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Placental mRNA in maternal plasma as a predictor of ectopic pregnancy

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Abstract

Objective—To measure and compare placental mRNA expression in the maternal circulation among women with intrauterine and ectopic pregnancies.

Methods—Plasma was collected from patients in early pregnancy at risk of ectopic pregnancy. Clinical information was prospectively collected and entered into a dedicated database. mRNA was isolated from maternal plasma and quantitative RT-PCR was performed to measure mRNA for human gonadotropin (hCG) and human placental lactogen (hPL). GAPDH mRNA expression was used as an internal control.

Results—Twelve women with ectopic pregnancy and 13 women with intrauterine pregnancy were enrolled. Patients with ectopic pregnancy were 6 times more likely to have undetectable levels of hPL mRNA (relative risk [RR] 6.36; 95% confidence interval [CI], 1.70–23.20; $P<0.01$). They were also 8 times more likely to have undetectable levels of hCG mRNA (RR 8.64, 95% CI, 1.30–57.10; $P<0.01$). mRNA copy numbers for hPL and hCG (normalized by GAPDH) were significantly lower in the ectopic group than in the intrauterine group.

Conclusion—Placental mRNA is present in the maternal circulation in significantly lower copies in cases of ectopic pregnancy compared with cases of intrauterine pregnancy. Measurement of placental mRNA in the maternal circulation may help to distinguish between intrauterine and ectopic pregnancies.

Keywords

Ectopic pregnancy; Intrauterine pregnancy; Placental mRNA

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Conflict of interest
The authors have no conflicts of interest.

1. Introduction

Ectopic pregnancy is a life-threatening condition affecting an estimated 1%–2% of all pregnancies and causing approximately 6% of all pregnancy-related deaths [1,2]. The diagnosis of ectopic pregnancy continues to be a clinical challenge for physicians; if the location of the pregnancy is not identified via ultrasound on initial presentation, an algorithm based on serial β-human chorionic gonadotropin (hCG) and serial ultrasound must be applied. Meanwhile, the ectopic pregnancy is at risk of rupture, with a possibility of maternal death [3]. The development of an accurate test to diagnose ectopic pregnancy is imperative because early detection and treatment would have a great impact on clinical outcome [4,5].

The presence of nucleic acids in the blood facilitates possibilities for the noninvasive diagnosis of different diseases. Levels of cell-free DNA have been correlated with response to treatment and presence of metastasis among patients with cancer [6]. Abnormalities of fetal DNA have also been studied in maternal blood samples, and a quantitative discrepancy of fetal DNA has been associated with pathologic pregnancy conditions such as fetal chromosomal abnormalities, pre-eclampsia, and abnormal placentation [7–9]. The presence of placental mRNA sequences (e.g. human placental lactogen [hPL] and β-hCG) is useful for monitoring placental function [10]. The specificity, independence for fetal gender, and stability of placental mRNA could have an important role for future diagnostic methods [11–15].

Maternal blood is in direct contact with the syncytiotrophoblast during pregnancy; this tissue constantly undergoes apoptosis and releases microparticles containing RNA and DNA into the maternal blood [16,17]. Similarly, invasive extravillous trophoblast (EVTB) undergoes apoptosis and may enter the maternal circulation. It is possible that the cellular environment encountered by EVTB in ectopic pregnancy differs from that of the normal placenta in that there is no decidua. Moreover, EVTB in ectopic pregnancy may undergo altered cell–cell interaction at the maternal–fetal interface, which might influence the rate at which placental RNA enters the maternal circulation. The aim of the present study was to measure and compare placental mRNA expression in the maternal circulation among women with intrauterine and ectopic pregnancies.

2. Materials and methods

Women in the first trimester of pregnancy were asked to participate in a pilot study at the University of Miami, Miller School of Medicine, Miami, USA, between October 1, 2009, and August 1, 2010. The study was approved by the Institutional Review Boards of the University of Miami; all participants provided written informed consent.

