VARIATION TENDENCY OF COAGULATION PARAMETERS IN DIFFERENT HYPOTHYROIDISM STAGES

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Abstract

The hemostatic balance is a complex system where the delicate equilibrium is regulated by several factors including hormones. Hypothyroidism, as a common disease in the general population, affects both the coagulation and fibrinolytic systems. However, the reliable clinical evidence is so far lacking and published data remain conflicting. According to the severity of the disease, we divided all study subjects into four groups: 50 controls, 47 patients displaying subclinical hypothyroidism, 41 patients displaying moderate hypothyroidism ($TSH \leq 50$ mU/L), and 53 patients displaying severe hypothyroidism ($TSH > 50$ mU/L). We investigated various coagulation parameters including: activated partial thromboplastin time (APTT), prothrombin time (PT), thrombin time (TT), fibrinogen (FIB) and international normalized ratio (INR). Compared with control subjects, patients with subclinical hypothyroidism displayed hypercoagulability, as reflected by shorter APTT levels and increased FIB levels. The patients with moderate hypothyroidism had only increased TT levels without any significant variation in the other studied parameters, which suggested neither obvious bleeding tendency nor clotting tendency. The patients with severe hypothyroidism displayed a bleeding tendency, as reflected by higher APTT, PT, TT, INR levels and decreased FIB levels. The patients with different hypothyroidism stages display various abnormalities of coagulation.

Key words: hypothyroidism, activated partial thromboplastin time, fibrinogen.

INTRODUCTION

Thyroid dysfunction has been associated with various abnormalities of coagulation, ranging from subclinical laboratory abnormalities to major haemorrhage or thromboembolism (Erem *et al.* 2002; Erem *et al.* 2003; Franchini 2004; Hofbauer and Heufelder 1997; Marongiu *et al.* 2004; Ozcan *et al.* 2003). Hypothyroidism is one of the most commonly experienced endocrine disorders, which may be either

subclinical or overt. The prevalence of subclinical disease was 4.3% and overt disease 0.3% (Garber *et al.* 2012).

The close relationship between thyroid failure and haemostasis has been studied, but it is still not very well understood. The number of studies investigating hemostasis in subclinical hypothyroidism is limited. In all studies, except one (Gullu *et al.* 2005), results concordant with hypercoagulability and hypofibrinolysis were observed, and it was suggested that subclinical hypothyroidism might cause an increase in risk for thrombosis (Akinci *et al.* 2007; Guldiken *et al.* 2005; Muller *et al.* 2001). Although former reports indicated that overt hypothyroidism was associated with a bleeding tendency, ranging from a mild mucocutaneous bleeding to a severe post traumatic or post surgical bleeding, the pathogenesis of this hemostatic defect remains unclear. Moreover, in all former studies, only Chadarevian *et al.* (Chadarevian *et al.* 2001) analyzed the pattern of variation of the fibrinolytic system depending on the severity of hypothyroidism, but this observation was done only in 51 patients, and 11 patients of them with subclinical hypothyroidism were not observed as an independent group. Therefore, we further studied the relationship between coagulation abnormalities and the degree of hypothyroidism by more carefully grouping.

PATIENTS AND METHODS

Patients

The study was carried out in the department of endocrinology at the Cangzhou Central Hospital affiliated to Hebei Medical University, China. 50 controls and 141 patients were prospectively selected. All patients including 116 females and 25 males were newly diagnosed with hypothyroidism and no replacement therapy. Inclusion criteria included thyroid stimulating hormone (TSH) levels > 4.2 mU/L and serum free thyroxine (FT4) concentration below the lower limit of

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the reference range or in the normal range. Exclusion criteria included: 1) age below 18 years, 2) presence of inflammatory disease, kidney disease, hepatic lesion, hematopathy, coronary heart disease, cerebral infarction, immune system disorder or any serious medical disease, which may affect the blood coagulation system, and 3) history of taking anticoagulant drugs or estrogen replacement before the study. 50 control subjects with normal thyroid function were recruited among patients attending our hospital for asymptomatic benign nodule, without ongoing medical or psychiatric illnesses.

All patients and controls were examined by the attending physician, who performed a complete physical examination, including height, weight, blood pressure, heart rate, respiratory rate, and body temperature, and recorded demographic and historical data. Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. A complete medical history was recorded. The present study protocol has been approved by the Cangzhou Central Hospital ethic committee.

