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CORRELATION OF SERUM LIPIDS, LIPOPROTEINS AND HOMOCYSTEINE LEVELS IN HYPOTHYROID PATIENTS OF NORTH - WEST INDIA

Biochemistry

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ABSTRACT

BACKGROUND: Thyroid diseases with accompanying dyslipidemia is widely recognized as a risk factor for various cardiovascular disease like atherosclerosis/myocardial infarction, which cannot be fully explained by the atherogenic lipid profile and plasma total homocysteine is an independent risk factor for the initiation of atherosclerosis.

OBJECTIVE: The aim of this study was to investigate the serum total homocysteine level in subclinical as well as overt hypothyroid patients of North/West Indians to observe the relationship between lipid, homocysteine and hypothyroidism.

MATERIAL AND METHODS: In the present study, 80 hypothyroid patients (40 sub clinical hypothyroidism and 40 overt hypothyroidism) and 40 normal healthy controls subjects of both sex, with a mean age range 20–50 years from North West Punjabi population were recruited in the present study for the evaluation of serum total homocysteine along with lipid and lipoproteins levels.

RESULTS: In the present a significant increase in total cholesterol ($P \leq 0.05$), triglycerides ($P \leq 0.001$), LDL- cholesterol ($P \leq 0.05$), and VLDL-cholesterol was observed in sub clinical and overt hypothyroid patients while a significant fall ($P \leq 0.001$) in both sub clinical hypothyroidism and overt hypothyroidism was reported in comparison to normal healthy subjects. A significant ($P \leq 0.001$) increase in total homocysteine level was recorded in overt hypothyroidism but non significantly increased was observed in total homocysteine levels in sub clinical hypothyroidism patients.

CONCLUSION: A strong relationship between serum homocysteine levels and lipid profile concentrations especially the concentrations of total cholesterol and triglycerides in hypothyroidism might increase cardiovascular risk. So, determination of serum levels of thyroid profile is recommended in subjects with unexplained Hyperhomocysteinemia.

KEYWORDS

Subclinical hypothyroidism, overt hypothyroidism, lipids, Lipoproteins, Total homocysteine (tHcy).

INTRODUCTION

Thyroid disease, such as hypothyroidism and hyperthyroidism, constitutes the most common endocrine abnormality is associated with various metabolic abnormalities, due to the effects of thyroid hormones on nearly all major metabolic pathways. Thyroid hormones, thyroxine (T_4), and triiodothyronine (T_3) play an important role in all major metabolic pathways. They regulate the basal energy expenditure through their effect on protein, carbohydrate, and lipid metabolism. This might be a direct effect or an indirect effect by modification of other regulatory hormones such as insulin or catecholamine's[1]. Many studies reported that thyroid hormones stimulate cholesterol synthesis by inducing 3-hydroxy-3-methyl-glutaryl coenzyme A reductase in the liver[2,3]. In addition thyroid hormones influence all aspects of lipid metabolism including synthesis, mobilization, and degradation. Furthermore, thyroid hormones affect lipoprotein lipase activity and thus, the hydrolysis of triglycerides into very-low density lipoprotein (VLDL) and Chylomicrons into fatty acids and glycerol[2]. Finally, thyroid hormones modulate lipid metabolism by upregulation of the low density lipoprotein (LDL) receptors, which results in enhanced catabolism of the LDL particles. Thyroid gland function regulates a wide range of metabolic events, it significantly affects lipoprotein metabolism and as a result cardio vascular disease (CVD) risk[4]. In addition, thyroid failure is accompanied by an increase in plasma homocysteine levels with its known adverse effect on the cardiovascular system[5]. Homocysteine a type of amino acid that is naturally found in blood plasma is not harmful at normal levels, but many lifestyle factors like smoking [6], coffee consumption[7] and excessive alcohol intake[8]. Lack of exercise, obesity[9] and stress are associated with hyperhomocysteinemia. Hyperhomocysteinemia induced injury to the arterial wall is one of the factors that can initiate the process of atherosclerosis, hence leads to heart attacks and strokes[10,11]. Several studies[12,13,14] have shown that homocysteine can inflict damage to the arterial wall via multiple destructive molecular mechanisms. So, the present study was designed to evaluate the changes in total homocysteine levels in sub clinical hypothyroidism (SCH) and overt hypothyroidism (OH) in North – West Punjabi population of India.

