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Research Article

Reference Intervals (RIs) of Lipid Parameters for Nepalese Population

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Abstract

Introduction: Reference interval (RIs) is the range of values provided by laboratory scientists in a convenient and practical form to support clinician in interpreting observed values for diagnosis, treatment and monitoring of a disease. Laboratories in Nepal uses RIs, provided in the kit inserts by the manufacturers or from the scientific literature, established for western/European population. It is well known that population across the globe differs physiologically, genetically; race, ethnically, lifestyle, food habits and diet which have great impact on the reference values. Thus, it is inappropriate to use RIs that do not represent the local population. This approach highlights for establishing reference values in Nepalese population using the IFCC-CRIDL guidelines published in (C28-A3). **Objectives:** The objective of this study is to analyze blood lipids concentration in apparently healthy Nepalese population to set up reference values for total cholesterol (TC), triglycerides (TG), High Density Lipoprotein-cholesterol (HDL-C) and Low Density Lipoprotein-cholesterol (LDL-C) and compare with the internationally recommended values. **Methods:** Reference individuals selected from healthy volunteers according to the IFCC/C-RIDL protocol in (C28 –A3). Volunteers were requested to avoid excessive physical exertion/exercise/excessive eating and drinking and fast overnight for 10-12 hour. Blood samples were collected from 120 subjects from each five centers of the country between 7:00-10:00 am, serum were separated and refrigerated at -20 in a cryo-vials. Finally, 617 samples were transported to Yamaguchi University, Graduate School of Medicine, Ube, Japan for analysis in dry Ice and 30 parameters were measured by fully automated biochemistry analyzer, Beckman Coulter (BC480) in the clinical laboratory. **Results:** A reference interval for each parameter was calculated from the 95% reference intervals ranging from 2.5% and 97.5% percentiles and, arithmetic mean $\pm$ 2 SD were also calculated. The 95% reference range for total cholesterol (2.53-6.14), triglyceride was(0.42-3.32),for HDL Cholesterol was (0.28-1.46), for LDL was(1.05-4.00) and for VLDL was (0.054-0.92) for Nepalese population. **Conclusion:** Nepalese clinicians can take into consideration of reference lipid values of this study for diagnosis, treatment and monitoring of disease.

Keywords: Reference value; Reference Interval; lipid profile; triglyceride; cholesterol; HDL-Cholesterol; LDL-Cholesterol

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Introduction

In 20th century the term "Reference Value "was first introduced by Ralph Grasbeck, Fellman and Nils-Erik Sarisin (Grasbeck and Saris, 1969). They published a paper entitled 'Normal Values and Statistics' as an initial study in the field of reference intervals (RIs) (Solberg, 1987). In subsequent years it was realized that the terminology of 'normal values' was not adequate and even partially incorrect, so the term 'reference values' came into use. From 1987 to 1991, the International Federation of Clinical Chemistry (IFCC) published a series of 6 papers, recommending that each laboratory should produce its own reference interval following the IFCC-CRIDL and CLSI guidelines (Petit and Solberg, 1987)

In spite of immense clinical importance of RVs, most laboratories across many developing countries including Nepal refers either from kit inserts provided by the manufacturers or from the scientific literature, which are based on Western/European population. It is well known that population across the globe differs physiologically, genetically, ethnically, geographically, lifestyle and food habits (frequency and type of food) which have great impact on the various biochemical analytes. Therefore, it is inappropriate to use RIs that do not represent the local population.

