

The Relationship Between Homocysteine and Autoimmune Subclinical Hypothyroidism

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Abstract

Objectives: The present study was to correlate the association of homocysteine level with various profile of autoimmune subclinical hypothyroidism patients.

Methods: A 40 subclinical hypothyroidism patients and 40 healthy control subjects were enrolled. The relevant laboratory parameters of patients who had been diagnosed with subclinical hypothyroidism due to Hashimoto's thyroiditis in the clinic and were in follow-up, and whose homocysteine level had been tested in addition to assessment of thyroid function, thyroid autoantibodies, lipid parameters, thyroid ultrasonography, and hs-CRP were recorded. Patients with a thyroid-stimulating hormone (TSH) level above the normal range but with a free thyroxin (fT4) level within the normal range were considered to have subclinical hypothyroidism.

Results: Mean TSH level of case and control group subjects were 6.71 ± 1.23 and 2.11 ± 0.34 μ IU/mL respectively. Which is extremely significant ($p < 0.0001$). Mean FT4 (ng/dL) level of case and control group subjects were 1.3 ± 0.38 and 1.4 ± 0.43 ng/dL respectively. Which is not significant differences ($p = 0.273$). Anti-Tg level of case and control group subjects were 221.6 ± 06.65 and 12.2 ± 04.24 IU/mL respectively. Which is extremely significant ($p < 0.0001$). There was significant higher anti-Tg level in subclinical hypothyroidism patients as compared to control subjects. Similarly, anti-TPO level of subclinical hypothyroidism (165.8 ± 05.17) patients were extremely significant ($p < 0.0001$) as compared to control (6.7 ± 02.65) subjects. Homocysteine was significantly associated with TSH level ($p = 0.028$), FT4 level ($p = 0.017$), anti-TPO level ($p = 0.000$), anti-Tg ($p = 0.024$) and Hs-CRP level ($p = 0.011$) in subclinical hypothyroidism patients.

Conclusions: Increased level of homocysteine is an independent factors for the diagnosis of autoimmune subclinical hypothyroidism.

Key words: Autoimmune Subclinical Hypothyroidism, Homocysteine, TSH level.

Introduction

Normal blood FT4 levels in the presence of elevated serum TSH levels are referred to as subclinical hypothyroidism. The incidence of

subclinical hypothyroidism in the population under study ranges from 3% to 15%. Statistics

indicate that subclinical hypothyroidism is more common in women and elderly adults [1].

The etiopathogenesis of subclinical hypothyroidism is the same as that of overt hypothyroidism. Autoimmunity (thyroiditis) is the most common cause of hypothyroidism. Other causes of primary hypothyroidism apart from autoimmunity include iodine deficiency and iatrogenic (radioactive iodine) [2]. Increasing age is an essential factor associated with decreased thyroid functioning in the elderly, which results in the overdiagnosis of subclinical hypothyroidism [3].

Subclinical hypothyroidism is a clinical condition associated with increased chronic inflammation, oxidative stress, and lipid peroxidation. Therefore, homocysteine, an atherosclerotic marker, may be hypothesized to be high in cases of subclinical hypothyroidism [4].

Homocysteine is an intermediate metabolite that emerges as a result of the metabolism of the amino acid methionine. The plasma concentration may vary due to genetic, pathological, or nutritional factors. Homocysteine causes endothelial dysfunction through damage to the elastin lamina and accumulation of lipoprotein-proteoglycan complexes in the vessel wall as a result of a chemical injury in the endothelial tissue [5, 6]. Homocysteinemia, which presents with high levels of homocysteine, is considered to be a risk factor for coronary artery disease [7, 8]. Objectives of our study was to evaluate the relation between homocysteine and autoimmune subclinical hypothyroidism.

MATERIAL & METHODS

The present study was conducted in the Department of Biochemistry, with the collaboration of Department of Radio-diagnosis, Sri Krishna Medical College & Hospital, Muzaffarpur, Bihar, India during a period from January 2022 to July 2022.

Inclusion criteria

A total of 80 cases, 40 patients older than 18 years of age and diagnosed with subclinical

hypothyroidism due to Hashimoto's thyroiditis, and 40 volunteers with no known disease, were included in the study.

Exclusion criteria

Those with known cardiovascular or cerebrovascular diseases, venous or arterial acute or chronic thrombus in any site of the body, acute-chronic kidney or liver diseases, serious surgical intervention, hereditary homocysteinemia, B12 or folic acid deficiency, individuals using any medication that may lead to high levels of homocysteine and those who smoked or consumed alcohol were excluded from the study.

