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Comparison of Fasting and Non Fasting Lipid Profile and Lipoprotein (a) in Healthy Adult Population

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ABSTRACT
Background: *Serum lipid profile and Lp(a) levels are routinely measured for cardiovascular disease risk prediction. This study aimed to compare the fasting and non fasting lipid profile and Lp(a) in healthy adults and to find any variation.*
Methods: *Data was examined from 200 healthy adults of 20-60 years after thoroughly examining and applying exclusion criteria, fasting after 10 hour of overnight fast and approx 2 hour after breakfast blood samples were drawn and subjected to testing for lipid profile and Lp(a).*
Result: *The variation of fasting and non fasting total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, Non HDL-cholesterol, total cholesterol/HDL-cholesterol ratio and lipoprotein(a) were found to be 2.03%, 9.50%, 1.24%, 5.20%, 2.24%, 0.84%, 3.15% respectively. The triglycerides variation of fasting and non-fasting was found to be statistically significant among all these. (p value<0.05).*
Conclusion: *Non fasting lipid profile and Lp(a) levels are appropriate for assessment of risk of cardiovascular diseases.*

Introduction

Cardiovascular disease (CVD) is a major health problem across the world for 30% of all deaths.[1,2] In India, 52% of deaths occur among those younger than 70 years, resulting in a considerable burden from cardiovascular diseases on working-age citizens.[3] Atherosclerosis leading to cardiovascular disease remains the major cause of death and premature disability globally. Major risk factors for atherosclerosis are: advancing age, male sex, dyslipidaemia, diabetes mellitus, hypertension, cigarette smoking, hypothyroidism, family history of CAD. Non-traditional risk factors are inflammatory markers, highly sensitive C-reactive protein (CRP), lipoprotein-associated phospholipase A2 (Lp-PLA2), lipoprotein(a), hyperhomocysteinemia, hyperuricemia.[4]Dyslipidaemia is considered one of major predisposing risk factor for atherosclerosis, American Academy of Clinical Endocrin-

ologists (AACE) recommends evaluation of all adults >20 years of age for dyslipidaemia every 5 years as part of a global risk assessment.[4]

Dietary lipids metabolise via Exogenous pathway (intestinal lumen) and Endogenous pathway (hepatic). Dietary lipids after going through metabolism, cholesteryl esters are hydrolysed and cholesterol is utilised for synthesis of cell membrane, steroid hormone and bile acids where appropriate.[5] Oversupply of free cholesterol leads to: i) Inhibition of HMG Co-A reductase, ii) Increased cholesteryl ester formation by ACAT and, iii) Inhibition of new LDL synthesis. LDL remains in circulation for about 3 days. LDL are also taken up extrahepatic tissue (eg. Macrophages) through the scavenger receptors or non-receptor mediated pinocytosis. Macrophages that become engorged with cholesteryl esters are called "foam cells" which are earlier lesions of atherosclerosis. Two thirds of the LDL is normally removed by LDL receptors and remainder by scavenger cell system.[6]

HDL particles undergo extensive remodelling in the plasma by a variety of lipid transfer proteins and lipases. Phospholipid transfer protein transfers phospholipids from other lipoproteins to HDL. After Cholesterol Ester Transfer Protein (CETP) mediated lipid exchange, the triglyceride enriched HDL becomes a much better substrate for Hepatic lipase (HL) which hydrolyses the triglycerides and phospholipids to generate smaller HDL particles that are catabolised faster.[6]

LDL contains cholesterol and a single protein or apolipoprotein apoB-100. LDL constitutes about 60% to 70% of total serum cholesterol. LDL is the major atherogenic lipoprotein, and is the primary target cholesterol lowering therapy.[7] HDL contains cholesterol and apo AII apolipoproteins. HDL constitutes, about 20% to 30% of total serum cholesterol. HDL is thought to protect against the development of atherosclerosis. Triglycerides are transported in the blood as chylomicrons following absorption from the intestine, or as a component of VLDL if synthesized by the liver.[7]VLDL is triglyceride- rich lipoprotein and constitutes about 10% to 15% of total serum cholesterol. VLDL has several apolipoproteins, including apoB100, apoCI, apoCII, apoCIII and apoE. VLDL is a precursor of LDL and partially degraded lipoproteins called VLDL remnants. Since both VLDL remnants and LDL are atherogenic, they may be combined to estimate risk prediction. The sum of VLDL + LDL is called non- HDL cholesterol.[7]

