



Establishment of reference interval for selected clinical chemistry parameters in apparently health adult in sekela and burie woredas, Ethiopia

Abstract

Background: Clinical laboratory reference ranges are vital events for clinical diagnosis, prognosis, treatment, and monitoring of any health complications. So locally established reference range is required to correctly interpret clinical laboratory results. So this study aimed to determine reference interval for selected biochemical tests for apparently healthy participants in Sekela and Burie Woredas, North West Ethiopia.

Methods: A community-based cross-sectional study was conducted among apparently healthy individuals in Sekela and Burie Woredas, Ethiopia from December 2019 to June 30, 2020 on a total of 360 healthy participants. The analysis was done with the Biosystem 25; a fully automated clinical chemistry analyzer. The data was analyzed using SPSS software. By use of Clinical Laboratory and Standards Institute (CLSI) guidelines, we determined reference ranges, at 95% reference intervals, following specific exclusion criteria.

Result: This study observed statistically significant differences between males and females in serum total cholesterol, serum triglyceride, LDL, urea and creatinine Reference intervals. The established reference intervals for females and males, respectively, were: LDL (mg/dl) 70.0-161.3 vs. 90.0-197.4, serum total cholesterol (mg/dl) 95.0-152.4 vs. 105.0-200, serum triglyceride (mg/dl) 75.4-152.0 vs. 100.4-176.7 mg/dl, blood urea nitrogen (mg/dl) 11-29.4 vs. 9.9-28, and serum creatinine (mg/dl) 0.54-1.1 vs. 0.61-1.2. The combined RIs for high density lipoprotein were 42-84.3 mg/dl, FBS 68-115 mg/dl.

Conclusion: The result the current study indicated that significant difference was among sex, with other studies done in Africa and Europe and with the company derived reference interval values for selected clinical chemistry parameters. Due to this fact, use of age and sex specific locally established reference intervals for clinical chemistry parameters is recommended.

Keywords: reference range, biochemical parameters, West Gojjam, Ethiopia

Abbreviations: TC: Serum Total Cholesterol, TG: Serum Triglyceride, LDL: Low Density Lipoprotein, HDL: High Density Lipoprotein, FBS: Fasting Blood Sugar, BUN: Blood Urea Nitrogen, CR: Serum Creatinine, CLSI: Clinical and Laboratory Standards Institute Milligram Per Deciliter, SPSS: Statistical Package for the Social Science, WHO: World Health Organization; IFCC: International Federation of Clinical Chemistry, RIs: Reference Intervalsp

Introduction

The International Federation of Clinical Chemistry defines “reference range” as the value obtained by observation or measurement of a particular type of quantity on a reference individual where the individual is selected using defined criteria [1-3]. Reference values are typically reported as Reference Ranges (RRs) comprising 95% of a healthy reference population; which are widely used in the process of providing medical diagnosis, therapeutic management, or other pathologic and physiological evaluations. For the interpretation

of clinical chemistry parameters, a reference range of a clinical chemistry parameter has a set of values [4]. The establishment of a reference range that is specific for the reference population is vital and international guidelines also recommended a reference range for all clinical laboratory tests [5]. Any healthy individual is different in different countries, in a different period in the same country, in different age groups and sex. The definition of health is a relative and not an absolute state [6]. Unless appropriate data for comparison is provided a patient’s laboratory result simply is not medically useful [7].

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For appropriate diagnosis, treatment, and follow-up of patients, the correct interpretation of the laboratory results is mandatory [1]. This is attained by knowing the normal reference intervals that have been established in the local setting considering climate, socioeconomic status, living style, and genetic makeup than using values from other areas of the world. In most African countries, however, reference intervals have not been adequately addressed. Adopting non-Ethiopian reference values for Ethiopians might be misleading. Hence the present study was carried out to establish normal reference intervals for the selected biochemical parameters in healthy adults in Sekela and Burie Woredas and compare the results against reference values being used currently [1,8].

Methods

■ Study area

The study was conducted in two woredas (Burie and Sekela Woreda which are located in West Gojjam, Amhara Regional State, Ethiopia). Based on the 2007 national census conducted by the Central Statistical Agency of Ethiopia (CSA), Sekela woreda has a total population of 13,86,91 populations and Bure has a total of 14,31,32 population.

■ Study design and period

A community-based cross-sectional study was conducted from December 2019 to June 30, 2020 among healthy adult populations in Burie And Sekela Administration city, Amhara, Northwest Ethiopia.

