Evaluation of Coagulation Profile in Children with Type 1 Diabetes Mellitus Using Rotational Thromboelastometry

Cigdem Binay¹ · Ayse Bozkurt Turhan² · Enver Simsek¹ · Ozcan Bor² · Olga Meltem Akay³

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Abstract The prothrombotic state in type 1 diabetes mellitus (T1DM) has been reported as a plausible cause of vascular complications. Rotational thromboelastometry (ROTEM) assay enables the global assessment of coagulation status. This study aimed to assess hypercoagulability in children with T1DM using ROTEM. A total of 43 T1DM children (20 females and 23 males) aged 2–18 years and age- and sex-matched 30 healthy control subjects were enrolled in the study group. ROTEM assays [intrinsic TEM (INTEM) and extrinsic TEM (EXTEM)] were used to measure and analyze coagulation time (CT), clot formation time, maximum clot firmness (MCF). Glycated hemoglobin levels (HbA1c), diabetic complications, platelet count, prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, and dimerized plasmin fragment D (D-dimer) were determined in the study group. The mean duration of T1DM diagnosis was 3.15 ± 2.49 years, and the mean HbA1c level was $8.94 \pm 1.88\%$ (74.29 ± 20.59 mmol/mol). None of the patients had macrovascular complications. Nephropathy was present in five patients. In the T1DM group, EXTEM-CT [80.00 (66.75–108.50)] was significantly lower, and EXTEM-MCF [65.00 (64.00–70.00)] and INTEM-MCF [65.00 (62.00–68.00)] were significantly higher than in the controls ($p < 0.001$, $p = 0.026$, and $p = 0.004$, respectively).

However, the duration of T1DM and the degree of metabolic control had no influence on these parameters. Platelet count, PT, aPTT, fibrinogen and D-dimer levels were comparable between the diabetic patients and the control group. There were statistically significant correlations between fibrinogen level and INTEM-MCF and EXTEM-MCF ($p < 0.001$, $p = 0.002$ and $r = 0.545$, $r = 0.454$, respectively). This study shows that decreased levels of CT and increased levels of MCF suggest hypercoagulability in patients with T1DM. Further studies are needed to confirm our findings on a larger number of diabetic patients.

Keywords Type 1 diabetes mellitus · Hypercoagulability · Children · Fibrinogen · Rotational thromboelastometry

Introduction

Type 1 diabetes mellitus (T1DM) has been shown to result in a hypercoagulable state that involves various components of clotting factors or pathways. The underlying mechanism of coagulation and fibrinolysis disorders in patients with T1DM is poorly understood and controversial [1–3]. A greater appreciation of the intimate interactions among endothelial integrity, coagulation and fibrinolytic factors, and platelets in T1DM can likely provide a greater understanding of the risk of developing cardiovascular disease and microvascular complications (e.g. retinopathy, nephropathy, and neuropathy) in patients with this disorder [4–6].

Currently, no laboratory assay can reliably screen for hypercoagulability or predict the occurrence of the hypercoagulable state. Thromboelastogram (TEG), first described in 1948 by Hartert, has been enhanced through the decades and become a valuable coagulation testing [7]. Whole blood rotational thromboelastometry (ROTEM) is a

✉ Cigdem Binay
cigdembinay@gmail.com

¹ Division of Pediatric Endocrinology, Department of Pediatrics, Osmangazi University School of Medicine, Eskisehir, Turkey

² Division of Pediatric Hematology and Oncology, Department of Pediatrics, Osmangazi University School of Medicine, Eskisehir, Turkey

³ Department of Hematology and Oncology, Osmangazi University School of Medicine, Eskisehir, Turkey

relatively new method to evaluate the whole process of blood coagulation from the beginning of clot formation to fibrinolysis; it maintains good correlation with conventional TEG [8, 9].

Although information from ROTEM is based on its performance in whole blood, ROTEM may still be superior to other routine screening tests performed in plasma without the cellular components of platelets and tissue, including hypocoagulability, hyperfibrinolysis, hypercoagulability and platelet dysfunction. Moreover, ROTEM is a useful tool for detecting these dynamic changes faster [10, 11].

Only limited data are available from previous research on the use of this method in detecting the hypercoagulable state in DM [12–14]. More studies are required to suggest that using ROTEM is more appropriate than other methods to evaluate the procoagulant condition. Nevertheless, it may be a more practical method that can conduct a global assessment of the coagulation system, including vascular endothelial damage. In this study, we aimed to assess the procoagulant condition using ROTEM in children with T1DM and to correlate ROTEM parameters with routine coagulation tests.

