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EVALUATION OF REFERENCE INTERVALS OF SERUM LIPID PROFILE FROM HEALTHY POPULATION IN WESTERN MAHARASHTRA

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ABSTRACT

Fasting samples of 914 subjects from healthy population were analyzed for total cholesterol, triglyceride and three major fractions of lipoproteins i.e. high-density lipoprotein cholesterol, low lipoprotein cholesterol and very low-density lipoprotein cholesterol. The values obtained were (in mg/dl) 165.7±30.2,88.36±31.2, 44.86 ±10.68, 101.66±29.8 and 18.11±7.35 respectively. When these subjects were grouped according to the age and sex,no appropriate differences were observed between most of the groups. Triglycerides were found to be low and HDL cholesterol was high in female when compared with male of similar age. Beyond age 40 years cholesterol level and low density lipoprotein cholesterol was found to be gradually increased in case of women. Minor difference was observed with dietary pattern. Present study suggests that clinical evaluation of patient should be made on the basis of these reference values for Western Maharashtra population.

KEY WORDS

Total cholesterol, Triglyceride, HDL cholesterol, Lipoprotein cholesterol Reference Intervals.

INTRODUCTION

The concept of reference intervals was introduced by international federation of clinical chemistry (IFCC) to avoid the problems with normal values and values obtained from an individual under clinical investigation (1). An important part of medical decision in diagnosis is dependent on comparison of patient related observations with reference values. Since the serum lipid levels even in healthy normal population are affected by a number of factors such as age, sex, racial differences, dietary factors, socio- economic status, geographic conditions which influence these values.

It is therefore essential to establish reference range of the values of serum lipids and lipoproteins for a given population in India. Few studies have been carried out in some population (2,3). The diversity in the findings though attributed to socio-economic status, nature of diet/dietary fat and genetic predisposition, the different methodology adopted also could not be ignored. In the light of guidelines suggested by National Cholesterol Education Programme (NCEP) (4) of USA it was also necessary to establish normal reference intervals for plasma lipids in various parts of India. In India laboratories across the country follow reference values which have been establish in western population even though diet, life style, and genetic pool is different. The serum lipid evaluation in Western Maharashtra population was carried out with this purpose, because Krishna Hospital is referral Centre of Karad Taluka population. Also to be best of our knowledge there is no documented data available on Maharashatrians biochemical reference interval therefore we took up this research project

The main objectives of this study are evaluating mean ±SD for lipid profile from Healthy population in western Maharashtra, find out any difference with respect to age and gender, calculate reference interval for given population and compare the reference interval values with other geographically different regions.

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MATERIALS AND METHODS

In two years study of 914 normal healthy subjects of western Maharashtra from lower to moderate-income families of various age groups either sex were selected using IFCC exclusion criteria : a) pathophysiological states – renal failure, congestive heart disease, chronic respiratory diseases, liver diseases, malabsorption syndrome, and nutritional anemias, b) Systemic deceases – hypertension, diabetes, c) Intake of pharmacologically active agents – alcohol, and tobacco, oral contraceptives, replacement or supplementation therapy eg. Insulin, d) Modified physiological states – pregnancy, psychological and mental disorders such as severe stress and depression, exercise or physical training, food intake prior to blood collection and e) Other factors – obesity (BMI>30 kg/m^2).

The healthy subjects obtained after applying the exclusion criteria were used for establishing the reference values. They were categorized according to age (<20yrs, 21 – 30, 31 – 40, 41 – 50, 51 – 60, 61 ≤ yrs) sex and diet (Vegetarian and non-vegetarian). Analysis was performed using the commercially available statistical software SPSS – 10.0. Since distribution of some of the factors were skewed (non Gaussian) the logarithmic transformations of observations noted under various factors (variables) were worked out (5,6). Mean ± SD of these log values was computed. The 95% reference interval for each of these factors was determined by taking mean ± 1.96 SD using these log values. Finally the antilog of these ranges were determined as reference intervals, as interpercentile interval. It is more commonly used and recommended by IFCC.

