Journal of Medical Diagnostic Methods
ISSN: 2168-9784

Santos et al., J Med Diagn Meth 2018, 7:3
DOI: 10.4172/2168-9784.1000277

Research Article Open Access

Comparing a Polymerase Chain Reaction (PCR) Based Analysis for Gender Determination in Early Stage of Pregnancy versus Sonography

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Received date: August 28, 2018; **Accepted date**: September 25, 2018; **Published date**: September 29, 2018

Abstract

Objective: The aim of this study was to evaluate two genes as candidates for fetal sex determination by PCR from blood samples during the first trimester of pregnancy and then contrast the results with the sonography in the second trimester.

Study Design: In the present work we evaluated blood from 70 women between 7 to 12 weeks of gestation. DNA extraction was done using a commercial kit. Two Y-chromosome DNA sequences were selected as targets for PCR reactions. The results obtained were compared with two sonographies during 12 and 24 weeks of pregnancy. The gender of the baby was confirmed after birth.

Results and Conclusions: The PCR test designed in this work allowed us to determine with 98.5% accuracy the sex of the fetus among the women in the first weeks of pregnancy. The interference of the sex predicted was found with multifetal pregnancy. This non-invasive technique for fetal sex determination can be applied on a regular basis during the first trimester of gestation as early and precise as a regular sonography.

Keywords Prenatal; PCR test; Sex fetal diagnose; Non-invasive

Introduction

Classically, early-fetal sex determination has been performed by techniques such as chorionic villus sampling or amniocentesis. These procedures, which can be performed after week 10 and 15 respectively, are invasive for both the mother and fetus, and increase the risks of miscarriages [1]. Non-invasive techniques such as ultrasonography rely on the observation of external genitalia. Therefore, the sonography cannot be performed until external genitalia is fully developed during the 12th week of gestation.

Denis Lo, reported for the first time the presence of cell-free fetal DNA (cffDNA) in maternal plasma [2]. This opened a new area for applying the technology called Non-invasive Prenatal Testing (NIPT). The most remarkable application of this is the detection of fetal trisomy 21 or RHD genotype and sex gender. This technique is highly sensitive and more specific compared to the classical invasive techniques. In addition, some authors maintain that the development of non-invasive molecular techniques can address prenatal treatments when the fetus is still developing [3].

Blood isolation for PCR do not lead miscarrying, so the technique is considering non-invasive. Invasive procedures such as amniocentesis, chorionic villous sampling are associated with miscarrying [4].

For some X chromosome-linked diseases, it is important to have an early diagnosis not only for treatment, but also to prepare the mother for the impact it might have. For instance, Congenital adrenal hyperplasia, Duchenne/Becker muscular dystrophy, Hemophilia, Adrenoleukodystrophy, Wiskott-Aldrich syndrome, and Severe combined immunodeficiency are the most frequent X-chromosome linked genetic diseases.

Fetal sex determination in maternal plasma targets the Y-chromosome specific–fragment sequence. This can be performed by Real Time PCR and target single copy gene *SRY*, as well as a multi-copy gene *TSPY* (*DYS14* marker sequence). Since this procedure was tested in different laboratories the amplifications of the Y-chromosome fragments become a reliable and non-invasive procedure for sexing the fetus using maternal blood. A previous report showed that *DYS14* with *SRY* genes amplification is highly specificity 100% and has 99.5% accuracy.

In this study, samples from pregnant women in different gestational weeks were analyzed to determine the sensitivity, specificity, and accuracy of a PCR based non-invasive prenatal test. We also studied possible causes of interference as well as the limitations of the technique. Finally, we compared the sexing result with two sonographies at 12 and 24 weeks. The ultimate goal was to confirm the accuracy of a PCR based non-invasive prenatal test for gender identification. We recovered data when the mother delivered the baby in order to confirm the prediction with the real sex.

Materials and Methods

Sample collection and plasma separation

Ten ml of peripheral blood from 70 pregnant women between 7 and 12 weeks of gestation was collected in tubes with 0.5 M EDTA. All volunteer participants signed a consent form prior to the extraction of blood and participation in this study. To assess possible interferences

with male cell free DNA in the blood, we requested the participants give us some relevant background information such as previous transplants, blood transfusions, mother's blood type, the gender of previous pregnancies, and whether they had a multiple pregnancy or not. Before delivery, we collected data about the sex of the baby, multifetal pregnancies, and sonography results.

To separate the cell-free fraction, the blood was centrifuged at 3000 g for 10 min. The upper plasma layer was carefully removed, transferred to a new tube and centrifuged again. The plasma was then transferred to a new tube and stored at -20ºC until further processing.

DNA extraction

Genomic DNA was extracted from 500 µl of plasma using QiaGen MinElute PCR Purification Kit following manufacturer's instructions. The extracted DNA was eluted in 30 µl of Tris-EDTA buffer.

Primers and PCR

For the PCR reactions, we amplified sequences in *SRY* gene forward: 5-CCCCCTAGTACCCTGACAATGTATT-3', reverse: 5-TGGCGATTAAGTCAAATTCGC-3'; probe: 5' FAM/AGCAGTAGAGCAGTCAGGGAGGCAGA/3IABkFQ-3' as previously reported [2]. To detect the *DYS14* gene we used a commercial kit from Primer Design (Catalogue Nº: yDNA-hu). The PCR reactions were set in a total volume of 25 µl with 10 µl of DNA solution. As a negative control we used DNA extracted from a non-pregnant female and as a positive control, diluted genomic DNA extracted from male blood.

