ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Establishment of reference intervals for the common renal and liver function clinical chemistry parameters among apparently healthy pregnant and non-pregnant women in South wollo zone, Amhara National Regional State, northeast Ethiopia.

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A research thesis submitted to the Department of Medical Laboratory Sciences, College of Health Science, Addis Ababa University, in partial fulfillment of Master of Science Degree in Clinical Laboratory Sciences (Clinical chemistry track).

November, 2019

Addis Ababa, Ethiopia.

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by Miftah MohammedAssen, entitled: **Establishment of reference intervals for the common renal and liver function clinical chemistry parameters among apparently healthy pregnant and non-pregnant women in South wollo zone, Amhara National Regional State, northeast Ethiopia** 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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Acknowledgement

I am grateful to thank Addis Ababa University for funding of this study. Special mention goes to my enthusiastic teacher and advisor Dr.Aster Tsegaye for her generously contribution to the study presented in this final thesis. I would also like to acknowledge instructor Samuel Kindie for his tremendous academic support and for being so dedicated to his role as my advisor. My sincere respect also goes for health extension workers that participated in the selection and mobilization process of study participants in the study area.

I must express my very profound gratitude to Amhara public health institute Dessie branch staff members for their kindly cooperation in sample analysis stage. Finally, I would like to pass my special affection for study participants for their willingness, my parents and to the many people, in many locations, who so generously contributed to the work presented in this study.

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Abbreviations

AAU	Addis Ababa University
AE	Adverse Event
ALB	Albumin
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
APHIDB	Amhara Public Health Institute Dessie Branch
AST	Aspartate Aminotransferase
BIL	Bilirubin
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
CI	Confidence Interval
CLSI	Clinical Laboratory Standards Institute
CREA	Creatinine
DADIS:	Division of AIDS,
DBIL	Direct Bilirubin
HBV	Hepatitis B Virus
HBsAG	Hepatitis B Surface Antigen
HCG	Human Chorionic Gonadotropin
HCV	Hepatitis C Virus
HGB	Hemoglobin
HIV	Human Immunodeficiency Virus
IFCC	International Federation for Clinical Chemistry
IU	International unit
KG	kilogram
L	Liter
LFT	Liver Function Test
M	meter

NCCLS	National Committee for Clinical Laboratory Standard.
OOR	Out of Range
RFT	Renal Function Test
SD	Standard Deviation
SPSS	Statistical Package for Social Sciences
S. Wollo	South Wollo
T.P	Total Protein
VCT	Voluntary Counseling and Testing

Abstract

Background: Physiological changes during pregnancy causes alterations in biochemical analytes concentration. Thus, pregnancy specific reference intervals (RI) are important for accurate diagnosis and treatment of cases. In Ethiopia, clinical laboratory test results are usually interpreted using values established on western population.

Objective: To establish RIs for common renal and liver function clinical chemistry parameters among apparently healthy pregnant and non- pregnant women in South wollo zone, Amhara National Regional State, northeast Ethiopia.

Methods: A community based cross sectional study was conducted on a total of 378 apparently healthy study participants randomly selected from south wollo zone, Ethiopia from April to June 2019. Data like medical history, physical examination and socio-demography were collected by using well organized questionnaire. After the exclusion of outliers using quartile method, Kolmogorov–Sminorv test was used to check its normality. The 95% RI with 95% confidence interval was established using the non-parametric method. The significance of differences between pregnant and non-pregnant women was evaluated using Mann–Whitney U test.

Result: There was statistically significant variation between pregnant and non-pregnant women in values of Albumin, T.Protein, ALP, urea and Creatinine but not for AST, ALT, bilirubin(direct) and bilirubin(total). Reference intervals established for pregnant women includes: albumin 26.14–42.87g/l, total protein 48.52–74.71 g/l, AST 2.4–43.6 U/L, ALT 0.94–28.35 U/L, ALP 21.2–337 U/L, bilirubin(direct) 0.03-0.32 mg/dl, bilirubin(total) 0.26-0.94 mg/dl, creatinine 0.29–0.87 mg/dl, urea 7.17–20.82 mg/dl. Albumin: 32.81–47.87, total protein: 56.71–83.9 U/L, AST: 4.2–37.1 U/L, ALT: 2.69–41.18 U/L, ALP: 3.22–278.7 U/L, bilirubin(direct) 0.1-0.51mg/dl, bilirubin(total) 0.24-1.06mg/dl, creatinine 0.44–1.00 mg/dl, urea 8.07–27.87 mg/dl for non-pregnant women.

Conclusion: The RIs provided by this study are decisive in interpreting clinical laboratory results for medical decision making and other health-related conditions. Physiological adaptations of pregnancy should be taken into account when interpreting LFT and RFT in a pregnant woman.

Key words: Reference interval, Clinical chemistry, Biochemical analytes, South wollo, Healthy, pregnant, non-pregnant.

1. INTRODUCTION

1.1 Background

The concept of ‘reference interval’ (RI) as known today was evolved by Grasbeck and Saris in the late 1969 with the intention of replacing an obscure ideas of normal values (1). Until the 1970s, before Grasbeck’s publications and the work of the International Federation for Clinical Chemistry (IFCC) expert panel, the term ‘normal values’ was usually used (2). The use of the term "normal”, however, has been discouraged since it can assume different meanings: (Gaussian distribution, most representative of a class, most suited for survival, that does not harm, conventional, ideal) (3). But, in practice, RI need to be established for specific physiologic conditions like pregnancy, ovulation or menopause, professional athletes and some pathologic conditions (4). Besides, it seems to imply that everything outside these range be abnormal while the way it is calculated does not absolutely guarantee it. Moreover, when the test results from different populations are examined, it is occasionally realized that what is normal for one group is not necessarily normal for another group. IFCC, therefore, has abandoned the term "normal range" and introduced a more appropriate word "reference interval". IFCC defines reference interval as the set of values in which 95% of the normal healthy population falls (5).

The biological components of the human organism are exposed to variation caused by physiological phenomena, genetic differences, environmental factors and diseases. A reasonable interpretation of laboratory results requires knowledge of the variation of these components in the individual under study or in one or more satisfactory defined sets or reference individuals (6). The existence of significant inter-and-intra population differences in RI obtained within and between countries in definite biochemical analytes (that is brought through different factors) is the inevitable presumption for the need of establishing population centered RI (7–11).

During pregnancy, a woman experience physiological and hormonal changes. The kidney also undergoes several anatomical and physiological changes(12)The plasma volume increases by as much as 50%; these changes results an alteration in the plasma constituent concentration. The large amount of estrogen, progesterone, placental lactogen and corticosteroids that are produced during pregnancy affect various metabolic, physiological and endocrine pathways. An increase in the rate of lipid metabolism over glucose utilization

with that of an increased resistance to angiotensin are some another manifestation of pregnancy. Physiological changes occurring in pregnancy affects nearly every organ system and the kidney is not an exception. As a result of these changes, many of the laboratory reference intervals of non-pregnant women are not appropriate for pregnant women. Similarly renal system undergoes multitudes of changes in function during pregnancy because of hormonal effects and increased metabolic load of the fetus. The glomerular filtration rate increases by up to 50 % in pregnancy, which is an indication of increased renal function. This increase in renal blood flow and glomerular filtration rate will attributable to an increased cardiac output, increase in progesterone and aldosterone. Thus the clearance of urea and creatinine increases and their plasma levels are lowered in pregnancy. That is why clinical chemistry RI derived only from pregnant women are assumed to has an important role in passing medical decision for pregnancy related health disorders(13).

Aspartate aminotransferase enzyme (AST) and Alanine aminotransferase enzyme (ALT) are found predominantly in liver. Thus, increases in these enzymes indicate hepatobiliary disease. In the hepatocytes, AST is cytoplasmic while ALT is intramitochodrial. Alkaline phospahtase (ALP) is actually a collection of isoenzymes, present throughout the body thought clinically relevant ALP is found in liver, bones and biliary duct. It is used to evaluate liver or bone disease. Normal ALP levels are assumed to vary greatly depending on gestational age mainly due to the production of placental and bone isoenzyme rather than elevation of hepatic isoenzyme Albumin which is a globular protein and is produced by the liver. It is used to binds negatively charged cations, hormones, conjugated bilirubin and medications with its primary function of maintenance capillary oncotic pressure. The decrease in serum albumin levels is attributed to pregnancy-related plasma expansion plus increased rate of catabolism) in pregnant women(14).

In all clinical practice, RI is accepted to be the most widely used decision making tool especially for clinical chemists and clinicians who depends on RIs for guidance. It has a wide scopes of application ranging from diagnosis of health disorders, evaluation of the toxicity of xenobiotic, disease staging to monitoring of treatment (1,15–17). The significant difference in the RI of clinical chemistry parameters among different countries and even population groups of the same country attributes the risk of unessential further investigations or default in the detection of the underlying disease or leads to wrong management of patient (18).

The RI of many clinical laboratory tests are established by cutting off the threshold values within which the test results of a specified percentage (usually 95%) of apparently healthy individuals would fall that in turn enforces the exclusion of the 2.5% of individuals with the lowest results and 2.5% of individuals with the highest values . The limiting observations for the RI are commonly the 2.5 and 97.5 percentile of the test result in test result distribution of reference samples(4).

Even though RI is arguably the most widely used decision making tool (6) in the clinical setups, no RI is completely right or wrong as the majority of them falls in central 95% of the reference population test result so that 5% of results from healthy people will fall outside of the established RI and will flagged as abnormal. The present study was done to provide reference interval (RI) on various biochemical variables by establishing RI for the selected clinical chemistry parameters for pregnant and non-pregnant women in south wollo zone, Northeast Ethiopia. It should also be put in mind that the comparison of laboratory test result of an individual to RI or decision limits solely is not the only and absolutely flawless way of interpretation of cases and final decision making point. It should be congruent with clinical presentations and other supporting diagnostic techniques.

In Ethiopia, no adequate numbers of community based age, geographical location and sex - stratified RI studies has been established in clinical chemistry settings especially for pregnant women and non-pregnant women (19). During pregnancy, maternal physiology undergoes many changes on cardiovascular, respiratory, renal, hepatic and gastrointestinal physiology that is mainly secondary to the effects of progesterone and estrogen that are produced by ovary and the growing placenta. Though is normal physiological phenomenon, it exhibits many biochemical alteration ranging from change of electrolyte concentrations to further alteration in cortisol metabolism that is why clinical chemistry test results during the period of pregnancy differ from reference intervals of non-pregnant women (20)

Adopting wrong RI may lead to unnecessary and potentially dangerous therapeutic actions without determining the real cause of the abnormality. Therefore, this study aimed to establish RIs for the common renal and liver function clinical chemistry parameters among apparently healthy pregnant and non-pregnant women in South wollo zone, Amhara National Regional State, northeast Ethiopia.

1.2 Statement of the problem

Around 80% of clinician's medical decisions are based on information provided by laboratory reports(21). Most laboratories for example in Pakistan , Saudi Arabia, china and Ethiopia rely on the references ranges provided by the manufacturer of the kit and/or developed by another reference laboratory (6). These adoption is as escaping mechanism from sort of challenging aspects (like selection of volunteer and well defined study subjects, sample collection) and due to the fact that certain tests require different RI for different age groups and for different samples.

Africans are striving about identifying effective prevention and treatment strategies to combat with the heavy burden of infectious as well as emerging non communicable disease (22).Also there are plentiful evidences claiming the use of their own method-specific RI for physicians and medical researchers especially in developing countries, But still there are inconsiderable tries to establish well standardized reference intervals of one's own (7). Pregnancy brings changes in hematological and biochemistry values. However, there are no adequate numbers of African RI for clinical management of such pregnant women. Moreover, as manifested by different studies (23–25)conducted on pregnant women some of these reference intervals adopted from principally Caucasian western population brought further misinterpretation of laboratory results and mismanagement of cases.

A study conducted in India on common liver function biochemical tests showed significantly lower and slightly higher concentrations of Serum bilirubin (direct and total) and serum alkaline phosphatase (ALP) in the second and third trimester respectively. Such changes in liver function tests during normal pregnancy can be misinterpreted as pathological and can hide or worsen preexisting disease while it could be due to physiological changes (25).

From a study conducted in different African countries on common biochemical tests like alanine aminotransferase (ALT), aspartate aminotransferase (AST) and bilirubin (direct and total) which are usually used during screening/enrollment and safety monitoring of trial participants showed out of range (OOR) values of up to 32% in Kintampo(Ghana) (10), up to 42% in Kenya (7) and up to 81% in Tanzania (9) which favors the degrees of ruining eligibility for enrolment into clinical trials and misinterpretations of AEs using the western RI values.

In many African population, the clinical laboratory RIs have not been established and non-locally derived RIs were usually being habituated in diagnostic laboratories and clinical trial studies to screen, diagnose and monitor pathological conditions. A number of studies showed variations between African and western population derived RIs (26–28) especially the upper reference limits of the majority of clinical chemistry parameters were higher for healthy African population than western population (7,19).

Most clinical laboratories in Ethiopia seemed to entirely depend on western derived RIs for disease diagnosis and management because of the absence of well-established local RIs. However, an ample of studies showed variations between African and western population RIs (26,27) and also between Ethiopian and other African countries plus western population(19,28,29).Moreover, owning to the absence of data on RI at a population level in Ethiopian, clinical laboratories were adopting reference values developed from populations of advanced countries by neglecting the reality that RI are dependent on different aforementioned factors.

Generally, few clinical biochemistry RI studies are conducted in certain areas of Ethiopia. If otherwise; they entirely concentrate on adults of larger age ranges. They recommended further studies and partitioning for pregnant and due attentions in RIs interpretations for them.

To the best of my knowledge, RIs had not been addressed adequately for pregnant and non-pregnant women in S.wollo zone, northeast Ethiopia. Thus, the aim of this study was to establish RIs for common renal and liver function clinical chemistry parameters among apparently healthy pregnant and non-pregnant women in S.Wollo zone, Northeast Ethiopia.

1.3 Significance of the study

In order to accurately ascertain what is healthy and normal, locally established RIs are necessary for laboratory tests in the population. Furthermore, clinical RIs in a population are needful in order to accurately assess the health status of the population, screening participants for enrolment into clinical trials and for monitoring the occurrence of adverse events (AE) during these trials.

Many of maternal physiological changes that occur during pregnancy affect clinical laboratory parameters. Reference values established from samples of non-pregnant women are, therefore, not valuable for clinical decisions for pregnant women. Hence, establishing one's own RI during pregnancy is monumental in order to isolate pathological conditions.

A test result by its own is of a little significance unless it is presented with the appropriate information for its interpretation, usually in the form of RI. In a general manner, medical decisions done on carefully determined reference intervals along with patient's clinical data yields escaping of patients misdiagnosis rates, unnecessary wastage of scarcity of valuable laboratory resources and ultimately and most importantly for better prognosis of patients. This study is of paramount importance being reference interval to the nearby medical laboratory professionals enabling them to provide complete laboratory reports. Pregnant and non-pregnant women will get better health service as their result would be interpreted based on the locally established reference intervals. It definitely supports clinicians in better diagnosis and management of their patients of different partitions and even for clinical researchers to appropriately recruit and follow up of their study subjects in clinical trial studies. Moreover this study will be used as baseline information for further other related studies.