Fasting blood samples were collected from all these subjects, serum was separated and all samples were analysed within 6 hrs of collection in our laboratory. Serum cholesterol (C)- By enzymatic method using cholesterol esterase, cholesterol Oxidase and peroxidase kit by Accurex Biochemical Pvt.Ltd. Triglyceride – By enzymatic method using lipoprotein lipase, glycerolkinase, glycerol phosphate, and Oxidase and peroxides kit by Accurex Biochemical (7). HDL- C & LDL-C: - Direct HDL-C &LDL-C by using HDL& LDL solubilizing a special detergent, cholesterol esterase, cholesterol Oxidase, & peroxidase Kit by DAIICHI pure chemicals Co. Ltd. Tokyo-Japan.

During the course of study there was no change in the equipment, reagent, Calibration standards and controls. Before starting the analysis the instruments were calibrated using calibrators and the controls run at normal concentrations of the analytes. As a part of external quality assurance our laboratory is enrolled in Vellor and EQAS (Biorad-USA) quality control programmes. All the measurements were done using Slim (Seac) semiautoanalyser.

RESULTS AND DISCUSSION

We have selected 1070 cases using NECP exclusion criteria. When the exclusion criteria based on laboratory examinations were followed we have deleted total 156 (14.58%) cases because fasting blood sugar level was found more than 110 in 74 (6.9%) cases, total cholesterol more than 250 mg%, TG more than 170 mg% and LDL more than 180 mg% in 82 (7.7%) cases were found. When we have selected the cases using NECP criteria still 14.58% population was unaware of

Table 1: Mean values and standard deviation (SD) of serum lipids and lipoproteins in mg/dl

Age groups (Years)	Sex	No	Total cholesterol Mean±SD	HDL-C Mean±SD	LDL-C Mean±SD	VLDL-C Mean±SD	Triglyceride Mean±SD
≤20	Male	19	156.3±30.5	42.3±10.1	96.1±26.8	17.7±5.8	90.4±29.3
	Female	18	161.3±37.2	48.8±16.4	94.7±31.2	14.8±6.7	74.7±33.9
21-30	Male	167	164.0±30.3	43.6±10.6	100.4±27.0	18.8±7.0	16.4±6.2
	Female	133	163.5±29.3	46.0±10.9	101.1±27.6	92.6±30.6	81.4±29.0
31-40	Male	133	166.9±29.3	43.7±10.32	101.9±29.1	19.2±7.9	90.5±29.3
	Female	122	165.3±30.3	45.9±9.4	101.9±27.1	17.7±8.1	85.4±32.1
41-50	Male	71	164.5±32.9	45.1±11.1	98.9±30.3	19.01±8.5	90.9±32.6
	Female	112	168.1±32.5	45.1±10.7	104.9±29.9	18.1±6.9	89.0±32.1
51-60	Male	99	165.8±31.4	43.0±10.6	102.6±25.3	17.8±6.1	89.1±30.2
	Female	69	168.6±31.4	47.9±11.4	101.4±27.2	18.5±8.7	87.8±30.0
≥61	Male	17	178.2±26.8	45.0±7.18	111.0±25.8	20.64±7.9	98.0±35.0
	Female	8	171.3±29.9	44.0±6.3	105.0±32.5	18.5±4.9	94.0±25.0

Table 2: Comparative value of Serum lipids & lipoproteins with other Indian studies

