Thrombosis Research 148 (2016) 45–49

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Full Length Article

Only minor changes in thrombin generation of children and adolescents with type 1 diabetes mellitus – A case-control study

Christina Cimenti *, Axel Schlagenhauf, Bettina Leschnik, Elke Fröhlich-Reiterer, Hildegard Jasser-Nitsche, Harald Haidl, Elisabeth Suppan, Gudrun Weinhandl, Maximilian Leberl, Martin Borkenstein, Wolfgang E. Muntean

Department of Pediatrics and Adolescent Medicine, Medical University of Graz, 8036 Graz, Austria

ARTICLE INFO

Article history:
Received 1 June 2016
Received in revised form 7 October 2016
Accepted 15 October 2016
Available online 17 October 2016

Keywords:
Hemostasis - blood coagulation test - diabetes mellitus
Type 1 - child – adolescent

ABSTRACT

Background: Micro- and macrovascular diseases are frequent complications in patients with diabetes. Hypercoagulability may contribute to microvascular alterations.
Objective: In this study, we investigated whether type 1 diabetes in children is associated with a hypercoagulable state by performing a global function test of coagulation – the thrombin generation assay.
Subjects: 75 patients with type 1 diabetes aged between 2 and 19 years were compared to an age-matched healthy control group. Diabetes patients were divided into high-dose and low-dose insulin cohorts with a cut-off at 0.8 U kg^{-1} d^{-1}.
Methods: Measurements were performed with platelet poor plasma using Calibrated Automated Thrombography and 1 pM or 5 pM tissue factor. Additionally, we quantified prothrombin fragments F1 + 2, thrombin-antithrombin complex, prothrombin, tissue factor pathway inhibitor, and antithrombin.
Results: Patients with type 1 diabetes exhibited a significantly shorter of lag time as well as decreased thrombin peak and endogenous thrombin potential compared to control subjects with 5 pM but not with 1 pM tissue factor. In high-dose insulin patients peak thrombin generation was higher and time to peak shorter than in low-dose patients. Thrombin-antithrombin complex was decreased in patients with type 1 diabetes, whereas prothrombin fragments F1 + 2 was comparable in both groups. Thrombin generation parameters did not correlate with parameters of metabolic control and the duration of diabetes.
Conclusions: Taken together, we found only minor changes of thrombin generation in children and adolescents with type 1 diabetes which - in contrast to type 2 diabetes - do not argue for a hypercoagulable state.

1. Introduction

Micro- and macrovascular diseases are frequent complications in patients with diabetes. Endothelial dysfunction leads to the subsequent development of atherosclerosis and thromboembolic complications which highly contribute to morbidity and mortality rates in patients with diabetes [1,2]. The contribution of the coagulation system to this condition is discussed controversially. In adults, there seems to be evidence that diabetes is associated with elevated clotting factors, enhanced coagulation activation and a decreased fibrinolytic capacity making the patients prone to a prothrombotic state [1–3]. Functionally active coagulation factors as well as inhibitors have been found in atherosclerotic lesions. Especially in the early phase of plaque formation these proteins have the potential to increase thrombin- generating capacity. These findings provide strong evidence that alterations in the coagulation system might promote vascular disease [4]. In contrast to the large number of studies in adult patients with diabetes, little is known about coagulation abnormalities and type 1 diabetes in children and adolescents. Children with diabetes are characterized by a short duration of diabetes without co-existing vascular complications, and the absence of possible confounders like long-term smoking, alcohol or drug abuse. This allows the investigation of the influence of the disease itself on hemostasis without bias such as consequent development of hypertension, dyslipidemia and malignant obesity frequently seen in adult patients with diabetes.

Abbreviations: aPTT, activated partial thromboplastin time; AT, antithrombin; CAT, calibrated automated thrombography; ETP, endogenous thrombin potential; F1 + 2, prothrombin fragments F1 + 2; FII, prothrombin; FVIII:C, factor VIII coagulant activity; IDDM, insulin-dependent diabetes mellitus; NIDDM, non-insulin-dependent diabetes mellitus; PAI-1, plasminogen activator inhibitor type 1; PPP, platelet-poor plasma; PT, prothrombin time; TAT, thrombin-antithrombin complex; TF, tissue factor; TFPI, tissue factor pathway inhibitor; TG, thrombin generation; tPA, tissue plasminogen activator; ttPeak, time to peak; VelIndex, velocity index; vWF, von Willebrand factor.

* Corresponding author at: Department of Pediatrics and Adolescent Medicine, Medical University Graz, Auenbruggerplatz 34/II, 8036 Graz, Austria.
E-mail address: christina.cimenti@medunigraz.at (C. Cimenti).

http://dx.doi.org/10.1016/j.thromres.2016.10.016
0049-3848/

Most studies investigating changes in hemostasis in patients with diabetes focus on single pro- and anticoagulant factors but only few studies report on the overall balance of coagulation. Thrombin is the central enzyme of coagulation. The measurement of the endogenous thrombin potential (ETP) quantifies the enzyme thrombin activity that can be triggered in the plasma in-vitro. It has been shown that this method is applicable to detect states of hypo - as well as hypercoagulability. It is sensitive to clotting factor deficiencies and reflects the effect of anticoagulant drugs [5–7]. Additionally, prothrombotic conditions like deficiencies in antithrombin (AT), protein C or protein S and APC-resistance as well as the influence of contraceptives on blood coagulation can be monitored [8]. The intraindividual variation rate of the ETP is with <5% notably different from the interindividual variation accounting for 15% in the normal population [8].

