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Unfractionated heparin and placental pathology in high-risk pregnancies: Secondary analysis of a pilot randomized controlled trial

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ABSTRACT

Introduction: Heparin is often prescribed during pregnancy with the intention of improving perinatal outcomes on the basis that it exerts an anticoagulant action in the inter-villous space. Accumulating in-vitro and *in-vivo* evidence indicates that heparin's beneficial effects in pregnancy may result from 'non-anticoagulant' effects including the promotion of angiogenesis.
Methods: To study the effect of heparin within the placenta, we performed secondary analyses on a pilot trial where 32 women with negative thrombophilia screens and second-trimester evidence of placental insufficiency were randomized to standard care or antenatal self-administration of unfractionated heparin (UFH) 7500IU twice-daily. Serial placental ultrasound images were reviewed and compared with histo-pathologic findings following delivery.
Results: There were no differences between the two arms in either the evolution of abnormal placental lesions on ultrasound ($p = 0.75$) or evidence of maternal vascular under-perfusion on histopathology ($p = 0.89$). In pregnancies considered at increased risk for adverse pregnancy outcomes based on previous history or abnormal serum marker screen, early (second-trimester) placental ultrasound, reflecting developmental pathology had better test characteristics (sensitivity 77.8%; positive predictive value 80.8%) for predicting adverse pregnancy outcomes than third-trimester ultrasound that is reflective of placental thrombotic injury.
Conclusions: Administration of UFH did not prevent the development or evolution of abnormal placental lesions on placental ultrasound or evidence of maternal vascular underperfusion on placental histopathology. Second-trimester placental ultrasound may be of value in predicting those at greatest risk of adverse outcomes.

1. Introduction

The concept of 'placental anticoagulation' as a treatment option for recurrent placental infarction was suggested over four decades ago [1]. Since then, there has been a steady rise in the prescription of heparin to pregnant women with the intent of improving perinatal outcomes, based on the premise that heparin is capable of reducing the risk of multi-focal placental thrombosis and infarction via its anticoagulant properties [2]. This hypothesis has been fuelled by studies suggesting that adverse perinatal events are accompanied by an increased maternal risk of thrombophilic disorders [3]. Investigators have suggested that heparin promotes successful implantation following *in-vitro* fertilization [4] and prevents recurrent miscarriages [5]. However, large high-quality randomized control trials (RCTs) show that low molecular weight heparin (LMWH) is no more effective than placebo in preventing unexplained recurrent miscarriages [6], and its use for this indication is therefore now discouraged, except in the subgroup of women with anti-phospholipid antibody syndrome [7,8].

By contrast, several RCTs demonstrate that LMWH reduces the risk of perinatal complications in women who screen negative for thrombophilia disorders [9,10]. A systematic review [11] and meta-analysis [12] have recently demonstrated significant reductions in the rates of severe pre-eclampsia, intrauterine growth restriction (IUGR) and perinatal mortality when high-risk women are allocated to LMWH injections. Despite almost 2000 participants involved in these studies, our pilot trial [13] was the only

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study to incorporate sonographic assessment of the placenta *in-vivo* and placental pathology following delivery. In a previous retrospective study [14] we demonstrated modest associations between sonographic lesions and placental infarction following delivery. Here we report the assessment of placental ultrasound findings and detailed placental pathology in the two arms of our pilot trial.

2. Methods

Following Research Ethics Committee approval (06-0246-A), a pilot RCT was conducted at Mount Sinai Hospital, Toronto, Canada between March 2007 and May 2010 where women with singleton pregnancies, a negative thrombophilia screen and at higher risk of having future placental dysfunction were randomized to unfractionated heparin (UFH) or 'standard care' using a central telephone randomization service.

2.1. Inclusion/exclusion criteria

Details are described in the original publication [13]. In brief, a participant was considered 'thrombophilia screen negative' if she was neither homozygous nor heterozygous for prothrombin-20210A or factor V Leiden R506Q mutations, and did not test positive for lupus anticoagulant or IgG cardiolipin antibody. [The original study didn't test for antithrombin III, protein C and S deficiencies or elevated factor VIII]. Women were deemed to be at increased risk for future placental dysfunction if they met at least two of the following criteria; abnormal placental biochemistry (assessed by reinterpretation of first- and second-trimester maternal serum screening for fetal aneuploidy) [15], abnormal placental morphology (assessed by real-time ultrasound imaging) [14,16], and abnormal uterine artery Doppler waveforms (assessed using colour flow mapping to obtain waveforms of the proximal uterine arteries by pulsed Doppler) [17]. These criteria were chosen as they were previously shown to be highly predictive of IUGR and preterm birth (PTB) [positive predictive value (PPV) of 74%] in a cohort study of 212 women at increased risk for adverse pregnancy outcomes (APO) [16].

