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Original Article

Small Dense Low-Density Lipoprotein Cholesterol as a Novel Biomarker of Coronary Heart Disease

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ABSTRACT

Objective: To compare serum small dense low-density lipoprotein cholesterol levels in coronary heart disease (CHD) patients and healthy controls.

Study Design: Comparative cross-sectional study.

Place and Duration of Study: Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology (AFIP), Rawalpindi Pakistan, in collaboration with AFIC Rawalpindi from Feb to Dec 2021.

Methodology: A total of 220 participants were selected for the study, 120 healthy controls and 100 CHD patients. Fasting blood samples after 12 hours of fast were collected for lipid profile and small dense low-density lipoprotein cholesterol (sdLDL-C) levels. SdLDL-C levels were analyzed by automated, standardized enzymatic assay on Siemens Advia 1800 automated chemistry analyzer using Ex Denka Seiken kits.

Results: Among the total participants, 154 (70%) were males, and 66(30%) were females. The median age of all participants was 55(IQR: 52 -56) years, while their median sdLDL-C was 0.93 (IQR: 0.56-1.08) mmol/L. Results showed that small, dense LDL cholesterol serum levels were significantly raised in CHD patients compared to healthy controls (p-value <0.05).

Conclusion: The current study showed that patients with CHD had elevated small, dense LDL-C levels compared to the healthy control group.

Keywords: Coronary heart disease, Novel biomarker, Small dense LDL cholesterol.

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INTRODUCTION

CHD is the most common condition globally, with high mortality and morbidity.[1] Over the past years, numeral studies have described its probable risk factors, including dyslipidemia, diabetes mellitus, smoking and hypertension, so that risk assessment of cardiovascular disease can be done at early stages.[2] Dyslipidemia increases the possibility of developing cardiovascular disease(CVD).[3] Classically, dyslipidemia is described by the reduced concentration of high-density lipoprotein cholesterol (HDL-C) and elevated concentration of triglycerides(TG) and low-density lipoprotein cholesterol(LDL-C).[4,5]

LDL cholesterol is heterogeneous, consisting of particles with different diameters, chemical composition and density.[5,6] LDL cholesterol with higher density and small-sized particles is designated as small dense low-density lipoprotein cholesterol (sdLDL-C). This LDL cholesterol having low density and large particle size is described as light and large LDL cholesterol, and medium-density LDL-C is between these two subtypes. SdLDL-C is more atherogenic as compared to LDL-C.[7] As a huge cost is spent on managing CVD, an early biomarker is being searched to predict CHD risk before the event occurs. It has been proved in many clinical and basic studies that sdLDL cholesterol is found to be a risk factor for atherosclerosis development, and raised levels of sdLDL cholesterol are related to CHD development.[8]

Some studies have proved small dense LDL cholesterol to be a superior marker for cardiovascular disease outcomes.[9] Recently, direct assay adjustable to auto analyzers for small dense LDL-C quantification has been developed.[10] Studies are available in the West, but no such research is available nationally. As sdLDL-C has been highlighted as a helpful new marker for coronary heart disease risk estimation. Hence this study has been planned to measure sdLDL-C levels in CHD patients and its comparison with healthy controls.

METHODOLOGY

It was a comparative cross-sectional study conducted at the Department of Chemical Pathology and Endocrinology, AFIP, Rawalpindi Pakistan, in collaboration with AFIC, Rawalpindi, from February to December 2021, with prior ethical approval by the

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The WHO calculator was used for sample size calculation based on the prevalence of CHD in Pakistan (6.25 %).[11] A total of 220 individuals were enrolled in our study, 120 healthy controls and 100 CHD patients through non-probability convenient sampling technique.

Inclusion Criteria: Patient of CHD with a documented history of myocardial infarction or angiographically proven CHD and for controls, healthy individuals without hypertension, CHD, DM, CKD, CLD, any malignancy, and not taking any drugs affecting lipid metabolism were included in the study.

Exclusion Criteria: Patients of less than 18 years and patients with chronic diseases (DM, CKD, CLD or any malignancy) were excluded from our study.