MATERIAL AND METHODS

The present case-control study was carried out in the Department of Biochemistry, Government Medical College - Amritsar in collaboration with Department of Medicine, Guru Nanak Dev Hospital- Amritsar on 80 confirmed SCH and OH subjects of both sex (Males and Females) in the age range of 20 - 50 years. These subjects were taken from general population attending the outdoor patients of Department of Medicine, Guru Nanak Dev Hospital- Amritsar.

Inclusion criteria: The confirmed case of hypothyroidisms in the age range of 20-50 years of both sex (Male and Female), body mass index (BMI) below 25 Kg/m^2 with TSH, T_3, and T_4 levels in the range of 15.01±5.42µIU/ml; 7.16± 0.93pg/ml and 5.98± 0.57ng/dl respectively were included in OH group and hypothyroidism patients in the age range of 20-50 years of both sex (Male and Female) with TSH, T_3, and T_4 levels in the range of 9.21± 4.02µIU/ml; 3.98± 0.53pg/ml and 3.15± 0.17ng/dl respectively were included in SCH group. Age, gender and BMI matched healthy people with normal thyroid functions were recruited as controls for the study. These subjects were recruited from rural and as well as from urban areas of Amritsar District of Punjab state.

Exclusion criteria: Obese people with BMI greater than 30 Kg/m^2, current smokers and alcoholics, diabetes mellitus, renal insufficiency (serum creatinine > 1.5 mg/dL) hepatic failure, diagnosed cases of hypothyroidism or those already on treatment, polyglandular disorders, thyroid cancer, people with a history of antipsychotic treatment were excluded.

Ethical Issues: The study protocol was approved by the institutional ethic committee. Study details & potential risks and benefits were explained to individuals taking part in the study and at least one attendant. A written informed consent and detailed history was obtained from all patients and controls before entering into the study.

Collection of Blood and Preparation of Serum

Venous blood samples (5 ml) were drawn from all the subjects following an overnight fast of 12 hrs all aseptic conditions in plain vacutainer from both the groups (control as well as SCH subjects). Plain vacutainer was kept at 37°C for 30 min and then centrifuged at 3000rpm for 15 min. A clear supernatant (serum) obtained was used for the analysis of various biochemical assays.

Biochemical assays

1. Estimation of Lipids and lipoproteins: Serum Total Cholesterol (Total Chol.), Triglyceride (TGs), and High-Density Lipoprotein Cholesterol (HDL-Chol.) levels were analyzed according to Watson (1960)[15], Fossati et al. (1982)[16], and Freidewald et al. (1972)[17], respectively using commercial kits procured from Transasia Bio-Medicals Ltd. Solan (HP) in Technical collaboration with ERBA diagnostic Mannheim, Germany. Very Low-Density Lipoprotein-Cholesterol (VLDL-Chol.) levels was calculated by dividing the triglyceride concentration with five. LDL-cholesterol was calculated according to the equation of Assmann et al. (1984)[18] as follows:

LDL-Cholesterol = Total cholesterol - (HDL-Cholesterol + Triglyceride/5).

2. Estimation of serum Triidothyronine (T_3), Thyroxine (T_4) and Thyroid Stimulating Hormone (TSH):- TSH, T_3, and T_4 levels in serum were analyzed by using ELISA techniques. The kits were purchased from Transasia Biomedical Private Limited, Mumbai (India). The concentrations are expressed in µIU/ml.

***3. Estimation of homocysteine level*:** The levels of total homocysteine (t-Hcy) were assayed by the aid of ELISA (Sandwich immunoassay technique) using commercial kit (CUSABIO, China) according to Primus et al. (1988)[19].

Statistical Analysis

All the continuous variables were expressed as mean ± SD. In addition, categorical variables were analyzed by chi-square test. All the results were discussed at 5% level of significance; P value < 0.05 was considered significant. Statistical Package for Social Sciences version 21.0 (SPSS Inc., Chicago, IL, USA,) was used for statistical analysis.

