

**LIPIDS AND ISCHEMIA-MODIFIED ALBUMIN IN MILD SUBCLINICAL HYPOTHYROIDISM:  
RESPONSE TO LEVOTHYROXINE REPLACEMENT**JASEEM T<sup>1</sup>, ANUPAMA HEGDE<sup>1\*</sup>, CHAKRAPANI M<sup>2</sup>, SATHISH RAO<sup>2</sup>, POORNIMA MANJREKAR<sup>1</sup>, RUKMINI MS<sup>1</sup><sup>1</sup>Department of Biochemistry, Kasturba Medical College, Manipal University, Manipal, Karnataka, India. <sup>2</sup>Department of Internal Medicine, Kasturba Medical College, Manipal University, Manipal, Karnataka, India. Email: anupama.hegde@manipal.edu

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**ABSTRACT**

**Objective:** Subclinical hypothyroidism (SCH) with thyroid-stimulating hormone (TSH) less than 10  $\mu$ IU/ml is a common finding discovered during routine thyroid function testing. Thyroxine substitution and its benefits to alleviate dyslipidemia and oxidative stress (OXs) markers at this stage are a matter of debate.

**Methods:** This study aimed to investigate the influence of thyroxine substitution on lipid profile and OXs markers in newly diagnosed SCH subjects. The study included a total number of 50 newly diagnosed (20 treated and 30 untreated), SCH subjects aged 20-50 years with (TSH<10  $\mu$ IU/ml), and free thyroxine (FT4) levels in the normal range. Patients on medications that could cause thyroid hormone dysfunction, diabetes mellitus, and current or pregnancy during the last 2 years were excluded from the study. Serum TSH, T3, T4, FT4, anti-thyroid peroxidase antibodies, total cholesterol (TC), high-density lipoprotein cholesterol (HDL), triglycerides (TG), low-density lipoprotein cholesterol (LDL), and ischemia modified albumin (IMA) were determined in all subjects at baseline and after 9 months.

**Results:** After thyroxine replacement, a significant decrease in TSH, LDL, IMA and an increase in FT4 were observed. The decrease in TC was not statistically evident. There was no significant change in T3, T4, TG, HDL, after treatment. The untreated group showed an insignificant increase only in TSH.

**Conclusion:** Thyroid substitution therapy has a favorable influence on lipid profile and OXs, where it particularly reduced LDL and IMA.

**Keywords:** Subclinical hypothyroidism, L-thyroxine, Lipids, Ischemia-modified albumin, Oxidative stress.

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**INTRODUCTION**

Subclinical hypothyroidism (SCH) is a common finding discovered during routine thyroid function testing with a prevalence reaching up to 10-20% worldwide [1-3]. SCH is a well-established clinical entity with biochemical evidence of cardiovascular risk similar to that of overt hypothyroidism in relation to atherogenic lipids and oxidative stress (OXs) markers when thyroid-stimulating hormone (TSH) levels are >10  $\mu$ IU/ml [4-6].

Recent prevalence studies [1-3] show that 80-90% of patients with SCH have TSH <10  $\mu$ IU/ml. Most of the studies taken up in the past decade did not categorize SCH subjects based on the degree of TSH elevation while concerning cardiovascular impact and management protocol in them. The data on this subdivision are scanty and evidence in favor of thyroxine therapy is not well established; hence, studies to assess the cardiovascular risk in the newly defined SCH patients are needed. Hence, this study aimed to study the influence of thyroxine substitution on lipids and OXs based on ischemia-modified albumin (IMA) in newly diagnosed SCH subjects with TSH<10  $\mu$ IU/ml.

**METHODS**

A total of 50 newly diagnosed SCH subjects aged 20-50 years with TSH<10  $\mu$ IU/ml and free thyroxine (FT4) levels in the normal range for a minimum period of 3 months (20 treated and 30 untreated) were followed prospectively for 9 months. Patients on medications that could cause thyroid hormone dysfunction, diabetes mellitus, and current or previous pregnancy in the last 2 years were excluded from the study. L-thyroxine (LT4) was administered at doses ranging from 25-100  $\mu$ g/day.