Lipoprotein (a) is a complex particle in human plasma that is assembled from one LDL molecule that carries all the lipid and one glycoprotein [apo(a)], which has a high degree of homology to plasminogen.[8,9] Lp(a) contains cholesterol, either in the free or esterified form, represents almost 40% of its total mass, with phospholipids making upto 17-24%, proteins 17-29% and triglycerides constitute 9%. Numerous epidemiological studies have shown that Lp(a) in plasma is a risk factor for variety of cardiovascular diseases including silent coronary artery disease (CAD), acute myocardial infarction (AMI), asymptomatic carotid atherosclerosis,[10,11] stroke, peripheral artery occlusive disease (PAOD) and abdominal aortic aneurysm.[12] Its incorporation into plaque and high affinity binding to glycosaminoglycans and fibronectin suggest a direct atherogenic action in combination with elevated cholesterol. The optimal level of Lp(a) should not be greater than 20mg/dl.[13-15] Modestly elevated Lp(a) levels of 20 mg/dl to 30 mg/dl are associated with 2 to 3 fold higher risk of myocardial infarction or restenosis following coronary angioplasty and bypass surgery.[14-16] A 10 fold increase is observed when Lp(a) level is >50mg/dl mostly in patients with high cholesterol levels. The risk of MI is 100 fold higher when Lp(a) is more than 55mg/dl and this is accompanied by low HDL and high ratio of total cholesterol to HDL.[17-19]

The first step in risk management is risk assessment by measurement of LDL cholesterol as part of lipoprotein analysis and identification of accompanying risk factors. The highest risk consist of CHD and CHD risk equivalent which includes other forms of atherosclerotic diseases,

diabetes, multiple risk factors that confer a 10 year risk >20%. Risk status in persons without clinically apparent CHD or other clinical forms of atherosclerotic disease determined by counting the risk factors. For those 2 or more risk factors, 10 year risk assessment is carried by using the Framingham scoring system to identify individuals whose short term risk warrants consideration of intensive treatment. Asian tend to have higher levels of triglyceride, lower HDL levels and higher levels of Lp(a). In addition the higher CHD risk in this population may be related to higher prevalence of metabolic syndrome, insulin resistance and diabetes.[20] Therefore, Asian Indians with dyslipidaemia should be treated as aggressively as if they had a CHD risk equivalent-similar to treatment of patients with diabetes or heart disease. Thus a total cholesterol level of < 200mg/dl desirable according to the Framingham model for those with 0 to 1 risk factor, the goal for the Asian Indian population should be <160mg/dl. An LDL level of <160mg/dl is appropriate for most Americans with 0-1 risk factor, but a level of < 100mg/dl is optimal for Asian Indians.[20] HDL levels of 60mg/dl are considered optimal in both whites and Asians Indians . HDL levels are considered low when they drop below 40 mg/dl in males and < 50mg/dl in females. The acceptable normal level of Triglycerides was decreased from < 200 mg/dl in ATP 11 report to < 150mg/dl in ATP 111 classification.[21,22]. National Cholesterol Education Program NCEP and European guidelines recommend doing Lipid profile in fasting blood specimen for assessment of CVD risk but these guidelines allow total and HDL-cholesterol in non fasting specimen as these lipids are not much different in fasting and non fasting specimen. In addition, non HDL- cholesterol (total cholesterol – HDL cholesterol), a secondary target of therapy in adult treatment panel III, may also be used in non fasting state.[23] A fasting sample is preferred if CVD risk assessment is based on total cholesterol, LDL-cholesterol or non-HDL cholesterol but HDL cholesterol, triglycerides, total cholesterol/ HDL cholesterol ratio and apolipoprotein a1 predict CVD when measured non fasting.[24] The most interesting part is that non fasting triglyceride levels may be even better predictor of cardiovascular risk as compared to fasting triglycerides.[25,26] Triglyceride increase step wise after fat diet, therefore, non fasting triglyceride would vary depending upon time after meal with highest levels 4-5 hr post prandially.[25] Further, the cut off levels of non fasting triglycerides for CVD risk have not yet be identified. It is important to compare serum lipid profile in fasting and at different time interval after a representative meal in terms of prediction of cardiovascular risk. As is true for fasting triglyceride, post prandial lipemia can be effected by ethnicity, alcohol consumption, and menopausal status and thus these factors should be considered in clinical practice.[27]

Material and Methods

This study was prospective open labelled observational study conducted on 200 normal healthy adults of age 20-60 years, attending outdoor and indoor departments, resident doctors and other staff members of Guru Nanak Dev Hospital, Amritsar during period of July 2013 to June 2014.Exclusion criteria includes persons with DM Hypertension, Dyslipidaemia, Persons with hypolipidemic agents due to any reason, Alcoholics, Smokers, Females on Oral contraceptive pills/hormone replacement therapy, pregnancy/ lactation. After selecting the subjects their informed and written consent was taken and detailed personal history and general physical examination was done specially for height, weight and signs of Atherosclerosis. Besides routine investigations, Liver function tests, Renal function tests, Thyroid function tests, ECG, two blood samples – one fasting after 10 hour overnight fast and another after breakfast (average 2 hr) were drawn and subjected to testing for lipid profile and Lp(a).

