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Establishment of reference intervals of oxidized low-density lipoprotein cholesterol in a population of West Bengal

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ABSTRACT

Background: Elevated oxidized low-density lipoprotein (Ox-LDL) was significantly associated with the atherosclerotic cardiovascular disease as well as retinal vascular diseases but there were no studies to show the reference range of Ox-LDL levels in any Indian population. **Aims and Objectives:** The aim of this study was to formulate the reference intervals of Ox-LDL cholesterol in a population of West Bengal. **Materials and Methods:** Ox-LDL levels of 434 apparently healthy individuals were estimated based on the history, clinical examination, and laboratory investigations. Statistical analysis was performed using the statistical package for the social sciences software. **Results:** The reference range of Ox-LDL levels for all ages was 34.1 ± 4.9 mol/L in healthy male and 34.5 ± 4.5 mol/L in healthy female. The 2.5th and 97.5th percentile values of Ox-LDL level in the reference population were 27 (0.90 CI = 26.4–27.6) and 42 (0.90 CI = 41.4–42.6), respectively. **Conclusions:** Reference intervals for Ox-LDL may help clinicians to predict the future risk for coronary heart disease and retinal vascular disease by taking medical decision limits and also open up the scope for further research for other laboratories to formulate their own reference interval of Ox-LDL.

Key words: Reference intervals; Oxidized low-density lipoprotein; Percentile

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INTRODUCTION

Increase low-density lipoprotein (LDL) and decrease high-density lipoprotein (HDL) are the established causes of coronary artery disease.[1] Elevated oxidized LDL (Ox-LDL) was significantly associated with atherosclerotic cardiovascular disease (ASCVD)[2-4] as well as retinal vascular disease.[5] Oxidized fraction of LDL induces more cholesterol accumulation in macrophages through rapid uptake by the scavenger receptor, which is not downregulated in response to increase intracellular cholesterol.[6]

The International Federation of Clinical Chemistry (IFCC) emphasized the necessity of every laboratory to have its own set of reference limits which helps the clinician in interpreting the observed values obtained from an individual under clinical investigations.[7] Unfortunately, many laboratories in India mostly followed the reference range mentioned on the kit literature which do not match with the Indian population. The reference values are obtained by observation or measurement of a particular type of quantity on reference individuals who are selected by the use of defined criteria for comparison with individuals under clinical investigation.[8]

Aims and objectives

There were studies to show the reference range of serum lipid parameters in a population of Kolkata, West Bengal[9] but no study has determined the reference interval of Ox-LDL cholesterol in this population. Hence, this study wants

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to prepare a reference range of Ox-LDL cholesterol in a population of West Bengal.

MATERIALS AND METHODS

A total of 434 apparently healthy subjects including 225 males and 209 females from West Bengal were enrolled in the 18-month long cross-sectional observational study. The age of reference individuals ranged from 21 to 58 years. Reference individuals were selected from those persons who accompanied the patients attending the outpatient department of ophthalmology for cataract operations in a medical college. The Institutional Ethics Committee approved the study and informed consent was obtained from all the study populations, in accordance with the Declaration of Helsinki. A detailed questionnaire on social status, family history, drug history, dietary habits, addiction to alcohol and smoking, and history of systemic and thromboembolic diseases was completed by the study participants. Patients with hypertension, diabetes mellitus, cardiovascular disease, high LDL cholesterol, low HDL cholesterol, and high homocysteine, renal disease, liver disease, hematologic and coagulation abnormalities, and on anti-oxidants, statins, and fenofibrate therapy were excluded from the study.

Measurement of Ox-LDL

Venous blood samples were collected into the tubes containing no anticoagulant after an overnight fasting from participants. Serum was separated from blood cells by centrifugation at 2000×g for 10 min. Measurement of circulating Ox-LDL was done by precipitation method.[10] Total cholesterol (cholesterol oxidase-peroxidase method), triglyceride (glycerophosphate oxidase-peroxidase method), HDL cholesterol (direct method), and LDL cholesterol (direct method) were measured by enzymatic assays.[11-14]

Statistical analysis

Of the strategies available for the selection of reference individuals, only the direct selection method agrees with the reference values concept as recommended by the IFCC.[7] Statistical analysis was performed using the statistical package for the social sciences software. The type of distribution was determined with the Kolmogorov–Smirnov test. Mean, standard deviation, median, and 2.5 and 97.5th percentile with the 0.90 confidence interval of each percentile values of Ox-LDL were presented.

RESULTS

The frequency distribution of Ox-LDL levels showed normal Gaussian distribution (Figure 1). The reference range of Ox-LDL levels for all ages was 34.1±4.9 mol/L in healthy males and 34.5±4.5 mol/L in healthy females (Table 1). The median values of Ox-LDL levels were 34 mol/L in males and 35 mol/L in females, respectively (Table 1). The 2.5th and 97.5th percentile values of Ox-LDL level in the reference population were 27 (0.90 CI = 26.4–27.6) and 42 (0.90 CI = 41.4–42.6), respectively (Table 2).

