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Original Article

Procoagulant versus anticoagulant factors in cirrhotic patients

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ABSTRACT

Background and study aim: Liver cirrhosis leads to decreased production of clotting factors that are generally all produced in the liver except factor VIII and von Willebrand factor. However, cirrhotic patients are not protected from thrombosis. The present study aimed to assess the procoagulant and anticoagulant factors in cirrhotic patients with and without bleeding and/or thrombotic events.

Patients and methods: A total of 102 adult subjects were enroled: 51 cirrhotic patients and 51 healthy controls. After full history taking with special attention given to thromboembolic and haemorrhagic events, platelet count, serum albumin, bilirubin, international normalised ratio (INR), PT, partial thromboplastin time (PTT), hepatitis B surface antigen (HBsAg), hepatitis B core (HBc) antibodies, hepatitis C virus (HCV) antibodies, factor VIII, protein C, Protac-induced coagulation inhibition percentage (PICI%) assay and abdominal ultrasound were performed for patients and controls. Upper gastrointestinal endoscopy was conducted for the patients.

Results: Compared with control subjects, factor VIII and factor VIII/protein C were significantly higher, while protein C and PICI% were significantly lower among patients.

Conclusion: Patients with liver cirrhosis may have a tendency for bleeding or thrombosis according to the balance of coagulant and anticoagulant status. PICI%, the assay that evaluated the functionality of the protein C anticoagulant system, was significantly lower in patients compared to control subjects. Accordingly, low PICI% and high factor VIII/protein C ratio can be taken as an index of hypercoagulability in cirrhotic patients.

Introduction

The balance of coagulation in normal conditions is ensured by the tight control of thrombin generation. This control results from two opposing drivers: The procoagulant and the anticoagulants. Among the procoagulant drivers, factor VIII plays a key role, being responsible together with other factors for boosting thrombin generation [1].

Liver cirrhosis is characterised by clinical bleeding and decreased levels of most procoagulant factors with the notable exceptions of factor VIII and von Willebrand factors (vWF) [2]. However, this is accompanied by a decrease in levels of naturally occurring anticoagulants as anti-thrombin and protein C [3]. The protein C system downregulates haemostasis because it inhibits thrombin as well as the essential cofactors Va and VIIIa. Thrombin initiates this inhibitory pathway by binding a membrane-associated protein, thrombomodulin, to activated protein C. Proteins C and S are vitamin K-dependent factors synthesised by hepatocytes. While they are often called anticoagulants, they primarily prevent normal endothelial cells from serving as a site of thrombin generation. Thus, patients with cirrhosis are not naturally auto-anticoagulated as previously believed. This concept is reinforced by clinical evidence indicating that they are not protected and may even be at an increased risk of thrombosis [4].

The basic laboratory tests of coagulation, prothrombin time (PT) and activated partial thromboplastin time (PTT) have been used to assess the risk of bleeding. However, their results are poorly correlated with the onset and duration of bleeding after liver biopsy or other potentially haemorrhagic procedures [5].

This coagulant imbalance associated with liver cirrhosis can be detected by measuring thrombin generation in plasma in the presence and absence of thrombomodulin [6]. An alternative method uses a snake venom extract, Protac. This acts as a surrogate activator of protein C. Results are expressed as a percentage of extract-induced coagulation inhibition measured as the amount

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of thrombin generated in the presence of versus the absence of the venom extract. Protac-induced coagulation inhibition percentage (PICI%) assay is the chromogenic assay, designed to globally evaluate the functionality of the protein C anticoagulant system. It is based on the ability of endogenous activated protein C generated after activation of protein C by snake venom extract (Protac) to reduce the tissue factor-induced thrombin generation [7].

The aim of this study was to assess the imbalance between procoagulant and anticoagulant factors in Egyptian cirrhotic patients with and without bleeding or thrombotic events using the new PICI% assay.

Patients and methods

A total of 102 adult subjects (51 cirrhotic patients and 51 controls) were included in this case–control study. All were recruited from the Internal Medicine Department, Hepatology inpatient and outpatient clinics of Ain Shams University Hospitals, and Faculty of Medicine, Misr University for Science and Technology (MUST). The study protocol conformed to the ethical guidelines of Ain Shams University and was approved by the local ethics committee of scientific research. Prior to initiation, every subject was informed about the aim of the study and they gave consent.

Subjects were divided into two groups:

Group "I"

Fifty-one patients with cirrhosis with a median age of 55 years (27 males and 24 females) were classified into three groups according to the severity of disease, which was estimated according to the Child–Pugh–Turcotte (CPT) score. Inclusion criteria included patients with cirrhosis diagnosed on the basis of clinical, laboratory and ultrasound results.