Methods

Hormone measurements, lipid profile and fasting glucose

The 12-h fasting blood samples were collected from every patient in the morning. Serum TSH, FT3, and FT4 levels were determined with full-automatic immune analyzer by electro-chemiluminescence method (cobas e601, Roche, Berlin, Germany), with normal reference ranges of 0.27–4.2 mU/L, 2–4.4 pg/mL, and 0.93–1.7 ng/dL, respectively.

The levels of triglyceride (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C) and

fasting glucose (FBG) were measured with full-automatic biochemistry analyzer (7600-120 HITACHI, Japan), with normal reference ranges of 0–1.7mmol/L, 2.8–6mmol/L, 0–3.12mmol/L, 0.8–1.5 mmol/L, and 3.6–6.1mmol/L, respectively.

Coagulation parameters

The activated partial thromboplastin time (APTT) mirrors abnormalities of the intrinsic (contact) pathway (i.e., deficiencies in factors VIII, IX, IX, and XII), whereas the prothrombin time (PT) reflects factor deficiencies of the extrinsic pathway (i.e., tissue factor and factor VII). Serum APTT, PT, TT, and FIB were performed with a computerized blood coagulation analyzer (CA 7000, Sysmex, Kobe, Japan), with normal reference ranges of 23–35s, 10–14s, 14–21s, and 2–4g/L, respectively. The international normalized ratio (INR) is the ratio of the PT of the patient to a normal (control) sample.

Statistical analysis

All data are expressed as means $\pm$ standard deviation (SD). We divided patients into three subgroups according to their TSH and FT4 levels before the study: subclinical hypothyroidism with elevated TSH levels and normal thyroid hormone, moderate hypothyroidism with TSH ≤ 50 mU/L, and profound hypothyroidism with TSH > 50 mU/L. All variables were checked by the Kolmogorov-Smirnov test. Results were evaluated by analysis of variance (Andreeva *et al.*) - Sheffe's F test. The level of statistical significance was set at $P < 0.05$.

RESULTS

The results for the different groups are showed in Table 1. The levels of TC, TG, and LDL-C had a

Table 1. Clinical and biological parameters of controls and patients according to the levels of FT4 and TSH

	Controls (Group 1)	Subclinical Hypothyroidism (Group 2)	Moderate Hypothyroidism (Group 3)	Severe Hypothyroidism (Group 4)	F	P
N	50	47	41	53		
Age (year)	50.30 $\pm$ 10.10	46.13 $\pm$ 10.35	52.22 $\pm$ 14.74	42.91 $\pm$ 12.30	10.966	0.053
BMI(kg/m ²)	23.77 $\pm$ 2.02	25.01 $\pm$ 2.88	24.13 $\pm$ 3.25	25.31 $\pm$ 2.52	5.412	0.062
SBP(mmHg)	124.86 $\pm$ 11.78	125.26 $\pm$ 12.68	130.26 $\pm$ 14.70	123.59 $\pm$ 17.17	1.049	0.374
DBP (mmHg)	78.39 $\pm$ 12.59	79.48 $\pm$ 8.43	76.87 $\pm$ 9.41	78.14 $\pm$ 11.60	0.224	0.879
TSH (mU/L)	1.63 $\pm$ 0.91	6.77 $\pm$ 2.24	20.05 $\pm$ 16.11	95.38 $\pm$ 9.84	970.468	0.000
FT4 (ng/dL)	1.26 $\pm$ 0.17	1.17 $\pm$ 0.18	0.68 $\pm$ 0.26	0.31 $\pm$ 1.03	21.346	0.000
TC (g/L)	1.18 $\pm$ 0.67	1.48 $\pm$ 0.72	1.80 $\pm$ 1.23	2.01 $\pm$ 1.22	4.865	0.003
TG (g/L)	4.75 $\pm$ 1.21	5.03 $\pm$ 1.10	5.14 $\pm$ 2.65	6.53 $\pm$ 1.88	7.656	0.000
HDL-C (g/L)	1.16 $\pm$ 0.28	1.02 $\pm$ 0.21	1.00 $\pm$ 0.27	1.16 $\pm$ 0.34	2.589	0.570
LDL-C (g/L)	2.78 $\pm$ 0.77	2.79 $\pm$ 0.96	3.13 $\pm$ 0.81	3.76 $\pm$ 1.22	7.721	0.000
FBG(mmol/L)	5.00 $\pm$ 0.52	4.69 $\pm$ 0.55	4.74 $\pm$ 0.39	4.72 $\pm$ 0.54	2.302	0.083

N, Number of patients; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TSH, thyroid stimulating hormone; FT4, free thyroxine; TC, triglyceride; TG, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; EBG, Fasting glucose.

