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Establishment of Reference Values of Small Dense Low Density Lipoprotein Cholesterolin Local Population

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ABSTRACT

Objectives: To establish reference interval of small dense low density lipoprotein cholesterol (sdLDL-C) in local population.
Study Design: Cross sectional study.
Place and Duration of Study: Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology (AFIP), Rawalpindi Pakistan, from Feb 2021 to Dec 2021.
Methodology: One hundred and twenty healthy reference individuals were enrolled in this study. Blood samples for lipid profile and sdLDL-C were collected after 12-hour fast. SdLDL-C levels were analyzed by automated standardized enzymatic assay on Siemens Advia 1800. The normality of distribution of data was determined by Kolmogorov Smirnov test. Data was analyzed using statistical package for social sciences (SPSS) version 21. Data being non parametric, median and inter quartile range (IQR) were used for expression of quantitative variables. For reference interval of sdLDL-C, 2.5th and 97.5th percentiles were used.
Results: A total of 120 healthy participants were enrolled in our study, out of which 84(70%) were male and 36(30%) were female. Median/IQR age of all participants were 43(30 - 51) years, while their median/IQR sdLDL-C level was 0.58(0.48 – 0.73) mmol/L. Reference values of sdLDL-C were 0.33 - 0.99 mmol/L at 2.5th and 97.5th percentiles respectively.
Conclusion: Reference interval for sdLDL-C is 0.33 - 0.99 mmol/L in local healthy population. It is recommended to look beyond conventional lipid profile and estimate sdLDL-C which might help in identifying a high risk population in absence of other known risk factors.

Keywords: Coronary Heart Disease, Reference values, Small Dense LDL Cholesterol.

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INTRODUCTION

Coronary Heart disease (CHD) is the chief cause of mortality and morbidity worldwide. It affects both genders and people of most racial and ethnic groups. In 2019 approximately 18.6 million deaths were attributed to cardiovascular disease globally.[1] More than 20 percent of deaths are attributed to coronary heart disease in Pakistan according to WHO statistics published in 2018.[2] Atherosclerosis is responsible for most CHD events, characterized by atheroma formation in the walls of coronary arteries, causing narrowing and reducing the blood supply to cardiac muscles.[3] Risk factors for atherosclerosis include smoking, hypercholesterolemia, diabetes mellitus, hypertension, obesity and positive family history of CHD.

Early identification of risk factors is required to decrease the incidence of cardiovascular events. In particular hypercholesterolemia which is related to the pathogenesis of atherosclerosis responsible for most cardiovascular disorders.[4] In the circulation, low density lipoprotein cholesterol (LDL-C) is the principal carrier of cholesterol. Raised concentrations of LDL cholesterol were found to be associated with an increased risk of developing cardiovascular diseases (CVD). LDL particles can be subcategorized based upon their size and density into large buoyant LDL cholesterol (lbLDL-C) and small dense LDL-cholesterol (sdLDL-c).[5] SdLDL-c particles are more atherogenic because of their high density and small size, which help them to easily penetrate the vascular endothelium and leads to atheroma formation.[6] The amount of cholesterol released from sdLDL-c particles is associated to the thickening and stiffening of the intima media and to increased risk of CVD.[7] Situations where LDL-C concentration alone is unable to predict residual cardiovascular risk, three factors whose measurement are helpful in this condition include size of particles, their number and concentration of cholesterol in different lipoproteins subclasses.[8] Individuals having normal LDL-C levels and considered to be at low CVD risk, sdLDL-C is able to

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predict incident CVD risk in these also. Techniques available for estimation of sdLDL-C until now were limited to arduous, semi quantitative and highly complex techniques. Semi quantitative approaches include ultracentrifugation, gradient gel electrophoresis and nuclear magnetic resonance imaging but they are laborious and time consuming therefore, not readily adaptable to many samples in a usual clinical laboratory environment.[9] Recently, direct assay adaptable to autoanalyzers for the quantification of sdLDL-C has been developed.

The National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP) considers the preponderance of sdLDL-C particles to be an evolving risk factor for CHD.[10] Small dense LDL-C is recently accepted as a risk factor and potential biomarker for development of CVD by the National Cholesterol Education Program (NCEPIII).[10] Studies are available in the west and their reference values have been established, but no such research is available at the national level. Since the blood levels of this parameter are affected by diet and exercise, it is pertinent to determine population based reference interval before this marker could be utilized for identification of subjects who are at increased risk of developing CHD. Currently there are no local/ national reference interval available for sdLDL-C. Therefore, this study has been planned to determine reference interval of sdLDL-C in local population.