Results and Discussion

Here we optimized a PCR based technique to determine the fetus gender during early stages of pregnancy, and correctly predicted the sex of the fetus before it could be detected by sonography. The distribution of gestational weeks points out that most of the participants of this study were in early stages of pregnancy, before 12 weeks.

To assess possible interferences and limitations of the technique, we analyzed factors such as the gender of a previous pregnancy, in particular males, since it was previously reported that male cells can remain in the maternal blood for 27 years postpartum. In addition, there is no data that shows the DNA or cells present in the blood of non-pregnancy women [4]. This indicates that the cffDNA in blood is not present for long periods after delivery. In fact, a previous pregnancy, are not correlated with the cffDNA in blood. We did not find any interference during the PCR assay. According to interviews, the mothers showed a high interest in knowing the baby's sex as early as possible and in particular in young mothers (24-34 years old).

In 70 analyzed samples, PCR technique correctly determined the sex of the fetus, 30 (42%) of these were male and 40 (56%) were female. A total of 70 cases were then confirmed at birth and the results compared with two different sonographies at 12 to 15 weeks and 20 to 24 weeks of pregnancy (Figure 1). In one case, the sex was predicted as male and the echographers later predicted a single female embryo in utero. The mother in this case reported a multifetal pregnancy during the first trimester, but only one of the fetus prospered. Therefore, we assume that the fetus was a male and released cffDNA in the mother's blood during the first trimester of pregnancy.

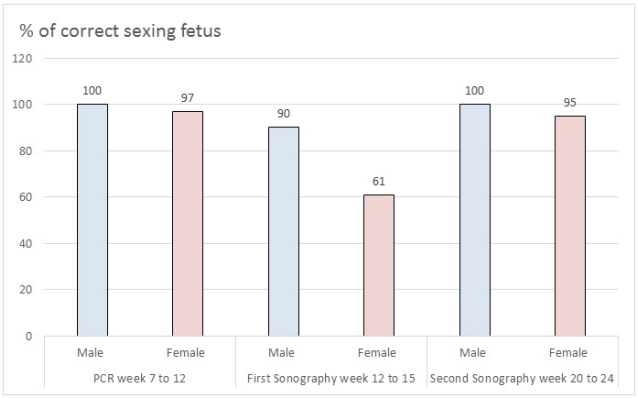

Figure 1: Percentage of well predicted sex of the fetus by PCR techniques and Sonography during the development of the baby.

According to Figure 2, the PCR technique was capable of discriminating a male fetus with 100% accuracy before the sonography and before the genitals were developed. *In vitro* Fertilization is commonly associated with multiple births. In these particular groups, PCR based sexing can lead to miss-determination in particular if fetal sexes are not the same.

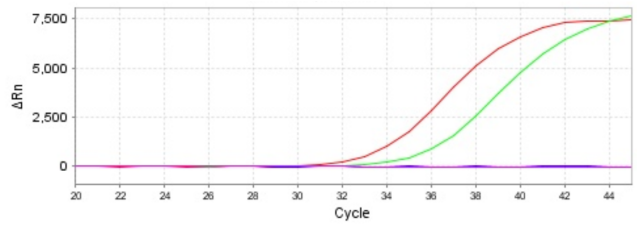

Figure 2: Amplification of cffDNA form male (XY genotype) green color, and female (XX genotype) violet color. No temple control in blue color, and genomic DNA as a C+ or positive control in red color.

In our study by PCR technique, a single blood samples showed amplification of a Y chromosome fragment and the second sonography pointed out a female sex fetus, which was confirmed in the newborn. In order to clarify this situation, we found that these mothers delivered more than one baby. Both did not have previous pregnancies. In the interviews, these mothers indicated that one of the fetuses did not prosper during the pregnancy.

In most studies, regardless of gestational age, concordance for males was not as good as for females, and thus there was a greater risk that males would be erroneously classified as females. This has a concordance with previous literature reviews and meta-analysis showing superiority in the PCR [5,6].

Conclusions

We demonstrated that the Real time PCR, based on Y-chromosome fragments detection, is more sensible for diagnosing the gender of the fetus before genital development using sonography. We did not find a direct relationship with the previous pregnancy and sex of the

newborn with the current sex fetus. The diagnosis of the sex can be achieved with high accuracy in the early stages of pregnancy in a non-invasive way.

Funding

All funding comes from the company Bioplex Montevideo Uruguay.

References

1. Bianchi DW (2012) From prenatal genomic diagnosis to fetal personalized medicine: Progress and challenges. Nat Med 18: 1041-1051.
2. Breveglieri G, Elisabetta B, Carlassara S, Cosenza LC, Pellegatti P, et al. (2016) Y chromosome identification in circulating cell-free fetal DNA using surface plasmon resonance. Prenat Diagn 36: 353-361.
3. Nanal R, Phillipa K, Soothill PW (2003) A classification of pregnancy losses after invasive prenatal diagnostic procedures: An approach to allow comparison of units with a different case mix. Prenat Diagn 23: 488-492.
4. Rijnders RJP, Christiaens GCML, Soussan AA, van der Schoot CE (2004) Cell-free fetal DNA is not present in plasma of non-pregnant mothers. Clin Chem 50: 679-681.
5. Colmant C, Morin-Surroca M, Fuchs F, Fernandez H, Senat MV (2013) Non-invasive prenatal testing for fetal sex determination: Is ultrasound still relevant?. Eur J Obstet Gynecol Reprod Biol 171: 197-204.
6. Wright CF, Wei Y, Higgins JP, Sagoo GS (2012) Non-invasive prenatal diagnostic test accuracy for fetal sex using cell-free DNA a review and meta-analysis. BMC Res Notes 5: 476.