

Study of Serum High-sensitivity C-reactive Protein in Subclinical Hypothyroidism

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ABSTRACT

Aim: The aim of the study is to see the difference between high-sensitivity C-reactive protein (hsCRP) in subclinical hypothyroidism (SCH) and controls and find an association between hsCRP and thyroid-stimulating hormone (TSH) in SCH.

Materials and methods: Totally, 60 subjects were selected for the study, which included 30 cases of SCH and 30 controls with normal thyroid status.

Results: The mean TSH levels were significantly elevated in SCH when compared with controls (9.20 ± 2.12 mU/mL; 2.26 ± 0.78 mU/mL; p -value: <0.0001 respectively). Significantly elevated hsCRP was observed in SCH when compared with controls (3.05 ± 1.78 mg/L; 0.62 ± 0.39 mg/L; $p < 0.0001$ respectively). As per stratification of cardiovascular disease (CVD) risk by hsCRP, 23% of SCH had high risk of developing CVD. Multivariate linear regression suggested that hsCRP is significantly and positively associated with SCH after adjusting for age and body mass index (BMI).

Conclusion: Elevated levels of hsCRP in SCH suggest inflammation as a possible factor for linking SCH and CVD.

Clinical significance: Progression to overt hypothyroidism and cardiovascular risk are the major implications of SCH. The hsCRP is not only an inflammatory marker, but also a stimulator of inflammation and predictor of CVD. The hsCRP indicates the cardiovascular risk associated with SCH. Hence, it can be used to screen SCH patients who are at a risk of developing CVD.

Keywords: Cardiovascular disease, High-sensitivity C-reactive protein, Subclinical hypothyroidism.

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INTRODUCTION

Subclinical hypothyroidism is a common health problem when compared with overt hypothyroidism, with an incidence of 3 to 8%.¹ The SCH is a condition associated with mild thyroid dysfunction, with elevated TSH and normal levels of thyroid hormones [free triiodothyronine (FT3) and free thyroxine (FT4)]. The SCH is usually asymptomatic and diagnosed either on normal screening of TSH or on evaluating nonspecific symptoms. Various studies have shown a clinical impact of SCH on metabolism, endothelial dysfunction, and various CVD risk factors.²⁻⁴

Numerous inflammatory markers have emerged as independent risk factors for CVD. High-sensitivity C-reactive protein, determined by high-sensitivity immunoassay, is now considered as an effective predictor of CVD than low-density lipoprotein cholesterol.⁵ The CRP is an acute phase reactant synthesized by the liver. It was proposed that CRP directly stimulates the inflammatory process by inducing the expression of adhesion molecules in vascular endothelial cells.⁶ The role of hsCRP for assessing the CVD risk in SCH is conflicting. Studies have shown elevated hsCRP levels in SCH,⁷⁻¹⁰ whereas in few studies, no significant difference was observed.^{11,12} This study is undertaken to observe if hsCRP can predict CVD risk in patients with SCH. The aim of the study was to estimate hsCRP in SCH and compare with euthyroid and also investigate the association between TSH and hsCRP in SCH.

MATERIALS AND METHODS

This is a comparative cross-sectional study done in MedCiti Institute of Medical Sciences. The study group included patients attending the outpatient department in the Department of Medicine. Thirty subjects were grouped as SCH based on thyroid status (an elevated TSH, and FT3 and FT4 in normal reference range). Freshly diagnosed cases with no history of medications

for thyroid disorders were grouped under SCH. Thirty age-matched volunteers with normal thyroid status were grouped as controls.

Patients suffering from diabetes, hypertension, tuberculosis, liver and renal disorders, congestive cardiac failure, and/or any other systemic infections were excluded. Patients on anti-inflammatory drugs, antibiotics, thyroid supplementation, and any medication that can affect thyroid status were excluded. Comorbidities were excluded based on self-reported questionnaire. Pregnancy was also accounted for exclusion from the study. Subjects with hsCRP >10 mg/L were excluded as it could be due to acute infections. A written informed consent was obtained from all the participants. Institutional ethics committee clearance was obtained.

