

Utility of Lipoprotein (a) as a marker of cardiovascular disease risk in hypothyroid patients.

Abstract

Aim: The aim of our study is to assess the utility of lipoprotein (a) as reliable cardiovascular risk marker in hypothyroid patients. This involved estimation and comparison of lipoprotein (a) levels between hypothyroid patients and healthy adults. To correlate lipoprotein (a) [Lp(a)] with thyroid stimulating hormone is the other objective of this study.

Study design: Case-control study

Place and the duration of the study: Department of Biochemistry and Department of General Medicine, Rajarajeswari Medical College and Hospital, Bengaluru, between January 2014 and May 2014.

Methodology: Forty one individuals aged between 18 and 55 years, who were newly diagnosed with hypothyroidism were our cases. Twenty nine age and sex matched healthy volunteers constituted the controls. Serum Thyroid stimulating hormone (TSH), Free Thyroxine (FT₄), Serum total cholesterol (TC), serum low density lipoprotein (LDL), serum high density lipoprotein (HDL), serum triglycerides (TGL) and serum Lipoprotein (a) were estimated by standard methods in cases and controls. The anthropometric data included measurement of weight and height of study subjects in order to calculate their body mass index (BMI).

Results: The mean $\pm$ SD of the lipid parameters (TC, LDL, VLDL, HDL and TGL) in both the groups are almost same and are at the upper limit of the reference range. The mean $\pm$ SD levels of Lp(a) in cases is 39.4 ± 26.5 mg/dl and 18.1 ± 7.4 mg/dl in controls. There was no

25 correlation between lipoprotein (a) and thyroid stimulating hormone in both cases and
26 controls.

27 Conclusion: Lipoprotein (a) levels are elevated in hypothyroidism and it can be considered
28 as a reliable marker to detect cardiovascular disease risk in hypothyroid patients. We
29 recommend Lp (a) estimation by a standard procedure in hypothyroid patients for detection
30 of CVD risk in these patients.

31 **Key words:** Lipoprotein (a) [Lp (a)], Hypothyroidism, Cardiovascular disease (CVD) risk,
32 Dyslipidemia, Thyroid stimulating hormone (TSH).

33

34 **Introduction**

35 Dysfunction and anatomic abnormalities of the thyroid are among the most common
36 diseases of the endocrine glands. Recent studies show that about two hundred million people
37 worldwide suffer from some form of thyroid disorder[1]. Hypothyroidism defined as low free
38 thyroxine [T_4] with a normal or high thyroid stimulating hormone (TSH) is one of the most
39 common disorders of thyroid gland[2]. The prevalence of hypothyroidism is 4% to 5% in the
40 developed countries[3]. Back home the picture is no better. According to a recent survey,
41 prevalence of hypothyroidism is 10.9% among urban Indian population with the women
42 being three times more prone to the disease than men[4].

43 Thyroid hormones (Triiodothyronine [T_3] and Thyroxine [T_4]), act through thyroid
44 hormone receptors widely distributed across all the cells of the body and help maintain
45 thermogenic and metabolic homeostasis. This includes regulating the synthesis, metabolism,
46 and mobilization of lipids[5]. Dyslipidemia is a common feature of an underactive thyroid.

47 In patients with overt hypothyroidism there is an increase in serum total
48 cholesterol(TC), low-density lipoprotein (LDL) cholesterol, apolipoprotein B, lipoprotein(a)
49 [Lp(a)] levels, and possibly triglyceride(TGL) levels also. An increase in low density
50 lipoprotein is due to reduced clearance of LDL from the plasma and also due to decrease in
51 the number and activity of LDL receptors. A reduced activity of lipoprotein lipase leads to
52 decreased clearance of triglycerides rich lipoproteins resulting in elevated levels of
53 triglycerides and very low density lipoprotein (VLDL)[6].

54 Dyslipidemia with other cardiovascular changes which include decreased cardiac
55 output and myocardial contractility, increased peripheral vascular resistance act
56 synergistically and subjects hypothyroid patients to an early and accelerated cardiovascular
57 disease risk. However, researchers have observed that the cardiovascular changes (both
58 hemodynamic and atherosclerotic) are reversible when the underlying thyroid disorder is
59 detected and treated at an early stage[7].

