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Regular Article

Long-term increased factor VIII levels are associated to interleukin-6 levels but not to post-thrombotic syndrome in patients with deep venous thrombosis

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ABSTRACT

Introduction: Increased FVIII levels are a well established risk factor for deep venous thrombosis (DVT), whose etiopathogenesis is not yet well understood. In this study, we aimed to evaluate the possibility that inflammatory markers and post-thrombotic syndrome (PTS) could contribute to FVIII levels in patients with a history of DVT.
Design and Methods: It is a case-control study that included 68 patients with DVT of the lower limbs 32 months after the acute episode, and 67 healthy adults as controls. We evaluated plasma levels of FVIII, VWF, D-dimer and serum levels of CRP, IL-6, IL-8, TNF-α in patients and controls. The presence of PTS was evaluated by the Villalta scale.
Results: Patients with DVT presented higher levels of FVIII, VWF and D-dimer when compared to controls ($P \leq 0.001$). Almost 50% of patients presented FVIII levels above 90th percentile. Furthermore, IL-6 (1.19 vs. 0.98 pg/mL, $P = 0.01$) and TNF-α (2.27 vs. 1.57 pg/mL, $P \leq 0.001$) were also higher in patients when compared to controls. In a linear regression multivariate model, VWF and IL-6 levels were independent factors associated with FVIII levels ($P \leq 0.001$). FVIII levels were not increased in patients with PTS. Patients with PTS showed higher levels of IL-8 when compared to patients without PTS (23.03 vs. 18.20 pg/mL, $P = 0.04$).
Conclusions: In conclusion, we demonstrated that DVT is associated with increased levels of inflammatory and coagulation markers, including FVIII, even a long time after the acute episode. Moreover, IL-6 levels were an independent factor associated with FVIII levels. Finally, PTS seems to be related to inflammatory cytokine IL-8, a proinflammatory and proangiogenic chemokine, but not to FVIII levels.

Introduction

Deep venous thrombosis (DVT) is a multifactorial disease, and increased levels of coagulation factor VIII (FVIII) have been established as a risk factor for this disease [1–5]. We previously demonstrated that increased levels of FVIII and von Willebrand factor (VWF) were associated with DVT in a cohort of Brazilian patients [6]. The main determinants of FVIII in plasma are VWF and the ABO blood group [7,8]. Although FVIII is an acute phase protein, increased FVIII levels can occur even in the absence of an acute phase response, determined by C-reactive protein (CRP) levels [9–12]. Patients with a history of DVT may also present persistently high levels of inflammatory cytokines, such as interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor-α (TNF-α) [13–19]. However, the relationship between these cytokines and FVIII levels was not previously investigated.

Post-thrombotic syndrome (PTS) is a long-term complication present in 20-50% of patients with DVT of the lower limbs, and is associated with an increased risk of DVT recurrence, morbidity, poor quality of life, and significant cost to healthcare system [20,21]. The role of coagulation factors such as FVIII or VWF in PTS is still unclear and controversial [22–26]. We have recently demonstrated that FVIII levels could remain higher in patients even after 10 years from the acute DVT episode, particularly among patients with severe PTS [27]. It is possible that PTS could contribute to maintain increased FVIII levels through a persistent inflammatory response.

Thus, we hypothesized that the inflammatory response detected long-term after the acute DVT episode could contribute to the persistence of increased FVIII levels. This association between inflammation and high FVIII levels would be particularly true among patients with PTS. In this setting, we evaluated plasma FVIII levels and serum cytokine levels in a case-control study that included patients

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with a history of DVT, and also investigated whether these coagulation markers and cytokines were associated with the presence and severity of PTS.

Design and Methods

Study Population

This case–control study included 68 patients with at least one episode of DVT of lower limbs attended at the Hemostasis and Thrombosis Clinic, at the University of Campinas - Brazil between April 2011 and July 2013. Inclusion criteria were a symptomatic and objectively confirmed DVT of the lower limbs, in the previous one to six years, treated with anticoagulants for at least 3 months.

In the period of the study, 353 consecutive patients were attended at the clinic after anticoagulant treatment for symptomatic DVT, but 285 could not participate in the study due to exclusion criteria. Reasons for exclusion were: DVT at non-lower limbs sites (N = 100), younger than 18 years old or older than 70 years old (N = 42), carriers of natural anticoagulant deficiency (N = 5), antiphospholipid antibody syndrome or systemic inflammatory diseases (N = 13), cancer (N = 40), infection (N = 2), liver failure (N = 9), renal failure (N = 8), under anticoagulant therapy (N = 53), DVT episode >6 years ago (N = 5), refusal to participate (N = 8). All acute episodes of DVT were confirmed by duplex ultrasonography. All patients had stopped anticoagulants at least one month before the enrollment for the study. The DVT episodes were classified as unprovoked or provoked according the presence of acquired risk factors for DVT (surgery, immobilization, pregnancy/puerperium, and contraceptive use) and not according to inherited risk factors.

