

“Study of association of serum lipid profile, IL-6, & ADMA levels with subclinical hypothyroidism”

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Abstract

Background & Objectives: Subclinical hypothyroidism (SCH) represents the mildest form of thyroid hormone deficiency and may be associated with adverse consequences. SCH is defined as an elevated serum thyrotropin (often referred to as thyroid-stimulating hormone, or TSH) level with normal levels of free thyroxine (FT₄) that affects up to 10% of the adult population. The identification of patients with subclinical hypothyroidism having an increased cardiovascular risk is of great importance in the present era. The aim of the study was to evaluate cardiovascular risk factors in patients with subclinical hypothyroidism.

Methods: 120 patients with subclinical hypothyroidism and 120 age and gender matched healthy euthyroid controls in the age group of 18-70 years were included in the study. Body mass index (BMI), lipid profile, and levels of serum biomarkers were estimated in both the groups and further compared using student t-test. Lipid profile was also correlated with the serum ADMA & IL-6 and the results were analyzed using Pearson's correlation coefficient.

Results: Patients with subclinical hypothyroidism had significantly higher levels of serum TSH, IL-6 and ADMA. Similarly, serum total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) were significantly increased and HDL-C was significantly decreased in SCH patients when compared to the same parameters of controls ($p < 0.001$). Significant positive correlations were found among lipid profile and serum IL-6 & ADMA.

Conclusions: The present study concluded that the SCH patients presented increased concentrations of cardiovascular disease (CVD) risk factors viz. IL-6, ADMA & lipid profile. The potential benefits of diagnosis and treatment of subclinical hypothyroidism may have possible advantages firstly by preventing the progression to overt hypothyroidism and secondly decrease the risk of death from cardiovascular disease by starting appropriate therapy to improve lipid parameters.

Key words: BMI, Subclinical Hypothyroidism, IL-6, ADMA, Cardiovascular risk

INTRODUCTION

Subclinical hypothyroidism is biochemically defined as an elevated serum thyrotropin (often referred to as thyroid-stimulating hormone, or TSH) level with normal levels of free thyroxine (FT₄). Albeit the connection between plain hypothyroidism and atherosclerosis has been confirmed, the one amongst SCH and atherosclerosis is problematic.¹ Subclinical hypothyroidism exists when serum thyroid levels are inside the reference range; however, serum thyrotropin levels are raised beyond the reference range. The finding of subclinical hypothyroidism is a biochemical determination exclusively found on thyroid capacity testing. In iodine-adequate population, subclinical hypothyroidism influences up to 10% of the populace, with the most noteworthy impact among ladies and old individuals.² Whether SCH has any obvious impact on serum lipid fractions has been dubious. Few reports have shown that patients with SCH have impaired lipid digestion, including raised serum levels of total cholesterol (TC) and low-density lipoprotein -cholesterol (LDL-C). Such changes are, by and large, perceived as hazardous factors for they result in atherosclerosis and coronary disease.³ SCH is an increased risk of cardiovascular disease, coronary artery disease, and coronary illness-related mortality. Moreover, patients with SCH in their middle years might present with mental disability, vague indications like exhaustion, and emotional episodes. Other cardiovascular hazard factors, for example, pulse changes and atherosclerosis have been connected with SCH. A change in lipid profile (LP) is one more result of SCH. Thyroid hormone influences lipid metabolism. Furthermore, various studies have observed that the lipid levels increase as TSH levels rise. Serum low-density lipoprotein-C (LDL-C) levels were seen to be higher in SCH patients in a few studies. Some of them found more raised serum total cholesterol (TC) levels in SCH patients, though others found lower serum TC levels in SCH patients.

Changes in blood high-density lipoprotein-C (HDL-C) and triglycerides (TG) levels under SCH have additionally yielded incongruous outcomes.⁴

It would be very unreasonable to say that SCH is related with cardiovascular disease (CVD), because of incongruous discoveries in past reports. Since inflammatory markers have arisen as an assessor of cardiovascular risk, they might give a superior perspective on the emerging hazard of cardiovascular disease. Interleukin-6 (IL-6), an inflammatory cytokine assumes a significant role in the pathophysiology of cardiovascular disease because of its expected connection with obesity, irritation, and coronary disease. IL-6 manages the development of C-reactive protein (CRP), an acute phase protein that is normally incorporated in the liver, which has been noticed to have a positive relationship with TSH in SCH patients.⁵