After taking informed consent, blood samples from selected participants were taken in fasting from an antecubital vein in a yellow-topped serum separator tube for lipid profile and sdLDL-C levels. Samples were sent to the laboratory and centrifuged within two hours. Separated serum was stored at -20°C till analysis. Fasting lipid profile was analyzed on random access fully automated chemistry analyzer Siemens Advia 1800. SdLDL-C was analyzed by automated, standardized enzymatic assay on Siemens Advia 1800 using Ex Denka Seiken kits. The assay utilised well-described enzymes and surfactants that selectively reacted with definite groups of lipoproteins.

Statistical Package for Social Sciences (SPSS) version 21.0 was used for the data analysis. The Shapiro Wilk test was applied to determine the determination of normality of the distribution of the data. Data being non-parametric, median and IQR were used to express quantitative variables. Mann Whitney U test was used for statistical comparison. The *p*-value lower than or up to 0.05 was considered as significant.

RESULTS

A total of 220 participants were enrolled in our study, out of which 154 (70%) were males, and 66 (30%) were females. The median age of all participants was 55 (IQR:52-56) years (Figure).

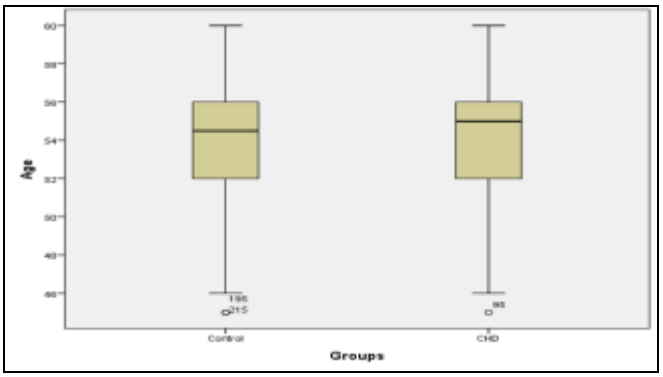

Figure: Distribution of Age in two groups.

While their median sdLDL-C level was 0.93 (IQR: 0.56-1.08) mmol/L as illustrated in Table-I. Results showed that serum levels of sdLDL-C were significantly raised (p<0.05) in patients with CHD compared to the healthy controls Group as shown in Table-II.

Table-I: Baseline Characteristics of Study Population (n=220)

Parameters	Median (IQR) (25th and 75th Percentiles)
Age (years)	55 (52-56)
BMI (kg/m2)	23.90 (23.50-24.10)
PGF (mmol/L)	5.2 (5.0-5.3)
Total Cholesterol(mmol/L)	4.25 (3.64-4.56)
Triglycerides(mmol/L)	1.36 (1.26-1.44)
HDL-C(mmol/L)	1.04 (0.96-1.12)
LDL-C(mmol/L)	2.64 (2.54-2.70)
sdLDL-C(mmol/L)	0.93 (0.56-1.08)

Body mass index; BMI, Plasma glucose fasting; PGF, High density lipoprotein cholesterol; HDL-C, Low density lipoprotein cholesterol; LDL-C, Small dense low density lipoprotein cholesterol; sdLDL-C.

Table-II: Comparison between Median (IQR) values of Coronary Heart Disease Patients and Healthy Controls (n=220)

Parameters	Healthy Controls(n=120)	Coronary Heart Disease (n = 100)	*p*-value
	Median (IQR) 25th and 75th percentiles	Median (IQR) 25th and 75th percentiles	
Age (years)	54 (52-56)	55 (52-56)	0.795
BMI (kg / m2)	23.90 (23.80-24.10)	23.80 (23.40-24.10)	0.123
Placental Growth Factor (mmol / L)	5.2 (5.0-5.4)	5.1 (5.0-5.3)	0.757
Total Cholesterol (mmol / L)	4.34 (3.78-4.57)	4.13 (3.58-4.54)	0.199
Triglycerides (mmol / L)	1.34 (1.26-1.39)	1.42 (1.26-1.48)	<0.001
HDL-C (mmol /L)	1.10 (1.04-1.16)	0.94 (0.87-0.99)	0.000
LDL-C (mmol /L)	2.64 (2.54-2.71)	2.64 (2.54-2.70)	0.623
sdLDL-C((mmol/L)	0.58 (0.48-0.73)	1.08 (1.06-1.09)	<0.001

Body mass index; BMI, Plasma glucose fasting; PGF, High density lipoprotein cholesterol; HDL-C, Low density lipoprotein cholesterol; LDL-

