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Research Article

Alterations in Lipid Fraction Levels in Subclinical Hypothyroidism in North Indian Population

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ABSTRACT
A data of serum lipid profile such as total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, very low density lipoprotein cholesterol and triglyceride from 100 patients in the age range of 15-65 years of both sex having subclinical hypothyroidism were compared with euthyroid controls to observe that whether subclinical hypothyroidism is associated with abnormal lipid levels or not in a population-based sample from Northern Indians study. A significant increase in triglycerides and very low density lipoprotein cholesterol levels were observed in patients of subclinical hypothyroidism with respect to euthyroid controls while a nominal increase in serum cholesterol, low-density lipoprotein and high-density lipoprotein levels were recorded. However, there was no statistical difference found in any of the lipid fraction levels with change in the severity of subclinical hypothyroidism. All these observation suggested that subclinical hypothyroidism did not have a marked impact on any of the fraction of lipids.

Key Words: Subclinical hypothyroidism, Thyroid-stimulating hormone (TSH), Lipid Profile.

INTRODUCTION
Subclinical hypothyroidism can be best defined as a high serum thyroid stimulating hormone (TSH) and normal serum total/free thyroxine (T_4), triiodothyronine (T_3) concentrations associated with few or no symptoms/signs of hypothyroidism. It is referred to as a state of mild thyroid failure and is essentially a laboratory diagnosis (Ayala *et al.,* 2000 and Cooper, 1987). Subclinical hypothyroidism is much more common than overt hypothyroidism (Danese *et al.,* 2000 and Tunbridge *et al.,* 1977). Therefore, early diagnosis and treatment may prevent the onset of overt hypothyroidism and its associated effects. Subclinical hypothyroidism may be associated with increased risk of coronary artery disease (CAD), peripheral vascular disease, and various biochemical abnormalities including increased LDL-C levels, increased total cholesterol and serum triglyceride values (Bhaskaran *et al.,* 2004). However, the results of lipid profile alterations in subclinical hypothyroidism are controversial in different studies; some of those showing positive correlation and prompt reversal of changes following treatment **(**Atthans *et al.,* 1988 and Monzani *et al.,* 2004) and while other refuting any correlation between the two (Danese *et al.,* 1996). The screening cost for subclinical hypothyroidism was examined in one of the study and it was found to be cost-effective, this study also included the identification and treatment of lipid abnormalities as a benefit of screening (Cooper *et al.,* 1984). The decision about whether to screen cases for this disorder is clouded by inconsistent evidence of any benefit from early treatment. A few trials have found that persons with subclinical hypothyroidism who are given L-thyroxine experience some improvements in their energy level and feelings of well-being (Nystrom *et al.,* 1988). These studies, however, had few participants, enrolled cases with preexisting thyroid disease resulting from thyroid ablation, and cases referred to specialists rather than the general population. It is not clear that these results can be generalized to individuals who would be identified solely through mass-screening efforts. The aim of our study was to determine whether lipid abnormalities in patients with subclinical hypothyroidism are more common as compared to euthyroid individuals as there are only few Indian studies related to lipid profile changes in subclinical hypothyroidism.

MATERIAL AND METHODS
Subjects
The present study was conducted on 100 (12 males, 88 females) subclinical hypothyroidism cases and equal number of (20 males, 80 females) healthy normal subjects in the age range of 15-65 years (Table 1 & 2).

The patients were screened at Gian Sagar Medical College, Banur, Punjab, India. The patients with elevated TSH levels (> 5.0μiu/ml) and normal total T_3/T_4 levels

were recruited in the study group while the patients suffering from overt hypothyroidism (primary/secondary), undergoing treatment with Thyroxine/anti thyroid drugs, at end stage renal disease, with post myocardial infraction, with congestive cardiac failure, with type 2 diabetes mellitus, undergoing treatment with anti lipidemic drugs, pregnant woman and women on oral contraceptives were excluded.

Blood Collection and Sample Preparation

10ml of blood each was withdrawn from patients of subclinical hypothyroidism and normal healthy subjects after overnight fasting with dry disposable syringe and needle, under all aseptic conditions by venepuncture in the anticubital vein in sterile, dry and acid washed vial. The blood samples were incubated at 37°C temperature for 25-30 minutes for proper clot formation and these blood samples then centrifuged at 3000rpm for 10 minutes for serum separation. This serum sample was used for various biochemical assays.