The SCH is defined as a condition where TSH levels are above the upper defined limits, while elevated FT3 and FT4 levels are not present.¹³ The normal ranges of thyroid hormones are: TSH: 0.4 to 5.5 μ LU/mL, FT4: 0.8 to 2.7 ng/dL, FT3: 2 to 4.4 pg/dL.¹⁰

Under strict aseptic technique and by venous puncture, 5 mL of blood sample was collected into properly labeled plain polystyrene tubes. The samples were collected, handled, and transported to the lab according to the guidelines given by the clinical and laboratory standards institute/National Clinical Chemistry Laboratory Standards.^{14,15} Blood samples were centrifuged at 10,000 rpm for 10 minutes and the serum was collected in vials and stored at -80°C until the analysis. Samples were analyzed for thyroid hormones and hsCRP. The analysis was done in a batch after thawing to room temperature.

Serum levels of TSH, FT3, and FT4 were estimated by competitive immunoassay using chemiluminescence technology on Siemens Advia Centaur CP. The reference range of TSH for apparently healthy individual was 0.4 to 5.5 μ U/mL. The assay range for serum TSH was 0.01 to 150 μ U/mL. The intra-assay coefficient of variance (CV) using Randox immunoassay control levels 1, 2, 3 with target values of 0.106, 2.4, 20.9 μ U/mL was 3.6, 4.2, and 4.6% respectively.

The reference range of FT3 for apparently healthy individual was 2.3 to 4.2 pg/dL. The assay range for serum FT3 was 0.2 to 20 pg/dL. The intra-assay CV using random immunoassay controls levels 1, 2, 3 with target values of 3.1, 9.41, 5.8 pg/dL was 5.2, 4.2, and 3.8% respectively. The reference range of FT4 for apparently healthy individual was 0.89 to 1.76 ng/dL. The assay range for serum FT4 was 0.1 to 12 ng/dL. The intra-assay CV using Randox immunoassay control levels 1, 2, 3 with target values of 0.779, 2.18, 3.84 ng/dL was 4.2, 4.6, and 3.2% respectively.

The serum hsCRP was estimated by particle-enhanced immunonephelometric assay on Hitachi Cobas fully automated analyzer. The assay range is from 0.15 to

20mg/L. The intra-assay CV using Randox levels 1, 2, 3 with a target value of 1.0, 2.8, 7.4 mg/L was 3.4, 4.8, and 3.8% respectively.

Sample Size

Sample size was calculated from the difference in mean and standard deviation of hsCRP in SCH and Controls from the study done by Czarnywojtek A et al.²¹ With an α error of 0.05 and Power of 80% using statistical software STATA 14 statistics/data analysis Stata Corp LP (www.stata.com) the estimated sample size was 27 in each group. Hence the sample size in our study was thirty subjects with SCH and 30 controls (27 is rounded off to 30).

The sample size in the present study is 30 in each group totaling to 60.

Statistical Analysis

The data were processed on MS Excel worksheet, and analysis was carried out using STATA 14 statistics/data analysis Stata Corp LP (www.stata.com). The normal distribution of the data was assessed by Kolmogorov-Smirnov test. The results were analyzed by Student's t-test. Univariate linear regression was performed with TSH as independent and hsCRP as dependent variables with age and BMI as covariates in the SCH group. Multivariate linear regression was done after adjusting for age and BMI. A two-tailed probability value of 0.05 was considered as statistically significant.

RESULTS

The study population included 60 subjects, of which 30 were euthyroids and 30 were SCH.

Of the 30 euthyroids included in the study, 23 were females and 7 were males. Of the 30 SCH, 19 were female and 11 were male. All the participants included were between 25 and 50 years of age. The TSH, FT3, FT4, and hsCRP were measured in each subject of both the groups.

