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Hypercoagulability detected by whole blood thromboelastometry (ROTEM®) and impedance aggregometry (MULTIPLATE®) in obese patients

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ABSTRACT

Introduction: Obesity has been associated with hypercoagulability and to increased risk of both arterial and venous thromboembolic events. Many different and complex changes in plasma coagulation factors have been described in patients with obesity. The aim of this case–control study is to evaluate hypercoagulability in a group of overweight and obese subjects by whole blood rotation thromboelastometry (ROTEM®) and impedance aggregometry (Multiplate®).

Methods: ROTEM® and Multiplate® analyses were performed in 80 subjects with a BMI ≥ 25 Kg/m^2, of whom 20 overweight [BMI = 25-29.9 Kg/m^2], 20 with I degree obesity [BMI = 30-34.9 Kg/m^2], 20 with II degree obesity [BMI = 35-39.9 Kg/m^2] and 20 with III degree [BMI > 40 Kg/m^2] and compared with 80 age and gender-matched normal weight healthy individuals.

Results: Thromboelastometry. In INTEM and EXTEM tests MCF and AUC were significantly increased in III degree obese compared with controls. MCF in FIBTEM was significantly higher in I, II and III degree obesity than controls ($p = 0.027$, 0.002 and <0.001, respectively). *Impedance aggregometry*. A significant difference in platelet aggregation was found between III degree obese subjects and healthy controls in each of the tests considered. A significant correlation between FIBTEM-MCF and aggregometry parameters with BMI, waist circumference, leptin levels and high sensitive-C reactive proteins was also found.

Conclusions: A relationship between hypercoagulability detected by whole blood thromboelastometry and aggregometry and increased fat mass is shown. Hypercoagulability also correlated with inflammatory markers. Point-of-care tests can be used to assess the degree of hypercoagulability and hyperaggregability in obese patients. Wider studies are needed to confirm our observations.

Introduction

It has been widely shown that obese patients are at higher risk for arterial and/or venous thrombotic events [1–3]. In particular, obesity is considered a risk factor for coronary and peripheral artery disease, stroke and venous thromboembolism, leading to a reduce life expectancy [1–3]. One of the mechanisms through which obese patients can develop thrombotic events is hypercoagulability. Obesity has been reported to be associated with both increased levels of clotting factors and the inhibition of the fibrinolytic pathway [4,5]. Several studies showed that elevated levels of coagulation factors such as prothrombin, factors VII and VIII, and fibrinogen were present in obese participants [6,7]. Moreover, platelets from obese individuals seem to be more reactive both at baseline and after aspirin, suggesting an innate platelet hyperaggregability [8]. More recently our group reported higher levels of microparticles and thrombin generation in overweight and obese patients than in normal weight subjects [9]. Although the results of this, and other studies, confirm the presence of a hypercoagulable profile in obese patients, they have a common limitation: since all these tests are performed in plasma, they do not take into account the interactions between plasma factors and phospholipid surfaces with other blood components in the clot formation. Moreover, they are poorly informative about clot stability and fibrinolysis. Recently, there has been a growing interest in the use of whole blood point-of-care devices either to study hypo- [10,11] as well as hypercoagulable [12,13] conditions. Thromboelastometry/graphy records the viscoelastic changes that occur during clot formation, providing a graphical representation of the fibrin polymerization and dissolution process [14]. The rate of fibrin polymerization as well as the overall clot strength is assessed. These apparatus enable a complete evaluation of the process of clot initiation, formation and stabilization and fibrinolysis, using whole blood with results available within 30 minutes. The Multiplate® analyzer (Roche Diagnostics GmbH, Mannheim, Germany) is a semi-automated computerized whole blood impedance aggregometer device

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with multiple electrode channels [15]. The aim of this case–control study was to investigate the effects of obesity on global hemostasis using whole blood rotation thromboelastometry (ROTEM®) and impedance aggregometry (Multiplate®) in order to evaluate the possibility to early detect hypercoagulability in these group of patients.

Methods

Population

The patients reported in this study were previously described elsewhere [9]. Briefly, all consecutive patients who were referred to the Centre for the Study and the Integrated Treatment of Obesity of the Padua University Hospital between January 2011 and December 2012 for a cardiovascular evaluation with a body mass index (BMI) ≥ 25 Kg/m2 were eligible. These subsequent exclusion criteria were considered: *i*) the presence of a prothrombotic condition (i.e. acute infections [defined as a documented or suspected pathological process occurred in the previous 3 months induced by a micro-organism and alteration of general and/or inflammatory and/or hemodynamic and/or tissue perfusion parameters [16], pregnancy or hormonal therapy, acute or chronic cardiovascular diseases, acquired or inherited thrombophilic condition, severe blood hypertension [≥160/100 mmHg], diabetes mellitus, recent surgery and/or cancer); *ii*) age ≤18-yrs; *iii)* previous objectively proven venous thromboembolism and/or conditions requiring indefinite anticoagulation and/or antiaggregation. Healthy volunteers, referred to our centre in the same study period, matched for age (±3 years) and sex with the cases, with BMI < 25 Kg/m^2, not experienced thromboembolic episodes and free from conditions potentially accounting for hypercoagulability were enrolled as controls. Metabolic Syndrome was defined following the National Cholesterol Education Program/Adult Treatment Panel III (NCEP/ATP III) criteria [17]. The study protocol was approved by the Institutional Ethical Committee of the University Padua Hospital.