■ Study population

The source populations were all healthy adult populations of the Burie and Sekela Woreda administrative city. Healthy adults age between 18-40 years of both sexes from the two Woredas were the study population.

■ Sample size and sampling techniques

CLSI recommends that the best means to establish a reference interval is to collect samples from a sufficient number of reference individuals to yield a minimum of 120 samples for analysis, by non-parametric means for each partition (e.g. sex, age range) with a power of 90%. In the current study, the maximum partition needed was female and male. Thus, two partition groups were needed ($2 \times 120=240$). For this study, a total of 360 study participants 170 participants from Sekela Woreda, and 210

participants from Burie Town were included. The study participants were selected using a systematic sampling technique by considering different Kebeles as a sampling frame and then households the final selection units.

■ Inclusion criteria

Healthy individuals (adults) with age >18 years of both sexes and within the age range of 18 to 40 years from the surrounding the two Woredas were included.

■ Exclusion criteria

1. Individuals with age <18 years.
2. Participants with known chronic illnesses like diabetes mellitus, chronic renal disease, hypertension, heart disease, anemia, thyroid, liver diseases, and cancer.
3. Individuals who had known infectious disease (HIV, HCV, HBV).
4. Individuals who had Hemo-parasite and intestinal parasites.
5. Individuals who have, history of blood transfusion <6 months.
6. Hospitalized persons, chronic diseases, and acutely ill individuals.
7. And Pregnant females were excluded from the study.

■ Operational definition

Healthy adults: Individuals (adults) age 18-40 years and without disease or disabilities based on clinical signs and symptoms plus laboratory investigations.

Reference intervals: Including two reference values defined by 95% (the two reference limits 2.5th and 97.5th percentile) for apparently healthy individuals.

Selected clinical Chemistry analytes: In this study refer to the biochemical analytes, Blood Urea, Serum Creatinine, LDL, HDL, TC, TG, and fasting blood sugar.

■ Data collection and laboratory analysis

Data collectors were trained for one day on the objective of the study, study procedures, confidentiality of information, procedure of physical examination, the procedure of blood sample collection and also how to approach and interview participants. The study participants who agreed to give written consent after being informed about the purpose of the study were

invited to come to Asrade Zewudie Memorable Primary Hospital for those from Burie Woreda administration and Abay Minch Health Center for those from Sekela Woreda administration were physically examined and interviewed. Social-demographic data and biological samples (urine, stool, and blood) were collected from those who fulfilled the eligibility criteria. Urinalysis, parasitological examinations and Serological tests for syphilis, hepatitis B, and C were performed at the site of collection. And radio-logical assessment is also taken to assure the absence of chronic and acute illness additional with laboratory tests.

About 10 ml of the blood sample was collected from each study participant using an SST tube in the morning from 7:30 AM to 10:00 AM. The collected blood samples were centrifuged at 2500 rpm (revolution per minute) for 5 minutes at the site laboratory department to separate serum from the whole blood. Then the separated blood sample was analyzed for the selected clinical chemistry tests by using biosystem 25 A (Biosystem, Spain), a fully automated clinical chemistry analyzer. The selected parameters were analyzed by using preferable methods of analysis for all selected analytes. Selected clinical chemistry parameters such as FBS, LDL, HDL, TG, TC, Urea, and Creatinine, values were done per the manufacturer's instructions.

■ Quality control

To ensure accuracy; each activity including taking clinical information, blood sample collection, transportation, and storage were based on Good Laboratory Practices (GLP) using Standard Operating Procedures (SOPs). Also, the accuracy and precision of the test results, all pre-analytical, analytical, and post-analytical precautions were carefully controlled. Besides, to maintain internal quality control, the equipment had been calibrated monthly by the type-Auto calibrator. Also, two levels (normal and pathological) of Internal Quality Control (IQC) samples were run along with the serum sample. The control sample results were to be interpreted using the Westgard multi-rule algorithm. The sample was analyzed after well understood the leaflet for each analyte by the principal investigator and senior laboratory technologists.

■ Data analysis and interpretation

Data was cleared, edited, checked for completeness manually, and entered to Software Package for the Social Science (SPSS) for version 20.0 for Windows' (SPSS Inc., Chicago, IL,

the USA) for analysis. After organizing and cleaning the data, frequencies, and percentages were calculated to all variables that are related to the objectives of the study, and the first quartile (Q.25), the median (Q.50), and the third quartile (Q.75) were determined. Then interquartile range was calculated (IQR) from the differences of third and first quartile s (Q.75-Q.25). Wilcoxon rank-sum test was also used to see whether the partition was needed between males and females. RIs were calculated following CLSI/IFCC guidelines using non-parametric methods. RI was estimated using 2.5 the percentile for the lower reference limit and 97.5th percentile for the upper reference limit.