2. LITERATURE REVIEW

2.1 The concept of Reference interval

A number of theories have been proposed to establish RIs for biological analytes. Moreover, many studies have been performed to assess the possibility of directly adopting elsewhere established RI. Multitudes of recommendations were propagated to verify the presence of significant variations among populations of different countries and even in population of the same countries. Even though these literatures cover a wide variety of approaches and findings, this review focus on three major themes that emerge repeatedly throughout the literature. These themes are: the concept of RI, Statistics used to establish RIs, reference interval studies Even though the literature presents these themes in a variety of context, this section will primarily focus on pregnancy as a factor to bring statistical difference of RI and degree of discrepancies or wrong conclusions in healthy and/or diseased isolation process while adopting not verified non-local RI.

In reality, interpretation of any entity needs a sort of standard or control based on which the status of someone will be compared and final conclusion will, therefore, be drawn. Similarly, the 95% RI values obtained will be used as a manifestation of healthiness and for comparison (29).Typically, it should be established for each laboratory test to delineate the range of values that would usually be encountered in a healthy population. This was supported by National Clinical chemistry laboratory standard (NCCLS) and by article reviewed on reference intervals (30) which further states that due to the fact that many RI are found to be dependent on several parameters, optimal RIs should be established by taking other significantly affecting factors like geographical location, pregnancy, ethnicity, life style and other factors in to consideration. These recommendations dictate the needs of using predefined reference populations whose test results will be used for comparison in attaining the demand of accurate RI for accurate diagnosis and monitoring of patients.

2.2 Statistics used to establish RI

Though, health is a relative condition that lacks a universal definition and defining what is assumed to be healthy and establishing such criteria employed to exclude unhealthy from healthy reference sample group is the first problem in any RI study, a variety of examinations, such as a history and physical and/or infectious diseases laboratory tests are used to evaluate the health of each reference individual and to keep unhealthy person from being included in the reference sample.

There are two types of sampling technique in the selection of reference sample groups (priori and posteriori).A priori sampling is a method that requires well-defined exclusion and partitioning criteria before the selection of the reference individuals. This method could be best applied to well-studied, established laboratory procedures with established methods, But in posteriori sampling, the process of exclusion and partitioning also takes place but in a different order (after sampling and analyte testing rather than before). This method is especially important for laboratory procedures that are new or poorly studied.

Concentrating to frequently existing values and removing certain outlier observations from apparently healthy populations' laboratory test results will bring about more values of purified reference distribution. The treatment of such outlier observations can be done by the quartile method, in which the range of the central 50% of the resulting distribution is calculated, and then subtracting 150% of this value from the 25th percentile and adding 150% of this value to the 75^{th} percentile for each test results of all partitions. Any values beyond these limits are considered to be treated as an outlier. Although Parametric(the central 95% boundaries are specified by the mean ± 2 standard deviation(SD), non-parametric(the central 95% boundaries are determined by trimming off the lowest and highest 2.5% of observations) for calculation of reference interval, NCCLS recommends and many clinical laboratories RI were being defined by non-parametric approach which focus on threshold values between which the test results of a specified percentage (usually 95%) of apparently healthy individuals would fall. The threshold or limiting values are usually the 2.5^{th} and 97.5^{th}percentile of the test result distribution in the reference population(4). In spite of the presence of such approaches, literature claimed that due attention is very important in the selection and defining of healthy individual and assessment of pre-analytical and analytical factors.

Roughly, an individual's test results and hence health status seems to be strictly evaluated based on the established RI. However an article on the need of establishing RI (6) and also on defining laboratory reference values and decision limits (29) contended that reference intervals are relatively inflexible. They also do not take into account certain special history or other patient characteristics or conditions like strict vegetarian while most of the communities are not vegetarians that could probably affects the results of the laboratory test under consideration.

Inevitable occurrence of exclusion of study participants promotes mobilization of more individuals to the study area. A research on establishment of Pediatric and Adult RIs of Canadians revealed total exclusion rates of 21% (for 3-18 years old subjects), 43% (for19-59 years old subjects) and 79% (for 60-69 years old subjects) on the basis of all exclusion criteria including outlier observations. This is supported by a cross sectional study at four eastern and southern Africa countries (Rwanda, Kenya, Zambia and Uganda) on hematology and biochemistry reference on adults; where about 20% and 10% exclusion rate were observed by physical examination and laboratory screening tests respectively (7). These and other studies claimed the existence of not more than 5% exclusion of observation from each analytes in consented subjects as outlier even though it could be relatively higher in elders.

2.3 Reference Interval studies

By its very nature, well defined, highly specific and sensitive RI may require partitioning of reference populations in to less heterogeneous group for each analyte. A study on biochemical marker reference study across Canadians addressed presence of significant age based variations of RI and the need of partitioning of data set during analysis for both liver and renal function tests (RFT)(31).

According to the a comparative study on serum liver function tests (LFT) levels in non-pregnant and Pregnant Women in La Riche, France, there was no significant difference in certain liver function test between non- pregnant and pregnant women. However, Serum albumin levels were significantly lower during all three trimester while serum ALP activity was significantly higher during the third trimester compared with non- pregnant women and during the second trimester compared with the first trimester. Serum ALT activity was slightly but insignificantly higher during the second trimester of pregnancy compared with non-pregnant women. Nevertheless, all serum ALT activity values remained below 35 IU/L,

the upper normal limit in their laboratory. Serum AST activity was not significantly different in pregnant and non-pregnant women. Total and indirect bilirubin concentrations were significantly lower during all three trimesters, as was direct bilirubin during the second and third trimesters(32).Similarly, according to a study in Gujarat, India(25)on evaluation of changes in liver function test in normal pregnant and control groups revealed slightly but insignificantly increased levels of serum ALT and AST activity in third trimester while Serum ALP activity was significantly higher in second and third trimester till it seemed to increase as pregnancy advances. On contrary, there was no significant change in serum total protein concentration whereas serum albumin concentration was significantly lower.

A study on the assessment of reference interval for clinical chemistry tests during normal pregnancy in Uppsala, Sweden found a change of most biochemistry analyte (AST, ALT,ALP, Albumin and urea) during normal pregnancy(33). It is, thus, of importance to use special reference values during pregnancy. The study conducted in North-Central Nigeria on pregnant women by using non-pregnant women as a control group revealed a significant ($P < 0.05$) difference in the level of urea and creatinine in first and second trimester. The progressive decrease in the levels of creatinine through the 3 trimesters of pregnancy is suggested to be due to an increase in glomerular filtration rate, probably due to increased cardiac output, renal blood flow and changes in fluid distribution(23).

A study on Laboratory reference intervals during pregnancy in Berlin, New York indicated large variations of majority of common clinical chemistry test results when compared with non-pregnant women that gestational age-specific reference intervals were necessary. Only a few parameters were unaffected during uncomplicated pregnancy.(34)

Another study on RFT Levels in normal pregnancy in Uttar Pradesh, India showed that serum urea and creatinine levels significantly decreased during the 1st trimester of pregnancy as compared to control group while the non-significant decrease level was observed in 3rd trimester of pregnancy. Comparison between 1st and 3rd trimester of pregnancy found that the serum urea concentration of 1st and 3rd trimester of pregnancy was not significant while significance difference was found between serum creatinine levels. The biochemical parameters serum urea and creatinine were affected by pregnancy in the 1st trimester more than the 3rd trimester(35).Another study from southern India on liver function tests in normal pregnancy stated that Serum albumin, AST and ALT were lower in pregnant mothers, whereas alkaline phosphatase were higher. A Kolmogorov-

Smirnov analysis showed normal distribution for alkaline phosphatase but not for albumin, AST and ALT (24).

According to a study on LFT in Nairobi, Kenya on normal Pregnant Women, they found that Serum ALT and albumin decreases while ALP increases significantly with gestational age. Meanwhile, AST and bilirubin fluctuate but remain within the normal ranges established for non-pregnant women. They concluded that, unless these normal gestation-related alterations are taken into account when evaluating LFT values in a pregnant woman, physiologic adaptations of pregnancy can be misinterpreted as pathologic or, alternatively, pathologic findings may not be recognized (36). Additionally on other related study on evaluation of changes in LFT of normal pregnant women in Shendi locality, Sudan depicted slightly decreased (not significant) serum ALT and AST activity in the first, second and third trimester than control pregnant women (37).

Direct application of non-locally established RI to our set up will bring unfavorable results of diagnosis. A consensus RI from a study of biochemistry reference intervals for healthy adults in eastern and southern Africa showed considerably wide range of RI than the adopted U.S RI(OOR value of 31% for TBIL, 41.6 for DBIL, 11.6% for AST, 11.8% for ALT, 2% for ALB and about 16.4% for TP). These otherwise healthy volunteers would be excluded or would require special exemption to participate in many clinical trials. When the division of acquired immunodeficiency syndrome AIDS(DAIDS) AE criteria were applied to the 12 analytes evaluated in their study for which applicable values exist, a total of 511 (24.3%) volunteers would have been considered to have had at least one laboratory-based AE with chemistry AE (7). This is supported by a cross sectional study in adults of middle belt Ghana where using of Clinical chemistry reference values based on the package inserts, it would have been found to screen out up to 25% of potential trial participants. Specifically when compared to values from reagent inserts, Ghanan's Out Of Range percentage (OOR %) was relatively wider being more higher for male than female and parameters like TP and TBIL had higher OOR% while ALB and DBIL had lower OOR% (10).

A multicenter cross sectional study conducted on reference intervals of routine clinical chemistry parameters among apparently healthy adults in Amhara national regional state, Ethiopia (19) revealed the presence of significant difference of all operationalized common renal and liver clinical chemistry parameter values by sex where males experienced higher values than females. There was observable clinical chemistry parameter RIs variation

between this study and studies conducted in northwest Ethiopia(28) . Similarly, RIs of all clinical chemistry parameters established by study in southwest Ethiopian (39) were not comparable with studies conducted in northwest Ethiopia (38) and southwest Ethiopia (39). Even though the study in Amhara region (19) encompass wider study area and wider age range (15-60), about 60%,83% and 91% of the participants were 18-20 years old aged, students and single respectively.

3. OBJECTIVE

3.1 General objective

- This study was intended to establish RIs for common renal and liver function clinical chemistry parameters among apparently healthy pregnant and non- pregnant women in South wollo zone, Amhara National Regional State, Ethiopia

3.2 Specific objective

- To compare RI common RFT and LFT of apparently healthy pregnant and non-pregnant women in S.wollo zone, Amhara national regional state, Ethiopia.
- To compare RI common RFT and LFT of apparently healthy pregnant women based on trimester in S.wollo zone, Amhara national regional state, Ethiopia.

4. HYPOTHESIS

H0: There is no statistically significant difference between reference values for commonly performed LFT and RFTs between pregnant and non-pregnant women as well as other studies or company derived kit insert values.

5. MATERIALS AND METHODS

5.1 Study area

Amhara region is located in northwestern Ethiopia between 9°20' and 14°20' North latitude and 36° 20' and 40° 20' East longitude (Figure 1) with an estimated land area of about 170,000 square kilometers being divided into 11 zones, and 140 woredas.

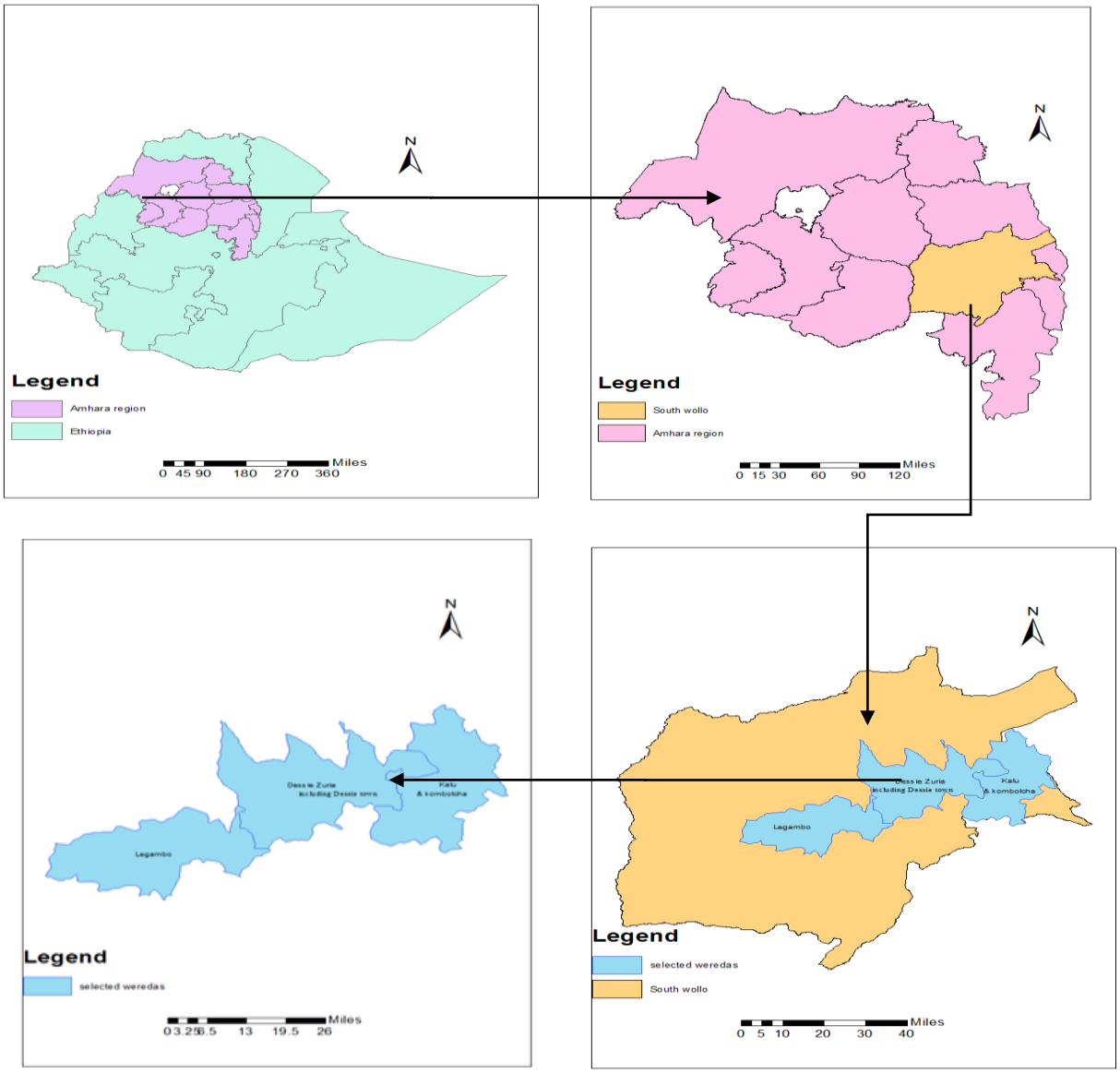

Figure 1.Map of study area

S.Wollo is found in Amhara National regional state having an area of 17,067.45 square kilometers. According to Federal Democratic Republic of Ethiopia Central Statistical Agency Population Projection in 2017 this Zone would be expected to have a total

population of 3,087132, of whom 1,528,769 are male and 1,558,363 female with 525,762 urban and 2,561,373 rural residents (40).