Blood samples were obtained from women in early pregnancy. Demographic and clinical data were prospectively entered into a computerized database. Participants were followed until they were definitively diagnosed. A visualized intrauterine pregnancy was defined as an intrauterine pregnancy identified via ultrasound, with a yolk sac or a fetal pole. Ectopic pregnancy was defined as either visualized (extrauterine gestational sac with yolk sac or embryonic cardiac activity identified via ultrasound, or an ectopic visualized at the time of surgery) or nonvisualized (rising hCG level after uterine evacuation) ectopic pregnancy.

Plasma samples from women with normal intrauterine pregnancy or ectopic pregnancy were used for RNA isolation and quantitative reverse-transcription polymerase chain reaction (RT-PCR). The JEG-3 human choriocarcinoma cell line was used as a positive control. Total RNA extraction from 5×10^6 JEG-3 cells was performed using the RNeasy Mini Kit (Qiagen, Valencia CA, USA), and total RNA extraction from patient samples using 2 mL of

plasma was performed using the QIAmp Circulating Nucleic Acid Kit (Qiagen). Reverse transcription of total RNA to cDNA was performed using the qScript cDNA Synthesis Kit (Quanta Biosciences, Gaithersburg, MD, USA). cDNA samples were then used to perform quantitative gene expression analysis (qPCR) of hPL and β-hCG using gene-specific TaqMan primer and probe sets (Biosearch Technologies, Novato, CA, USA). The gene encoding GAPDH was used as a housekeeping control gene. Multiplexed qPCR reactions were prepared using Perfeta Multiplex qPCR Super Mix (Quanta Biosciences) with a final concentration of 300-nM forward and reverse primers, and a 200-nM probe for each gene. The thermal profile used for the multiplexed hPL/β-hCG/GAPDH gene expression analysis was as follows: 1 cycle at 95 °C for 3 minutes, followed by 40 cycles at 95 °C for 15 seconds and 60 °C for 1 minute. All gene expression data were collected and analyzed. The primer and probe sequences for each of the genes used are listed in Table 1.

Transcript copy numbers of each target gene were quantified via absolute quantification. To establish standard curves for each target gene, serial dilutions were prepared from single-stranded synthetic DNA oligonucleotides (IDT Technologies, San Diego, CA, USA) corresponding to the amplicons from each target, ranging from 1×10^9 to 1 copy. Polymerase chain reaction efficiencies between 95% and 105% for each standard curve were deemed acceptable for use of target gene quantification. Raw threshold values for each gene target were used in concordance with the standard curve prepared for respective genes to determine the copy number present in each patient sample. The copy numbers for each target gene were then normalized for relative quantification by dividing the target gene copy number by the housekeeping gene copy number.

Continuous data were compared using the Student *t* test if the distribution of samples was normal; if the sample distribution was asymmetric, the Mann–Whitney *U* test was used. $P<0.05$ was considered to be statistically significant. All statistical calculations were performed using SigmaStat (SPSS, Chicago, IL, USA).

3. Results

Of the 25 participants, 12 were diagnosed with ectopic pregnancy, while 13 were found to have viable intrauterine pregnancies. The clinical characteristics of the participants are shown in Table 2. Women in the ectopic group were similar to those in the intrauterine group with regard to age, gravidity, parity, and estimated gestational age based on last menstrual period. Levels of hCG were significantly higher in the intrauterine group than in the ectopic group (35 696 ± 22 000 mIU/mL vs 2704 ± 2981 mIU/mL; $P=0.01$).

mRNA for hPL could not be detected in 10 of the 12 women with ectopic pregnancy, compared with 1 of the 13 participants with intrauterine pregnancy (specificity 92%; sensitivity 83%; positive predictive value [PPV] 91%; negative predictive value [NPV] 86%). Patients with ectopic pregnancy were 6 times more likely to have undetectable levels of hPL mRNA (relative risk [RR] 6.36; 95% confidence interval [CI], 1.70–23.20; $P<0.01$). mRNA for hCG could not be detected in 11 of the 12 participants with ectopic pregnancy, compared with 3 of the 13 women with normal intrauterine pregnancy (specificity 77%; sensitivity 92%; PPV 78%; NPV 91%). Patients with ectopic pregnancy were 8 times more likely to have undetectable levels of hCG mRNA (RR 8.64; 95%CI, 1.30–57.10; $P<0.01$).

mRNA copy numbers for both hPL and hCG (normalized by GAPDH) were significantly lower in the ectopic pregnancy group than in the intrauterine pregnancy group (Table 2).