■ Ethical considerations

The study was conducted after ethical approval was obtained from the Research and Ethics Institutional Review Board of Debre Markos University College of Health Science, Department of Medical Laboratory Science. Informed written consent was also obtained from each study participant before the actual data collection. Participants were informed of the risks and benefits of the study, their right to withdraw anytime, how confidentiality is maintained using codes, and their right to get their results for free. An Individual's clinically significant laboratory test analysis for tests was linked to the responsible doctor for further diagnosis and treatment accordingly.

Results

■ Social-demographic characteristics

A total of 360 healthy young adults were recruited to establish the RI of the selected clinical chemistry parameters from voluntary study participants in Sekela and Buurie Woredas, Gojjam, Amhara National Regional State, Ethiopia. Out of 360 study participants, 188 (52.2%) were males and the rest 172 (47.8 %) were females. The study participants had a mean age of 27 years with a proportion of 242 (67.2%) from 21-29 years, 102 (28.3%) from 31-39 years, and 16 (4.4%) from 18-20 years. The median age of the study participants was 28 years. In the current study most of the participants were in the age range 21-20. 242 (67.2%), and 247 (68.6%) from urban residents (TABLE 1).

■ Reference intervals of selected clinical chemistry parameters

In the current study, we have observed statistically significant differences between males

TABLE 1. Social-demographic characteristics of the study participants in Sekela and Burie Woredas, Ethiopia, 2020 (n=360).

Variables		Absolute number	Percentage
Sex	Male	188	52.2
	Females	172	47.8
Age	18-20	16	4.4
	21-29	242	67.3
	30-40	102	28.3
Resident	Urban	247	68.6
	Rural	93	31.2
Occupation they concern to do	Farmers	44	12.2
	Driver	57	15.8
	Hotel employee	45	13.2
	NGO	40	11.8
	Governmental	120	33.3
	House wife	30	8.33
	Private/self	24	6.7

TABLE 2. The 95% RIs of selected clinical chemistry parameters for the study participants in Sekela and Burie Woredas, Amhara regional state, Ethiopia, 2020 (n=360).

Analyze	Sex	Mean	Median	95% CI of Mean		Min	Max	25 th -95 th + percentile	2.5 th ,97.5 th + percentile, RI	p value to sex
				LB	UB					
FBS	C	88.6	60	87	90	65	125	75-100	68,115	0.215
	M	89.7	89	87.5	91.9	65	125	76.5-102	68,116.1	
	F	87.3	85	85	89.5	65	125	75-99	68.7,115.7	
BUN	C	19.1	18	18.5	19.6	8	32.5	14.7-23	11,29	0.023
	M	18.8	19	18	19.6	8	32.5	14.7-23	9,9,28	
	F	19.3	18	18.4	20.2	8	32	14.6-23.5	11,29.4	
CR	C	0.82	0.8	0.8	0.84	0.48	1.3	0.68-0.94	0.54,1.2	0.04
	M	0.86	0.81	0.84	0.89	0.51	1.3	0.71-1.0	0.61,1.2	
	F	0.77	0.74	0.74	0.8	0.48	1.3	0.64-0.87	0.54,1.1	
HDL	C	57.5	54	56.1	58.9	37	88.4	48-69.6	42,84.3	0.985
	M	57.4	54	55.5	59.3	37	88.4	48.5-69.4	42,84.1	
	F	57.6	54	55.6	59.6	38	88.4	49-69.6	42,85.6	
LDL	C	127	124.6	123.5	130.5	56.3	230	104.7-147.5	70.2,189.6	0.014
	M	140.7	143.2	135.9	145.6	66	230	111.3-164.2	90,197.4	
	F	112	110	108	116.1	56.3	189.6	91.5-127.4	70,161.3	
TG	C	122.7	119.8	120.2	125.3	50	214.4	106.0-142.1	81.9,160.4	0.004
	M	130.6	130.3	127.2	134	93.7	214.4	109.9-146.1	100.4,176.7	
	F	114.1	112	110.6	117.7	50	160.4	100-133.3	75.4,152.0	
TC	C	134	135	131	136.8	65	229.4	110-150	100,190	0.013
	M	144.3	138.8	140.1	148.8	98	229.4	122-155	105,200	
	F	122.6	122	119.6	125.6	65	190	106-137.8	95.0, 152.4	

BUN: Blood Urea Nitrogen, CR: Serum Creatinine, HDL: High-Density Lipoprotein, LDL: Low-Density Lipoprotein, TC: Serum Total Cholesterol, TG: Serum Triglyceride, FBS: Fasting Blood Sugar, LB: Lower Bound, UB: Upper Bound, CI: Confidence Interval, RI: Reference Range, Min: Minimum, Max: Maximum, C: Combined, M: Male, F: Female. p-value <0.05 considered as statistically significant.

and females in clinical chemistry parameters' total cholesterol, triglyceride, and serum Low-reference intervals like urea, creatinine, serum Density Lipoprotein (LDL) (p value <0.05).