Pathophysiology of atherosclerosis shows a relationship with inflammatory markers, which along with dyslipidemia may speed up the atherogenesis, in later life in patients of SCH. IL-6 and C-reactive protein (CRP) are provocative markers that are dependable and notable indicators of cardiovascular disease. CRP is a biochemical means for the determination of coronary artery disease. IL-6 is a cytokine and a laid out provocative marker that potentiate the action of CRP.⁶ The intracellular concentration of asymmetric dimethylarginine (ADMA), an endogenous NOS inhibitor, as well as the transport of L-arginine and the rate, at which this amino acid is degraded by arginase, appear to be crucial variables in nitric oxide production control. This methylarginine appears to be the risk factor for a wide range of conditions, including hypertension, cardiovascular disease, diabetes, hypothyroidism, and hypertensive disorders of pregnancy.⁷

The serum levels of TSH were significantly higher in SCH patients, whereas FT3 and FT4 levels were within the normal range. TC, triglycerides, and LDL-C levels were significantly higher in patients group; while the HDL-C levels were significantly lower in SCH patients.⁸ Serum concentration of FT4 was not significantly different between the groups.⁹ Serum levels of TG, total cholesterol, and LDL-C were higher in the SCH group than the control group.¹⁰ Participants with SCH had considerably greater serum total cholesterol (TC) and low-density lipoprotein (LDL-C) values than those without SCH.⁴ Previous study showed that ADMA level was positively correlated with TSH and LDL-C levels when compared to the control group.¹ Similarly, IL-6 levels were also higher in SCH patients.⁸ The present study was carried out to evaluate the cardiovascular disease risk association with the subclinical hypothyroidism; with key on the analysis of cardiovascular disease risk markers and inflammatory markers.

MATERIALS AND METHODS:

The present study was conducted at Shree Guru Ram Rai Institute of Medical and Health Sciences, Dehradun, Uttarakhand and Adesh Medical College and Hospital, Shahabad, Haryana, India. A total of 120 patients with SCH in the age group of 18-70 years & an equal number of age and sex matched healthy controls were included in the study. The patients were included on the basis of elevated serum TSH level > 6.5 mU/L, normal FT₃ (2.0 – 4.0 pg/mL), normal FT₄ (0.7 – 1.7 ng/dL), healthy controls with normal thyroid functions whereas, obese people with BMI > 30 Kg/m²; smokers & alcoholics, patients with diabetes mellitus, renal disorders, hepatic failures, thyroid cancers along with patients already diagnosed cases of hypothyroidism or those already on treatment, history of antipsychotic drugs, estrogen therapy were excluded from this study. Written informed consent was obtained from all subjects prior to the study sampling and the above study was approved by IEC.

After 12 hours of fasting, about 5 ml of venous blood sample were collected in plain tube from both the groups. Serum was separated and preserved at –80°C for the subsequent analyses. Serum free T3 (FT3), free T4 (FT4) and TSH and cardiovascular risk factors; Interleukin-6 (IL-6) and asymmetric dimethyl arginine (ADMA) were measured by enzyme linked immunosorbent assay (ELISA). Lipid parameters (triglycerides, total cholesterol and high density lipoprotein cholesterol) were analysed by fully autoanalyzer (EM-360). Low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) were calculated by Friedwald's formula.¹¹ BMI was calculated using the equation (body weight in kilograms divided by body height in meters squared) (kg/m²).¹¹

The statistical analysis was done using SPSS (version 26.0). The descriptive analysis of BMI, lipid profile, thyroid profile, serum IL-6 & ADMA were expressed in terms of mean and standard deviation. Student's t-test was applied to study the comparative analyses. The correlation analysis among the lipid profile, BMI, serum IL-6 & ADMA were studied by Pearson's correlation coefficient. $p < 0.05$ were considered statistically significant.

RESULTS:

Total 120 patients (87 females and 33 males) with subclinical hypothyroidism were enrolled for the study and compared with 120 euthyroid controls (77 females and 43 males). There was a statistically significant difference in age between both the groups. There was a statistically significant difference in BMI ($23.35 \pm 2.21 \text{ kg/m}^2$) between patients with SCH and the healthy control group ($22.32 \pm 2.29 \text{ kg/m}^2$) ($p < 0.001$) (Table 1). TSH levels were significantly higher in the SCH group as compared to the control group (9.57 ± 6.84 vs 2.97 ± 0.91). FT4 levels were not significantly different in the SCH group (13.81 ± 18.5 vs 13.55 ± 2.52). Similarly FT3 concentration was not significantly different in SCH patients compared with the control group (4.27 ± 0.82 vs 4.51 ± 1.2). TC, TG, HDL-C, and LDL-C levels were significantly different in SCH patients as compared to controls. Total cholesterol was significantly higher in the SCH group compared to the control group (190.66 ± 42.08 vs 164.67 ± 7.4) ($p < 0.001$). Other parameters of lipid profile like triglycerides were also significantly higher in the SCH group while serum concentration of HDL-C was significantly lower in the SCH group. The serum ADMA levels were significantly higher in the SCH group compared to the control group (748.88 ± 262.82 vs 108.99 ± 33.47) ($p < 0.001$) (Table 2) and the serum levels of IL-6 were significantly higher in the SCH patients compared to the controls (397.24 ± 66.78 vs 84.42 ± 35.02) (Table 2). There was a statistically significant positive correlation of TSH