Lipid	Sex	Present Study		Data from Gujarat Population by Jhala et al (4)		Data from Kolkata Bengali Population by Goswami and Bandyopadhyay (3)	
		Number of patients with age group	Mean ±SD	Number of patients with age group	Mean ±SD	Number of patients with age group	Mean ±SD
Serum Cholesterol	M	≤ 20 (19)	156.31± 30.5	00 – 15 (16)	150.30 ±25.68	21 –30(36)	169.6 ± 31.4
		21-30 (167)	164.02 ±30.3	16 – 45 (482)	179.27 ±37.50	31 –40(132)	178.4 ±31.4
		31-40 (133)	169.9 ±29.3	45+ (341)	181.44 ±40.47	41 –50(326)	194.5 ±28.2
		41-50 (71)	164.5 ±32.9	51 –60(306)	194.9 ±32.2		
		51-60 (99)	165.8 ± 31.4	61 –70(146)	186.7 ±29.5		
		≥ 61 (17)	178.2 ± 26.8	71+(52)	180.2 ±28.2		
	F	≤ 20 (18)	161.3 ± 37.2	00 –15 (55)	143.16 ±25.08	21 –30(34)	168.1 ±27.1
		21-30 (133)	163.5 ± 29.3	16 – 45 (252)	107.46 ±30.66	31 –40(85)	175.5 ±47.7
		31-40 (122)	165.3 ± 30.5	45+ (183)	194.87 ±37.63	41 –50(108)	194.3 ±30.7
		41-50 (112)	168.1 ± 32.5	51 –60(92)	196.4 ±29.9		
		51-60 (69)	168.6 ± 31.4	61 –70(62)	199.2 ±43.6		
		≥ 61 (08)	171.3 ± 29.9	71+(18)	204.4 ±27.7		
Serum Triglyceride	M	≤ 20 (19)	90.4 ± 29.8	00 – 15 (16)	74.81 ±19.78	21 –30(36)	138.1 ±37.2
		21-30 (167)	92.6 ± 30.6	16 – 45 (482)	119.56 ±62.75	31 –40(132)	136.2 ±40.1
		31-40 (133)	90.5 ± 29.3	45+ (341)	122.08 ±61.20	41 –50(326)	135.4 ±36.0
		41-50 (71)	90.9 ± 32.6	51 –60(306)	134.6 ±45.1		
		51-60 (99)	89.1 ± 30.2	61 –70(146)	128.2 ±43.1		
		≥ 61 (17)	98.2 ± 35.1	71+(52)	117.5 ±45.1		
	F	≤ 20 (18)	74.7 ± 33.9	00 –15 (55)	94.69 ±33.95	21 –30(34)	100.7 ±39.4
		21-30 (133)	81.4 ± 29.6	16 – 45 (252)	100.19 ±46.91	31 –40(85)	130.5 ±38.6
		31-40 (122)	85.4 ± 32.1	45+ (183)	121.52 ±28.26	41 –50(108)	130.4 ±40.8
		41-50 (112)	89.0 ± 32.1	51 –60(92)	133.3 ±38.5		
		51-60 (69)	87.8 ± 30.2	61 –70(62)	134.5 ±49.9		
		≥ 61 (08)	94.2 ± 25.1	71+(18)	115.5 ±49.8		
Serum HDL - C	M	≤ 20 (19)	42.3 ±10.1	00 – 15 (16)	45.0 ±25.7	21 –30(36)	49.5 ±10.1
		21-30 (167)	43.6 ±10.6	16 – 45 (482)	44.0 ±37.5	31 –40 (132)	52.2 ±11.6
		31-40 (133)	43.7 ±10.3	45+ (341)	44.3 ±40.5	41 –50 (326)	51.9 ±9.8
		41-50 (71)	45.1 ±11.1	51 –60 (306)	51.8 ±10.2		
		51-60 (99)	43.0 ±10.6	61 –70 (146)	51.6 ±8.1		
		≥ 61 (17)	45.0 ±7.18	71+ (52)	50.5 ±8.5		
	F	≤ 20 (18)	48.8 ±16.4	00 –15 (55)	36.7 ±25.08	21 –30 (34)	57.5 ±8.9
		21-30 (133)	46.0 ±10.9	16 – 45 (252)	48.7 ±30.7	31 –40 (85)	54.0 ±10.8
		31-40 (122)	45.9 ±9.4	45+ (183)	49.7 ±37.6	41 –50 (108)	59.6 ±4.9
		41-50 (112)	45.1 ±10.7	51 –60 (92)	55.4 ±9.5		
		51-60(69)	47.9 ±11.4	61 –70 (62)	53.6 ±13.2		
		≥ 61 (08)	44.0 ±6.3	71+ (18)	49.5 ±6.6		
Serum LDL -C	M	≤ 20 (19)	96.1 ±26.8	00 – 15 (16)	90.7 ±25.7	21 –30 (36)	97.8 ±27.1
		21-30 (167)	100.4 ±27.0	16 – 45 (482)	109.3 ±37.5	31 –40 (132)	104.2 ±28.7

