Fetal Sex Determination Using Cell-Free Fetal DNA in Pregnant Women: Diwaniyha Local Study

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Abstract
Objective :Our Interest has been sparked through the viable use of plasma and serum for molecular diagnostics. women's plasma and serum have each covered circulating DNA. and this substance has passed through molecular examination. We checked out whether or not fetal DNA become contribution with inside the maternal plasma and serum, that is a circumstance that takes place further all through pregnancy. Materials and methods: Plasma and serum were rapidly treated in order to extract the DNA from them. A tricky Y-PCR test was used to detect circulating male fetal DNA from pregnant girls carrying male babies using DNA from the plasma, serum, and nucleated blood cells of 43 pregnant girls. Results: In 40 maternal plasma samples, fetal-derived Y sequences had been found. Only 10 L of the samples had been used to get those findings. Only five (17%) of the 30 samples produced a wonderful Y sign while DNA from nucleated blood cells removed from a similar quantity of blood changed into employed. None of the 10 non-pregnant manipulate ladies, nor any of the thirteen ladies wearing woman babies, had wonderful plasma, serum, or nucleated blood tests. Conclusion: We concluded that free circulating fetus DNA could be used for gender determination or other possible molecular studies related to the fetus during pregnancy period.
Keywords: Fetal ,PCR, Pregnancy, DNA, Iraq

1. Introduction

It is now well-known that nucleated cells can go back and forth between the mother and fetus in both directions[1, 2]. In a clinical setting, fetal genetic material can be obtained by the transfer of fetal cells into mother blood for non-invasive prenatal diagnostics[3]. Most fetal cells are eliminated two to three months after birth[4]. It has been demonstrated that some women continue to have fetal hematopoietic progenitor cells even decades after giving birth by using sensitive PCR assays and cell sorting[5-7].
For many years it's been not placed as know how that a few cells from the growing fetus make their manner into maternal circulation[8], and that this procedure begins off evolved within side the early weeks of first trimester[9]. Naturally, while opportunity technology for prenatal prognosis had been being explored, the usage of fetal cells for noninvasive prenatal prognosis got here on pinnacle of the listing[10]. After years of intermediate achievement and failure, the hobby in fetal cells circulating in mother blood has come complete circle[11].
Cell-loose fetal DNA may be detected in maternal circulation, just as fetal cells can be found there[12]. Fetal DNA has been shown to be present at excessive amounts in maternal plasma using a quantitative PCR technique[6]. This statement suggests that the noninvasive prenatal prognosis of some conditions[7], including sex-related illnesses and fetal hemolytic disorder caused by Rh blood institution incompatibility, may also be possible using plasma fetal DNA examination[13].
Little is known about the factors that determine the amount of circulating fetal DNA, other than the fact that it tends to increase as gestation goes on, especially toward the end of pregnancy[14].
One of the research branch is the kinetics of fetal DNA clearance will allow estimate of the cost of fetal DNA launch into maternal circulation if a steady scenario is used. The cost of clarity may also provide data on the usefulness of fetal DNA measurement inside the examination of the dynamic strategies involved inside the management of circulating DNA during pregnancy[15].
The fact that certain fetal cells find their way into the mother's bloodstream and that this system starts to develop in the first few weeks of the first trimester has long been common knowledge[16]. Naturally, when prenatal prognosis technology options were being investigated, employing fetal cells for noninvasive prenatal prognosis rose to the top of the list[17].

2. Subjects and Methods

Subject

With 45 pregnant women, after the child became delivered, 2 ml of the mother's peripheral blood became drawn into an EDTA tube. Prior to the cesarean segment and hours after birth, five ml of maternal blood became drawn right into a tube with EDTA and kept at 20 °C for DNA extraction later.

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Sample preparation

Blood samples from subjects were collected at a single post-shipment time point and processed as described later. A researcher receives a series of pre- and post delivery samples, which are promptly brought to the laboratory for quick processing for subjects recruited for the serial sampling work and for an examination of the function of plasma nucleases. Plasma is removed from blood samples after they have been centrifuged at 3,000 g and is then placed into clear polypropylene tubes. The supernatants from the re-centrifugation of the plasma samples at 3,000 g have been collected into gleaming polypropylene tubes. The samples were kept at —20°C until further processing.