Committee on Reference Intervals and Decision Limits (C-RIDL) of the IFCC initiated a worldwide multicenter study on references values in 2011 facilitating the implementation of country-specific RI by providing a common protocol. (Ceriotti, 2007). Thus, as a part of the worldwide multicenter study, with limited literature on Nepalese population-specific RIs, this study is undertaken to determine the RIs for a lipid parameters in healthy adult Nepalese volunteers (Ozarda and Ichihara, 2013)

Ishemic Heart Disease and Cardiovascular disease are the leading cause of morbididty and mortality throughout the world. (Flaherty and Ford, 2008) Incidence of coronary artery disease increases with advancing age in men above 40 and postmenopausal women. Recently the prevalence of these disorders is also reported in younger individuals (Stamler and Daviglus (2000**)** Rrelationship of lipids and other risk factors with cardiovascular and cerebrovascular events are established. Lipoproteins for predicting coronary and cerebrovascular diseases is not fully understood (Strong and Malcom, 1999). During past two decades, expert panels from western and eastern countries including National Cholesterol Education Programme (NCEP) of U.S (Bachoric and Ross, 1995) have released guidelines for preventing mortality from coronary artery disease. Expert committee has defined the appropriate medical decision cut off points for serum total cholesterol, high density lipoprotein-cholesterol (HDL), low density lipoprotein-cholesterol (LDL) and triglycerides for their population. This NCEP ATP III criterion has been shown in Table 1. The reference intervals established in western population that usually does not match with Nepalese population especially in case of lipid profile (WHO, 1985) as serum lipids levels are much dependent upon genetic background, ethnicity and dietary pattern of a particular population. Most laboratories still do not have comprehensive data, especially ranges that are specific for their typical patient populations. Therefore, clinical laboratories should establish reference ranges for serum lipids based on local healthy population (Wayne, 2000)**.** Individual laboratories should pool data of minimum 120 samples in generating reference values for both as a theoretical concept and as a practical approach (Horowitz, 2012). Thus the objective of this study was to analyze concentration of blood lipids in 532 apparently healthy Nepalese populations from around the country to set up reference values for Total Cholesterol, Triglycerides, HDL-Cholesterol and LDL-Cholesterol to compare these with the globally recommended reference intervals.

Materials and Methods

A total of 617 reference individuals were selected from apparently healthy volunteers from community, colleges, hospitals, and clinical laboratories of five developmental region of Nepal, according to the IFCC/C-RIDL protocol in (C28 –A3). (27) The study design was approved by Nepal Health Research Council (NHRC), Institutional Review Board (IRB). Age, sex, height, weight, abdominal circumference, smoking history, alcoholic history and exercise habits are included in the general health questionnaire.

Table 1: International Classification of Lipid as recommended by WHO and NCEP in (mg/dl)

Classification	Total Cholesterol	Triglyceride	HDL Cholesterol	LDL Cholesterol
Desirable	<200	<150	>60	<130
Borderline	200-239	200-399	35-59	130-159
High	>240	>399	-	>160
Low	-	-	<35	-

(Source**:** Warnick *et al*., 2002)

Inclusion Criteria

Healthy volunteers aged 18-65 years who understood the objective and importance of the study were selected as reference individuals.

Exclusion Criteria

i) Individuals on regular drug therapy for chronic disease (diabetes, hypertension, thyroid disorder, dyslipidemia, gout, depression, renal disease, cardiovascular diseases, coronary bypass graft ii) within two weeks' recovery from acute disease requiring hospitalization, or surgery iii) pregnancy or within one year of delivery iii) smoker, alcoholic, hormone therapy, women on oral contraceptive. Volunteers were requested to avoid excessive physical exertion/exercise/excessive eating and drinking and fast overnight for 10-12 hour. The blood samples were collected from 120 subjects from each five centers of the country between 7:00-10:00 am, serum were separated and refrigerated at -20 in a cryo-vials. Finally, 617 samples were transported to Yamaguchi University, Graduate School of Medicine, Department of Laboratory medicine, Ube, Japan for analysis in dry-Ice and lipid parameters were measured by fully automated biochemistry analyser, Beckman Coulter (BC480) in the Clinical Laboratory.

Sample Collection centres

Fig. 1 shows the sample collection centres (Biratnagar, Dharan, Janakpur, Kathmandu, Pokhara, Nepalganj). Those centres belong to five developmental regions of Nepal.

Results

Out of total 617 apparently healthy subjects 532 were reference individuals of which 256 were females and 276 were males. The age of reference individuals ranged from 18-65years.Average age was 38.73±12.24yrs.

