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The coagulation profile of preterm delivery

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ABSTRACT

Introduction: Hypercoagulation was suggested to be involved in preterm birth etiology; however, the coagulation state of preterm parturients remains unelucidated. The study aim was to evaluate the haemostatic system of pregnant women with premature uterine contractions (PUC).
Materials and Methods: The cohort study population consisted of 76 healthy pregnant women admitted with regular PUC. The study group included 38 women who experienced preterm birth; 14 of them had preterm premature rupture of membranes (PPROM). The control group included 38 women who eventually had term delivery. Groups were matched for maternal age, number of births and gestational age at admission. Blood samples were tested for haemostatic parameters and coagulation activation markers.
Results: Significantly shorter PT and aPTT were documented in the study compared to control group (25.7 ± 2 vs. 27.4 ± 2.7 seconds, P = 0.003, and 9.96 ± 0.5 vs. 10.1 ± 0.4 seconds, P = 0.05, respectively), although differences in absolute values were small. There was no significant difference between the two groups in levels of: fibrinogen, D-dimer, protein C-global, free protein S antigen, factor VIII activity, Von Willebrand factor, plasminogen activator inhibitor-1, prothrombin fragments F1 + 2 (PT F1 + 2), tissue factor and tissue factor pathway inhibitor.
Women with PPROM had significantly lower PT F1 + 2 levels compared to those who had preterm delivery with intact membranes (351 ± 99 vs. 561 ± 242 pmol/L, P = 0.003).
Conclusions: Shortened PT and aPTT, reflecting increased thrombotic activity in maternal plasma, could serve as a marker of real preterm labor in women with premature uterine contractions. Further prospective studies in a larger cohort are warranted to validate these findings.

Introduction

Normal pregnancy is described as a hypercoagulable state. Previous studies demonstrated an increase in the levels of procoagulant factors along with a decline in anticoagulant intensity and fibrinolysis during gestation [1–5]. Hypercoagulation may be further aggravated by hereditary or acquired thrombophilias which could be a ground for pregnancy complications [6–8]. Pathological studies of placentas obtained from women with recurrent miscarriages and particularly those with thrombophilic risk factors have demonstrated increased fibrin deposition, infarcts and microthrombi within placental vessels [9–11]. Moreover, single maternal nucleotide polymorphisms of the coagulation gene tissue plasminogen activator, factor V and factor VII were found to be associated with preterm delivery [12,13], leading to the assumption that hereditary or acquired thrombophilias together with normal physiological changes in pregnancy may enhance the risk for adverse pregnancy outcomes [14,15].

Prior to delivery the cervix softens and dilates, and uterotonins, such as prostaglandins, are released, resulting in vigorous contraction of the underlying myometrial cells [16]. Approximately 10-12% of pregnant women develop premature uterine contractions (PUC) leading to preterm birth, however, not all PUC cause preterm birth. Nevertheless, about 40% of preterm birth cases are complicated with preterm premature rupture of membranes (PPROM). The biophysical pathways initiating PPROM are myometrial activation and genital tract protease stimulation, causing membrane extracellular matrix degradation, membrane rupture and uterine contractions [17].

Although preterm labor has a major impact on maternal and neonatal morbidity and mortality, the trigger initiating preterm birth is, in most cases, unidentified [18]. Previous studies suggested inflammation and coagulation factors, specifically the thrombin-antithrombin (TAT) complex [19–21] and elevated tissue factor (TF) levels [21–23], to be

Abbreviations: PUC, Premature uterine contractions; PPROM, Preterm premature rupture of membranes; PTL, Preterm labor; aPTT, Activated partial thromboplastin time; PT, Prothrombin time; PT F1+2, Prothrombin Fragments F1+2; TAT, Thrombin anti thrombin; PAI 1, Plasminogen activator inhibitor-1; VWF-Ag, Von Willebrand factor antigen; fPS, Free protein S antigen; TF, Tissue factor; TFPI, Tissue factor pathway inhibitor; PET, pre-eclampsia toxemia; PIH, pregnancy induced hypertension; IUGR, Intrauterine growth restriction.

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etiologically involved in the development of preterm birth, implicating thrombin as an uterotonic agent. Despite the diverse previous studies, the issue of coagulation state in preterm parturients remains debatable.

The identification of women with PUC who are at risk of preterm delivery is essential and challenging. Nowadays, the key to treat and prevent preterm labor is its early diagnosis, which is obvious when associated with PPROM, but obscure in cases with a closed cervix and intact membranes. Moreover, the dilemma whether "to treat or not to treat" emerges from the uncertainty that premature labor will develop, on one hand, and from the need to tailor specific treatment, which could be harmful for both the mother and the neonate, on the other hand.

The aim of the current study was to evaluate the haemostatic parameters in pregnant women with premature uterine contractions and to establish potential coagulation markers that could serve as diagnostic tools for identifying women at risk of developing preterm labor.

Material and Methods

The study was approved by the local IRB and all patients signed a detailed informed consent form before being recruited to the study. The population of this prospective cohort study included 76 healthy pregnant women admitted to the Labor and Delivery unit of Rambam Health Care Campus with regular premature uterine contractions (PUC) before 37 weeks of gestation. Inclusion criteria were singleton pregnancy between 22 to 35 weeks of gestation, without evidence of vascular disease (PET, PIH or IUGR), without a history of preterm labor, premature separation of the placenta or past thromboembolic events and without documented thrombophilia. Blood samples were collected from the recruited women; the clinical outcome and the week of gestation at delivery were recorded. All deliveries resulted in live viable newborns. The study group consisted of 38 women with PUC who experienced preterm birth, defined as a delivery at less than 37 weeks of gestation. Twenty four of them were admitted with intact membranes and 14 parturients had preterm premature rupture of membranes (PPROM). The control group included 38 women who had PUC but eventually gave birth at term. Demographics of both groups were matched.