Written informed consent was taken from all subjects. The study was approved by the Institutional Ethics Committee. Fasting serum TSH, T3, T4, FT4, anti-thyroid peroxidase (anti-TPO) antibodies, total cholesterol (TC), high-density lipoprotein cholesterol (HDL), triglycerides (TG), low-density lipoprotein cholesterol (LDL), IMA, and atherogenic index of plasma (AIP) were determined in all subjects at baseline and after 9 months.

**Laboratory parameters**

Thyroid function tests were performed by electrochemiluminescence assay. Anti-TPO antibody was measured using enzyme-linked immunosorbent assay (ELISA) kits with a normal range of 0.1-34.0 IU/L. TC, TG, HDL, LDL-C direct levels were estimated using Roche kits in fully automated biochemistry analyzer.

**IMA estimation**

IMA was estimated by colorimetry using the method developed by Bar-Or *et al.* [7]. The absorbance of the assay mixture was read at 450 nm using ELISA reader. IMA was reported in absorbance units.

AIP was calculated using the formula [ $\log$  [TG/HDL cholesterol]], and value over 0.5 has been proposed as the cutoff point indicating atherogenic risk [8]. The association between TG and HDL cholesterol reflected by this ratio depicts the balance between atherogenic and protective lipoproteins.

**Statistical analysis**

Comparisons between the patients at two-time points were performed using paired t-test for normally distributed data and Wilcoxon signed-rank test for nonparametric distribution. A  $p$ <0.05 was considered statistically significant.

## RESULTS

Thyroid function tests of both treated and untreated groups at induction and after 9 months of follow-up were compared (Table 1). Following thyroxine replacement, there was a significant decrease in TSH and increase in FT4 and T4, whereas no significant changes were observed in T3, anti-TPO values. Thyroid autoimmunity was evident in 17 (85%) of subjects in the treated group. The untreated group showed an increase in TSH and in FT4 which was not statistically significant

Lipid and OXs markers were compared in both treated and untreated groups (Table 2). In the treated group, a significant decrease in LDL was observed. There was no significant change in TC, TG, HDL, and AIP after treatment. IMA an indicator of OXs was also reduced after LT4 replacement. The untreated group had no significant alteration in any of the estimated parameters.

## DISCUSSION

This study explores the effects of LT4 replacement therapy on OXs level and lipid profile in patients with SCH. LT4 substitution showed the favorable effect on lipid profile in SCH subjects of the present study. Earlier studies have shown inconsistent results. Few of the studies have reported no change in TC, TG, LDL, and HDL [9,10], whereas few showed a significant decrease in TC and LDL after LT4 replacement [11,12]. Majority of the studies [13] seem to have no significant effect on serum HDL and TG expects for few [14,15]. A significant decrease in LDL and a non-significant decrease in TC after the restoration of euthyroid state were observed in the present study.

**Table 1: Thyroid profile in levothyroxine-treated and untreated groups at baseline and after 9 months**

Parameters	Untreated group (n=30)		Treated group (n=20)	
	Baseline	After 9 months	Baseline	After 9 months
Gender (M/F)	(4/26)		(1/19)	
Age (years)	37±8		35±9	
TSH (μIU/ml)	6.1±1.69	7.46±5.08	6.7±1.7	2.16±1.9*
FT4 (ng/dL)	1.09±0.26	0.962±0.13	0.97±0.73	1.71±0.43*
T3 (ng/mL)	1.06±0.13	1.03±0.16	1.01±0.15	1.12±0.28
T4 (μg/dL)	7.57±1.65	7.20±1.45	6.78±1.6	8.92±2.6*
Anti-TPO (IU/ml) <sup>#</sup>	12.46±7.3	13.7±10	283 (349)	234 (272)

Values are the mean±SD. Replacement effects of L-T4 were analyzed by paired t-test and by \*Wilcoxon signed-rank test for nonparametric distribution.