Sixty-seven healthy controls were selected according to age, gender, ABO blood group and the same exclusion criteria used for patients, including personal history of venous thromboembolism. This study was approved by the local Ethics Committee on Human Research and a written informed consent was obtained from all study participants.

Laboratory Methods

After the patients fasted overnight, venous blood samples (18 mL) were collected from all participants, drawn from the antecubital vein into Vacuette® tubes (Greiner Bio-One, Austria): 0.129 mmol/L trisodium citrate tube, ethylenediaminetetraacetic (EDTA) tube, and Z Serum Sep Clot Activator tube. Samples were immediately centrifuged for 20 minutes at 1500 *g*, and plasma/serum were immediately frozen and stored at -80 °C.

FVIII Activity

FVIII activity was measured by a one-stage clotting assay with FVIII-deficient plasma (*Siemens, Marburg, Germany*) as recommended by the manufacturer. The FVIII tests were performed in duplicate on automated coagulation analyzer (*BCS XP, Siemens, Marburg, Germany*). Laboratory normal range was 62.0 to 151.0 IU/dL.

VWF Antigen

VWF antigen levels were measured in plasma by enzyme-linked immunosorbent assay (ELISA), as previously described [28]. Laboratory normal range was 53.4 - 173.0 IU/dL.

D-dimer

D-dimer plasma levels were performed by immunoturbidimetric analysis, as recommended by the manufacturer, on an automated coagulation analyzer (*BCS XP, Siemens, Marburg, Germany*). Laboratory normal range was ≤0.55 mg/L.

Inflammatory Cytokines

Commercial ELISA kits were used to measure serum levels of IL-8 (BD OptEIATM, BD Biosciences Pharmingen, San Diego, USA), IL-6 and TNF-α (Quantikine, R&D Systems, Minneapolis, USA), in accordance with the manufacturer's protocols. Normal ranges were 12.3 to 315.8 pg/mL, 0.44 to 9.96 pg/mL, and 0.55 to 2.81 pg/mL, respectively. Serum high sensitive CRP (hs-CRP) levels were determined by a nephelometric method (*Siemens, Marburg, Germany*), on Siemens BN ProSpec analyzer. Normal range was <0.50 mg/dL.

ABO Blood Group

ABO blood group was determined by agglutination and adsorption-elution test.

Diagnosis and Classification of PTS

All patients were examined by the same investigator, and the PTS clinical evaluation was performed on the same day of blood collection. Presence and severity of PTS was evaluated by the Villalta scale [29]. The scale consists of five patient-rated venous symptoms (pain, cramps, heaviness, paresthesia, pruritus) and six clinician-rated physical signs (pretibial edema, skin induration, hyperpigmentation, pain during calf compression, venous ectasia, redness), which are each rated on a four-point scale (0 = none, 1 = mild, 2 = moderate, 3 = severe). Points are summed to produce a total score (range: 0-33). Subjects are classified as having PTS if the score is ≥5 or presence of venous ulcer. Among patients with PTS, a Villalta score of 5-9 was consistent with mild PTS, a score of 10-14 was consistent with moderate PTS and a score of ≥15 or presence of venous ulcer was consistent with severe PTS.

Statistical Analysis

Continuous variables were described as median and interquartile range. Medians between patients and controls were compared by the Mann-Whitney test, and categorical variables were compared by the Fisher's exact test. Parameters potentially associated with FVIII levels were evaluated in a linear regression univariate and multivariate model. A P value < 0.05 was considered statistically significant. All analysis was performed using the R Foundation for Statistical Computing, version 3.0.1.

Results

Study Population

Sixty-eight patients were included, 22 male and 46 female. The median age was 44 years and timing after the last DVT episode was in median 32 months (range 6-72) at the day of the enrollment in the study. The control group consisted of 67 subjects (23 male and 44 female) with a median age of 42 years. There was no significant difference between patients and controls related to gender, age, ethnicity and ABO blood group (Table 1). DVT was spontaneous in 42 (61.8%) patients. In the remaining 26 patients (38.2%), DVT-associated risk factors were hormonal contraceptive use (n = 20), surgery (n = 4), and immobilization (n = 2). Regarding DVT localization, 56/68 (82.4%) were proximal DVT and 12/68 (17.6%) were distal DVT.

Importantly, 10/68 (14.7%) of DVT patients had a history of prior DVT episodes (recurrent DVT). However, recurrent DVT patients did not show significant differences in coagulation markers and inflammatory cytokines levels when compared to non-recurrent DVT patients (data not shown).