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Original Article

Biochemistry Section

Evaluation of Dyslipidemia, Lipid Ratios and Atherogenic Index as Cardiovascular Risk Factors in Overt and Subclinical Hypothyroid Patients

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ABSTRACT

Background: Thyroid disorders are known to cause metabolic disturbances. Dyslipidemia, one of the important modifiable cardiovascular risk factors is known in patients with overt hypothyroidism (OHT) and is also reported in sub clinical hypothyroidism (SHT).

Aims: To evaluate dyslipidemia, lipid ratios and atherogenic index in patients with OHT and SHT in comparison with healthy controls and between the hypothyroid patients.

Material and Methods: The study included 36 patients with OHT, 36 patients with SHT and 39 euthyroid controls. OHT was diagnosed with thyroid stimulating hormone (TSH) level of >15 mU/L and thyroxine (T4) <55µg/L, while SHT was diagnosed with TSH levels between 5-15 mU/L and T4 levels between 55-135µg/L. T3, T4, TSH, total cholesterol (TC), triglycerides (TG) and high-density lipoprotein cholesterol (HDL) were estimated. Low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL–C), non-HDL-cholesterol (non HDL-C), lipid ratios and atherogenic index (AI) was calculated.

Statistical Analysis: Differences between groups were tested with analysis of variance (ANOVA). Spearman correlation analysis was performed to study the association between parameters. Statistical analysis was performed using SPSS for Windows 11.5 program. Statistical significance was considered at $p<0.05$.

Results: TC, TG, LDL-C, VLDL-C were significantly elevated in patients with OHT, compared to SHT patients and controls ($p<0.01$). Patients with SHT had significantly higher LDL-C than controls ($p<0.01$). HDL-C was significantly lower in OHT and SHT patients compared to controls ($p<0.01$). Non HDL-C, lipid ratios including TC/ HDL- C, TG/HDL-C, LDL-C/ HDL-C and AI were significantly higher in OHT and SHT patients than controls ($p<0.01$). In patients with OHT, T4 correlated with TC ($r = - 0.37$, $p < 0.05$) and non HDL-C ($r=- 0.34$, $p<0.05$); TSH correlated with TC ($r=0.42$, $p<0.05$) and LDL-C ($r=0.44$, $p<0.05$). No correlation for T4 or TSH with any of the lipid parameters was observed in patients with SHT.

Conclusion: Dyslipidemia was observed in patients with OHT and also in SHT. Evaluation of lipid parameters including non HDL-C, lipid ratios and AI helps in better identification of cardiovascular risk, especially in patients with SHT, who were found to have normal TC, TG and VLDL-C levels.

Key Words: Atherogenic risk, Dyslipidemia, Hypothyroidism, Lipid ratios, Atherogenic index

INTRODUCTION

Thyroid disturbances, diagnosed in sub clinical or clinical forms constitute the most common endocrine abnormality. It is now well-established that patients with both overt hypothyroidism and subclinical hypothyroidism are at an increased risk of developing cardiovascular disease, which is mainly attributed to hemodynamic alterations as well as due to atherosclerosis [1-3]. Several factors are known to contribute to this increased risk, with dyslipidemia being one of the important contributors.

Thyroid hormones influence various metabolic processes, including lipid metabolism, changes in thyroid hormones are known to affect lipid profile, resulting in dyslipidemia, which is a common metabolic abnormality in patients with overt hypothyroidism (OHT) and also reported in sub clinical hypothyroidism (SHT) [4].

A traditional atherogenic lipid profile is characterized by increased total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG) and decreased high-density lipoprotein cholesterol (HDL-C) [5]. In addition to

the traditional lipid parameters, non HDL-C, lipid ratios including TC / HDL-C, TG/HDL-C, LDL-C/HDL-C [6] and Atherogenic Index (AI), calculated as logarithm of molar ratio of TG to HDL-C (log TG/HDL) [7] are used to assess the risk of atherosclerosis. Although atherosclerosis risk is generally accepted to be present in patients with OHT [8], whether patients with SHT are also associated with a similar risk of atherosclerosis is debatable [9].

Therefore, in the present study, serum levels of individual lipids were estimated and atherogenic risk due to dyslipidemia was evaluated using individual traditional lipid parameters and other lipid risk factors obtained by calculation from the quantified lipids in patients with OHT and SHT. The changes in the atherogenic profile between the hypothyroid patients; between OHT group and controls and between SHT group and controls were evaluated. The focus of the present study was to evaluate cardiovascular risk factors in hypothyroidism with emphasis on subclinical hypothyroid group.