\*p<0.05 after L-T4 replacement therapy. TSH: Thyroid-stimulating hormone, FT4: Free thyroxine, Anti-TPO: Anti-thyroid peroxidase antibodies

**Table 2: Lipids and IMA in levothyroxine-treated and untreated groups at baseline and after 9 months**

Parameters	Untreated group (n=30)		Treated group (n=20)	
	Baseline	After 9 months	Baseline	After 9 months
TC (mg/dL)	167±28	173±35	159±45	145±36
TG (mg/dL)	114±46	127±43	111±25	116±45
HDL (mg/dL)	44±8	44±10	41±7	40±6
LDL (mg/dL)	117±28	119±38	127±32	109±33*
AIP	0.36±0.19	0.44±0.20	0.39±0.11	0.47±0.18
IMA (ABSU)	0.70±0.18	0.75±0.32	0.80±0.33	0.53±0.16*

Values are the mean±SD. Replacement effects of L-T4 were analyzed by paired t-test. \*p<0.05, TG: Triglycerides, HDL-c: High-density lipoprotein cholesterol, TC: Total cholesterol, LDL-c: Low-density lipoprotein cholesterol, AIP: Atherogenic index of plasma, IMA: Ischemia-modified albumin, ABSU: Absorbance units

In untreated SCH subjects, there was no significant variation in any of the biochemical parameters except for a further elevation in TSH. The percentage alteration in TSH and FT4 was 23% and 12%, respectively, after 9 months. Karmisholt *et al.* [16] reported that a 40% increase in TSH and 15% decrease in FT4 from the initial values can be considered significant in untreated stable SCH with TSH initially up to 12 mU/L in an 1-year follow-up.

IMA as measured using the ACB test is currently the most promising biomarker for early detection of ischemic stress [17]. Recent studies have reported a strong association of IMA with oxidative stress (OXs) and its generation depends on the extent of OXs [18]. Studies suggested that elevated IMA levels can be a clinically useful marker of oxidative damage to protein and OXs in hypothyroidism [19]. However, results of IMA in SCH are inconsistent and inconclusive [20-22]. In the present study, LT4 replacement in SCH patients caused a significant decrease in IMA levels. Our results contradictory to the finding of Erem *et al.* [23], wherein serum IMA levels did not decrease significantly after replacement. Ma *et al.* [19] reported a significant positive association between IMA levels and TPOAb in overt hypothyroid subjects and its reduction after LT4 replacement. Elevated anti-TPO in Hashimoto's thyroiditis is found to be associated with OXs; similarly, hyperlipidemia of any cause is also reported to be associated with an increase in OXs and IMA levels [18,24,25]. In the present study, coexistence of elevated anti-TPO and high cholesterol levels (total and LDL) was found to be associated with high IMA levels at baseline which reduced on LT4 replacement.

The mechanism of OXs in hypothyroidism seems to be multifactorial because thyroid hormone (T3) is associated with the regulation of prooxidant and antioxidant balance [26]. Direct effects of thyroid hormones on the regulation of antioxidant enzymes, protein, and vitamin are the proposed mechanisms associated with increased OXs [27,28]. The plausible explanations for altered OXs markers in SCH are attributed to the direct effects of TSH on OXs and inflammatory processes [29]. In contrast to this hypothesis, other studies supported the concept that OXs itself can alter circulating thyroid function parameters and can trigger the autoimmune process resulting in underactive thyroid condition [30,31].

The current study differs from most of earlier studies with respect to the TSH cutoff considered and recruitment of relatively young subjects without pre-existing alterations and other comorbidities at baseline. Smaller sample size and replacement therapy which are not placebo-controlled are the major limitations of this study. Estimation of albumin adjusted-IMA would have provided further insights into the level of IMA. As our study group did not have any other complications except for slight alteration in TSH, this limitation is not likely to affect the conclusions drawn.

