Thrombelastography monitoring of resistance to enoxaparin anticoagulation in thrombophilic pregnancy patients

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Abstract The anticoagulant effect of enoxaparin is readily observed by Thrombelastography (TEG®), particularly on the reaction time (R) to form a clot, and is completely reversed by heparinase. In this study, recalcified citrated whole blood with heparinase (CNHR) and without (CNR), along with TEG R time, was used to derive a delta R (CNR – CNHR). This delta R (ΔR) was then used to measure enoxaparin anticoagulation, which was correlated by linear regression (r² = 0.806) with plasma anti-Xa in 48 thrombophilic pregnancy patients. In a follow up study whole blood from 15 thrombophilic and 15 normal pregnancy subjects was titrated ex vivo with enoxaparin and TEG ΔR determined. Linear dose responses (all r² > 0.9) of ΔR versus plasma enoxaparin concentration were obtained for each subject. A large variation in slope was observed for both thrombophilic (7 fold, 217 to 1588 s ΔR/unit anti-Xa) and normal (3 fold, 788 to 2758) pregnancy subjects. The average slope for the thrombophilic group (710 s ΔR/unit anti-Xa) was significantly (P = 0.002) lower than the normal pregnancy group (1354 s), indicating resistance to enoxaparin anticoagulation in the thrombophilic group. This technique may help gauge the appropriate dose of enoxaparin for each individual, check for residual anticoagulation before invasive procedures, and perhaps help screen for thrombophilic subjects.

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KEYWORDS
Thrombophilic; Pregnancy; Low molecular weight heparin; Anti-Xa; Recurrent pregnancy loss

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Introduction

Pregnancy complications may be secondary to several hypercoagulable states, including anti-phospholipid syndrome, dysfibrinogenemia, anti-thrombin III, protein C and S deficiencies, factor V Leiden mutation, and hyperhomocystinemia [1-3]. Frequently these thrombophilic patients will have several cofactors including hypo-fibrinolytic conditions due to PAI-1 polymorphisms [4]. These thrombophilic states are associated with deep vein thrombosis, pulmonary embolism, unexplained spontaneous abortion, and pregnancy induced hypertension. Low molecular weight heparin (LMWH) is often used for treatment of parturients with these conditions, but a rapid test is not available for monitoring therapy [5-8]. As the thrombophilic patient approaches term, concerns arise regarding the coagulation status for regional anesthesia or surgical delivery [9]. Although not correlated with bleeding complications, anti-Xa assays remain the mainstay of recommended monitoring for LMWH therapy [10,11]. Compared to anti-Xa assays, thromboelastograph (TEG) is a rapid simple test measuring the shear elasticity of blood and has been shown to be sensitive to LMWH anticoagulation [12,13]. This study was designed to compare a novel TEG assay to standard plasma anti-Xa assays for monitoring LMWH therapy in thrombophilic obstetric patients.

Methods

Human subjects

After IRB approved informed consent, 48 pregnant patients identified as thrombophilic by the High Risk Obstetric Department (HRO), either with or without unexplained spontaneous abortion, were enrolled as they presented into this study. Enoxaparin therapy of 40 to 120 mg/day, subcutaneously, along with once per day aspirin, unless contraindicated, was initiated per HRO protocol. Diagnosis for thrombophilia included screening for anti-phospholipid antibodies, a variety of auto-antibodies, protein C and S deficiencies, factor V Leiden, prothrombin G202210A polymorphism, plasminogen activator inhibitor 4G/4G or 4G/5G polymorphism, anti-thrombin III deficiency, activated protein C resistance, methylenetetrahydrofolate reductase C677T polymorphism, individual or family history of thrombosis, and history of pregnancy induced hypertension and edema.

Assays

Two 5 ml citrated Vacutainer® tubes (Becton Dickinson, Franklin Lakes, NJ) of blood were drawn at 16-20 weeks gestation, 24-28 weeks gestation, and at term for each patient. Interval from last dose of enoxaparin to sampling was 4 to 6 h according to patient interview. The first tube was centrifuged
1000 ×g for 15 min, the plasma collected and recentrifuged 1200 ×g for another 15 min to prepare plasma. Plasma samples were stored at −70 °C until assayed by the Hemostasis Reference Laboratory (Canada) using a chromogenic method. The procedure is performed in the presence of excess antithrombin III (ATIII) to compensate any existing deficiency of this protein. The ATIII-enoxyaparin complex inhibits a quantity of added factor Xa. The amount of paranitroaniline released from a factor Xa chromogenic substrate, measured at 405 nm, is inversely proportional to the amount of enoxaparin present in the plasma as determined on a standard titration curve. The second tube was assayed in duplicate using a TEG5000 Hemostasis Analyzer (Haemoscope Corporation, Niles, IL). Citrated whole blood was allowed to clot by recalcification. Initial clot formation denoted as TEG reaction times (R) were obtained with calcified native blood plus heparinase (CNHR) and calcified native blood without heparinase (CNR). The duplicate times were averaged and the difference between heparinase and regular recalculated citrate whole blood TEG R times (ΔR = CNR − CNHR) were compared to anti-Xa assays by linear regression analysis.