Table 2. Coagulation parameters of controls and patients according to the levels of FT4 and TSH

	Controls (Group 1)	Subclinical Hypothyroidism (Group 2)	Moderate hypothyroidism (Group 3)	Severe hypothyroidism (Group 4)	<i>F</i>	<i>P</i>
APTT	26.85±3.07	25.12±2.34	28.11±4.05	29.82±3.62	11.580	0.000
PT	11.17±0.74	11.56±1.04	11.47±1.03	11.66±0.74	2.698	0.048
TT	16.35±1.02	16.39±1.38	17.01±1.28	17.66±0.92	10.802	0.000
INR	0.93±0.07	0.97±0.81	0.96±0.82	0.97±0.06	2.800	0.042
FIB	2.62±0.67	2.72±0.64	2.47±0.65	2.10±0.43	7.664	0.000

APTT, activated partial thromboplastin time; PT, prothrombin time; TT, thrombin time; INR, international normalized ratio; FIB, fibrinogen.

Table 3. Multiple comparisons of coagulation parameters for 4 groups (*P*)

	APTT	PT	TT	INR	FIB
Group 1 and 2	0.040	0.070	0.903	0.053	0.468
Group 1 and 3	0.124	0.162	0.025	0.095	0.310
Group 1 and 4	0.000	0.007	0.000	0.008	0.000
Group 2 and 3	0.002	0.689	0.058	0.783	0.131
Group 2 and 4	0.000	0.642	0.000	0.752	0.000
Group 3 and 4	0.042	0.353	0.031	0.524	0.016

significant increase ($P=0.003$, $P=0.000$ and $P=0.000$, respectively), that was observed in patients as the degree of hypothyroidism worsened. There were no significant differences among the 4 subgroups for mean Age, BMI, SBP, DBP, HDL and FBG.

Differences of the coagulation characteristics among the 4 subgroups are summarized in Table 2 and Table 3. Compared with control subjects, patients with subclinical hypothyroidism had shorter APTT levels ($P=0.040$), whereas a distinct feature was encountered in those who had severe hypothyroidism ($P=0.000$). There was no statistically significant difference in APTT between controls and moderate hypothyroid patients. A significant decrease in mean fibrinogen levels was observed in patients as the degree of hypothyroidism worsened. Although there was no statistically significant difference in FIB between subclinical hypothyroidism and controls, the FIB of patients with subclinical hypothyroidism were significantly higher than euthyroid patients. Levels of INR, PT and TT were significantly higher during severe hypothyroidism compared with controls. In patients with moderate hypothyroidism, except higher TT levels, APTT, FIB, INR and PT were similar to controls.

DISCUSSION

The clinical relationship between thyroid disorders and the hemostatic system was first defined in the beginning of the last century (Squizzato *et al.* 2005). Various acquired abnormalities of the coagulation-fibrinolytic system have been reported in patients with thyroid dysfunction (Erem *et al.* 2003; Franchini 2004,