Materials and Methods

Study Population

A total of 43 T1DM children (20 females and 23 males) aged 2–18 years and age- and sex-matched 30 healthy control subjects were enrolled in the study group conducted from May 2012 to August 2013. The study protocol was approved by the Clinical Research Ethics Committee of the Osmangazi University School of Medicine (80558721/86). Written informed consent was obtained from parents after they had been informed about the aim and procedures of the study. None of the patients with diabetes and control subjects had a personal or first-degree family history of bleeding, thrombophilia or thrombosis, were taking any medications, or had any acute infection or chronic illness. Demographic data and anthropometric parameters of the patients with diabetes and control subjects were recorded. Daily insulin requirements of patients with diabetes were determined. Glycemic control was based on glycated hemoglobin (HbA1c) measured in whole blood. HbA1c values were categorized using the International Society for Pediatric and Adolescent Diabetes clinical practice consensus guidelines cut points for good control ($<7.5\%$) and poor control ($>9.5\%$) regardless of age [15]. The T1DM patients were subdivided into three groups according to their duration of diabetes (≤ 1 , 1–5, and ≥ 5 years). Nephropathy was diagnosed when the urinary albumin excretion rate exceeded 30 mg/24 h. Retinopathy was

diagnosed in the presence of microaneurysms and hemorrhages in the fundoscopic examination.

Clinical Assessment and Biochemical Measurements

Height was measured in a standing position, without shoes and socks, using a wall-mounted stadiometer (Harpender, Holtain, Crymych, UK) sensitive to 0.1 cm. With the participants in light clothing, weights were measured using a portable and calibrated scale (SECA762; Vöge & Hakle, Hamburg, Germany) sensitive to 0.1 kg. Body mass index (BMI) was calculated as weight (kg) divided by height (m)². Height, weight, and BMI were expressed as a standard deviation score using 2007 growth reference percentiles for Turkish children and adolescents [16, 17]. According to the data from The National High Blood Pressure Education Program Working Group, systolic and diastolic hypertension was defined as a blood pressure of >95 th percentile (p) [18]. All blood samples were obtained after overnight fasting during the same phlebotomy procedure required for routine testing at the time of an office visit. Preanalytical variables such as proper sample procurement, processing, and storage were provided adequate attention through the International Society on Thrombosis and Haemostasis (ISTH) recommendations [19]. Specimens for ROTEM and coagulation tests were drawn into tubes containing 3.2% buffered sodium citrate (0.129 mol/L) as the anticoagulant (9:1). Specimens for complete blood count were drawn into tubes containing ethylenediaminetetraacetic acid. Complete blood count was measured using a Beckman Coulter LH750 machine (Kraemer Blut. Brea, CA, USA). Prothrombin time (PT, s), activated partial thromboplastin time (aPTT, s), fibrinogen (mg/dL), and dimerized plasmin fragment D (D-dimer, mg/L) tests were performed immediately on a Siemens BCS XP machine (Tem International, Marburg, Germany). HbA1c levels were measured by Roche Tina-quant[®] III assay on the Hitachi Modular P analyzer. The method was based on the turbidimetric inhibition immunoassay. HbA1c results were reported by clinical laboratories worldwide in Système International (SI) units (mmol/mol) and Diabetes Control and Complications Trial (DCCT) units (%). Serum levels of triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were determined enzymatically using standard methods. Lipid profiles were compared with the reference percentiles for children and adolescents [20]. Dyslipidemia was defined as TC, TG, and LDL-C of ≥ 95 th p, and HDL-C of ≤ 5 th p.

ROTEM Analysis

ROTEM was performed according to the manufacturer's guidelines using a ROTEM[®] Coagulation Analyzer (Tem Innovations GmbH, Munich, Germany). The two standard

ROTEM assays, intrinsic TEM (INTEM) and extrinsic TEM (EXTEM) were performed. In these assays, the intrinsic and extrinsic coagulation pathways are triggered, respectively [21, 22]. In INTEM, coagulation is activated with 20 IL of contact activator (partial thromboplastin–phospholipid from rabbit brain extract and ellagic acid, INTEM; Tem Innovations GmbH, Munich, Germany). In EXTEM, coagulation is activated by 20 IL of tissue factor (TF, tissue thromboplastin from rabbit brain extract, EXTEM; Tem Innovations GmbH, Munich, Germany). The mean parameters obtained were clotting time (CT, s), clot formation time (CFT, s), and maximum clot firmness (MCF, mm). CT is the time from the beginning of the coagulation analysis until an increase in amplitude of 2 mm, which reflects the initiation phase of the clotting process. CFT is the time taken for the amplitude of the TEM to increase from 2 to 20 mm and reflects the propagation phase of whole blood clot formation. MCF is the maximal amplitude reached during ROTEM [21], and it correlates with platelet count and function and with the concentration of fibrinogen [23]. A “hypercoagulable profile” was defined as a shorter CT, shorter CFT, and/or higher MCF than the corresponding values in healthy controls [21, 24].