SUBJECTS AND METHODS

Subjects: Thirty-six OHT (33.6± 9.6 years; 10 males) and 36 SHT patients (34.4± 10.0 years; 2 males) were recruited from the outpatient department of Endocrinology and Metabolism, Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati, Andhra Pradesh, India. Thirty-nine healthy euthyroid subjects (32.9± 7.1 years; 10 males) were included as controls for the study. The diagnosis of OHT was based on the finding of high serum thyroid stimulating hormone levels (TSH>15mU/L) and low thyroxine (T4) levels (T4<55µg/L), while SHT was diagnosed as having high TSH levels (5-15 mU/L) associated with normal T4 levels (55-135µg/L). Smokers, alcoholics, subjects with chronic or acute diseases, diabetes, hypertension, hepatic, renal diseases, inflammatory diseases, pregnant women, postmenopausal women, endocrine diseases other than OHT and SHT, subjects on lipid lowering drugs and antioxidant vitamin supplements were excluded. The study was approved by the institutional ethics committee. Informed consent was obtained from all the participants.

Methods: After overnight fasting, venous blood samples were drawn from all the participants into additive free tubes. Samples were allowed to clot and serum was separated by centrifugation. Serum samples were stored in aliquots at –80°C (Thermo Fischer Scientific, USA) until biochemical analysis. Thyroid profile was assessed by using radioimmuno assay kits for triiodothyronine (T3) and thyroxine (T4) (Bhaba Atomic Research Centre, Mumbai, India). TSH was measured by using immunoradiometric assay kits (Bhaba Atomic Research Centre, Mumbai, India). Lipid profile was estimated using commercial kits from Aspen laboratories (Delhi, India) for TC, Accurex (Thane, India) for TG by enzymatic methods. HDL-C kits were obtained from Beckman Coulter (Galway, Ireland). LDL-C level was calculated using Friedewald equation [10]. Non-HDL-C was calculated by subtracting HDL-C concentration from TC concentration. Various lipid risk factors were calculated as the ratios of TC/ HDL-C, TG/ HDL-C and LDL-C/ HDL-C. The AI was calculated as logarithm of TG/HDL-C [7].

The estimations were performed on Wizard automatic gamma counter WallacOy, Turku, Finland (Radioactive assays for thyroid profile), Beckman-Coulter Synchron CX9 fully automated clinical chemistry analyzer, USA (Clinical chemistry assays for lipid profile).

STATISTICAL ANALYSIS

All data were expressed as mean ± SD. The differences between groups were tested with analysis of variance (ANOVA) test followed by a post-hoc Bonferroni and Tukey's comparison between multiple groups, as appropriate. Spearman correlation analysis was performed to determine the association between various parameters in each patient group. Statistical analysis were performed using SPSS for Windows 11.5 program (SPSS Inc, Chicago, IL, USA). Statistical significance was considered at $p<0.05$.

RESULTS

The general characteristics and estimated biochemical parameters of the three groups studied are shown in [Table/Fig-1], the calculated lipid parameters, lipid ratios and AI of the groups are shown in [Table/Fig-2]. BMI was significantly elevated in both OHT and SHT when compared with the controls, but there was no significant difference in BMI between OHT and SHT patients [Table/Fig-1]. There was a significant elevation in TC, TG [Table/Fig-1], LDL-C and VLDL-C [Table/Fig-2] in OHT patients when compared to SHT patients and controls. In patients with SHT, only LDL-C was found to be significantly increased when compared to controls. HDL-C levels were significantly reduced in both OHT and SHT groups when compared to controls, but there was no significant difference in HDL levels between OHT and SHT groups [Table/Fig-1]. The lipid ratios, non HDL-C and AI were significantly elevated in both OHT and SHT patients when compared to controls, with a higher elevation found in OHT group than that observed in SHT group [Table/Fig-2].

Correlation analysis between the variables in OHT and SHT are shown in [Table/Fig-3]. We found no correlation of T3 with variables studied in OHT and SHT groups ($p>0.05$). In patients with OHT, there was a significant negative correlation of T4 with TC ($r=-0.37$, $p=0.02$) and non-HDL-C ($r=-0.34$, $p=0.04$) [Table/Fig-2]. TSH had significant positive correlations with TC ($r=0.42$, $p=0.01$), LDL-C ($r=0.44$, $p=0.007$) and lipid risk factors non-HDL-C ($r=0.39$, $p=0.01$) and LDL-C/HDL-C ratio ($r=0.37$, $p=0.02$). There were no significant correlations of T4 and TSH with any of the lipid variables in SHT patients [Table/Fig-3].