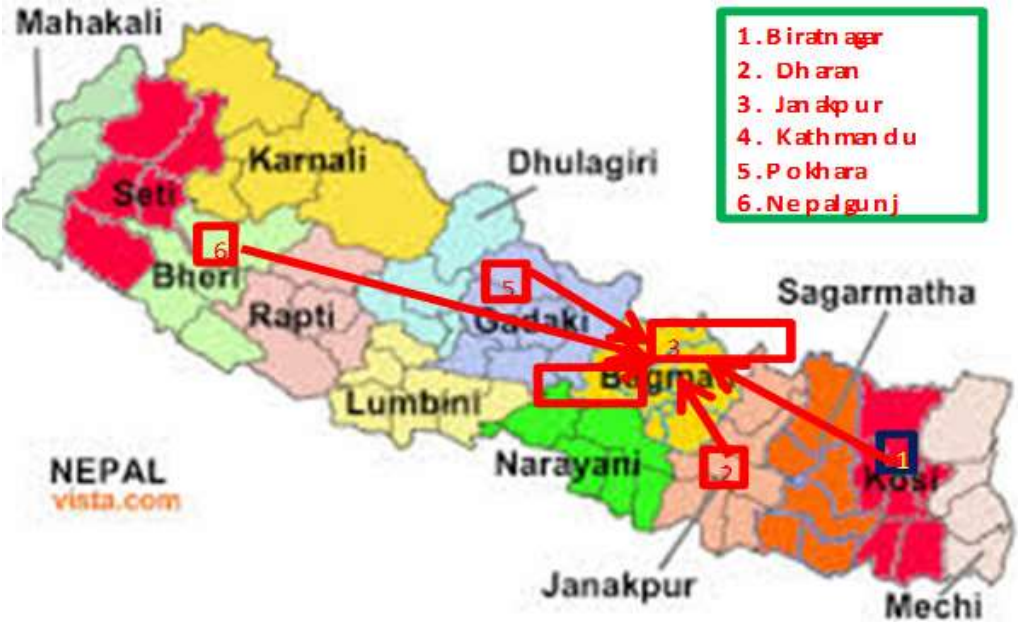

Fig 1: Shows sites of sample collection from five developmental regions of Nepal

Table 2: Classification of lipid concentrations as recommended by WHO and NCEP in (mmol/L) Mg/dL of total cholesterol(TC), HDL-C and LDL-C has been converted into mmol/L (38.67 x mmol/L=mg/dL). While mg/dL TG is converted to mmol/L (88.57xmmol/L)=mg/dL.

Classification	Total Cholesterol	Triglyceride	HDL Cholesterol	LDL Cholesterol
Desirable	<5.16	<1.7	>1.55	<3.36
Borderline	5.16-6.1	2.25-4.5	0.9-1.52	3.36-4.11
High	>6.20	>4.5	-	>4.13
Low	-	-	<0.90	-

A total of 617 serum samples were included in the analysis process, 85 samples were excluded applying Latent Abnormal Value Exclusion (LAVE). Remaining 532, Male 276 and Female 256 were analyzed by parametric and non parametric methods.

We have calculated mean and Standard deviation for cholesterol, triglyceride, HDL, LDL and VLDL which are tabulated in Table 3. A reference interval for each lipid parameter was calculated from the 95% reference intervals ranging from 2.5% and 97.5% percentiles and, arithmetic mean + 2 SD were also calculated. The results are shown in Table 2, and Characteristics in Table 3 for respective parameters. Reference values should be based on percentiles determined from well-defined population samples. So 95% reference range for total cholesterol (2.53-6.14), triglyceride was (0.42-3.32), for HDL Cholesterol was (0.28-1.46), for LDL was (1.05-4.00) and for VLDL was (0.054-0.92) for Nepalese population.