Procedures

The relevant laboratory parameters of patients who had been diagnosed with subclinical hypothyroidism due to Hashimoto's thyroiditis in the clinic and were in follow-up, and whose homocysteine level had been tested in addition to assessment of thyroid function, thyroid autoantibodies, lipid parameters, thyroid ultrasonography, and hs-CRP were recorded.

In addition, the patients' height, weight, body mass index (BMI), and gender were recorded. The hospital real-time health data analysis system was used to collect the parameters.

The diagnosis of Hashimoto's thyroiditis was made based on positive findings of one or both of anti-thyroid peroxidase (anti TPO) and anti-thyroglobulin (anti-Tg) among the laboratory parameters and/or an ultrasonographic result consistent with thyroiditis (heterogeneous parenchymal echogenicity of thyroid) [9].

Following a diagnosis of Hashimoto's thyroiditis, patients with a thyroid-stimulating hormone (TSH) level above the normal range but with a free thyroxine (fT4) level within the normal range were considered to have subclinical hypothyroidism.

The individuals selected as the control group had TSH, fT4, anti-Tg, and anti-TPO measurements within the normal range, and a diagnosis of Hashimoto's thyroiditis was discarded during ultrasonographic examination.

Laboratory parameters

Blood samples were collected between 8:00 and 10:00 in the morning following 12 hours of fasting. All of the samples were assayed in the same laboratory with the same kit within a maximum of time interval of 1-2 hours.

TSH (normal range: 0.5-4 μ IU/mL), fT4 (normal range: 0.9-1.2 ng/dL), anti-TPO (normal range: 0-9 IU/mL), and anti-Tg (normal range: 0-4 IU/mL) levels were measured by using an electrochemiluminescence immunoassay method.

Homocysteine levels were also measured by using an electrochemiluminescence method.

Ultrasonographic findings of thyroid

Thyroid ultrasonography was performed by a single individual using a 7.5-mHz probe and a Logic 7 ultrasonography device (GE Healthcare, Inc. Chicago, IL, USA). The examination included evaluation of the size, echogenicity, and vascularization of the thyroid gland, the presence of a nodule, and assessment of peripheral lymph nodes.

RESULTS

In the present study, a total of 40 subclinical thyroid patients were enrolled. 40 subjects were selected as control group. Out of 40 subclinical hypothyroid subjects, 35% were males and 65% were females. Mean age of case (subclinical hypothyroid) and control group subjects were 38.68 ± 13.46 and 35.46 ± 10.74 years respectively. Which is not statistically significant ($p=0.364$). Similarly, mean BMI of case and control group subjects were 29.57 ± 4.98 and 24.27 ± 3.38 kg/m² respectively. Which is extremely significant differences ($p<0.0001$). Mean SBP of case and control group subjects were 126.12 ± 7.28 and 121.47 ± 11.29 mmHg respectively. Which is statistically significant differences ($p=0.031$). Similarly, Mean DBP of case and control group subjects were 67.59 ± 7.16 and 62.32 ± 5.44 mmHg respectively. Which is highly statistically significant differences ($p=0.0004$). Chronic disease was not seen in any subjects of case and control group.

Table 1: Distribution of demographic and clinical findings in the study population.

Variables	Subclinical hypothyroidism	Control	p-value
Gender (N=40)			
Male	14(35%)	18(45%)	0.364
Female	26(65%)	22(55%)	0.364
Age (years)	38.68 ± 13.46	35.46 ± 10.74	0.2405
BMI (kg/m²)	29.57 ± 4.98	24.27 ± 3.38	<0.0001
SBP (mm Hg)	126.12 ± 7.28	121.47 ± 11.29	0.0316
DBP (mm Hg)	67.59 ± 7.16	62.32 ± 5.44	0.0004
Chronic disease			
Present	00	00	
Absent	40	40	

In the present study, mean TSH level of case and control group subjects were 6.71 ± 1.23 and 2.11 ± 0.34 μ IU/mL respectively. Which is extremely significant ($p<0.0001$). Mean FT4 (ng/dL) level of case and control group subjects were 1.3 ± 0.38 and 1.4 ± 0.43 ng/dL respectively. Which is not significant differences ($p=0.273$). Anti-Tg level of case and control group subjects were 221.6 ± 06.65 and 12.2 ± 04.24 IU/mL

respectively. Which is extremely significant ($p<0.0001$). There was significantly higher anti-Tg level in subclinical hypothyroidism patients as compared to control subjects. Similarly, anti-TPO level of subclinical hypothyroidism (165.8 ± 05.17) patients were extremely significant ($p<0.0001$) as compared to control (6.7 ± 02.65) subjects. Similarly, mean total cholesterol level of subclinical hypothyroidism (196.45 ± 44.28)