Biochemical Assays:

Lipid Profile:

- Total cholesterol: Serum cholesterol level was assayed as per the method given by Allain *et al.,* 1974.
- Triglycerides: Serum triglyceride level was estimated by using enzymatic GPO-PAP method given by Mcgowan *et al.,* 1983.
- HDL Cholesterol: HDL-C was determined by the method given by Burstein *et al* ., 1970
- LDL Cholesterol: LDL-C was analyzed by applying the method of Bates *et al.,* 1989.
- VLDL- cholesterol: VLDL-C was estimated by using the method of Lowenstein *et al.,* 1984.

T_4, T_3 and TSH Estimations:

A fully automated Immunoflorescence immunoassay analyzer (Tosoh, AIA -360) was used for the estimation of T_3, T_4 and TSH.

Statistical Analysis

Numerical data was presented as mean ± S.D. The statistical difference was evaluated by Students‘t’test. The difference from normal healthy control subjects was considered significant at $p<0.05$ (Rosner 2000).

RESULTS

TSH, T_3 and T_4

The results of TSH, T_3 and T_4 are summarized in Table 3. A significant increase ($p<0.001$) was observed in TSH levels in patients with subclinical hypothyroidism in comparison to normal healthy subjects whereas significant decrease ($p<0.001$) was seen in the levels of T_4 in cases with subclinical hypothyroidism with respect to euthyroid controls l subjects (from 9.50 ± 2.80 µg/dl to 5.13 ± 1.80µg/dl) and a nominal increase was found in T_3 levels in subclinical hypothyroidism patients in comparison to euthyroid controls.

Lipid Profile

The results of TSH, T_3 and T_4 are summarized in Table 3. A statistical significant increase ($p<0.001$) observed in serum triglyceride levels in subclinical hypothyroidism patients with respect to euthyroid controls. The mean value of triglyceride in the patients of subclinical hypothyroidism was 174.78 ± 32.92 mg/dl and mean serum triglyceride levels in euthyroid controls was 149.10±27.62 mg/dl. Furthermore, the mean VLDL level in cases with subclinical hypothyroidism was 32.82±8.66 mg/dl compared to Euthyroid controls with mean VLDL level 24.09±5.43 mg/dl. It was found to be statistically significant with $p<0.001$. A non significant change was observed in serum cholesterol, LDL-C and HDL-C levels in patients with subclinical hypothyroidism with respect to controls (Table 4).

Furthermore, no statistical difference was also seen in any of the lipid fraction by comparing the TSH levels with the range of 5.0-9.99 µiu/ml and TSH levels above 10 µiu/ml (Table 5).

DISCUSSION

In the present study, we observed a significant increase ($p<0.001$) in the levels of triglyceride and VLDL-C in subclinical hypothyroidism cases with respect to control subjects (Table 4). Hypertriglyceridemia, a well known risk factor for cardiovascular diseases like atherosclerosis. So, there may be some risk involved in cases of subclinical hypothyroidism whereas no significant changes was seen in serum cholesterol, HDL-C and LDL-C levels of subclinical hypothyroidism cases in comparison to control healthy subjects.

The correlation between lipid profile changes and overt hypothyroidism is well established. However, lipid profile alterations in subclinical hypothyroidism are controversial; some studies showing positive correlation and prompt reversal of changes following treatment (Atthans *et al.,* 1988 and Monzani *et al.,* 2004) and few studies refuting any correlation between the two (Houston and Pearson, 2004). The results of the present study contrasts previous study in which it was observed that subclinical hypothyroidism was associated with

Table 1. Distribution of normal healthy controls and Patients of subclinical hypothyroidism on the basis of sex.

Sex	Healthy Control subjects (n)	Patients of subclinical hypothyroidism (n)
Male	20	12
Female	80	88
Total	100	100

Table 2. Distribution of normal healthy controls and Patients of subclinical hypothyroidism on the basis of age.

