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Hemoglobin, Vitamin D, and Lipids in Subclinical Hypothyroid Patients – Do the Anti -Thyroid Autoantibodies Titer Matters?

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ABSTRACT

Hashimoto's thyroiditis, the commonest cause of hypothyroidism has been proposed as a risk factor for atherosclerosis independent of thyroid function. The aim of this study was to investigate whether Hashimoto's thyroiditis validated by their anti-TPO positivity have any effect on hyperlipidemia, Hemoglobin, vitamin D independent of thyroid function in newly diagnosed subclinical hypothyroid patients (SCH) subjects with TSH < 10μIU/ml. 40 newly diagnosed SCH and 40 healthy euthyroid controls were included in this study. Based on anti-TPO status, the SCH subjects was divided into TPO positive and negative groups. Serum lipid, hemoglobin and vitamin D levels were determined and compared between among the TPO positive and negative SCH subjects. Subjects with and without anti-TPO had significant differences in levels of low-density lipoprotein, total cholesterol, and nonHDL cholesterol. In correlation analysis, anti-TPO showed statistically significant association with Hb, LDL and vitamin D. Subclinical hypothyroidism with thyroid autoimmunity is associated with a marginal decrease in hemoglobin and elevation in LDL. Whether this holds, any bearing on promoting cardiovascular risk needs to be considered further

Keywords: Anti -Thyroid Autoantibodies, Hemoglobin, Lipids, Subclinical hypothyroidism, Vitamin D.

INTRODUCTION

Subclinical hypothyroidism (SCH) is a common finding discovered during routine thyroid function testing, which is more prevalent than overt dysfunction reaching up to 10-20% worldwide. [1-3] In iodine-replete areas, hypothyroidism is most commonly caused by Hashimoto's thyroiditis (HT). [4] In HT the immune system attacks the thyroid gland and the resulting inflammation often lead to an underactive thyroid gland. [5] The key biochemical characteristic of HT is the presence of thyroid peroxidase antibody (anti-TPO) in 90-95% subjects. [6] An elevated anti-TPO is reported in 70-80% of SCH subjects. [4]

HT is an independent cardiovascular risk factor in the overt hypothyroid state. [7] SCH with elevated anti-TPO has an increased risk of progression to overt state reinforces its measurement, whether elevated titers influence metabolic markers independent of thyroid function is not clear. The current study was designed to assess the impact of elevated anti-TPO on hemoglobin, vitamin D and lipids in subclinical hypothyroid subjects.

MATERIALS AND METHODS

A total of 40 newly diagnosed SCH subjects with (TSH<10μIU/ml) and FT4 levels in the normal range and 40 healthy subjects as the control group

(group 1) were included in the study. Based on cut-off for positive anti-TPO titer as 34 IU/ml, the patients in the subclinical hypothyroid group were divided into TPO –VE (group 2) and positive +VE (group 3). Patients on medications affecting thyroid function, diabetes mellitus, vitamin supplements and current or previous pregnancy in the last 2 years were excluded from the study. Written informed consent was obtained from all subjects enrolled. The institutional ethical committee approved the work.

Fasting Serum TSH, T3, T4, FT4, anti-TPO, Total cholesterol (TC), High-density lipoprotein (HDL), Triglycerides (TG), low-density lipoprotein (LDL), nonHDL cholesterol (N-HDL-C) Vitamin-D were determined in all subjects using Roche kits in fully automated biochemistry analyzer by the electrochemiluminescence assay (ECLIA).Hb was estimated by Beckman coulter.

Statistical Analysis

All parameters were expressed as Mean ± SD. Statistical analysis was performed by ANOVA followed by Tukey's multiple comparison test. Correlation of anti- TPO with study parameters analysis by spearman's correlation. A '***p***' value less than 0.05 was considered statistically significant.