DNA Extraction from Plasma Samples

In order to prepare plasma or serum samples for PCR, a modified version of Emanuel and Peska's procedure was used. A 0.5 mL Eppendorf tube containing nine 200 L plasma or serum was heated at 99 °C for five minutes on a warming block. The heated pattern was then centrifuged in a microcentrifuge at maximum speed, and 10 L of the clean supernatant were utilized for PCR[18]. In total, 50 l of extracted DNA, 20 pmol of every primer (Y1.7, Y1.8, GAPDH forward, and GAPDH reverse), and 25 l of HotStarTaq Master Mix (containing 2.five gadgets of HotStarTaq DNA polymerase, 1 x PCR buffer with 1.five mM MgCl2, and 200 M of every dNTP (Promega, USA) has been used for all PCR reactions as used by [19].

Primers	Forward sequence	Reverse sequence	Reference
FMR 1	CCCTGATGAAGAACTTGTATCTC	GAAATTACACACATAGGTGGCACT	[20]
SRY1	CTAGACCGCAGAGGCGCCCAT	TAGTACCCACGCCTGCTCCGG	[20]

The thermal cycle went as follows: HotStarTaq DNA polymerase was first activated at 95 °C for 5 minutes, then there were 40 cycles of denaturation at 94 °C for 1 minute, annealing at 61 °C for 40 seconds, and extension at 72 °C for 1 minute, with the last extension taking place at 72 °C for 10 minutes. Using the same circumstances and varying the number of PCR cycles, 30, 40, and 50 cycles, the number of cycles was optimized. After being stained with Ethidium Bromide, PCR amplification products were separated by 2% agarose gel electrophoresis and then documented by ultraviolet light.

3. Results

The basis gain the use of circulating free cell-DNA as opposed to the conventional prenatal diagnosis methods, which include CVS and amniocentesis.The sample method used for maternal plasma is non-invasive and poses no risk to the mother or her unborn child. Another benefit is its accuracy in detecting fetal intercourse from 7 weeks of gestation forward. 10,eleven Amniocentesis is most useful when performed after 15 weeks of gestation[21, 22].

The Y-PCR –based method turned into used to perform the suitable extent of heated polasma or serumsamples for PCR amplification turned into determined. A shown in figure 1 and 2 , the results confirmed the gender of fetus by molecular approach that is reliable and low cost method[23, 24].

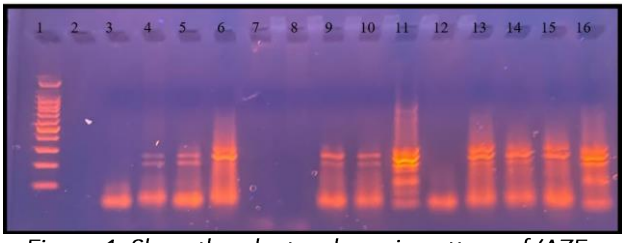

Figure 1: Show the electrophoresis pattern of (AZF a ,AZF b , AZF c and SRY region). 2 % Agarose stained with Ethidium Bromide. Lane 1: DNA ladder 100bp,Lane 2-16 : samples.

Maternal plasma or serum samples had been amassed from forty three ladies who had been among 12 and forty weeks pregnant. There had been 30 male and thirteen girl fetuses. Among the 30 ladies bearing male fetuses, Y-superb indicators had been detected in 24 plasma samples and 21 serum samples whilst 10 µL of the samples turned into used for PCR (parent and table). When DNA from nucleated blood-cells turned into used for Y-PCR, superb indicators had been detected in best 5 of the 30 cases (table). None of the thirteen ladies bearing girl fetuses, and none of the 10 non-pregnant manipulate ladies, had a superb Y sign whilst plasma, serum, or mobile DNA turned into amplified.

