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Utility of Lipoprotein (a) as a Marker of Cardiovascular Disease Risk in Hypothyroid Patients

N. Chandrika[1*], S. M. R. Usha[2], H. V. Shetty[3] and Victoria Kshetrimayum[2]

[1]Department of Biochemistry, Chamarajanagar Institute of Medical Sciences, Chamarajanagar, India.
[2]Department of Biochemistry, Rajarajeswari Medical College and Hospital, Bengaluru, India.
[3]Rajarajeswari Medical College and Hospital, Bengaluru, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author HVS designed the study. Author SMRU wrote the protocol and managed the analysis of the study. Author NC wrote the first draft of the study, performed the statistical analysis and managed the literature searches. Author VK was instrumental in collecting and processing the samples. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of our study was to assess the utility of lipoprotein (a) as a 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 was 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

**Corresponding author: E-mail: dr.chandrikar@gmail.com;*

constituted the controls. Serum Thyroid stimulating hormone (TSH), Free Thyroxine (FT_4), 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 ± SD of the lipid parameters (TC, LDL, VLDL, HDL and TGL) in both the groups were almost same and were at the upper limit of the reference range. The mean ± SD levels of Lp(a) in cases was 39.4±26.5 mg/dl and 18.1±7.4 mg/dl in controls. There was no correlation between lipoprotein (a) and thyroid stimulating hormone in both cases and controls.

Conclusion: Lipoprotein (a) levels are elevated in hypothyroidism and it can be considered as a reliable marker to detect cardiovascular disease risk in hypothyroid patients when estimated by a standard method.

Keywords: Lipoprotein (a) [Lp (a)]; hypothyroidism; Cardiovascular Disease (CVD) risk; dyslipidemia; Thyroid Stimulating Hormone (TSH).

1. INTRODUCTION

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

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

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

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

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

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

2. METHODOLOGY

Ours is a case-control study. The research project was carried out in Rajarajeswari Medical College and Hospital, Bengaluru, between

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

In a clot activator containing nVac tube 5 ml fasting blood sample was collected from each of the study subject. The serum was separated and the following biochemical tests were run: Serum Thyroid stimulating hormone (TSH), Free Thyroxine (FT_4), Serum total cholesterol (TC), serum low density lipoprotein (LDL), serum high density lipoprotein (HDL), serum triglycerides (TGL) and serum Lipoprotein (a). Serum very low density lipoprotein, (VLDL) was calculated using the formula VLDL= TGL/5.

TSH and FT_4 were estimated by Chemiluminescence immunoassay method on Fully automated Maglumi 1000 analyser.

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

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

2.1 Lipoprotein (a) Assay

2.1.1 Biochemistry of lipoprotein (a)

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

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

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

Lp (a) Assay: Lipoprotein (a) can be measured by Enzyme linked immunosorbent 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].

In our study 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 are associated with increased risk of cardiovascular disease.

2.2 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.

3. RESULTS

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

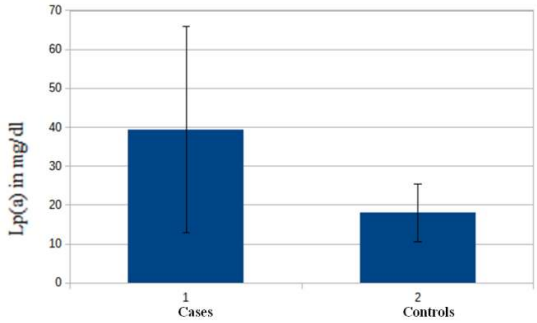

Fig. 1. Lipoprotein (a) levels in cases and controls

Table 1 is compilation of mean and standard deviation values of biochemical and anthropometric parameters in two groups of our study, the cases and the controls.

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

4. 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 celluar actions through binding to thyroid hormone nuclear receptor in 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 Fig. 2.

Table 1. Descriptive statistics in study population

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

*$p < 0.01$ **$p < 0.0001$

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

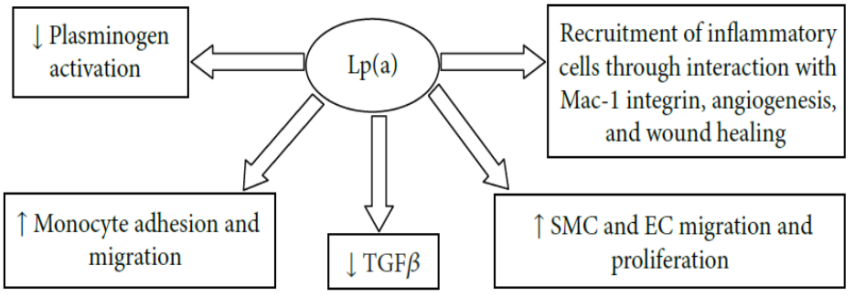

Fig. 2. Mechanism of anti fibrinolytic and atherogenic activity of Lp(a)
**TGFβ- Transforming growth factor beta, *SMC-Smooth muscle cell, *EC-Endothelial cell*

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 ± 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 ± 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 ± 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. [19] 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. 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. [20] 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 6-20 mU/L and group II with TSH 21-40 mU/L) varied minimally and were found to be on the upper limit of the reference range. 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 21 mU/L.

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

Fig. 1 depicts the large difference in Lp(a) values between cases and controls. The mean ± 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. [22] where in the Lp(a) in hypothyroids was 27.02±0.58 mg/dl and 18.65±0.72 mg/dl in healthy controls. Our findings are also supported by the observations made by Pop-Radu Cristina Corina et al. [23], in their study, Lp(a) in

hypothyroids was 48.33±28.1.6 mg/dl and 30.5±10.04 mg/dl in healthy controls.

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

There are studies by Zoe Efstathiadou [24] and A. Beccaria [25] who have demonstrated that the lipoprotein (a) levels have reverted to normal levels once euthyroid status has been restored in hypothyroid individuals. 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

5. CONCLUSION

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 method for detection of CVD risk in hypothyroid patients.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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