RESULTS

Comparison of thyroid profile and study parameters between the study groups, summarized in Table.1 and Table.2 respectively. There was no significant difference in any of the estimated parameters except for TSH between group 1 and 2. TC, LDL, N-HDL-C was significantly higher in patients with elevated anti-TPO (Group 3) than the healthy controls (Group 1). When compared between anti-TPO positive and negative cases (group 2 v/s group 3), TC, LDL, N-HDL was found significantly higher in-group 3. A significant reduction in hemoglobin was observed only in-group 3. Correlations between anti-TPO and other study parameters were summarized in Table 3. Anti-TPO showed a significant positive correlation with LDL and a negative correlation with Vit-D and Hb in the subclinical hypothyroid group.

DISCUSSION

In the current study SCH subjects with and without autoimmunity as categorized by the presence of anti-TPO, titers were enrolled to compare their thyroid profile and metabolic parameters. Findings related to lipid parameters

Table 1: Thyroid profile in Anti-TPO Antibody Negative (TPO – VE), anti-TPO Antibody Positive (TPO + VE) subclinical hypothyroid subjects, and control group

Parameters	Group 1 control	Group 2 TPO – VE (< 34 IU/ml) SCH Subjects	Group 3 TPO + VE (≥ 34 IU/ml) SCH Subjects
N	40	21	19
M/F	13/27	7:14	4:15
Age (yrs.)	36 ± 9	34 ±10	34 ± 9
TSH (ìIU/ml)	2.12 ± 0.78	6.18 ± 1.4	6.10 ± 1.3
FT4 (ng/dl)	1.2 ± 0.35	1.1 ± 0.18	1.0 ± 0.25
T4 (µg/dL)	7.8 ±1.09	7.6 ± 1.3	7.2 ± 1.5
Anti-TPO (IU/ml)	-	9.8(5.4- 12.7) #	214(107-475) #

Values represented as mean±SD and #median and interquartile range. Statistical analysis was performed by ANOVA followed by Tukey's multiple comparison test. * P< 0.05 Group 1 Vs Group 3; # P< 0.05 Group 2 Vs Group 3. T3 -free triiodothyronine; T4-thyroxine; FT4-Free thyroxine; TSH-Thyroid stimulating hormone; TPO Ab - Anti-Thyroid Peroxidase Antibodies.

Table 2: Study Parameters between Anti-TPO Negative (TPO - VE) and Anti-TPO Positive (TPO + VE) cases of subclinical hypothyroid subjects and control group

Parameters	Group 1 control	Group 2 TPO – VE (< 34 IU/ml) SCH Subjects	Group 3 TPO + VE (≥ 34 IU/ml) SCH Subjects
HB (g/dl)	13.5 ± 1.3	12.9 ± 1.1	12.4 ± 1.1 *
TC (mg/dl)	175 ± 23	173 ± 33	194 ± 30 * †
TG (mg/dl)	102 ± 37	105 ± 42	108 ± 23
HDL (mg/dl)	48 ± 12	47 ± 11	45 ± 16
LDL (mg/dl)	112 ± 22	119 ± 34	138 ± 24 * †
N-HDL-C (mg/dl)	127 ± 22	126 ±32	149± 31 * †
Vitamin - D (ng/ml)	17.3 ± 11	16.2 ± 6.5	14.1 ± 6.3

Values represented as mean + SD. Statistical analysis was performed by ANOVA followed by Tukey's multiple comparison test. * P< 0.05 Group 1 Vs Group 3; † P< 0.05 Group 2 Vs Group 3. Hb- hemoglobin ; TC - total cholesterol; LDL-C-low-density lipoprotein cholesterol; HDL-C - high-density lipoprotein cholesterol; TG - triglyceride; N-HDL-C- Non HDL cholesterol. TPO Ab - Anti-Thyroid Peroxidase Antibodies; Vit-D -vitamin D.

in SCH are highly inconsistent in earlier studies with values either higher or similar to the euthyroid group.[8-10] Earlier studies have reported that thyroid autoimmunity may have effects on hyperlipidemia independent of thyroid function. [11, 12] Whereas Mazaheri *et al* [13] observed lipid alterations in relation to autoimmunity only in subjects with anti-TPO levels higher than 1000 IU/ml. In this study, TC, LDL, and N-HDL-C levels of TPO (+) patients were found to be significantly higher when compared to TPO (-) cases and controls (Table 2).