1) Estimation of Total Cholesterol in serum (enzymatic method – cholesterol oxidase /peroxidise) –the assay was carried out by

using A25 bio system auto analyser. Its reference range was Upto 200mg/dl – desirable, 200 – 239 mg/dl – borderline high and > 240 mg/dl – High .

2) Estimation of serum Triglycerides (Enzymatic method glycerolphosphate/ peroxidise). This assay was carried out using A25 bio system auto analyser. Reference range was Upto 150 mg/dl – Normal, 150 – 199 mg/dl – Borderline high , 240 -249 mg/dl – High and > 500 mg/dl – Very High .
3) Estimation of serum High Density Lipoprotein – Cholesterol (direct detergent method) . The assay was carried out by using A25 bio system auto analyser. Reference range Upto 35 mg/dl – High risk, > 60 mg/dl – Low risk.
4) Estimation of serum Low Density Lipoprotein Cholesterol .Serum LDL Cholesterol is calculated by Friedewald equation LDL cholesterol = Total cholesterol – (HDL cholesterol + Triglycerides). Serum LDL cholesterol id was estimated by direct method when TG values were > 400 mg/dl
5) Non HDL cholesterol was calculated by Total cholesterol – HDL cholesterol.
6) TC/ HDL ratio was calculated.
7) Estimation of serum Lipoprotein (a) (Turbidimetric immunoassay).This assay was carried out by using A25 bio system auto analyser. Reference values 0-30mg/dl.

Statistical Analysis

All statistical analysis was performed using SPSS version 21.0 for windows. Frequency distribution, mean and standard deviation were calculated for various qualitative and quantitative variables respectively. Independent student 't' test was applied to mean values of fasting and non fasting total cholesterol, triglyceride, HDL cholesterol, LDL cholesterol, T.chl/HDL ratio, non HDL cholesterol and Lp(a) to find out whether the difference was statistically significant or not. The difference was considered statistically significant when the result was $p<0.05$.

Results

Study group comprised of total 200 healthy adults of age 20 – 60 years. It included 127 males and 73 females with 63.5% and 36.5% respectively. The mean age of study population was 28.61 years. The mean weight was 60.02 kg. The mean height was 1.65m. the mean BMI was found to be 22.04 kg/m^2. The mean systolic and diastolic BP were 122.25 and 80.28 mm of hg respectively. In age group 20-30 years males and female were 102 (51%), 45(22.5%) respectively and total no. of subjects was 147(73.5%). In age group 31-40 years males and females were 14(7%) and 27(13.5%) and total no. of subjects were 41(20.5%). In age group 41-50 years among 11(5.5%) subjects males and females were 10(5%) and 1(0.5%). In age group 51-60 years among total no. of 1 subject (0.5%) there was only 1 male (0.5%) and 0 female (0%). The mean +- S.D. values of fasting and non fasting total cholesterol was found to be 222.40+- 61.7 mg/dl and 217.88+-63.08 mg/dl respectively with the variation of 2.03%. The p value was < o.4694 and it was not statistically significant. The mean+- S.D. values of fasting and non fasting triglyceride levels were found to be 176.90+-75.10 and 193.71+-79.65 respectively with the variation of 9.50%, p value (0.0305) which was considered to statistically significant. The values of mean +-S.D. of fasting and non fasting HDL-C were 46.79+-5.68 and 46.21+-6.02 respectively with the variation of 1.24%, p value (0.3223) and it was considered not to be statistically significant. The values of mean+- S.D. of fasting and non fasting LDL-C were 140.22+-52.40 and 132.93+-51.87 with a variation of 5.20%, p value (0.1628) and it was not statistically significant. The values of mean +- S.D. of fasting and non fasting non HDL-C were found to be 175.60+-59.09 and 171.67+-60.28 respectively with a variation of 2.24%, p value (0.5106) which was not statistically

significant. The values of mean +- S.D. of fasting and non fasting T. Cholesterol / HDL- C were found to be 4.74+-1.10 and 4.70+-1.15 respectively with the variation of 0.84%, p value (0.7224) and it was not found to be statistically significant. The values of mean +- S.D. of fasting and non fasting Lp(a) were found to be 12.68+-2.17, 12.28+-2.19 respectively with the variation of 3.15%, p value (0.0673) and this was not considered to be statistically significant.

