Ind J Clin Biochem (July-Sept 2012) 27(3):290–295
DOI 10.1007/s12291-012-0208-9

ORIGINAL ARTICLE

To Establish the Reference Intervals of Lipid Profile in Punjab

Vaneet Kaur · Minni Verma · Amandeep Kaur · Shalini Gupta · Kamaljit Singh

Received: 4 December 2011 / Accepted: 26 March 2012 / Published online: 19 April 2012

Abstract Overnight fasting samples of 1,031 apparently healthy people of Punjab visiting the hospital over a period of 3 years were tested for serum lipid profile. The mean ± SD of serum total cholesterol, triglycerides, high density lipoprotein cholesterol, low density lipoprotein cholesterol and very low density lipoprotein cholesterol in mg/dl were 182.2 ± 33.9, 122.4 ± 33.4, 44.1 ± 6.8, 113.9 ± 32.0, 24.6 ± 7.1 respectively. When these subjects were grouped according to age and sex, no appreciable difference was observed between most of the groups. Serum triglycerides were found to be low and HDL-C was high in females when compared with males of similar age and vice versa. With advancing age, total cholesterol and low density lipoprotein cholesterol levels were found to be higher in women. The present study suggests that the obtained lipid values should be taken into consideration during clinical evaluation.

Keywords Lipid profile · Reference intervals · Cholesterol · Triglycerides · Age · Gender

Introduction

Health of an individual is conceptually different in different countries, in the same country at different times and in the same individual at different ages. It is thus a relative or absolute state. This means that the condition of individuals must be related to or compared with reference data. It is thus the central role of the laboratory scientist to aid the clinician in interpreting the observed values by providing the relevant reference values and presenting them in a convenient and practical form. The concept of reference interval was introduced by International Federation of Clinical Chemistry (IFCC) to avoid the problems with normal values and values obtained from an individual under clinical investigations [1]. According to IFCC, it is necessary for every laboratory to have its own set of reference limits. However, in India most of the laboratories follow the reference intervals established in the western population; these usually do not match with the Indian population especially noted in case of lipid profile. In clinical chemistry, the use of the term lipids generally refers to lipoprotein metabolism and atherosclerosis, a cause of coronary heart disease (CHD) [2]. Available global data have clearly established the relationship of lipids and other risk factors with cardiovascular and cerebrovascular events [3–11]. It is therefore essential to establish a reference range of the values of serum lipids for a given population as only a few studies have been carried out in other regions of India [12–15].

Materials and Methods

A total of 1,031 individuals were tested for lipid profile which included serum total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and serum triglycerides. They (reference individuals) included healthy employees of the companies on the panel of our hospital who came for routine health

V. Kaur · M. Verma · A. Kaur · S. Gupta · K. Singh
Department of Biochemistry, Gian Sagar Medical College and Hospital, Ramnagar, Distt. Patiala, Rajpura, India

V. Kaur (✉)
House No 176, Sector 16 A, Chandigarh 160015, India
e-mail: kaurvnt@gmail.com

check-up. Samples were drawn after 12 h of overnight fasting and serum was separated for various parameters of lipid profile to be analyzed. Reagent kits for all lipid parameters were procured from Narmis diagnostics Ltd., Croydon, Surrey, United Kingdom.

Serum total cholesterol was measured by enzymatic CHOD–PAP method with lipid clearing agent using cholesterol esterase, cholesterol oxidase and peroxidase.

High density lipoprotein cholesterol was measured by a homogeneous method for directly measuring HDL-C levels in serum without the need for any off-line pre-treatment or centrifugation steps.

Serum triglycerides were measured with an enzymatic colorimetric method involving lipoprotein lipase, glycerol kinase, glycerol-3-phosphate oxidase and finally in the presence of peroxidase (POD), hydrogen peroxide oxidizes aminoantipyrine and *N*-ethyl-*N*-(2-hydroxy-3-sulfopropyl)-3-methylaniline into a red compound. VLDL and LDL were calculated using Friedewald's formula [16]. To maintain accuracy and precision, ERBA norm and path were run daily as a part of internal quality control program. As a part of External Quality Assurance Scheme (EQAS), our laboratory is enrolled with CMC Vellore. The samples were analyzed on HITATCHI-912 autoanalyzer and were interpreted with the help of a statistician. The study protocol was approved by the ethical committee of Gian Sagar Medical College and Hospital.