The largest ethnic group reported in S. Wollo was the Amhara (99.33%); all other ethnic groups made up 0.67% of the population. Amharic accounts the first spoken language by (98.65%).Majority (70.89%) were Islamic religion followers while 28.8% of the population followed Ethiopian Orthodox Christianity.

Dessie is a city and a zone located in the Amhara region at a latitude and longitude of 11°8′N 39°38′E, north-eastern Ethiopia with an elevation between 2,470 and 2,550 meters above sea level. Based on Federal Democratic Republic of Ethiopia Central Statistical Agency Population size Projection, Dessie administration has a total population of 223,077, of whom 110,260 are men and 112,817 women.

Kombolcha is a city and administration in north-central Ethiopia. It is located in the S. Wollo Zone of the Amhara Region. It has a latitude and longitude of 11°5′N 39°44′E with an elevation between 1842 and 1915 meters above sea level. Based on Federal Democratic Republic of Ethiopia Central Statistical Agency Population size Projection, Kombolcha administration has a total population of 122,033, of whom 61,151 are male and 60,882 women; 91,831 are urban inhabitants living in town of Kombolcha, the rest of population is living at rural kebeles around Kombolcha.

Kalu is one of the woredas in the Amhara region of Ethiopia and Part of the S.Wollo zone. The altitude of this woreda ranges from 800 meters above sea level in the lowlands border. Based on Federal Democratic Republic of Ethiopia Central Statistical Agency Population size Projection, this woreda has a total population of 219,228, of whom 110,683 are male and 108,575are female.

Legambo is one of the woredas in the S.wollo zone with an elevation of between 1500 to 3700 meters. Based on Federal Democratic Republic of Ethiopia Central Statistical Agency Population size Projection, the woreda has a total population of 189,898 of whom 93,111 are male while 96,787 are found to be female (41,42).

Four study woredas (Dessie administration, Kombolcha administration, Legambo woreda and Kalu woreda) were selected taking in to account of their density of residents and altitude. The study included were those individuals who were living at the aforementioned four areas and showed willingness to participate in the study.

5.2 Study design and period

A community based cross-sectional study to determine RI for common renal and liver function clinical chemistry parameters on apparently healthy pregnant and non-pregnant women was conducted from April to June 2019 among healthy pregnant and non-pregnant women in South Wollo zone, Amhara National Regional State, Northeast Ethiopia.

5.3 Population

5.3.1 Source population

The source populations were all apparently healthy pregnant and non-pregnant women of S. Wollo Zone, Amhara National Regional State, northeast, Ethiopia.

5.3.2 Study population.

Apparently healthy volunteer pregnant and non-pregnant women in the study area thatfulfill the eligibility criteria were the study population.

5.4 Inclusion and exclusion criteria

5.4.1 Inclusion criteria

Inclusion into the study was based on willingness of all pregnant women and non pregnant women between the ages of 15-60 years old. Generally the same inclusion and exclusion protocol illustrated by Committee on reference intervals and decision limits international federation for clinical chemistry and laboratory medicine (IFCC) and NCCLS document C28-A2 was applied with some modification to meet with the local population. Accordingly, participants included in the study were:

- Those who were feeling well.
- Healthy pregnant and non-pregnant women that was available at the selected households.

5.4.2 Exclusion criteria

The participants were excluded if any of the following was observed:

- Age less than 15 years old.
- Subjects with hemoparasite infection.
- Dehydrated individuals.
- Individuals who received blood transfusion within the previous 3 month.
- Human Immunodeficiency virus (HIV), Hepatitis C Virus (HCV), Hepatitis B Virus (HBV) and syphilis positives individuals.
- Lipemic and hemolysed sample.
- Observable mental illness.
- Individuals with regular strenuous physical exercise.
- Individuals working with hazardous chemicals (kerosene and acids)
- Individuals that are taking dispensable or indispensable drugs.
- Hypertensive individuals.
- Individuals with high grade fever with sort of discomforts.
- Chronic Smokers and alcohol drinkers.
- History of chronic liver or kidney disease.
- Malnourished (BMI<17.5Kg/M^2) (43).
- Known diabetes on oral therapy or insulin.
- History of being a hospitalized or otherwise seriously ill during the previous 4 weeks.
- Acutely ill as per the recommendations of WHO (44).

5.5 Study variables

5.5.1 Dependent variable

Common clinical biochemistry parameters for renal function (urea and creatinine) and LFT (albumin, total protein, AST, ALT, ALP, bilirubin (direct and total) are the dependent variables.

5.5.2 Independent variables

Age, geographical location (altitude), BMI, life style (nutritional factors), ethnicity, pregnancy and residence are the independent variables.

5.6 Measurement and data collection

5.6.1 Sample size calculation and sampling method

According to NCCLS recommendation and for the ease of using conventional statistical methods, a minimum number of 120 study subjects by class or sub-class (partition) are required. The Clinical Laboratory Standards Institute/ International Federation for Clinical Chemistry (CLSI/IFCC) recommended a non-parametric 95% RI with 90% confidence interval using at least120 study subjects. However, in order to attain the minimum sample size requirement, the maximum possible numbers of subjects being excluded should be added. Therefore, based on a cross sectional study in four African countries to produce comprehensive RI, about 30% total exclusion rate was observed. Moreover, about a total of 42% total exclusion rate was also observed in population based cross sectional RI establishment study in northwest Ethiopia (38). Therefore, this study used the maximum possibility of exclusion rate (which is 42%) to empower the possibility of attaining minimally required sample size (120 subjects). Therefore, about 42 % of 120 subjects had been added to 120 subjects to yield a total of 171 study participants. Moreover, about maximum of 10% outlier from all analytes was reported in large scale community based RI study in Canada (31). Thus, 10% of 171 subjects were again added to give a total 189 study subjects to be incorporated in each partitioning classes. Finally a total of 378(from 189x2) reference sample group were incorporated (enrolled) in the study. Taking altitude and residence difference in to account and also ease of accessibility of the areas two study woredas from lowlands and another two from the highlands was randomly selected from S.wollo zone (Table 1).

Table 1Total numbers of female population and corresponding numbers of sampled individuals in each of selected areas of S.woll zone Ethiopia,2019 .

Sr. No	Woreda	Estimated numbers of female population(41).	Name of selected kebeles	Number of enrolled reference individuals	
				Pregnant	Non-pregnant
1	Dessie administration	112,817	01	29	28
			04	29	28
2	Kombolcha administration	60,882	03	15	15
			05	15	15
3	Kalu	108,575	03	27	27
			06	27	27
4	Legambo	96,787	04	23	24
			07	24	23
Total				189	189

Then specific study kebele was selected using lottery method for each study woredas. The determined sample size was allocated for each selected woredas proportionally to their population size. The households from each kebele was addressed using convenience sampling method till it brings the predefined sample size for each partition keeping in mind that the minimum sample size will be absolutely maintained. Available pregnant and non-pregnant woman who showed willingness to participate were recruited and a maximum of one individual per partition per house hold was included in the study.

5.6.2 Data collection procedure

The regional, zonal and woreda (district) health bureaus were communicated about the purpose of the study. Awareness creation was done for concerned laboratory professionals, clinicians, health extension workers, and other health care providers about the general purpose and study procedures to be followed. The list of selected kebeles was distributed to the health extension workers. Then the purpose of the study was communicated to the participants and their willingness was confirmed. Volunteers with no easily identified prominent chronic and acute illness were scheduled to the nearby health institution.

In the health center, those participants in accordance with CLSI/IFCC recommendations with respect to patient selection and preparation like subjects who abstain the preceding

overnight correctly (2) was screened by obtaining medical history and symptom-directed physical examination by clinicians. Besides, anthropometric measurements like height, weight and blood pressure were recorded. Data like socio-demographic characterstics was collected from each participants using structured questionnaire via face to face interview. After completion of the interview, all eligible respondents were requested to give about 5ml of venous blood, urine and stool sample. In order to minimize diurnal variation, all samples were collected before noon (9.00–11.30 am) and were processed within 2 hours.

The sample was collected by medical laboratory professionals according to SOPs. Samples for biochemical analysis were allowed to stand for at least 30minutesand then centrifuged at 3000 rpm for 10 min. Using the sample they provided, study participants were further screened like intestinal parasites using concentration technique, blood film examination for hemoparasite infection, urine analysis (dipstick, microscopy and pregnancy), diabetes mellitus (using sensocard), using serological tests for viral diseases as HIV, HCV, syphilis and HB_SAg.

All participants who were positive for the above mentioned screening tests were excluded from the study but linked to the nearby health institutions for diagnosis and teratment. Results of HIV test were given to all participants after post-test counseling by trained HIV counselor. Physical examination findings and Results of laboratory examination for screening tests were provided for the participants. Serum sample that was used as a reference (sero-negative) in every way was subsequently aliquoted in another tube. Then, it was further placed in icebox and transported to the nearby central laboratory, Amhara public health institute Dessie branch (APHIDB) in the afternoon. It had been analyzed within 8 days after collection for common liver and renal function clinical chemistry parameters (AST, ALT, ALP, albumin, TP, bilirubin (direct and total), creatinine and urea). If testing was inevitable to be delayed, serum was stored frozen at -20^{o}c and was subjected to a single freeze thaw cycle at the time of analysis.

5.6.3 Specimen collection, processing, analysis and interpretation

A. Stool

Stool Specimen was collected at the right time using the correct technique and equipment, and be delivered to the laboratory in a timely manner. A stool (feces) sample can provide valuable information about our illness. Bacteria, viruses, and parasites are expelled in the stool during and after illness. The detection of ova, larvae, throphozoite, cyst or adult stage of intestinal parasite presumes that the individual is infested with intestinal parasites Protozoan trophozoites, cysts, oocysts, and helminthic eggs and larvae were seen and identified using a wet mount identification technique and concentration technique as well. Systematically the entire cover slip was scanned using the 10× objective till something suspicious is seen in which, a higher magnification may be necessary. (See annex I. section A)

B. Urine specimen

Examination of urine is an indispensable part of evaluation of patients with impaired kidney function, particularly proteinuria, hematuria, urinary tract infection, nephrolithiasis and other renal diseases. This simple chemical test performed in routine urinalysis rapidly provides important information about primary kidney disorder and systemic diseases. Examination of urine sediment provides valuable information about renal parenchyma. It involves macroscopic examination, chemical examination and microscopic examination of urine. Positive Chemical test results of urine analysis predisposes further microscopic examination of urine. Together with microscopic examination of urine an individual is ruled out for renal disease and some biochemical disorder diseases (45). (See annex I. Section B)

C. Blood specimen

Venin puncture is one of the most routinely performed invasive procedures for blood sample collection and is carried out to obtain blood for diagnostic purposes. Blood sample was collected using vein puncture. Use of the evacuated blood collection system was preferable because it allows the blood to pass directly from the vein into the evacuated tube eliminating the need for specimen transfer. The sample was centrifuged and then aliqoted. (See annex I.SECTION C)

Screening tests for HIV, HBsAG, and HCV, *Treponema palladium* was done by rapid serological test kits. (See annex I section D,E,F,G). Biochemical analysis for clinical

chemistry parameters such as albumin, ALT, AST, ALP, BUN, CREA, T.Protein and bilirubin (direct and total) was done using clinical chemistry auto analyzer (A25 Bio system, Biosystems S.A, Spain) as per the manufacturer's instructions and Standard operating procedures (SOP).Methods for all analysis could be traceable to International Federation of Clinical Chemistry (IFCC) standards Clinical chemistry parameters were determined by the methods/techniques described in table 2.All measurements were reported in their respective SI units.

5.7 Data quality assurance

5.7.1 Data collection tool quality assurance

The anticipated good quality of study participants was addressed through exhaustively organized questionnaire, appropriate history taking, physical examination and laboratory examinations in order to recruit healthy individuals. The validity of the questionnaire was assessed through translation, pre-testing followed by subsequent remedial action. All responsible participants were well equipped with all the study perspectives. All pre-analytical, analytical and post-analytical phases of quality assurance cycle were maintained.

5.7.2 Pre-analytical

Standard Operating Procedures (SOPs) were followed for sample collection, processing, storage and handling of the sample. Appropriate physical examination, screening of the subjects for acute and chronic diseases was performed. Proper orientation was given clearly for the candidates before sample collection and to avoid some factors such as strenuous exercise, eating, drinking and medication. Reassuring the study subjects immediately prior to sample collection was addressed and strict adherence to SOP was made during sample transportation and sample preparation to evade from losing sample integrity and hemolysis (46).

5.7.3 Analytical

Internal quality control was done for each parameter by using two quality Control levels. The laboratory strived to comply with the principles of Good Clinical Laboratory Practice protocols(47). Between-run and within run precision for the analytes was done using 20 measurements made on both the same and separate days using normal control samples (Table 2).

Table 2.Methods used (traceability) and analytical precisions for selected assays .

Analytes	**Method**	**unit**	**APHLDB within-run precision**		**Manufacturer's within-run precision**		**APHLDB between-run precision**		**Manufacturer's between-run precision**	
			mean	%CV	Mean	CV%	mean	%CV	Mean	%CV
Albumin	Bromocresol green-Succinate Buffer	g/l	4.4	0.06	4.5	1.2	4.2	1.3	4.5	1.6
Total protein	Biuret/endpoint	g/l	51.4	1.1	51.8	1.0	51.1	0.9	51.8	1.1
AST	IFCC Modified without pyridoxal phosphate	U/l	47.6	0.41	38	1.4	47.3	5.3	38	5.9
ALT	IFCC Modified without pyridoxal phosphate	U/l	40	1.6	43	1.8	40.5	4.9	43	5.3
ALP-DEA	p-Nitrophenyl phosphate. Diethanolamine	U/l	110	1.0	117	1.1	110.3	4.2	117	4.5
Bilirubin (direct)	Dizotizedsulfanilic	mg/dl	0.85	0.8	0.77	1.2	0.86	1.5	0.77	2.3
Bilirubin(total)	Dizotizedsulfanilic	mg/dl	2.13	2.1	0.59	3.0	2.09	3.8	0.59	3.6
Urea	Enzymatic – UV Kinetic	mg/dl	26.2	2.9	42	3.3	26.4	4.2	42	4.3
Creatinine	Jaffe –Kinetic	mg/dl	1.65	2.6	1.7	2.9	1.63	3.0	1.7	3.9

The mean and coefficient of variation (CV) was calculated for each analyte. Coefficient of variation (CVs) was compared to those mentioned in the reagent inserts. The machine was calibrated as per standards recommended by the manufacturer. Each activity like blood sample collection, transportation, storage and analysis was based on good laboratory practices using standard operating procedures (SOPs) to ensure result quality. The control

sample results were interpreted using Westgard multi-rule algorithm. The control sample results had to be within acceptable ranges prior to testing reference individual sample. Moreover, the laboratory has been participating in external quality assessment programs like an onsite evaluation by Ethiopian public health institute (EPHI) and External Quality Assessment (EQA) Proficiency Testing (PT) schemes by Randox International Quality Assessment Scheme (RIQAS) (48).The centralized measurement site (APHIDB) was used to eliminate variations due to differences in analytical methods. Therefore, APHIDB did act as the central laboratory for receiving samples and performing the assays collaboratively.