Dyslipidemia in SCH is often associated with altered LDL. OXs in SCH if not given due attention can cause oxidation of LDL resulting in oxidatively modified LDL, a potent proatherosclerotic mediator. The results of the current data demand conduction of large-scale prospective studies with more potent markers to elucidate the role of thyroid autoimmunity on lipids and OXs and to define the role of L-T4 therapy on atherogenic lipids and OXs in SCH subjects with mildly elevated TSH.

## CONCLUSION

Thyroxine substitution therapy had a favorable influence on lipid profile and OXs, where it significantly reduced both LDL and IMA.

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## REFERENCES

- Garber JR, Cobin RH, Gharib H, Hennessey JV, Klein I, Mechanick JI, et al. Clinical practice guidelines for hypothyroidism in adults: Cosponsored by the American association of clinical endocrinologists and the American thyroid association. *Thyroid* 2012;22:1200-35.
- Pearce SH, Brabant G, Duntas LH, Monzani F, Peeters RP, Razvi S, et al. 2013 ETA Guideline: Management of subclinical hypothyroidism. *Eur Thyroid J* 2013;2(4):215-28.
- Unnikrishnan AG, Menon UV. Thyroid disorders in India: An epidemiological perspective. *Indian J Endocrinol Metab* 2011;15 Suppl 2:S78-81.
- Rodondi N, Bauer DC. Subclinical hypothyroidism and cardiovascular risk: How to end the controversy. *J Clin Endocrinol Metab* 2013;98(6):2267-9.
- Gaurav G, Preeti S, Pradeep K, Rachna S. Cardiovascular risk in patients with mild to severe subclinical hypothyroidism. *Asian J Pharm Clin Res* 2016;9:183-5.
- Cooper DS, Biondi B. Subclinical thyroid disease. *Lancet* 2012;379(9821):1142-54.
- Bar-Or D, Lau E, Winkler JV. A novel assay for cobalt-albumin binding and its potential as a marker for myocardial ischemia—a preliminary report. *J Emerg Med* 2000;19(4):311-5.
- Millán J, Pintó X, Muñoz A, Zúñiga M, Rubiés-Prat J, Pallardo LF, et al. Lipoprotein ratios: Physiological significance and clinical usefulness in cardiovascular prevention. *Vasc Health Risk Manag* 2009;5:757-65.
- Anagnostis P, Efstathiadou ZA, Slavakis A, Selalmatzidou D, Poulasouchidou M, Katargari S, et al. The effect of L-thyroxine substitution on lipid profile, glucose homeostasis, inflammation and coagulation in patients with subclinical hypothyroidism. *Int J Clin Pract* 2014;68(7):857-63.
- Aksoy DY, Cinar N, Harmanci A, Karakaya J, Yildiz BO, Usman A, et al. Serum resistin and high sensitive CRP levels in patients with subclinical hypothyroidism before and after L-thyroxine therapy. *Med Sci Monit* 2013;19:210-5.
- Adrees M, Gibney J, El-Saeity N, Boran G. Effects of 18 months of L-T4 replacement in women with subclinical hypothyroidism. *Clin Endocrinol (Oxf)* 2009;71(2):298-303.
- Razvi S, Ingole L, Keeka G, Oates C, McMillan C, Weaver JU. The beneficial effect of L-thyroxine on cardiovascular risk factors, endothelial function, and quality of life in subclinical hypothyroidism: Randomized, crossover trial. *J Clin Endocrinol Metab* 2007;92(5):1715-23.
- Villar HC, Saconato H, Valente O, Atallah AN. Thyroid hormone replacement for subclinical hypothyroidism. *Cochrane Database Syst Rev* 2007;3:CD003419.
