Original Article

A comparison of cell-free placental messenger ribonucleic acid and color Doppler ultrasound for the prediction of placental invasion in patients with placenta accreta

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Abstract

Background: The aim of the present study was to comparison between cell-free placental messenger ribonucleic acid (mRNA) and Doppler ultrasound for the prediction of placental invasion in women with placenta accreta.

Materials and Methods: In this cross-sectional study, 50 pregnant women at risk for placenta accreta underwent color Doppler and assessment of cell-free placental mRNA. Real-time reverse-transcription polymerase chain reaction was used for measurement of cell-free placental mRNA in maternal plasma. Based on the findings at cesarean delivery and histological examination, patients were divided into two groups of women with and without placenta accrete. To compare of the mean of mRNA levels between the two groups we used independent *t*-test and to compare of the mean of age and gestational age at sonography we used Mann-Whitney test. For determination of sensitivity and specificity and the cut-off point of mRNA levels we used the receiver operating characteristic curve.

Results: A total of 50 women with a mean age of 30.24 ± 4.905 years entered the study and 12 (24%) patients were diagnosed with placenta accreta. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of Doppler ultrasound were 83.3%, 78.9%, 56% and 94%, respectively. Results of our study showed if we consider a cut-off point equal to 3.325, with sensitivity and specificity of 0.917 and 0.789, respectively and the sensitivity, specificity, PPV and NPV of mRNA with were cut-off point of 3.325 were 91.7%, 78.9%, 57.9% and 96.8%, respectively.

Conclusions: Cell-free mRNA is an acceptable, easy made, functional test with sensitivity, specificity, PPV and NPV more than Doppler ultrasound for diagnosis and prediction of incidence of placenta accrete and we recommend the use of cell-free mRNA test for diagnosis of placenta accreta.

Key Words: Cell-free placental messenger ribonucleic acid, color Doppler, placenta accreta

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INTRODUCTION

Placenta accreta refers to an abnormality of placental implantation in which the anchoring placental villi attach to myometrium rather than decidua, resulting in a morbidly adherent placenta.[1] Placenta accreta is much more common than placenta increta and percreta.[2]

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The pathogenesis of placenta accreta is not known with certainty. The most common theory is that defective decidualization (thin, poorly formed, or absent decidua) related to previous surgery or to anatomical factors (endocervix, lower uterine segment, endosalpinx, uterine anomaly) allows the placenta to attach directly to the myometrium.[3]

The presence of placenta accreta is associated with major pregnancy complications and is thought to be becoming more common, due to a number of factors including rising maternal age at delivery and an increasing proportion of deliveries by cesarean. Placenta accreta affects approximately 1 in 533 pregnancies.[2] The risk of placenta accreta in future deliveries after cesarean section is 0.4-0.8%. It occurs in 15% of patients with placenta previa.[4] Patients above 35 years of age, who have had placenta previa and also a cesarean section, have 40% chance of placenta accrete.[5] In patients with placenta previa and multiple cesarean sections, the risk is 60-65%.[6] In the year 2002, American College of Obstetricians and Gynecologists estimated that incidence has increased 10-fold over the past 50 years.[7]

Placenta acctera is associated with catastrophic and life-threatening maternal and neonatal complications and it is very important to diagnosis of placenta acctera before emergency symptoms. Prenatal diagnosis is vital for timely management so that possible treatment can be conducted under more controlled conditions. In a review of 20 case records of women with morbidly adherent placenta during year 2001-2006, 85% of patients underwent hysterectomy and there were 30% maternal deaths. Furthermore, 55% of the newborns were preterm and the perinatal mortality was 33.3%.[8]

Ultrasound and Doppler are first-line methods used to diagnose placenta accreta. However, their reported sensitivity and specificity vary among studies, even when similar criteria are used.[9,10]

But, there are evidences that fetal and/or placental messenger ribonucleic acid (mRNA) can be used for diagnosis of placenta accreta and prediction of placental invasion.

Fetal and/or placental mRNA in maternal plasma has been detected during pregnancy and such mRNA tends to be stable against degradation.[11] A quantitative study of plasma mRNA suggested that the quantitative analysis of placental mRNA in maternal plasma may be a useful method to monitor placental status.[12]

In a study by Masuzaki *et al*. demonstrated that real-time quantitative reverse-transcription polymerase chain reaction (RT-PCR) is a sensitive method to monitor changing mRNA concentrations resulting from apoptotic effects in the placenta and to evaluate invading conditions of the trophoblastic villus and could be used to monitor the efficacy of methotrexate therapy for placenta percreta. In addition, placental mRNA may be useful as a predictive marker for hysterectomy in patients with placenta previa associated with placenta accreta.[13]

Doppler ultrasound has a high cost and requires an expert radiologist. However, many cases, remains without diagnosis and missing is probable because this method is based on individual skill but RNA measurement is a simple, affordable, accessible, easy and without the need for an expert individual and it can be replaced with Doppler ultrasound for diagnosis of placenta accreta.

