit

5.1 Regular exercise (at least once per week for 1 year)

1. yes
2. No

5.2 Average hr/day of standing_____

5.3 What is your staple food?

1. Injera
2. Porridge
3. Fish
4. Other, specify _____

5.4 Fruit, fruit juice consumption

1. No
2. Every day
3. Every other day
4. Once a week
5. Once a month
6. Others specify _____

5.5 Vegetables consumption (weekly)_____

1. No
2. Every day
3. Every other day
4. Once a week
5. Once a month
6. Others specify _____

5.6 Frequency of consumption of foods from animal sources (weekly)_____

1. No
2. Every day
3. Every other day
4. Once a week
5. Once a month
6. Others specify _____

6 Have you ever practiced and/or exposed to the following?

6.1 Chronic illness

1. No
2. TB
3. DM
4. Hypertension
5. Other _____

6.2 History of congenital transmitted diseases

1.	No	4.	HBV
2.	Syphilis	5.	HCV
3.	HIV	6.	Other_____

6.3 Blood transfusion

1.	No	2.	yes

6.4 Surgical procedure in the past 6 month

1. NO
2. Yes

JUMMA UNIVERSITY

INSTITUTE OF HEALTH

SCHOOL OF MEDICAL LABORATORY SCIENCE

QUESTIONNAIRE FOR ADULTS

1. **Anthropometric measurement**

1.1 weight in the nearest 0.1 Kg _____

1.2 Height in the nearest 0.1 cm _____

2. **Identification**

2.1 Code _____

2.2 Address _____

3. **Socio-demographic information**

3.1 Age in years _____

3.2 Sex

1. Male
2. Female

3.3 For how money years have you been here _____

3.4 Occupational status

1. Merchant
2. Employee
3. Former
4. Daily laborer
5. Student
6. Others -----

3.5 Educational Status

1. Illiterate
2. Read and write
3. Grade 1-4

4. Grade 5-8
5. Grade 9-12
6. College/University

3.6 Marital status

1. Unmarried
2. Widowed
3. Married
4. Divorced

3.7 Religion

1. Muslim
2. Catholic
3. Orthodox
4. Protestant
5. Others_____

3.8 Ethnicity

1. Oromo
2. Kaffa
3. Amhara
4. Tigrea
5. Dawuro
6. Others-_____

3.9 Family size _____

3.10 Annual household income (in ETB)_____

4. Life style factors and nutritional habit

4.1 Regular exercise (at least once per week for 1 year)

1. yes
2. No

4.2 Average hr/day of standing_____

4.3 Do you currently smoke any tobacco products, such as cigarettes?

1. No
2. Yes, sometimes
3. Yes, daily

4.4 Have you ever consumed an alcoholic drink such as beer, wine or other alcoholic drinks?

1. No
2. Yes, before 12 months
3. Yes, till now

4.5 If you have consumed during the past 12 months, how much frequently have you had at least one alcoholic drink?

1. Daily
2. 5-6 days per week
3. 1-4 days per week
4. 1-3 days per month
5. Less than once a month

4.6 What is your staple food?

1. Injera
2. Porridge
3. Fish
4. Other, specify _____

4.7 Vegetables consumption (weekly)_____

1. No
2. Every day
3. Every other day
4. Once a week
5. Once a month
6. Others specify ____

4.8 Frequency of consumption of foods from animal sources (weekly)_____

1. No
2. Every day
3. Every other day
4. Once a week
5. Once a month
6. Others specify ___

5. Have you ever practiced and/or exposed to the following?

5.1 History of chronic illness

1. No
2. TB
3. DM
4. Hypertension
5. Other _____

5.2 History of STD/STI A.

1. No
2. Syphilis
3. HIV
4. HBV
5. HCV
6. Other_____

5.3 Blood donation in the past 4 month

1. No
2. Yes

5.4 Blood transfusion in the past 1 year

1. No
2. Yes

5.5 Surgical procedure in the past 6 month

1. No
2. Yes

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Laboratory Results

1. RFT
    a. Cr----------mg/dl
    b. BUN----------mg/dl
    c. Uric acid----------mg/dl
2. LFT
    a. AST----------u/l
    b. ALT----------u/l
    c. ALP----------u/l
3. lipid profile
    a. TC----------mg/dl
    b. TG----------mg/dl
    c. HDL----------mg/dl
4. Fast blood glucose level----------mg/dl

Annex III. Consent,and information sheet

Information sheet and a consent/assent form

English version

Name of Investigator: Tesfaye Demie

Postal Address: P.O. Box 378

Cell phone: 0917978123

E-mail: tesfishde12@gmail.com

Supervisors:

Jimma university P.O. Box 378 Jimma, Ethiopia.