- Mutlu S, Parlak A, Aydogan U, Aydogdu A, Soykut B, Akay C, et al. The effect of levothyroxine replacement therapy on lipid profile and oxidative stress parameters in patients with subclinical hypothyroid. *Arch Pharm Res* 2013.
- Asranna A, Taneja RS, Kulshreshtha B. Dyslipidemia in subclinical hypothyroidism and the effect of thyroxine on lipid profile. *Indian J Endocrinol Metab* 2012;16 Suppl 2:S347-9.
- Karmisholt J, Andersen S, Laurberg P. Variation in thyroid function tests in patients with stable untreated subclinical hypothyroidism. *Thyroid* 2008;18(3):303-8.
- Gaze DC. Ischemia modified albumin: A novel biomarker for the detection of cardiac ischemia. *Drug Metab Pharmacokinet* 2009;24(4):333-41.
- Duarte MM, Rocha JB, Moresco RN, Duarte T, Da Cruz IB, Loro VL, et al. Association between ischemia-modified albumin, lipids and inflammation biomarkers in patients with hypercholesterolemia. *Clin Biochem* 2009;42(7-8):666-71.
- Ma SG, Yang LX, Bai F, Xu W, Hong B. Ischemia-modified albumin in patients with hyperthyroidism and hypothyroidism. *Eur J Intern Med* 2012;23(6):e136-40.
- Reddy SV, Suchitra MM, Pradeep V, Alok S, Suresh V, Bitla AR, et al. Ischemia-Modified albumin levels in overt and subclinical hypothyroidism. *J Endocrinol Invest* 2015;38(8):885-90.
- Roy S, Banerjee U, Dasgupta A. Effect of sub clinical hypothyroidism on C-Reactive protein and ischemia modified albumin. *Mymensingh Med J* 2015;24(2):379-84.
- Oncel M, Kiyici A, Onen S. Evaluation of the relationship between ischemia-Modified albumin levels and thyroid hormone levels. *J Clin Lab Anal* 2015;29(6):427-31.
- Erem C, Suleyman AK, Civan N, Mentese A, Nuhoglu I, Uzun A, et al. Ischemia-Modified albumin and malondialdehyde levels in patients with overt and subclinical hyperthyroidism: Effects of treatment on oxidative stress. *Endocr J* 2015;62(6):493-501.
- Rostami R, Aghasi MR, Mohammadi A, Nourooz-Zadeh J. Enhanced oxidative stress in Hashimoto's thyroiditis: Inter-relationships to biomarkers of thyroid function. *Clin Biochem* 2013;46(4-5):308-12.
- Nanda N, Bobby Z, Hamide A. Oxidative stress in anti thyroperoxidase antibody positive hypothyroid patients. *Asian J Biochem* 2012;7:54-8.
- Mishra P, Samanta L. Oxidative stress and heart failure in altered thyroid states. *Sci World J* 2012;2012:741861.
- Mancini A, Di Segni C, Raimondo S, Olivieri G, Silvestrini A, Meucci E, et al. Thyroid hormones, oxidative stress, and inflammation. *Mediators Inflamm* 2016;2016:6757154.
- Gaurav G, Preeti S, Pradeep K, Rachna S. Scope of inflammatory markers in subclinical hypothyroidism. *Asian J Pharm Clin Res* 2015;8(6):24-7.
- Dardano A, Ghiadoni L, Plantinga Y, Caraccio N, Bemi A, Duranti E, et al. Recombinant human thyrotropin reduces endothelium-dependent vasodilation in patients monitored for differentiated thyroid carcinoma. *J Clin Endocrinol Metab* 2006;91(10):4175-8.
- Nadol'nik LI. Stress and the thyroid gland. *Biomed Khim* 2010;56(4):443-56.
- Baser H, Can U, Baser S, Yerlikaya FH, Aslan U, Hidayetoglu BT. Assessment of oxidative status and its association with thyroid autoantibodies in patients with euthyroid autoimmune thyroiditis. *Endocrine* 2015;48(3):916-23.