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Thrombin generation post elective caesarean section: Effect of low molecular weight heparin

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ABSTRACT

Introduction: Caesarean section (CS) is a significant risk factor for venous thromboembolism.. Low molecular weight heparin (LMWH) is commonly used for thromboprophylaxis post emergency caesarean delivery. However, no consensus exists regarding LMWH thromboprophylaxis following elective caesarean section. Measures of thrombin formation may indicate the full anticoagulant activity of LMWH in this setting.
Materials and methods: Anti-Xa, tissue factor pathway inhibitor (TFPI), thrombin anti-thrombin complex (TAT) and endogenous thrombin potential (ETP) were measured in twenty healthy women who received 4,500 IU tinzaparin 6 hours post CS (CS1), twenty women who received 4,500 IU tinzaparin at 10–12 hours post delivery (CS2) and twenty women post spontaneous vaginal delivery (SVD).
Results: Prior to initiation of LMWH, TAT levels at 6 hours post delivery were significantly higher in the CS1 and CS2 groups than the SVD group ($P<0.002$); TAT levels were significantly reduced up to 24 hours post LMWH treatment despite declining anti-Xa levels ($P<0.001$). In CS1, peak thrombin and ETP were significantly reduced following LMWH prophylaxis ($P<0.0001$; $P<0.002$) and reverted to pre-delivery levels 10 hours post LMWH. TFPI levels mirror anti-Xa levels during the 24 hours following LMWH treatment in CS1 group with peak levels coinciding with peak anti-Xa levels 4 hours post injection.
Conclusion: In women post caesarean section, anti-Xa levels do not reflect the full anticoagulant effects of LMWH. *In-vivo* thrombin production (TAT) is effectively reduced even when anti-Xa levels are negligible. LMWH thromboprophylaxis in this healthy cohort of patients appears to have a sustained effect in reducing excess thrombin production post elective caesarean section.

Introduction

Venous thromboembolism (VTE) is a leading cause of maternal mortality [1,2]. After an elective caesarean section (CS), the risk of developing post partum VTE is increased (OR 2.2 CI 1.5 -3.2) compared to women who undergo vaginal delivery; mortality associated with post partum VTE following caesarean delivery is increased ten-fold [3,4]. Low Molecular Weight heparin (LMWH) is used extensively for the treatment and prophylaxis of VTE in pregnancy.

The use of LMWH thromboprophylaxis in low risk women post elective caesarean section is still controversial. Current guidelines do not recommend LMWH thromboprophylaxis in this setting without any additional risk factors [5, 6]. However, the CEMACH report showed risk factors were absent in more than twenty percent of women who died resulting from venous thromboembolism [1].

Currently, there is no randomised control trial addressing the issue of LMWH thromboprophylaxis after elective CS in women with no additional risk factors. A decision analysis study comparing 7-day LMWH thromboprophylaxis with none post elective caesarean section suggested that even at low incidence of VTE, benefits of LMWH exceed the risks [7]. In our centre, women are routinely given a fixed dose of LMWH (tinzaparin 4,500 IU) per day post all caesarean delivery.

LMWH activity is commonly assessed by anti-Xa activity in blood taken 3-4 hrs post administration; however there is an increasing appreciation of the contribution of the anti-IIa effect to efficacy of LMWH. This is particularly important in LMWH such as tinzaparin, where the ratio of anti-Xa/anti-IIa is relatively low [8]. In recent years it has been reported that heparin also acts by increasing tissue factor pathway inhibitor (TFPI) release from the vessel wall [8]. Measures of *in vivo* thrombin formation (*eg* thrombin-antithrombin complex (TAT)) [9,10] and of the enzymatic thrombin activity that can be triggered in plasma, (endogenous thrombin potential (ETP)) as well as TFPI levels may give a better indication of the full anticoagulant activity of LMWH than just anti-Xa alone.

The aim of this study is to compare thrombin activation in women post elective caesarean delivery with women post vaginal delivery and to determine the full anticoagulant effects of LMWH therapy in the first 24 hours post elective caesarean section.

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Materials and methods

Patients and study design

Sixty healthy women having given full informed written consent were recruited for this study at Cork University Maternity Hospital. Forty women undergoing elective caesarean section were recruited. In our centre, patients received 4500 IU tinzaparin per day usually at 6 or 10 hours post caesarean delivery, depending on clinician preference. In this study, twenty women received 4,500 IU tinzaparin at 6 hours post caesarean section (CS1); the remaining twenty women received 4,500 IU tinzaparin at 10–12 hours post delivery (CS2). Twenty patients who underwent spontaneous vaginal delivery with no complications were also recruited (SVD group). Women with any additional risk factor for VTE such as personal or family history of VTE or thrombophilia, BMI > 29, cigarette smokers were excluded from this study. Ethical approval was obtained from the Cork Teaching Hospital Ethics Committee.

Sample size calculations were based on ETP data. A recent report suggested that peak thrombin levels in uncomplicated pregnancy are reported to be 500 nm ± 100 nm (SD), and may be reduced by approximately 15-20% in pregnancy following LMWH treatment [11,12]. Based on these figures, the sample size required to detect a significant difference in thrombin production between the caesarean section group and the control group would be 17 (in each group) (probability of Type I error of 0.05, and a probability of Type II error of 0.2). Therefore, twenty patients were recruited in each group.