Cod of volunteer----------

Purpose of study

This study is need to sample and analyze blood for clinical chemistry tests from apparently healthy individual and the results will be used to establish reference values in the region.

Participation

Participation is voluntary and one is free to reject. If you choose to participate, you have to read and understand the contents of this document then I will provide you before sample collection.

Those with limited capacity to consent will be excluded from the study. Participants will include those who will have lived in the region for a period not less than six months.

Sample collection

This study will involve 10ml blood collection from the vein with a sterile needle and a syringe. This will only be done once for the entire study.

Risks and discomforts

Participants may experience some pain during venipuncture and there is no risks expected.

Benefits and costs

Participants will not spend any money on this study and also will not get any money. In case the participant is found with any clinical condition he/she will be informed upon his/her own request. This study will be of benefit to the hospital and entire community since its success will aid in proper clinical decision making and treatment of patients.

Confidentiality

Results will be confidential.

Enquiries

For further explanation and queries contact the investigator on above address. For more about your rights contact the Ethical committee of Jima University.

I……do agree that I have read, been explained to, allowed to ask questions

concerning this study, understood and consented.

Signature of volunteer/ parent ……Date……..

I have clearly explained the above study to the participant and he/she has understood and consented.

Signature of researcher……..Date……

Introduction and consent form Amharic version
መግቢያ ደብዳቤ እና ስምምነት ቅጽ

መርማሪ ስም: ተስፋዬ ደሜ

የፖስታ አድራሻ: 378

የእጅ ስልክ: 0988468734

ኢ-ሜይል: tesfishde12@gmail.com

የጥናት ዓላማ

በዚህ ጥናት ያደም ናሙና ከጤናማ ግለሰብ በመዉሳድ የክሊኒካል ኬሚስትሪ ያጤናኛ ሰው ያዉጤት መጠን(Normal Renge) ለማወቅ ይሆናል። እናም ውጤቶቹ በክልሉ ውስጥ ማጣቀሻ እሴቶች ለማቋቋም ጥቅም ላይ ይውላል.

መካፈል/ታሰትፎ

ተሳትፎ የሚያደርጉት በፈቃደኝነት ነው እናም ላለመቀበል ይችላሉ። ለመሳተፍ ከመረጡ ይህን ሰነድ ማንበብ አና ይዘቱን መረዳት አለቦት ።

ለመስማማት ውስን አቅም ያላቸው ሰዎች ከጥናት ተነጥለው ይወጣሉ። ተሳታፊዎች ከስድስት ወር በላይ በክልሉውስጥ ያኖሩ ሰዎችን ያካትታል።

የናሙና ስብስባ

ይህ ጥናት 10ml ደም መሰብሰብን የሚያካትት ስሆን ይህም አንድ ጊዜ ብቻ ይከናወናል።

በጤና ላይ ሊያጋጥማቸው የሚችል አደጋ

ለተሳታፊዎች በመርፌ ሲወጉ ከምሰመዉ ትንሽ ህመም በቀር የሚጠበቀው ምንም አደጋ የለም።

ጥቅሞች እና ወጪዎች

ተሳታፊዎች በዚህ ጥናት ላይ ምንም ገንዘብ አያወጡም; እንድሁም ተሳታፊው አጋጠሚ ህመም ብገኝበት አዉቃው በግዜ እንድታከም ይረደዋል። የዚህ ስኬት ተገቢ የክሊኒካል ውሳኔ ሰጭነት እና ለታካሚዎች ሕክምና ይረዳዋል በተጨማርም በዚህ ጥናት ለሆስፒታል እና ለ መላው ማኅበረሰብ ጥቅም ይሆናል።.

ምስጢራዊነት

ውጤቶች በሚስጢር ይጠበቃሉ።

ከሚከተሉት

ተጨማሪ ማብራሪያ እና መጠይቆች ከላይ አድራሻ ላይ መርማሪው ያነጋግሩ. የእርስዎ መብቶች ተጨማሪ ለማግኘት ጂማ ዩኒቨርሲቲ ኤቲካል ኮሚቴ ያነጋግሩ.