Current guidelines endorse the assessment of thyroid status in the work-up of anemia [14, 15] Hypothyroidism adversely affects hematological system causing anemia by its effects on erythropoiesis. [16, 17] The frequency of anemia in SCH is as high as that in the overt state. In the present study, the mean Hb percentage was significantly decreased in TPO (+) SCH (Table 2). Wang *et al* [18] have also reported significantly severe Hb deficiency in Anti-TPO positive patients.

There has been growing evidence of the relationship between vitamin D insufficiency and autoimmune thyroid diseases. [19] We observed lower mean vitamin D levels in TPO (+) SCH, however, the difference did not reach statistical significance. Studies [20, 21] have reported a higher prevalence (92%) of vitamin D insufficiency in HT and its correlation with anti-TPO. In contrast, studies [22, 23] hypothesized that vitamin D deficiency can trigger the autoimmune process resulting in the pathogenesis of underactive thyroid condition. Randomized placebo-controlled trials demonstrated the ameliorative effects of vitamin D supplementation on anti-TPO in autoimmune thyroiditis. [24,25] Even though the exact mechanisms responsible were not clear, the results highlighted the beneficial effects of vitamin D supplementation in attenuation of the risk and adverse outcomes. SCH groups 2 and 3 were merged and analyzed. (Table 3) anti-TPO showed a significant negative correlation with Hb, vitamin D and a positive correlation with LDL.

The current study differs from the earlier studies with respect to the TSH cutoff considered for SCH (4.12-9.9 ìIU/ml) and recruitment of relatively young subjects. The primary limitation of this study is the small sample size and measurement of anti-

Table 3: Correlation of anti- TPO with study parameters in subclinical hypothyroid subjects (n=40)

Parameters	r	*p*
Age (yrs.)	0.081	0.624
TSH (ìIU/ml)	-0.036	0.829
FT4 (ng/dl)	-0.032	0.926
T4 (µg/dL)	-0.184	0.263
HB (g/dl)	-0.346	0.031*
TC (mg/dl)	0.294	0.069
TG (mg/dl)	0.123	0.456
HDL (mg/dl)	-0.057	0.731
LDL (mg/dl)	0.31	0.050*
N-HDL (mg/dl)	0.264	0.104
Vitamin - D (ng/ml)	-0.476	0.002*

T3 -free triiodothyronine; T4-thyroxine; FT4-Free thyroxine; TSH-Thyroid stimulating hormone; Hb- hemoglobin ; TC - total cholesterol; LDL-C-low-density lipoprotein cholesterol; HDL-C - high-density lipoprotein cholesterol; TG - triglyceride; N-HDL-C- Non HDL cholesterol. TPO Ab - Anti-Thyroid Peroxidase Antibodies; Vit-D -vitamin D. * '*p*' value less than 0.05.

TPO in euthyroid subjects would have added further information on thyroid autoimmunity.

Earlier studies showed that patients with autoimmune-mediated clinical and subclinical hypothyroidism display significantly elevated circulating inflammatory markers, [26,27] Oxidative stress markers, [28] Lipid Parameters and Lipoprotein (a), [29] carotid intima-media thickness (CIMT) [30] Insulin and obesity [11] suggesting that, autoimmune processes by itself may employ a major impact on endothelial dysfunction. We believe that the combined results of earlier studies on and the present work, provide compelling evidence for the association of elevated anti-TPO with intermediate cardiovascular risk biomarkers. This would be an indication for therapy with levothyroxine replacement and its effects on these biomarkers.

CONCLUSION

Subclinical hypothyroidism with thyroid autoimmunity is associated with a marginal decrease in hemoglobin and elevation in LDL. Whether this holds, any bearing on promoting cardiovascular risk needs to be considered further

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