Moreover, in order to ensure the accuracy and precision of the test results, all pre-analytical, analytical and post-analytical precautions were taken into consideration. The Bio-system clinical chemistry auto-analyze (Spain) and the protocols were under regular control of the Ethiopian public health institute (EPHI).Furthermore, all the laboratory staff received equipment and procedure (protocol) training from highly trained personnel. The two quality control levels (pathological/abnormal and non-pathological/normal) were run on daily basis till it falls within the acceptable ranges before testing samples. As a means of measuring the overall precision of the method used, laboratory errors that may emerge from day to day, factors like change of operators, reagents and surrounding operating conditions, within run and between run precision was performed. The result was compared with precision limits claimed in the analyte reagent insert kits. As clearly depicted in table 2, majority of the clinical chemistry tests were within the precision limits indicated by the reagent manufacturer which basically augment the reliability of the reference values documented in this study.

5.7.4 Post analytical

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

5.8 Data analysis and interpretation

The data was revised and checked for completeness manually and entered to EPI Info version 3.5.3 (CDC, USA) statistical software and then transferred to Statistical Package for Social Sciences SPSS version 20 (IBM, USA) software for analysis. The data was partitioned in to two groups; pregnant and non-pregnant women. Even though there was a trial to avoid the presence of outlier test results through intensive prior clinical and laboratory test screening of healthy reference samples, there was a possibility of observing few outliers. Thus at the beginning the data was visualized for the presence of outliers within each group. This was done by displaying the data in a histogram and inspecting the valuable information about the reference distribution data, like possible outliers, the presence of bimodal or poly modal peaks and erroneous values. But, actual exclusion of those outliers was done by the quartile method, in which the range of the central 50% of the resulting distribution was calculated, and then subtracting 150% of this value from the 25th percentile and adding 150% of this value to the 75th percentile for each test results of all partitions. Any values beyond these limits were considered outliers. This will attempt to balance the unreasonably too widening or too narrowing of RI establishment. Values below the lower and above the upper cut-offs were outliers and were not considered for further analysis (49).

After all outliers were excluded, descriptive statistics were employed to determine the mean, median and 95% range of each analytes. Reference intervals were calculated in accordance with CLSI/IFCC guideline using non-parametric methods. Nonparametric methods do not make assumptions as to the specific form of the underlying distribution of the data. Nonparametric methods are reasonable for large samples, at least 120 observations. This calculation is straightforward. The data was ordered and 2.5^{th} and 97.5^{th} percentiles were used to form the 95% reference interval with 95% confidence interval (CI) in this study. The 95% RIs was estimated using reference limits at 2.5^{th} percentile for the lower reference limit and 97.5^{th} percentile for the upper reference limit. Reference interval was value between the 2.5th and 97.5th percentile inclusive. Kolmogorov–Sminorv (KS) test was used for all test results of each partition to check its normality in SPSS data analysis software. All the observed data of each analytes were found to be not normally distributed. The presence of significant differences between pregnant and non-pregnant women was evaluated using the Mann–Whitney U test and P –<0.05 claimed the presence of statistically significance difference.

The newly established RI was subsequently compared to or displayed together with values currently in use by the central laboratory (Amhara Public Health Institute Dessie Branch). and with findings of a study conducted in few African countries like middle east Ghana, Zimbabwe, eastern, southern and eastern Africa, and also with Hong kong and US Massachusetts General Hospital (MGH) (50). In addition, it was also be analogized with the nearby studies in Amhara national regional state, North West Ethiopia, south west Ethiopia. The percentages of participants with out of range (OOR %) values were then calculated with the reagent insert kit RI as a template.

5.9 Ethical consideration

The study was conducted after ethical approval was obtained from Research and Ethics review board of the Addis Ababa University. It also got support letter from federal, regional and zonal health bureaus. Informed written consent and/or assent were obtained from each study participant before the actual data collection. Individual's positive for the screened infections and other disease conditions had been linked to nearby government health institution for further diagnosis and treatment accordingly. Information obtained at any point of the study was kept confidential.

5.10 Dissemination of Result.

As a research is of no use unless it gets to the people who need to use it, the final finding of this study will be submitted to the department of medical laboratory sciences of AAU and APHIDB. It will also be accessible for decision makers, researchers, clinicians, and patients as requested as possible through presentations and printout materials. The findings will also be published on peer reviewed journals.

5.11 Operational definition.

- **Apparently healthy pregnant women:** Individuals with age ≥ 15 years without disease based on clinical sign and symptom plus laboratory investigations and positive urine human chorionic gonadotropin hormone test result.
- **Apparently healthy non-pregnant women:** Individuals with age ≥ 15 years having negative urine human chorionic gonadotropin test result, without disease based on clinical sign and symptom plus laboratory investigations.
- **Common liver function clinical chemistry parameters:** albumin, total protein, AST, ALT, ALP, bilirubin (direct and total).

- **Common renal function clinical chemistry parameters**: urea and creatinine
- **Kebele:** the smallest administrative unit in Ethiopia.
- **Observed value:** the value of a particular type of quantity obtained by observation or measurement of a test subject.
- **Population based RI:** are RIs derived from a group of systematically selected reference individuals from the population
- **Reference individual:** a person selected for testing on the basis of well-defined criteria
- **Reference population:** The selected population that the reference individuals belonged to.
- **Reference sample group:** a group of selected reference individuals from a reference population on which the reference intervals are determined.
- **Reference value: T**he value obtained by the observation or measurement of a particular type on a reference individual.
- **Reference distribution:** is the distribution of reference value and involves reference sample group and adequate statistical methods
- **Reference limits:** a value derived from the reference distribution and used for descriptive purposes
- **Woreda:** an administrative unit which consists of certain numbers of kebeles

6. WORKFLOW

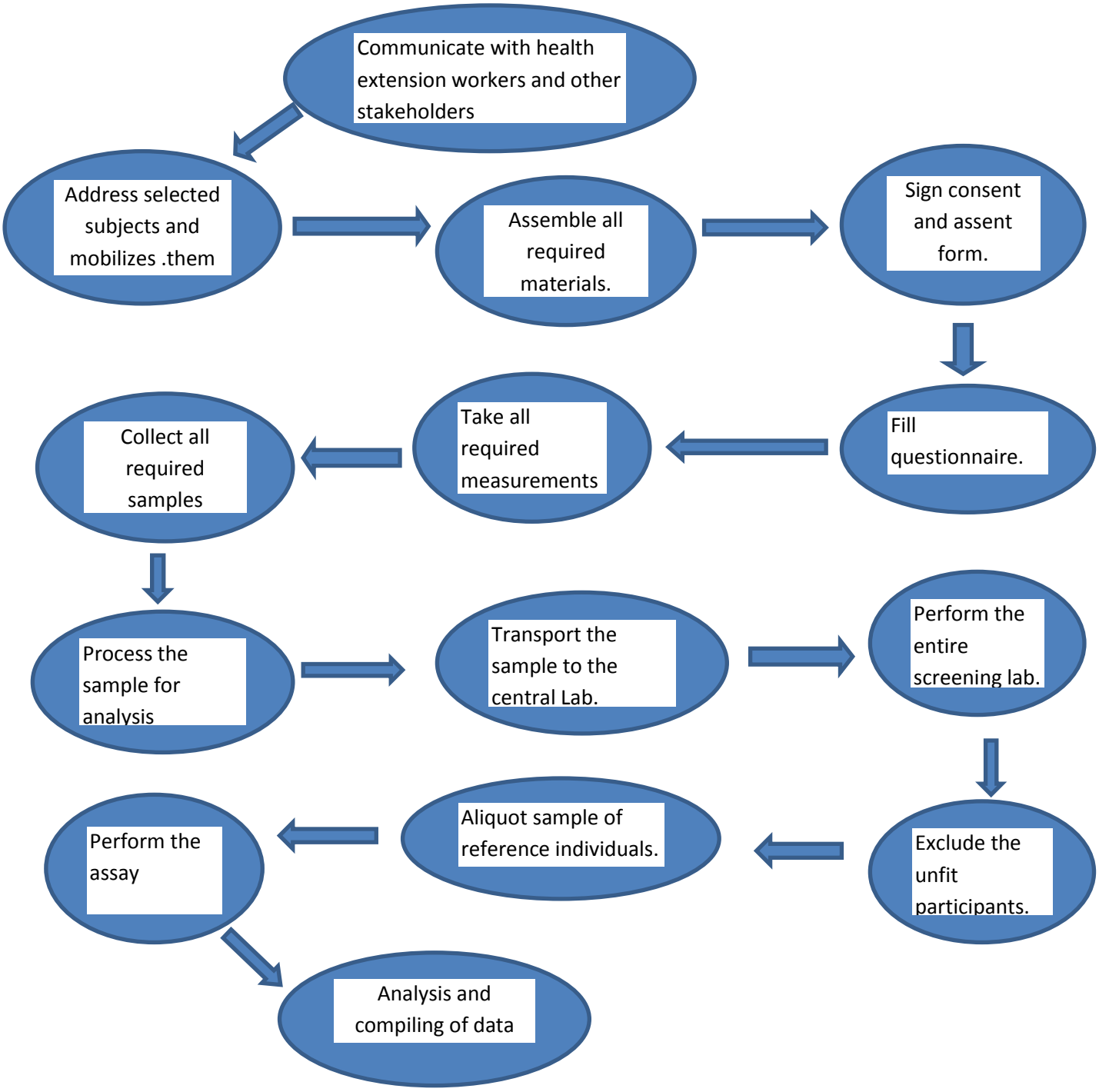

Figure 2.Diagrammatic illustration of workflow.

7. RESULT.

7.1 Screening results.

A total of 378 apparently healthy adult women (189 pregnant and 189 non-pregnant) were consented and screened to establish the RI of common liver and renal function clinical chemistry parameters from S.wollo zone, northeast Ethiopia. Of these participants, a total of 24(12.6 %)pregnant were excluded as a result of positive laboratory test results for HIV,HBV,HCV and syphilis in a magnitude of 6(3.1%), 5(2.6%),2(1.05%) and 3(1.5%) respectively and 8(4.2%) by clinical history, physical examination and others while 31(16.4%)non-pregnant were excluded as a result of positive laboratory test results for HIV,HBV,HCV and syphilis in a magnitude of 6 (3.1%), 5(2.6%),1(0.5%) and 4(2.1%) respectively and 15(7.9%) by clinical history, physical examination and others. After screening, those who were positive for HIV,HBV, HCV, syphilis, blood parasites and other disease conditions were referred to the nearby governmental health care institutions and were not included in the study. A total of 165pregnant and 158 non-pregnant participants who met the eligibility criteria were enrolled and became a reference sample group in this study.

7.2 Socio-demographic characteristics

The mean and median age of all the study participants at the study entry period was 27.2(SD: 6.6) and 27(Inter quartile range (IQR: 23-30) years, respectively. The mean age and mean gestational age of pregnant were 27.4(SD: 4.4) years and 19.7(SD: 7.7) weeks respectively. The mean weight, mean height and mean body mass index (BMI) for pregnant women were 62(SD:4.3) Kg,1.63(SD:0.05) meter and 23.3(SD:1.7) kg/m^2respectively whereas 59(SD:3.9) kg ,1.62(SD:0.06) meter and 22.4(SD:1.5)kg/m^2 respectively for non-pregnant women. Around one-fourth of the participants 86(26.6%) and 73(22.6%) were single and at least high school completed respectively (Table 3).

Table 3.Socio-demographic characteristics of Study participants from S.wollo zone, northeast Ethiopia,2019.

Variables	Pregnant		Non-pregnant	
Age in years	**Number**	**%**	**Number**	**%**
16-20	6	3.6	26	16.4
21-29	115	69.6	83	52.5
30-39	39	23.6	32	20.2
40-60	5	3.0	15	9.4
Marital status				
Single	5	3.1	81	51.2
Married	160	96.9	77	48.8
Pregnancy status				
Primigravida	51	30.9	NA	NA
Multigravida	114	69.1	NA	NA
Occupation				
Employed	23	14.5	25	15.8
House wife	92	55.7	43	27.2
students	3	1.8	34	21.5
Others	47	30.3	56	35.4
Religion				
Muslim	96	58.1	97	61.3
Orthodox	60	36.3	56	35.4
Others	9	5.6	5	3.2
Educational status				
Illiterate and elementary Completed	109	66.1	95	60.1
High School Completed and Above	56	33.9	63	39.9
Residence				
Urban	118	71.5	102	64.6
Rural	47	28.5	56	35.4

%: Percentage

7.2 Reference intervals for commonly performed liver and renal function clinical chemistry tests.

As shown in table 4, the overall mean values for pregnant and non-pregnant women respectively were albumin (34.1 vs.41.5g/l), T.protein (62.0 vs.71.8 g/l), AST (18.8vs. 20.8U/L), ALT(13.5vs.17.6 U/L), ALP (171vs.142 U/L), bilirubin(direct)

(0.17vs.0.19mg/dl), bilirubin(total) (0.60 vs.0.58mg/dl), urea (13.0 vs16.8mg/dl)and creatinine(0.58vs. 0.73mg/dl). The respective RIs for pregnant and non-pregnant women were as follows: ALB 26.1-42.8 g/l vs. of 32.8-47.8 g/l, T.protein 48.5-74.7 g/l vs. 56.7-83.9 g/l, AST2.4-43.6 U/L vs. 4.2-37.1 U/L, ALT 0.9-28.3 U/L vs. 2.6-41.1 U/L, ALP 21.2-337 U/L vs. 3.2-278.4 U/L, bilirubin(direct) 0.03-0.32mg/dl vs 0.1-0.51mg/dl, bilirubin(total) 0.26-0.94mg/dl vs. 0.24-1.06mg/dl, Urea 7.1-20.8mg/dl 8.0-27.8mg/dl and Creatinine 0.29-0.87mg/dl vs. 0.44-1.00mg/dl..

The study showed the presence of statistically significant differences between pregnant and non-pregnant women in majority of commonly performed liver and renal function clinical chemistry parameters except for AST, ALT, bilirubin(direct) and bilirubin (total). Pregnant women had significantly lower values than non-pregnants in most of the analytes. Pregnant women had significantly ($P<0.05$) lower value of albumin, total protein, Urea and creatinine whereas significantly ($P<0.05$) higher value of ALP than non-pregnant women.The calculated mean, median, 95% CI for mean and 2.5^{th}-97.5^{th} percentile range (RI) of common liver and renal function clinical chemistry tests for pregnant and non-pregnant apparently healthy adults in S. Wollo Zones, Amhara national regional state, northeast Ethiopia were summarized in tables 4.

Table 4. The calculated Mean,median,95%CI for mean and 2.5th-95th percentile RI of common liver and renal function tests in relation to pregnancy status of healthy adult women in south wollo zone, Amhara National Regional State, Northeast Ethiopia, 2019.