Group "II" or control group

This group included 51 healthy individuals with a median age of 49 years (28 males and 23 females).

Exclusion criteria included subjects using any drugs known to interfere with blood coagulation, subjects with ongoing bacterial infections, hepatocellular carcinoma or extra-hepatic malignancy as well as pregnancy and childhood (age <18 years).

All included subjects were subjected to full medical history taking, thorough clinical examination, laboratory investigations and abdominal ultrasound. Upper gastrointestinal endoscopy was conducted for the patients. The oesophageal varices were graded as large (Grade III–IV) or small (Grade I–II) based on Paquet's grading system [8].

Laboratory investigations included:

1. Complete blood picture.
2. Liver function tests and diagnostic laboratory investigations for cirrhosis including:
   a. Liver enzymes (aspartate amino-transferase (AST), alanine amino-transferase (ALT) and alkaline phosphatase (ALP)).
   b. Laboratory investigation needed for CPT score:
      - Total and direct bilirubin.
      - Serum albumin.
      - Coagulation profile, prothrombin profile (PT and international normalised ratio (INR)) and PTT.
   c. Additional laboratory investigations needed to calculate the model for end-stage liver disease (MELD) and the model for end-stage liver disease excluding INR (MELD-XI).
3. Kidney function tests including: Serum creatinine level and blood urea nitrogen (BUN).
4. Laboratory investigations for the cause of cirrhosis: Viral markers: hepatitis B surface antigen (HBsAg), hepatitis B core antibody (HBcAb) (total) and hepatitis C virus antibody (HCVAb).
5. Specific investigations:

All included patients and control groups were subjected to:

1. Factor VIII assay: a chromogenic assay expressed as percentage of normal plasma activity, which was set to 100%. The normal range was 60–150%.
2. Protein C assay: a chromogenic assay expressed as percentage of normal plasma activity, which was set to 100%. The normal range was 70–140%. The factor VIII/protein C ratio was then calculated with a normal range of 0.857–1.07.
3. PICI% assay: a chromogenic assay designed to globally evaluate the functionality of the protein C anticoagulant system, based on the ability of endogenous activated protein C generated after activation of protein C by a snake venom extract (Protac) to reduce the tissue factor-induced thrombin generation. The amount of thrombin was evaluated by recording changes in optical density (OD) at 405 nm in the presence (A) or absence (B) of Protac after adding a thrombin-specific chromogenic substrate. Briefly, 10 μL of the plasma sample and 40 μL of the diluent were incubated with either the activator A or the activator B (45 μL) for 120 s, before the thromboplastin reagent (50 μL) was added. After 90 s of incubation, 50 μL of the substrate was added, and a change in OD at 405 nm was recorded for 45 s. Results were expressed as PICI%, calculated by the following equation: PICI% = $([B - A]/B) \times 100$, where B and A are the OD for plasma tested in the absence (B) or presence (A) of Protac. The smaller the PICI% value, the greater the procoagulant imbalance. Testing was performed by a fully automated coagulation analyser (ACL 10000; Instrumentation Laboratory, Bedford, MA, USA) according to the manufacturer's specification. To minimise methodological variability, all samples were tested in a single occasion. The normal range was 80–90%.

Blood for the routine laboratory investigations was drawn by clean venipuncture, collected in suitable vacuum tubes. Blood for the specific laboratory investigations was also drawn by clean venipuncture but specifically collected in vacuum tubes containing sodium citrate as anticoagulant in the proportion of 1/9 parts of anticoagulant/blood. Blood was centrifuged within 50 min from collection at 2880*g* for 10 min at room temperature. Platelet-free plasma was then harvested and each sample was equally divided into two sets. Factor VIII and protein C assay were performed freshly on one set within 30 min at the coagulopathy laboratory of the Ain Shams University Hospitals, while the PICI% was then performed on the other set after quick storage at −70 °C until tested (duration of storage ranged from 7 to 30 days).

Statistical analysis

All data were statistically analysed using the SPSS software package (IL, USA, version 12). Continuous variables were expressed as median and interquartile ranges, tested for statistical significance with the non-parametric Mann–Whitney (M–W) and Kruskal–Wallis (K–W) tests. The chi-squared test (χ^2) was used for comparison between two or more independent percentages. The correlation between values was assessed by means of the Spearman rho correlation test. P values of 0.05 or less were considered as statistically significant. P-values $\leqslant 0.001$ were considered as