patients were significantly difference ($p=0.0505$) as compared to control (179.5 ± 30.87) subjects. Mean triglycerides (107.2 ± 07.76 mg/dL) of case group patients were extremely significant ($p<0.0001$) as compared to control group subject (87.6 ± 05.78). Mean LDL cholesterol level (117.5 ± 31.3 mg/dl) of case group was highly significant ($p=0.0015$) as compared to control group subjects (97.7 ± 21.8 mg/dl). Similarly, mean HDL cholesterol level (48.92 ± 13.8 mg/dL) was very significant ($p=0.0021$) as compared to control group subjects. Mean non-HDL level (146.15 ± 40.37 mg/dL) of case group was

extremely significant (<0.0001) as compared to control group (119.62 ± 32.44) subjects. Mean Hs-CRP level (3.12 ± 01.24 mg/L) of case group was extreme significantly lowered ($p<0.0001$) as compared to control group subjects (6.21 ± 01.65). Similarly, mean homocysteine level (10.34 ± 03.28 $\mu\text{mol/L}$) of case group was extreme significantly higher ($p<0.0001$) as compared to control group subject (0.62 ± 0.23 $\mu\text{mol/L}$). On ultrasonography, all the control group subjects were homogenous. While in case group only 2(5%) subjects were homogenous and all 48(95%) subjects were heterogenous.

Table 2: Distribution of laboratory findings in the study population.

Variables	Subclinical hypothyroidism	Control	p-value
TSH ($\mu\text{IU/mL}$)	6.71 ± 1.23	2.11 ± 0.34	<0.0001
FT4 (ng/dL)	1.3 ± 0.38	1.4 ± 0.43	0.273
Anti-Tg (IU/mL)	221.6 ± 06.65	12.2 ± 04.24	<0.0001
Anti-TPO (IU/mL)	165.8 ± 05.17	6.7 ± 02.65	<0.0001
Total cholesterol (mg/dL)	196.45 ± 44.28	179.5 ± 30.87	0.0505
Triglycerides (mg/dL)	107.2 ± 07.76	87.6 ± 05.78	<0.0001
LDL cholesterol (mg/dL)	117.5 ± 31.3	97.7 ± 21.8	0.0015
HDL cholesterol (mg/dL)	48.92 ± 13.8	59.45 ± 15.71	0.0021
Non-HDL (mg/dL)	146.15 ± 40.37	119.62 ± 32.44	<0.0001
Hs-CRP (mg/L)	3.12 ± 01.24	6.21 ± 01.65	<0.0001
Homocysteine ($\mu\text{mol/L}$)	10.34 ± 03.28	0.62 ± 0.23	<0.0001
Ultrasonography			
Homogeneous	2(5%)	40(100%)	<0.00001
Heterogeneous	38(95%)	0	

In the present study, when clinical and demographical findings were correlated with homocysteine level in case group patients. We found that the TSH level ($p=0.028$), FT4 level

($p=0.017$), anti-TPO level ($p=0.000$), anti-Tg ($p=0.024$) and Hs-CRP level ($p=0.011$) were significantly associated with clinical findings of subclinical hypothyroid patients.

Table 3: Clinical and demographic findings associated with homocysteine.

Variables	Subclinical hypothyroidism	p-value
	R	
Age	0.173	0.286
BMI	0.124	0.446
Systolic blood pressure	0.214	0.185
Diastolic blood pressure	0.176	0.277
TSH	0.348	0.028
FT4	-0.376	0.017
Anti-Tg	0.356	0.024

Anti-TPO	0.578	0.000
Total cholesterol	-0.276	0.085
LDL-cholesterol	-0.231	0.152
HDL-cholesterol	-0.104	0.523
Triglycerides	-0.187	0.248
Non-HDL	-0.281	0.079
Hs-CRP	0.397	0.011

Discussions

Subclinical hypothyroidism (SCH) is characterized by elevated serum thyroid-stimulating hormone (TSH) levels (above normal) and normal FT4 levels [10].

In the present study, the homocysteine, hs-CRP, and LDL cholesterol levels were observed to be higher and the HDL cholesterol level was lower in the autoimmune subclinical hypothyroidism group compared with the healthy control group. In the subclinical hypothyroidism group, a positive correlation was determined between homocysteine level and hs-CRP, anti-TPO, and anti-Tg levels. In the regression analysis performed, the HDL cholesterol, hs-CRP, and homocysteine levels were each found to be an independent risk factor for subclinical hypothyroidism [4].

Homocysteine (Hcy) is a sulfur-containing amino acid, which is the intermediate product of methionine demethylation. Hyperhomocysteinemia is a well-known independent risk factor for atherosclerosis and coronary heart disease [11, 12]. Recent investigations and our previous studies demonstrated that the blood homocysteine level was increased in patients with both subclinical hypothyroidism and hypothyroidism [13, 14]. However, the potential pathophysiological mechanism underlying the increase of homocysteine level in thyroid hormone deficiency state has not been fully elucidated. It has been speculated that the increase of homocysteine was at least partially due to the decrease of renal function [15], because of the evidence that homocysteine was negatively correlated with kidney function in general population [16,17].