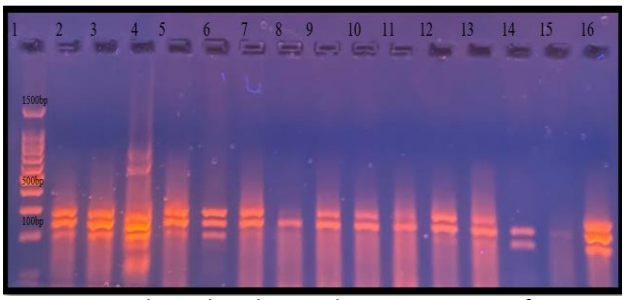

Figure 2: Show the electrophoresis pattern of (AZF a ,AZF b , AZF c and SRY region). 2 % Agarose stained with Ethidium Bromide. Lane 1: DNA ladder 100bp,Lane 2-16 : samples.

4. Discussion

Our findings show that maternal plasma or serum contain fetal DNA. It is hence also conceivable to use maternal plasma or serum to identify fetal genetic material for non-invasive liquid investigation. Ironically, plasma is the material that

is robotically discarded during the first stages of many DNA-extraction methods and also following the density-centifugation step that many researchers use for non-invasive prenatal diagnosis. This might be one of the reasons why the prior investigation on the presence of fetal DNA in maternal plasma has been discontinued.

For biochemical testing of chromosomal aneuploidies and neural-tube abnormalities, several centers use maternal serum. There is a method that can be included into current screening programs that allows DNA-based prognosis to be performed on blood samples.

Based on our research, maternal plasma or serum containing as little as 10 L can nevertheless include fetal DNA. In evaluation to DNA from nucleated blood cells taken from a similar quantity of complete blood, the detection price is drastically greater. This discovery factors to a relative fortification of fetal nucleic acid in maternal plasma or serum—a phenomenon similar to the relative enrichment of tumor DNA in polasma or serumof most cancers patients. 7,eight Low fetal DNA series detection charges for DNA from nucleated blood cells.

resulted from utilizing only 100 ng of DNA, as opposed to 1 g in our previous investigation. 12 We chose 100 ng because it was the typical quantity of DNA retrieved using our genomic DNA-extraction technique from 10 L of whole blood. With the use of this technique, we are able to assess the relative detectability of fetal DNA in 10 l of plasma or serum as well as the mobility of 10 l of whole blood.

Although the rates of fetal DNA detection in 10 L polasma or serumare currently too high at 80% and 70%, respectively, those figures may probably be raised. For example, 1 mL of maternal blood or serum will result in a 100-fold increase in the amount of genetic material that will be transferred to the fetus.