METHODOLOGY

It was a cross sectional study conducted at the department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology, (AFIP), Rawalpindi from February 2021 to Dec 2021 with prior approval by Institutional Review Board (IRB) of AFIP, Rawalpindi, vide number MP-CHP-5/READ-IRB/21/441.Non probability convenient sampling technique was used. Sample size was calculated in line with Clinical and Laboratory Standards Institute (CLSI) guidelines, document C 28-A2, 2000.[11,18] According to Clinical and Laboratory standard Institute (CLSI) guidelines, for determination of reference interval for any parameter the recommended sample size is 120. All laboratories follow these guidelines for reference interval studies.[11,18]

Inclusion Criteria: Detailed questionnaire was applied for collection of data after obtaining informed consent with fulfillment of inclusion criteria that included both genders, age 18 to 60 years, healthy controls without Hypertension, CHD, Diabetes Mellitus (DM), Chronic Kidney Disease (CKD), Chronic Liver Disease (CLD), any malignancy and not taking any drugs affecting lipid metabolism. Reference population included were having following desirable lipid status as proposed by NCEP guidelines ATP (III), Total cholesterol TC <5.2mmol/L (200mg/dl), Triglycerides <1.7 mmol/L (150mg/dl), high density lipoprotein (HDL) cholesterol >1.0mmol/L (40mg/dl) in men and >1.3mmol/L (50mg/dl) in women and LDL cholesterol < 3.4mmol/L(130mg/dl).[10]

Exclusion Criteria: Age less than 18 years, patients with chronic diseases (DM, CKD, CLD or any malignancy), and those who are having lipid profile levels above the cutoff values as proposed by NCEP were excluded from our study. Serum samples from 120 healthy reference individuals were collected after a 12 hour overnight fast for lipid profile and small dense LDL cholesterol (sdLDL-C) levels. Separated serum was stored at -20^{o}C till analysis. Fasting lipid profile and sdLDL-C were analyzed on Siemens Advia 1800 by automated standardized enzymatic assay (sLDL-Ex Seiken kits).

Data was analyzed by Statistical Package for Social Sciences software (SPSS) version 21. Kolmogorov Smirnov (K-S) test was utilized to determine the normality of distribution of data. As data was non parametric, median and inter quartile range (IQR) were used for expression of quantitative variables. For reference interval of sdLDL-C 2.5th and 97.5th percentiles were computed using the formula 0.025(n+1) and 0.975(n+1), which corresponded to rank number 3 and 118 respectively with 90% confidence interval(CI).

RESULTS

A total of 120 healthy reference individuals were enrolled in our study, out of which 36(30%) were female and 84(70%) were male. Median/IQR age of all participants was 43(30 - 51) years, while their median/IQR BMI was 23.40(22.70 – 24.02) kg/m^2. Median/IQR values for total cholesterol were 4.14(3.58 - 4.54) mmol/L, for triglycerides 0.83(0.64 – 0.99)mmol/L, for HDL-C 1.36(1.32 – 1.45) mmol/L, for LDL-C 2.48(2.21 – 2.54) mmol/L and for sdLDL-C 0.58(0.48 – 0.73) mmol/L. Reference values of sdLDL-C were 0.33 - 0.99 mmol/l at 2.5th and 97.5th percentiles respectively.

Table-I: Baseline Characteristics of Study Population (n=120)

Parameter	Median (IQR) (25th – 75th)
Age (years)	43.00 (30.00 – 51.00)
BMI (Kg/ m²)	23.40 (22.70- 24.02))
Total Cholesterol (mmol/ L)	4.14 (3.58 – 4.54)
Triglycerides (mmol/ L)	0.83 (0.64 – 0.99)
HDL-C (mmol /L)	1.36 (1.32 – 1.45)
LDL -C(mmol / L)	2.48 (2.21 – 2.54)
sdLDL-C (mmol / L)	0.58 (0.48 – 0.73)

Body Mass Index; BMI, High density lipoprotein cholesterol; HDL-C, Low density lipoprotein cholesterol; LDL-C, Small dense low density lipoprotein cholesterol; sdLDL-C

Table-II: Non Parametric Determination of sdLDL-C Reference Interval (n=120)