Variables	OHT (n=36)	SHT (n=36)	Euthyroid controls (n=39)
Age (years)	33.6 ± 9.6	34.4 ± 10.0	32.9 ± 7.1
BMI (kg/m2)	24.7 ± 3.0*	23.6 ± 3.8*	20.2 ± 1.2
Systole (mmHg)	109.1 ± 11.0	110.6 ± 12.7	113.8 ± 8.4
Diastole (mmHg)	72.2 ± 7.6	72.1 ± 8.5	74.6 ± 5.5
T3 (µg/L)	0.62 ± 0.30*, #	1.18 ±0.36	1.34 ± 0.41
T4 (µg/L)	24.0 ± 13.0*, #	85.6 ± 19.0	85.9 ± 20.3
TSH (mIU/L)	55.2 ± 33.3*, #	9.30 ± 3.1*	2.20 ± 1.1
TC (mg/dL)	209.9 ± 54.8*, #	162.6 ± 31.8	149.3 ± 29.7
TG (mg/dL)	174.4 ± 94.2*, #	126.5 ± 35.6	123.5 ± 37.9
HDL-C (mg/dL)	35.9 ± 5.1*	36.3 ± 5.09*	44.1 ± 3.9

[Table/Fig-1]: Mean ± SD of age, BMI, and Biochemical parameters of hypothyroid patients and euthyroid control subjects

*p<0.01 when compared with euthyroid controls, #p<0.01 when compared with sub-clinical hypothyroid patients. BMI: body mass index

Variables	OHT (n=36)	SHT (n=36)	Euthyroid controls (n=39)
LDL-C (mg/dL)	139.0 ± 47.7*,#	100.9 ± 27.7*	80.4 ± 28.8
VLDL-C (mg/dL)	34.8 ± 18.8*,#	25.3 ± 7.1	24.7 ± 7.5
Non-HDL-C (mg/dL)	173.2 ± 55.1*,#	126.2 ± 29.6*	105.2 ± 30.0
TC/HDL-C ratio	5.88± 1.48*,#	4.49± 0.79*	3.41± 0.76
TG/HDL-C ratio	2.17± 1.27*,#	1.52± 0.38*	1.23± 0.36
LDL/HDL-C ratio	3.89± 1.27*,#	2.80± 0.77*	1.85± 0.72
AI (SI units)	0.27± 0.20*,#	0.17± 0.10*	0.07± 0.10

[Table/Fig-2]: Mean ± SD of calculated lipid parameters, lipid ratios and AI of hypothyroid patients and euthyroid control subjects

*p<0.01 when compared with euthyroid controls, #p<0.01 when compared with sub-clinical hypothyroid patients. AI: atherogenic index

Variables	Correlation with T4 r		Correlation with TSH r	
	OHT	SHT	OHT	SHT
TC	-0.37*	0.091	0.42*	0.127
TG	-0.31	0.105	0.03	0.189
HDL-C	-0.26	-0.088	0.25	0.155
LDL-C	-0.28	0.018	0.44*	0.015
VLDL-C	-0.31	0.105	0.03	0.189
Non-HDL-C	-0.34*	0.061	0.39*	0.094
TC/HDL-C	-0.24	0.122	0.29	-0.093
TG/HDL-C	-0.23	0.148	-0.05	0.174
LDL/HDL-C	-0.17	0.098	0.37*	-0.134
AI	-0.25	0.148	0.005	0.164

[Table/Fig-3]: Correlations of serum T4 and TSH with lipids and coronary lipid risk factors in patients with overt and subclinical hypothyroidism

R (correlation coefficient), *(statistically significant), AI: atherogenic index

DISCUSSION

The present study was undertaken to estimate various lipid parameters in patients with overt and sub clinical hypothyroidism and compare them with euthyroid controls for evaluation of atherogenic risk. Patients with OHT had significantly elevated TC, TG, LDL-C and VLDL-C when compared to euthyroid subjects. Similar findings were reported in earlier studies in OHT patients [6, 11-13]. Although there is a decreased activity of HMG CoA reductase which is a regulatory enzyme in cholesterol biosynthesis, cholesterol levels were found to be increased in hypothyroid patients [4]. Decreased cholesterol clearance [11], as well as, reduced activity of LDL receptors, which results in decreased receptor mediated clearance of LDL-C particles have been proposed to be the mechanisms leading to hypercholesterolemia and elevated LDL-C levels in patients with OHT [4,14]. Hypertriglyceridemia in hypothyroidism is attributed to decreased activity of lipoprotein lipase, resulting in decreased clearance of triglyceride-rich lipoproteins [4]. Besides, these factors which cause dyslipidemia, other effects of thyroid hormones such as decreased adrenergic regulation of lipolysis [15], may also contribute to the lipid disturbances observed in hypothyroidism.