Comparasion of Fasting and Non Fasting Values of Triglycerides in Study Group

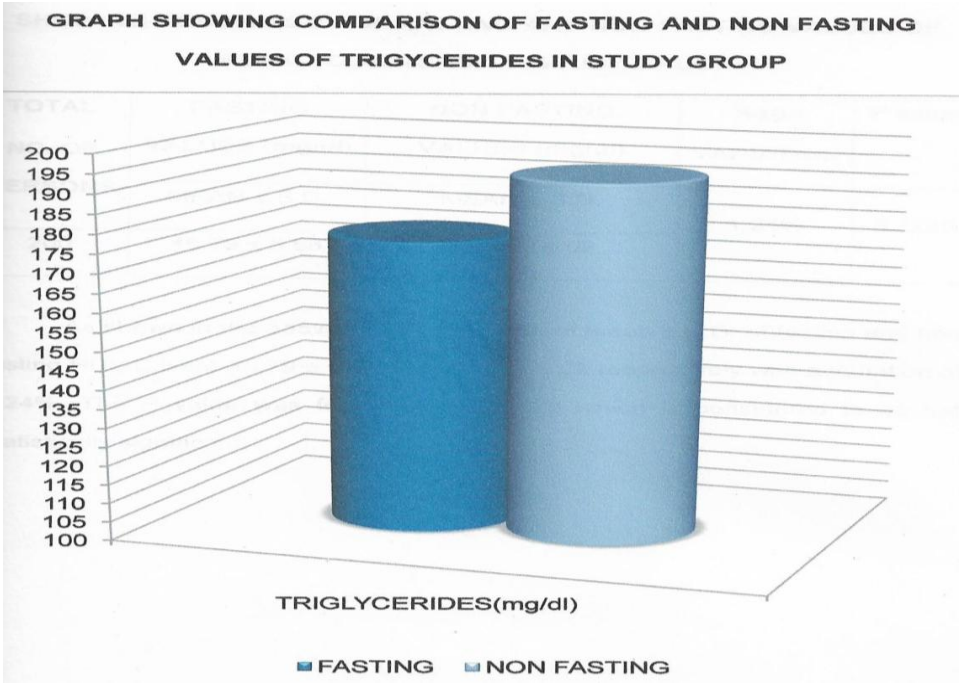
TOTAL NO. OF PERSONS	FASTING LEVELS (mg/dl)	NON FASTING LEVELS (mg/dl)	%age VARIATION	P value
	MEAN+-S.D.	MEAN+-S.D.	9.50%	0.0305
200	176.90+-75.10	193.71+-79.65		

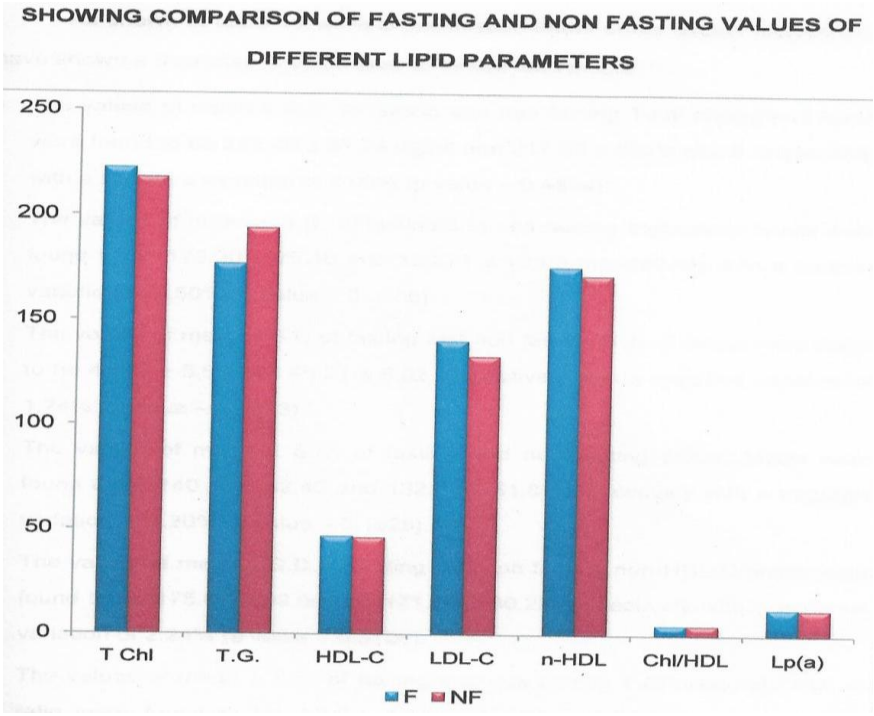
Comparasion of Fasting and Non Fasting Values of Lp (a) in Study Group