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1. Zhou, Y., et al., Analysis of cell-free fetal DNA in 16,843 pregnant women from a single center in China using targeted sequencing approach. Placenta, 2022. 122: p. 18-22.
2. Zhou, J., et al., Simulated confined placental mosaicism proportion (SCPMP) based on cell-free fetal DNA fraction enrichment can reduce false-positive results in non-invasive prenatal testing. Prenat Diagn, 2022. 42(8): p. 1008-1014.
3. Zhong, L.P.W. and R.W.K. Chiu, The Next Frontier in Noninvasive Prenatal Diagnostics: Cell-Free Fetal DNA Analysis for Monogenic Disease Assessment. Annu Rev Genomics Hum Genet, 2022. 23: p. 413-425.
4. Zhao, G., et al., [The value of re-sampling for patients who had failed non-invasive prenatal testing due to low cell-free fetal DNA fraction]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi, 2022. 39(2): p. 135-138.
5. Werner, B., K. Warton, and C.E. Ford, Endogenous cell-free DNA in fetal bovine serum introduces artifacts to in vitro cell-free DNA models. Biotechniques, 2022. 73(5): p. 219-226.
6. Wang, J., et al., [The effect of maternal HBV DNA levels on HBV intrauterine transmission and fetal distress]. Zhonghua Gan Zang Bing Za Zhi, 2022. 30(8): p. 873-878.
7. Turriff, A.E., C.M. Annunziata, and D.W. Bianchi, Prenatal DNA Sequencing for Fetal Aneuploidy Also Detects Maternal Cancer: Importance of Timely Workup and Management in Pregnant Women. J Clin Oncol, 2022. 40(22): p. 2398-2401.
8. Mahdi Mortazavipour, M., R. Mahdian, and S. Shahbazi, The current applications of cell-free fetal DNA in prenatal diagnosis of single-gene diseases: A review. Int J Reprod Biomed, 2022. 20(8): p. 613-626.
9. Lo, Y.M.D., Discovery of Cell-Free Fetal DNA in Maternal Blood and Development of Noninvasive Prenatal Testing: 2022 Lasker-DeBakey Clinical Medical Research Award. JAMA, 2022. 328(13): p. 1293-1294.
10. Laufer, B.I., et al., Placenta and fetal brain share a neurodevelopmental disorder DNA methylation profile in a mouse model of prenatal PCB exposure. Cell Rep, 2022. 38(9): p. 110442.
11. Kwan, A.H.W., et al., Genome-Wide Cell-Free DNA Test for Fetal Chromosomal Abnormalities and Variants: Unrestricted Versus Restricted Reporting. Diagnostics (Basel), 2022. 12(10).
12. Xu, C., et al., Genetic deconvolution of fetal and maternal cell-free DNA in maternal plasma enables next-generation non-invasive prenatal screening. Cell Discov, 2022. 8(1): p. 109.
13. Saito Reis, C.A., et al., Fetal DNA Causes Sex-Specific Inflammation From Human Fetal Membranes. Front Physiol, 2022. 13: p. 901726.
14. Rink, B.D., B.K. Stevens, and M.E. Norton, Incidental Detection of Maternal Malignancy by Fetal Cell-Free DNA Screening. Obstet Gynecol, 2022. 140(1): p. 121-131.
15. Richter, A.E., et al., Altered neurodevelopmental DNA methylation status after fetal growth restriction with brain-sparing. J Dev Orig Health Dis, 2022. 13(3): p. 378-389.
16. Persson, F. and H.S. Cuckle, Consequences of imprecision in fetal fraction estimation on performance of cell-free DNA screening for Down syndrome. Prenat Diagn, 2022. 42(4): p. 512-517.
17. Meng, R., et al., Genome-wide analysis of methylation in rat fetal heart under hyperglycemia by methylation-dependent restriction site-associated DNA sequencing. PLoS One, 2022. 17(5): p. e0268117.
18. Al-Hilail, D.W., W.S. Al-wazni, and M.A. Al-

Askeri, Association of the IL-4 gene polymorphisms with developing of renal diseases and bacterial urinary tract infections. HIV Nursing, 2022. 22(2): p. 3243–3248-3243–3248.
19. Abdulwahab Ati Al-Askeri, M., Correlation between single nucleotide polymorphism rs1345365 and risk of diabetes mellitus in Iraqi patients: Cross-section study of AD diwaniyha governorate. Materials Today: Proceedings, 2022. 65: p. 2709-2712.
20. Miura, K., et al., Clinical application of fetal sex determination using cell-free fetal DNA in pregnant carriers of X-linked genetic disorders. J Hum Genet, 2011. 56(4): p. 296-9.
21. Torres Aguilar, M.R., et al., Contingent prenatal screening for frequent aneuploidies with cell-free fetal DNA analysis. Taiwan J Obstet Gynecol, 2021. 60(4): p. 745-751.
22. Liu, Y., et al., Comparison of Genome-Wide DNA Methylation Profiles of Human Fetal Tissues Conceived by in vitro Fertilization and Natural Conception. Front Cell Dev Biol, 2021. 9: p. 694769.
23. Lee, S., et al., DNA Methylation and gene expression patterns are widely altered in fetal growth restriction and associated with FGR development. Anim Cells Syst (Seoul), 2021. 25(3): p. 128-135.
24. Kwak, S.H., et al., Sequencing Cell-free Fetal DNA in Pregnant Women With GCK-MODY. J Clin Endocrinol Metab, 2021. 106(9): p. 2678-2689.