4. Discussion

In the present study, hPL mRNA and β-hCG mRNA were undetectable in most of the patients with ectopic pregnancy, whereas they could be isolated from most patients with intrauterine pregnancy. If these findings can be confirmed in a larger group of women, testing for placental mRNA in maternal plasma could become a useful tool for distinguishing normal intrauterine pregnancy from ectopic pregnancy.

At present, one can only speculate on the etiology behind the findings. Although placentation is relatively similar in ectopic and intrauterine pregnancies, there are certain differences such as the absence of decidua formation by the tube and the impossibility of trophoblast formation and differentiation within the tube [18]. We hypothesize that there is a decreased blood supply in ectopic pregnancy, which may affect the transport of EVTB into the maternal circulation, with a subsequent decrease in levels of mRNA in maternal plasma. Furthermore, the decreased blood supply could be caused by not only the lack of space for the trophoblast to develop but also the structural differences of the tubal vessels.

Limitations of the present study were the small sample size and the significant difference in hCG levels between the 2 groups. Moreover, only women with intrauterine or ectopic pregnancies were investigated; women who experienced spontaneous abortion were not included. In addition, previous studies have shown that placental mRNA levels are higher in the cellular component of maternal blood than in the plasma during early pregnancy [19]. In the present study, only the plasma component—not the cellular component, which may behave differently—was analyzed. Future studies should evaluate both cellular and plasma mRNA.

In summary, lower levels of placental mRNA were detected in the maternal blood of women with ectopic pregnancies compared with women who had intrauterine pregnancies. The measurement of placental mRNA in the blood of women at risk for ectopic pregnancy may become a useful test for distinguishing ectopic from intrauterine pregnancies.

Synopsis

Measurement of placental mRNA in the maternal circulation may help to distinguish between intrauterine and ectopic pregnancies.

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Table 1

Primer and probe sequences for the genes used in the study

Gene name		Sequence
hPL	Forward	5'-CATGACTCCCAGACCTCCTTC-3'
	Reverse	5'-TGCGGAGCAGCTCTAGATTG-3'
	Probe	5'-FAM-TTCTGTTGCGTTTCCTCCATGTTGG-BHQ1-3'
hCG	Forward	5'-CTACTGCCCCACCATGACCC-3'
	Reverse	5'TGGACTCGAAGCGCACATC-3'
	Probe	5'-CAL560-CCTGCCTCAGGTGGTGTGCAACTAC-BHQ1-3'
GAPDH	Forward	5'-CCACTCCTCCACCCTTGAC-3'
	Reverse	5'-ACCCTGTTGCTGTAGCCA-3'
	Probe	5'-CAL610-TTGCCCTCAACGACCACTTTGTC-BHQ2-3'

Table 2

Clinical characteristics and mRNA levels of women with intrauterine or ectopic pregnancies [a]

Characteristic	Intrauterine group (n=13)	Ectopic group (n=12)	*P* value
Age, y	30 ± 6	28 ± 5	0.43
Gravidity	2 (1–6)	2 (1–10)	0.78
Parity	1 (1–3)	1 (0–8)	0.57
Gestational age, wk	7 ± 4	7 ± 3	0.84
hCG, mIU/mL	35 696 ± 22000	2704 ± 2981	0.01
Relative copy number of hCG mRNA	68 ± 149	2 ± 9	0.03
Relative copy number of hPL mRNA	285 ± 651	35 ± 285	0.05

Abbreviations: hCG, human chorionic gonadotropin; hPL, human placental lactogen.

[a] Values are given as mean ± SD or median (range) unless otherwise indicated.