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COMPARISON OF TWO METHODS OF ESTIMATION OF HIGH DENSITY LIPOPROTEIN CHOLESTEROL: DIRECT VERSUS INDIRECT METHOD

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ABSTRACT

Background: High density lipoprotein cholesterol (HDL c) can be estimated in two ways. One is direct, homogenous assay method which is less cumbersome but more expensive. The second one, indirect method is more time consuming but less expensive. However, as far as accuracy is concerned, it is not known that which method is better. Methods: A total of 70 serum samples were analysed by both direct & indirect method. The mean values along with their standard deviation were tabulated and compared by student's t test. Findings: No significant difference in the mean HDL c levels was observed whatever be the level of total cholesterol. The main limitation of the study is that the number of sample analysed is very less. However, this study may be considered as a pilot study as no study report showing this type of comparison is available. Conclusion: In a country like India, where a lot of people belong to below poverty line, estimation of HDL c can be done by indirect method without compromising the quality of the result.

INTRODUCTION

HDL_C is the smallest and densest lipoprotein particle containing high proportion of protein, mostly apolipoproteins, Apo I and Apo II. It transports cholesterol mostly to the liver by reverse cholesterol transport. They are synthesized in the liver as complexes of apolipoproteins and phospholipids. Free cholesterol in plasma gets esterified by an enzyme, lecithin cholesterol acyl transferase (LCAT) and is then sequestrated into the core of HDL particle increasing them in size. HDL thus transport cholesterol present in the peripheral tissue to liver and Is removed by HDL receptors such as scavenger receptor B_1 (SRB_1). The cholesterol delivered in the liver is excreted after conversion into bile acids. HDL also transports cholesterol to adrenals, ovaries and testes, which is important for the synthesis of steroid hormones (1).

Epidemiological and clinical studies have demonstrated a strong negative correlation between high density lipoprotein cholesterol (HDL c) concentrations in serum and the incidence of coronary heart disease (CHD) especially myocardial infarction. Pathological studies have shown that increased HDLc concentration plays a protective role against the development of myocardial infarction (2). According to third report of National Cholesterol Education Program (NCEP), HDL_C level is considered to be low when its concentration is below 40 mg%, and high when its concentration equals or above 60 mg% (3). The estimation of HDL is also important to determine LDLc concentration (low density lipoprotein concentration), high level of which is also known to be significantly associated with CHD. LDLc level is determined by indirect method using Friedewald equation (4) which is as follows:

[LDL cholesterol]= [Total cholesterol] – [HDL cholesterol] – [triglyceride/5].

Thus, HDLc estimation is highly important. In fact, reliability in measurement of all parameters of lipoprotein profile is important according to NCEP (3).

HDLc level can be estimated in two ways. One is direct homogenous assay method (5) based on selective solubilizing effect on HDL. Another is an indirect method (6) where using phosphotungstic acid, all lipoproteins other than HDL is precipitated and HDL present in the supernatant is estimated. The direct method is less time consuming but more costly (Rs. 45 – 50/test) whereas the indirect method is more time consuming and less expensive (Rs. 8 – 10/test). Studies have been reported that significat difference exist in estimation of LDLc level by two different methods, but no report regarding accuracy of two methds in HDLc estimation has been noted so far.

Hence this study was undertaken to estimate HDLc level in serum by direct and indirect method and to compare the data to find out whether any significant difference exist among the two methods.

MATERIALS AND METHODS

This study was conducted in the Department of Biochemistry, NRS Medical College and Hospital from July to November, 2010. A total of 70 patients from different out patients' departments of this hospital were referred to Department of Biochemistry for estimation of lipid profile. These patients (Both sexes and all age groups) are included in this study. The study was approved by appropriate authority.

The patients were advised to come after 10 – 12 hrs of overnight fasting. Blood was collected from each of them in a plain vial, which was centrifuged to separate serum. This serum was used to estimate serum cholesterol and HDL c by both direct and indirect method.

HDL estimation by direct method:

HDL_C level was estimated directly by end point immunoinhibition method using kit from logotech, where antihuman β lipoprotein antibody was allowed to bind to all lipoproteins except HDL_C which was measured by cholesterol esterase and oxidase.