60 Lipoprotein (a) [Lp (a)] is an LDL like particle in which apoprotein B100 is linked by
61 a single interchain disulfide bridge to a unique glycoprotein apoprotein (a) [Apo (a)]. It has
62 both prothrombogenic and proatherogenic properties. Lipoprotein (a) for long has been a
63 non-traditional cardiovascular disease (CVD) risk marker. Infact an increase in Lp (a) level is
64 the most common inherited lipid disorders in subjects with premature coronary heart disease
65 (CHD)[8]. This feature of Lp(a) makes it an apt marker to detect hypothyroid patients
66 susceptible to early CVD due to multiple factors.

67 There have been quite a few studies of Lp (a) in hypothyroidism in the recent past. Each
68 contradicting the other, thus building up the curiosity about Lp(a) role and its behaviour in
69 hypothyroidism. So in the current study the search is for Lp(a) levels in newly diagnosed
70 hypothyroid subjects and to look for the correlation between Lp(a) and TSH, if any.

71 **Methodology**

72 Ours is a case-control study. The research project was carried out in Rajarajeswari
73 Medical College and Hospital, Bengaluru, between January 2014 and May 2014. In the
74 General Medicine out patient department forty one adults with the following criteria were
75 identified and classified as cases: newly diagnosed overt hypothyroid patients aged between
76 18 and 55 years, without any history of medical or surgical illness. Twenty nine, age and sex
77 matched apparently healthy volunteers were inducted into this study as controls. Drugs like
78 ascorbic acid, androgens, estrogen, aspirin, statins and fenofibrate affect Lp(a) levels and
79 none of the subjects in our study were on any these drugs. A written and an informed consent
80 was taken from all the participants of this study. Also the project was granted the Institutional
81 Ethics committee approval.

82 In a clot activator containing nVac tube 5 ml fasting blood sample was collected from
83 each of the study subject . The serum was separated and the following biochemical tests were
84 run. Serum Thyroid stimulating hormone (TSH), Free Thyroxine (FT₄), Serum total
85 cholesterol (TC), serum low density lipoprotein (LDL), serum high density lipoprotein
86 (HDL), serum triglycerides (TGL) and serum Lipoprotein (a). Serum very low density
87 lipoprotein,(VLDL) was calculated using the formula $VLDL = TGL/5$.

88 TSH and FT₄ were estimated by Chemiluminescence immunoassay method on Fully
89 automated Maglumi 1000 analyser.

90 Serum total cholesterol was estimated by cholesterol oxidase peroxidase method,
91 serum LDL by direct photometric method, HDL by Immuno FS and serum triglycerides was
92 measured by glycerol phosphate oxidase -peroxidase method . All these lipid parameters
93 were processed on fully automated Mindray BS 300 analyser.

94 Apart from biochemical parameters, the anthropometry which included weight and
95 height of study subjects was also recorded according to standard protocol. The Body mass
96 index of each individual was calculated as weight in kilogram divided by the square of height
97 in meters.

98 **Lipoprotein (a) assay:**

99 Biochemistry of Lipoprotein (a): Lp (a) is a plasma lipoprotein produced in the liver, very
100 similar in structure and density to LDL. It has a cholesterol rich TGL core encapsulated by a
101 layer of phospholipid and free cholesterol [9]. It has a single molecule of apoprotein B
102 attached to the surface.

103 Lp (a) contains structurally unique protein apo (a) , the size of which is genetically
104 determined and highly variable. Apo (a) contains multiple repeated corresponding
105 plasminogen kringle (K) domains which are similar in sequence to plasminogen. The
106 variation in number of K sequences in apo (a) give rise to Lp(a) isoform heterogeneity. Most
107 commonly the Lp(a) carried in particles with smaller size apo (a) are those associated with
108 CVD and subclinical atherosclerosis.

109 The apo(a) gene has 10 different types of plasminogen-like K4 domains, referred to as
110 K4 type 1 through type 10. The first type (type1) and K4 types 3-10 repeats are present in
111 only one copy each; K4 type 2 is present as multiple copies. The encoding sequences vary
112 from 3-48 copies. It is the variable K4 type 2 repeats that are responsible for the heterogeneity
113 in size variation in the apo(a) glycoprotein [10]. Lp(a) size is inversely related to Lp(a)
114 plasma levels[11].