The aim of the present study was to comparison between cell-free placental mRNA and Doppler ultrasound for the prediction of placental invasion in women with placenta accreta.

MATERIALS AND METHODS

The present cross-sectional study was conducted during the period from April 2012 to February 2013 in clinics of the Obstetrics and Gynecology Department of Isfahan University of Medical Science at Al-Zahra and Shahid Beheshti Hospitals at the city of Isfahan, Iran. In this study, 50 women referred to obstetrics and gynecology clinics were participated. Patients were enrolled sequentially by convenient sampling method. All patients had written informed consent.

Inclusion criteria were: (1) Singleton pregnancy of >28 weeks of gestation; and (2) clinical risk factors for placenta accreta, namely previous history of one or more cesarean deliveries, placenta previa, prior curettage or endouterine surgery. Exclusion criteria were pre-eclampsia, preterm labor, intrauterine growth restriction and patients taking a tocolytic agent or those with uterine bleeding at or after blood sampling, since these complications may increase the level of cell-free placental mRNA.[14]

During the perinatal care of patients, transabdominal, transvaginal and Doppler ultrasound was performed for evaluation of placental location. Color Doppler ultrasound criteria suggestive of placenta accreta were: Turbulent or diffuse blood flow through placental lacunae; vessels crossing the interface disruption site.[14]

A volume of 5 mL of peripheral blood was collected from each patient at 32 weeks of gestation and blood

samples was centrifuged at 3500 rpm for 10 min at 4°C and supernatants were separated. For RNA extraction we used TRIzol LS Reagent method.[15] Total RNA was eluted with 15 μL of RNase-free water. DNase treatment was carried out to remove any contaminating deoxyribonucleic acid (DNA) (RNase-Free DNase Set; Qiagen). Then RT-PCR was performed to access complementary DNA (cDNA) (First Strand cDNA Synthesis Kit, Fermentas).

Human placental lactogen (hPL) was selected as representative placental mRNAs and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was measured as a housekeeping gene. The hPL primer sequences were 5'-CATGACTCCCAGACCTCCTTC-3′ (sense) and 5′-TGCGGAGCAGCTCTAGATTG-3′ (antisense). The GAPDH primer sequences were 5′-GAAGGTGAAGGTCGGAGT-3′ (sense) and 5′-GAAGATGGTGATGGGATTTC-3′ (antisense). PCR assay (SYBR green, Qiagen, Germany) was performed on a step one plus Applied Biosystem to measure the mRNA concentration in maternal plasma. Calibration curves for hPL mRNA ranged from 1 × 107 to 1 × 101 copies/mL and the curve for GAPDH mRNA from 1 × 1010 to 1 × 104 copies/mL.[11] Each sample was analyzed in triplicate with thermal cycling condition: denaturation at 95°C for 15 min, annealing and extension at 60°C for 1 h. Plasma concentrations of cell-free hPL mRNA and of cell-free GAPDH mRNA were measured and compared to standard curve to measure the quantity of free mRNA in maternal plasma.[15]

Then, at 37 weeks of pregnancy, patients were undergone elective cesarean delivery. During the cesarean section placenta accreta was diagnosed if there was absence of a plane of cleavage between the placenta and myometrium, difficulty in placental Detachment from the uterus or if part of it remained attached or visible invasion of the bladder.[14] If the patients had uncontrollable bleeding hysterectomy was performed.

Placenta accreta was confirmed histologically in specimens by the absence of decidua or the presence of smooth muscle fibers in contact with placental villi. Based on the cesarean or histological diagnosis, patients were classified into two groups of with and without placenta accreta.

The data presented as mean ± standard deviation for continuous variables and number (percent) for categorical ones. To compare of the mean of mRNA levels between the two groups we used independent *t*-test and to compare of the mean of age and gestational age at sonography we used Mann-Whitney test. For determination of sensitivity and specificity and the cut-off point of mRNA levels we used the receiver operating characteristic (ROC) curve. All analyses were performed using Statistical Package for Social Sciences version 20 (SPSS Inc., Chicago, IL, USA) and $P < 0.05$ were considered to be significant.