DISCUSSION

Our study results revealed that serum levels of sdLDL-C were significantly raised in CHD patient group in comparison to healthy controls. It also exhibited that patients with CHD had reduced HDL-C concentration and increased triglyceride levels. However, the two study groups observed no significant difference between LDL-C. It had been explained that subcontinent Asian Indians have classical dyslipidemia described by reduced HDL-C levels and high triglycerides with almost normal levels of LDL cholesterol when contrasted with the western population, who were found to have raised levels of LDL cholesterol.[11,12] Patients in our study also have normal range LDL-C concentration.[13,14]

These findings showed that sdLDL-C level is a clinically helpful biomarker that could be utilized to estimate the future event of CHD even in subjects with serum levels of LDL-C within the normal range. In general practice, for CHD prevention, sdLDL-C is a likely therapeutic target.[15]

Median sdLDL-C concentrations were significantly elevated in patients with CHD in comparison to those not having CHD (1.08 vs 0.58 mmol/L, $p < 0.05$). These findings are consistent with earlier studies.[16,17]

A newly established assay for serum sdLDL-C would be easily adjustable to mass screening in common practice, so some prospective research work has evaluated the association between serum levels of sdLDL-C and CHD risk.[18,19] A multiethnic study of atherosclerosis (MESA) showed a positive association between serum levels of sdLDL-C and CHD risk but observed it as a risk factor in non-diabetics onl.[20] Ai *et al*.[10] revealed that sdLDL-C concentrations were raised in those individuals with CHD compared to those without CHD (0.83 vs 0.63 mmol/L). St Pierre *et al*.[21] findings explained that sdLDL-C is a strong and independent predictor of CHD in the initial follow-up period of seven years, supporting the present study.

Hoogeveen *et al*.[19] observed in their study that serum levels of sdLDL-C were strongly associated with a more atherogenic lipid profile and were found to be elevated in diabetic patients than in nondiabetic patients (49.6 vs 42.3 mg/dL, $p<0.001$). For individuals who were supposed to be at lower cardiovascular risk based on their serum LDL-C, serum levels of sdLDL-C were related to the incidence of CHD in ARIC study participants. The ARIC and SUITA study both demonstrated that subjects with raised serum levels of sdLDL-C were at a significantly higher risk of developing CHD than those with decreased levels of sdLDL cholesterol, supporting the present study.[21]

In a study by Higashioka *et al*.[22] in 2019, the relation of serum levels of sdLDL-C with incident CHD was also strong after modification for well-identified risk factors for cardiovascular disease, including serum sdLDL-C levels. Their study exhibited that levels of sdLDL-C were strongly related to the development of CHD.

Similarly, in a study piloted by Goel *et al*.[23] in 2016 in India mean sdLDL-C concentration was raised in patients having coronary heart disease than in those without CHD (16.3 ± 6.8 mg/dl in the CHD group vs 10.1 ± 5.7 mg/dl in the control group, $p<0.001$). In addition, Mohan *et al*.[24] observed that patients with CAD had elevated levels of sdLDL-C compared to healthy controls (16.7 ± 11.1 vs 7.2 ± 6.8 mg/dl).

In this study, we observed that sdLDL cholesterol levels were raised in patients with CHD. For patients at interme-diate risk of CHD, estimation of sdLDL-C is more clinically useful. Individuals having raised sdLDL-C levels might be assigned more insistent treatment protocols.

In the general population, the predictive ability for risk assessment of CHD could be improved by including sdLDL-C into a model consisting of known risk factors.[24] Estimation of sdLDL-C would be helpful for evaluation of the future risk of CHD even in those patients having normal range LDL-C levels. It may be assumed that raised serum LDL-C levels should be reduced intensively to prevent incident CHD. Further research is needed to elucidate whether the reduction of disease burden due to CHD would sdLDL-C be an appropriate target for intervention or not.

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CONCLUSION

The current study showed that patients with CHD had elevated small dense LDL-C levels compared to the healthy control group, although having comparable LDL-C levels. Serum sdLDL-C is a valuable biomarker that can be used to assess the future onset of CHD even in subjects with normal serum LDL-C levels.

Conflict of Interest: None.

Author's Contribution

IS: Literature search, data analysis and concept, MA:, ZHH: Data interpretation, AY: Study design, MUM: Proof reading, MA: Data collection.

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