115 Lp (a) Assay: Lipoprotein (a) can be measured by Enzyme linked immunosorbent
116 assay (ELISA), immunoturbidimetric, immunonephelometric, radial immunodiffusion and

electro immunoassays. Lipoprotein (a) levels can be expressed in terms of its constituents, such as cholesterol, protein mass and mass of apo (a) or B-100 [12].

The immunoassays with apo(a) sizes smaller than the size of the apo(a) in calibrators tend to underestimate Lp(a) concentration in samples, whereas Lp(a) concentration in samples with larger apo(a) isoforms are overestimated [13]. This can be overcome by using 5-point calibrator which takes into account the heterogeneity of the Lp(a) molecule in the assay and also by quantification of Lp(a) by measuring cholesterol content [14].

We estimated Lp (a) by particle enhanced immunoturbidimetric method. The calibration curve was obtained with 5 calibrators (5-point calibration) before processing the patient samples. The samples were processed on Mindray BS 300 fully automated analyser. The reference range of Lp (a) is < 30 mg/dl. Values >30 mg/dl associated with increased risk of cardiovascular disease.

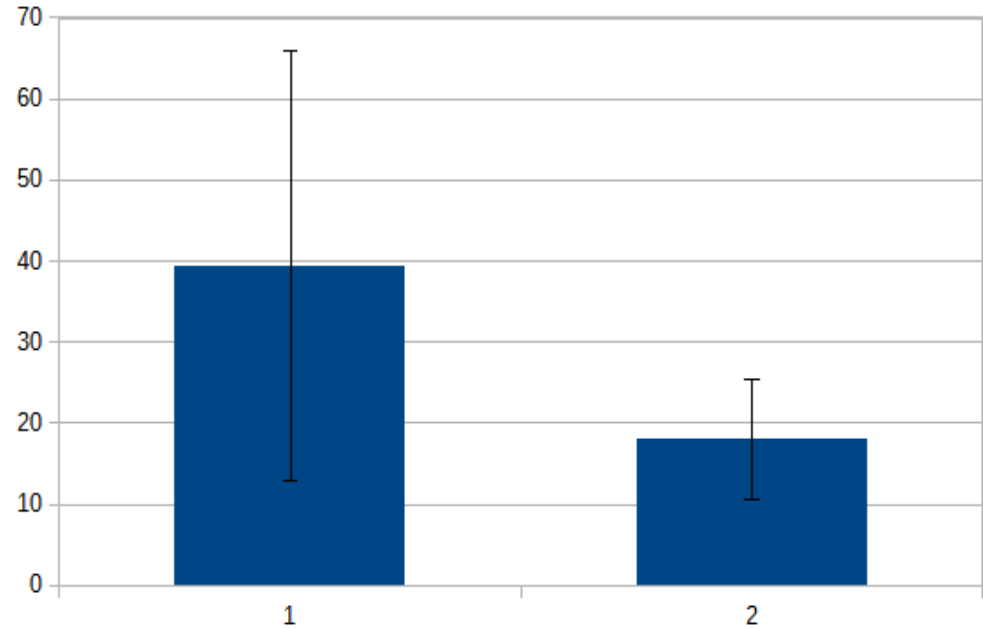
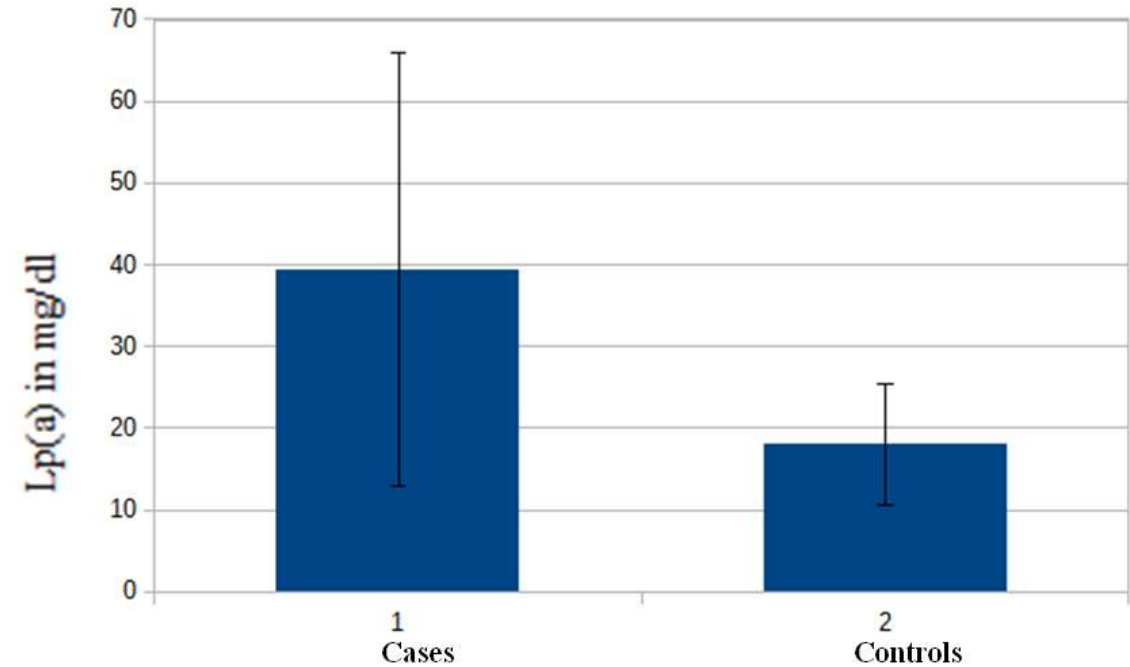
Statistical analysis:

The data was compiled and analysed for both cases and the controls. The biochemical parameters were expressed as mean and standard deviation (SD) . Correlations between the parameters within the group were done using Pearson's correlation. The variables between cases and controls were compared using student 't' test. All statistical analysis was done at 5% level of significance.

Results

We begin analysing the data by comparing the mean lipoprotein (a) levels between the cases and the controls. Figure 1 represents and highlights the significant difference in the principle analyte of this study, lipoprotein (a) in both the groups.

Figure 1: Lipoprotein (a) levels in cases and controls



153

154

155

156

157

158

159

160 Table 1 is compilation of mean and standard deviation values of biochemical and
 161 anthropometric parameters in two groups of our study, the cases and the controls. **Table**
 162 **1 :Descriptive statistics in study population**

163

Sl no.	Parameter	Mean $\pm$ SD	Mean $\pm$ SD
		Cases	Controls
		n=41	n=29
1	Serum Total Cholesterol (mg/dl)	180.6 $\pm$ 55.9	184.0 $\pm$ 38.1
2	Serum High Density lipoprotein (mg/dl)	47.2 $\pm$ 19.5	46.4 $\pm$ 8.9
3	Serum Low Density lipoprotein (mg/dl)	107.9 $\pm$ 31.2	104.5 $\pm$ 31.8
4	Serum Very low Density lipoprotein (mg/dl)	31.3 $\pm$ 19.0	31.1 $\pm$ 13.6

5	Serum triglycerides (mg/dl)	154.9 ± 96.2	156.0 ± 68.6
6	Thyroid stimulating hormone (mU/L)	11.9 ± 19.9	2.23 ± 0.9*
7	Free Thyroxine (T ₄) (pg/ml)	3.3 ± 15.5	13.8 ± 1.88*
8	Lipoprotein (a) (mg/dl)	39.4 ± 26.5	18.1 ± 7.4**
9	BMI	25.9 ± 5.2	25.9 ± 7.2
*p < 0.01 **p < 0.0001			

164

165

166 The second table, Table 2 is a tabulation of Pearson's correlation outcome between Lp (a) and
167 TSH in both the groups.

168

169

170

171 **Table 2: Pearson's correlation between Lp(a) and TSH in both cases and controls**

Sl no	Pair	Pearson's correlation	'p' value
1	Serum Lipoprotein (a) v/s TSH in cases	0.070	0.663
2	Serum Lipoprotein (a) v/s TSH in controls	0.056	0.767

