Haemostatic functions and metabolic profile of subclinical hypothyroid and hypothyroid patients.

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Abstract

Objectives: Both hypercoagulable and hypocoagulable states have been proposed for hypothyroidism, whether in overt or subclinical spectrum. The status of haemostatic functions, metabolic profile and their relationship in hypothyroid disorders need to be evaluated. Methods and Material: This prospective case control study was undertaken in 30-50 years old female subclinical and hypothyroid patients. Haemostatic functions like bleeding time (BT), clotting time (CT), prothrombin time (PT), activated partial thromboplastin time (APTT), platelet count and metabolic parameters like plasma glucose and lipid levels and clinical variables like blood pressure and body mass index were noted and compared. In addition the strength of correlation of TSH, T3, T4, lipid profile with the haemostatic functions was evaluated. Results: Both groups of patients were obese, normotensive with normal haemostatic parameters. The platelet count correlated with TSH in subclinical hypothyroid patients and with T3 levels in hypothyroid patients. Although within normal range, total cholesterol and LDL cholesterol levels were higher and postprandial plasma glucose (PPPG) levels lower in hypothyroid patients compared to subclinical hypothyroid patients. A positive correlation was seen between TSH and LDL, PPPG levels, between fT3 and BMI, and also of antiTPO with total cholesterol, LDL, Fasting plasma glucose (FG) in hypothyroid patients. The BMI was negatively associated with fT3 levels in subclinical hypothyroid patients. Conclusion: This study found normal haemostatic and metabolic functions in both subclinical and hypothyroid patients. Although within normal range, hypothyroid patients had higher total and LDL cholesterol. TSH and antiTPO levels correlated with LDL levels in these patients. Correlation of platelet count with TSH in subclinical hypothyroid and T4 levels in hypothyroid patients advocate a difference in mechanism involved. Therefore it can be connoted that thyroid status influences metabolic profile, and platelet count.

Keywords: hypothyroid disorders; haemostatic functions; metabolic profile

Introduction

The haemostatic and metabolic profile may both contribute to vascular abnormalities in both hypothyroid patients and in those in the subclinical stage of thyroid hypofunction. Both an increased and decreased coagulopathy has been reported. Increases in fibrinogen and homocysteine level, thrombin activatable fibrinolysis inhibitor (TAFI), FVIII, Von Willebrand factor (vWF), FVIIIC, carotid artery intima media thickness advocated a hypercoagulable state1-5. Alternately an increased bleeding tendency and a decreased fibrinogen and FVIII C in subclinical hypothyroid patients have been found6. Gullu et al reported decreased FVIII and vWF in both and increased BT, CT, APTT, PT in overt hypothyroid patients which reversed after treatment with

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levothyroxine7. Others have however reported no difference or alteration in haemostatic function with no change in APTT, PT, Fibrinogen, FVII, FVIII, IX, X, vWF, antithrombin III, protein C, protein S, tissue plasminogen activator(tPA), plasminogen activator inhibitor (PAI-I) in subclinical hypothyroid patients and euthyroid controls8, 9.

It is also known that the altered lipid profile may lead to atherosclerosis and increased intima media thickness of blood vessels and influence the coagulation and fibrinolytic status in hypothyroid disorders10, 11. Blood glucose levels along with obesity and abnormal blood pressure are additional cofactors contributing to altered vascular morphology and dysfunction12.

In this background this study was undertaken in both subclinical hypothyroid and hypothyroid patients, to evaluate haemostatic functions and risk factors of vascular dysfunction like increased body weight, blood pressure, lipid profile, plasma glucose levels and thyroid function of these patients. Further the idea was to establish a link if any between the alteration in metabolic and haemostatic function in hypothyroid disorders.

Material and methods

The open and prospective case controlled pilot study was conducted in the department of Physiology, Pathology, Neurology and Medicine. Thirty subclinical hypothyroid female patients (TSH>5m IU/L, normal T3, T4), and same number of hypothyroid patients, 30-50 years of age were recruited from the medicine out patient department (OPD), and later followed up in the thyroid clinic.