RESULTS

A total of 50 women with a mean age of 30.24 ± 4.905 years entered the study and 12 (24%) patients were diagnosed with placenta accreta. The mean age of patients without placenta accreta was 29.84 ± 5.006 years and in patients with placenta accreta was 31.50 ± 1.311 years and the difference was not significant $P = 0.312$.

Doppler ultrasound correctly diagnosed 10 cases of 12 patients (88.3%) with placenta accreta and results for eight patients (21.1%) were incorrect diagnosed. About 30 patients (78.9%) were correctly diagnosed from the 38 patients without placenta accreta [Table 1 and 2].

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of Doppler ultrasound were 83.3%, 78.9%, 56% and 94%, respectively.

In this study, we intend to use of the mRNA levels as a prognostic factor for placenta accreta. To determine the best amount of mRNA, with the highest sensitivity and specificity, ROC curve method was

Table 1: Patient baseline characteristics and mRNA levels

Variable	With placenta accreta (*n*=12)	Without placenta accreta (*n*=38)	*P* value
Age (year)	31.50±1.311	29.84±5.006	0.312
GA at sonography (week)	30.33±1.923	30.42±1.840	0.887
Gravity (number)	3.25±1.138	3.05±1.138	0.628
Pariety (number)	1.83±1.193	1.53±0.862	0.599
History of C/S (number)	1.75±1.055	1.42±0.826	0.380
History of curtage (number)	0.58±0.669	0.53±0.797	0.576
mRNA (MoM)	6.02±1.550	2.83±0.648	<0.001

*Data are mean±SD. GA: Gestational age; C/S: Cesarean section; MoM: Multiple of the median; SD: Standard deviation; mRNA: Messenger ribonucleic acid

Table 2: Doppler ultrasound results in patients with and without placenta accreta

Variable	Doppler ultrasound results (%)		Total (%)
	Positive	Negative	
Placenta accreta			
Yes	10 (83.3)	2 (16.7)	12 (100)
No	8 (21.1)	30 (78.9)	38 (100)
Total	18 (100)	32 (100)	

used. In the method of ROC, if the curve is closer to the upper left corner of the graph, ROC analysis also will be better. In our study, the curve was closed to the upper left corner of the graph. It means the power of mRNA was acceptable as a predictor of placenta accreta Graph 1.

If the area under the ROC curve for each value is closer to 1, the ability of investigated factor for the separation of patients with and without complication is higher. Thus, if the area under the curve is between 0.5 and 0.7, indicates poor clinical accuracy, between 0.7 and 0.9 indicates moderate accuracy and if is more than 0.9 indicates a high clinical accuracy. In this study, the area under the curve was 0.94. Therefore, mRNA had high power for prediction of cases with and without complication.

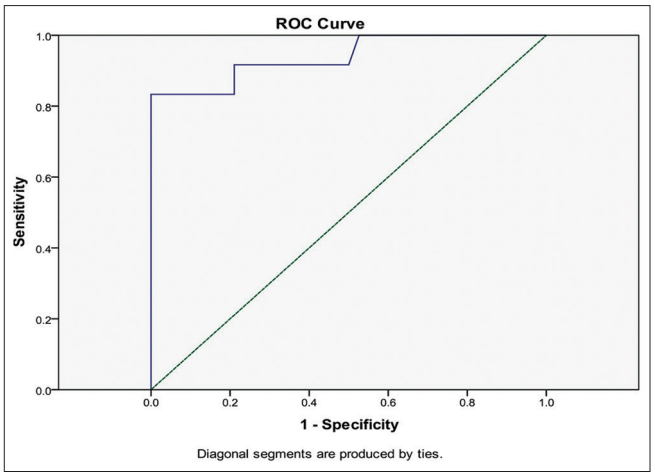
The best cut-off point must be create the best balance between sensitivity and specificity. Sensitivity and specificity values consistently act opposite to each other. For this reason, the researcher must determine what amounts of sensitivity and specificity are suitable for the cut-off point. Due to the sensitivity and specificity of the two columns in the table we could choice the best cut-off point with the highest sensitivity and specificity.

Results of our study showed if we consider a cut-off point equal to 3.325, with sensitivity and specificity of 0.917 and 0.789, respectively.

As it has been shown in Table 3, 48% of patients with mRNA < 3.325 had negative sonography, 7 (14%) patients had positive sonography and negative mRNA, 8 (16%) patients had negative sonography and positive mRNA and 11 (22%) had negative sonography and mRNA [Table 3].