Demographic and biochemical parameters of SCH and controls are shown in Table 1.

Table 1: Biochemical parameters of controls and SCH

	Controls (n = 30) mean \pm SD	SCH (n = 30) mean \pm SD	p-value
Age (yrs)	32.2 \pm 6.9	34.1 \pm 9.1	0.36
BMI (kg/m ²)	26.5 \pm 5.7	28.5 \pm 3.9	0.11
FT3 (pg/dL)	3.25 \pm 0.59	3.14 \pm 0.27	0.39
FT4 (ng/dL)	1.46 \pm 0.32	1.34 \pm 0.13	0.06
TSH (μ U/mL)	2.26 \pm 0.78	9.20 \pm 2.21	<0.0001*
hsCRP (mg/L)	0.62 \pm 0.39	3.05 \pm 1.78	<0.0001*

*p-value<0.05 significant;SD: Standard deviation

Table 2: Univariate linear regression for the effect of TSH, age, and BMI on hsCRP in SCH

Variable	β_0 (constant)	β (coefficient)	SE	95% CI	p-value
TSH	-2.28	0.58	0.11	0.34 to 0.82	<0.001*
Age	1.57	0.04	0.035	-0.3 to 0.11	0.24
BMI	5.18	-0.07	0.08	-0.24 to 0.09	0.38

*p<0.05 significant; SE: Standard error; CI: Confidence interval

Table 3: Multivariate linear regression for the effect of TSH, age, and BMI on hsCRP in SCH

Variable	β_0 (constant)	β (coefficient)	SE	95% CI	p-value
Constant	-2.38				
TSH		0.56	0.13	0.31 to 0.85	<0.001*
Age		0.001	0.03	-0.58 to 0.06	0.96
BMI		0.002	0.06	-0.13 to 0.14	0.35

*p<0.05 significant; SE: Standard error; CI: Confidence interval

In our study, no significant difference was observed between age, BMI, FT3, and FT4 in the study groups. The mean TSH levels of the SCH were significantly higher than that of the controls (9.20 ± 2.21 ; 2.26 ± 0.78 $\mu\text{U/mL}$; p-value: <0.0001 respectively). In addition to the TSH levels, significantly higher levels of the hsCRP were observed in SCH when compared with controls (3.05 ± 1.78 , 0.62 ± 0.39 mg/L; p<0.0001) respectively.

Risk stratification of CVD for hsCRP as recommended by the American Diabetes Association (ADA)/Centers for Disease Control and Prevention (CDC) and National Academy of Clinical Biochemistry (NACB) experts is <1, 1 to 3, >3 mg/L for low, moderate, and high risk respectively.^{16,17} In our study, 24 (80%) and 6 (20%) of controls were at low and moderate risk of developing CVD respectively. In SCH, 8 (24%), 15 (53%), and 7 (23%) were at low, moderate, and high risk of developing CVD respectively.

Univariate linear regression, for the effect of TSH, age, and BMI on hsCRP in SCH, are represented in Table 2.

Univariate analysis showed a significant and positive association of hsCRP with TSH in SCH ($\beta = 0.58$), although age and BMI were associated with hsCRP, but were not statistically significant ($\beta = 0.04$, -0.07 , p-value 0.24, 0.38 respectively). Though insignificant in univariate analysis, age and BMI were included in multivariate analysis.¹⁸

Multivariate linear regression for the effect of TSH on hsCRP after adjusting for age and BMI in SCH are represented in Table 3.

Multivariate analysis showed hsCRP is significantly and positively associated with SCH after adjusting for age and BMI ($\beta = 0.56$, p<0.001). The hsCRP was not associated with age or BMI after adjusting for TSH and BMI and TSH and age ($\beta = 0.001$, 0.002, p-value 0.96, 0.35) respectively. The β -coefficient shows the magnitude of relationship between independent and dependent variables.