2006; Franchini *et al.* 2010a; Franchini *et al.* 2010b; Franchini *et al.* 2009; Hofbauer and Heufelder 1997; Mina *et al.* 2007). These abnormalities may range from subclinical laboratory abnormalities to clinically important hemostasis disorders. Our knowledge of the effect of hypothyroidism on the coagulation system is based mainly on older studies performed exclusively in patients with overt hypothyroidism. Therefore, we investigated the profile of coagulation parameters in patients across varying states of hypothyroidism, as well as in controls, to identify any prominent changes. We found that patients with subclinical hypothyroidism had shorter APTT levels compared with controls and overt hypothyroidism respectively. Similarly, in a recent study including patients with subclinical hypothyroidism, Muller *et al.* (Muller *et al.* 2001) found slightly, but not statistically significantly, decreased levels of APTT. Many studies (Aboud and Ma 2001; Hron *et al.* 2006; Legnani *et al.* 2006; Stephan *et al.* 2003; ten Boekel *et al.* 2003; Tripodi *et al.* 2004) had provided interesting evidence that a short APTT might be considered as either a marker or a risk factor for hypercoagulability and expected to reflect an excess of these coagulation factors. Mina *et al.* (Mina *et al.* 2010) showed that the level of most APTT associated clotting factors were elevated in short APTT test samples (i.e., FV, FVIII, FIX, FXI, FXII). The shortened APTTs have been associated with high levels of biochemical markers of thrombin generation and fibrin deposition such as prothrombin fragment, thrombin-antithrombin complex, and D-dimer (Korte *et al.* 2000; Ten Boekel and Bartels 2002), as well as with a poor prognosis for thrombosis and mortality in patients admitted over time to a general medical center. The procoagulant imbalance detected by a shortened APTT is associated with an increased risk of venous thromboembolism independently of the presence of inherited thrombophilia and of factor VIII levels (Tripodi *et al.* 2004). On the other hand, we observed that patients with subclinical hypothyroidism had higher FIB than controls, which corroborate previous reports describing increased FIB (Chadarevian *et al.* 1999; Dorr *et al.* 2006; Muller *et*

al. 2001). In fact, while increased fibrinogen value can alter the rheological structure of blood by inducing an increase in plasma viscosity (Lord 2007). Thus, the potential hypercoagulable state described in our patients with subclinical hypothyroidism might add to the risk for thrombotic, atherosclerotic vascular disease.

The other main finding of our study is that there is a distinct pattern of variation of the coagulation system in different hypothyroidism states. We observed that patients with moderate hypothyroidism had only increased TT levels without any significant variation in the other studied parameters compared with controls, which suggested neither obvious bleeding tendency nor clotting tendency. Similarly, Chadarevian *et al.* (Chadarevian *et al.* 2001) proposed that the degree of hypothyroidism was associated with different effects on coagulation. However, they found decreased fibrinolysis in patients with moderate hypothyroidism (TSH ≤ 50 mU/L), which was in conflict with our finding. In their study, the patients with moderate hypothyroidism included a small number of patients and did not except for 11 patients with subclinical hypothyroidism, which might provide one explanation for the contradictory results. We analyzed that moderate hypothyroidism may be a transition period of variation of the coagulation parameters. In addition, the finding may provide one explanation for rarely major hemorrhage and thromboembolism in subclinical and overt hypothyroidism. We found that patients with severe hypothyroidism were associated with increased PT, APTT, INR and TT, and decreased FIB, which were in agreement with those of Gullu *et al.* (Gullu *et al.* 2005) and suggested that these patients present an increased capacity of degradation of normal amount of fibrin.

Our study has certain strengths and limitations. The main strength is the strict inclusion criteria, assessing haemostatic parameters depending on the severity of the disease, which enabled us to have a more thorough understanding to the different hypothyroidism patients' coagulation status. The main limitation is the relatively small sample size. Our results have not been confirmed using other commercial reagents for the APTT test. Variable responsiveness of commercial reagents to hypocoagulability is already known. A similar variability in response to coagulability can be anticipated, but comparative observations are lacking. Shortened PT/ APTT values may result from either a poor quality venipuncture (activated sample), or cold activation of the sample (in vitro activation from factor XII activation of factor VII) that occurs if the plasma

sample is stored at cold temperatures (above freezing) for several hours or increased factor VIII levels. We avoided poor quality venipuncture cold temperatures for sample. However, the factor VIII was not measured in this study. Moreover, we did not assess whether the levothyroxine treatment would improve variation of coagulation parameters.

In conclusion, we found the patients with subclinical hypothyroidism display a prothrombotic tendency, while the risk for bleeding may increase in patients with overt hypothyroidism, as revealed by the presence of various abnormalities of coagulation. The principal mechanism that affects the hemostatic balance is deficiency of thyroid hormones. However, the relationship between hormone levels and hemostasis is complex and undefined. Future large observational and intervention studies are needed to provide more definitive information on the clinical relevance of this association and the possible impact of the pharmacological treatment of the hormonal dysfunction on coagulation-fibrinolytic abnormalities. Moreover, molecular pathogenesis-oriented experimental and molecular studies are needed to explain the degree and type of coagulation/fibrinolytic abnormalities in patients with subclinical hypothyroidism and overt hypothyroidism.

Conflict of interest

The author declares that he has no conflict of interest concerning this article.

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