172

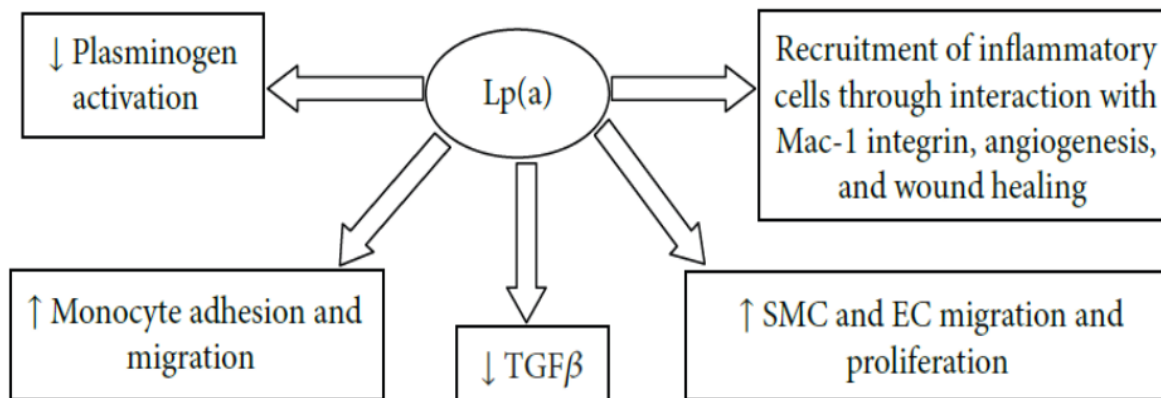
Discussion

The majority of the signs and symptoms in hypothyroidism is attributed to the effect the thyroid hormone has on the cardiovascular system. The heart relies mainly on Triiodothyronine (T_3). T_3 exerts its cellular actions through binding to thyroid hormone nuclear receptor on the cardiac myocyte which in turn mediates induction of thyroid hormone response elements in the promoter regions of positively regulated genes [5]. As mentioned earlier thyroid hormones influence lipid metabolism. T_3 upregulates LDL receptors by controlling the LDL receptor gene activation. In clinical or overt hypothyroidism total cholesterol and LDL levels are increased due to decreased LDL receptor activity. An increased TGL rich lipoproteins (VLDL and IDL) and HDL_2 as a result of decreased lipoprotein and hepatic lipase activities respectively is reported [15,16].

The influence of thyroid hormones depletion on cardiac myocyte and lipid metabolism collectively bring about the following cardiovascular changes which are decreased cardiac output, decreased myocardial contractility, increased peripheral vascular resistance and risk of premature atherosclerosis [17].

Lp (a) is ascribed an independent cardiovascular risk marker.. The mechanism of proatherogenic and prothrombogenic effects of Lp (a) are summarised in Figure 2.

Figure 2 : Mechanism of Anti fibrinolytic and atherogenic activity of Lp(a)



*TGFβ- Transforming growth factor beta , *SMC-Smooth muscle cell, *EC-Endothelial cell

High Lp(a) levels are commonly detected in patients with premature coronary heart disease, stroke, peripheral vascular disease and myocardial infarction[18]. We therefore chose to estimate Lp(a) in newly diagnosed overt hypothyroid patients who are at high risk for an early cardiovascular disease risk.

In table 1 all the biochemical parameters and BMI expressed as mean $\pm$ SD are compared between hypothyroid patients (cases) and healthy controls. The mean TSH level is raised and FT4 level is decreased in cases, a prerequisite to diagnose hypothyroidism. The mean BMI levels in both cases and controls are almost the same. Incidentally, both the groups contain subjects who fall in overweight/obese category. The mean $\pm$ SD of BMI values of cases and controls are 25.9 ± 5.2 and 25.9 ± 7.2 respectively. Its interesting to notice that the mean $\pm$ SD of the lipid parameters (TC, LDL, VLDL, HDL and TGL) in both the groups are almost same and are at the upper limit of the reference range. This is an unusual finding, as majority of the studies so far comparing hypothyroids with healthy

individuals have reported a significantly higher levels of lipid parameters in hypothyroids compared to healthy people[6]. The probable explanation for this is that the volunteers of apparently healthy control group are of higher BMI (25.9 ± 7.2) and since obesity predisposes to lipid abnormalities the lipid picture is similar to that of hypothyroids.

Leonidar H.Duntas et al, have stated in their article that the influence of sub-clinical hypothyroidism (SCH) on lipids is directly proportional to the degree of TSH elevation and becomes more significant with the progression from SCH to overt disease[19]. This means that a linear increase in TC and LDL levels is observed with increasing TSH. Based on this, is the study by Archana Prakash et al, who have studied lipid profile at varying degrees of TSH levels in overt hypothyroid patients. In their study, the TC and LDL levels in two groups of overt hypothyroid patients (group I with TSH 6-20mU/L and group II with TSH 21-40mU/L) varied minimally and were found to be on the upper limit of the reference range[20]. This is in support of our study where we have observed only mild elevation in TC and LDL levels in the group of overt hypothyroids whose TSH levels range between 6 and 21mU/L.