RESULTS

1. ***Anthropometric Measurements:*** The anthropometric measurements of SCH, OH and normal healthy control subjects are summarized in the Table- 1. The body weight, height, age, BMI, Blood pressure systolic and blood pressure diasystolic was measured and all were statistically non significant.
2. ***Thyroid Profile:*** A significant increase was observed in TSH by 189.62% (p≤0.001) and 372.01% (p≤0.001) in SCH and OH respectively. A nominal increase by (11.79%) & 8.24% was recorded in T_3 & T_4 levels respectively in SCH and a significant increase from 3.56 ± 0.71 to 7.16 ± 0.93 pg/ml by 101.12% in T_3 and from 2.91 ± 0.15 to 5.98 ± 0.57 ng/dl by 105.49% was observed in T_4 levels in OH (Table-2)
3. **Lipids and lipoproteins:** A significant increase was observed in total cholesterol (from 160.21 ± 12.36 to 288.29 ± 19.01 mg/dL) by 20.41% (p≤0.05) & 79.94% (p≤0.001), triglycerides (118.67 ± 7.40 to 211.94 ± 21.63 mg/dL) by 46.45% (p≤0.005) & 78.59% (p≤0.001), LDL- cholesterol(97.57 ± 9.90 to 288.29 ± 19.01 mg/dL) by 29.02% (p≤0.005) & 119.31% (p≤0.001) and VLDL –cholesterol (from 23.73 ± 3.52 to 42.38 ± 5.75 mg/dL) by 46.43% (p≤0.05) & 78.59% (p≤0.001) in SCH and OH respectively w. r. t. normal healthy control subjects levels while a significant fall was reported in HDL –cholesterol (from 38.91 ± 5.92 to 31.92 ± 5.52 mg/dL) by 5.88% & 81.21% in SCH and OH respectively in comparison to normal healthy control subjects (Table-2).
4. ***Total Homocysteine:*** A significant increase was observed in total Homocysteine (from 9.69 ± 1.96 to 17.56 ± 2.34 µmol/L by 81.21% (p≤0.001) in overt hypothyroidism1 and a non significant increase was recorded from 9.69 ± 1.96 to 10.26 ± 1.39 by 5.88% in SCH patients (Table-2).

DISCUSSION

Lipid and lipoproteins: Thyroid disorders are known to influence lipid metabolism. OH and SCH is associated with increased risk of CVDs like atherosclerosis/myocardial infarction, attributed to increased total cholesterol and LDL-cholesterol levels[4]. Elevation of plasma LDL-cholesterol is due to impaired clearance of LDL lipoprotein, probably reflecting decreased LDL- receptor expression[20]. Literature reports[21,22] revealed that inconsistent results have been reported regarding the association between SCH, serum lipids and cardiovascular disease. Vierhapper, 2000 et al[23] reported that there were no significant differences in serum total cholesterol, LDL-cholesterol, triglycerides, or HDL-cholesterol between patients with SCH and the euthyroid control group similarly in 2004, Hueston and Pearson[24] reported that SCH was not associated with alterations in total cholesterol, LDL-cholesterol, triglycerides, or HDL-cholesterol while a number of studies showed that total cholesterol, LDL-Cholesterol, triglycerides were elevated but no associations were observed between serum TSH and HDL-Cholesterolin SCH compared with respect to controls[25, 26, 27] where as Lai *et al*, 2011[28] reported a significantly higher triglycerides and decrease in HDL-cholesterol levels in Chinese adults with SCH patients in comparison to euthyroid individuals. In the present study, we observed a significant increase in total cholesterol (from 160.21 ± 12.36 to 288.29 ± 19.01 mg/dL) by 20.41% (p≤0.05) & 79.94% (p≤0.001), triglycerides (118.67 ± 7.40 to 211.94 ± 21.63 mg/dL) by 46.45% (p≤0.005) & 78.59% (p≤0.001), LDL- cholesterol(97.57 ± 9.90 to 288.29 ± 19.01 mg/dL) by 29.02% (p≤0.005) & 119.31% (p≤0.001) and VLDL –cholesterol (from 23.73 ± 3.52 to 42.38 ± 5.75 mg/dL) by 46.43% (p≤0.05) & 78.59% (p≤0.001) in SCH and OH respectively w. r. t. normal healthy control subjects levels while a significant fall was reported in HDL –cholesterol (from 38.91 ± 5.92 to 31.92 ± 5.52 mg/dL) by 5.88% & 81.21% in SCH and OH respectively in comparison to normal healthy control subjects was observed in the present study could be due the alteration of thyroid gland functions and hence could be responsible for the phathophysiology of various cardiovascular diseases like atherosclerosis/myocardial infarction.