Hydrogen peroxide produced by the enzyme reactions with HDLc, yields a blue color complex upon oxidative condensation of F DAOS (N ethyl N (2 hydroxyl 3sulfopropyl) 3,5 dimethoxy 4 fluroaniline, sodium salt) and 4 aminoantipyrine in the presence of peroxidase. The absorbance of the blue color complex produced is measured at the wavelength of 600nm.

HDL_C estimation by indirect method:

HDL_C level was measured indirectly using kit from Accurex (India).

High Density Lipoproteins are separated from other lipoproteins fractions by treating serum with phosphotungstic acid and magnesium chloride. HDL remains in solution while all other lipoprotein fractions are precipitated. Cholesterol content of the supernatant solution is estimated by enzymatic method.

Serum cholesterol level was measured by CHOD/ PAP method using kit from logotech (India).

All the data were classified into 3 groups on the basis of total serum cholesterol concentrations. Subjects with cholesterol level below 150mg% constituted Group 1 (n = 12). Those with cholesterol level between 150-220mg% and above 22omg% constituted Group2 (n= 36) and Group 3 (n = 22) respectively. The results of HDL c by direct and indirect method were

expressed in mean ± SD. They were compared by student's t test and the results were considered as significant when $p < 0.05$.

RESULT AND DISCUSSION

A total number of 70 patients (29 male and 41 female) were included in the study. Their age group ranged from 26 years to 67 years. Table 1 shows Mean & Standard deviation of HDLc level estimated by direct and indirect method grouped according to different level of serum cholesterol. In none of the group the difference was found to be significant.

HDLC is commonly measured in the following conditions.

A) To screen primary & secondary hyperlipidaemia. The lowest age reported to have CHD is 20 years. Hence adults aged 20 yr or above should have their fasting lipoprotein profile measured once every 5 year. (7)

B) To monitor risk for atherosclerosis especially CHD & stroke. One of the well accepted methods for risk assessment is the Framingham Risk Score which use an algorithm that includes age, gender, total cholesterol, HDL, history of hypertension & cigarette smoking (8).

C) To monitor treatment of hyperlipidaemia. Statin group of drug used to treat hypercholesterolemia is also known to increase level of HDL.

CHD is emerging as a serious problem in developing countries, including India. It is expected to be the single most important cause of death in India by 2015. There is considerable increase in prevalence of CHD both in urban & rural areas though the increase is more in former cases. Some of risk factors of CHD are modifiable, one of which is dyslipidaemia. Each 1% decrease in serum cholesterol (2-3mg / dl) results in approximately 2% decrease in CHD incidence , a relation of considerable clinical & public health significance (9).

Moreover, Cholesterol lowering Atherosclerosis study demonstrated the benefit of cholesterol lowering even in people with normal or moderately increased cholesterol concentration (185-240 mg%) and those with established disease(10).

CONCLUSION

India caters a total population of 1, 181.4 million (2008) of which 60 % population are over 20 yr age group. Thus a huge population need screening for lipid profile of which a lot live below poverty line & quiet a number can not afford excess expenditure for health as they live a hand to month existence. Hence HDLC estimation by indirect method can be used as cost effective

screening option. Laboratories should have the two options of measurement of HDLC (direct & indirect) so that the patients do not have to face the economic burden of investigation unnecessarily. The limitation of this study is less number of samples analysed. As hypercholesterolaemia is more important as a risk factor of CHD, more number of samples should be included in group 3 to come to a proper conclusion.

Table 1: Mean & Standard deviation of HDLc level estimated by Direct and Indirect method

Serum Cholesterol level	HDL c by direct method (Mean ± S.D.)	HDL c by indirect method (Mean ± S.D.)
Group 1 <150mg% (n=12)	36.33 ± 4.72	38.7 ± 2.48
Group 2 150 - 220 mg% (n = 36)	42.82 ± 5.41	42.27 ± 4.83
Group 3 >220 mg% (n = 22)	42.9 ± 8.3	43.54 ± 7.98
Total (n = 70)	42.21 ± 6.59	42.37 ± 5.96

p> 0.05 i.e. non significant in all 3 groups

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