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Full Length Article

Mechanisms of hypercoagulability in nephrotic syndrome associated with membranous nephropathy as assessed by thromboelastography

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ABSTRACT

Introduction: Thromboelastography (TEG) was performed to assess potential hypercoagulability in Nephrotic syndrome (NS) patients with membranous nephropathy (MN) and to explore correlated factors contributing to hypercoagulable status

Materials and Methods: 101 MN patients, 61 minimal change disease (MCD) patients and 20 healthy controls met the inclusion criteria. The MN and MCD patients were stratified into two layers according to serum albumin (SALB) levels (<20 g/l or 20–30 g/l). Primary outcome measures included reaction time (R), α-angle, maximum amplitude (MA) and coagulation index (CI). TEG parameters of four patient subgroups were analyzed in factorial designed ANOVA with factors disease and SALB.

Results: By linear regression analysis, TEG parameters in MN patients correlated with SALB ($P < 0.01$) and the ANOVA for factorial designed data confirmed that the main effects of factors SALB and disease were both statistically significant. Besides, comparison between control group and patient subgroups showed that R value in normal controls was significantly higher than that in MN subgroups, but was not statistically different from that in MCD subgroups. NS patients (MCD, MN) had significantly higherα-angle, MA and CI values than healthy controls ($p < 0.05$).

Conclusions: MN patients tend to be more hypercoagulable than normal and MCD patients. Hypercoagulability in MN patients involves the whole thrombotic processes acceleration (activated intrinsic pathway, fibrinogen, platelet function and fibrin-platelet interaction), whereas hypercoagulable state in MCD patients may be that the coagulation factors are not fully activated. Greater efforts should be made to prevent hypercoagulability especially for MN patients with severe hypoalbuminemia.

1. Introduction

Nephrotic syndrome (NS) patients are prone to thromboembolic events. It is presently thought that the mechanisms are possibly associated with the involvement of multiple factors in the blood coagulation pathway [1–3]. Membranous nephropathy (MN) is the pathological type of NS associated with the highest incidence of thromboembolic events [2–4]. Hypercoagulability is defined as abnormalities in any of the steps that promote coagulation and is important part of early diagnosis of thromboembolic disease. Compared with other pathological types, the difference and the underlying mechanisms of MN-related hypercoagulability are not fully understood. Thromboelastography (TEG) is a blood coagulation assay that can dynamically monitor the entire blood coagulation cascade. Thus far, TEG has been widely used in various clinical fields, such as cardiovascular surgery, trauma, and oncology [5,6]. However, no controlled studies have been reported on the mechanisms of hypercoagulability in NS. Therefore, we retrospectively evaluated the records of TEG in NS patients including the pathological types MN and minimal change disease (MCD). The mechanisms underlying the hypercoagulable state in NS patients with two different pathological types were explored, and the relevant factors influencing the changes in coagulation function in MN patients were analyzed.

2. Material and Methods

2.1. Study Population

This study retrospectively involved inpatients with NS who both had undergone renal biopsy with the diagnosis of MN and MCD in our hospital and had taken TEG examination from 2012 to 2014. Inclusion criteria: (1) urinary protein ≥3.5 g/24 h and serum albumin <30 g/L; (2) aged 18 to 70 years; (3) normal liver function; and (4) no previous history of thrombosis or hemorrhagic disease. Exclusion criteria:

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(1) secondary renal disease; (2) presence of risk factor(s) for hypercoagulable state, such as the acute phase of infection, recent trauma, surgery, or cancer, and pregnancy; (3) administration of hormones, anticoagulants, or diuretics within one month; (4) treatment with plasma or albumin infusion, plasma exchange, or hemodialysis. A total of 116 MCD patients and 171MN patients received TEG examination. There were 12 MCD patients and 43 MN patients who did not meet the diagnostic criteria for NS, 31 MCD patients and 25 MN patients receiving hormones or anticoagulant therapy prior to admission, 12 MCD patients aged <18 years, and 2 MN patients with abnormal liver function who were excluded from the study. In total, 61 NS patients with MCD and 101 NS patients with MN were included, while 20 healthy adults volunteered as normal controls. The MN and MCD patients were stratified into two layers according to serum albumin levels (<20 g/l or 20–30 g/l). There were 32 MN patients and 27 MCD patients in SALB <20 g/l level, whereas 69 MN patients and 34 MCD patients in SALB 20–30 g/l level. All renal-biopsy-patients had signed the informed consent of *Renal Clinical Database Establishment* when hospitalized, allowing their data for clinical research. A description of the study flow is depicted in Fig. 1.

2.2. Physical and Clinical Data

We collected physical and clinical data from101 MN patients and 61MCD patients. These data included Body height, body mass, systolic blood pressure (SBP), diastolic blood pressure (DBP), hemoglobin (Hbg), platelet count(PLT), liver function, serum total protein, serum albumin (SALB), serum creatinine (Scr), cholesterol(CH), triglycerides(TG), serum IgG and 24-h urine protein excretion (UPr).

Body mass index (BMI) was calculated in formulae as follows: $BMI = weight\ (kg) / [height\ (m)]^2$.