TABLE 3. Comparison of clinical chemistry parameter RIs of this study against manufacturer ranges and other similar studies.

Analyte	Sex	Current	Instrumental	Ethiopia	Tanzania	Ghana	Batswana	Uganda
FBS (mg/dl)	C	68,115	70-126	NA	53.1-95.1	64.8-115.2	NA	NA
	M	68,116.1		NA	42.2-103.1	63-113.4	NA	NA
	F	68.7,115.7		NA	41.5-100	66.6-118.8	NA	NA
BUN (mg/dl)	C	11,29	Aug-28	Nov-41	9.1-29.5	16.2-102.6	NA	4.6-15.5
	M	9.9,28	Aug-29	Dec-43	9.3-29.8	16.2-111.6	NA	4.7-15.8
	F	11,29.4	Jul-23	10-38.7	8.8-27.4	16.2-97.2	NA	4.4-14.2
CR (mg/dl)	C	0.54,1.2	0.5-1.1	0.47-1.12	0.48-1.02	0.56-1.3	NA	0.5-1.2
	M	0.61,1.2	0.6-1.1	0.48-1.13	0.54-1.09	0.64-1.4	NA	0.6-1.2
	F	0.54,1.1	0.5-1.0	0.47-1.09	0.45-0.92	0.53-1.3	NA	0.5-0.9
HDL (mg/dl)	C	42,84.3	>35	NA	NA	NA	11.9-28.8	NA
	M	42,84.1	>35	NA	NA	NA	10.6-28.6	NA
	F	42,85.6	>34	NA	NA	NA	12.1-29.7	NA
LDL (mg/dl)	C	70.2,189.6	<100	NA	NA	NA	21-80.6	NA
	M	90,197.4		NA	NA	NA	19.8-82.3	NA
	F	70,161.3		NA	NA	NA	23.2-78.7	NA
TG (mg/dl)	C	81.9,160.4	<150	36-215.6	39-281	7.2-40	23.6-80.6	39-281
	M	100.4,176.7		37-221.9	40-299	7.2-40	19.8-82.9	39-299
	F	75.4,152.0		35.3-201.5	34-206	7.2-37.8	25.2-98.7	34-206
TC (mg/dl)	C	100,190	<200	80.4-206.6	95.5-213.8	36-97.2	38.9-95.4	91-233
	M	105,200		78.1-211.6	89.7-218.3	32.4-90	39.6-97.4	90-235
	F	95.0,152.4		83.6-202.7	109-212.7	37.8-100.8	38.2-102.1	100-235

NA: Not Addressed in this study, RIs: Reference Intervals

But we do not observe statistically significant differences between males and females in clinical chemistry parameters' RIs like HDL cholesterol and fasting blood sugar (p value >0.05).

Except for HDL and BUN, all selected clinical chemistry parameter values were higher in males than females. **TABLE 2** summarizes the 95% RI, the 95% CI of the mean, median, maximum, and minimum findings of the five selected clinical chemistry parameters.

Discussion

Nowadays, most developing countries like Ethiopia are facing the burden of communicable and non-communicable diseases [9]. In this regard, clinical laboratory attempts to play a major role in providing valuable information for the prevention, diagnosis, and management of life-threatening diseases. Currently, most of the Ethiopian Hospitals and other health care institutions used western derived reference intervals for disease diagnosis and management due to a lack of locally established RIs for

biochemical parameters. However, several studies showed variations between African and western population derived RIs [10,11]. Therefore, establishing local reference intervals is a vital thing for adequate medical care and the prevention of related health issues. According to the current study finding, the reference intervals for all parameters are different based on different demographic and lifestyle variations; especially, high variation is seen in lipid profile findings of the participants when we compare with different previous studies in Africa [12-14], and other studies were done in the western population, (USA and manufacturer represent the western population) [15].