DISCUSSION

Subclinical hypothyroidism, a mild thyroid dysfunction disorder, has clinical significance due to its high prevalence, risk of progression to overt hypothyroidism, and associated CVD risks. High-sensitivity C-reactive protein is a marker of underlying proinflammatory processes and a strong predictor of CVD. In our study, we observed elevated levels of hsCRP in SCH compared with euthyroids. Similar results were observed in the studies done by Roy et al,⁷ Syamsunder et al,⁸ Yu et al,⁹ and Mhto et al.¹⁰

Roy et al,⁷ in their study, concluded that low-grade inflammation starts in the early stages of hypothyroidism, resulting in elevated hsCRP. Syamsunder et al⁸ studied sympathetic vagal imbalance in SCH and stated that a concomitant increased sympathetic and decreased vagal activities are associated with low-grade inflammation, which is directly linked to hsCRP. Studies have shown elevated levels of other inflammatory markers, such as procalcitonin, erythrocyte sedimentation rate, and interleukin (IL)-6 in SCH.^{19,20} Czarnywojtek et al,²¹ in their study, suggested that the elevation of hsCRP in SCH is due to the interaction of IL-6 with tumor necrosis factor- α and IL-1. Other mechanisms of CRP elevation in SCH were suggested as due to the slowing down of overall metabolic rate, slower uptake of CRP by target cells, and decreased rate of clearance.

La Vignera et al,²² in their study, had shown that SCH affects vascular health by increasing oxidative stress and endothelial dysfunction. Mild thyroiditis is associated with low thyroid dysfunction. The resulting inflammation rises hsCRP independent of other factors.⁹ Studies have also shown that hsCRP is not only produced by liver, but also from atherosclerotic plaques activated by vascular cells, suggesting its role in systemic and tissue inflammation.²³

It is further stated that TSH >10 $\mu\text{U/mL}$ is associated with more elevated cardiovascular risk. In our study,

we observed nine subjects with TSH > 10 μ U/mL; all had hsCRP > 2.5 mg/L indicating that increasing TSH increases cardiovascular risk. As per the risk stratification of CVD for hsCRP recommended by the ADA/CDC and NACB experts, in our study, 7 (23.3%) of SCH were at high risk for developing CVD. This is in accordance with the study done by Sharma et al,²⁴ where 21.6% of SCH were at high risk for developing CVD.

Yu et al⁹ performed multiple linear regression and confirmed significant positive correlation between hsCRP and TSH after adjusting for potential confounder. A significant increase in odds ratio for SCH in progressive hsCRP quartiles was observed, suggesting a dose response effect. In our study, multivariate linear regression had confirmed a positive association between TSH and hsCRP in SCH after adjustment for age and BMI.

Conflicting reports exist that relate the association of TSH with hsCRP in SCH. Hueston et al¹² found no significant association between hsCRP and TSH in SCH after adjusting for potential confounders. But, it was suggested that absence of association between inflammatory markers and TSH does not rule out cardiovascular risk, as it could be due to other mechanisms like autoimmunity. Aksoy et al¹¹ did not find any association between hsCRP and TSH in SCH. But, hsCRP levels correlated with weight-related parameters, such as adiposity, insulin resistance, and metabolic syndrome suggesting inflammation as a link between SCH and cardiovascular risk.

To conclude, SCH usually goes underdiagnosed, but still is associated with various cardiovascular risks. Our results show a positive association between TSH and hsCRP after adjusting for age and BMI, highlighting cardiovascular risk in SCH.

CLINICAL SIGNIFICANCE

Clinical impact of SCH is its progression to overt hypothyroidism and risk for CVD. Hence, TSH should be included in routine screening. The hsCRP is an inflammatory marker, stimulator of inflammation, and predictor of CVD. The hsCRP levels highlight the cardiovascular risk in SCH, and hence, can be used to screen SCH patients. Thus, treatment can be started at the earliest to ward off future cardiovascular events.

Being a cross-sectional study, the etiology of SCH and the following further events could not be done.

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