BASIC SCIENCE: OBSTETRICS

Quantification of cell free fetal DNA in maternal plasma in normal pregnancies and in pregnancies with placental dysfunction

Medhat S. Alberry, MRCOG; Deborah G. Maddocks, PhD; Medhat A. Hadi, PhD; Helmi Metawi, MD; Linda P. Hunt, PhD; Sherif A. Abdel-Fattah, MD; Neil D. Avent, PhD; Peter W. Soothill, PhD

OBJECTIVE: To assess the normal levels of free fetal DNA in maternal plasma through pregnancy compared with those in pregnancies complicated with placental dysfunction manifested by preeclampsia and/or fetal growth restriction.

STUDY DESIGN: Maternal blood samples from 138 singleton male pregnancies were divided into 3 groups; normal pregnancies (77), preeclampsia (49), and fetal growth restriction (12). Royston and Wright's methods were used to calculate gestational age-related reference limits of free fetal DNA in the 3 groups. The DYS14 gene of the Y chromosome was quantified and compared in maternal plasma by using real-time quantitative polymerase chain reaction.

RESULTS: Free fetal DNA in normal pregnancies increased with gestational age. Results were significantly higher in preeclampsia and fetal growth restriction groups than in normal pregnancy and were higher in severe preeclampsia than in milder disease.

CONCLUSION: Free fetal DNA is a potential marker for placental dysfunction in pregnancy. Large prospective studies are now needed to investigate its role in the prediction of pregnancy complications and severity and or timing of delivery.

Key words: fetal DNA, fetal growth restriction, normal pregnancy, preeclampsia

Cite this article as: Alberry MS, Maddocks DG, Hadi MA, et al. Quantification of cell free fetal DNA in maternal plasma in normal pregnancies and pregnancies with placental dysfunction. Am J Obstet Gynecol 2009;200:98.e1-98.e6.

The discovery of cell free fetal DNA (ffDNA) circulating in maternal plasma has and continues to revolutionize the field of noninvasive prenatal diagnosis both in research and clinical care.[1] The use of this technology in the diagnosis of fetal gender and RhD genotype was integrated into the National Health Service (NHS) in the United Kingdom less than 10 years after its discovery.[2] Its use has also been described in the diagnosis of single-gene defects, including cystic fibrosis, beta thalassemia, and myotonic dystrophy; however, the reliability of these tests within a clinical service and the cost-effectiveness of this approach remain to be validated.[3-5]

There is a lot of evidence that the molecular ffDNA investigations can be used in different physiologic and pathologic conditions during pregnancy. The accuracy of the normal range is therefore important. Previous studies have suggested that pregnancies with conditions associated with placental dysfunction (preeclampsia [PE] and fetal growth restriction [FGR]) have higher concentrations of ffDNA than normal.[6,7] This has also been observed in other conditions, including trisomy 21, preterm labor, hyperemesis gravidarum, and fetomaternal hemorrhage.[8-10] Although early work on ffDNA used conventional polymerase chain reaction (PCR), the introduction of real-time quantitative PCR enabled the detection and quantification of ffDNA with higher sensitivity and specificity. Also, the initial work used a Y chromosome gene called SRY, which has only 1 copy per Y chromosome, and approaches using the DYS14 gene would be expected to have a higher sensitivity and accuracy because there are 10 copies on each Y chromosome.[11,12]

PE is a common and serious complication affecting 3-5% of pregnancies occurring in the second half of pregnancy and can result in maternal and fetal mortality or morbidity. It is diagnosed by high blood pressure and proteinuria with or without edema and/or elevated liver and renal function blood tests or low platelet counts. The pathogenesis of PE is only incompletely understood, but it is generally accepted that the earliest pathologic change is in the uteroplacen-

From the Departments of Fetal Medicine (Mr Alberry and Dr Abdel-Fattah) and Obstetrics and Gynecology (Prof Soothill), University of Bristol, St Michael's Hospital; the Centre for Research in Biomedicine (Dr Maddocks), Faculty of Applied Sciences, University of the West of England; the Department of Clinical Sciences (Dr Hunt), Institute of Child Life and Health, University of Bristol at South Bristol; and the Centre for Research in Biomedicine (Prof Avent), Faculty of Applied Sciences, University of the West of England, Bristol, UK, and the Department of Obstetrics and Gynecology (Mr Alberry and Drs Hadi and Metawi), Ain-Shams University Maternity Hospital, Cairo, Egypt.

Received Jan. 24, 2008; revised May 13, 2008; accepted July 28, 2008.

Reprints not available from the authors.

This study was funded by the European SAFE Network of Excellence.