Results

1. The present study is a retrospective study in which the lipid profile has been analysed in a total of 1,031 individuals (age range 21–85 years) who attended the clinical biochemistry lab for a regular check up. All these individuals were selected after following the IFCC exclusion criteria as mentioned in Table 1 [17].
2. Out of 1,031 individuals, 501 were males and 530 were females. The mean age for males was 50.1 ± 14.3 years and for females was 48.5 ± 11.8 years. The mean age for these individuals was 49.3 ± 13.1 years. No significant difference was observed for age in males and females (Table 2).
3. The mean, median, standard deviation, range (minimum–maximum value) and confidence limits for mean for Total-C, HDL-C, LDL-C, VLDL-C, TGs, Total-C/HDL-C ratio, LDL-C/HDL-C ratio along with *P* values are shown in Table 3. Decade wise analysis for all the above stated parameters is shown in Table 4. A comparison of the values of various lipid parameters in our study according to the reference values being followed in our lab is shown in Table 5.
4. The overall mean total cholesterol levels were significantly higher ($P = 0.032$) in females (184.4 ± 32.3 mg/dl) as compared to males (179.9 ± 35.3 mg/dl) and the overall mean cholesterol was 182.2 ± 33.9 mg/dl. The median value of 180 mg/dl in males and 185 mg/dl in females were quite similar to the observed mean value of cholesterol. The confidence limits for mean were also higher in females (181.69 to 187.22 mg/dl) as compared to males (176.82 to 183.03 mg/dl). Also, the range observed for total cholesterol was 98–270 mg/dl in males and 100–305 mg/dl in females (Table 3). Decade wise analysis of cholesterol levels showed a steady increase from 2nd to 4th decade in males after which a steady fall was observed whereas in females a steady increase has been observed till 70 years of age. No significant difference was observed in decade wise analysis for mean total cholesterol levels in both males and females (Table 4). The number of patients with total cholesterol levels outside the reference range of our lab (130–200 mg/dl) is increasing with age and more in males than in females with maximum number in 4th decade in both males and females and increasing in females in the 5th decade and onwards (Table 5).
5. The overall mean HDL-C level in females was higher (45.1 ± 6.7 mg/dl) as compared to males (43.1 ± 6.8 mg/dl) with a significant p value (<0.001). The median value in females (45 mg/dl) was also higher than in males (43 mg/dl) and the values were quite similar to mean values. The confidence limit in males and females was 42.50–43.70 and 44.56–45.70 mg/dl

Table 1 Exclusion criteria based on history and clinical examination for defining reference individuals

Diabetes mellitus	Excessive body weight
Dyslipidemias	Smoking
Hypertension	Alcohol abuse
Cardiovascular disease	Pregnancy
Medication	Strenuous exercise
Renal disease	Caffeine intake
Endocrine disorders	Use of OCPs
Liver obstruction	

Table 2 Mean levels of age for both males and females

Sex	Mean	N (number)	SD
Male	50.11	501	14.3
Female	48.96	530	11.8
Total	49.31	1,031	13.1

Table 3 Lipids (mg/dl) in healthy population of Punjab

		N	Mean	Median	SD	Standard error	95 % confidence interval for mean		Minimum	Maximum	*P* value
							Lower boundary	Upper boundary			
T-CHOL	Male	501	180	180	35.3	1.57	176.82	183.03	98	270	
	Female	530	184	185	32.3	1.4	181.69	187.22	100	305	0.032
	Total	1,031	182	182	33.9	1.05	180.18	184.33	98	305	
HDL-C	Male	501	43.1	43	6.8	0.3	42.5	43.7	24	84	
	Female	530	45.1	45	6.7	0.29	44.56	45.7	21	78	<0.001*
	Total	1,031	44.1	44	6.8	0.21	43.72	44.56	21	84	
LDL-C	Male	501	113	112	32.9	1.47	109.75	115.54	38	199	
	Female	530	115	115	31.2	1.35	112.55	117.88	37	228	0.198
	Total	1,031	114	113	32	0.99	112	115.93	37	228	
VLDL-C	Male	501	24.3	24	6.6	0.29	23.75	24.91	10	48	
	Female	530	24.8	24	7.4	0.32	24.24	25.51	9	80	0.216
	Total	1,031	24.6	24	7	0.22	24.18	25.04	9	80	
TGs	Male	501	122	120	32.6	1.45	118.89	124.62	44	240	
	Female	530	123	121	34.2	1.48	120.15	126	44	402	0.528
	Total	1,031	122	121	33.4	1.04	120.39	124.48	44	402	
T-CHOL/HDL-C	Male	501	4.25	4.16	0.98	0.04	4.16	4.34	1.67	8.35	
	Female	530	4.16	4.17	0.89	0.03	4.08	4.23	2.08	8.29	0.113
	Total	1031	4.2	4.16	0.93	0.02	4.15	4.26	1.67	8.35	
LDL-C/HDL-C	Male	501	2.67	2.6	0.89	0.04	2.6	2.75	0.76	6.31	
	Female	530	2.6	2.57	0.8	0.03	2.53	2.67	0.57	5.53	0.159
	Total	1,031	2.64	2.59	0.85	0.02	2.58	2.69	0.57	6.31	

