FETAL SEX DETERMINATION USING MATERNAL PERIPHERAL BLOOD CIRCULATION IN THE EARLY WEEKS OF PREGNANCY

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Abstract- The usage of new methods in biology has revolutionized scientific research like PCR that amplifies specific segments of DNA and agarose gel electrophoresis that estimating the sizes of the PCR products. The non-invasive determination of fetal sex, that include extraction of cell-free fetal DNA in maternal circulation and amplification of X and Y chromosomes genes that lets us to determine fetus gender and study some fetal genetic traits and disorders like X-linked genetic disorders during 5th to 10th weeks of pregnancy. We have studied cell-free fetal DNA existence in maternal circulation, it appears the release of cell free fetal DNA in maternal blood through placenta. Ultrasound examination is one of non-invasive reliable fetus sex determination but it works at second trimester of gestation and other invasive methods threaten the health of the mother and her baby.

Keywords- PCR; Fetus; Sex determination; Peripheral blood

I. INTRODUCTION

Presence of fetal nucleic acid in maternal blood has offered a new approach to non-invasive fetal sex determination that avoids the risks of conventional invasive techniques, such as chorionic villus and amniocentesis sampling [1,2]. The availability of a noninvasive fetal sex determination would reduce unintentional fetal losses and would likely be welcomed by pregnant women carrying fetuses at risk for disorders [3]. Ultrasound examination is a method for Fetal sex determination in 11 weeks’ gestation, but fetal sex cannot be determined by ultrasound examination in 7.5% to 50% of pregnancies at 11 weeks’ gestation, and this decreases to 3% to 24% at 13 weeks [3].

A subsequent prospective study shown that fetal sex could be determined by ultrasound 90% of the period, with 86% accuracy from 11 to 14 weeks’ gestation [3]. Fetal DNA can be used as a reliable tool for fetal Rh status, fetal sex determination and fetus with and X-linked genetic disease and Sex chromosome aneuploidy include Turner syndrome (45,X and variants), Kleinfelter syndrome (47,XY and variants), and 47,XXX and 47,YYY genotypes [4,5]. Existence of nucleated fetal cells in maternal blood is a recognized phenomenon that after a one high number of cycle PCR, fetal DNA will apparent in 80% of the maternal blood samples and reputable detection of fetal DNA may be potentially useful for the noninvasive fetal sex determination and diagnosing genetic disorders [6]. The existence of cell-free circulating Y chromosome DNA sequences in the blood of pregnant women was first reported in 1997. Since that description, many groups spearheaded all over the world have validated the initial finding that Y chromosome sequences can be amplified and used to identify male fetuses [3]. Using PCR tests on Y linked genes, such as SRY, several studies have shown high detection sensitivity rates (above 95%) also in the first trimester of pregnancy, with specificity approaching 100% [7]. For increasing the amplification of quantitative measurements of the low copy number of fetal DNA in the first trimester of pregnancy, there are two different type methods. The first with conventional PCR techniques, achieving 95% sensitivity rates already in the first trimester and the second with real-time PCR, represent a 10-fold lower detection threshold compared to the single-copy sequences used in the previous.

II. MATERIALS AND METHODS

The peripheral blood specimens were obtained from 55 pregnant women at 5 and 10 weeks of pregnancy of Shahrekord, Iran for this cross-sectional study. The samples were transported to the laboratory in sterile plastic falcon tubes (15 ml) under refrigerated conditions and stored frozen at -20°C until analysis. The whole blood DNA was extracted from 15 ml specimens using a DNA Extraction Kit (QIAGEN Hilden, Germany) according to the manufacturer’s procedure. The yield of DNA was quantified after electrophoresis in 1% agarose gel containing 0.5 µg/ml of ethidium bromide.

The primers of PCR reaction were as follow, F: 5'-ATAAGTATCGACCTCGGAAG-3', R: 5'-GCACTTCGCTGCAGATACCAGAAG-3'. The primers sequence was obtained from McKeown et al, 1999 and the size of its amplicon is 93bp [8]. PCR was carried out in 25 µl total reaction volumes, each containing 2.5 µl of 10X PCR buffer, 1.5 mM MgCl₂.
100 ng of template DNA, 0.2 µM of each primer, 0.2 µl dNTPs, and 1 unit of Taq DNA polymerase (Fermentas, Germany). The amplification reaction consisted of 5 min of predenaturing at 95°C, followed by 30 cycles of 1 min denaturation at 94°C, 1 min annealing at 62°C and 1 min extension 72°C, and final extension at 72°C for 5 min. The samples were amplified in a Gradient Palm Cycler (Corbett Research, Australia). The PCR amplification products was separated by electrophoresis in 1.5% agarose gel at 100 V for 30 min in Tris-borate-EDTA (TBE) buffer, visualized by ethidium bromide staining, illuminated by UV transilluminator and images were obtained in UVIdoc gel documentation systems (UK). A 100bp DNA ladder (Fermentas) was used as a size reference for PCR assay.

III. RESULTS

After sampling of 55 pregnant women at 5 and 10 weeks of pregnancy and DNA extraction we amplified extracted fetus DNA sequence and Analysis of PCR products for the determination of the gender of fetus on 1.5% agarose gel revealed 93bp fragment on agarose gel shows that pregnant women carrying a male fetus and if it doesn’t have any amplified male DNA mother is carrying a female fetus.