Homocysteine: Thyroid diseases also have a profound influence on a variety of biochemical processes, some of which may have secondary effects on the tHcy metabolism. Thyroid hormones markedly affect riboflavin metabolism, mainly by stimulating flavokinase and thereby the synthesis of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). Conceivably, these metabolic changes may affect tHcy metabolism because FMN and FAD serve as cofactors for enzymes involved in the metabolism of vitamin B_6, cobalamin, and folate. Circulating tHcy concentrations in hypothyroidism can rise through reduced activity of the flavoprotein methylene tetrahydrofolate reductase (MTHFR), an enzyme involved in the catalysis of tHcy and its remethylation to methionine. Hypothyroid individuals can be defective in converting riboflavin to the co-enzyme FAD, and consequently, deficient in MTHFR activity[29]. In the present study, a non significant increase was observed in tHcy levels by 5.88% in SCH and a significant increase was recorded in tHcy levels 81.21% (p≤0.001) in OH (Table-2). The results observed in the present study are in agreement with the literature reports[,30,31,32]. In conclusion a strong relationship between serum homocysteine levels and lipid profile concentrations especially the concentrations of cholesterol in overt hypothyroidism patients may increase cardiovascular risk. So, determination of serum levels of thyroid profile is recommended in subjects with unexplained hyperhomocysteinemia and hypercholesterolemia.

Table-1 Anthropometric parameters and distribution of SCH, OH patients and normal healthy controls.

Anthropometric Parameter	Healthy Controls	Sub Clinical Hypothyroidism Patients	Overt Hypothyroidism Patients
Number of subjects	40	40	40
Male	25	22	24
Females	15	18	16
Age, (years)	34.5 ± 10.3a	35.5 ± 11.1	37.40 ± 8.11
Weight (kg)	65.74 ±11.43	65.93±9.13	66.12±10.94
Height (cm)	151.74 ±11.62	149.24 ± 11.62	155.17 ±9.13
BMI (Kg/m2)	23.96 ± 2.53	24.07 ± 2.39	23.29 ± 3.31
Blood pressure systolic (mmHg)	123.19 ± 0.34	124.11 ± 0.43	120.87 ± 0.16
Blood pressure Diasystolic (mmHg)	84.18 ± 4.41	85.15± 5.34	84.18 ± 4.32

[a]Values are expressed as Mean ± S.D of 40 observations

Table-2 Changes in serum thyroid Profile (TSH, T_3, T_4) in SCH, OH patients and normal healthy controls.

Biochemical Parameter	Healthy Controls (n = 40)	Patients with SCH, (n = 40)	Overt Hypothyroidism Patients (n = 40)
TSH (µIU/ml)	3.18 ± 0.91[a]	9.21 ± 4.02 (+189.62%)***	15.01 ± 5.42 (+372.01%)***
T_3 (pg/ml)	3.56 ± 0.71	3.98 ± 0.53 (11.79%) NS	7.16 ± 0.93 (101.12%)***
T_4 (ng/dl)	2.91 ± 0.15	3.15 ± 0.17 (8.24%)NS	5.98 ± 0.57 (105.49%)***

[a]Values are expressed as Mean ± S.D of 40 observations

[b]Values in parentheses represent percentage changes in SCH and OH w. r. t. normal healthy control subjects.

*** P < 0.001

Table-3 Changes in fasting glucose, serum lipids, lipoproteins and Total Homocysteine levels in SCH, OH patients and normal healthy controls.

Biochemical Parameter	Healthy Controls (n = 40)	Patients with SCH, (n = 40)	Overt Hypothyroidism Patients (n = 40)
Fasting Blood Glucose (mg/dL)	79.85 ± 7.72	81.29 ± 10.12 (+1.80%) NS	78.94 ± 9.64 (+0.11%) NS
Total Cholesterol (mg/dL)	160.21 ± 12.36	192.91 ± 13.41 (+20.41%)*	288.29 ± 19.01 (+79.94%)***
Triglyceride (mg/dL)	118.67 ± 7.40	173.79 ± 9.18 (+46.45%)**	211.94 ± 21.63 (+78.59%)***
LDL-Cholesterol (mg/dL)	97.57 ± 9.90	125.89± 17.20 (+29.02%)*	213.99± 21.40 (+119.31%)***
HDL-Cholesterol (mg/dL)	38.91 ± 5.92	32.27 ± 6.71 (-17.06%)*	31.92 ± 5.52 (+17.96%)*
VLDL (mg/dL)	23.73 ± 3.52	34.75 ± 4.32 (+46.43%)**	42.38 ± 5.75 (+78.59%)***
Total Homocysteine (µmol/L)	9.69 ± 1.96	10.26 ± 1.39 (+5.88%) NS	17.56 ± 2.34 (+81.21%)***

[a]Values are expressed as Mean ± S.D of 40 observations

[b]Values in parentheses represent percentage changes in SCH and OH w. r. t. normal healthy control subjects.

* P < 0.05, ** P < 0.01, *** P < 0.001, NS: Non significant

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