DISCUSSION

Association between circulating Ox-LDL and ASCVD is already proven.[15] Hartley et al., have described the role of Ox-LDL and anti-Ox-LDL antibodies in atherosclerosis.[16] Jiang et al., highlighted the mechanisms of Ox-LDL-mediated endothelial dysfunction and its consequences for the development of atherosclerosis.[17] Ox-LDL also triggers changes in oxidative stress and inflammatory biomarkers in human macrophages.[18] The Ox-LDL-induced atherosclerosis may be responsible for the retinal venous occlusion in the absence of other risk factors.[19] We have described the prevalence of Ox-LDL in retinal venous occlusion in a population of West Bengal.[20]

A simple laboratory result of a patient is useless without appropriate data for comparison. Reference intervals

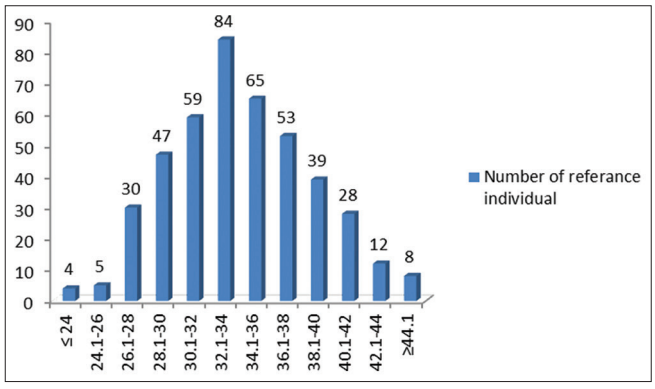

Figure 1: Frequency distribution of oxidized low-density lipoprotein level (mol/L) in a population of West Bengal

Table 1: The of Ox-LDL levels in a population of West Bengal

Group	n	Mean±SD (mol/L)	Median (mol/L)	Min-Max	2.5th percentile	97.5th percentile
Male	225	34.1±4.9	34	19–49	27	42
Female	209	34.5±4.5	35	22–48	26.2	42.3
Total	434	34.3±4.7	34	19–48	27	42

Ox-LDL: Oxidized low-density lipoprotein

Table 2: Percentile values of Ox-LDL level (mol/L) with 0.90 confidence interval in a population of West Bengal

Group	Percentile	Percentile value	Lower confidence limit	Upper confidence limit
Male	2.5th	27	26.1	27.9
	97.5th	42	41.1	42.9
Female	2.5th	26.2	25.3	27.1
	97.5th	42.3	41.4	43.2
Total	2.5th	27	26.4	27.6
	97.5th	42	41.4	42.6

Ox-LDL: Oxidized low-density lipoprotein

denote the normative values of laboratory parameters that can be used by diagnostic centers for clinical diagnosis. As India is a land of racial and ethnic diversity, it is mandatory to establish reference intervals specific to a particular Indian population.[8]

Limitations of the study

Decade-wise reference intervals could not be formulated due to low sample size.

CONCLUSIONS

Reference intervals for Ox-LDL may help clinicians to predict the future risk for coronary heart disease and retinal vascular disease by taking medical decision limits and also open up the scope for further research for other laboratories to formulate their own reference interval of Ox-LDL.