In a follow up study, four 5 ml citrated Vacutainer tubes of blood were drawn from 15 thrombophilic pregnancy patients (>12 h after last dose of enoxaparin) and 15 normal pregnancy patients, in their 2nd or 3rd trimester. These four tubes were combined and 2.5 ml aliquots titrated with enoxaparin in 0.1 unit/ml increments from 0 to 0.5 anti-Xa units/ml whole blood. Hematocrits were obtained using a PlateletWorks® analyzer (Helena Laboratories, Beaumont, TX) and used to calculate plasma concentrations of enoxaparin by the following formula.

Plasma enoxaparin = added enoxaparin/(100−HCT)/100

After duplicate TEG ΔR assays of each titrated sample the remaining blood was centrifuged to prepare plasma for storage at −70 °C till chromogenic anti-Xa assay.

Statistics

Unpaired T-test, linear regression analysis and graphical representations were done with StatView 4.0 software (SAS Institute, Cary, NC).

Results

As shown in Fig. 1, linear regression indicates an agreement (r² = 0.806) between anti-Xa levels and TEG ΔR in obstetric patients on enoxaparin therapy sampled at 16-20 weeks gestation, 24-28 weeks gestation, and at term. Levels ranged from undetectable (recorded as 0) up to 1.58 anti-Xa units/ml plasma.

Since considerable scatter in the ΔR versus measured anti-Xa plasma levels was observed, a follow up study was carried out with normal and thrombophilic subjects' blood. Ex vivo titration of normal and thrombophilic pregnancy patient blood samples gave a linear dose responses (r² > 0.9), as shown in Fig. 2A and B for representative samples. A

<table>
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<th>Table 1: Patient thrombophilic diagnosis</th>
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AntiPL = anti-phospholipid antibodies, anti-mitochondrial antibodies = AMA, protein C and S deficiencies = PC or PS, factor V Leiden = FVL, plasminogen activator inhibitor 4G/4G or 4G/5G polymorphism = PAI4G/4G, anti-thrombin III deficiency = ATIII, methylenetetrahydrofolate reductase C677T polymorphism = Homo or Hetero-MTHFR, family history of deep vein thrombosis = FamHxDVT, history of spontaneous abortion = HxSAB, history of pregnancy induced hypertension and edema = PIH, History of deep vein thrombosis = HxDVT, History pulmonary embolism = HxPE, and idiopathic thrombotic purpura = ITP.

comparison of the calculated enoxaparin, on the basis of hematocrit, to measured plasma enoxaparin gave a slope of 0.92 \((r^2 = 0.987\) indicating good agreement. A large variation in the dose response slopes was observed for both normal (\(>3\) fold ranging from 788 to 2758 \(s \Delta R/\text{unit anti-Xa}\)) and thrombophilic (\(>7\) fold ranging from 217 to 1588 \(s \Delta R/\text{unit anti-Xa}\)) pregnancy blood samples. The absolute \(R\) times, measured in the presence of heparinase, were not significantly different \((P=0.32, \text{unpaired } T\text{-test})\) between the thrombophilic \((R=478 \pm 101 \ s)\) and normal \((R=460 \pm 199 \ s)\) pregnancy subjects. The average slope for the thrombophilic group \((\Delta R\) of 710 ± 468 \(s/\text{unit anti-Xa}\)) was significantly \((P=0.002, \text{unpaired } T\text{-test})\) lower than the normal pregnancy subjects \((\Delta R\) of 1354 ± 548 \(s/\text{unit anti-Xa}\)). The average slope of \(\Delta R\) versus anti-Xa plasma levels for these 15 thrombophilic subjects is higher than the \(\Delta R/\text{unit anti-Xa}\) for the original 48 thrombophilic pregnancy cases as observed in Fig. 1. This may reflect that some of the samples shown in Fig. 1 were collected at later times in the pregnancy when hypercoagulable state for these subjects no matter what the primary coagulation or fibrinolytic defect. This has been shown in previous studies by elevated thrombin anti-thrombin III or prothrombin fragment plasma levels in thrombophilic pregnancy subjects as reviewed by Greer [3]. One study in particular [14] indicated resistance to thrombomodulin anticoagulation is observed in thrombophilic pregnancy even in the 1st trimester, which again may indicate a general hypercoagulable state. The \(\Delta R\) values correlate well with anti-Xa levels with a linear dose response in titrated individual blood samples. This also allows an approximation of the degree of anticoagulation, which varies greatly between patients. Risk stratification has been suggested to be useful in deciding the appropriate low molecular weight heparin therapy [6]. Clinical trials would be needed to determine if this point of care test could help in deciding an effective dose, monitoring effectiveness throughout pregnancy, and assuring the absence of anticoagulation at term when epidural or other invasive procedures may be employed. It is also possible that this resistance to anticoagulation could be useful in diagnosing thrombophilia and its severity. Such a relatively inexpensive point of care test could be used in mass screening for thrombophilias, which is not currently recommended, due to the high cost of genetic testing [15].

References