In addition a control group of 30, age and sex matched euthyroid individuals randomly selected from the hospital and academic staff was taken. They did not have any past history of thyroid disease and matched the selection criteria. Their BMI and thyroid function data was taken for standardization and comparison. Pregnant and postmenopausal women, smokers, subjects with history suggestive of addictions, diabetes, stroke, cardiovascular disease, diagnosed hypertension, malignancy, history of drug intake like sulphonylurea, lithium, amiodarone, ethionamide, iodine, phenyl butyrate, vitamin E, vitamin C, hormonal replacement therapy, past radiotherapy, were excluded from the study.

Study protocol

The protocol was approved by the institutional ethical committee. Guidelines for biomedical research on human subjects (2000) were followed13. After taking informed consent a complete medical history was taken, patient clinically examined to rule out other illness. A thyroid profile on two occasions 6 months apart was done to ensure that there was no reversibility pattern and the patients fulfilled the selection criteria. Once included the patient’s anthropometry measurements like height, body weight was noted and BMI was computed. Blood pressure both systolic and diastolic was noted on more than one occasion and an average noted.

Metabolic and haemostatic investigations

These included a thyroid profile with TSH, fT3, fT4 using Cobase 411 kits, electrochemiluminence method and anti TPO by ELECYS-2010, Roche/Hitachi, Germany 2004 were used.

Lipid profile and FPG, PPPG was measured. Haemostatic profile included PT using Neoplateine C1 kit diagnostic stago, France and APTT by CELIN kit, tulip diagnostic, Goa. BT by duke’s method; CT by Lee White, and platelet count using an automated analyzer, SYSMEX XT-Japan was done. Each test sample was compared to a normal “control” taken at the same time as per lab specifications.

Statistical Analysis

The data was analyzed using the SPSS statistical software version 17.0. One way analysis of variance, Levene’s test followed by Tukey’s test for multiple comparisons was used. For nonparametric tests Krushal Wallis and Mann Whitney U test were used. Welch test followed by Dunnett’s T3 multiple comparisons were done when homogeneity of variance condition was violated. Unpaired student t- test was used to compare variables measured in two groups. Pearson’s and Spearman’s methods were used to assess the strength of correlation of TSH, T4, T3 anti TPO, lipid profile and blood pressure with the haemostatic functions. The p value < 0.05 was considered significant.

Result

The profile of patients and their thyroid status is reported earlier14. The hypothyroid patients were older compared to subclinical hypothyroid patients and had higher BMI and body weight compared to euthyroid controls. There were significant differences in the thyroid status between the three groups.

Thyroid levels and Haemostatic function

The haemostatic functions were normal and comparable in the two groups of patients, table 1. Correlation of platelet count with TSH showed a significant result with in subclinical hypothyroid patients (r=0.384; p=0.036). The platelet count also correlated with T4 levels in hypothyroid patients(r=0.577, p=0.0008). All others correlations
of TSH, FT$_4$ and FT$_3$ with other haemostatic functions were not significant. There was no significant correlation between the antibody level and the haemostatic function in the two groups of patients.

**Thyroid levels and metabolic status**

Although within the normal range, the total cholesterol, LDL level were higher and PPPG was lower in hypothyroid compared to subclinical hypothyroid patients table 2. TSH levels showed a significant positive correlation with LDL levels and PPPG levels in hypothyroid patients (r= 0.429, p=0.018; r=0.365, p=0.047 respectively). The BMI was negatively associated with FT$_3$ levels (r= 0.4957, p=0.005), in subclinical but positively with hypothyroid patients(r=0.4908, p=0.005) respectively. The antibody levels (antiTPO) showed a significant relationship with total cholesterol, LDL, FPG(r=0.500, p=0.005; r=0.628, p=0.000; r=0.374, p=0.042 respectively) in hypothyroid patients.

**Metabolic status and haemostatic function**

Correlation of lipid profile (TC, LDL, TG, and HDL), plasma glucose levels (FPG, PPPG) and blood pressure with PT, APTT or platelet count did not show any significant result.

