European Journal of Obstetrics & Gynecology and Reproductive Biology 146 (2009) 165–168

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

Assessment of the female fetal DNA concentration in the plasma of the pregnant women as preeclampsia indicator—Preliminary report

Engel Karina [a], Płonka Tomasz [a], Marek Bilar [a,*], Orzińska Agnieszka [b], Brojer Ewa [b], Ronin-Walkowska Elżbieta [a]

[a] Department of Feto-Maternal Medicine, Pomeranian Medical University, 1 Unii Lubelskiej Street, 71-252 Szczecin, Poland
[b] Department of Immunohematology and Immunology of Transfusion Medicine, Institute of Hematology and Transfusion Medicine, 14 Gandhi Street, 02-776 Warsaw, Poland

ARTICLE INFO

Article history:
Received 22 January 2009
Received in revised form 26 April 2009
Accepted 15 June 2009

Keywords:
Fetal DNA
Preeclampsia
Female fetuses

ABSTRACT

Objectives: This research was designed to analyze the presence of fetal female DNA, expressed in copy number, in the plasma of the pregnant woman with preeclampsia-complicated pregnancy.
Study design: Twenty-four pregnant women with female fetuses identified by means of ultrasound scanning were enrolled in this pilot study. The study group consisted of 12 pregnant women with symptoms of preeclampsia, with 12 healthy women, matched for gestational age, as controls.
Results: Mean DNA number of genomic equivalents per reaction in the group was 201 geq/PCR (from 44.9 to 375) and increased over time after onset of PE, which was the reason for pregnancy termination. In the group of women with preeclampsia, a notably higher DNA copy number in comparison to the control group was noted ($p = 0.0003$ *U* Mann–Whitney test).
Conclusions: The pilot study presented in this work confirms that also in the case of preeclampsia-complicated pregnancy with female fetuses it is possible to implement the method of fetal DNA quantification. Use of the presented methods confirms that in severe preeclampsia-complicated pregnancies an increase of the number of DNA genomic equivalents per reaction in comparison to the control group is observed. Due to the small study group further research on the described issue is vital, but this study proves that it is also feasible among women carrying female fetuses.

1. Introduction

Traditional methods of fetal DNA testing for the prenatal diagnostics include chorion biopsy, and amnio- and cordocentesis; however, these invasive methods pose a risk of complications in the range of 0.5–2% [1]. Current diagnostics allows also for the non-invasive molecular analyses of the fetal-derived alleles on total extracellular DNA derived from the extracellular fraction in the peripheral blood of the pregnant women [2].

Presence of the fetal DNA in the maternal circulation results in the constant active remodeling of the placenta, with ongoing constant apoptosis and replacement of the placental cells at the maternal–fetal interface and transplacental leakage of the fetal cells (trophoblast, lymphocytes, granulocytes, nucleated red blood cells and stem cells). Fetal DNA becomes detectable from the fifth month of the pregnancy [3]. Between the 11th and 17th week of pregnancy the fetal DNA in the serum accounts for the 3.4% of total maternal DNA, gradually rising to reach the level of approximately 6.2% between the 37th and 40th week of pregnancy. Fetal DNA quantified using SRY gene as a marker in the plasma of the healthy pregnant women varies from 25.5 genome equivalents in the first trimester of pregnancy to 292 genome equivalents in the third [4]. It has been indicated that the concentration of the fetal DNA, assessed basing on the SRY analyses, reaches maximum levels in the 33rd to 36th week of pregnancy [5]. After birth, its concentrations decrease rapidly, as a result of the increased renal filtration [6].

An anomalous course of the pregnancy due to the malfunction and injury of the maternal–fetal interface (aneuploid pregnancy, 21 trisomy, preterm labor, hyperemesis gravidarum) results in the increased release of the fetal cells and free DNA from the apoptotic cells in the placenta directly to the pregnant women's circulation [3,5,7–9]. Both qualitative and quantitative measurements of the fetal DNA might be used as a dynamic marker of the feto-maternal well-being. Analyses of the extracellular presence of the fetal DNA in the plasma of the pregnant women by implementation of the real-time PCR technique is also helpful during early pregnancy for detection of Rh antigens among Rh negative women [10–12]. It is also possible to detect an abnormal number and structure of fetal chromosomes. Such a diagnostics allows the identification of chromosomal trisomies (e.g., [13,18,21]), and both recessive (cystic fibrosis, congenital adrenal hyperplasia) and dominant

* Corresponding author. Tel.: +48 91 425 3292; fax: +48 91 425 33 09.
E-mail address: marcobil@mp.pl (M. Bilar).

0301-2115/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.ejogrb.2009.06.016

(myotonic dystrophy, achodroplasia, Huntington's disease) autosomal diseases [13,14]. Real-time PCR method might not only be used solely for detection of the fetal DNA but also, with appropriate standards, quantitative measurements are possible [15]. Such an application allows for identification and prediction of preeclampsia symptoms, several weeks prior to the symptomatic disease [16]. Due to low invasiveness of the analysis, assessment of the fetal DNA from the mother's peripheral blood might become an important element of the non-invasive prenatal diagnostics for an array of genetic diseases.

Real-time quantitative polymerase chain reaction is method sensitive enough to detect DNA equivalent of a single target cell.

Most of the fetal DNA analyses of the blood of the pregnant women is based on identification of the Y chromosome as the marker for the male fetus development, usually absent in the female body, allowing for identification of male-sex linked genetic diseases in the fetus.

Early, non-invasive, reliable and inexpensive investigation seems important from the point of view of the clinical practice, with the chief limitation being related to the possibility of application for male fetuses only. Such an identification is possible by relatively uncomplicated diagnostically detection of Y chromosome genes, e.g., SRY in the blood of the pregnant women.

