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Case report

THE SIGNIFICANCE OF PLACENTAL CHROMOSOMAL COMPOSITION IN THE OCCURRENCE OF FALSE POSITIVE RESULT IN NON- INVASIVE PRENATAL TESTING: A CASE REPORT HIGHLIGHTING POSITIVE RESULT FOR TRISOMY 21

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Abstract

Introductioion: The article presents a case report of a false-positive result (FPR) for Trisomy 21 (T21) identified through a non-invasive prenatal test (NIPT) during pregnancy, which may arise from biological factors that lead to discrepancies between NIPT outcomes and the actual condition of the fetus.

T21 is the most frequently detected chromosomal anomaly, constituting 30% of findings obtained through NIPT. This study investigated the clinical application of NIPT following a positive T21 result.

Material and methods: Two distinct prenatal tests were conducted for the same pregnancy. The initial NIPT employed Harmony reagents with Ariosa cell-free DNA System, and was followed with confirmatory amniocentesis which involved quantitative florescent PCR analysis. Second NIPT utilized the VeriSeq NIPT Solution V2 from Illumina and was followed with confirmatory amniocentesis analysis and placental tissue analysis post-delivery both using quantitative fluorescent PCR and an oligonucleotide array.

Results: This study demonstrated that both NIPT tests produced consistent results, thereby clarifying the notion of a 'false positive' result attributed to Confined Placental Mosaicism (CPM).

Conclusion: Understanding the implications of FPR for T21 regardless of the lowest risk of CPM is essential when considering diagnostic options.

Keywords: non-invasive prenatal test, NIPT, pregnant woman, false positive result, fetus, placenta

Introduction

Cell-free DNA (cfDNA) represents a subset of circulating nucleic acids, first identified and described by Mandel and Metais in 1948[1]. Currently, millions of pregnant women utilize non-invasive prenatal testing each year, a practice that emerged following the 1997 discovery of cell-free fetal DNA (cffDNA) in maternal plasma, which revolutionized prenatal diagnostics[2]. This simple and low-risk screening method, enhanced by advancements in next-generation sequencing, was initially designed to identify prevalent aneuploidies, including Trisomy 21, 18, and 13, from cffDNA extracted from maternal serum[3]. In maternal blood, cfDNA is primarily derived from the mother, with the fetal component (cffDNA) accounting for approximately 10-20% of the total. cffDNA is specific to pregnancy, as it is rapidly eliminated from maternal circulation within hours

post-delivery, yet it reflects the complete fetal genotype originating from the placenta. Consequently, the foundation of NIPT lies in its ability to serve as a maternal blood test performed in early pregnancy, significantly refining the risk assessment for Trisomy 21 and minimizing the necessity for invasive procedures such as chorionic villus sampling (CVS) or amniocentesis (AC)[4]. Regarding T21, NIPT demonstrates significantly superior performance compared to combined screening, achieving a sensitivity exceeding 99% with a false positive rate of 0.1% in a routinely screened first trimester population. Furthermore, it has been established that the introduction of NIPT has led to a notable decrease in the rate of invasive testing, dropping from 70% to 48%[5].

A significant number of studies related to non-invasive prenatal testing (NIPT) express considerable optimism regarding its high sensitivity and specificity for detecting trisomy 21 (T21). However, a decline in these values when assessing other chromosomal abnormalities, including trisomy 13 (T13), trisomy 18 (T18), sex chromosome aneuploidies (SCAs), rare aneuploidies (RATs), and microdeletion/microduplication syndromes is noted. Nearly 10% of women with a positive NIPT for T21 and approximately 85% with a positive result for RATs were misled into believing their child had a chromosomal abnormality, which could occur due to a confined placental mosaicism. The trophoblast utilized in NIPT exhibits considerably higher and distinct mutation rates compared to the cells of the fetus, which contributes to the occurrence of false positive results in NIPT[6].

How discrepancies between NIPT outcomes and the actual fetal genotype - resulting in either false negatives or false positives - can stem from technical issues or various biological factors is elaborated, thereby underscoring the complexities inherent in NIPT. One biological factor contributing to the difference between the fetal genotype and the placental trophoblast, which may result in false positive or false negative outcomes, is either confined placental mosaicism or true-fetal mosaicism, leading to feto-placental discordance[7].

When interpreting NIPT results, it is essential to consider all pertinent information regarding the pregnancy. Given that NIPT is classified as a screening test, its positive predictive value (PPV) cannot reach 100%. Therefore, it is crucial to confirm any high-risk results through invasive testing before making any irreversible decisions concerning the pregnancy[7]. The challenge arises in determining the appropriate course of action following a high probability NIPT result for trisomy 21, 18, and 13 in relation to prenatal diagnosis. The decision to conduct chorionic villus sampling CVS or amniocentesis to validate a positive NIPT result remains a topic of debate. Current guidelines appear to be influenced by gestational age. Both procedures are regarded as safe, with a miscarriage rate of less than 0.5%. CVS involves obtaining a sample from the placenta, while amniocentesis is recognized for providing a more accurate representation of fetal DNA. Several bodies have suggested that amniocentesis is the preferred option following normal ultrasound findings, whereas both CVS and amniocentesis may be considered in cases with abnormal ultrasound results[8].

