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COMPARATIVE STUDY OF LDL CHOLESTEROL BY DIRECT METHOD VERSUS FRIEDEWALD FORMULA (FF) CALCULATED METHOD IN LOCAL POPULATION

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Abstract

Recent time has seen a lot of studies being conducted to study various risk factors for cardiovascular diseases. LDL cholesterol is one such risk factor that has garnered a lot of attention. Most of the clinical practitioners are depending on laboratory reports where LDLC is done by Friedwald's formula (FF) for their diagnosis. However, with evolving times and technology it is necessary to utilize advanced versions of available tests for accurate diagnosis and categorizing patients as higk risk coronary heart disease (CHD) patients. The aim is to conduct a comparative study of LDLC by direct method versus Friedewald's formula (Calculated LDLC).

A significant difference in LDL and calculated LDL was seen in case of borderline high category of cholesterol (200-239) where p (value)=0.000 which is less than level of significance 5%.

Keywords: Total cholesterol, Direct LDLC , LDL calculated, Triglycerides

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Introduction:

LDL is a very important parameter when it comes to detecting coronary heart diseases (CHD). However it has been observed that 80 – 90 % of labs do LDL by calculation method though LDL direct method kits are available commercially. In a bargain to reduce the cost of test, LDL is calculated by Friedewald's method where LDL is mostly underestimated. Most of the labs in the area of study are giving LDL value calculating using Friedewald formula when triglyceride levels are upto 500 ml/dl, after reports are issued with the remark that "**LDL cannot be calculated**" instead of doing it by direct method. Even renowned pathology laboratories do Direct LDL only if TG levels are above 300 or if it is a mandatory part of a health package. The most common reason for the labs to avoid doing LDL tests is the high cost of the reagents . Even HDL cholesterol is done by precipitation methods in many labs thus increasing the chances of technical errors. In such conditions, where HDL reading is erroneous, calculated LDL values go for a toss.

One of the key indicators of cardiovascular risk is the LDL content, Keevil *et al*.,(2007). Kannan *et al*.,(2014), in their studies concluded that at lower levels of LDL and high TG, calculated LDL by FF can underestimate LDL (in comparison to directly tested LDL). However, for higher LDL strata, FF overestimates LDL. In clinical laboratories, direct assays are currently used. However,

evaluations of these assays have only been done in small cross-sectional or retrospective studies, and there is little data comparing the association of directly measured LDL-C versus Friedewald estimation in association with clinical events, Nauck *et al*.,(2002). They further concluded and emphasised on the use of homogenous LDL-C estimation to FF method. The Friedewald equation is frequently used to predict LDL-C to guide treatment; however, compatibility with direct measurement has received comparatively little investigation, particularly at values of 70 mg/dl now targeted in high-risk patients, Martin *et al*.,(2013).

Studies by Scharnagl *et al*.,(2001), Jun *et al*.,(2004) and Sibal *et al*.,(2010), have also emphisised on the fact that when LDL levels are low and TG levels are high, the FF method may underestimate LDL.

Material and method:

The study was conducted in a private standalone pathology lab and 12 – 14 hours of fasting blood was collected. After centrifugation, the serum was used to analyze Cholesterol, Triglycerides, Direct HDL and Direct LDL and the readings were compared with LDL readings calculated by **Friedewald formula (FF)** where,

LDL = Total Chol – Trig/5 (VLDL) - HDL.

Patients with recent episode of Myocardial infarction (MI) were exempted from the study.

Blood collection and laboratory measurements

Before the blood sample is taken, a tourniquet (elastic) is placed tight on the upper arm. It causes blood to build up and fill the veins, so that the blood sample can easily be taken. In order to prevent bacteria, the skin is cleaned with 70% alcohol swabs before the blood-sample is taken. After collection of the sample, proper pressure was applied at the site with fresh cotton gauze further replacing it with a band - aid. After collection the samples were centrifuged at 3000 rpm for 15 to 20 mins, the serum separated, and were immediately analyzed on Erba chem 5 plus V2 semi - automatic biochemistry analyzer.

Estimation of Cholesterol was done by CHO/POD method. Estimation of triglycerides was done by GPO/POD method. Estimation of HDL was done by Direct method (POD).Estimation of LDL cholesterol was done by selective stabilization technique.

Study population

A total of 576 subjects were studied for fasting lipid profile levels. 3 ml of 12-14 hours fasting sample was collected in plain vacutainer. The patients were advised to follow intake of routine diet for minimum of 3-4 days.

Diagnostic criteria

The study was conducted using fasting 12-14 hours fasting samples for lipid profile levels Normal ranges for these parameters according to the test kit manual are mentioned in table below.

Tests	Sex	Chol.	Tg.	HDL	LDL
Normal ranges	Male	130-200	36-165	30-74	50-150
	Female	130-200	36-165	30-74	50-150

Normal ranges of Serum Lipid profile according to NCEP guidelines are mentioned in table 2. Adult treatment Panel III Recommendation by NCEP

Cholesterol	Desirable	Less than 200 mg/dL
	Borderline	200-239 mg/dL
	High	More than 239 mg/dL
Triglycerides	Normal	Less than 150 mg/dL

	Borderline	150 - 199 mg/dL
	High	200 - 499 mg/dL
	Very high	More than 500 mg/dL
HDL (good cholesterol)	Desirable	More than 39 mg/dL
	Low	Less than 40 mg/dL
LDL cholesterol(Bad cholesterol)	Optimal	Less than 100 mg/dL
	Near/above optimal	100 - 129 mg/dL
	High	130-189 mg/dL

Results:

A total of 594 participants were involved in the study. Among the 594 participants, 302 were females and 292 were males making it to 50.8 and 49.2 percent respectively.