Parameters	Unit	Pregnant				Non-pregnant				p-value	Combined pregnant and non-pregnant			
		Mean	Median	95% CI for mean	2.5th-97.5th Percentile Range(RI)	Mean	Median	95% CI for mean	2.5^{th} - 97.5^{th} percentile range(RI)		Mean	Median	95% CI for mean	2.5th-97.5th Percentile Range(RI)
*Albumin	g/l	34.1	34.11	33.4,34.7	26.1-42.8	41.5	41.8	40.9,42.1	32.8-47.8	0.0001	37.5	37.9	36.9,38.1	26.6-46.9
*T. protein	g/l	62.0	62.0	60.9,63.1	48.5-74.7	71.8	72.0	70.8,72.9	56.7-83.9	0.0001	66.7	67.7	65.7,67.6	49.4-82.3
AST(SGOT)	U/l	18.8	16.8	17.2,20.3	2.4-43.6	20.8	19.2	19.0,21.7	4.2-37.1	0.151	19.6	17.9	15.5,20.6	2.9-42.1
ALT(SGPT)	U/l	13.5	12.1	12.4,14.4	0.9-28.3	17.6	16.0	16.1,19.1	2.6-41.1	0.052	14.7	13.7	13.9,15.5	1.5-31.4
*ALP-DEA	U/l	171	157	157.9,184.3	21.2-337	142.0	138	131.6,152.5	3.2-278.4	0.045	157.1	146.0	148.6,165.6	8.8-320
Bilirubin (direct)	mg/dl	0.17	0.16	0.14,0.19	0.03-0.32	0.19	0.18	0.17,0.21	0.06-0.38	0.205	0.17	0.18	0.15,0.19	0.04-0.36
Bilirubin (Total)	mg/dl	0.6	0.64	0.58,0.63	0.26-0.94	0.58	0.61	0.55,0.62	0.24-1.06	0.227	0.59	0.63	0.57,0.61	0.24-0.96
*Urea	mg/dl	13.0	12.5	12.4,13.6	7.1-20.8	16.8	16.4	16.0,17.5	8.0-27.8	0.0001	14.7	14.5	14.2,15.2	7.5-24.9
*Creatinine	mg/dl	0.58	0.6	0.56,0.60	0.29-0.87	0.73	0.73	0.71,0.75	0.44-1.00	0.0001	0.65	0.66	0.63,0.67	0.33-0.97

*parameters with statistically significant differences based on pregnancy status.

7.3 Ninety five percent Confidence intervals for the established lower and upper reference limits.

The 95% CI for lower and upper reference limits of established RIs in clinical chemistry parameters were calculated and presented in table 5 below.

Table 5.The 95% CI for upper and lower reference limits of common liver and renal function clinical chemistry tests of healthy pregnant and non-pregnant women in S.wollo zone Amhara national regional state, northeast Ethiopia, 2019.

Analytes	**Unit**	**Pregnancy status**	**RI percentile range)**	**Lower reference limit 95% CI**	**Upper reference limit 95% CI**
Albumin	mg/dl	Combined	26.6-46.9	(26.1,27.46)	(46.3,47.7)
		Pregnant	26.1-42.8	(24.0,27.06)	(41.8,46.02)
		Non-pregnant	32.8-47.8	(31.85,35.23)	(46.95,49.26)
T. protein	mg/dl	Combined	49.4-82.3	(48.52,51.6)	(80.86,84.47)
		Pregnant	48.5-74.7	(41.2,50.77)	(72.73,77.07)
		Non-pregnant	56.7-83.9	(55.84,60.17)	(81.48,87.54)
AST(SGOT)	U/l	Combined	2.9-42.1	(0.89,6.25	(39.05,43.83)
		Pregnant	2.4-43.6	(0-4,84)	(40.76,45)
		Non-pregnant	4.2-37.1	(0.89,8.6)	(34.41,43.83)
ALT(SGPT)	U/l	Combined	1.5-31.4	(0.6,3.46)	(30.07,32.38)
		Pregnant	0.9-28.3	(0,3.18)	(25.87,31.0)
		Non-pregnant	2.6-41.1	(0,4.76)	(36.75,42.2)
ALP-DEA	U/l	Combined	8.8-320	(3.14,35.0)	(304,329)
		Pregnant	21.2-337	(3,37)	(316,404)
		Non-pregnant	3.2-278.4	(0,30)	(249,307)
Bilirubin(Direct)	mg/dl	Combined	0.04-0.36	(0.02,0.50)	(0.31,0.42)
		Pregnant	0.03-0.32	(0,0.08)	(0.29,0.37)
		Non-pregnant	0.06-0.38	(0.01,0.12)	(0.31,0.45)
Bilirubin(Total)	mg/dl	Combined	0.24-0.96	(0.15,0.26)	(0.9,1.0)
		Pregnant	0.26-0.94	(0.14,0.29)	(0.9,0.96)
		Non-pregnant	0.24-1.06	(0.12,0.28)	(0.99,1.11)
Urea	mg/dl	Combined	7.5-24.9	(6.28,8.13)	(23.28,26.34)
		Pregnant	7.1-20.8	(3.83,7.90)	(19.5,23.95)
		Non-pregnant	8.0-27.8	(5.08,10.14)	(25.3,29.18)
Creatinine	mg/dl	Combined	0.33-0.97	(0.34,0.44)	(0.86,0.99)
		Pregnant	0.29-0.87	(0.23,0.39)	(0.75,0.91)
		Non-pregnant	0.44-1.00	(0.41,0.51)	(0.93,1.04)

ALT: Alanine aminotransferase; ALP-DEA: Alkaline phosphatase-diethanolamine buffer; AST: Aspartate aminotransferase;CI: confidence interval; dl:Deciliter; g: Gram; L: liter; mg: Milligram; RI: Reference interval; SGOT: Serum glutamate oxaloacetate transaminase; SGPT: Serum glutamate pyruvate transaminase; U: Unit

7.4 Comparability of established RI with other studies in Ethiopia, Africa and with kit inserts claiming RIs.

The study assessed the comparability of the current RI with studies from other parts of Ethiopia, Africa, USA and China. As shown in Table 8, no consistent pattern was seen among the various studies. The current study was relatively close to the company derived values which are available for the non-pregnant women only. Lower limit of urea is lower than the company value (8 vs. 15 mg/dl) though higher than Zimbabwe (3.9gm/dl) and Ghana (5.4gm/dl). Bilirubin direct and total upper limit values were almost 2x lower when compared to the company value but close to the RI from Zimbabwe and USA study (total Bilirubin for example, 0.2-1.11 for the current study vs. 0.3-1.0 mg/dl). The upper limit of total protein was comparable to the company value and the other studies (except a study from Amhara region and Zimbabwe). In Table 7, the proportion of out of range values (OOR %) segregated by pregnancy status is displayed based on the company based RIs which is being utilized by the Amhara public health institute Dessie branch laboratory. Accordingly, large OOR values were detected especially for pregnant women for the RIs of Albumin, Urea, Total protein and Bilirubin (direct).

Table 6: The calculated median and 95% RI for common RFT and LFT on trimester based of pregnants in south wollo zone Ethiopia, 2019.

Parameters	Median(2.5^{th} – 97.5^{th})						p-value		
	Trimester 1		Trimester -2		Trimester 3				
	N	median(95%RI)	N	median(95%RI)	N	median(95%RI)	b/n 1^{st} & 2^{nd}	b/n 1^{st} & 3^{rd}	b/n 2^{nd} & 3^{rd}
Albumin	29	35.5(26.6-45.28)	99	34.1(26.16-42.85)	25	32.35(26.21-39.82)	0.739	*0.043	0.062
T. protein	28	65.9(39.17-76.33)	96	61.4(46.50-74.63)	23	61.84(48.53-70.74)	0.603	0.873	0.953
AST(SGOT)	27	19.1(5.1-53.6)	95	17.4(2.5-53.69)	25	16.82(1.41-55.85)	0.904	0.652	0.776
ALT(SGPT)	29	13.8(2.32-)62.3)	97	13.43(0.69-46.80)	26	12.21(2.34-34.86)	0.952	0.558	0.588
ALP	28	162.5(38.5-322.75)	99	148.0(18.41-330.55)	26	212.0(44.3-395.95)	0.957	0.114	*0.019
Bilirubin (direct)	24	0.28(0.06-0.54)	94	0.25(0.03-0.52)	26	0.27(0.02-0.47)	0.685	0.827	0.998
Bilirubin (total)	27	0.65(0.26-1.1)	100	0.64(0.25-0.95)	25	0.63(0.15-0.94)	0.834	0.665	0.810
Urea	26	12.9(6.82-27.43)	98	7.37(12.7-52.85)	24	12.52(4.2-18.7)	0.792	0.939	0.559
Creatinine	27	0.61(0.15-0.88)	97	0.57(0.23-0.86)	26	0.61(0.30-0.84)	0.994	0.970	0.991

*parameters with statistically significance difference between trimesters.

Table 7. OOR (N) and OOR (%) of current RI study as compared to APHIDB used RI (adopted from leaflet)

Analytes	Unit	Pregnancy status	DRHRL adopted RI	Current study RI	OOR (N)	OOR (%)
Albumin	g/l	Pregnant	35-50	26.1-42.8	99	60%
		Non-pregnant	35-50	32.8-47.8	38	24%
Total Protein	g/l	Pregnant	60-83	48.5-74.7	55	35.7
		Non-pregnant	60-83	56.7-83.9	6	3.7%
AST	U/l	Pregnant	0-40	2.4-43.6	7	4.2%
		Non-pregnant	0-40	4.2-37.1	4	2.5%
ALT	U/l	Pregnant	0-41	0.9-28.3	11	6.6%
		Non-pregnant	0-41	2.6-41.1	3	1.8%
ALP-DEA	U/l	Pregnant	0-270	21.2-337	28	16.9%
		Non-pregnant	0-270	3.2-278.4	4	2.5%
Bilirubin (direct)	mg/dl	Pregnant	0-0.2	0.03-0.32	39	23.6%
		Non-pregnant	0-0.2	0.06-0.38	48	29%
Bilirubin (Total)	mg/dl	Pregnant	0-2	0.26-0.94	16	9.6%
		Non-pregnant	0-2	0.24-1.06	8	4.8%
Urea	mg/dl	Pregnant	15-39	7.1-20.8	114	69%
		Non-pregnant	15-39	8.0-27.8	84	53%
Creatinine	mg/dl	Pregnant	0.5-1.2	0.29-0.87	51	30.9%
		Non-pregnant	0.5-1.2	0.44-1.00	12	7.5%

OOR: out of range; N: number; %: percentage

8. DISCUSSION

This study aimed to establish reference intervals for common liver and renal function clinical chemistry parameters among apparently healthy pregnant and non- pregnant women in South wollo zone, Amhara National Regional State, Ethiopia. It had an intention of serving as a bench-mark for the interpretation of such clinical laboratory results in the routine healthcare process and in clinical trials studies. Reference intervals for clinical chemistry parameters have an indispensable role in the assessment of the health status of an individual, evaluation of disease prognosis, in therapeutic drug monitoring and in selecting appropriate study participants in clinical trials for certain population (1,15,16). Even so nearly 80% of physicians' medical decisions relay on information provided by laboratory reports that are interpreted based on RI values. There is paucity of reference interval studies done in Ethiopia for the common clinical chemistry parameters especially for pregnant women. In spite of the fact that many of the methods that clinical laboratories adopted are more than enough from an analytical perspective, the reference intervals that a company claims require careful inspection and subsequent verification of its transference. Clinicians were facing a challenge in absolutely deciding the health status of an individual by correlating with such unverified RIs. Clinical researchers experienced wrong inclusion, exclusion and interpretation of their study subjects in their clinical trial studies.

The results obtained from this study revealed that though the out of range proportions vary, most analytes reference values varied from values fixed as a reference on the insert package to be used for clinical managements in the study area both for pregnant and non-pregnant women. This anticipated difference for population in different placement confirms the recommendations of the manufacturers and NCCLSI for each laboratory to establish their own reference values using their own local population (4).

Besides the presence of analogy among this study for the respective RIs and other similar studies in Africa and across the world, there were also few observable clinical chemistry parameter RIs differences inconsistently for each test in each country. However, relatively higher and wider RI for albumin, total protein, ALT and AST was observed in studies conducted in northwest Ethiopia (35), southwest Ethiopia (39), and middle belt of Ghana (10), Zimbabwe (52), south and eastern Ethiopia (7), USA (50) and Hong Kong China (51) than the current study in non-pregnant women.

As clearly summarized in table 8, lower reference limit of albumin for non-pregnant women in this study was comparable with study conducted in middle belt of Ghana (10), Zimbabwe(52), south and eastern Africa (7),USA (50) and manufacturer claimed RI while lower than studies conducted in Amhara region (19) and Hong kong China (51). Similarly, the upper reference limit was comparable with RI findings of related studies conducted in middle belts of Ghana (10) and Hong Kong China (51) but lower than reference limit of study conducted in Amhara region (19), Zimbabwe (52),south and eastern Africa (7) and USA (50).

In non-pregnant women the lower reference limit of T.protein was comparable with related studies done in north west Ethiopia (35), Amhara region (19), middle belts of Ghana (10),southern and eastern Africa(7),USA(50) and manufacturer but slightly lower than studies in southwest Ethiopia (37),Zimbabwe (52), and Hong Kong China (51) considering that the upper reference limit was analogous with manufacturer claimed value and studies operated in northwest Ethiopia (35),southwest Ethiopia (37),Ghana (10),USA (50) whilst higher than studies done in Hong Kong China (51).

The lower reference limit of bilirubin (direct) of non-pregnant women in this study was comparable with similar bilirubin RIs study conducted in Gojjam zone northwest Ethiopia (28), other sites in Amhara national regional state (19), southern and eastern Africa (7),USA (50), china (51)and reagent insert kit claiming RI but slightly lower than study conducted in middle belt of Ghana (10) which could be due to different analytical methods used in Ghana for bilirubin analysis(3-5, dichlorophenyl-diazonium-tetraforoborate). Even though the upper reference limit value of bilirubin (direct) become similar in some way with related studies in Zimbabwe (52) and USA (50), it is slightly lower than that of Gojjam zone northwest Ethiopia(28),other sites in Amhara region(19), southern and eastern Africa (7) and Hong Kong China (51). Lower RI value of bilirubin(total) is observed in non-pregnant women of this study than study conducted in Gojjam zone northwest Ethiopia (28), Amhara region (19), middle belt of Ghana (10) and south and eastern Africa (7).

Moreover, the lower and upper reference limit of this study for AST, ALT and ALP was slightly higher than and comparable in the order given with manufacturers declared limit whereas slightly lower than RI studies done in most of the aforementioned countries. This nearly similarity probably was due to similar IFCC methods used by all laboratories I the aforementioned countries.

Although the lower and upper reference limit of urea in this study was similar in the same way with related studies done in Amhara region (19), USA (50), Hong kong China (51), it was slightly lower and higher than manufacturers stated RI and studies done in Zimbabwe (52) and Ghana (10) respectively.

In spite of a matching value of lower and upper reference limit for creatinine in this study was observed with related studies done in Amhara region (19) and Hong Kong China (51),slightly higher lower reference limit was observed than similar studies conducted in northwest Ethiopia (28),southwest Ethiopia (39) and USA (50) and slightly lower upper reference limit than studies in southwest Ethiopia (37),south and eastern Africa (7),middle belt of Ghana (10),USA (50)and manufacturer stated reference limit. Factors like demographic variation, ethnic and genetic difference, nutritional behaviors, culture, life style and seasonal differences might be entities which contribute for such relatively inconsistent values of clinical laboratory RIs among apparently healthy women across population of the same country and among different nations.