*p value less than 0.05 is significant

respectively (Table 3). Decade wise analysis of HDL-C levels shows a fall with advancing age in both males and females with a significant change in males ($P = 0.027$, Table 4). The numbers of females with HDL-C levels below the reference range of our lab (30–80 mg/dl) are maximum in the 7th decade (Table 5).

6. The overall mean and median value of LDL-C was higher in females (mean 115.2 ± 31.2 mg/dl and median 115 mg/dl) as compared to males (mean 112.6 ± 32.9 mg/dl and median 112 mg/dl and median 112 mg/dl) but the difference was not significant ($P = 0.198$). The confidence limits for mean ranged from 109.75–115.54 mg/dl in males and 112.55–117.88 mg/dl in females (Table 3). The mean LDL-C level increased steadily from 2nd to 4th decade in both males and females after which a fall was observed in both 5th and 6th decades and then a rise in 7th decade in males. Similarly, in females, fall in mean LDL-C level was observed in 5th and 7th decades and a rise in 6th decade. No significant change was observed in mean LDL level in both males and females (Table 4). The number of patients with LDL level outside the reference range (up to 150 mg/dl) is increasing with age in both males and females with maximum in 4th decade in males and in the 7th decade in females (Table 5).
7. The overall mean and median values of serum triglycerides was higher in females (mean 123.0 ± 34.2 mg/dl and median 121 mg/dl) than in males (mean 121.7 ± 32.6 mg/dl and median 120 mg/dl) with no significant change ($P = 0.528$). The confidence limits for mean was also higher in females (120.15–126.00 mg/dl) than in males (118.89–124.62 mg/dl, Table 3). Decade wise analysis of serum triglycerides revealed an increase in both males and females till the 4th decade followed by a decrease in the 5th decade and an increase in the 6th decade but a decrease in 7th decade onwards in males and an increase in females. A significant change ($P = 0.040$) in decade wise analysis of triglycerides in females was observed. The number of patients with triglycerides levels outside the reference range (up to 150 mg/dl) is also increasing with age in females with maximum number in the 6th decade.
8. The changes observed in VLDL levels in both males and females reflect the changes in triglycerides.

Table 4 Decade wise analysis of lipids (mg/dl) in healthy population of Punjab

Age group		21–30	31–40	41–50	51–60	61–70	>70	Total	*P* value
(Years)	Males	57	71	132	117	93	31	501	
N	Females	54	137	186	96	49	8	530	
Mean	Males	176.6 ± 32.2	182.9 ± 33.8	187.1 ± 38.5	177 ± 35.2	175 ± 35.2	174.1 ± 26.6	179.9 ± 35.3	0.07
T-CHOL ± SD	Females	174.9 ± 31	182.1 ± 29	186.8 ± 32.5	186.1 ± 32.5	189.3 ± 39.1	184.2 ± 39.4	184.4 ± 32.3	0.16
Mean	Males	45 ± 6.9	44.5 ± 5.9	42.7 ± 6.O	43.1 ± 7.3	41.7 ± 8.0	41.6 ± 5.1	43.1 ± 6.8	0.02*
HDL-C ± SD	Females	44.5 ± 8.2	45.3 ± 6.1	44.4 ± 7.0	46 ± 5.2	46.4 ± 7.1	42.6 ± 7.6	45.1 ± 6.7	0.18
Mean	Males	107.8 ± 28.3	114.1 ± 34.9	119.9 ± 35.8	109.7 ± 32.0	1O8.1 ± 32.3	111.3 ± 25.3	112.6 ± 32.9	0.06
LDL-C ± SD	Females	108 ± 28.1	114.7 ± 27.7	117.2 ± 30.5	115.6 ± 35.4	116 ± 37.1	115 ± 31.1	115.2 ± 31.2	0.59
Mean	Males	119.7 ± 32.4	120.3 ± 31.7	125.9 ± 32.2	121.2 ± 36.2	123.4 ± 29.2	107.9 ± 30.1	121.7 ± 32.3	0.13
TGs ± SD	Females	112.6 ± 37.5	119.7 ± 36.7	126.5 ± 32.4	122 ± 29.6	130.8 ± 36.9	133.3 ± 26.1	123 ± 34.2	0.04*
T-CHOL/HDL-C ± SD	Males	3.9 ± 0.8	4.1 ± 0.9	4.4 ± 1.0	4.1 ± 0.9	4.3 ± 1.1	4.2 ± 0.8	4.2 ± 0.9	0.06
	Females	4 ± 0.9	4 ± 0.7	4.2 ± 0.9	4 ± 0.8	4.1 ± 1.0	4.3 ± 0.8	4.1 ± 0.8	0.22
LDL-C/HDL-C ± SD	Males	2.4 ± 0.7	2.6 ± 0.8	2.8 ± 0.9	2.6 ± 0.8	2.6 ± 0.9	2.7 ± 0.7	2.6 ± 0.8	0.06
	Females	2.5 ± 0.8	2.5 ± 0.6	2.6 ± 0.8	2.5 ± 0.8	2.5 ± 0.9	2.7 ± 0.6	2.6 ± 0.8	0.64