Blood sampling

At each time point, venous blood (4.5 mls) was taken from the ante-cubital fossa with minimum venous stasis using 3.13% sodium citrate as anticoagulant. In the CS1 group, bloods were also taken at 6, 10, 16, 24 and 30 hours post delivery. This corresponded to pre-dose, 4, 10, 18 and 24 hours post tinzaparin administration. A subset of ten patients had blood sampling pre-delivery. In the CS2 group, blood was taken at 6 and 10 hours post delivery. In the SVD group, blood samples were taken at 6, 10, 16 and 24 hours post delivery.

Samples were centrifuged at 4 °C for 20 minutes at 2000 × *g*. The resulting platelet poor plasma was carefully removed, aliquoted into cryotubes, snap frozen and stored in cardbox cryoboxes at −80 °C until assay. All samples were processed and stored within 1 hour of phlebotomy.

Laboratory Methods

Anti-Xa activity was measured using chromogenic substrate assay with tinzaparin used as standard (Hyphen BioMed, France). Plasma levels of thrombin-antithrombin III complex (Enzygnost® TAT Micro, Siemens, Marburg, Germany) and tissue factor pathway inhibitor (Quantikine®, R&D Systems, Minneapolis, USA) were measured with commercially available enzyme linked immunosorbent assays (ELISA).

Endogenous thrombin potential (Thrombinoscope™, Synapse BV, Maastricht, Netherlands), was measured as previously described [11]. Briefly, 80 μls of plasma was incubated with 20 μls of platelet poor plasma reagent containing 5pM of Tissue factor. Thrombin generation was initiated by addition of fluorogenic thrombin substrate (Fluca® Thrombinoscope™, Maastricht, Netherlands) and quantified by thrombin calibration standard. Fluorescence was measured at 20 second intervals for 60 minutes or until thrombin generation was completed. Peak thrombin production and area under the thrombin generation curve (ETP) was determined and reported for each sample.

Data Analysis

Data was first assessed for normal distribution using histogram and Normal Probability Plot. Data on TAT was positively skewed and log transformed to approximately normalize the data. 24-hour pharmacokinetic profiles were constructed for each variable and data was analysed using Repeated Analysis of Variance (Repeated ANOVA) with time post delivery (6, 10, 16, 24, 30 hours post delivery), LMWH prophylaxis and mode of delivery as factors. Interactions between factors were also examined. Post hoc tests (Least Significant Difference) were used to determine significant differences between the patient groups. In all cases $P<0.05$ was considered statistically significant. Pearson's correlation analysis was used to correlate TFPI, ETP and TAT (log transformed data) with anti-Xa activity.

Results

Patient Demographics

Sixty women were recruited (twenty patients in the CS1 group, twenty patients in CS2 group and twenty patients in the SVD group). There were no significant differences with respect to age, parity, BMI and gestation at delivery within these three groups (Table 1). All recruited women were non-smokers, had uncomplicated singleton pregnancies and had no personal or family history of VTE or known thrombophilia. Indications for elective caesarean in CS1 group were repeat caesarean delivery (n = 14), breech presentation (n = 5) and previous third degree tear (n = 1). Indications for elective caesarean in CS2 group were repeat caesarean delivery (n = 15) and breech presentation (n = 5). None of the patients in this study had any particular surgical difficulties, abnormal blood loss, spinal haematoma or bleeding complications following the LMWH prophylaxis.

Thrombin Generation and TFPI levels in Post Elective Caesarean Section and Normal Vaginal Delivery

Thrombin Antithrombin (TAT) complex

Repeated ANOVA showed that LMWH prophylaxis ($P<0.05$), mode of delivery ($P<0.001$) and time post delivery ($P<0.0001$) all significantly affected TAT levels in the post partum period. In addition, a significant interaction occurred between LMWH prophylaxis and time post delivery ($P<0.01$). This means that LMWH prophylaxis altered the profile of TAT levels over the immediate post partum period. In the SVD group, a significant decrease in TAT levels was observed during the 24 period following delivery ($P<0.0001$) reaching a minimum at 24 hours post partum. Both CS groups showed significantly higher levels of TAT prior to initiation of LMWH prophylaxis compared with the same time points in the SVD group ($P<0.002$) (6 hours post delivery), ($P<0.03$) (10 hrs post delivery). LMWH prophylaxis significantly reduced TAT levels in the CS1 group compared with the CS2 group at 10 hours post delivery ($P<0.01$). Levels remained decreased in the CS1 group compared with pre-LMWH ($P<0.0001$) and were similar to levels observed in the SVD group at the same time points except at 24 hours post delivery. 24 hours post delivery levels were significantly higher in the CS1 group compared with the SVD group ($P<0.01$). For the TAT results, geometric mean

Table 1
Patient Demographics. Values presented as mean(SD). NS-not significant.

Mean (±SD)	CS1	CS2	SVD	P-value
	n = 20	n = 20	n = 20	
Age	31.9 (4.6)	31.4(3.2)	31.5(4.5)	NS
Parity	1.1(0.7)	1.0(0.6)	1.1(0.8)	NS
BMI	23.9(2.2)	23.7(2.3)	23.9(2.6)	NS
Gestation at delivery	39.4(0.5)	39.0(0.5)	39.2(0.6)	NS