Mean arterial pressure (MAP) was calculated in formulae as follows: $MAP = (SBP\ (mmHg) + DBP\ (mmHg) \times 2) /3$.

The estimated glomerular filtration rate (eGFR) was calculated according to the Modification of Diet in Renal Disease (MDRD) formula:

$$\begin{aligned}&\text{for males, } eGFR(ml/min/1.73\ m^2)\\&= 186 \times \left((serum\ creatinine(\mu mol\ /L)/88.\ 4)^{-1.154}\right)\\&\quad \times age(years)^{-0.203};\ \text{for females, } eGFR(ml/min/1.73\ m^2)\\&= 186 \times \left((serum\ creatinine(\mu mol/L)/88.\ 4)^{-1.154}\right)\\&\quad \times age(years)^{-0.203} \times 0.742.\end{aligned}$$

2.3. Routine Coagulation Parameters

Activated partial thromboplastin time (APTT) and prothrombin time (PT).

2.4. TEG Analysis

Coagulation status was assessed via TEG using citrated whole-blood samples (2.0 ml sample volume per tube; 3.8% citrate in 9:1 blood to citrate ratio). For each TEG assay, citrated whole blood (1 ml) was pipetted into to a vial containing 1% kaolin, which was inverted 5 times to ensure mixing of kaolin with the blood. Then, 340 μl kaolin-activated citrated whole blood was transferred to the TEG cup to which 20 μl of 0.2 mol/l $CaCl_2$ had been preloaded for recalcification. The TEG analyzer was stopped 40–60 minutes after reaching maximum amplitude at 37 °C. Parameters included the following: (1) reaction time (R) - time from the start of the test to a TEG amplitude of 2 mm, reflecting the combined effect of coagulation factors involved in the initiation of hemostasis; (2) α-angle- the angle between the tangent line (drawn from the split point to the curve) and the horizontal base line, representing the acceleration of fibrin build-up and cross-linking; (3) maximum amplitude (MA)- indicative of the strength of clot that reflects the cross interaction between platelet functions and coagulation; (4)coagulation index (CI), which represents the overall coagulation profile and is calculated from the R, K, α-angle, and MA values. The typical TEG profile for hypercoagulability is characterized by short R time and highα-angle, MA, CI [7].

2.5. Statistical Analysis

Data analysis was performed using Statistical Package for Social Science (SPSS) 17 statistical software. Normally distributed continuous variables were expressed as mean ± standard deviation and non-normally distributed variables were expressed as median (interquartile range). For continuous data, statistical analysis in patient groups and healthy control group was made by t-test, Mann–Whitney U test, Kruskal-Wallis test or ANOVA as appropriate depending on the normality and levels of the outcome variable. TEG parameters: CI, R, MA and α-angle values of four patient subgroups were analyzed in factorial designed ANOVA with factors disease (two levels: MCD, MN) and SALB (two levels: <20 g/l, 20–30 g/l). Interaction between disease and SALB was included. Categorical variables were compared using the using the $\chi 2$ test. Linear regression analysis was used to detect any relation between TEG parameters and clinical data. P values less than 0.05 were considered statistically significant.

3. Results

3.1. Clinical Features of 101 NS Patients with Pathological MN

Data of 101 NS patients with pathological MN on age, gender, BMI, MAP, Hbg, PLT, SALB, Scr, BUN, UA, eGFR, TC, TG, UPr, IgG, C3 and Renal pathology were presented in Table 1.

3.2. Linear Regression Analysis Between TEG Parameters and Clinical Variables Presented in Table 1 in 101 MN Patients

To identify the correlated factors influencing TEG parameters (R, α-angle, MA and CI) in MN patients, linear regression analysis was performed on the variables presented in Table 1. The results showed that the TEG parameters of MN patients were mainly correlated with serum albumin. As shown in Fig. 2, CI, α-angle and MA were inversely correlated with serum albumin level ($r = -0.628$, $P < 0.001$; $r = -0.552$, $P < 0.001$; $r = -0.369$, $P < 0.001$). R was positively correlated with serum albumin level ($r = 0.432$, $P < 0.001$). Besides, we also found CI was positively correlated with 24-hour urine protein excretion($r = 0.378$, $P < 0.001$) in MN patients. No significant correlation was observed between TEG parameters and age, BMI, MAP, MN stage, or levels of Hbg, PLT, CH, TG, serum IgG, or C3.

3.3. Subject Characteristics

Study above showed that hypoalbuminemia is a risk factor for hypercoagulability in MN patients. We divided MN and MCD patients into two subclasses by the levels of SALB. Table 2 presents detailed stratified data of MN and MCD patients in different subgroups based on SALB (<20 g/l or 20–30 g/l) and clinical features of heathy volunteers. NS patients had significantly higher BMI, MAP, CH, TG and lower SALB, eGFR than healthy controls. MN and MCD patients showed no significant difference in SALB and UPr at the same SALB level. Besides,the whole study population showed no significant differences in age, gender and PLT ($P > 0.05$).

3.4. Routine Coagulation Test Parameters

The five groups showed no significant difference in PT value. In terms of APTT value, there was no significant difference among low