*p value less than 0.05 is significant

Table 5 Categorisation of lipids into normal/abnormal according to the reference range of our lab

Age	Sex		T-CHOL		HDL-C		LDL-C		VLDL-C		TG	
			Count	%	Count	%	Count	%	Count	%	Count	%
21–30	Male	Normal	37	64.9	56	98.2	54	94.7	57	100	44	77.2
		Abnormal	20	35.1	1	1.8	3	5.3	–	–	13	22.8
	Female	Normal	41	75.9	52	96.3	53	98.1	53	98.1	43	79.6
		Abnormal	13	24.1	2	3.7	1	1.9	1	1.9	11	20.4
31–40	Male	Normal	46	64.8	71	100	62	87.3	71	100	56	78.9
		Abnormal	25	35.2	–	–	9	12.7	–	–	15	21.1
	Female	Normal	100	73	136	99.3	123	89.8	134	97.8	113	82.5
		Abnormal	37	27	1	0.7	14	10.2	3	2.2	24	17.5
41–50	Male	Normal	63	47.7	129	97.7	105	79.5	132	100	93	70.5
		Abnormal	69	52.3	3	2.3	27	20.5	–	–	39	29.5
	Female	Normal	114	61.3	184	98.9	156	83.9	186	100	142	76.3
		Abnormal	72	38.7	2	1.1	30	16.1	–	–	44	23.7
51–60	Male	Normal	78	66.7	115	98.3	107	91.5	116	99.1	90	76.9
		Abnormal	39	33.3	2	1.7	10	8.5	1	0.9	27	23.1
	Female	Normal	60	62.5	96	100	80	83.3	96	100	78	81.3
		Abnormal	36	37.5	–	–	16	16.7	–	–	18	18.8
61–70	Male	Normal	59	63.4	84	90.3	84	90.3	93	100	73	78.5
		Abnormal	34	36.6	9	9.7	9	9.7	–	–	20	21.5
	Female	Normal	31	63.3	49	100	40	81.6	49	100	28	57.1
		Abnormal	18	36.7	–	–	9	18.4	–	–	21	42.9
>70	Male	Normal	25	80.6	31	100	30	96.8	31	100	27	87.1
		Abnormal	6	19.4	–	–	1	3.2	–	–	4	12.9
	Female	Normal	6	75	7	87.5	6	75	8	100	6	75
		Abnormal	2	25	1	12.5	2	25	–	–	2	25

9. The overall mean Total Chol/HDL-C ratio was higher in males (4.25 ± 0.98) than in females (4.16 ± 0.89). The mean Total Chol/HDL-C ratio also increased steadily in males and females with maximum in the 4th decade in males and in the 7th decade in females.

10. The overall mean LDL-C/HDL-C ratio was higher in males (2.67 ± 0.89) than in females (2.60 ± 0.80). The mean LDL/HDL ratio also increased steadily till the 4th decade in both males and females followed by a fall in the 5th decade and then again a rise there after.