hormone with lipid profile (TC, TG, LDL and VLDL) while significant negative correlation was observed with HDL cholesterol and ADMA level among patients with SCH (table 6).

DISCUSSION:

Hypothyroidism is a chronic condition characterized by varying degrees of thyroid dysfunction and metabolic consequences. With a normal blood FT4 concentration, SCH is defined as an increase in serum TSH above the top limit of the reference range.¹² In developed countries, cardiovascular illnesses are the main cause of death. Atherosclerosis is a disease with many causes and smoking, deranged lipid profile, diabetes mellitus and hypertension are all known risk factors.¹³ Furthermore, multiple case-control, cross-sectional, and prospective investigations have consistently shown that ADMA is a significant risk factor for vascular disease progression.¹⁴ In addition to validating the previous findings that untreated SCH in patients is linked with a cluster of minor pro-atherogenic anomalies, the results of this case-control prospective study that evaluated the premature indicators of vascular atherosclerosis in SCH patients is another interesting aspect of our research. The levels of ADMA, an early indicator of endothelial dysfunction, were considerably higher in SCH patients in the study.¹⁵

The present study found a statistically significant increase in BMI in patients with subclinical hypothyroidism (SCH) when compared with the control subjects ($p < 0.001$), which is in concordance with the previous studies.^{5, 18, 19} whereas few studies had contradictory findings as compared with the findings of our study.^{20, 21} A statistically significant increase in the levels of serum lipid profile was observed in patients with subclinical hypothyroidism (SCH) as compared to control subjects ($p < 0.001$). This is consistent with the finding of other studies.^{18, 22} whereas few studies have ruled out such changes.^{20, 23}

The present study demonstrated a statistically significant increase in the ADMA & IL-6 in patients with subclinical hypothyroidism (SCH) as compared to control subjects ($p < 0.001$), which is similar to the finding of the previous studies.^{15, 20-21, 24-25} while contradictory to some of the previous findings.²⁶⁻²⁸

CONCLUSION:

The present study showed statistically significant increase in the atherosclerotic risk factors viz. BMI & serum lipid profile. Similarly, the cardiovascular risk markers; ADMA & IL-6 were also elevated significantly in patients with SCH compared with the control group. Hence the estimation of serum biomarker ADMA & IL-6 can be considered in the diagnosis of re CVD in SCH patients.

Conflict of Interest: Nil.

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REFERENCES:

1. Arpaci D, Karakece E, Tocoglu AG, Ergenc H, Guroglu G, Ciftci IH et al. Endocan, TGF-beta, and ADMA as risk factors for endothelial dysfunction and possible vascular disease in patients with subclinical hypothyroidism. *Ann Clin Lab Sci.* 2016 Dec 1;46(6):601-7. PMID: 27993872.
2. Gupta G, Sharma P, Kumar P, Itagappa M. Study on subclinical hypothyroidism and its association with various inflammatory markers. *J Clin Diagn Res.* 2015 Nov;9(11):BC04.
3. Ejaz M, Kumar P, Thakur M, Bachani P, Naz S, Lal K, et al. Comparison of Lipid Profile in Patients With and Without Subclinical Hypothyroidism. *Cureus.* 2021 Aug 19;13(8):e17301.
4. Biondi B, Cappola AR, Cooper DS. Subclinical hypothyroidism: a review. *JAMA.* 2019 Jul 9;322(2):153-60.
5. Turhan S, Sezer S, Erden G, Guctekin A, Ucar F, Ginis Z et al. Plasma homocysteine concentrations and serum lipid profile as atherosclerotic risk factors in subclinical hypothyroidism. *Ann Saudi Med.* 2008 Mar;28(2):96-101.
6. Zoccali C. Asymmetric dimethylarginine (ADMA): a cardiovascular and renal risk factor on the move. *Journal of hypertension.* 2006 Apr 1;24(4):611-9.
7. Uppu SD, Gupta M. Association between C-reactive protein and interleukin-6 levels in subclinical hypothyroid patients. *Journal of Datta Meghe Institute of Medical Sciences University.* 2018 Oct 1;13(4):195.
8. Ahmed A, Abeer AA, Al-Raof AB, Yasser M. Role of changes in some adipokines with obesity in relation to thyroid function in early diagnosed patients with subclinical hypothyroidism. *The Medical Journal of Cairo University.* 2018 Dec 1;86(December):4657-65.
9. Nik MH, Darabi M, Ziaee A, Hajmanoochehri F. Serum phospholipase A2-IIA, hs-CRP, and lipids in women with subclinical hypothyroidism. *International journal of endocrinology and metabolism.* 2014 Jul;12(3).
10. El-Hefnawy KA, Elsaid HH. Assessment of some inflammatory markers and lipid profile as risk factors for atherosclerosis in subclinical hypothyroid patients. *The Egyptian Journal of Internal Medicine.* 2019 Jun;31(2):115-21.
11. Shah AK, Sarin M, Karunanand B, Mohapatra S, Bhat SA. Association of hormonal status with anthropometric and biochemical parameters in women with polycystic ovary syndrome. *The Journal of Community Health Management* 2017;4(1):30-4.
12. Udovic M, Pena RH, Patham B, Tabatabai L, Kansara A. Hypothyroidism and the heart. *Methodist DeBakey cardiovascular journal.* 2017 Apr;13(2):55.
13. Rodondi N, Newman AB, Vittinghoff E, Rekenire N, Satterfield S, Harris TB et al. Subclinical hypothyroidism and the risk of heart failure, other cardiovascular events, and death. *Archives of internal medicine.* 2005 Nov 28;165(21):2460-6.
14. Böger RH. Asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, explains the "L-arginine paradox" and acts as a novel cardiovascular risk factor. *The Journal of nutrition.* 2004 Oct 1;134(10):2842S-7S.
15. Türemen EE, Çetinarslan B, Sahin T, Cantürk Z, Tarkun I. Endothelial dysfunction and low grade chronic inflammation in subclinical hypothyroidism due to autoimmune thyroiditis. *Endocrine journal.* 2011;1103090544-.
16. Laway BA, War FA, Shah S, Misgar RA, Kotwal SK. Alteration of lipid parameters in patients with subclinical hypothyroidism. *International journal of endocrinology and metabolism.* 2014 Jul;12(3).
17. Jayasingh IA, Puthuran P. Subclinical hypothyroidism and the risk of hypercholesterolemia. *Journal of family medicine and primary care.* 2016 Oct;5(4):809.
18. Geng H, Zhang X, Wang C, Zhao M, Yu C, Zhang B et al. Even mildly elevated TSH is associated with an atherogenic lipid profile in postmenopausal women with subclinical hypothyroidism. *Endocrine Research.* 2015 Jan 2;40(1):1-7.

19. Harada PH, Buring JE, Cook NR, Cobble ME, Kulkarni KR, Mora S et al. Impact of subclinical hypothyroidism on cardiometabolic biomarkers in women. *Journal of the endocrine society*. 2017 Feb 1;1(2):113-23.
20. Zhu C, Gao J, Mei F, Lu L, Zhou D, Qu S et al. Reduction in thyroid-stimulating hormone correlated with improved inflammation markers in Chinese patients with morbid obesity undergoing laparoscopic sleeve gastrectomy. *Obesity Surgery*. 2019 Dec;29(12):3954-65.
21. Cerbone M, Capalbo D, Wasniewska M, Alfano S, Mattace Raso G, Oliviero U et al. Effects of L-thyroxine treatment on early markers of atherosclerotic disease in children with subclinical hypothyroidism. *Eur J Endocrinol*. 2016 Jul 1;175(1):11-9.
22. Abdel-Gayoum AA. Dyslipidemia and serum mineral profiles in patients with thyroid disorders. *Saudi medical journal*. 2014;35(12):1469.
23. Akinci B, Comlekci A, Yener S, Demir T, Bayraktar F, Yuksel F et al. The alteration of serum soluble CD40 ligand levels in overt and subclinical hypothyroidism. *Hormones-Athens*. 2007 Oct 1;6(4):327.
24. Oztas E, Erkenekli K, Ozler S, Aktas A, Buyukkagıncı U, Uygur D et al. First trimester interleukin-6 levels help to predict adverse pregnancy outcomes in both thyroid autoantibody positive and negative patients. *Journal of Obstetrics and Gynaecology Research*. 2015 Nov;41(11):1700-7.
25. Ozcan O, Cakir E, Yaman H, Akgul EO, Erturk K, Beyhan Z et al. The effects of thyroxine replacement on the levels of serum asymmetric dimethylarginine (ADMA) and other biochemical cardiovascular risk markers in patients with subclinical hypothyroidism. *Clinical endocrinology*. 2005 Aug;63(2):203-6.
26. Monzani F, Caraccio N, Meucci G, Lombardo F, Moscato G, Casolaro A et al. Effect of 1-year treatment with interferon- 1b on thyroid function and autoimmunity in patients with multiple sclerosis. *European journal of endocrinology*. 1999;141:325-31.
27. Duyu A, Çıtak EÇ, Ak E, Küpeli S, Küpeli BY, Bayram İ et al. Prevalence and related factors of euthyroid sick syndrome in children with untreated cancer according to two different criteria. *Journal of Clinical Research in Pediatric Endocrinology*. 2018 Sep;10(3):198.
28. Hermenegildo C, Medina P, Peiró M, Segarra G, Vila JM, Ortega J et al. Plasma concentration of asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, is elevated in hyperthyroid patients. *The Journal of Clinical Endocrinology & Metabolism*. 2002 Dec 1;87(12):5636-40.