Statistical Analysis

SPSS v. 20.0 for Windows was used to analyze raw data. The Shapiro–Wilk test was used to demonstrate that the sample data came from a normally distributed population. The baseline characteristics of the study population were described by frequency and descriptive analyses. The parametric independent sample *t* test and one-way ANOVA test were used for separate group comparisons with normal distribution. Group comparisons with non-normal distribution were analyzed using the non-parametric Mann–Whitney U test. Multiple comparisons were made using Kruskal–Wallis analyses. Correlations between variables were analyzed with Spearman’s rho analysis test. All data were expressed as mean $\pm$ standard deviation or median at Q1 and Q3. $p < 0.05$ was considered statistically significant.

Results

A total of 73 cases were enrolled in the study; 43 patients (mean age 10.97 ± 4.52) were in the T1DM group and 30 patients (mean age 10.07 ± 3.48) were in the control group. The mean HbA1c level of the 43 T1DM patients was $8.94 \pm 1.88\%$ DCC (74.29 $\pm$ 20.59 mmol/mol SI), and the mean duration of DM was 3.15 ± 2.49 years.

Patients with diabetes were on a basal/bolus insulin regimen consisting of insulin lispro and glargine in total daily doses ranging from 0.7 to 1.5 Unit/kg/day. Comparison of the demographic findings and hematological parameters of the T1DM patients and the control group is presented in Table 1. No significant difference in age and sex was found between the groups. None of the T1DM patients and control individuals had history or clinical signs of clinical thrombotic events. Hematological parameters affecting the ROTEM (erythrocytes, leucocytes, platelets, fibrinogen, and D-dimer) were compared in the T1DM patients and the controls. No significant difference in the serum fibrinogen and D-dimer levels, erythrocyte, leucocytes, and platelet counts was found between T1DM patients and the controls ($p = 0.853$, $p = 0.158$, $p = 0.061$, $p = 0.556$, $p = 0.656$, respectively).

Good, fair, and poor metabolic control were defined in 14 (33%), 13 (30%), and 16 (37%) patients with diabetes, respectively. In the T1DM group, four (9.3%) patients were hypertensive and six (13%) patients had dyslipidemia (hypercholesterolemia in four patients, hypertriglyceridemia in two patients). Microvascular complication (nephropathy) was present in five (11%) patients. None of the patients in the study group had macrovascular complications. None of the control group had hypertension or dyslipidemia.

ROTEM parameters are presented in detail in Table 2. In the INTEM assay, MCF value was significantly higher in the T1DM patients than in the control group ($p = 0.004$). In the EXTEM assay, a similar significant increase in MCF value ($p = 0.026$) and decrease in CT value ($p < 0.001$) were found in the T1DM group compared with the control group. However, the degree of metabolic control had no influence on INTEM-CT, CFT, MCF ($p = 0.611$, $p = 0.327$, and $p = 0.603$, respectively), and EXTEM-CT, CFT and MCF ($p = 0.916$, $p = 0.259$, and $p = 0.369$, respectively). The duration of T1DM had also no influence on INTEM-CT, CFT, MCF ($p = 0.159$, $p = 0.321$, and $p = 0.098$, respectively), and EXTEM-CT, CFT and MCF ($p = 0.605$, $p = 0.056$, and $p = 0.980$, respectively). Furthermore, we could not demonstrate any association among nephropathy, dyslipidemia, and coagulation parameters in ROTEM analysis.

There were statistically significant correlations between fibrinogen level and INTEM-MCF and EXTEM-MCF ($p < 0.001$, $p = 0.002$ and $r = 0.545$, $r = 0.454$, respectively). The correlation between fibrinogen level and MCF are shown in Fig. 1. Neither EXTEM-CT ($p = 0.707$ $r = -0.059$ for fibrinogen; $p = 0.328$ $r = -0.153$ for platelets), nor INTEM-CT ($p = 0.158$ $r = -0.207$ for fibrinogen; $p = 0.634$ $r = -0.075$ for platelets) were correlated with fibrinogen or platelet count.