Lipid	Sex	Present Study		Data from Gujarat Population by Jhala et al (4)		Data from Kolkata Bengali Population by Goswami and Bandyopadhyay (3)	
		Number of patients with age group	Mean ±SD	Number of patients with age group	Mean ±SD	Number of patients with age group	Mean ±SD
		31-40 (133)	101.9 ±29.1	45+ (341)	114.3 ±40.5	41 –50 (326)	120.8 ±25.9
		41-50 (71)	98.9 ±30.3	51 –60 (306)	121.8 ±26.3		
		51-60 (99)	102.6 ±25.3	61 –70 (146)	113.8 ±27.5		
		≥ 61 (17)	111.2 ±25.8	71+ (52)	109.5 ±25.6		
	F	≤ 20 (18)	94.7 ±31.2	00 –15 (55)	82.5 ±25.8	21 –30 (34)	94.2 ±42.0
		21-30 (133)	101.1 ±27.6	16 – 45 (252)	103.2 ±30.66	31 –40 (85)	100.4 ±36.7
		31-40 (122)	101.9 ±27.1	45+ (183)	121.3 ±30.64	41 –50 (108)	114.8 ±31.2
		41-50 (112)	104.9 ±29.9	51 –60 (92)	199.9 ±27.6		
		51-60 (69)	101.4 ±27.2	61 –70 (62)	122.8 ±36.2		
		≥ 61 (08)	105.0 ±32.5	71+ (18)	136.3 ±19.9		
Serum VLDL	M	≤ 20 (19)	17.7 ±5.8	00 – 15 (16)	25.7 ±14.9	21 –30 (36)	23.3 ±5.8
		21-30 (167)	18.8 ±7.0	16 – 45 (482)	37.5 ±23.33	31 –40 (132)	22.0 ±6.4
		31-40 (133)	19.2 ±7.9	45+ (341)	40.5 ±25.0	41 –50 (326)	21.9 ±5.7
		41-50 (71)	19.01 ±8.5	51 –60 (306)	21.7 ±7.1		
		51-60 (99)	17.8 ±6.1	61 –70 (146)	21.3 ±6.3		
		≥ 61 (17)	20.6 ±7.9	71+ (52)	20.2 ±7.1		
	F	≤ 20 (18)	14.8 ±6.7	00 –15 (55)	25.0 ±18.9	21 –30 (34)	16.4 ±6.2
		21-30 (133)	16.4±6.2	16 – 45 (252)	30.7±19.5	31 –40 (85)	21.1±6.0
		31-40 (122)	17.7±8.1	45+ (183)	37.6±25.1	41 –50 (108)	19.9±6.1
		41-50 (112)	18.1±6.9	51 –60 (92)	21.1±6.1		
		51-60 (69)	18.5±8.7	61 –70 (62)	22.8±7.2		
		≥ 61 (08)	18.5±4.9	71+ (18)	18.6±7.9		

increased blood glucose level or dyslipidemia, which is very alarming in this area.

Mean values and standard deviation (SD) of total cholesterol, serum Triglyceride, HDL, VLDL, &LDL cholesterol for all 914 subjects is shown in Table 1. The levels of triglyceride and total cholesterol and cholesterol in HDL, LDL and VLDL fraction for all the 914 subjects were 88.36 ± 31.15 mg%, 165.67± 30.83, 44.86 ± 10.68 mg% , 101.66 ± 29.8 & 18.11± 7.35 mg% respectively. In 472 males the values were 105.7 ± 55.4 mg%, 165.9 ± 9.8 mg%, 42.7 ± 10.3 mg%, 102.3 ± 30.0 mg% and 21.2 ± 11.2 mg% respectively and 442 females the values were 85.31 ± 34.06 mg%, 165.95 ± 31.93 mg%, 46.10 ± 10.78 mg%, 102.15 ± 28.69 and 17.45 ± 7.36 mg% respectively. No difference was observed in TG levels between the male and female population and in different age group except in less than 20 years group where the females showed a significant lower level ($p< 0.001$), VLDL – C levels followed the same pattern. Though the average HDL – C was higher in female the same change was not observed beyond 60 years. The females showed high Total cholesterol levels beyond 40 years with a simultaneous increase in LDL–C. Table 1 showed High HDL – C level in females below 60 years which probably protect them from CAD (8). The gradual increase in total Cholesterol and in later stage in LDL –C beyond 60 years could place the females on more vulnerable position compared to males in relation to CAD. When compared with the data available from other regions of India the difference were observed in various lipid fractions.