Discussion

The levels of total cholesterol, LDL-C, HDL-C and serum triglycerides are routinely requested by all the clinicians and so form an important profile in almost all the diagnostic laboratories. The recommended procedure by different authorities like IFCC, NCEP [18, 19] to identify, collect and measure enough samples from a sufficiently large population is not feasible for most laboratories, which thus have to rely on literature data or manufacturers' insert sheets [20]. There is a great variation of plasma lipid levels in different populations and usually are affected by age, sex, food habits, lifestyle, socio–economic status, race, heredity etc. Traditionally, Punjabi food is rich in fat and high in calories. Numerous reports are available in literature relating to serum plasma lipids, lipoprotein (a), apolipoproteins and their subtractions as important risk assessment parameters for atherosclerosis causing cardiovascular and cerebrovascular disorder [21].

Salient features of our study include that total mean cholesterol level in females(184.4 ± 32.3 mg/dl) was significantly higher ($P = 0.032$) than in males (179.9 ± 35.3 mg/dl) with an elevated consolidated interval (98–270 mg/dl and 100–305 mg/dl) as compared to our laboratory reporting values (130–200 mg/dl).When compared with a similar study done in western Maharashtra [13], total mean cholesterol level in males (165.7 ± 9.8 mg/dl) and females (165.95 ± 31.93 mg/dl) was similar but less as compared to the present study. Asharvaid et al. also conducted a study in healthy Indian population in 2005 revealing increased total mean cholesterol level in both males (199 ± 37.54 mg/dl) and females (196 ± 36.13 mg/dl) as compared to that in the present study. The increased level of total cholesterol in Punjabi population can be explained by the excessive consumption of ghee in food.

Also, the mean HDL-C level was also significantly higher in females ($P < 0.001$) than in males with a fall with advancing age in both males and females. The level of HDL-C in females has also been found to decrease with advancing age (>60 years) in the study done on lipid profile in western Maharashtra [13] (44.0 ± 6.3 mg/dl) as well as in Kolkata [12] (49.5 ± 6.6 mg/dl) in the same age group. Also, with increasing age, the number of females having HDL-C level lower than the reference range is increasing with maximum in the age group above 70 years. A high HDL-C level in females before the menopausal age probably protects them against atherosclerosis.

The overall mean LDL-C level was higher in females (115.2 ± 31.2 mg/dl) than in males (112.6 ± 32.9 mg/dl) but the difference was not significant (Table 3). High LDL-C levels in females (133.4 ± 34.8 mg/dl) have been seen as compared to males (116.5 ± 27.6 mg/dl) in a similar study done in Kolkata [12] in 2003. On the contrary, the mean LDL-C levels in the study conducted on lipid profile in Andhra Pradesh [15] reveals lower level of LDL-C in females (99.2 ± 30.6 mg/dl) as compared to males (102.4 ± 29.5 mg/dl). Decade wise analysis also reveals an increase in LDL-C levels with age in both males and females but the change is not significant (Table 4). Similar changes have also been observed in decade wise analysis of LDL-C levels in Bengali population of Kolkata [12]. Also, the LDL-C levels outside the reference range are increasing with age with maximum in the 4th decade in males and in the 7th decade in females.

The gradual increase in Total cholesterol and LDL-C levels with age could place the females in a more vulnerable position as compared to males in relation to atherosclerosis.

In the present study, no significant difference has been observed in serum triglycerides levels between males and females which resembles the pattern observed in the study conducted in western Maharashtra. Decade wise analysis of triglycerides reveals an increase in their level in females with age and the change is significant ($P = 0.040$). Similar pattern has been observed in various Indian studies conducted in western Maharashtra by Durgawale et al. [13] and in Kolkata by Goswami and Bandyopadhyay [12]. Serum VLDL-C level follows the same pattern as Serum triglycerides.

It is also observed that the values of the present study follow a similar pattern as in some of the foreign countries [22]. 3,044 elderly Japanese–American men from Honolulu Heart program showed the mean concentration of their lipid patterns as follows:

TC 189, HDL-C 51, LDL-C 109, TG 147 mg/dl ([13], Kolkata). The mean values of the various parameters of the present study did not differ much from the above mentioned values.

Also, the increased number of study samples showing values of the various lipid parameters outside the reference range being followed in our lab indicates that the lipid values of the present study should be taken into consideration for further evaluation.