Blood Samples and Laboratory Assays

After informed consent 9 mL of venous blood was collected from patients and controls with 21-gauge needles, without applying venostasis, into syringes pre-filled with 1 mL of sodium citrate 109 mM, after overnight fasting. Prothrombin time (PT, %), activated partial thromboplastin time (aPTT, sec.) and fibrinogen levels (mg/dl) were performed by standard methods on the BCT-Analyser (Dade Behring, Marburg, Germany). White blood cells and platelet counts were measured on the Sysmex Counter XE-2100 (Dasit Spa, Milan, Italy). Plasma levels of Interleukin-6 (IL-6, ng/L), TNFα (ng/L) and leptin (ug/L) were measured by ELISA (R&D Systems, Minneapolis, MN, USA). High-sensitive C reactive protein (hs-CRP) was measured by a turbidimetric assay on the Integra 800 analyzer (Roche).

Viscoelastic clotting measures were performed by ROTEM® (Tem International GmbH, Munich, Germany) tests according to the manufacturer's protocol. In particular, whole blood (WB) was incubated at 37 °C in a heated cup. Within the cup is suspended a pin connected to an optical detector system. The cup and pin are oscillated relative to each other through an angle of 4°45". As fibrin forms between the cup and pin, the transmitted impedance of the rotation of the pin is detected at the pin and a trace generated. All investigations were performed within two hours after blood collection and, once initiated, the blood coagulation was allowed to run until 60 min. Extrinsic coagulation cascade was studied with EXTEM test (ex-TEM®; Tem International GmbH) and intrinsic coagulation cascade was studied with INTEM test (in-TEM®; Tem International GmbH). The influence of fibrinogen on clot firmness was estimated with the platelet-inactivating FIBTEM test (fib-TEM®; Tem International GmbH). The following ROTEM parameters were analyzed: i) Clotting time (CT, sec), corresponding to the time from the beginning of the coagulation analysis until an increase in amplitude of 2 mm. The CT reflects the initiation phase of the clotting process; ii) Clot Formation Time (CFT, sec), the time between an increase in amplitude of thromboelastogram from 2 to 20 mm; Maximum Velocity (MaxV, mm/min), the peak of the first derivative of the thromboelastographic clotting curve. The CFT and MaxV reflect measures of the propagation phase of WB clot formation; iii) Maximum Clot Firmness (MCF, mm), the maximum amplitude in millimeters reached in thromboelastogram. The MCF correlated with the platelet count and function as well as with the concentration of fibrinogen. The MCF quantifies the maximum clot firmness of the established WB coagulum; iv) Area Under the Curve (AUC, mm*100), defined as the area under the velocity curve, that is the area under the first derivative curve ending at a time point that corresponds to MCF [18]. "Hypercoagulable profile" was defined as CT or CFT shorter and/or MCF, MaxV or AUC higher than in the healthy controls.

Aggregometry impedance measure in WB was performed on the Multiplate® function analyser [15]. Test cells of this device incorporate a duplicate sensor for acceptance sampling and the results, given as Area Under the aggregation Curve (AUC, AU*min), are calculated as the mean values of the two curves. The platelets, after activation by different agonists, adhere to the two copper wires of each of the two independent measuring units. During analysis, the instrument continuously measures the changes of the electrical resistance (called "impedance") between the electrodes that is proportional to the amount of platelets adhering to each couple of electrodes. Platelets were stimulated in three different ways: *i)* using TRAP-6 via the thrombin receptor (TRAP test - Roche Diagnostics GmbH, Mannheim, Germany); *ii)* via arachidonic acid, checking cyclooxygenase-dependent aggregation (ASPI test - Roche Diagnostics GmbH, Mannheim, Germany). This test is sensitive to acetylsalicylic acid, non-steroidal anti-inflammatory drugs or other inhibitors of platelet cyclooxygenase; *iii)* using ADP via the ADP receptor (ADP test - Roche Diagnostics GmbH, Mannheim, Germany).

Statistical Analysis

Statistical analysis was performed using the PASW Statistics 17.0.2 (SPSS Inc.) for Windows. The sample size calculation was based on pilot observations and the following assumptions: i) Expected increase in MCF of ≥3 mm; ii) Expected SD of 4.0 mm (1); iii) power = 95%; iv) alpha = 0.05. Based on these assumptions we needed a group of at least 42 obese patients. Continuous variables were investigated for normality using the Shapiro-Wilk test. Normally distributed variables were summarized as mean (±SD) and Student's t-test was performed, whereas non normally distributed variables were summarized as medians with interquartile ranges (IQR) and Mann–Whitney U-test was conducted. Bonferroni corrections were used to correct for multiple comparisons. Pearson's correlation analysis was used to detect significant associations between the parameters analyzed. A "p" value <0.05 was considered statistically significant.

Results

Eighty consecutive patients with a BMI ≥ 25 Kg/m^2 were included in the study. Twenty (25%) patients were overweight (BMI = 25-29.9 Kg/m^2); 20 (25%) were I degree obese (BMI = 30-34.9 Kg/m^2); 20 (25%) were II degree obese (BMI = 35-39.9 Kg/m^2) and 20 (25%) were III degree obese (BMI ≥ 40 Kg/m^2). Twenty-two patients were excluded: 6 were under treatment with anticoagulation or antiplatelet therapy before admission into the study; 5 had experienced a venous thromboembolic event; 2 were taking hormonal therapy; 2 had an acute infection; 4 had a cardiovascular disease; 3 were diabetics. The main clinical and laboratory characteristics of the study population are shown in Table 1. No significant difference in age, sex, PT, aPTT, platelet count and white blood cells among each subgroup of cases and controls was observed. Second and III degree obese patients had significantly higher levels of fibrinogen than controls. In I degree obese individuals