Calculation of Rank Numbers of Percentiles		
Lower	0.025(120+1) = 3.01	Rank No 3
Upper	0.975(120+1) = 117.97	Rank No 118
Original Values Corresponding to these Rank Numbers		
Lower Limit (mmol/L)	2.5 percentile	0.33
Upper Limit (mmol/L)	97.5 percentile	0.99
Rank Numbers and Values of the 0.90 Confidence Limits		
Lower Reference Limits	Rank No 1 and 7	Values 0.25 and 0.36
Upper Reference Limits	Rank No 114 and 120	Values 0.98 and 1.06
Summary		
sdLDL-C Lower Reference Limit (mmol/L)		0.33(0.25 to 0.36)
sdLDL-C Upper Reference Limit (mmol/L)		0.99(0.98 to 1.06)

DISCUSSION

Health associated reference values for every laboratory analyte are universally required for the interpretation of medical laboratory results. In our study, we established reference values of small dense LDL-C in local population. Median/IQR of sdLDL cholesterol was 0.58(0.48 – 0.73) mmol/L and reference values of sdLDL-C were 0.33 to 0.99 mmol/L at 2.5th and 97.5th percentile respectively.

Median age in our study group was 43.50(13) years whereas mean age group in study conducted by Ai *et al.,* was 57.1±9.7 years[12] and in study carried out by Goel *et al.,* it was 51.2±11.7 years[13] and in study conducted by Hoogeveen *et al.,* in 2014 it was 62.83±5.67 years.[8] In our study 68% were males and 32% were females while in study by Ai *et al.,* 55% were males and 45% were females,[12] in Goel *et al.,* study 80% were males and 20% were females.[13] Median BMI in our study was 23.40(1.3) kg/m², while mean BMI in Ai *et al.,* study[12] was 28.2±4.3 kg/m² and in Goel *et al.,* study[13] was 24.9±3.1 kg/m².

A prospective study conducted by Ai *et al.,* in 2010 for establishment of reference interval in Framingham offsprings study cycle.[6] Reference interval established was 0.43 – 1.21mmol/L and mean sdLDL-C level was 0.82±0.39 mmol/L.[12] The difference in reference values could be explained on basis of dietary habits and lifestyle. Higher values in this population could be due to utilization of saturated fats and carbohydrates.

In 2016, Goel *et al.,* observed an association between sdLDL-C and CAD in North Indian patients and mean sdLDL-C level was 0.77±0.38mmol/L with an interval of 0.39 to 1.15mmol/L in healthy controls. The difference in values could be explained on basis of difference in dietary habits and lifestyle modifications.[13] In Koba *et al.,* 2008 study mean sdLDL-C was 0.74±0.41 with a range of 0.33 – 1.16 mmol/L in healthy control group.[14] In ARIC study by Hoogeveen *et al.,* 2014 mean sdLDL-C level was 1.12 ±0.53 with a range of 0.58 – 1.66 mmol/L by direct measurement.[8] In SUITA study by Arai *et al.,* in 2013 mean sdLDL-C was 0.16 – 0.72 mmol/L.[15]

A study conducted by Fernandez-Cidon *et al.,* in Spain in 2017 on reference interval of sdLDL-C in Mediterranean population, with a sample size of 79 participants and their reference interval obtained was 0.04 to 0.47 mmol/L.[16] Their method was different, uses lipoprint LDL system. Evaluation of method specific reference values instead of using universal reference interval in a clinical setting improves the decision making process facilitating the risk stratification for CVD. Reference intervals are very important in preventive medicine.

In prospective study conducted by Higashioka *et al.,* 2019 Hisayama study median sdLDL-C level was 0.85mmol/L.[17] In recent years, the incidence of atherosclerosis and CAD is increasing which may be due to adverse changes in lifestyle such as poly unsaturated fatty acid (PUFA) deficient diet, physical inactivity and a higher genetic predisposition. The incidence of CHD events has considerably reduced via management with the therapeutic lifestyle changes with statins or statins combination with fibrates/niacin.[18] Multicentric studies should be conducted. A cut off value should be established, as in future this biomarker can be used for risk stratification of CHD patients.

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CONCLUSION

Reference interval for sdLDL-C is 0.33 to 0.99 mmol/L in local healthy population. It is recommended to look

beyond conventional lipid profile and estimate sdLDL-C which might help in identifying a high risk population in absence of other known risk factors.

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Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

IS & MA: Data acquisition, data analysis, critical review, approval of the final version to be published.

ZHH & MUM: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

AY & MY: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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