Elizebeth N.Pearce et al in her article has mentioned that TGL, VLDL and HDL levels in overt hypothyroid patients can be normal or elevated[21] and this is substantiated in our study where TGL, VLDL and HDL levels have remained within the normal reference range.

Figure 1 depicts the large difference in Lp(a) values between cases and controls. The mean $\pm$ SD levels of Lp(a) in cases is 39.4 ± 26.5 mg/dl and 18.1 ± 7.35 mg/dl in controls. Similar findings have been reported by Ramachandran Kaliaperumal et al, where in the Lp(a) in hypothyroids was 27.02 ± 0.58 mg/dl and 18.65 ± 0.72 mg/dl in healthy controls [22]. Our findings are also supported by the observations made by Pop-Radu Cristina Corina

et al, in their study, Lp(a) in hypothyroids was $48.33 \pm 28.1.6$ mg/dl and 30.5 ± 10.04 mg/dl in healthy controls [23].

The increased levels of lipoprotein (a) in hypothyroid individuals reflects the premature atherogenesis in these individuals.

There are studies by Zoe Efstathiadou, and A beccaria who have demonstrated that the lipoprotein (a) levels have reverted to normal levels once euthyroid status has been restored in hypothyroid individuals [24,25]. This finding is encouraging as the study can be taken a step forward, where Lp (a) levels can be re-estimated in patients with hypothyroidism after a stipulated period of treatment and we recommend such initiative as there is dearth of these kind of studies in south Indian population

To conclude lipoprotein (a) levels are elevated in hypothyroidism and it can be considered as a reliable marker to detect cardiovascular disease risk in hypothyroid patients. The possibility of Lp(a) being an early marker even before the onset of dyslipidemia needs to be evaluated. We recommend Lp (a) estimation by a standard procedure in hypothyroid patients for detection of CVD risk in these patients.

References

1. Komaravolu R. Thyroid awareness on world thyroid day. Biotechn Asia .2015. Accessed May 25th 2015. Available: www.ign.org/world-thyroid-day-2015.html
- 2 Grotzke M. Thyroid gland .In: Bishop ML, Fody EP, Schoeff LE editors. Clinical Chemistry Techniques, principles Correlations, Sixth edition ,Wolters Kluwer health/Lippincott Williams & Wilkins; 2010. page -495.

258 3.Hoogendoorn EH, Hermus AR, de Vegt F, Ross HA, Verbeek AL, Kiemeney LA et al.
 259 Thyroid function and prevalence of anti-thyroperoxidase antibodies in a population with
 260 borderline sufficient iodine intake: influences of age and sex. Clin Chem. 2006 Jan;
 261 52(1):104-11. PMID: 16254196

262 4. Unnikrishnan AG, Kalra S, Sahay RK, Bantwal G, John M, and Tewari N. Prevalence of
 263 hypothyroidism in adults: An epidemiological study in eight cities of India. Indian J
 264 Endocrinol Metab. 2013 Jul-Aug; 17(4): 647–652. DOI:10.4103/2230-8210.113755. PMCID:
 265 PMC3743364.

266 5.Jameson JL, Mandel SJ, Weetman AP. Disorders of the Thyroid Gland . In: Kasper, Fauci,
 267 Hauser, Longo, Jameson, Loscalzo editors. Harrisons Internal Medicie. 19th edition. Mc Graw
 268 Hill-Education. 2015.

269 6. Rizos CV, Elisaf MS and Liberopoulos EN. Effects of Thyroid Dysfunction on Lipid
 270 Profile.The Open Cardiovascular Medicine Journal. 2011; 5:76-84.
 271 DOI:10.2174/1874192401105010076 . PMCID: PMC3109527

272 7.Klei I, Dazi S. Thyroid disease and the heart. Circulation. 2007;116:1725-1735. DOI:
 273 10.1161/CIRCULATIONAHA.106.678326.