Table 1 Comparison of demographic and laboratory findings between the T1DM group and the control group

	T1DM group (n = 43)	Control group (n = 30)	<i>p</i>
Age (years)	10.97 ± 4.52	10.07 ± 3.48	0.277
Sex (girl/boy)	20/23	14/16	0.990
BMI SD (kg/m ²)	0.57 ± 0.95	0.41 ± 1.08	0.570
Hypertension (n)	4 (9.3%)	0 (0%)	N/A
Dyslipidemia (n)	6 (13%)	0 (0%)	N/A
Nephropathy (n)	5 (11%)	0 (0%)	N/A
Erythrocyte (×10 ⁶ µL)	4.77 ± 0.47	5.00 ± 0.54	0.061
Leucocytes (µL)	7100 (5900–7800)	6750 (6375–8500)	0.556
Platelet (×10 ³ µL)	264.00 (220.00–280.00)	268.50 (248.00–299.25)	0.656
Fibrinogen (mg/dL)	3200 (2600–4200)	3250 2400–5050)	0.853
PT (s)	12.04 ± 0.76	12.09 ± 0.98	0.841
aPTT (s)	31.89 ± 2.17	32.57 ± 1.43	0.114
D-dimer (mg/L)	0.29 ± 0.17	0.36 ± 0.22	0.158

Data are expressed as the means ± standard deviations or medians (Q1–Q3), as appropriate

BMI-SD body mass index standard deviation, *N/A* not assessed, *PT* prothrombin time, *aPTT* activated partial thromboplastin time, *D-dimer* dimerized plasmin fragment D

Table 2 Rotation thromboelastometry parameters in the T1DM group and the control group

	T1DM group (n = 43)	Control group (n = 30)	<i>p</i>
INTEM			
CT (s)	159.58 ± 31.59	176.07 ± 37.97	0.056
CFT (s)	74.00 (66.00–85.25)	76.00 (61.00–91.00)	0.590
MCF (mm)	65.00 (62.00–68.00)	62.00 (59.00–65.00)	0.004
EXTEM			
CT (s)	80.00 (66.75–108.50)	113.00 (96.00–126.00)	<0.001
CFT (s)	83.00 (66.00–105.00)	86.50 (65.00–97.50)	0.964
MCF (mm)	65.00 (64.00–70.00)	64.00 (61.00–67.25)	0.026

Data are expressed as the means ± standard deviations or medians (Q1–Q3), as appropriate

INTEM intrinsic thromboelastometry, *EXTEM* extrinsic thromboelastometry, *CT* coagulation time, *CFT* clot formation time, *MCF* maximum clot firmness, *ML* maximum lysis

Discussion

The current knowledge on the effects of T1DM on the prothrombotic state and endothelial dysfunction has been explained by several mechanisms, and a strong association between procoagulant state and development of vascular complications in T1DM [25–27] has been suggested. Oxidative stress, polyol pathway/protein kinase C system, and activation of advanced glycation end products are considered to play a role [28, 29]. Increased platelet activity related to enhanced protein kinase C activity, increased calcium (Ca²⁺) ATPase activity, and enhanced adhesion molecule expression has been shown to be an important factor in hypercoagulability [30–32]. Moreover, in previous studies, the levels of fibrinogen, factor VII, prothrombin fragment 1 + 2, thrombin–antithrombin complexes, and D-dimer were also found to be higher in T1DM patients than in the controls [2, 33]. Prothrombotic

disorder in T1DM has been suggested to concern mainly the endothelial dysfunction because of abnormally increased levels of soluble intracellular adhesion molecule, vascular cell adhesion molecule, von Willebrand factor (vWF), and plasminogen activator inhibitor [34–36].

The hypercoagulable state in this wide spectrum is difficult to be detected by standard coagulation tests. ROTEM is a blood coagulation test used to evaluate components of clot initiation, formation, and dissolution, as well as the stability and strength of clots in whole blood or plasma [37–39]. In the case of PT and aPTT, these assays were designed to evaluate the deficiencies of clotting factors and are generally sensitive to low concentrations of these factors. They are not routinely used for screening the prothrombotic state. The results of the present study yielded significant hypercoagulability in T1DM patients as detected by ROTEM. This hypercoagulability was detected by the presence of an accelerated clot formation, as evidenced

Fig. 1 The correlation between fibrinogen level and INTEM-MCF (a), EXTEM-MCF (b)

by the shortening of CFT and an increase in clot strength, as evidenced by the increase in MCF. In the present study, ROTEM assays revealed that, in the INTEM assay, MCF value was significantly higher in T1DM patients than in the control group. In the EXTEM assay, a similar significant increase in MCF value and a decrease in CT value were found in T1DM patients compared with the control group.