![Figure 1. Gel electrophoresis of sex determination PCR (Lane 1 shows fermentas 100bp DNA molecular marker, Lanes 2 and 3 are negative (women DNA) and positive (men DNA) controls, respectively, Lanes 4 and 7 are positive samples for male gender of fetus, and Lanes 5 and 6 are negative PCR samples for female gender of fetus).](image)

IV. DISCUSSION

We have developed finding of free fetal DNA in the maternal blood circulation at each gestational week of the first trimester of pregnancy [6, 7]. And the most important observation in this discussion is the very high concentration of fetal DNA in maternal blood [6]. Our data explain that fetal DNA can be finding in as little as 10 µL maternal serum and plasma. The detection rate is more than that for DNA from nucleated blood cells extracted from the same volume of whole blood [2]. We have confirmed that the absolute concentration of fetal DNA in maternal plasma is as good as in maternal serum [6]. The high concentration of fetal DNA in maternal blood has permitted us to detect reliably the presence of fetal DNA [6]. The precise cellular origin of cell-free fetal DNA in maternal blood is currently unknown [9]. The first real maternal blood circulation of the placenta occurs later with deeper invasion of the spiral arteries erosion and endometrium [4]. A typical pregnancies associated with placental damage cause liberate of increased fetal cells and release of free DNA directly from dying cells in the placenta to the maternal blood aneuploid pregnancy (trisomy 21), toxemia of pregnancy, preterm labor, hyperemesis gravidarum etc. A few weeks before the onset of preeclampsia increased fetal erythroblastic trafficking, increased fetal DNA concentration, even increased maternal DNA itself have been observed [10]. False-negatives and false-positives were not generated at all trimesters of pregnancy among pregnant women’s [1]. Use of maternal blood for the detection of fetal DNA for non-invasive prenatal diagnosis may be possible [2]. Nucleic acid amplification test like PCR enable the finding of low concentrations of organism or rare DNA in specimens [11]. Then we were able to find fetal SRY sequences before the 7th week of gestation, thus indicating that fetal genetic study in maternal blood could be used in the first trimester [6]. Lo et al, in 1997, were the first researcher that showed PCR with SRY gene primers on blood from women pregnant with male fetuses [9]. Our results demonstrate that fetal DNA is present in maternal this could supposedly reduce the number of invasive actions required for diagnosis of such diseases since prenatal diagnosis might thus be performed for male fetus’s only, significantly avoiding invasive actions for female fetuses [1]. The assumed sources of fetal DNA in maternal blood are fetal cells, trophoblast and/or via the passive diffusion of DNA [4]. The standard number of fetal cells in maternal circulation in normal pregnancies was 1.2 cells/ml [6]. The discovery of fetal DNA in maternal blood has disclosed new and exciting possibilities for the noninvasive prenatal determination of fetal blood group status [12]. Techniques have focused on sex determination and paternally inherited alleles for fetus autosomal dominant disorders. Although, positive identification of paternal inherited normal allele would not directly help in diagnosing of autosomal recessive disease [10]. The SRY sequence was never find in women, detected in all pregnant women with male fetus and did not show any existence of SRY when the previous child had been male but the present fetus was female [10]. Our method provides a definitive way to identify sex chromosome imbalances when both Y and X chromosomes are present [5]. Fetal Rh D status
has been anticipated with 99.5% accuracy [10]. Intrauterine finding could also lead to a sooner and optimized treatment of fetal in this group and of other illnesses like CAH. In addition one problem in introducing a technology that detects fetal sex as early as 6-8 weeks of gestation is the feasibility of abortion based on sex selection for social purposes [13]. A patient who carries a fetus at risk of congenital adrenal hyperplasia could benefit from this early determination as suggested by Rijnders et al [4]. We did not find any false-positive results from women who had formerly carried a male fetus but were carrying a female fetus at the time of blood sampling in this study [6]. We found prenatal diagnosis of sex-linked disorders; fetal rhesus D status determination in sensitized rhesus-negative pregnant women, autosomal dominant disorders wherein the father carries the mutation; and autosomal recessive disorders in which the mother and father carry different mutations [6]. The identification of fetal sex can be very useful for the managing of X-linked genetic diseases, as in the file of fragile X and hemophilia [14]. Prenatal diagnosis in pregnant women that carrying an X-linked genetic disorder might thus allow the limitation of invasive diagnostic procedures to male fetuses only [11]. Precision of the technique is higher than 97% when the assessment is performed in the 7th week of pregnancy. It is known that the concentration of fetal DNA in maternal blood increases with gestational age and therefore, the older the gestational age, the easier the diagnosis of fetal gender.

However, for reasons still unknown, there seem to be periods during pregnancy when there is no smallest amount concentration of fetal DNA for finding [14]. Newly, some researchers were able to show the detection of fetal RNA in the diagnosis of fetal gender by real-time PCR starting from 5 weeks of pregnancy has good sensitivity and excellent specificity. We recommend a repeat examination from the 7th week of gestation for a definitive diagnosis [14].

In addition to DNA in maternal blood this detection may represent a valuable device for the study of fetal gene expression related to different phases of intrauterine life, that is, the study of certain gene types that are expressed and silenced according to gestational semesters [14]. Cell-free fetal DNA extracted from maternal blood includes, among others, such autosomal recessive disorders as hemoglobinopathies and cystic fibrosis, when father and mother and carriers of mutations [9].

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REFERENCES


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