Previous studies found that subclinical hypothyroid individuals had systolic dysfunction

after exertion and diastolic dysfunction at rest and that restoring euthyroidism cured these anomalies. Normalization of heart function with euthyroidism resulted in a decrease in isovolumic relaxation time, a decline in pre-ejection period/ejection time, an increase in the early diastolic/late diastolic mitral flow velocity ratio, and a rise in the left ventricular ejection fraction ratio [18,19]. Age-related diseases such as atherosclerosis, coronary heart disease, and neurological conditions are affected by intricate physiological changes in the hypothalamic-pituitary-thyroid axis [20]. Traditional cardiovascular risk factors, such as a rise in homocysteine and low-density lipoprotein-cholesterol (LDL-C), as well as the emergence of a procoagulant state, are aggravated by thyroid failure [21,22]. Cardiovascular disease was more common in men with subclinical hypothyroidism under 50 compared to men with euthyroidism [22]. The most common cardiovascular diseases with subclinical hypothyroidism include coronary artery disease, arterial hypertension, hypercoagulation, atrial fibrillation, and heart failure.

In our study, the homocysteine level was determined to be higher in the group with subclinical hypothyroidism due to Hashimoto's thyroiditis compared with the healthy control group. Deicher et al., [23] Turhan et al., [24] and Sengul et al. [25] also reported a homocysteine level higher in a subclinical hypothyroidism group. While some studies only reported on the homocysteine level in the subclinical hypothyroidism group, other studies noted that increased homocysteine levels may be associated with hyperlipidemia. In our study, in addition to the homocysteine level, the hs-CRP level was also determined to be significantly higher in this group.

Furthermore, a positive correlation was observed between the levels of homocysteine and hs-CRP. This indicates that an elevated homocysteine level may be associated with chronic inflammation. Additionally, a positive correlation was also determined between the TSH level and the homocysteine level. This suggests that the homocysteine level is closely associated with thyroid functions. The determination of a significant reduction in the homocysteine level after treatment with levothyroxin given to euthyroid patients with Hashimoto's thyroiditis by Owecki *et al.* [26] suggests that increased homocysteine levels may also be influenced by thyroid function impairments that do not increase to clinical significance.

Additionally, our findings revealed a positive correlation between the homocysteine level and anti-TPO and anti-Tg levels. This correlation suggests that an increased homocysteine level may be closely associated with autoimmunity in the autoimmune subclinical hypothyroidism group. No significant correlation was determined between the homocysteine level and lipid parameters in the subclinical hypothyroidism group. However, a significant increase in the LDL cholesterol level and a significant reduction in HDL cholesterol were determined in the subclinical hypothyroidism group compared with the healthy control group. There may be an association between homocysteine level and lipid parameters that did not reach the level of significance.

From a pathophysiological standpoint, the mechanisms of the changes are derived from the role of the thyroid hormones on metabolic parameters, including lipoprotein metabolism [27]. With an increase in TSH levels, there was an increase in cholesterol, triglycerides, and LDL-c, and a decrease in HDL-c levels; this association has a linear character [28]. People with subclinical hypothyroidism have a higher increased risk compared to euthyroid patients of developing hypercholesterolemia, increased levels of LDL-c and CRP, and elevated diastolic blood pressure [29]. The major cardiovascular risk factors are diabetes, central obesity, dyslipidemia, elevated

LDL-c levels, and high blood pressure [30], which entails an increased risk of atheromatosis and myocardial ischemia. However, the etiology of hypothyroidism does not seem to influence these cardio-vascular metabolic parameters. For example, antithyroid peroxidase antibodies have not been positively associated with cardiovascular risk in patients with subclinical hypothyroidism [31]. Subclinical hypothyroidism is common in medical practice, and its diagnosis should consider demographics relative to the TSH reference range in the healthy population. According to the literature, in a considerable proportion of patients, subclinical hypothyroidism can be physiologically reversible, without any medication in this regard, but there are also persistent, progressive forms, mainly against the background of chronic autoimmune thyroiditis. Once subclinical hypothyroidism is detected, the patient requires periodic medical evaluations to allow risk stratification [32,33].

Conclusions

The present study concluded that the homocysteine is significantly associated with TSH, FT4, anti-TPO, anti-Tg and Hs-CRP level in subclinical hypothyroid patients. Hence, increased level of homocysteine is an independent factor for the diagnosis of autoimmune subclinical hypothyroidism.

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