SEX

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	F	302	50.8	50.8	50.8
	M	292	49.2	49.2	100.0
	Total	594	100.0	100.0	

Paired Samples Statistics

Cholestrol_category			Mean	N	Std. Deviation	Std. Error Mean
Desirable	Pair 1	LDL	82.7426	413	23.94782	1.17840
		CALCULATED LDL	74.5935	413	298.72612	14.69935
Boderline High	Pair 1	LDL	114.1859	116	29.95373	2.78113
		CALCULATED LDL	132.5461	116	31.04376	2.88234
High	Pair 1	LDL	1264.3854	48	7782.31584	1123.28054
		CALCULATED LDL	1275.7170	48	7723.32482	1114.76592

Paired Samples Test

Cholestrol_category			Paired Differences					t	df	Sig. (2-tailed)
			Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
						Lower	Upper			
Desirable	Pair 1	LDL - CALCULATED LDL	8.14905	297.32656	14.63048	-20.61066	36.90876	.557	412	.578
Boderline High	Pair 1	LDL - CALCULATED LDL	-18.36021	44.34319	4.11716	-26.51551	-10.20490	-4.459	115	.000
High	Pair 1	LDL - CALCULATED LDL	-11.33154	118.40009	17.08958	-45.71134	23.04826	-.663	47	.511

Parametric student t-test is applied to examine significant difference in LDL and calculated LDL among the categories of cholesterol it is seen that in case of borderline high category of cholesterol (200-239) there is a significant difference in LDL and calculated LDL as p (value)=0.000 which is less than level of significance 5%. Mean LDL =114.89 with SD ± 29.95 and Mean calculated LDL =132.56 with SD ± 31.04.

Paired Samples Statistics

Triglycerides_category			Mean	N	Std. Deviation	Std. Error Mean
Normal	Pair 1	LDL	89.8807	367	45.08541	2.35344
		CALCULATED LDL	88.6202	367	318.28203	16.61419
Mild Hyper TG (Borderline high)	Pair 1	LDL	101.2667	202	33.64942	2.36757
		CALCULATED LDL	104.0916	202	38.85468	2.73381
Moderate Hyper TG (High)	Pair 1	LDL	99.3067	3	44.27038	25.55951
		CALCULATED LDL	20.9000	3	8.06277	4.65504
Severe Hyper TG (Very high)	Pair 1	LDL	10873.7480	5	24138.71864	10795.16315
		CALCULATED LDL	10760.8160	5	23986.66148	10727.16113

Paired Samples Test

Triglycerides_category			Paired Differences					t	df	Sig. (2-tailed)
			Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
						Lower	Upper			
Normal	Pair 1	LDL - CALCULATED LDL	1.26052	317.58107	16.57760	-31.33877	33.85981	.076	366	.939
Mild Hyper TG	Pair 1	LDL - CALCULATED LDL	-2.82490	33.96371	2.38968	-7.53696	1.88715	-1.182	201	.239
Moderate Hyper TG	Pair 1	LDL - CALCULATED LDL	78.40667	42.27964	24.41016	-26.62178	183.43512	3.212	2	.085
Severe Hyper TG	Pair 1	LDL - CALCULATED LDL	112.93200	191.04357	85.43728	-124.27992	350.14392	1.322	4	.257

Discussion:

Several studies have focused on promoting estimation of Direct LDL(homogeneous method) to FF method. Study by Kannan *et al*.,(2014) culminated that estimating LDL by direct method provided adequate information for identifying coronary heart disease (CHD) as well as in achieving the treatment goal. In the our study it was observed that when cholesterol was in the borderline high category i.e. (200-239) there is a significant difference in LDL and calculated LDL. Similarly when the triglycerides were in the range of 200 - 499, there was a vast difference between direct LDL and LDL by FF method. In this case LDL by FF was underestimated. However since the sample size was less for the mentioned category, significant value could not be calculated. Thus through our study we conclude that it is very much important to do LDL by direct method rather than FF for proper management of treatment.

Limitations:

1. LDL particle size.
2. Female hormones effect on LDLC direct to Calculated values.
3. Non-fasting LDL direct and Calculated LDL also need to be analysed.
4. More focus should also be given for an annual lipoprotein and homocysteine level analysis as they help in accurate diagnosis of CVD.

Conclusion

The object of our research was to study the Lipid profile with respect to Direct LDL verses LDL calculated value. Grundy *et al*.,(2004) through their studies have concluded that LDL-C plays an important role in the prevention of CVD. Hence it is very important to do direct LDL rather than calculating LDL by FF method as direct LDL is more accurate as compared to LDL by FF.Also more emphasis should be given to regular apolipoprotein detection to improve and prevent CHD.

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