From all 12 patients with placenta accreta, we found no patients with both negative mRNA and Doppler ultrasound at the same time, 1 (8.3%) patient with placenta accreta had negative mRNA and positive Doppler ultrasound, 2 (16.7%) patients with placenta accreta had negative Doppler ultrasound and positive mRNA and 9 (75%) patients with placenta accreta had positive Doppler ultrasound and mRNA [Table 4].

As it has shown in Table 5, if mRNA cut-off point is considered 3.325, 11 patients (91.7%) from 12 patients with placenta accreta were diagnosed correctly. Also among the 38 healthy patients, 30 patients (78.9%) were diagnosed correctly as normal, but 8 patients (21.1%) were diagnosed incorrectly [Table 5].



Graph 1: Receiver operating characteristic curve of sensitivity and specificity of messenger ribonucleic acid

Table 3: Doppler and mRNA results of all patients (with and without placenta acctera) in the study

Variable	Results of doppler ultrasound (%)		Total (%)
	Negative	Positive	
Results of mRNA test			
Negative 3.325>	24 (48)	7 (14)	31 (62)
Positive 3.325<	8 (16)	11 (22)	19 (38)
Total	32 (64)	18 (36)	50 (100)

mRNA: Messenger ribonucleic acid

Table 4: Doppler and mRNA results of the patients with placenta acctera

Variable	Results of doppler ultrasound (%)		Total (%)
	Negative	Positive	
Results of mRNA test			
Negative 3.325>	0	1 (8.3)	1 (8.3)
Positive 3.325<	2 (16.7)	9 (75.0)	11 (91.7)
Total	2 (16.7)	10 (83.3)	12 (100)

mRNA: Messenger ribonucleic acid

Table 5: Predictive value of mRNA in patients with placenta acctera

Variable	Patients with placenta accreta (%)		Total
	Negative	Positive	
Results of mRNA test			
Negative 3.325>	0	1 (8.3)	1 (8.3)
Positive 3.325<	2 (16.7)	9 (75.0)	11 (91.7)
Total	2 (16.7)	10 (83.3)	12 (100)

mRNA: Messenger ribonucleic acid

Results of our study showed the sensitivity, specificity, PPV and NPV of mRNA with were cut-off point of 3.325 were 91.7%, 78.9%, 57.9% and 96.8%, respectively.

DISCUSSION

In contrast to popular belief that the placenta forms an impermeable barrier between mother and child, there is bidirectional traffic between the fetus and the mother during pregnancy.[16] Multiple studies have shown that both intact fetal cells and cell-free fetal nucleic acids cross the placenta and circulate in the maternal bloodstream.[17]

Cell-free fetal nucleic acids can be detected in the maternal circulation during pregnancy, potentially offering an excellent method for early non-invasive prenatal diagnosis.[18] Wright, Chitty have mentioned that cell-free fetal RNA in maternal blood as safer, acceptable and functional antenatal testing.[19]

In the present study, we compared the sensitivity, specificity, PPV and NPV of cell-free placental mRNA with Doppler ultrasound to diagnosis of placenta accreta.

In our study, we found that the sensitivity of Doppler ultrasound was 83.3% and specificity was 78.9% for diagnosis of placenta accreta. In our study, PPV and NPV were 56% and 94%, respectively.

In studies with more than 30 subjects, the sensitivity and specificity of ultrasound for detection of placenta accreta were 77-90% and 71-98%, respectively.[20]

A study by Yang *et al*. investigated the value of transvaginal sonographic findings of intraplacental lacunae for predicting adherent placenta and clinical outcome in patients with placenta previa totalis and a history of cesarean section. They found the sensitivity, specificity, PPV and NPV of diagnosing adherent placenta were 86.9%, 78.6%, 76.9% and 88.0%, respectively. They also found the sensitivity, specificity; PPV and NPV of diagnosing placenta increta or percreta were 100%, 97.2%, 93.8% and 100%, respectively.[21]

The Shih *et al*. study evaluated color Doppler ultrasound of 170 women with placenta previa, of whom 39 had previa-accreta confirmed at cesarean delivery. The sensitivity and specificity of color Doppler for diagnosis of placenta accreta was 92% and 69%, respectively.[22]

In study by Chou *et al*. the sensitivity of color Doppler imaging in the diagnosis of placenta previa-accreta was 82.4% (14/17) and the specificity was 96.8% (61/63). The PPVs and NPVs were 87.5% (14/16) and 95.3% (61/64), respectively.[23] Another study showed the sensitivity of grayscale sonography was 33% and Doppler was 100% with PPV of 78%.[24]

In our study, we found that the sensitivity of Doppler ultrasound was 83.3% and specificity was 78.9% for diagnosis of placenta accreta. In our study PPV and NPV were 56% and 94% respectively.