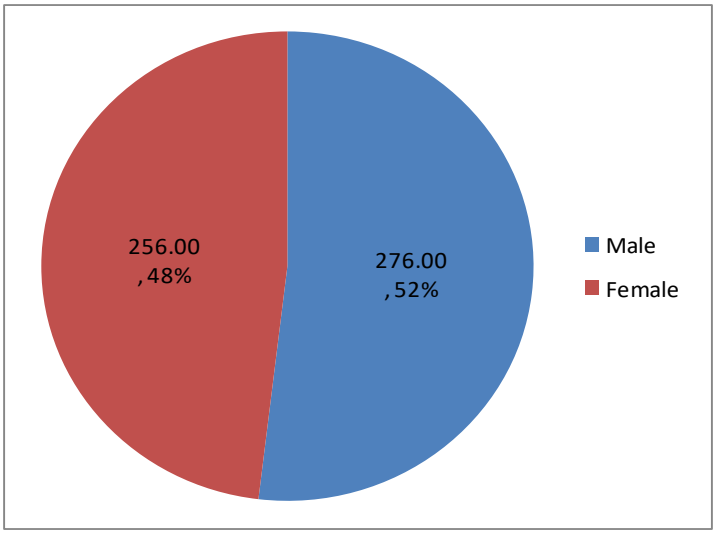

Fig. 2: Shows distribution of Male and Female recruited in the reference study.

Table 3: Reference Intervals (RIs) for Lipid Parameters in healthy Nepalese Population of age 18-65 years.

SN	Parameters	Unit	M+F		Male		Female	
			LL	UL	LL	UL	LL	UL
1.	Total.Cholesterol (TC)	mmol/L	2.53	6.14	2.52	6.43	2.50	5.88
2.	Triglyceride(TG)	mmol/L	0.42	3.32	0.47	3.82	0.41	2.92
3.	HDL-Cholesterol	mmol/L	0.28	1.46	0.30	1.43	0.26	1.51
4.	LDL-Cholesterol	mmol/L	1.05	4.00	1.04	4.20	1.09	33.81

LL: Lower Limit, UP: Upper Limit

Table: 4: Reference Intervals used in Tribhuvan University Teaching Hospital (TUTH) Maharajgunj, Kathmandu

	Parameters	Unit	Reference Intervals	
			LL	UL
1.	Glucose(Glu)	mmol/L	3.5	6.1
2.	T.cholesterol (TC)	mmol/L	3.5	5.1
3.	Triglyceride(TG)	mmol/L	0.5	1.8
4.	HDL-C	mmol/L	0.80	1.6
5.	LDL-C	mmol/L	1.05	4.00

Table 5: Distribution of Lipid profiles among healthy individuals

		Cholesterol	Triglyceride	HDL	LDL
Number (N)		532	532	532	532
Mean		4.11	1.40	0.79	2.36
Median		4.05	1.23	0.80	2.29
Std. Deviation		0.95	0.78	0.29	0.78
Minimum		1.82	0.27	0.18	0.58
Maximum		7.88	4-62	1.87	6.37
Percentiles	2.5	3.39	0.80	0.57	1.81
	97.5	6.08	3.34	1.33	4.00

Discussion

Lipid parameters cholesterol, LDL, HDL, and triglycerides are measured routinely in almost all clinical laboratories. Most of the laboratories refer to literature data or manufacturers insert sheets for interpretation of observed value (Solberg and Stamm, 1991) or reference range published by National Cholesterol Education Program (NCEP). It is difficult for most of the laboratories to collect ample number of samples from a sufficiently large reference/healthy individual as recommended by authorities like IFCC, C-RIDL, NCEP (Fonarow, 1994). Factors like dietary habits of people, lifestyle, heredity factors like ethnicity, race along with the other factors as mentioned earlier like age, sex, geography etc have great impact on reference intervals. In the present study we have tried to establish reference ranges of fasting lipid parameters for apparently healthy Nepalese population, which have not been ever studied.