The article discusses a detailed case report concerning a false-positive result for T21 detected via a non-invasive prenatal test throughout pregnancy. This occurrence may stem from biological factors that result in discrepancies between the outcomes of NIPT results and the true condition of the fetus.

Case report

The patient was referred to Bio Save Cells for performing the initial prenatal testing and providing advice and support during the various genetic tests that followed to clarify the

preliminary NIPT results. In collaboration with the patient referring physician, Bio Save Cells was tasked with overseeing the pregnancy to ascertain the source of the identified genetic condition.

This case involved a spontaneous pregnancy, the third for the woman, who conceived at the age of 40. The patient maintained a normal lifestyle and had never smoked. Both partners were healthy without personal medical history of relevance for the tested condition during pregnancy. Their first child died at the age of two due to glioblastoma, which was diagnosed a year after birth, while their second pregnancy resulted in a healthy child.

The NIPT was performed at the patient's request and in consultation with her referring physician at gestational age 10 weeks and 5 days. The results indicated a positive finding for T21, with 8.2% cell-free fetal DNA (cffDNA). At 12 weeks and 2 days, a first-trimester screening was performed, revealing a completely normal ultrasound examination, including the confirmed presence of the nasal bone and a nuchal translucency measurement within the normal range.

Amniocentesis was recommended for diagnostic confirmation, and the analysis yielded a normal karyotype. At the request of the patient, following counseling regarding the potential for a positive result related to the affected finding and the possibility of CPM, a second NIPT utilizing a different technology was conducted. This result also indicated Trisomy 21, consistent with the initial NIPT. A second amniocentesis, employing array technology and quantitative PCR in addition to standard karyotyping, was performed to rule out rare mosaic findings for T21. As anticipated, the results were normal for the tested chromosomal conditions, aligning with the findings from the first amniocentesis. The second-trimester screening yielded completely normal results, and throughout the pregnancy, no complications arose. The pregnancy ended at 39 weeks gestation with the delivery of a healthy girl, with no indications of trisomy 21 or other abnormal findings. A placental sample was collected and analyzed using array technology, revealing placental mosaicism.

This case study adopts a distinctive methodology to attain its objective, which is in alignment with the recent research findings across various studies. These studies confirm the objective and strengthen the conclusions reached.

Discussion

The main challenge arises in determining the appropriate course of action following a high probability NIPT result for trisomy 21, 18, and 13 in relation to prenatal diagnosis. There is ongoing debate on whether to do amniocentesis or chorionic villus sample in order to confirm a positive non-invasive prenatal testing (NIPT) result. The gestational age of the fetus appears to influence current guidelines, which assume that both treatments are safe with a chance of miscarriage of less than 0.5%. While amniocentesis is recognized for providing a more accurate assay of fetal DNA, chorionic villus sampling involves taking a sample from the placenta. Various organizations have recommended that amniocentesis be the preferred method when ultrasound results are normal, while both chorionic villus sampling and amniocentesis may be considered in instances of abnormal ultrasound findings[8]. In a case presentation by Bonanni *et al*. in 2022, amniocentesis was deemed the most effective diagnostic method following normal ultrasound findings in the context of a positive NIPT result for trisomy 13, in order to mitigate the risk of complications from prenatal management (CPM). It is recommended that, in the absence of detected anomalies during ultrasound, chorionic villus sampling may be advised solely for trisomy 21, as the associated risk of CPM in such cases is approximately 1-2%, comparable to the risk of mosaicism in the general population. Conversely, the risks of CPM for trisomy 18 and 13 are significantly higher, at 3-4% and 22%, respectively[9]. Statistically, a low risk of aneuploidy

indicated by first-trimester screening and the absence of fetal structural anomalies can lead to an increased likelihood of false positive results in non-invasive prenatal testing. The occurrence of persistently false positive results due to confined placental mosaicism is plausible, as even though the UK best practice recommends quantitative fluorescent polymerase chain reaction (QF-PCR) testing that considers both cell lineages in chorionic villus sampling, it may predominantly reflect the cytotrophoblast. Genetic counseling should encompass the risks and benefits associated with both CVS and amniocentesis, as well as the implications of CPM, emphasizing the necessity for accurate interpretation of results[10].

Conclusion

The optimal diagnostic tool to consider following a positive NIPT result remains somewhat ambiguous, as it largely depends on gestational age and the specific chromosomal abnormality identified. Regarding Trisomy 21, which is considered a chromosomal abnormality with the lowest mosaic rate, in the absence of ultrasound finings, the possible limitation of this chromosomal abnormality to the placenta should always be considered.

This distinctive approach, which eliminates the possibility of fetal findings after initial positive T21 on non-invasive test, suggests that when feasible and in alignment with the comprehensive condition of the pregnant woman and her autonomy, the preferred method for invasive confirmation following a positive result for Trisomy 21 (T21) should be amniocentesis. This recommendation aims to eliminate any uncertainties regarding confined placental mosaicism.

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Contribution to authorship

The author is accountable for the preparation and organization of the paper, initially communicating the results to the pregnant woman and her referring gynecologist, and subsequently designing the strategy and guiding the completion of the case in collaboration with the pregnant woman and the medical team involved in prenatal diagnosis and screening.

Conflict of interest statement. None declared.

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