This study showed the presence of significant difference for majority of common renal and liver function clinical chemistry parameter values by pregnancy status that agreed with other similar studies in Africa. Majority of common LFT and RFT clinical chemistry parameter values were lower among pregnant than non-pregnant women except for AST, ALT, bilirubin (direct and total). Whilst NCCLS recommends a minimum of 120 reference individuals in each partition for the statistical tool validity, most clinical chemistry reference intervals of the few similar studies on pregnant women are unfortunately established based on trimester scales. Although such amount of participants were not maintained RI was computed in order to look for such instance based on trimester level. However, as depicted in table 6, only a few parameters were found to show statistically significant variation (albumin: between first and third trimester and ALP: between second and third trimester). Moreover, the trend of analyte concentration as gestational age advance was examined. Nevertheless, there was no linear association for all analytes concentration in relation to gestational age except that of Alp that showed moderate association as gestational age advances (Spearman’s rho of 0.7).

The finding of significantly lower albumin values in pregnant than non-pregnant women in this study was slightly lower than currently used by the laboratory as the laboratory directly adopted RI given for non-pregnant to use it as a reference frame. The lower reference interval for non-pregnant as compared to pregnant could be probably due to alteration of plasma fluid distribution and hemodilution with that of high demand of albumin by the growing fetus. This lower albumin RI values agreed with results from related studies in Kenya (36), India (24), France (53), Guagarat India (25) and north central Nigeria (23).This decrease in serum albumin concentration could be attributed to pregnancy-related plasma expansion with hemodilution while increased ALP activity with pregnancy could be ascribed to production of placental isoenzyme and fetal bone marrow development (20).

Even though it was slightly fluctuated within RIs of non-pregnant women, an observation in the absence of significant difference between pregnant and non-pregnant women for AST and ALT in this study coincided with similar study conducted in France(48), north central Nigeria (23) and Kenya (36). However, result of slightly lower value of ALT activity in pregnant in this study agreed with similar studies in Sudan(37) unlike a slightly but not significantly increased value in studies of Guajarati, India (25) and France (48).Unlike significantly lower value of Bilirubin (total) from a study conducted in France (48), this study and studies from Shendi,India (37) and Punjab, India (54) depicted no change but slightly decreased in value of Bilirubin (direct and total) between pregnant and non-pregnant women. According to a study and finding of significant and progressive decrease in the levels of urea and creatinine in all trimester in northen central Nigeria (23) as was support by this study, which demonstrated lower urea and ceatinine values though not partitioned by trimester. These higher and lower values were different across studies that could be the result of demographic and racial differences beside the variability of analytical methods, equipment types and reagents being used. Moreover, variations in such clinical chemistry parameters results could be mainly due to the effects of progesterone and estrogen that are produced largely by ovary and placenta to allow the fetus to grow but at the same time attributed to the increased in cardiac output, renal plasma flow and glomerular filtration rate during pregnancy which intern increases urea and creatinine excretion in pregnant than non-pregnant women (20).

The most common prerequisites for clinical chemistry parameters in their screening or enrolment of participants and monitoring their safety during clinical trials are liver and renal function tests. In addition, in regarding to management of medical clients, a patient in need

of an appropriate treatment could be negated whereas the one that should not will get ineffectual treatments far as wrong RIs are implemented. Since many of clinical trial studies uses non locally but alternatively reagent insert kit provided RI sets, studies conducted in African countries encountered problems of excluding an appropriate participants in the clinical trial study when they applied such unfit elsewhere established reference values. To exemplify, studies conducted in Kintampo (Ghana) (10) experienced up to 32% exclusion rate when adopting western derived intervals for recruitment of clinical trial study participants , up to 42% in Kenya (7) and to 81% in Tanzania (9). In the same way, the proportion of %OOR values for commonly evaluated LFT and RFT; ALT, AST, bilirubin(direct) bilirubin (total),Urea and Creatinine during screening or enrollment and safety monitoring of participants in the clinical trial studies was up to 6.6%,4.2%,23.6%, 9.6, 69% and 30.9% respectively for pregnant and 1.8%,2.5%,29%, 4.8%, 53% and 7.5% respectively for non-pregnant women based on currently deserved insert kit reference interval as compared to result of this study. Moreover, higher %OOR values were also observed in albumin (60%), total protein (35%) and bilirubin (direct) (23.6%) for pregnant and albumin (24%), total protein(3.7%)and bilirubin (direct) (29%) for non-pregnant women. This means that based on insert kit claimed RIs applied in the study area, up to 60%,for example for total protein, in pregnant women that could be of potential study participants would have been declared as having abnormal results or if enrolled, would be reported as having adverse events (AEs). The theme is on how such significant numbers of eligible study participants would wrongly either have been affirmed as abnormal results or admitted participants would be reported as having adverse events. These all favor an implication in need of establishing locally established population based RIs for their valid uses in medical care setups.

9. STRENGTH AND LIMITATION OF THE STUDY

9.1 Strength of the study

The strength of the study was that the samples were community based who were thoroughly assessed by clinicians diagnosing a wide range of conditions together with the laboratory results. Moreover, samples were analyzed in a laboratory that has been participating in external quality assessment programs like an onsite evaluation by Ethiopian Public Health Institute and External Quality Assessment (EQA) Proficiency Testing (PT) schemes by Randox International Quality Assessment Scheme (RIQAS).The centralized measurement site (APHIDB) was used to eliminate variations due to differences in analytical methods

9.2 Limitation of the study

This study was limited to determination of RI for albumin, total protein, AST,ALT,ALP, bilirubin(direct and total), urea and creatinine only on pregnant and non-pregnant women and was unable to determine RI based on trimester level for pregnant in detail. Moreover, the reference interval is compared among different countries that may use different methods of test analysis for different biochemical analytes other than used in this RI study.

10. CONCLUSION AND RECOMMENDATION

10.1 Conclusion

In the current study majority of the common renal and liver function clinical chemistry parameters reference intervals of healthy pregnant and non-pregnant women in S.wollo zones exhibit some differences from the references values drawn from African countries and western population. There was significant variation for the majority of common liver and renal function biochemistry parameters between pregnant and non-pregnant women. The significant difference in the majority of liver and renal function clinical chemistry parameters (higher in non-pregnant than pregnant) in the reference values of albumin, total protein, ALP, urea and creatinine in this study are consistent with different studies. Only few parameters were unaffected during normal pregnancy. Reference intervals established from samples of non-pregnant women are not necessarily applicable for passing medical decision for pregnant. Since the awareness of such variations are important in the interpretation of RFT and LFTs results and thus in management of renal and liver diseases at pregnancy, it will be paramount important to use special reference values specific to pregnancy.

10.2 Recommendation

Unless such significance difference in reference values distributions are taken in to considerations, any of the so called physiologic adaptations of pregnancy would either be misstated as pathologic or, markedly abnormal results may not be realized. Because of that, health care providers and all concerned bodies should give curious attention in interpreting pregnancy related clinical chemistry test results interpretation process. Besides, further related studies should be undertaken in full scale study to see the enlarged scope of such variations and improve quality of health related services. However, these biochemical parameters reference intervals established for S.wollo area could be of invaluable for patient diagnosis, treatment, follow ups, clinical trial studies and also for other stakeholders in need of it.

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12. ANNEX

Annex I: Lab SOPs

Section A: SOP for stool examination.

Examination of fresh specimens permits the observation of motile trophozoites, but this must be carried out without delay. Liquid (diarrheic) specimens (which are more likely to contain trophozoites) should be examined within 30 minutes of passage (not within 30 minutes of arrival in the laboratory!), and soft specimens (which may contain both trophozoites and cysts) should be examined within one hour of passage. If delays cannot be avoided, the specimen should be preserved to avoid disintegration of the trophozoites. Formed specimens (less likely to contain trophozoites) can be kept for up to one day, with overnight refrigeration if needed, prior to examination. Specimens preserved in 10% formalin can be tested directly (wet mount or can be concentrated prior to further testing. Concentration procedure separate parasites from fecal debris and increase the chances of detecting parasitic organisms when these are in small numbers. Sedimentation techniques use solutions of lower specific gravity than the parasitic organisms.

Materials required

1. Disposable (sterile container with sufficiently large opening, only to be used once; volume: ≥125 ml)
2. dry, clean, leak proof container with screwable lid
3. Spatula (separate or integrated)
4. Container label including patient-specific ID and a space to note down the patient's name and the time of stool production
5. plastic bag per container, in which the container can be stored after stool collection
6. Clean paper for stool collection
7. Gloves for handling of the sample in the laboratory

Procedure

1. Place a clean wide mouth container (for example empty plastic food container/one-liter ice cream carton, or a potty) in the toilet bowl or place a clean newspaper or plastic wrap over the toilet seat opening (If the stool is very watery this may not be possible).

2. Pass the stool into the potty, plastic container or onto newspaper or plastic wrap.
3. Place small scoopfuls of the stool into the specimen container using the spoon built into the lid of the specimen container (or the wooden stick, if supplied). Try to make sure that any parts of the stool which appear bloody, slimy or watery are put into the specimen container.
4. Do not overfill the specimen container (the fill line indicates the required amount). Try not to spill the stool on the outside of the specimen container. If this happens clean the outside of the specimen container with soap and warm water, then wash your hands thoroughly with soap and warm running water and dry.
5. . Put on the specimen container lid and screw on tightly. Wash your hands thoroughly with soap and warm running water and dry.
6. Dispose of the remaining stool in the potty, plastic container or newspaper into the toilet.
7. Wash your hands thoroughly with soap and warm running water and dry.
8. Deliver the sample to the personnel requested you to provide the sample it as soon as possible

Fresh stool should be examined, processed, or preserved immediately. If not processed immediately, preserve the specimen as soon as possible in 10% formalin. Insure that the specimen is mixed well with the preservative as formed stool needs to be well broken up.

To prepare a wet mount, obtain a microscope slide and the stool specimen. Take a small amount of the specimen and place it on a microscope slide. If the stool specimen is still somewhat solid, add a drop or two of saline to the specimen and mix and seal cover slide. Protozoan trophozoites, cysts, oocysts, and helminthic eggs and larvae may be seen and identified using a wet mount identification technique.

Section B: SOP for Urine specimen analysis.

Analysis of urine specimens is useful in monitoring the effectiveness of treatment of chronic problems, and in screening for asymptomatic conditions. There are three portions of a complete urine analysis: the appearance of the urine, the dipstick evaluation, and the microscopic examination. Proper collection and transport of specimens is critical to the quality of results produced by the laboratory. The validity of all diagnostic information produced in the lab is contingent on the quality of the specimen received. Consequences of

poorly collected and /or poorly transported specimens include failure to isolate the causative organism, and recovery of contaminants or normal flora, which could lead to improper treatment of the patient.

Materials required

Clear, dry, chemically-clean containers of minimum 50ml capacity with tight-fitting lids and label free space.

Procedure

1. Wash hands with soap and water.
2. If uncircumcised, retract the foreskin.
3. Wipe the end of penis with tissue. As you start to urinate, allow small amount urine to pass in to the toilet bowl to clear urethral contamination.
4. After the urine stream is well established, urine should be passed into a sterile, screw-cap plastic cup. The container should be half-full (approximately 50 ml).
5. Pass the remaining urine into toilet.
6. Screw the lid on the cup tightly
7. Transport the specimen immediately to the laboratory. If transport delay of >2 hrs is anticipated, use container with boric acid solution (max volume is 20 ml).
8. Refrigerate (up to 2 hrs.) if transport is delayed

Procedure for female

1. Wash hands with soap and water.
2. With one hand spread the folds of skin (labia) apart until the urine is voided into a sterile screw-cap container.
3. Wipe the urethral meatus from front to back.. As you start to urinate, allow small amount urine to pass in to the toilet bowl to clear urethral contamination.
4. After the urine stream is well established, urine should be passed into a sterile, screw-cap plastic cup. The container should be half-full (approximately 50 ml).
5. Pass the remaining urine into toilet.
6. Screw the lid on the cup tightly
7. Transport the specimen immediately to the laboratory. If transport delay of >2 hrs is anticipated, use container with boric acid solution (max volume is 20 ml).
8. Refrigerate (up to 2 hrs.) if transport is delayed

Use a fresh urine sample within 1 hour of collection or a sample that has been refrigerated; bring to room temperature and mix specimen

Dip a reagent strip into well-mixed urine, then remove it, blot, and compare each reagent area on the dipstick with the corresponding color control chart within the established time frame. Correlate color comparisons as closely as possible using good lighting and screen for PH, leukocyte, nitrite, glucose, ketone, uroblilinogen, bilirubin,blood, protein. Microscopic examination of urine sediment is also performed for detection of bacteria, WBC, RBC, casts and crystals.

Section C: SOP for blood sample collection

Blood specimen collection is performed routinely to obtain blood for a variety of laboratory testing conditions such as electrolyte imbalances, to screen for risk factors like high cholesterol levels, to assess for infections and to monitor the effects of treatments and medications.

Required materials

1. blood tube like SST tube
2. Vacutainer needle
3. Vacutainer needle holder
4. Cotton /Gauze/adhesive
5. Disinfectant like Alcohol Swab
6. Tourniquet
7. Disposable gloves
8. Sharp container like safety box for transport

Procedure

1. Identification of the subject is important.
2. Explain the procedure to him/her and ask if he/she is fasting and any other necessary data prior to collection
3. Label the tube with the patient's particulars
4. Put tourniquet on the patient about 3-4 inch above the venin puncture site
5. Ask patient to form a fist so veins are more prominent
6. After finding the vein, clean the venin puncture site with alcohol using circular motion.
7. Allow the area to dry
8. Assemble needle and vacuum tube holder

9. Insert the collection tube into the holder until the tube reaches the needle
10. Remove cap from needle
11. Use thumb to draw skin tight about 1-2 inch below the venin puncture site
12. Hold the skin tight through
13. "Insert the needle, bevel side up, into the vein
14. Push the tube completely onto the needle. Blood should begin to flow into the tube until vacuum tourniquet is exhausted
15. Release the tourniquet
16. After opening the patient's hand, place dry gauge over the venin puncture site and slowly remove the needle Step 13 Apply mild pressure to the pad
17. Apply bandage or continue applying mild pressure until bleeding has stopped
18. Properly dispose of all contaminates supplies in sharp / biohazard container

Section D. HBsAg (RAP4579) Rapid Test

Principle of the assay

The RAP4579 HBsAg Rapid Test employs chromatographic lateral flow device. Colloidal gold conjugated monoclonal antibodies reactive to HBsAg (sAb-Au) are dry-immobilized onto a nitrocellulose membrane strip. When the sample is added, it migrates by capillary diffusion through the strip rehydrating the gold conjugate. If present, HBsAg will bind with the gold conjugated antibodies forming particles. These particles will continue to migrate along the strip until the Test Zone (T) where they are captured by anti-HBs antibodies immobilized there and a visible red line appears. If there is no HBsAg in sample, no red line will appear in the Test Zone (T). The gold conjugate will continue to migrate alone until is captured in the Control Zone (C) from immobilized goat, anti-mouse IgG antibody and aggregating in a red line, which indicates the validity of the test.

Reagent and materials provided

1. HBsAg Test device in aluminum pouch with desiccant.
2. Package Insert

Section E. The HCV Rapid Test Cassette (Serum/Plasma)

The HCV Rapid Test Cassette (Serum/Plasma) is a qualitative, membrane based immunoassay for the detection of antibody to HCV in serum or plasma. The membrane is pre-coated with recombinant HCV antigen on the test line region of the cassette. During testing, the serum or plasma specimen reacts with recombinant HCV antigen conjugated colloid gold. The mixture migrates upward on the membrane chromatographically by capillary action to react with recombinant HCV antigen on the membrane and generate a colored line. Presence of this colored line indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a colored line will always appear at the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred.