Blood samples were collected in sterile tubes with sodium citrate 3.2% and centrifuged at 3500 G for 15 minutes. Fibrinogen, prothrombin time (PT) and activated partial thromboplastin time (aPTT) were evaluated in fresh plasma samples. The remaining plasma underwent additional centrifugation at 3500 G for 5 minutes and was then frozen in aliquots at -70 ± 5 °C. These aliquots were thawed in a warm bath at 37 °C for 15 minutes and used for the measurement of tissue factor (TF), tissue factor pathway inhibitor (TFPI), D-dimer, prothrombin fragments 1 + 2 (PT F1 + 2), Von Willebrand factor (VWF) antigen, factor VIII activity, protein C global assay, free protein S (fPS) and plasminogen activator inhibitor-1 (PAI 1).

PT, aPTT, fibrinogen, D-dimer, VWF antigen and proC global assays were performed on the Sysmex CA7000 analyzer (Siemens) using recombinant human thromboplastin Dade Innovin, Actin FS, Thrombin Reagent, Innovance D-dimer and VWF-Ag for PT, aPTT, fibrinogen, D-dimer and VWF antigen assays, respectively (Dade Behring Marburg GmbH). The proC global assay (Siemens) was performed as described previously [24]. Levels of free protein S antigen were determined on the Sysmex CA7000 analyzer, using STASTACHROM, STA-Liatest Free Protein S (Diagnostica Stago). Levels of coagulation factor VIII activity were evaluated by a 1-stage assay using factor VIII deficient plasma (HemosIL Instrumentation Laboratory) on the ACL-9000 analyzer (HemosIL). PT F1 + 2 concentration was measured by an enzyme immunoassay (ELISA) using Enzygnost* F1 + 2 (monoclonal antibodies, Siemens). TF level was determined with IMUBIND Tissue Factor ELISA kit (American Diagnostica Inc). TFPI free levels were measured using a one-step ELISA method (Asserachrom free TFPI, Diagnostica Stago).

Statistical Analysis

Results were expressed as means ± SEMs. Paired 2-tailed t test was used to assess differences in continuous data. Pearson correlation was applied for testing the eventual association between the parameters. Data were analyzed with Excel software (version 2010, Microsoft) and GraphPad Prism (GraphPad Software Inc, version 4.03, 2005). A P value of ≤ 0.05 was considered to be statistically significant.

Results

The demographic characteristics of parturients in the study and control groups are detailed in Table 1. Both groups were matched for maternal age and for number of previous births. Furthermore, the week of gestation when women had premature uterine contractions (PUC) was similar in preterm and term labor groups. There were statistically significant differences in the gestation week at delivery and in newborn weight between the preterm and term labor groups (34.6 ± 2 vs. 38.9 ± 1.1 weeks, and 2.4 ± 0.47 vs. 3.15 ± 0.31 Kg, respectively; $P < 0.0001$). All the women delivered live viable neonates with a birth weight suitable to their gestation week.

The coagulation profile of patients with and without preterm labor is presented in Table 2.

A significantly shorter aPTT was documented in patients who delivered prematurely (study group) than in the control group (25.7 ± 2 vs. 27.4 ± 2.7 seconds, respectively; $P = 0.003$). Similarly, PT was shorter in the study group compared to the controls (9.96 ± 0.5 vs. 10.1 ± 0.4 seconds, respectively; $P = 0.05$). The levels of other haemostatic markers such as fibrinogen, D-dimer, factor VIII activity, VWF antigen and TF antigen were slightly elevated in the preterm labor group compared to the term labor group, while protein C global and free protein S antigen levels were moderately lower in the preterm labor group. Despite a more marked trend for coagulation activation observed in the preterm labor group compared to the term labor group, these differences failed to reach statistical significance. There was no significant difference between the two groups in the level of PAI-1, PT F1 + 2 and free TFPI.

Table 3 presents data on the coagulation profile of patients who developed preterm labor. Within this group, prothrombin F1 + 2 levels were found to be significantly lower in women with preterm premature rupture of membranes (PPROM) than in those without rupture of membranes (351 ± 99 vs. 561 ± 242 pmol/L, respectively; $P = 0.003$). However, no significant difference between the two groups in levels of any other evaluated haemostatic parameters was found.

The lag time between a clinical manifestation of premature uterine contractions and time of delivery in the study and control groups was expressed as the number of days between appearance of contractions and delivery. No correlation was documented between the lag time and the level of aPTT at admission, used for the evaluation of coagulation activity (Fig. 1).

Discussion

Preterm delivery is a well-recognized gestational complication affecting both the mother and the newborn. The exact mechanisms underlying this challenging state have not been fully investigated.

Previous studies suggested that hypercoagulation could be associated with preterm labor. [12–15].

The aim of the current study was to identify the coagulation factors that would discriminate between women with premature uterine contractions (PUC) who are at risk of preterm labor and those who would eventually continue their pregnancy to term.

The present study revealed significantly shorter PT and aPTT in women with PUC who had preterm labor compared to those with normal term delivery, implying that both the intrinsic and the extrinsic pathways are activated in the preterm labor.