The current study showed that there is a significant difference in some of the selected clinical chemistry parameter values like LDL, serum creatinine, BUN, TC, and serum triglyceride regarding the sex of the study participants (p value <0.05). Most clinical chemistry parameter values like LDL, CR, TC, and TG were higher among males than females except for BUN; females had higher BUN value

than males which is consistent with previous reports of studies in Ethiopia [10,16], and other studies done in African and European countries [14,15,17]. On the opposite, the current study findings were significant differences for the majority of clinical chemistry parameter values by sex although the higher and lower values were different across studies for both sexes [18-20] (**TABLE 3**).

According to the current study finding, RI for selected clinical chemistry tests were established as BUN 11-29.4 mg/dl for females and 9.9-28 mg/dl for males, serum creatinine 0.54-1.1 mg/dl for females and 0.61-1.2 mg/dl for males, serum total cholesterol 95.0-152.4mg/dl for females and 105.0-200 mg/dl for males, serum triglyceride 75.4-152.0 mg/dl for females and 100.4-176.7 mg/dl for males, serum LDL 70.0-161.3 mg/dl for females and 90.0-197.4 mg/dl for males, serum HDL 42-84.3 mg/dl and FBS 68-115 mg/dl. Further details of sex-specific mean with 95% confidence interval, median with IQR (25th and 75th percentile), and ranges are also shown (**TABLE 2**).

In our study finding, the RIs determined for RFT (CR and BUN) were significantly similar to the study conducted in Amhara National Regional State, Ethiopia [16], and a study conducted in Tanzania [17]. On the other hand, the RIs determined for RFT (CR and BUN) are significantly high relative to the previous study finding done in Gojjam, northwest Ethiopian [10] and the study in Mekelle, northern Ethiopia [21], especially on the finding of the lower boundary. The RI finding of serum creatinine in the current study was consistent with the study conducted in Uganda [13], Tanzania [17], and Ghana [14]. But the RI of BUN in this study was significantly high relative to the study finding in Uganda [13] and significantly low relative to the study finding in Ghana [14] (**TABLE 3**).

According to our study finding, the RIs of lipid profiles was somewhat different from the previous studies in Ethiopia in this area [16]. These inconsistencies may be due to demographic differences; lifestyle variations, ethnic differences, geographic longitude differences, and availability of analysis methods, types of equipment, and reagents being used may affect the value of clinical chemistry parameters in these different studies [22]. The RI of TC determined in this study was nearly similar to the finding of the study conducted in Uganda [13] and Tanzania [17], but significantly higher relative to the study conducted in Batswana [12] and Ghana [14]. On the other hand; the

RI of serum triglyceride in the current study was significantly high relative to the finding of the previous study conducted in Uganda [13], Tanzania [17], and Ghana [14]. Similarly, the finding of the RI of HDL in this study was significantly high relative to the study conducted in Botswana [12] (**TABLE 3**).

In the current study, the RIs of fasting blood sugar is also determined and was consistent with the previous study done in Mekelle city, Tigray, northern Ethiopia [21], in Gilgel Gibe Field Research Center, Southwest Ethiopia [23] and the study conducted in Ghana [14]. On the opposite, the current finding was different from the study conducted in Batswana which reports that a significant difference between sex [12] (**TABLE 3**).

In general, several studies and international guidelines recommended that local clinical laboratory RIs should be established for each homogenous population and health care management systems [3,13,24-26]. In this study, the dietary pattern and the geographical variation of study participants were not considered because of difficulty getting standardize based on international guidelines of the CLSI guidelines.

Conclusion

In general the locally established RIs in the current study for selected clinical chemistry parameters were BUN 11 mg/dl-29.4 mg/dl for females and 9.9 mg/dl-28 mg/dl for males, serum creatinine 0.54 mg/dl-1.1 mg/dl for females and 0.61 mg/dl-1.2 mg/dl for males, serum total cholesterol 95.0 mg/dl-152.4mg/dl for females and 105.0 mg/dl-200 mg/dl for males, serum triglyceride 75.4 mg/dl-152.0 mg/dl for females and 100.4 mg/dl-176.7 mg/dl for males, serum LDL 70.0 mg/dl-161.3 mg/dl for females and 90.0 mg/dl-197.4 mg/dl for males, serum HDL 42 mg/dl-84.3 mg/dl and FBS 68 mg/dl-115 mg/dl (**TABLE 2**).

Recommendation

A significant reference interval variation was seen between the current study and other Ethiopian and African studies. Therefore, further local and national studies are recommended, with consideration of differences in age variation, geographic variation, ethnic group variation, and lifestyle variation. In general, it is recommended that all health care institutes should use a locally determined reference range.

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