እኔ ..... ገላፃ ተደርጓልኛል, ኣንብቤዋለሁ ጥያቄዎችን መጠየቅ ፈቅደዉልኛል ።

በዚህ ጥናት ለማሳተፍ ተረድቸለው እናም ፍቃደኛ ነኝ።

ፈቃደኛ / ወላጅ ፊርማ..... ቀን ....

እኔ ከላይ ያለዉን ጥናት ለተሳታፊዉ በግልጽ ገልጫለሁ፡ እናም በመረዳት ፈቀደኛ ሆኖዋል።

ተመራማሪ ፊርማ ....ቀን.....

Introduction and consent form Afaan oromoo version

Ibsa Hirmaatota qo'annotif unka Afaan Oromoo.

Guciin kun wareen qu'niichatti fe'aan hirmataniif yoo ta'u halichi adeemsa qu'anichaa irriga ibsamee booda wanti itti aanu hundu ifa godhama.

Mataduree qu'anna-Qorannon bu'a laaboraatori kiliinikaala keemistirii warren fayyaa qaban irratti naanno kiba lixa ithopiyaatti hojjetamu.

Maqaa qorata Tesfaye Demie

Lak.bilbila;0917978123

Imeela;tesfishde12@gmail.com

Dhimi qu'anicha-Bu'aan laboratory kaneen warra fayaa qabanii hagam akka ta'ee murteesufii gara fulduraaf fayyaaf dhibamaan adaan basuuf akka toluuf yoo ta'u, bu'aan isaas warra naanotiif bay'ee gudaadha..

Sodaafi miidha qabu

Seraf namusa wal'ansa fayya waan hordufinuuf want nama sodaachisu hinjiru, hata'u malee yeroo dhigni fuudhamu dukubiin tinno bakka lilmoon seenteetti dhaga'aamu mala ,ta'us yoosu bada.

Faayida qu'anichaafi kanfalti hirmaataf godhamu

Qu'anichi bu'aa gudda sabaaf busa innis tajajila dhugaa akka argatan tasisa. Qu'anicha irratt hirmaachuun kanfaliti tokkolle hin qabuu.haatu malee bu'aan qoranno wanta batalumatti deebi'uuf tajaajila wadhansa argachuu ni danda'a.

Icciiti qu'anicha

Wantooni qu'anichaan argaman hundi icciitin eeggamu.akkasumas ragaawan fudhatama hundinu maqaa keesanin oso hin ta'in lakkofisa koodii addaa ta'een wanta'eef kanas kan beekan warreen ragicha guraan qofaadha.

Mirga fe'aan hirmachu

Qu'annoo kana irratti hirmaachun fe'a gutuu kee qofa ta'usaa beektee yeroo barbaadeetti dhiisu akka dandeesu mirgi kee guutudha.qu'anno kanarati hirmaachufi hirmaachu baatun kee gara fulduraf tajaajila argaturatti rakkoo tokkolee hin fidu.

Qu’anicha ilaalate gaafille qabadan hundaa yeroo barbaadanit tisso mataarati bareefameen aba qu’anicha gafachu nidandeesan.itti annisees qu’anno kanaaf hirmana gootanif galani keya guddaadh. Galatoomaa.

Anni ---------- Yaanni armaan olii hunduma dubbisse ,ibsiis naaf godhame,wa’ee qu’anichaas akkan gaafi barbachisu gafadhu naaf eyyamamee jira, wanta naa galeef waligaltee koo kennee jira.

Mallattoo hirmaata/warra saa----------guyyaa----------

Waa’ee qu’anicha ibsa barbachisa hundaa wantan godheef itti hirmaachuuf amanee fudhaate waligaltee kennee jira.

Mallattoo qu’ataa----------guyyaa----------

DECLARATION

I, the undersigned, declare that this research thesis is my own work and it has not been presented in other universities, colleges or other institutions for similar degree or other purpose. Where other peoples work has been used, it has been carefully acknowledged and referenced in accordance with the requirements.

Name of the Principal investigator	**Signature**	**Date**
	_____	_____
Approval of the first Advisor	**Signature**	**Date**
	_____	_____
Approval of the Second Advisor	**Signature**	**Date**
	_____	_____
Approval of the Assessor	**Signature**	**Date**
	_____	_____