Reference values should be based on percentiles determined from well-defined population samples. Comparison of the values of serum total cholesterol (TC), Triglyceride (TG) low density lipoproteins (LDL) high density lipoproteins (HDL), very

Table 3: Reference Interval of Lipid Profile

Age Group (Yrs)	Sex	No. of Subjects	Total Cholesterol	Triglycerides	HDL-C	LDL-C	VLDL-C
≤ 20	*M*	*18*	*122.7-187.1*	*62.0-115.9*	*32.2-52.8*	*69.8-122.5*	*12.9-25.75*
	F	18	130-191.4	46-100.7	32.4-66.4	62.0-122.5	9.01-13.5
21-30	M	161	133-191.4	61.8-122.2	33-52.5	72.78-128.2	12.16-24.15
	F	131	134.9-190.1	54.6-108.0	35.4-56.7	73.8-127.1	11-21.33
31-40	M	117	135.5-191.9	62.0-117.5	34.04-53.95	74.8-130	12.2-23.4
	F	114	134.6-192.3	55.8-114.3	36.8-55.2	73.4-128.5	10.6-21.8
41-50	M	66	130.3-198.2	60.7-120.8	35-56.7	68.6-132.3	12.3-23.3
	F	106	134.9-197.2	57.3-119.7	34.4-55	73.8-136.1	11.5-23.8
51-60	M	96	134.6-190.1	62.9-115.6	33-53	76.2-126.8	12-24
	F	65	135.8-200.9	59.7-116	34.6-59.7	73.6-131.5	11.8-26.4
≥ 61	M	14	146.6-203.1	61.8-138.4	36.4-51	81.8-139.3	12.52-29.2
	F	07	125-160.95	68.3-120.8	36.5-51.6	67.4-140	13.6-23.7

low density lipoproteins (VLDL) of different age groups of both sexes has been made with the other Indian studies and shown in Table 2.

The differences were observed in TG and VLDL-C values. Mean value of serum TGs in the present study is 88.36 ±31.15 mg% and mean VLDL-C is 18.11±7.35 mg% which are significantly lower (P<0.05) than those from Gujrati and Bengali population. Low VLDL-C may be due to the low TG in the studied population, this in turn decreases VLDL-C in the serum. Gujrati and Bengali populations in contrast to western Maharashtra population consume more ghee and perform less physical activity in relation to the calorie intake. The mean total cholesterol in the present study is 165.67± 30.83 mg% which is comparable with Gujrati and Bengali study and other studies in India. HDL-C is comparable with Gujrati population but it is significantly lower than Bengali population. This may be because of different diet pattern as fish intake is high in Bengali which increase HDL-C level in the blood.

Table 4: Different ratios determined from healthy population

Age Group (Yrs)	*Sex*	*TG/HDL*	*LDL/HDL*	*TC x TG/HDL*
≤ 20	M	1.3-3.1	1.531-3.2	186.3-527.2
	F	1.1-2.6	1.104-3.2	122.7-415.9
21-30	M	1.3-3.2	1.5-3.4	190-550.8
	F	1.1-2.7	1.5-3.2	167.5-446.7
31-40	M	1.22-3.1	1.6-3.5	177.0-538.6
	F	1.2-3.1	1.5-3.1	163.3-485.3
41-50	M	1.2-3	1.4-3.2	198-535.6
	F	1.2-2.6	1.5-3.4	180.3-534.6
51-60	M	1.3-3	1.6-3.3	213.6-514
	F	1.4-3.1	1.4-2.9	181.6-463.6
≥ 60	M	1.4-3.4	1.7-3.5	191-539.5
	F	1.4-3.1	1.5-3.2	197.7-612.4
AVERAGE RATIOS		1.26-3	1.486-3.258	180.75-513.68

It is suggested that the lipid values of the present study should be taken as base parameters and the clinical evaluation should be made on the basis of these findings for Western Maharashtra. Reference values which were based on percentiles determined from this well defined population samples are shown in Table 3. Predicting the risk factors for CAD, relative ratios of different fractions of plasma lipids along with main parameters are gaining importance to rule out dyslipidemia. The ratio of TG\HDL ,TGx C\HDL (triad index) are shown in Table 4. This can be the basis for the assessment of CAD . There is one study on Indian Population (9), which is a hospital-based study, while our approach to find out reference interval is in Healthy Population.

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