274 8. Genest J Jr, Jenner JL, McNamara JR et al. Prevalence of lipoprotein (a) [Lp(a)] excess in
 275 coronary artery disease. Am J Cardiol. 1991 May 15;67(13):1039-145.PMID: 1827234

276 9.Rainwater DL, Ludwig MJ, Haffner SM, VandeBerg JL. Lipid and lipoprotein factors
 277 associated with variation in Lp(a) density. Arterioscler Thromb Vasc Biol. 1995
 278 March;15(3):313-319. PMID: 7749840

- 279 10. Van der Hoek YY, Wittekoek ME, Beisiegel U, Kastelein JJ, Koschinsky ML. The
280 apolipoprotein(a) kringle IV repeats which differ from the major repeat kringle are present in
281 variably-sized isoforms. Hum Mol Genet. 1993 April;2(4):361-366.
- 282 11. Boerwinkle E, Menzel HJ, Kraft HG, Utermann G. Genetics of the quantitative Lp(a)
283 lipoprotein trait. III. Contribution of Lp(a) glycoprotein phenotypes to normal lipid variation.
284 Hum Genet. 1989 April;82(1):73-8.
- 285 12. Marcovina SM, Albers JJ. Lipoprotein (a) measurements for clinical application. J Lipid
286 Res. 2016 Apr; 57(4): 526-537. PMID:26637278.
- 287 13. Boffa MB, Marcovina SM, Koschinsky ML. Lipoprotein (a) as an emerging risk factor of
288 atherothrombosis. Principles from bench to bedside. In: Davidson MH Toth PP, Maki KC.
289 Editors. Therapeutic lipidology. New Jersey. Humana Press. 2007
- 290 14. Lipoprotein (a). In: Ballantyne CM. Clinical Lipidology: A companion to Braunwald Heart
291 Disease. 2nd edition. Elsevier. 2014.
- 292 15. Nikkila EA, Kekki M. Plasma triglyceride metabolism in thyroid disease. J Clin
293 Invest. 1972; 51: 2103-14.
- 294 16. Lam KS, Chan MK, Yeung RT. High-density lipoprotein cholesterol, hepatic lipase and
295 lipoprotein lipase activities in thyroid dysfunction-effects of treatment. Q J Med. 1986; 59:
296 513-521.
- 297 17. Klein I. Endocrine disorders and cardiovascular disease. In: Zipes DP, Libby P, Bonow R,
298 Braunwald E, eds. Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine. 7th
299 ed. Philadelphia, Pa. W.B. Saunders; 2005: 2051-2065.

- 18.Valentine RJ, Grayburn PA, Vega GL, Grundy SM. Lp(a) lipoprotein is an independent, discriminating risk factor for premature peripheral atherosclerosis among white men. Arch Intern Med. 1994 April 11;154(7):801-6.
- 19.Duntas LH, Wartofsky L. Cardiovascular disease risk and subclinical hypothyroidism: Focus on lipids and new emerging factors. What is the evidence? Thyroid. 2007; 17(11): 1075-1084
- 20.Prakash A, Lal AK.Serum lipids in Hypothyroidism: Our experience.Indian Journal of Clinical Biochemistry. 2006; 21 (2): 153-155.
- 21.Pearce EN. Update in Lipid Alterations in Subclinical hypothyroidism. J Clin Endocrinol Metab, February 2012, 97(2):326–333.
- 22.Kaliaperumal R, William E, Selvam T, Krishnan S. Relationship between Lipoprotein(a) and Thyroid Hormones in Hypothyroid Patients. Journal of Clinical and Diagnostic Research. 2014 Feb; Vol-8(2):37-39. DOI: 10.7860/JCDR/2014/7817.4001
- 23.Corina PC, Mirela G. Lipoprotein(a) Levels in Thyroid Disorders. Acta Medica Marisiensis. 2013;59(2):81-84. DOI: 10.2478/amma-2013-0018
- 24.Efstathiadou Z, Bitsis S, Milionis HJ, Kukuvtis A, Bairaktari ET, Elisaf MS and Tsatsoulis A. Lipid profile in subclinical hypothyroidism:is L-thyroxine substitution beneficial? European Journal of Endocrinology. 2001;145: 705–710.
- 25.Becerra A, Bellido D, Luengo A, Piedrola G, De Luis DA. Lipoprotein (a) and other lipoproteins in hypothyroid patients before and after thyroid replacement therapy. Clin Nutr. 1999;18(5):319-322.