In this study we assessed TG by means of Calibrated Automated Thrombography (CAT), and compared it to conventional clotting parameters FII, AT, and tissue factor pathway inhibitor (TFPI) as well as activation markers of coagulation (TAT and F1 + 2).

2. Materials and methods

2.1. Patient recruitment

This case-control study was approved by the local research ethics committee of the Medical University of Graz (OHRP IRB number 00002556, protocol number 21-480 ex 09/10); written informed consent was obtained from all of the parents and assent obtained from all of the children. Patients were recruited when attending the annual review at the pediatric diabetes clinic at the University Children's Hospital of Graz. Patients presented with diabetes onset, prediabetes, MODY (Maturity Onset Diabetes of the Young) and patients with evidence of diabetic hypertension were excluded from the study. Furthermore, patients with acute or chronic illness, except diabetes associated diseases (Hashimoto thyroiditis and/ or coeliac disease), or medication other than insulin or thyroxin were excluded. Based on this sample, healthy children with normal coagulation screening were recruited as sex and age-matched control group. In our pediatric study population only 2/39 female patients with type 1 diabetes took oral contraceptives. 1 patient used a combined oral contraceptive pill (COCP) containing estradiol and progestin, 1 patient took a progestogen-only pill (POP) containing desogestrel. In both cases duration of this medication was >6 but <12 months. No events of thrombotic nor bleeding disorders have been reported in the medical history of all children and juveniles of our study population. 3% of our cohort have declared that they smoke regularly. Chronic alcohol or drug abuse was absent in our study population.

We investigated parameters of TG determined by CAT (Thrombinoscope, Maastricht, The Netherlands). Additionally, we quantified prothrombin fragments F1 + 2, TAT, FII, AT, and TFPI. Correlation analysis was performed using HbA_{1c}, plasma glucose, cholesterol including HDL and LDL and triglycerides. Screening for microalbuminuria as a parameter of microvascular disease was performed in all patients by timed (overnight) collection. Microalbuminuria was defined as at least two increased urine albumin tests with an elevated albumin excretion rate (AER >20 μg/min) within 3–6 months.

Patients with type 1 diabetes were divided into two subgroups depending on the daily insulin dose. Cut-off value was set below the mean daily insulin dose at 0.8 U kg^{-1} d^{-1}.

2.2. Pre-analytics

Venous blood was collected into plastic tubes containing sodium citrate (3.2% weight/volume, 0.01 M end concentration) using S-Monovette tubes (Sarstedt, Nümbrecht, Germany). Immediately after collection, platelet-poor plasma (PPP) was prepared by centrifugation of whole blood at 2800*g* for 10 min at room temperature and stored at −70 °C until further examination.

2.3. Reagents and devices for analysis of TG

Fluobuffer contained 20 mM HEPES and 60 mg/L bovine serum albumin, both purchased from Sigma (St. Louis, MO, USA). The fluorogenic substrate *Z*-Gly-Gly-Arg-amino-methyl-coumarin (Bachem, Bubendorf, Switzerland) was solubilized in pure dimethyl sulfoxide, which was purchased from Sigma to give a concentration of 100 mM. Calcium chloride was purchased from Merck (Darmstadt, Germany) and dissolved in deionized water to give a 1 M solution. The PPP-reagent and the thrombin calibrator were purchased from Thrombinoscope BV (Maastricht, The Netherlands).

2.3.1. Measurement of in vitro plasma TG by means of CAT

TG was measured continuously in PPP by means of CAT as described by Hemker et al. [9]. We performed TG measurements for each study subject in triplicate. Thrombin generation was triggered with 1 pM or 5 pM tissue factor (TF), respectively.

2.4. Activation markers

F1 + 2 and TAT complex were determined using commercially available ELISA systems (Enzygnost F1 + 2 and Enzygnost TAT, Siemens Healthcare Diagnostics, Erlangen, Germany).

2.5. Coagulation factors and inhibitors

TFPI was measured in the plasma using the Actichrome TFPI activity assay (American Diagnostica, Greenwich, CT, USA). TFPI antigen levels were determined by means of the Immubind Total TFPI ELISA Kit (American Diagnostica, Stamford, CT, USA).

FII was determined using coagulation factor II deficient plasma (Dade Behring, Marburg, Germany). 50 μL of a complete thromboplastin (Thromborel S, Dade Behring, Marburg, Germany) were added to 50 μL of the citrated plasma samples. The Behring Coagulation Timer from Behring Diagnostics (Behring Diagnostics GmbH, Marburg, Germany) was used for the determination of FII levels.

AT was measured using the “Hitachi 917” chemistry analyzer (Boehringer Mannheim GmbH, Mannheim, Germany) with Antithrombin III reagent (Roche/Hitachi, Holliston, MA).

2.6. Measurement of HbA_{1c}

EDTA anticoagulated blood samples were used to measure HbA_{1c} by HPLC chromatography on the Adams HA-8160 analyzer (Menarini Diagnostics, Florence, Italy).

2.7. Data analysis

Based on ETP data in an adolescent control group previously published by our group [10] we calculated an a-priori sample-size of n = 58 (alpha = 0.05, beta = 10, expected difference 10%, group allocation 1:1). In expectation of drop-outs and data incompleteness we decided to include a total of 75 participants each group.

Samples were tested for normal distribution of parameters with Shapiro-Wilk's W test and were further analyzed either by Student's *t*-test or Mann-Whitney *U* test. The Spearman rank correlation test was used to analyze correlations between the various parameters. Pearson product-moment correlation coefficient was used to measure the degree of linear dependence. Descriptive statistical analysis was performed using a commercially available software program (NCSS, version 07.1.5, NCSS LLC, Keysville, Utah). P-values of 0.05 or less were considered significant.