Results of our study also showed that the Sensitivity, specificity, PPV and NPV for mRNA test were 91.7%, 78.9%, 57.9% and 96.8%, respectively. As can be seen, the sensitivity, PPV and NPV of cell-free mRNA is greater than the Doppler ultrasound. Specificity of Doppler ultrasound and mRNA are equal in our study.

The median multiples of the median (MoM) value of cell-free placenta mRNA in the present study was significantly higher in women with confirmed placenta accreta compared with those confirmed without placenta accreta. Moreover, the levels of cell-free placental mRNA were significantly higher in patients with placenta increta or percreta compared with those who had simple accreta diagnosed at cesarean delivery. This is consistent with a study of Miura *et al*.[25] and Masuzaki *et al*.[13] that detected higher levels of cell-free placenta mRNA in women with placenta accreta who underwent hysterectomy.

Placental mRNA has been detected in maternal plasma during pregnancy and was stable against degradation.[26] Significantly higher concentrations of plasma mRNA found in pregnant compared with non-pregnant women suggested that placental mRNA in maternal plasma could be a valuable method to monitor placental status.[27]

Fetal hematopoietic cells and placenta can contribute to the pool of cell-free fetal/placental mRNA detected in maternal circulation.[16]

In a study by Miura *et al*. 28 singleton pregnant women with placenta previa were classified into the following four groups: 16 women with placenta located at a posterior part of the uterine wall (Group A); 4 each with placenta located at the anterior wall without (Group B) or with (Group C) previous cesarean section; and the other 4 with a history of previous cesarean section and who had the placenta located at an anterior part of uterine wall and underwent a cesarean hysterectomy (Group D). The MoM (range) values of cell-free PL mRNA in the control group and Groups A to D were 1.00 (0.39-2.35), 2.04 (0.91-3.93), 2.58 (1.90-3.90), 3.50 (1.20-4.30) and 6.28 (5.24-7.63), respectively. The concentration of cell-free mRNA was significantly higher in Group D than in Groups A, B, or C (Mann-Whitney's U-test, $P < 0.05$).[25]

In a study by El Behery *et al.* (2010), level of cell-free placenta mRNA in patients with placenta accreta was

significantly higher than without placenta accreta. In their research, the median MoM values of cell-free placental mRNA in the women diagnosed with and without placenta accreta were 6.50 (range: 5.40-7.75) and 2.60 (range: 2-3.95) respectively ($P < 0.001$).[14] In present study, we found similar results to the study of El Behery *et al*.

In the present study, the median MoM values of cell-free placental mRNA in the women diagnosed with and without placenta accreta were 6.02 ± 1.550 and 2.83 ± 0.648 with a significant difference between two groups ($P < 0.001$).

The increased level of cell-free placental mRNA in the maternal plasma of patients with placenta accreta is thought to result from the presence of thin deciduas at the lower uterine segment and the possibility of direct uteroplacental transfer of cell-free placental mRNA molecules resulting from a connection between the placenta and maternal circulation.[14]

Previous studies have shown that cell-free mRNA test can be used for prediction of other dangerous gynecologic conditions. Reddy *et al*. (2008) investigated the effect of labor and placental separation on the shedding of cell-free mRNA in normal pregnancy and pre-eclampsia. The results of study showed that levels of placental cell-free mRNA were significantly increased in labor in both normal pregnancy and pre-eclampsia and were still high 24 h after delivery in the pre-eclamptic women.[28]

Results of other studies showed that cell-free placental mRNA levels used in combination with ultrasound and color Doppler might increase the diagnostic accuracy to predict placental invasion in pregnant women with suspected placenta accreta.

CONCLUSION

Cell-free mRNA is an acceptable, easy made, functional test with sensitivity, specificity, PPV and NPV more than Doppler ultrasound for diagnosis and prediction of incidence of placenta accreta. In contrast, Doppler ultrasound is operator dependent and there is the possibility of missing of patients. With attention to the catastrophic complications of placenta accreta this reason is very important. Thus, according to the results of our study and other similar studies, it is recommended the use of cell-free mRNA test for diagnosis of placenta accreta.

The study was limited by its small sample size, making it difficult to draw a definite conclusion. Further large scale studies are necessary to verify its usefulness as a predictor of partial placenta accreta with the possibility of conservative treatment or of placental invasion with the possibility of radical surgery without hemorrhage.

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