2.2. Interventions

Women randomized to the UFH arm self-administered 7500IU (0.3 cc) subcutaneous UFH into the lower abdomen or lateral thighs on a rotating basis, using an insulin syringe twice-daily from the time of recruitment (18 + 0 to 23 + 6 weeks) to 34 weeks of gestation or delivery, whichever occurred first. Compliance was assessed by examination of injection sites at each visit. Women randomized to the 'standard care' arm did not receive placebo injections, although eight of these women took low-dose aspirin (LDA) until 34 completed weeks of gestation. All women received standard maternal–fetal surveillance for high-risk pregnancies.

2.3. Study endpoints

2.3.1. Primary

Difference in the proportion of placentas with evidence of maternal vascular under-perfusion (MVU) between the two arms.

2.3.2. Secondary

Test characteristics of second- and third-trimester ultrasound for predicting placental lesions and APO.

2.4. Outcome assessment

2.4.1. Sonographic evaluation of placental morphology

Placental morphology was initially assessed at recruitment, by real-time ultrasound imaging using criteria developed in earlier studies [14,16,18]. Thereafter, serial examinations included placental texture and were performed on all participants at least every 4 weeks, by sonographers trained by JK. Ultrasound examinations included fetal growth, biophysical profile and Doppler studies to guide clinical management. Images were archived to the hospital digital image storage system. Two series of anonymized placental ultrasound images per participant – those obtained at recruitment and just before delivery – were retrieved for each participant and independently reviewed by RD and JK, both blinded to clinical outcomes and placental pathology. Ten random images were again selected for re-reporting by both reviewers. There was 100% agreement between reviewers, excluding intra- and inter-observer variation. The criteria used for identification of abnormal placental texture, were echogenic cystic lesions (ECL) representing inter-villous thrombi [18,19] or jelly-like appearance with turbulent utero-placental flow, visible due to lack of normal villous development [20]. ECLs were defined by the maximum diameter of their hypo-echoic centre being $\geq$1 cm, using previously validated criteria [18].

2.4.2. Placental histopathology

A perinatal pathologist (SK), blinded to treatment allocation, examined 31/32 placentas that were submitted for histo-pathologic examination. Placental weights and dimensions were recorded and the weight percentile and gross morphology were documented as previously described [21,22]. Following formaldehyde fixation for 48 h, placentas were sliced into 1–2 cm thick sections for macroscopic identification of pathological lesions and an estimate of the percentage of tissue involved. Where a gross lesion category occupied 2% or less of the tissue and was not centrally placed, it was not considered significant. Grossly visible lesions were sampled in addition to samples of normal villous parenchyma, chorionic plate, the maternal surface, umbilical cord and a roll of fetal membranes. These samples were wax-embedded for H&E evaluation. The original publication [13] contained a preliminary description of the placental pathology. For purposes of this study, placental pathology was re-interpreted using standard definitions [22–28] and grouped into three distinct categories; (1) pathology related to MVU, (2) other significant pathology (apart from MVU) and (3) normal or non-significant pathology, based on a previously published classification [29]. These placental lesions and their clinical significance are summarized in Table 1.

2.4.3. Adverse pregnancy outcome (APO)

This was defined as one or more of the following: small for gestational age (SGA) baby [birth weight less than the 10th centile for gestational age and sex, at increased risk of perinatal morbidity and mortality regardless of evidence of placental dysfunction] [30], IUGR (evidence of SGA + abnormal umbilical artery Doppler findings), spontaneous or induced PTB (before 32 weeks), placental abruption, pre-eclampsia or HELLP syndrome, intrauterine fetal death (IUFD) or early neonatal death (within seven days of life). To avoid serious infant morbidity and mortality, in cases of absent or reversed end-diastolic blood flow in the umbilical artery, delivery is recommended under 32 weeks when venous Dopplers become abnormal and at 32 weeks even if the venous Dopplers remain normal [30].

2.5. Statistical analysis

Descriptive statistics are presented as medians and interquartile ranges. Chi-squared and Fisher's exact tests were used to determine