Table 1: Comparison of Anthropometric parameters among SCH

variable	Case		Control		p-value
	Mean	SD	Mean	SD	
Age (years)	36.57 ± 11.01		34.0 ± 8.5		<0.004
BMI(kg/m ²)	23.35 ± 2.21		22.32 ± 2.29		< 0.001

Table 2: Comparison of cardiovascular markers in SCH patients.

Variables	Cases (n=120)		Controls (n=120)		p-value
	Mean	SD	mean	SD	
ADMA (ng/ml)	748.88 ± 262.82		108.99 ± 33.47		<0.001
IL-6 (pg/ml)	397.24 ± 66.78		84.42 ± 35.02		<0.001

Table 3: Correlation of lipid profile with ADMA & IL-6 in patients with SCH

Variables	ADMA		IL-6	
	r	p	r	p
TC (mg/dl)	0.31	< 0.001	0.36	< 0.001
TG (mg/dl)	0.55	< 0.001	0.617	< 0.001
HDL-C(mg/dl)	-0.46	< 0.001	-0.548	< 0.001
LDL-C (mg/dl)	0.510	< 0.001	0.586	< 0.001
VLDL-C (mg/dl)	0.556	< 0.001	0.617	< 0.001

Table 4: Comparison of lipid profile among SCH patients and the control

Parameters	Cases (n=120)		Control (n=120)		p-value
	Mean	SD	Mean	SD	
TC	190.66	42.08	164.68	7.41	<0.001
TG	247.50	115.90	99.88	21.37	<0.001
HDL-C	45.39	11.97	60.02	7.26	<0.001
LDL-C	194.78	59.84	124.63	10.90	<0.001
VLDL-C	49.50	23.18	19.98	4.27	<0.001

TC: total cholesterol, TG: triglycerides, HDL: high-density lipoprotein, LDL: low-density lipoprotein, VLDL: very low-density lipoprotein

Table 5: Comparison of thyroid profile among SCH patients and the control

Parameters	Cases (n=120)		Control (n=120)		p-value
	Mean	SD	Mean	SD	
FT ₃ (pg/mL)	4.28	0.83	4.52	1.21	0.076
FT ₄ (ng/dL)	13.81	18.50	13.55	2.53	0.879
TSH (mU/L)	9.58	6.84	2.98	0.91	<0.001

FT₃: free triiodothyronine, FT₄: free thyroxine, TSH: thyroid stimulating hormone

Table 6: Correlations of thyroid profile with lipid profile & ADMA in patients with SCH

	TC	TG	HDL	LDL	VLDL	FT ₃	FT ₄	TSH	ADMA
TC	1	0.55	-0.27	0.87	0.55	0.00	-0.11	0.18	0.31
TG		1	-0.59	0.86	1.00	-0.04	-0.03	0.32	0.55
HDL			1	-0.62	-0.59	-0.02	-0.04	-0.32	-0.46
LDL				1	0.86	-0.01	-0.07	0.31	0.51
VLDL					1	-0.04	-0.03	0.32	0.55
FT ₃						1	0.05	-0.12	-0.15
FT ₄							1	-0.01	0.07
TSH								1	0.55
ADMA									1

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)