**Discussion.**

This study showed normal haemostatic functions of bleeding time, clotting time; prothrombin time, APTT and platelet count in subclinical hypothyroid patients and hypothyroid patients with no intergroup variations. Similarly Erem did not find any changes in coagulation and fibrinolysis parameters between hypothyroid and subclinical hypothyroid patients. In the Tromso study, the haemostatic functions were similar in subclinical hypothyroid patients and controls, although TSH level was a negative predictor of FIX activity. Gullu et al on the other hand found an increase in BT, CT, PT APTT in overtly hypothyroid patients compared to euthyroid controls and a decrease in factor VIII and vWF activity in both group of patients, which seems to change following LT$_4$ therapy. Likewise Yango et al reported an increase in APTT in short term severe hypothyroidism. In another study a decreased PT, but similar APTT, fibrinogen levels, platelet count in hypothyroid patients compared to controls was mentioned. Moreover there were no differences between clinically hypothyroid and subclinical hypothyroid patients.

We found that only in subclinical hypothyroid patients the platelet count was significantly related to the TSH levels. Subclinical hypothyroidism due to Hashimoto’s thyroiditis has been shown to be associated with thrombocytopenia which improves with levothyroxine treatment. Also in a recent study an increased mean platelet volume and platelet count ratio has been expressed as an independent risk factor for vascular access failure. Therefore the estimation of platelet count in patients is gaining importance to identify potentially at risk patients for vascular abnormalities. However studies have also counseled that TSH levels do not affect coagulation factors. Li et al have advocated FT$_4$ to be an independent risk factor for prothrombin time, normalized ratio. However we did not find any significant correlation between haemostatic functions and FT$_4$ levels but in our hypothyroid group of patients the platelet count correlated with FT$_4$ values. Similarly a positive relationship between FT$_4$ and vWF, VIII, protein C, S and negative between antithrombin and TSH was found in a study done in children. The authors recommend that as the anticoagulation proteins were also affected, hypothyroid patients should be monitored for risk of thrombosis. Analyzing all these studies it can be said that both pro and anti coagulant protein synthesis may be affected by hypothyroidism. The net effect could depend on many factors, the TSH, T$_4$ and FT$_3$ levels. In addition the role of autoimmune mechanisms independent of the underlying thyroid pathology is known to influence the haemostatic balance. Nevertheless we did not find any significant association between antibody levels and haemostatic function tests. Our patients did not have hyperlipidemia or abnormal blood glucose levels, or high blood pressure. However in hypothyroid patients, the total cholesterol and LDL levels were higher than subclinical hypothyroid patients. In hypothyroid patients, LDL and higher PPPG were affected by TSH and both total and LDL cholesterol and FPG were related to the antibody titre. Therefore the autoimmune mechanism may have influenced the lipid and glucose profile but the effect was not enough to influence the haemostatic function tests.

Recent studies propose the role of obesity, hyperlipidemia, platelet activity and tissue factor expression in the coagulation and fibrinolytic balance. Obesity is linked to the expression of prothrombotic molecules, plasminogen activator inhibitor, increased platelet activation and TF expression. Hyperlipidemia is related to oxidant stress, interaction of platelet CD36, increased tissue factor expression and prothrombotic events. A recent study however has conveyed that it is a prothrombotic state of subclinical hypothyroidism which improves with LT$_4$.

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Controversially, a low level of soluble CD40 ligand was found in autoimmune thyroiditis with both overt and subclinical hypothyroidism, it was associated with decreased platelet activity and improved with thyroxine treatment. An earlier study also found that patients with moderate hypothyroidism had decreased fibrinolytic activity while those with severe hypothyroidism had a tendency toward increased fibrinolytic activity. A balance between TF/FVIIa and TFPI (tissue factor pathway inhibitor) can lead to either haemorrhage or thrombosis. Therefore the plurimetabolic states in different degree of hypothyroidism may influence synthesis, degradation of various proteins associated with the coagulation mechanisms.

