

Original article:**Haemostatic functions and metabolic profile of subclinical hypothyroid and hypothyroid patients.****Abstract** Mahajan AS¹, Mahaur R², Singh T³, Jain AK⁴, Dhanwal DK⁵, Gupta M⁶.

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, T₄, T₃, 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 T₄ 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 (FPG) 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

Bangladesh Journal of Medical Science Vol. 17 No. 04 October '18. Page : 532-536

DOI: <http://dx.doi.org/10.3329/bjms.v17i4.38312>**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), FVIIC, 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

1. Aarti Sood Mahajan, Physiology Department, MAMC
2. R Mahaur, Physiology Department, MAMC
3. T Singh, Pathology Department, MAMC
4. AK Jain, Physiology Department, MAMC
5. DK Dhanwal, Medicine Department, MAMC & Lok Nayak Hospital
6. M Gupta, Neurology Department, GIPMER

Correspondence to: Aarti Sood Mahajan, Director Professor, Department of Physiology, Maulana Azad Medical College, New Delhi.e-mail aartis_mahajan@yahoo.co.in

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 controls^{8,9}.

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 disorders^{10,11}. 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 > 5 mIU/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, electrochemiluminescence 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 Neoplastine 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, FT₄ and FT₃ 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 FT₃ 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 patients⁹. In the Tromso study, the haemostatic functions were similar in subclinical hypothyroid patients and controls, although TSH level was a negative predictor of FVII activity⁸. 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 LT₄ therapy⁷. Likewise Yango *et al* reported an increase in APTT in short term severe hypothyroidism¹⁵. 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 patients¹⁶.

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 treatment¹⁷. 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 failure¹⁸. 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 factors¹⁵. Li *et al* have advocated FT₃ to be an independent risk factor for prothrombin time, normalized ratio¹⁹. However we did not find any significant correlation between haemostatic functions and FT₃ levels but in our hypothyroid group of patients the platelet count correlated with FT₄ values. Similarly a positive relationship between FT₄ 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 thrombosis²⁰. 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, T₄ and FT₃ levels. In addition the role of autoimmune mechanisms independent of the underlying thyroid pathology is known to influence the haemostatic balance⁶. 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 auto immune 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 expression²¹. Hyperlipidemia is related to oxidant stress, interaction of platelet CD36, increased tissue factor expression and prothrombotic events^{22,23}. A recent study however has conveyed that it is a prothrombotic state of subclinical hypothyroidism which improves with LT₄

treatment²⁴. 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 treatment²⁵. 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 activity²⁶. A balance between TF/FVIIa and TFPI (tissue factor pathway inhibitor) can lead to either haemorrhage or thrombosis²⁷. 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.

Table 1. Comparison of platelet count, BT, CT, PT and APTT in subclinical hypothyroid and hypothyroid patients.

| Parameter | Group 1-SH (n=30) | Group 2-H (n=30) | P value |
|---|----------------------|---------------------|---------|
| | Mean± SD | Mean± SD | |
| Platelet count (lac/ mm ³) | 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 |

Group1, 2, consist of subclinical hypothyroid (SH) and hypothyroid patients (H). * indicates significance (p<0.05).

Table2. Metabolic profile of subclinical hypothyroid and hypothyroid patients.

| Parameters (mg/dl) | Group 1-SH (n=30) | Group 2-H (n=30) | P value |
|-----------------------|----------------------|---------------------|---------|
| | Mean± SD | Mean± SD | |
| 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 thyroxine 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 S.Y, Kim SH, Park KS, Park SW, Cho YW. Regression of the increased common carotid artery intima media thickness in subclinical hypothyroidism after thyroid 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, Cetinarlan B, Shin T, Canturk Z, Tarkun I. Endothelial dysfunction and low grade chronic 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 Sci* 2016; **2**:43-7
15. Yango J, Alexopoulou O, Eeckhoudt 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, Kang SW, 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, Daruwala V. Immune thrombocytopenia (ITP) secondary to Hashimoto'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, Tapci AE, Alioglu B. Procoagulant and anticoagulant factors in childhood hypothyroidism. *Int J Endocrinol* 2012. 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, Valiyaveetil 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, Giral P, Ankril 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.