References

1. Solberg H. International federation of clinical chemistry, expert panel on theory of reference values: approved recommendation on the theory of reference values. Part 1—The concepts of

reference values. J Clin Chem Clin Biochem. 1987;25(337–42): 639–44.
2. Rifai N, Bachori K, Albers JJ. Lipids, Lipoproteins and Apolipoproteins. In: Border BG, editor. Tietz fundamentals of clinical chemistry. Philadelphia: Saunders an Imprint of Elsevier; 2006. p. 462–93.
3. Austin MA, McKmight B, Edwards KL, Bradley CM, McNeely MJ, Psaty BM, Brunzell JD, Motulsky AC. Cardiovascular disease mortality in familial forms of hypertriglyceridemia: a 20 year prospective study. Circulation. 2000;101:2777–82.
4. Ingelsson E, Schaefer EJ, Contois JH, McNamara JR, Sullivan L, Keyes MJ, et al. Clinical utility of different lipid measures for prediction of coronary heart disease in men and women. JAMA. 2007;298:776–85.
5. Cutri BA, Hime NJ, Nicholls SJ. High-density lipoproteins: an emerging target in the prevention of cardiovascular disease. Cell Res. 2006;16:799–808.
6. Chein KL, Hsu HC, Su TC, Sung FC, Chen MF, Lee YT. Lipoprotein (a) and cardiovascular disease in ethnic chinese: the Chin-Shan community cardiovascular cohort study. Clin Chem. 2008;54:285–91.
7. Chopra V, Wasir HS. Implications of lipoprotein abnormalities in Indian patients. J Assoc Physicians India. 1998;46:814–8.
8. Hippisley-Cox J, Coupland C, Vinogradova Y, Robson J, Minhas R, Sheikh A, Brindle P. Predicting Cardiovascular risk in England and Wales: prospective deviation and validation of QRISK2. Br Med J. 2008;336:1475–82.
9. Grundy SM, Parternak R, Greenland P, Smith S Jr, Fustex V. Assessment of cardiovascular risk by use of multiple risk factor assessment equation: a statement for health care professional from the American Heart Association and the American College of Cardiology. Circulation. 1999;100:1481–92.
10. Misra A, Luthra K, Vikram NK. Dyslipidemia in Asian Indians: determinants and significance. JAPI. 2004;52:137–42.
11. O'Connor PJ, Gary RJ, Maciosek MV, Fillibrandt KM, DeFor TA, Alexander CM, Weiss TW, Teutsch SM. Cholesterol levels and statin use in patients with coronary heart disease treated in primary care settings (CDC original research document). Prev Chronic Dis Public Health Res Pract Policy. 2005;2:1–17.
12. Goswami K, Bandyopadhyay A. Lipid profile in middle class bengali population of Kolkata. Indian J Clin Biochem. 2003; 18(2):127–30.
13. Durgawale P, Patil S, Shukla PS, Sontakke A, Kakade S, Yadav S. Evaluation of reference intervals of serum lipid profile from healthy population in western Maharashtra. Indian J Clin Biochem. 2009;24(1):30–5.
14. Das M, Saikia M. Estimation of reference interval of lipid profile in Assamese population. Indian J Clin Biochem. 2009;24(2): 190–3.
15. Malati T, Mahesh MRU. Reference intervals for serum total cholesterol, HDL-cholestrol, LDL-cholestrol, triglycerides, Lp (a), apolipoprotein A-i, A-ii, C-ii, C-iii and E in healthy south Indians from Andhra Pradesh. Indian J Clin Biochem. 2009; 24(4):343–55.
16. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low density lipoprotein cholesterol without the use of the preparative ultracentrifuge. Clin Chem. 1972;18:449.
17. Villanova PA. National Commmittee for Clinical Laboratory Standards: how to define determine and utilize reference intervals in the clinical laboratory proposed guidelines. 2nd ed. NCCLS Document;028–A2.
18. Solberg HE, Stamm D. Approved recommendation on the theory of reference values. Part 4. Control of analytical variation in the production, transfer and application of reference values. Eur J Clin Chem Clin Biochem. 1991;29:531–5.
19. Fonarow GC. National Cholesterol Education Program. Second report of the expert panel on detection evaluation and treatment of high blood cholesterol in adults (Adult Treatment Panel II). Circulation. 1994;89:1329–445.
20. Furruqh S, Anitha D, Venkatesh T. Estimation of reference values in liver function test in health plan individuals of an urban south Indian population. Indian J Clin Biochem. 2004;19(2): 72–9.
21. D'Agostino RB Sr, Vasan RS, Pencina MJ, Wolf PA, Cobain M, Massaro JM, et al. General cardiovascular risk profile for use in primary care: the Framingham heart study. Circulation. 2008;117: 743–53.
22. Yano K. Distribution and correlates of lipids and lipoproteins in elderly Japanese–American men the Honolulu Heart Program. Atheroscler Thromb Vasc Biol. 1996;16:1356–564.