**Conclusion.**
Both group of our patients had normal haemostatic functions of BT, CT, PT, APTT, lipid profile and blood glucose parameters. The metabolic functions of plasma glucose and lipid did not relate with haemostatic functions. The autoimmune mechanisms affected lipid and plasma glucose profile in hypothyroid patients but not the haemostatic function. Platelet count being positively correlated with TSH in subclinical hypothyroid and with T4 in hypothyroid may indicate that mechanisms may be different in the two groups of patients. Screening subclinical and hypothyroid patients for metabolic abnormalities, identifying at what point of metabolic dysfunction is the haemostatic function likely to be affected is the future scope and relevance of this study.

| Parameter                | Group 1-SH (n=30) | Group 2-H (n=30) | P value |
|--------------------------|-------------------|------------------|---------|
| Platelet count (lac/mm³) | 2.36±0.64         | 2.40±0.63        | 0.823   |
| BT (minute)              | 2.50±0.62         | 2.78±0.73        | 0.109   |
| CT (minute)              | 4.30±1.21         | 4.50±0.95        | 0.480   |
| PT (s)                   | 12.37±0.81        | 12.50±0.68       | 0.493   |
| APTT (s)                 | 29.73±1.96        | 29.83±1.98       | 0.845   |

Group 1, 2, consist of subclinical hypothyroid (SH) and hypothyroid patients (H). * indicates significance (p<0.05).

| Parameters (mg/dl)       | Group 1-SH (n=30) | Group 2-H (n=30) | P value |
|--------------------------|-------------------|------------------|---------|
| S. Cholesterol           | 170.15±22.50      | 186.95±26.69     | 0.011*  |
| S. Triglycerides         | 131.03±10.39      | 137.30±16.07     | 0.079   |
| S. LDL                   | 100.79±14.38      | 119.09±16.44     | 0.000*  |
| S.HDL                    | 53.05±3.55        | 52.39±3.11       | 0.451   |
| FPG                      | 86.67±10.38       | 90.43±8.46       | 0.129   |
| PPPG                     | 121.60±11.34      | 119.43±9.96      | 0.044*  |

Group 1, 2, consist of subclinical hypothyroid (SH) and hypothyroid patients (H) respectively. FPG, PPPG is fasting and postprandial plasma glucose.

* indicates significance (p<0.05).
References.

1. Cakal B, Cakal E, Demirbas B, Ozkaya M, Karaahmetoglu S, Serter R, et al. Homocysteine and fibrinogen change with L-thyroxine in subclinical hypothyroid patients. J. Korean Med Sci 2007; 22:431-35.

2. Cetinkalp S, Tobu M, Karadeniz M, Buyukkececi F, Yilmaz C. The effect of hormone replacement treatment on thrombin activatable fibrinolysis inhibitor activity level in patients with Hashimoto thyroiditis. Inter Med 2009; 48:281-85.

3. Debeij J, Cannegieter SC, Zaane B, Smit JWA, Corssmit EPM, Rosendaal FR, et al. The effect of changes in thyroid and thyroid stimulating hormone levels on the coagulation system. J Thrombosis Haemostasis 2010; 8:2833-44.

4. Muller B, Tsakiris DA, Roth CB, Guglielmetti M, Staub JJ, Marbet GA. Haemostatic profile in hypothyroidism as potential risk factor for vascular or thrombotic disease. Eur J Clin Invest 2001; 31(2): 131-37.

5. Kim SY, Kim SH, Park KS, Park SW, ChoYW. Regression of the increased common carotid artery intima media thickness in subclinical hypothyroidism after hormone replacement. Endocrine J 2009; 56(6):753-58.

6. Squizzato A, Romualdi E, Buller HR, Gerdes VE. Clinical review: Thyroid dysfunction and effect on coagulation and fibrinolysis: A systematic review. J Clin Endocrinol Metab 2007; 92(7):2415-20.

7. Gullu S, Sav H, Kamal N. Effect of levothyroxine treatment on biochemical and hemostasis parameters in patients with hypothyroidism. European J of Endocrinology 2005; 152:355-61.

8. Jorde R. Haemostatic functions in subjects with mild subclinical hypothyroidism. The Tromso study. Thromb Haemost 2006; 95:750-51.

9. Erem C. Blood coagulation, fibrinolytic activity and lipid profile in subclinical thyroid disease: Subclinical hyperthyroidism increases plasma factor X activity. Clin Endocrinol (oxf) 2006; 64(3):323-29.