0002-9378/$36.00 • © 2009 Mosby, Inc. All rights reserved. • doi: 10.1016/j.ajog.2008.07.063

TABLE 1
The patient characteristics of cases and controls enrolled in the study

	Controls	Preeclampsia	FGR
Number	77	49	12
Age mean (range, y)	32.3 (18-42)	35.8 (18-43)	29.6 (20-40)
Parity median (IQR)	2 (3-1)	2 (2.5-0)	1 (2.5-0)
Gestational age median (IQR, wk)	35 (37.6-24)	34 (36-31)	31 (33.7-28.2)
Prepregnancy weight mean (range, kgs)	70.1 (53-98)	70.6 (49-101)	72.3 (52.5-96)
Prepregnancy height mean (range, cms)	167.7 (153-182)	161.7 (150-178)	166 (159-173)

FGR, fetal growth restriction; *IQR*, interquartile range.

Alberry. Quantification of cell ffDNA in normal pregnancies and pregnancies complicated with placental dysfunction. Am J Obstet Gynecol 2009.

tal circulation.[13,14] FGR is a common cause of perinatal mortality and morbidity. A growth-restricted fetus is usually defined as birthweight or ultrasound measurements smaller than the 10th percentile for gestational age. The prognosis and management is determined by the cause, and the use of Doppler is vital to indicate the cause of the growth restriction, because many small fetuses are simply normally small. Repeated ultrasound and Doppler assessment of placental and fetal circulation are essential in the diagnosis and management of FGR fetuses secondary to placental dysfunction.[15] Both PE and FGR are associated with placental vascular dysfunction that leads to ischemia and apoptosis of trophoblastic cells, which may increase the release of ffDNA in maternal plasma.[16] Furthermore, recent work has been aimed at evaluating ffDNA as a predictor of PE and as a marker for the severity of the disease. This showed that ffDNA levels were elevated before the onset of the clinical PE and may show a relationship to the severity of the disease.[17,18]

Our aim was to use the DYS14 target to establish a reference range for normal pregnancies and to confirm elevated levels in PE and pregnancies with FGR (identified to be caused by placental dysfunction by the use of Doppler).

Materials and Methods

Pregnant women attending the St Michael's Hospital, Bristol, UK, or the Obstetrics and Gynecology Department, Ain-Shams University Maternity Hospital, Cairo, Egypt, were recruited for the study. The patients were approached in the Fetal Medicine Unit, high-risk antenatal clinic, in the Day Assessment Unit and in the Antenatal ward "admitted patients." They were recruited after written informed consent. Women with singleton male pregnancies (n = 138) were recruited (over a period of 18 months in 2006-2007) as being normal pregnancies (controls n = 77), PE (n = 49), or FGR (n = 12). The median (upper quartile-lower quartile) of the gestational age (in weeks) in the 3 groups was 35 (37.6-24) for the controls, 34 (36-31) for the PE group, and 31 (33.7-28.2) for the FGR group (Table 1).

Recruitment took place when the investigator was present in the Fetal Medicine Unit. However, the investigator was called to any other department or clinic whenever patients with PE or FGR were present. The criteria of diagnosis were checked by the investigator before recruitment. All recruited patients consented, as they had already received the diagnosis of clinical disease.

PE was defined as a sustained increase in diastolic blood pressure ≥ 90 mm Hg, with persistent proteinuria (more than a trace using dipstick urine analysis) in the absence of urinary tract infection. A 24-hours assay of proteinuria was performed to assess PE severity. Edema was not used as a diagnostic criterion because it is not a constant feature of PE. Severe PE was defined as a blood pressure of ≥ 170 systolic or ≥ 110 diastolic on 2 occasions in addition to significant proteinuria (+3 proteinuria or ≥1 g/L) or a systolic blood pressure ≥ 150 or diastolic blood pressure ≥ 100 mm Hg on 2 occasions with + 2 proteinuria AND at least 2 signs or symptoms of imminent eclampsia, such as headache, epigastric pain, blurred vision, or deranged blood and biochemical tests.[13,19]

FGR was diagnosed when the fetal abdominal circumference (AC) and/or estimated fetal weight were below the 10th percentile for gestational age,[20] with a decrease or arrest of growth on repeated scans AND abnormally high pulsatility index (PI) in the umbilical artery Doppler (above 95th percentile for gestational age).[21]

One 10-mL blood sample was collected per pregnancy into ethylenediaminetetraacetic acid collecting tubes. Each blood sample was collected at 1 point in the pregnancy in all groups (when PE or FGR were diagnosed in the case groups). The samples were centrifuged at 1600 *g* for 10 minutes, the supernatant plasma was separated and then recentrifuged at 16,000 *g* for 10 minutes and the resulting plasma was aliquoted into 1.1 mL volumes in 2-mL plastic tubes for DNA extraction. The plasma aliquots were stored at -80°C. Thirteen of the UK samples (1 FGR, 3 PE, and 9 controls) had to be stored in a -20°C freezer for 3 days because of a temporary breakdown of the -80°C freezer, but they did not become unfrozen. These samples were marked to be distinguished when we interpreted the results. Apart from the above, the same sampling and storage protocols were followed in Egypt and the samples were shipped to Bristol on dry ice and arrived frozen after 48 hours.