TOTAL NO. OF PERSONS	FASTING LEVELS (mg/dl)	NON FASTING LEVELS (mg/dl)	%age VARIATION	P value
	MEAN+-S.D.	MEAN+-S.D.	3.15%	0.0673
200	12.68+-2.17	12.28+-2.19		

Comparasion of Fasting and Non Fasting Values of Different Parameters

LIPID SUBCLASS	FASTING LEVELS (mg/dl) MEAN+-S.D.	NON FASTING LEVELS (mg/dl) MEAN+-S.D.	%age VARIATION	P value
T. CHOL	222.40+-61.74	217.88+-63.08	2.03%	0.4694
TG	176.90+-75.10	193.71+-79.65	9.50%	0.0305
HDL-C	46.79+-5.68	46.21+-6.02	1.24%	0.3223
LDL-C	140.22+-52.40	132.93+-51.87	5.20%	0.1628
Non HDL-C	175.60+-59.09	171.67+-60.28	2.24%	0.5106
T CHO/HDL-C	4.74+-1.10	4.70+-1015	0.84%	0.7224
Lp(a)	12.68+-2.17	12.28+-2.19	3.15%	0.0673





Discussion

This study has found insignificant negative variation between fasting and non fasting values of all the lipid subclasses i.e. T Chl, LDL-C, HDL-C, Non HDL-C, Chl/HDL-C and Lp(a) except Triglyceride which have shown a positive statistically significant variation. A cross sectional study in 2003-2006 in Denmark on Copenhegen general population showed that maximum changes after normal food and fluid intake from fasting levels were – 0.2mmol/l for total cholesterol, -0.2mmol/l for lower density lipoprotein, -0.1mmol/l for HDL cholesterol, and 0.3mmol/l for triglycerides.[28] A large community based cross sectional study over a period of 6 month in 2011 in Canada has found out that variation among mean cholesterol subclass levels were less than 2% for total cholesterol and HDL-C, <10% for calculated LDL-C and <20% for triglycerides among individuals with various fasting times. It concluded that fasting times showed little association with lipid subclass levels which suggests that fasting for routine lipid levels is largely unnessecary.[29]

In another prospective study, in 26,330 healthy women over an 11 year follow up period showed that except for triglycerides, lipid conc. differed minimally (<5%) for fasting vs. non fasting.[30] A decrease in total cholesterol, HDL and LDL is observed for upto 4 hours after a standard meal.[28] A decrease in lipids levels after a meal is perhaps converse to what would be expected, due this is due to dilutionary effect of water contained in the food. Triglycerides are increased for six to eight hours after a standard meal. Peak non fasting TG levels four hours after a meal are reported to be a strong predictor of cardiovascular events and insulin resistance and risk equations may be developed based on these levels in the future.[28,29]Measuring non-fasting TG levels may provide additional information for determining cardiovascular risk

Summary

Serum Lipid profile is measured for cardiovascular risk prediction and has now become almost a routine test. The difference between fasting and non fasting values of different subclasses of lipids i.e. T.- Chl, LDL – C,HDL – C, non HDL-C , Chl./HDL-C was found to be statistically insignificant ($p>0.05$), so it concluded that non fasting sample can be considered for these lipid parameters for cardiovascular risk assessment. The difference between fasting and

non fasting values of TG was statistically significant (p<0.0.5).so postprandial TGs are better predictor of cardiovascular risk, hence post prandial sample can also be considered for TG for as CVD risk assessment. Difference between fasting and non fasting Lp (a) has also found not to be statistically significant (p>0.05), hence showing a positive co-relation with other lipid sub-classes. Non fasting lipid test as concluded would be appropriate in following clinical scenarios: CVD risk assessment, initial investigation of lipid levels (unless the patient has a history of familial hyperlipidaemia), monitoring lipid levels over time, monitoring response to lipid lowering treatment (unless patient has high TGs) and testing for any reason in patients who are hard to reach or have low motivation for undergoing a fasting test.

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