MCF is a measure of clot strength and is the valuable parameter to assess fibrinogen activity, platelet count, and function [23, 40–42]. The result of the present study yielded increased MCF levels both in EXTEM and INTEM assays. In addition, previous studies suggested that a strong correlation exists between MCF value and fibrinogen level and platelet count in patients with cancer, Behcet's disease, or thalassemia [23, 40–43]. Our findings are in line with the available data. With the present investigation, we identified the value of fibrinogen in T1DM patients, since MCF was correlated with this parameter. CT is the time from the beginning of the coagulation analysis until the initiation phase of the clotting process. In this study, EXTEM-CT was also found to be short, indicating the prothrombotic tendency of the T1DM group. These findings are consistent with the current knowledge of hypercoagulability in these patients. However, there were no statistically significant correlations between CT and other laboratory parameters. Therefore, MCF appears to be the important parameter for identifying hypercoagulopathy in T1DM patients.

Our data suggest no association between the degree of metabolic control and ROTEM parameters. Consensus on the reported data on the role of glycemic control in prothrombotic disorders in T1DM is lacking [1, 5, 28, 32]. Some studies proposed the enhanced thrombotic risk in patients with poor glucose control [5, 29, 32, 44], whereas

others [1, 4] found no relation in glycemic control, consistent with our findings. We also found no significant difference in patients' duration of DM. By contrast, Davi et al. [45] showed that platelet activation presented as an early event (<6 weeks) in T1DM children. Romano et al. [36] studied 40 children with T1DM and showed increased plasma levels of vWF, tissue plasminogen activator, and prothrombin fragment 1 + 2 in <1 year duration of DM. In our cohort, 43 T1DM patients were enrolled in the study group, and we suggest that with larger number of patients, it would be more reliable to determine the influence of these parameters. Moreover, hypercoagulability has been suggested to play a role in the development of diabetic vascular complications. However, we could not demonstrate any relationship with complications because we only had a small number of patients with nephropathy.

Only limited data are available from previous research on the use of ROTEM in detecting the hypercoagulable state in DM [12–14]. Burke et al. [14] and Delis et al. [46] showed hypercoagulability in T1DM patients with end-stage renal disease using TEG for evaluating the coagulation status in the intra-operative period. There is evidence in the literature regarding type 2 DM and changes in coagulation status, and includes factors such as hypercoagulability, reduced clot permeability and decreased fibrinolysis [47]. A recent study examined clot formation using TEG in 90 poorly controlled type 2 diabetic patients [48]. Healthy individuals had well-structured elongated fibers, while type 2 diabetic patients had results that varied from a hypocoagulable state to a hypercoagulable profile suggesting increased clot strength. Many patients in this study were on anticoagulant treatment, which might account for the variable results. Most type 2 DM patients have

cardiovascular comorbidities, including hyperlipidemia and hypertension. These comorbidities and/or medication use may also play an important role in coagulation status in type 2 DM. Another study on TEG parameters for evaluating coagulation status demonstrated subtle changes in the coagulation system in type 2 DM, indicating the activation of the tissue factor pathway [12]. The study also described hypercoagulability, as EXTEM-MCF was found to be slightly elevated in patients with diabetes. In line with our results, increased MCF indicates increased clot strength, and fibrinogen glycation may cause structural changes in fibrin fibers.

To our knowledge, only one report has been conducted on ROTEM analysis of pediatric patients with diabetes. To assess the potential hypercoagulability during ketoacidosis in children, Tran et al. [49] performed TEG analyses on blood samples from a cohort of patients with acute and resolved diabetic ketoacidosis and a control group. However, they found that, in T1DM patients with ketoacidosis, TEG was not consistent with hypercoagulability. As mentioned by the authors, the study was limited by the small number of patients ($n = 15$) enrolled in the study group.

This study has some limitations. The number of patients and controls was relatively low. We had no patient suffering from macrovascular complication and had only five patients with nephropathy as a microvascular complication. Thus, we could not determine the relation between the ROTEM parameters and the consequences of diabetes. At this point, the results could not be supported by clinical findings.

In conclusion, using the relatively new ROTEM technique, our study presented thromboelastographic evidence of the prothrombotic state in T1DM. Although this tendency was not supported by clinical findings in this study, note that children with T1DM are prone to the development of thrombosis. Moreover, ROTEM analysis measures clotting in whole blood and gives more valuable information on coagulation cascade than conventional coagulation tests. Further studies are needed to confirm our findings on a larger number of children with diabetes and to determine the relevance of this analysis in predicting premature atherothrombosis, cardiovascular disease, or microvascular complications.

Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

Informed Consent Informed consent was obtained from all individual participants included in the study.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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