10. Turemen EE, Cetinsaran B, Shin T, Canturk Z, Tarluk I. Endothelial dysfunction and low grade inflammation in subclinical hypothyroidism due to autoimmune thyroiditis. Endocrine J 2011; 58(5):349-54.

11. Rizos CV, ElisaF MS, Liberopoulos EN. Effect of thyroid dysfunction on lipid profile. The open cardiovascular medicine Journal 2011; 5:78-84.

12. Iwen KA, Schroder E, Brabant G. Thyroid Hormone and the metabolic syndrome. Eur Thyroid J 2013; 2(2):83-92.

13. Ethical guidelines for biomedical research on human participants. Indian Council of Medical Research, New Delhi, 2006. Available from http://www.icmr.nic.in/

14. Mahaur R, Mahajan AS, Jain AK, Singh T, Dhanwal DK, Gupta M. Role of event related potentials in evaluation of cognitive function in subclinical hypothyroid patients. MAMC J Med Sci2016;2:43-7

15. Yango J, Alexopoulos O, Eekhoudt S, Hermans C, Daumerie C. Evaluation of the respective influence of thyroid hormone and TSH on blood coagulation parameters after total thyroidectomy. European Journal of Endocrinology 2011; 164:599-603.

16. Mohamed- Ali MS, Ahmed RO. Coagulation profiles in hypothyroid and hyperthyroid female patients in Sudan. Saudi Med J 2008; 29(9): 1289-93.

17. Shin DH, Rhee SY, Jeon HJ, Park JY, KangSW, Oh J. An increase in mean platelet volume/platelet count ratio is associated with vascular access failure in hemodialysis patients. PLoS ONE 2017; 12(1): e0170357. doi:10.1371/journal.pone.0170357.

18. Tahir H, Sheraz F, Sagi J, Daruwalla V. Immune thrombocytopenia(ITP) secondary to Hashomoto’s thyroiditis: Role of levothyroxine in improving the clinical outcome of ITP. Journal of investigative medicine high impact case reports 2016;4(2):1-4. DOI:10.1177/2324709616647085.

19. Li Li, Guo CY, Yang J, Jia E, Zhu T, Wang L, et al. Negative association between free triiodothyronine level and international normalized ratio in euthyroid subjects with acute myocardial infarction. Acta Pharmacol Sin 2011; 32(11): 1351-56.

20. Kilic N, Dallar Y, Simsek E, Karamercan S, Tapei AE, Alioglu B. Procoagulant and anticoagulant factors in childhood hypothyroidism. Int J Endocrinol2012. doi 10.1155/2012/156854.

21. Samad F, Ruf W. Inflammation, obesity and thrombosis. Blood 2013; 122(20): 3415-22.

22. Owen AP, Byrnes JR, Mackman N. Hyperlipidemia, tissue factor, coagulation and simvastatin. Trends Cardiovasc Med 2014; 4(3):95-98.

23. Podrez EA, Byzova TV, Febbraio M, Salomon RG, Ma Y, Valiyaveettil M, et al. Platelet CD36 links hyperlipidemia, oxidant stress and prothrombotic phenotype. Nature Medicine 2007; 13(9):1086-95.

24. Lupoli R, Di Minno MN, Tortora A, Scaravilli A, Cacciapuoti M, Barba L, et al. Primary and secondary hemostasis in patients with subclinical hypothyroidism: effect of levothyroxine treatment. J Clin Endocrinol Metab 2015; 100(7):2659-65; doi 10.1210/jc.2015-1726.

25. Akinsi B, Comlekci A, Yener S, Demir T, Bayraktar F, Yuksel F, et al. The alteration of serum soluble CD40 ligand level in overt and subclinical hypothyroidism. Hormone 2007; 6(4): 327-33.

26. Chadarevian R, Bruckert E, Leenhardt L, Giraud P, Ankri A, Turpin G. Components of the fibrinolytic system are differently altered in moderate and severe hypothyroidism. J Clin Endocrinol Metab, 2001; 86(2): 732–37.

27. Tatsumi K, Mackman N. Tissue Factor and atherothrombosis. J Atheroscler thromb 2015; 22(6): 543-49.