In the present study, patients with SHT had significantly elevated LDL cholesterol levels when compared to controls. There are studies which have reported similar elevated LDL-C [11] in SHT and also, studies which have found no change in LDL-C in SHT patients [16]. It was reported earlier that the effects of hypothyroidism on lipid metabolism are more evident in patients with higher TSH levels [17]. Accordingly, in the present study, levels of TC, TG, VLDL-C and LDL-C in patients with OHT were found to be significantly higher when compared to patients with SHT. The lower T4 levels and the higher TSH levels observed in OHT subjects compared to SHT patients can be considered to be responsible for the more pronounced dyslipidemia observed in OHT subjects. This is supported by significant correlation observed for T4 with TC, TSH with TC and LDL-C in OHT patients and no significant correlation of T4 and TSH with any of the lipid variables being observed in SHT patients.

HDL-C levels in overt and subclinical hypothyroid patients in the present study, were found to be significantly lower when compared to controls. Decreased HDL-C has been reported previously in OHT [13] and SHT [18] groups. It is well-known that thyroid hormones have multiple effects on lipoprotein metabolism. In addition, to its effects on other lipids, thyroid dysfunction was found to exert significant effects on HDL-C metabolism. It has been described that several genes coding for proteins involved in the intravascular metabolism of HDL-C are regulated by thyroid hormones. Also, Apo A1, which is the

main constituent of HDL cholesterol, is expressed at lower levels under hypothyroid conditions [19]. However, studies on HDL-C in hypothyroidism have reported normal levels [11] and also elevated HDL-C levels [20] in hypothyroidism. The differences in the various studies may be explained as an outcome due to the influence of genetic and environmental factors on the balance between reduced synthesis and catabolism of HDL – Apo A1 in hypothyroid subjects; as a result of which, HDL levels may be low, normal and even increased [19].

Functional cardiovascular abnormalities and atherosclerosis have been reported in hypothyroidism and patients with overt, as well as, subclinical hypothyroidism were found to be associated with increased risk of CVD [8]. The dyslipidemia pattern in OHT subjects, in the present study, was clearly evident in the form of elevated TC, TG, LDL-C, VLDL–C and decreased HDL–C. On the other hand, patients with SHT had significant elevation in LDL–C only and a significant decrease in HDL–C compared to euthyroid subjects. Lipid parameters such as non HDL–C, and lipid ratios, TC/HDL-C, TG/HDL-C, LDL-C/HDL– C, are considered as better indicators of cardiovascular risk [21]. The atherogenic index (AI), a surrogate marker of small LDL-C particle size, has been proposed as an atherogenic risk factor [7]. In the present study, non HDL-C, TC/HDL-C, TG/HDL-C, LDL-C/HDL-C and AI were significantly elevated in SHT patients compared to euthyroid subjects, indicating increased cardiovascular risk in these patients despite relatively less severe dyslipidemia as evidenced by a significant increase in only LDL-C and a significant decrease in HDL-C. Elevated LDL-C and decreased HDL-C levels are also well known independent risk factors for cardiovascular diseases, which indicates the presence of cardiovascular risk in SHT patients, as found in this study. Patients with OHT had significantly elevated non HDL-C, TC/HDL-C, TG/HDL-C, LDL-C/ HDL-C and AI compared to patients with SHT indicating a more pronounced dyslipidemia in OHT group.

CONCLUSION

In conclusion, it was observed in the present study that patients with overt hypothyroidism had more pronounced individual lipid disturbances when compared to subclinical hypothyroid patients. Although patients with SHT had similar TC, TG, and VLDL-C levels when compared to controls, further evaluation using other lipid parameters, showed significantly elevated lipid ratios and non HDL-C thus predisposing these patients to an increased cardiovascular risk. Findings of the present study indicate that dyslipidemia, which is an important yet modifiable cardiovascular risk factor may not always be evident in the form of individual traditional lipid parameters. Hence, inclusion of lipid ratios in evaluation of lipid disturbances helps in better risk stratification, especially in patients with sub clinical hypothyroidism.

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