Age Range (Years)	Healthy control subjects (n)	Patients of subclinical hypothyroidism (n)
15-25	21	22
26-35	27	32
36-45	15	19
46-55	22	13
56-65	15	14
Total	100	100

Table 3. Changes in T_3, T_4 and TSH levels in normal healthy controls and patients of subclinical hypothyroidism

Biochemical Assays	Healthy control subjects (n=100)	Patients of subclinical hypothyroidism (n=100)
T_3 (ng/ml) (Normal range: 0.55-1.70 ng/ml)	$1.29 \pm .103^{a}$	1.34 ± 0.88^{NS}
T_4 (µg/dl) (Normal range: 4.2-12.0 µg/dl)	9.5 ± 1.80^{a}	5.13 ± 0.88^{a} ***
TSH (µiu/ml) (Normal range: 0.35-5.5 µiu/ml)	1.98 ± 0.51^{a}	8.23 ± 2.48^{a} ***

A: values are Mean ± S.D. of 100 observations
NS: non significant w.r.t. Control
****p<0.001 w.r.t. Control*

Table 4. Changes in lipid profile in normal healthy controls and Patients of subclinical hypothyroidism

Biochemical Assays	Healthy control subjects (n=100)	Patients of subclinical hypothyroidism (n=100)
Total cholesterol (mg/dl)	161.83 ± 30.96[a]	181.58 ± 35.16 [a NS]
Triglycerides (mg/dl)	149.10 ± 27.62 [a]	174.78 ± 32.92 [a] ***
LDL (mg/dl)	101.37 ± 13.91 [a]	112.77 ± 24.56 [NS]
HDL (mg/dl)	40.21 ± 12.49 [a]	42.99 ± 17.10 [a]
VLDL (mg/dl)	24.09 ± 5.43 [a]	32.82 ± 8.66 [a] ***

A: values are Mean ± S.D. of 100 observations
NS: non significant w.r.t. Control
****p<0.001 w.r.t. Control*

Table 5. Changes in lipid profile in TSH levels with the range of 5.0-9.99 µiu/ml and above 10 99 µiu/ml.

Lipid Profile	TSH (µiu/ml)	
	5.0-9.99	**>10.0**
Total cholesterol (mg/dl)	171.33± 35.51 [NS]	183.21± 35.57[NS]
Triglycerides (mg/dl)	143.14± 43.61 [NS]	157.00± 42.39 [NS]
LDL (mg/dl)	92.65± 25.35 [NS]	123.64± 23.01 [NS]
HDL (mg/dl)	43.03± 18.70 [NS]	38.79± 12.68 [NS]
VLDL (mg/dl)	28.57± 8.77 [NS]	31.48± 8.64 [NS]

NS: non significant

raised LDL-C levels and thus had larger cardiovascular risk (Bakker *et al.*, 2001). Literature reporting hypercholesterolemia to be more common condition in cases with subclinical hypothyroidism may simply reflects that hypercholesterolemia is a common condition in general population. A significant increase in serum triglycerides and VLDL levels in the present study are in agreement with the report by Vierhapper *et al.*, (2000) that hypothyroid cases showed alteration in pattern of triglyceride kinetics as removal of both endogenous and exogenous triglycerides is markedly reduced and this change seems to account for the hypertriglyceridemia associated with thyroid hypofunction. There is also decrease in plasma post heparin lipolytic activity in hypothyroid state accounting for the raised triglyceride levels (Nikkila and Kekki, 1972). The above factors may play some part in raising the level of serum triglycerides in cases of subclinical hypothyroidism (Miura *et al.*, 1994). On the other hands, raised levels of TSH did not reflect any statistical increase or decrease in levels of various lipid profile parameters (Table 5) suggesting that severity of subclinical hypothyroidism had no significant impact on lipid profile.

A double-blind study, demonstrated that levo-thyroxine replacement in cases with subclinical hypothyroidism had beneficial effects on LDL-C levels and also reduced the clinical symptoms of sub clinical hypothyroidism significantly (Meier *et al.*, 2001). This is further supported by other workers who reported that cardiovascular mortality of 9%–31% can be reduced via improvement in levels of LDL and serum triglycerides (Luboshitzky *et al.*, 2002). So patients of subclinical hypothyroidism receiving levo- thyroxine therapy can be benefited, considering serum triglycerides as important risk factor.

So it can be concluded that patients with subclinical hypothyroidism did not have a marked impact on lipids fractions like total cholesterol, LDL-C and HDL-C levels whereas the raised levels of triglyceride and VLDL-C in patients with subclinical hypothyroidism suggested that subclinical hypothyroidism might be associated with increased risk of some amount of cardiovascular risk, that could be improved on giving L-thyroxine.

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