The 95% reference range for serum total cholesterol (2.53-6.14), triglyceride was (0.42-3.32), for HDL Cholesterol was (0.28-1.46) and for LDL was (1.05-4.00) mmol/dL. In the current study upper limit of the reference range of the lipid profile are higher than manufacturer's reference values. Lower limit of reference values observed in this study, Total Cholesterol (TC) 2.53 mmol/L, Triglyceride (TG) 0.42 mmol/L, High Density Lipoprotein Cholesterol (HDL-C) 0.28 mmol/L, Low Density Lipoprotein Cholesterol (LDL-C) 1.05 mmol/L. lower limit of all lipid parameters values are lower than the reference value used in the Tribhuvan university teaching hospital (TUTH) except LDL-C which shows same value as observed. The upper limit of reference values for total cholesterol (6.14 mmol/L), triglycerides (3.32 mmol/L), HDL (1.46 mmol/L) and LDL (4.00 mmol/L) in the studied population which shows higher values for TC and TG but lower in HDL and same value for LDL-C.as shown in Table 3. The observed slightly wider range of lipid profile of our study than the recommended reference range is probably due to the sedentary lifestyle of people along with modern habit of junk food. Carbohydrate is the chief content in every food and lack of exercise mainly in urban area. There is rapid urbanization and ethnic diversity, population of Nepal is mixed regarding food composition and dietary habits. So, it is concluded that that lipid profile pattern of Nepalese population is different from that of western and European population. There are many study reports available in literature related to serum/plasma lipids as important risk factors for atherosclerosis and similar research were carried out in Punjab in Assam (Das and Saikia, 2009) in Maharashtra (Durgawale *et al*., 2013) in Ahemdabad, Gujrat (Patel *et al*., 2013), Andhra Pradesh (Malathi *et al*., 2009) due to the large variability of the food habits. Lipid profile as it is influenced by biological entities of a population comparison between data is little difficult in real terms. We have made an attempt to compare our results with other studies. While comparing with (Goswami *et al*., 2003). Total cholesterol and LDL levels were found to be higher in the present study, whereas TG levels were low compared to (Reddy *et al*., 2006)

The observed value from this study and other published reports from India (Ashavaid and li wang., 2005, 1988) China (Li *et al*., 2005) Japan (Carrol *et al*., 1993) and US (Burchfie *et al*., 1996) revealed highest total cholesterol in American of all races (>5.16 mmol/L) followed by China, Japan and India seem to have relatively lower average cholesterol compared to other populations. Nepalese population seems to have relatively wider reference intervals for all lipid parameters as compared to study reported from Americans, Europeans, Japanese and Chinese populations (Concepcion *et al*., 1994)

Our study had significantly wider values than (Burchfie *et al*., 1994) study except for LDL (Concepcion *et al*., 1994)

the significant difference between these populations groups could be due to the environment, temperature difference, mainly composition of food and different dietary habits. The high HDL levels in the above foreign studies were in harmony with results obtained in the longevity syndromes (Aurora O *et al*., 1994) in which high values for HDL are a frequent finding. Contrastingly, as compared to the current study, the total cholesterol and TG upper reference limits were higher, whereas the HDL-C upper limits is lower and LDL-C has same value. Due to the present evidence of difference in the observed reference intervals and reference provided by the manufacturers, we urge clinical laboratories to determine their own reference intervals, taking into account the eating habits, genetics, lifestyle, environmental, inherent characters and other factors which influence reference intervals for local population. Diversity of commercial test kits (even using the same analysis technique) in addition to the various sample selection methods, generates large numbers of variation in reference intervals that prevent proper comparison with observed value. Therefore, the standardization and consensus in the evaluation is important factors that should be followed in future studies.

Despite the reported values, the rigorous process of selection of healthy individuals hinders proper comparison with other standard reference range. In the present study some characteristics of the study population like eating habits, ethnicity was not considered which could influence the alteration of plasma lipid concentrations. This may be considered as the limitation of our study.

Conclusion

Nepalese clinicians can take into consideration reference lipid values of this study for diagnosis, treatment and monitoring of disease. There is a great need for reference ranges of other parameters like apolipo-proteins, not included in this study to be established. The finding of this study opens an avenue for similar studies to be carried out in other geographical regions for various other parameters It can be suggested that lipid values obtained in this study can be used as the reference value, based on which clinical correlation can be made. Clinicians of Haryana should take into consideration reference lipid values of this study for clinical evaluation.

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