Both in our own studies and in research published by other authors the association between the fetal DNA concentration in the maternal plasma and clinical symptoms of preeclampsia were noted [16–19]. Due to methodological difficulties the research in this field was largely focused on pregnant women with symptoms of preeclampsia and male fetuses. Publications by Brojer et al. [20], used for diagnostics of the serological conflict and analysis of chimerism after the bone marrow transplant method by Alizadeh et al. [21] with analysis of null and insertion–deletion polymorphisms, allowed the authors of this work to perform the pilot study with analysis of the amount of the fetal DNA in pregnant women carrying female fetuses with the pregnancy complicated with symptomatic preeclampsia.

2. Aim of the work

This research was designed to analyze the presence of fetal female DNA, expressed in the number of genomic equivalents (geq), in the plasma of the pregnant woman with preeclampsia-complicated pregnancy.

3. Materials and methods

The study was granted approval by the local ethical committee. Each participant consented to take part in the study.

From February 2003 to November 2007, 24 pregnant women with female fetuses identified by means of the ultrasound scanning were enrolled in this pilot study. The study group consisted of 12 pregnant women with symptoms of preeclampsia and 12 healthy women, matched for gestational age.

Mean age for the pregnant women enrolled for this study was 28.6 years (minimum 22 years, maximum 35 years). Mean time of pregnancy termination was 34.1 week (minimum 5 weeks, maximum 40 weeks). Mean birth weight was 1655 g (minimum 490 g, maximum 3400 g). In four cases, intrauterine growth retardation (IUGR) was observed. Inappropriate blood flow in the umbilical artery recorded by the Doppler ultrasound examination (AEDF) was observed in three cases. "Notch" sign in the uterine artery was noted in seven pregnant women of the study group.

All women included in the study group presented with severe form of preeclampsia (PE), according to the ACOG criteria. It was diagnosed with onset of arterial hypertension with concomitant proteinuria after the 20th week of pregnancy. Severe form of PE is identified with systolic arterial blood pressure of >160 mmHg, diastolic of 110 mmHg, with proteinuria of (+2/+3). Fetal hyportrophy (intrauterinal growth retardation—IUGR) was defined as values <2 SD from the appropriate gestational age in the ultrasound examination. For this purpose, the scanning device Voluson E8 was used, with examination being performed directly before blood sample collection and termination of pregnancy.

No chromosomal aneuploidy was identified among neonates from both the study and healthy groups.

Venous blood was collected from each participant between the 22nd and 40th week of pregnancy, during routine obstetric follow-up, with approximately 10 ml blood from the cubital vein taken to vacuum tubes with gel barrier containing EDTA (Vacutainer-Becton Dickinson, Pharmouth, UK). Blood was centrifuged $3000 \times g$ (Centrifuge 5416-Eppendorf) not later than 1 h after collection. Plasma was carefully transferred to the fresh tubes and frozen at −80 °C. Before DNA isolation, plasma was centrifuged $16{,}000 \times g$. From the fathers, 5 ml of blood was collected in the manner described above, into EDTA containing tubes.

DNA from the whole blood of the pregnant women and the father of her child was extracted manually (QiaAmp Blood Mini Kit, Qiagen, Hilden, Germany) and plasma DNA of the pregnant women was isolated automatically (Nuclisens easyMag, BioMerieux, Boxtel, ND).

Quantitative real-time PCRs (real-time PCRs) were carried out in 25 μl volume in monoplex assays containing: 12.5 μl PCR buffer (Applied Biosystems, USA), 200 nM appropriate fluorescent probes (Applied Biosystems, USA), and 300 nM appropriate primers using ABI Prism 7700 (Applied Biosystems, USA). Real-time PCR conditions of quantification of 12 polymorphisms useful for that study were optimized.

One of 30 polymorphisms, described in other studies, was used as fetal marker: 6 null alleles (S03, S06, S15b, GSTM1, GSTT1, RHD) and 10 insertion/deletion polymorphisms (ACE, S01, S04, S05, S07, S08, S09, S10, S11, S14, S18 RHC/c) [4,12,20,21]. Firstly, genomic DNA from parental blood was tested to identify a specific marker present only in the father and absent in the mother. Once the fetal marker was identified, the real-time PCR specific for that polymorphism was performed in four replicates using DNA extracted from maternal plasma.

Standard amplification curves of each real-time PCR were plotted for all specific polymorphisms found. Real-time PCRs with an artificial mixture of DNA were prepared by serial dilution of appropriate positive DNA (at concentrations of 2500, 500, 100, 20, and 4 geq per reaction) in negative DNA at a constant concentration of 12,500 geq per reaction. The concentration and purity of each DNA sample were measured spectrophotometrically at 260 and 280 nm. Ct value for 1 geq/PCR was between 37.63 and 40.515 (Table 2). The slope of the regression curve varied from −3.221 to −3.530 (except S11b—3.766), and the correlation coefficient R^2 from 0.984 to 0.999 (Table 2). Fetal DNA concentrations were estimated from the standard curve for a paternally inherited polymorphism. The slopes of the regression curves of the polymorphism used for analysis of the concentration of fetal DNA in women with preeclampsia and the matched control were similar.

4. Results

As shown in Table 1, mean time of PE onset was 32.2 of pregnancy and ranged from the 22nd to 40th week of pregnancy. Mean age of the pregnant women was 28.6, while mean uric acid serum concentration was 5.5 mg/dl. Additionally, in three women in the analyzed group, except for the preeclampsia symptoms, intrauterinal growth retardation (IUGR) was observed. Doppler