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REFERENCES

1. Rifai N, Bachori K and Albers JJ. Lipids, lipoproteins and apolipoproteins. In: Border BG, editor. Tietz Fundamentals of Clinical chemistry. Philadelphia, PA: Saunders an Imprint of Elsevier; 2006. p. 462-493.
2. Zhang YC, Tang Y, Chen Y, Huang XH, Zhang M, Chen J, et al. Oxidized low-density lipoprotein and C-reactive protein have combined utility for better predicting prognosis after acute coronary syndrome. Cell Biochem Biophys. 2014;68(2):379-385. https://doi.org/10.1007/s12013-013-9718-1
3. Gómez M, Valle V, Arós F, Sanz G, Sala J, Fiol M, et al. Oxidized LDL, lipoprotein (a) and other emergent risk factors in acute myocardial infarction (FORTIAM study). Rev Esp Cardiol. 2009;62(4):373-382. https://doi.org/10.1016/s1885-5857(09)71664-0
4. Johnston N, Jernberg T, Lagerqvist B, Siegbahn A and Wallentin L. Oxidized low-density lipoprotein as a predictor of outcome in patients with unstable coronary artery disease. Int J Cardiol. 2006;113(2):167-173. https://doi.org/10.1016/j.ijcard.2005.11.006
5. Idrees, Z, Dooley I, Power B, Yeung AM and Dwyer MH. Levels of oxidized low-density lipoprotein and high sensitive C-reactive protein in retinal vascular occlusions: A pilot study. J Adv Med Pharm Sci. 2014;1(1):24-29. https://doi.org/10.9734/JAMPS/2014/11341
6. Steinbrecher UP, Parthasarathy S, Leake DS, Witztum JL and Steinberg D. Modification of low density lipoprotein by endothelial cells involves lipid peroxidation and degradation of low density lipoprotein phospholipids. Proc Natl Acad Sci U S A. 1984;81(12):3883-3887. https://doi.org/10.1073/pnas.81.12.3883
7. Solberg H. International federation of clinical chemistry (IFCC), scientific committee, clinical section, expert panel on theory of reference values, and international committee for standardization in haematology (ICSH), standing committee on reference values. Approved recommendation (1986) on the theory of reference values. Part 1. The concept of reference values. J Clin Chem Clin Biochem. 1987;25(5)337-342.
8. Malati T. Whether western normative laboratory values used for clinical diagnosis are applicable to Indian population? An overview on reference interval. Indian J Clin Biochem. 2009;24(2):111-122. https://doi.org/10.1007/s12291-009-0022-1
9. Goswami K and Bandyopadhyay A. Lipid profile in middle class Bengali population of Kolkata. Indian J Clin Biochem. 2003;18(2):127-130. https://doi.org/10.1007/BF02867378
10. Ahotupa M, Ruutu M and Mantyla E. Simple methods of quantifying oxidation products and antioxidant potential of low density lipoproteins. Clin Biochem. 1996;29(2):139-144. https://doi.org/10.1016/0009-9120(95)02043-8
11. Rifai N, Bachorik PS and Albers JJ. Lipids, lipoprotein and apolipoprotein. In: Burtis CA and Ashwood R, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia, PA: W.B. Saunders Company; 1999. p. 806-861.
12. Mc Gowan MW, Artiss JD, Standbergh DR and Zark B. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. Clin Chem. 1983;29(3):538-542.
13. Sugiuchi H, Uji Y, Okabe H, Irie T, Uekama K, Kayahara N, et al. Direct measurement of high-density lipoprotein cholesterol in serum with polyethylene glycol-modified enzymes and sulfated alpha-cyclodextrin. Clin Chem. 1995;41(5):717-723.
14. Armstrong V and Seidel D. Evaluation of a commercial kit for the determination of LDL-cholesterol in serum based on precipitation of LDL with dextran sulphate. Arztl Lab. 1985;31:325-330.
15. Gao S, Zhao D, Wang M, Zhao F, Han X, Qi Y, et al. Association between circulating oxidized LDL and atherosclerotic cardiovascular disease: A meta-analysis of observational studies. Can J Cardiol. 2017;33(12):1624-1632. https://doi.org/10.1016/j.cjca.2017.07.015
16. Hartley A, Haskard D and Khamis R. Oxidized LDL and antioxidized LDL antibodies in atherosclerosis-novel insights

and future directions in diagnosis and therapy<sup/>. Trends Cardiovasc Med. 2019;29(1):22-26.

https://doi.org/10.1016/j.tcm.2018.05.010

17. Jiang H, Zhou Y, Nabavi SM, Sahebkar A, Little PJ, Xu S, et al. Mechanisms of oxidized LDL-mediated endothelial dysfunction and its consequences for the development of atherosclerosis. Front Cardiovasc Med. 2022;9(1):925923.

    https://doi.org/10.3389/fcvm.2022.925923

18. Lara-Guzmán OJ, Gil-Izquierdo Á, Medina S, Osorio E, Álvarez-Quintero R, Zuluaga N, et al. Oxidized LDL triggers changes in oxidative stress and inflammatory biomarkers in human macrophages. Redox Biol. 2018;15:1-11.

    https://doi.org10.1016/j.redox.2017.11.017

19. Lahiri KD, Gupta AK and Biswas UK. A study on oxidized-LDL cholesterol in normolipidemic retinal vein occlusion. Asian J Med Sci. 2023;14(5):71-74.

    https://doi.org/10.3126/ajms.v14i5.51305

20. Lahiri KD, Gupta AK and Biswas UK. A study to determine the prevalence of oxidised low-density lipoprotein in retinal venous occlusion in a population of West Bengal. Asian J Med Sci. 2023;14(10):79-82.

    https://doi.org/10.3126/ajms.v14i10.54565

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KDL- Definition of intellectual content, literature survey, prepared the first draft of manuscript, implementation of the study protocol, data collection, data analysis, manuscript preparation, and submission of the article; **KDL**, **AKG**, **UKB**- Concept, design, clinical protocol, manuscript preparation, editing, and manuscript revision; **KDL**, **AKG**, **UKB**- Design of study, statistical Analysis and Interpretation; **KDL**, **AKG**, **UKB**- Review Manuscript; **KDL-** Review manuscript; **KDL, UKB**- Literature survey and preparation of figures; **KDL-** Coordination and manuscript revision.

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