Reagent and materials provided

The test cassette contains recombinant HCV antigen conjugated colloid gold and HCV antigen coated on the membrane.

Section F. Syphilis Rapid Test

The Biopanda Syphilis Rapid Test Cassette is a qualitative membrane based immunoassay for the detection of TP antibodies (IgG and IgM) in whole blood, serum, or plasma. In this test recombinant Syphilis antigen is immobilized in the test line region of the cassette. After specimen is added to the specimen well it reacts with Syphilis antigen coated particles in the test. This mixture migrates chromatographically along the length of the test and interacts with the immobilized Syphilis antigen. The double antigen test format can detect both IgG and IgM in specimens. If the specimen contains TP antibodies, a coloured line will appear in the test line region, indicating a positive result. If the specimen does not contain TP antibodies, a coloured line will not appear in this region, indicating a negative result. To serve as a procedural control, a coloured line will always appear in the control line region, indicating that proper volume of specimen has been added and membrane wicking has occurred.

Test procedure

1. Ensure specimen and test kits are brought to room temperature before testing.
2. Open the foil wrapped pouch and remove the cassette. Place the cassette on a flat, clean surface. Use test immediately after opening.

3. Using a dropper provided transfer 1 drops of the serum or plasma or 2 drops of the whole blood samples to the sample well on the cassette followed by 1 drop of buffer.
4. Read results at 5 minutes.
5. Results read after 20 minutes are considered invalid. Dispose of the cassette safely after testing.

Section G: HIV rapid test.

I. HIV 1/2 STAT-PAK™ ASSAY

Biological principles of the test

The Chembio HIV 1/2 STAT-PAK™ Assay employs a unique combination of a specific antibody binding protein which is conjugated to colloidal gold dye particles and HIV-1/2 antigens which are bound to the solid phase membrane. The venous or capillary (finger stick) whole blood, serum or plasma is applied to the SAMPLE (S) well of test device followed by the addition of Running Buffer. The Buffer facilitates the lateral flow of the specimen and test reagents and promotes the binding of the antibodies to the antigen. The specimen/buffer mixture migrates along the test strip by capillary action, reconstituting the conjugate. If present, the antibodies bind to the colloidal gold conjugated antibody binding protein. In a reactive sample, the dye conjugated-immune complex migrates on the nitrocellulose membrane and is captured by the antigens immobilized in the TEST (T) area producing a pink/purple line. In the absence of HIV-1 and HIV-2 antibodies, there is no pink/purple line in the TEST (T) area. The sample continues to migrate along the membrane and produces a pink/purple line in the CONTROL (C) area containing immunoglobulin G antigens. This procedural control serves to demonstrate that specimen and reagents have been properly applied and have migrated through the device.

Materials provided

Each Kit contains the components to perform 20 tests:

20 STAT-PAK™ Individually Pouched Test Devices

20 Copies of Subject Information Notice

20 Disposable 5µL Sample Loops

1 HIV Running Buffer (5mL)

1 Product Insert for the HIV 1/2 STAT-PAK™ Assay

II. ABON HIV 1/2/O Tri-Line Human Immunodeficiency Virus Rapid Test Device.

Test principle:

The HIV 1/2/O Tri-line Human Immunodeficiency Virus Rapid Test Device test strip is pre-coated with HIV-1 and subtype O antigens on T1 test line and HIV-2 antigen on T2 test line. Firstly, specimen and then buffer is added to the specimen well, thus starting the migration of the specimen/buffer. The specimen/buffer passes the conjugate pad which contains a mixture of HIV-1 envelope and capsid antigens and HIV-2 envelope antigen. These detection antigens are conjugated to latex particles. If present, the HIV-1 of HIV-2 antibodies reacts and bind to the detection antigen-conjugate. The antibody/antigen-conjugate mixture then migrates further and binds to antigens present on the test lines. If the specimen contains antibodies to HIV-1, the specimen will bind to the T1 test line and produce a line, if specimen contains antibodies to HIV-2, the specimen will bind to the T2 test line. As liquid continues to migrate down the test strip, the control line will appear. If the control line is present, in addition to either or both test lines, then the test is reactive for HIV1/2 antibodies. If the specimen does not contain HIV-1 or HIV-2 antibodies, no colored lines will appear for either of the test lines region indicating a non-reactive result. Please note that the appearance of coloured lines at T1 and T2 is highly unlikely to be indicative of co-infection with HIV-1 and HIV-2 but rather is a result of cross-reactivity between antigens. A colored line will appear in the control line region if the migration of liquid has been successful and must be present for the test to be valid. Its presence does not confirm sufficientspecimen addition

III. SD BIOLINE HIV-1/2 3.0.

This test is an immunochromatographic assay for the differential and qualitative detection of all isotypes (IgG, IgM, IgA) antibodies specific to HIV-1 including subtype O and HIV.

Materials provided

1. Cassettes individually foil pouched with a desiccant, 30

2. Assay diluent 4 ml (buffer), 1 bottle.

3. Instructions for use.

Specimen collection

Prior to specimen collection, provide test subjects with Subject Information Notice and pre test counseling according to CDC Guidelines for Rapid HIV Testing.The Chembio HIV 1/2 STAT-PAK™ Assay is performed on fingerstick whole blood, venous whole blood, serum or plasma specimens. Fingerstick Whole Blood: Prepare to perform the fingerstick blood collection procedure. Clean the finger of the person being tested with an antiseptic wipe. Allow the finger to dry thoroughly or wipe dry with a sterile gauze pad. Using a sterile lancet, puncture the skin just off the center of the finger and wipe away the first drop with sterile gauze and avoid squeezing the fingertip to accelerate bleeding as this may dilute the blood with excess tissue fluid. Collect the sample from the second drop touching the disposable Sample Loop provided to the drop of blood until the Sample Loop is full. Test immediately, following Test Procedure Instructions. Venous Whole Blood: Draw blood following laboratory procedures for obtaining venous blood. Collect sample in a tube containing citrate, heparin, or EDTA. Be sure the tube of blood is well mixed. Serum or Plasma: Draw blood following laboratory procedures for obtaining serum or plasma specimens. Collect specimen in a tube not containing any anticoagulant (serum), and in a tube containing citrate, heparin, or EDTA (plasma). Collect specimen in a clean container following standard laboratory procedures. Venous whole blood, serum and plasma specimens may be tested immediately after collection. If specimens are not tested immediately, refrigerate them at 2 to 8°C (36 to 46°F) following collection. These specimens should be tested within 3 days of collection. If specimens are not tested within 3 days of collection, serum or plasma specimens should be frozen at -20°C (-4°F) or colder.

Annex II: Information sheet for adults (≥18 years)

Project tittle: Establishment of RIs for common renal and liver function clinical chemistry parameters among healthy pregnant and non-pregnant women in South wollo, Amhara National Regional State, Ethiopia.

Project PI: Miftah Mohammed (BSc, Medical Laboratory Sciences, Addis Ababa University)

Sponsor: Addis Ababa University and 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 Clinical Chemistry reference intervals for Ethiopians aged ≥15years pregnant and non pregnant women in South wollo zone Ethiopia.

Purpose of the research:

The health laboratory plays an indispensable role in the health care system. It supports diagnosis (to rule in or rule out a diagnosis), monitoring of response to treatment, epidemiological surveillance, prevention as well as Research (to understand the pathophysiology of a particular disease process). Especially there is lack of local reference interval for indigenous population and local quality control materials. Therefore, the purpose of this proposed study is to establish Clinical Chemistry reference Intervals for Ethiopians aged ≥ 15years in various localities of Ethiopia.

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

Procedures:

After agreeing that you can take part, one or more of our research staff will ask you some questions which will take up to 15 minutes. Your weight, height and vital signs will be measured. You will be asked to provide urine and fresh stool on a particular container we provide. We will also collect 5 ml venous blood (about 1 table spoon) from you by sterile-disposable vacutainer tube and needle (5ml in plane tube. We will conduct laboratory

examination to determine different hematological, serological, parasitological and clinical chemistry parameters.

Confidentiality:

The information obtained during the study will remain confidential. Disclosure of any of the data to third parties other than those allowed in the Informed Consent form will not be permitted. The results of the research study may be published, but participants' names or identities will not be revealed. To maintain confidentiality, the investigator will keep records in locked cabinets in a locked room at the office and the results of the tests will be coded to prevent identification of the volunteers. Access to data entered into computerized files will be permitted only for authorized personnel directly involved with the study and will be password protected. Individual-specific information may be provided to responsible local medical personnel only with your permission. Urine, stool and blood collected will not be used for other purposes. The leftover samples will be stored at the 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. However, there might be some minimal risk and discomfort when we take venous blood. Nevertheless, we will try to minimize the discomfort as much as possible, as the blood samples will be taken by experienced laboratory professionals.

Safety:

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

Benefits:

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

Incentives:

Any positive finding in your 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.

1. Mr.Miftahmohammed ----------0912357741

Annex III: Consent form for adults (≥18 years (English)

Code No._____

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

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

_____years

No.	Questions	Responses
	Part III. SOCIO-DEMOGRAPHIC INFORMATION	
5	Educational status	1. Illiterate 2. Read and write 3. Primary (1-8) 4. Secondary (9-12) 5. College diploma/degree and above

6	Occupation	1. Student 2. House wife 3. Government employee 4. Private employee 5. Farmer 6. Gas station 7. Others (specify) _____
7	Marital status	1. Single 2. Married 3. Divorced 4. Widowed 5. Not applicable (children)
8	Religion	1. Orthodox Christian 2. Muslim 3. Protestant 4. Catholic 5. Others (Specify) _____
9	Residence	1. Rural 2. Urban
	Part IV. Clinical information	
	Questions 24-28 for female participant who are pregnant specify	
10	Gestation _____(weeks)	
11	Parity _____	
12	Iron supplementation:	1. Yes 2. No
13		
14	Folate supplementation	1. Yes 2. No
15	Iron and folate combined supplementation	1. Yes 2. No
	Questions 30-31for female participant who are not pregnant	
16	Do you have <1 years old breast feeding child	1. yes 2. No
17	Are you at menstruation by now?	1. Yes 2. No
18	Did you take any type of drug for any illness for the last three month?	1. Yes 2. No
19	If yes to Q29, what type of drug? (more than one	1. Anti-protozoa

	answer possible)	2. Anti-helminthic 3. Anti-allergy 4. Birth control pills 5. Anti-bacterial 6. Anti-TB 7. Anti-pain 8. Other_____ (specify)
	History of common diseases	
20	History of diabetes	1. Yes 2. No
21	History of Hypertension	1. Yes 2. No
22	History of Blood transfusion for the last 1 year	1. Yes 2. No
23	Any history of blood transfusion	1. Yes 2. No
24.	History of Hospital Admission for the last 1 year	1. Yes 2. No
25.	History of Surgical procedure for the last three years?	1. Yes 2. No
26.	History of chronic gastritis	1. Yes 2. No
27.	History of Malaria for the last 6 month	1. Yes 2. No
28.	History of TB for the last two years	1. Yes 2. No
29.	History of Cancer	1. Yes 2. No
30.	History of Cardiac illness	1. Yes 2. No
31.	History of Bleeding disorders	1. Yes 2. No
32.	History of allergy	1. Yes 2. No
33.	History of Wheezing	1. Yes 2. No

Part V. Nutritional habit and your life style

	Part V. Life style/Habit Continued…	
36	Do you have the habit of physical Exercise?	1. Yes 2. No
37	If yes, how many times do you do the exercise per week?	
	Part VI. Anthropometric measurement	
38	Body temperature	_____ ^{o}

39	Height (in cm)	_____
40	Weight (in kg)	_____
41	Blood pressure (mm Hg)	_____
42	Body mass index(BMI)	_____kg/m^2

❖ We thank you for your cooperation!

Interviewer's Name_____ Signature_____ Date_____

Annex V. Laboratory result format

Code _____Region _____

Woreda_____/ city /_sub city _____Kebele _____

Stool examination

- Consistency_____
- Direct_____
- Formol ether concentration _____
- **Urine analysis**

Dipstick:

protein___Glucose___Bilu____spG____ketone___leuco___urob___blood___nitrite___Ph_

- Microscopy_____
- HCG (for female) _____
- Blood film _____
- Clinical chemistry test (attach print out)
- HBsAG_____
- HCV_____
- T.Pallidium_____
- HIV_____

Annex VI: 18 ዓመትና ከዚያ በላይ ለሆኑ አዋቂዎች መረጃ.

የፕሮጀክቱ ርዕስ፡ ‘‘እድሜአቸው አምስት ዓመትና ከዚያ በላይ ለሆኑ ኢትዬጵያውያን የጤናማ ሰው ደም ውስጥ የሚገኙ የክሊኒካል ላቦራቶሪ ምርመራዎች መጠን ሪፈረንስ ኢንተርቫል የሚሰራ ጥናት‘‘

የፕሮጀክቱ ዋና ተመራማሪ: አቶ ሚፍታህ መሀመድ

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

ስፖንሰር (ወጪውን የሸፈነው)፡የአድስ አበባ ዩኒቨርሲቲና የፌደራል ሳይንስና ቴክኖሎጂ ሚኒስቴር እና

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፡፡ የጤናማ ሰው ደም ውስጥ የሚገኙ የክሊኒካል ኬሚስትሪ ምርመራዎች መጠን ሪፈረንስ ኢንተርቫል እድሜአቸው አስራ አምስት ዓመትና ከዚያ በላይ ለሆኑ ነፍሰጡር የሆኑና ያላሆኑ ኢትዮጵያውያን ላይ ለመስራት ጥናት እያካሄድኩኝ 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 የለም፡፡ ስለሆነም የዚህ ጥናት ዓላማ የጤናማ ሰው የክሊኒካል ኬሚስትሪ ውጤት ማወዳደሪያ ሪፈረንስ ኢንተርቫል እድሜአቸው አስራ አምስትና ከዚያ በላይ ለሆኑ ነፍሰጡር የሆኑና ያላሆኑ ኢትዮጵያውያን ላይ መሥራትነው፡፡

እርስዎም ለዚህ ጥናት ተመርጠኃል፡፡ ስለዚህ በዚህ ጥናት እንዲሳተፉና የጤናማ ሰው የክሊኒካል ኬሚስትሪ ውጤት ማመዳደሪያ ሪፈረንስ ኢንተርቫል ለመስራት አስተዋፅዎ እንዲያደርጉ ተጋብዘኃል፡፡ ስለዚህ የዚህ ጥናት ውጤት ኢትዮጵያ ውስጥ የአዋቂሰዎች ጤናን ለማሻሻል ይረዳል፡፡

የጥናቱ አካሄድ:

በጥናቱ ለመሳተፍ ከተስማሙ የጥናቱ አባል/አባላት 15 ደቂቃ የሚወስድ ጥያቄ ይጠይቁዎታል፡፡ ክብደት፣ቁመት፣የክንድ እና የደም ግፊት ልኬት ይወሰዳል፡፡ ሽንትና አይነምድር በምንሰጠው እቃ እንድትሰጡን እንጠይቃለን፡፡፣በተጨማሪም 5 ሚሊሊትር በንፁህ ቫኩቴይነ ርብልቃጥ እና መርፌ እንቀዳና 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 ነገርግን 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 በአዲስአበባ ዩኒቨርስቲ ህክምና ላቦራቶሪ ትምህርት ክፍል ደህና ቦታ ተቀምጠው ለተጨማሪ ምርመራዎች እንደአስፈላጊታቸው ጥቅም ላይ ይውላሉ፡፡ በመጨረሻም ተሰርቶባቸውየ ተራረፉ 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 መነዳትና 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 የጤንነት ሁኔታ ማወቅ ይቻላል፡፡ የጤናማ ሰው የክሊኒካል ኬሚስትሪ ውጤት ማመዳደሪያ ሪፈረንስ ኢንተርቫል እድሜአቸው አስራአምስትና ከዚያ በላይ ለሆኑ ነፍሰጡር የሆኑና ያላሆኑ ኢትዮጵያውያን የጤና ሁኔታ ለማሻሻል ይረዳል፡፡

በጥናቱ ለመሳተፍ ማትጊያ:

ከዓይነምድር፣ ሽንት እና ደም ምርመራ ጤናማ ያልሆነ ውጤት ከተገኘ በአቅራቢው ወደሚገኝ ጤና ተቋም ይላካሉ፣ የላቦራቶሪ ውጤቶቹን በነፃ ያገኛሉ፡፡ ይሁንእንጂ በዚህ ጥናት ለመሳተፍም ሆነ ለመድሃኒት ክፍያ አይሰጥም፡፡ ስለተሳትፎዎ ግን እናመሰግናለን፡፡

ያለመሳተፍ መብት:

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

ጥያቄ ካለ ለማነጋገር:

ምንም ዓይነት ጥያቄ ካለ የዓይነምድር፣ ሽንት እና የደም ናሙና የሰጡትን ሰው መጠየቅ ይቻላል፡፡

አቶ ሚፍታህ መሀመድ----------0912357741

Annex VII: 18 ዓመት እና ከዚያ በላይ ለሆኑ አዋቂዎች የስምምነት ቅፅ)

ኮድ._____

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

የዓይነምድር 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/ዓመተምህረት)

ያልተማሩ ከሆኑ;

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

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

ስልክ ቁጥር_____

የተመራማሪው ስም፣ ቀንና ፊርማ

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

Annex-VII: የአማርኛ ቃለ-መጠይቅ

በጤና ባለሞያዎች የሚሞላ ቃለመጠይቅ

መመሪያ፡

በቅድሚያ ይህንን ቃለመጠይቅ ለመሙላት ለሰጡን ጊዜና ትብብር አድናቆቴን እገልፃለሁ፡፡የዚህ ቃለመጠይቅ አላማ “በላቦራቶሪ ውስጥ የጤናማ ሰው ደም ውስጥ የሚገኙ የክሊኒካል ኬሚሰትሪ ምርመራዎች መጠን ሪፈረንስ ኢንተርቫል እድሜአቸው አስራ አምስት ዓመትና ከዚያ በላይ ለሆኑ የህብረተሰብ ክፍሎች ለመስራት“ መረጃ ለመሰብሰብ ነው፡፡የዚህ ጥናት ሃሳቡን ያመጡት የጥናቱ ዋና ተመራማሪዎች መስፍን ፍስሃ እና ሚፈታህ ሙሃመድ ሲሆኑ ጥናቱም በአዲስ አበባ ዩኒቨርስቲ፣ጤና ሳይንስ ኮሌጅ፣ህክምና ላቦራቶሪ ሳይንስ ትምህርት ክፍል ስር በመሆን ድጋፍ ይደረግልታል፡፡የጥናቱን ወጪ የሸፈነው ትምህርት ሚኒስቴር ነው፡፡ስለሆነም የእርስዎ ቅን ትክክለኛ መልስ በሰዓቱ መስጠት የዚህን ጥናት ስኬት ይወስናል፡፡ስለሆነም ይህንን ቃለመጠይቅ ሃቀኝነትና ሃላፊነት በተሞላው መንገድ እንዲሞሉ በትህትና እንጠይቃለን፡፡

አመሰግናለሁ!!!

ክፍል 1. አጠቃላይ መረጃ

ኮድ_____ ክልል _____ ዞን_____ ወረዳ_____

ከተማ/ክፍለከተማ _____ቀበሌ _____

ክፍል 2. የግል መረጃ

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

ክፍል 3. ማህበራዊና ኢኮኖሚያዊ መረጃ		
ቁጥር.	ጥያቄ	ምላሽ
6	የትምህርት ደረጃ	1. ያልተማሩ 2. ማንበብና መፃፍ 3. አንደኛ ደረጃ (1-8) 4. ሁለተኛ ደረጃ (9-12) 5. ኮሌጅ ዲፕሎማ/ዲግሪ እና ከዚያ በላይ
7	ሥራ	1. ተማሪ 2. የቤት እመቤት 3. የመንግስት ሠራተኛ 4. የግል ተቀጣሪ 5. ገበሬ 6. ሌላ ካለ ይግለፁ _____
8	የጋብቻ ሁኔታ	1. ያላገቡ 2. ያገቡ 3. የተፋቱ 4. ባል/ሚስት የሞተባቸው 5. አይመለከታቸውም (ህፃናት)
9	ሃይማኖት	1. ኦርቶዶክስ ክርስቲያን 2. ሙስሊም 3. ፕሮቴስታንት 4. ካቶሊክ 5. ሌላ ካለ ይግለፁ _____
10	መኖሪያ ቦታ	2. ገጠር 2. ከተማ
	ጥያቄ 12-17 ለተማሪዎች ተጨማሪ ጥያቄዎች	
	ክፍል 4. የጤና መረጃ	
	ከ 24-29 ያሉት ጥያቄዎች ለነፍሰጡር ሴቶች ብቻ ነው	
11	ከፀነሱ ስንት ጊዜዎ ነው?	_____(ሳምንት)
12	ለስንተኛ ጊዜ ነው የፀነሱት?	_____
13	ተጨማሪ የብረት ንጥረ ነገር ይወስዳሉ?	1. አዎን 2. የለም
14	ተጨማሪ ፎሌት ንጥረ ነገር ይወስዳሉ?	1. አዎን 2. የለም
15	ተጨማሪ የብረት ንጥረ ነገርና ፎሌት ይወስዳሉ?	1. አዎን 2. የለም

16	ከአንድ አመት በታች የሆነው ጡት የሚጠባ ልጅ አለዎት?	1. አዎን 2. የለም
ከመድሃኒት ጋር የተያያዙ ጥያቄዎች		
17	ባለፉት ሶስት ወራት ለማንኛውም ዓይነት ህመም ማንኛውንም ዓይነት መድሃኒት ወስደኃል?	1. አዎን 2. የለም
18	ለተራ ቁጥር-30 መልስዎ ወስጃለሁ ከሆነ የትኛውን ዓይነት መድሃኒት ነው ወሰዱት? (ከአንድ በላይ መልስ ይቻላል)	1. ፀረ-ፕሮቶዞአ 2. ፀረ-ሄልሚንትስ 3. ፀረ-አለርጂ 4. የወሊድ መከላከያ ኪኒን 5. ፀረ-ባክቴሪያ 6. ፀረ-ቲቢ 7. የባህል መድሃኒት 8. ሌላ ካለ ይግለፁ _____
የሚከተሉት የህመም ዓይነቶች አሞዎት ያውቃል?		
19	የስኳር ህመም አለብዎት?	1. አዎን 2. የለም
20	የደም ግፊት ህመም አለብዎት?	1. አዎን 2. የለም
21	ባለፈው 1 ዓመት ደም ተሰጥቶዎ ያውቃሉ?	1. አዎን 2. የለም
22	ባለፈው 3 ወር ውስጥ ደም ለግሰው ያውቃሉ?	1. አዎን 2. የለም
23	በማንኛውም ጊዜ ደም ተሰጥቶዎ ያውቃሉ?	1. አዎን 2. የለም
24	ባለፈው 1 ዓመት ሆስፒታል ተኝተው ያውቃሉ?	1. አዎን 2. የለም
25	ባለፉት 3 ዓመታት የቀዶ ህክምና ተደርጎልዎ ያውቃሉ?	1. አዎን 2. የለም
26	የቆየ የጨጓራ ህመም አለብዎት?	1. አዎን 2. የለም
27	ባፉት 6 ወራት የወባ ህመም አጋጥሞዎት ያውቃሉ?	1. አዎን 2. የለም
28	ባለፉት 2 ዓመታት የቲቢ ህመም ታመው ያውቃሉ?	1. አዎን 2. የለም
29	ካንሰር ህመም አለብዎት?	1. አዎን 2. የለም
30	የልብ ህመም አለብዎት?	1. አዎን 2. የለም
31	የመድማት ችግር/ህመም አለብዎት?	1. አዎን 2. የለም
32	አለርጂ (የሰውነት መቆጣት) አለብዎት?	1. አዎን 2. የለም
33	የመተንፈስ ችግር (ሲተነፍሱ ሲርሲር የሚል ድምፅ)አለብዎት?	1. አዎን 2. የለም

34	የኩላሊት ህመም አለብዎት?	1. አዎን 2. የለም
35	የደም ማነስ ችግር አለብዎት?	1. አዎን 2. የለም
36	የጉበት ህመም አለብዎት?	1. አዎን 2. የለም
37	የእንቅርት ህመም አለብዎት?	1. አዎን 2. የለም
38	በቤተሰብ ውስጥ ስር የሰደደ ህመም (ስኳር፣የደም ግፊት) የታመመ አለ ?	1. አዎን 2. የለም
	የህመም ስሜትና ምልክት	
39	የህመም ስሜት አለዎት?	1. አዎን 2. የለም
40	በተራቁጥር 46 መልስዎ አዎ ከሆነ ምን አይነት ስሜት ይሰማዎታል?	_____
41	የወር አበባ ላይ ነዎት? **(ለሴት ተሳታፊዎች ብቻ)**	1. አዎን 2. የለም
	በጤና ባለሙያዉ ምልክታ የሚሞላ	
42	ከተሳታፊዉ የትኛዉን የህመም ስሜት እና ምልክት (የጤና ችግር) ተመልክተዋል?	1. ትኩሳት(የሙቀት መጠኑ ከ 37^{0}c በላይ የሆነ) 2. የሰውነት ፈሳሽ ድርቀት(ዲሀይድሬሽን) 3. ጭንቀት 4. የአዕምሮ ውስንነት 5. ከቦታ ቦታ ለመንቀሳቀስ የሚያዳግት የአካል ጉዳት 6. ሌላ ካለ_____

የሚከተሉትን ምን ያህል ይበላሉ/ይጠቀማሉ (√ይህን ምልክት ያስቀምጡ)							
		በቀን አንድ ጊዜ (ሁልጊዜ)	በቀን ከ1 ጊዜ በላይ	በሳምንት ከ 2 እስከ 3 ጊዜ	በሳምንት 1 ቀን	አልፎ አልፎ (ለምሳሌ፣ ለበዓል፣ልዩ ዝግጅቶች ሲኖሩ)	ተጠቅሜ አላውቅም
43	አልኮል						
44	ጫት						
45	ሲጋራ						

	ክክፍል 5. የቀጠለ የህይወት አመራርና ልምዶች	
46	የሰውነት እንቅስቃሴ የማድረግ ልምድ አለዎት?	1. አዎን 2. የለም
47	መልስዎ አለኝ ከሆነ በሳምንት ለምን ያህል ጊዜ ይንቀሳቀሳሉ?	_____
48	ዘወተር ከነዳጅ እና ኬሚካሎች ጋር ግንኙነት አለዎት?	1. አዎን 2. የለም
	ክፍል 6. ክብደት፣ቁመት፣የክንድ፣የሰውነት ሙቀትና የደም ግፊት ልኬት	
49	ቁመት	_____ሴንቲሜትር
50	ክብደት	_____ኪሎግራም
51	የክንድ መሃለኛው ክፍል ዙሪያው (MUAC)	_____ሴንቲሜትር
52	የደም ግፊት (በሚሊሜትር ሜርኩሪ)	_____(mm Hg)
53	የሰውነት ሙቀት መጠን	_____(^{0}c)

❖ ስለትብብርዎ እናመሰግናለን!

ቃለ መጠይቅ የተደረገበት ቀን:_____

ቃለ መጠይቁን ያካሄደው ስም_____ ፊርማ_____

Annex VIII: Table 7 Comparison of common RFT and LFT RIs of healthy non-pregnant women in S.wollozone, Amhara National regional state, Northeast Ethiopia with manufacturer RIs and other similar studies.

Analyte	Unit	Manufacturer (APHIDB) used RI	RI of Current study	Northwest Ethiopia (28)	Southwest Ethiopia (39)	Amhara region (19)	Middle belt of Ghana (10)	Zimbab-we (52)	South &eastern Africa (7)	USA (MGH) (50)	Hong kong (China) (51)
Albumin	g/l	35-50	32.8-47.8	NA	NA	36-61	33.5-50.4	41-55	35-52	35-55	37.6-48.6
Total protein	g/l	60-83	56.7-83.9	53.2-86.0	76-80	56-94.7	55.2-86.9	68-94	58-88	55-80	63.5-79.0
AST(SGOT)	U/l	0-40	4.2-37.1	6.0-32.1	12.0-59.9	10.0-43.8	13-48	12-40	14-60	0-35	11-26
ALT(SGOT	U/l	0-41	2.6-41.1	3.0-30.0	10.1-54.0	4.3-37.0	6-51	5-35	8-61	0-35	7-39
ALP-DEA	U/l	0-270	3.2-278.4	40.0-236	70.4-384.4	89.0-381	82-293	39-131	48-164	NA	36-105
Bilirubin(direct)	mg/dl	0-0.2	0.06-0.38	0.01-0.71	NA	0.01-0.49	0.04-0.2	0.0-0.3	0.02-0.51	0.1-0.3	NA
Bilirubin (Total)	mg/dl	0-2	0.24-1.06	0.21-2.19	NA	0.08-0.91	0.15-1.5	0.2-1.1	0.16-2.16	0.3-1.0	0.2-0.9
Urea	mg/dl	15-39	8.0-27.8	NA	NA	10.0-38	5.4-32.4	3.9-15.4	NA	10-20	11.5-32.0
Creatinine	mg/dl	0.5-1.2	0.44-1.00	0.24-1.08	0.32-1.32	0.47-1.09	0.53-1.24	0.5-1.1	0.53-1.23	0-1.5	0.6-0.93

ALT: Alanine aminotransferase; ALP-DEA: Alkaline phosphatase-diethanolamine buffer; AST: Aspartate aminotransferase; dl: Decilitre; g: Gram; L: liter; mg: milligram; RI: Reference interval; SGOT: Serum glutamate oxaloacetate transaminase; SGPT: Serum glutamate pyruvate transaminase; U: Unit; APHIDB: Amhara public health institute Dessie branch

*Eastern and Southern Africa: Rwanda, Uganda, Kenya and Zambia.

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: Miftah Mohammed Assen (B.Sc.)

Signature:_____

Date of submission:_____

This proposal has been submitted with our approval as advisors.

Advisor 1. Samuel Kindie (MSc, PhD candidate)

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Place: Addis Ababa, Ethiopia.

Advisor 2. Aster Tsegaye (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia