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CASE REPORTS

Investigational Management for a Positive NIPT Result - Case Report

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Abstract

Non-invasive prenatal testing (NIPT), since its introduction in 2011, has revolutionized prenatal screening, becoming widely used globally and replacing traditional screening methods in developed countries. The accuracy of NIPT in detecting aneuploidies is extremely high and there has been a recent trend towards improving NIPT for detecting microdeletion and microduplication syndromes, monogenic diseases, fetal sex determination, and RH genotyping. Significant progress has been made in molecular analysis techniques of fetal DNA, including methods such as massively parallel sequencing, RNA-based testing, digital PCR, and single nucleotide polymorphism analysis.

We present the case of a 32-year-old patient who, at 12 weeks of gestation, had a non-invasive prenatal test result showing a maternal 22q11.2 deletion. Following genetic consultation, further investigations were conducted to stratify the fetal risk of inheriting the microdeletion syndrome. As a result, microarray CGH cytoarray from amniotic fluid was performed, and no 22q11.2 deletion was detected. In this case, complete elucidation of the origin of the deletion found in NIPT could not be achieved, as it would require arrayCGH testing for the mother, a test that was not performed due to financial reasons.

Given the high rate of genetic syndromes with potential impact on fetal development and familial psychological impact, we wish to emphasize the necessity of financial support from the state to introduce non-invasive prenatal testing into the list of reimbursed analyses covered by health insurance. This would enable superior testing and, implicitly, genetic prevention of all pregnancies, facilitating appropriate risk stratification of pregnancies in our country.

Keywords: Non-invasive prenatal testing, microdeletion, DiGeorge Syndrome, aneuploidies, fetal fraction

Rezumat

Testele prenatale neinvazive (NIPT) odată cu apariția lor în anul 2011 au revoluționat screeningul prenatal, ajungând să fie utilizate la scară mondială, iar în țările dezvoltate au reușit să înlocuiască metodele de screening tradiționale. Fidelitatea NIPT pentru depistarea aneuploidiilor este extrem de ridicată, în ultima perioada s-a observat o tendința spre perfecționarea testelor prenatale neinvazive în detectarea sindroamelor de microdeleție și microduplicație, a bolilor monogenice, în depistarea sexului fetal, dar și în genotiparea RH. În acest sens, se fac progrese mari în ceea ce privește tehnicile de analiză moleculară a ADN-ului fetal, folosind metode precum MPS (massively parallelsequencing), testare bazată pe ARN, PCR digital, SNP(single nucleotide polymorphism). Prezentăm cazul unei paciente în vârsta de 32 de ani căreia la vârsta gestaționala de 12 săptămâni rezultatul testului prenatal neinvaziv a arătat o deleție 22q11.2 maternă. În urmă consultului genetic, s-a decis continuarea

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investigațiilor pentru stratificarea riscului fetal de a moșteni sindromul de microduplicație. Astfel, s-a practicat microarray CGH cytoarray din lichidul amniotic, care nu a detectat deletie de tip 22q11.2. În acest caz elucidarea completă a originii deleției descoperite în cadrul NIPT nu a putut fi realizată, acest lucru presupunând testarea arrayCGH pentru mama, test care însă nu a fost realizat din motive financiare.

Având în vedere rată crescută a sindroamelor genetice cu potențiale debilitante asupra produsului de concepție, dar și cu impact psihologic familial, dorim să subliniem necesitatea sprijinului financiar din partea statului pentru introducerea testării prenatale neinvazive în grilă de analize decontate de casă de asigurări de sănătate, astfel se va putea realiza o testare și implicit o prevenție superioară a tuturor sarcinilor, generându-se, o stratificare adecvată pe grade de risc a sarcinilor și în țara noastră.

Cuvinte cheie: Testele prenatale neinvazive (NIPT), microdeleție, Sindrom DiGeorge, aneuploidii, fracție fetală.

INTRODUCTION

In recent years, methodologies for prenatal screening have undergone significant advancements, thereby facilitating an enhanced stratification of the risks associated with aneuploidies, including sex chromosome aneuploidies (SCAs), such as Turner's Syndrome which is the most widespread SCAs encountered in female fetuses[1]. Great efforts have also been made for detection of subchromosomal anomalies, and monogenic disorders.

In conducting a brief overview of the developmental and implementation stages of prenatal screening methods, it is noteworthy that for the first time in 1984, an inversely proportional relationship between the level of maternal alpha-fetoprotein and the risk of trisomy 21 was identified. Subsequently, other serological markers such as beta hCG and unconjugated estriol were considered. Following this, additional serological markers were introduced alongside the measurement of various sonographic indices, for example nuchal translucency.[2]

Since 1997, with the discovery of fetal DNA fragments in plasma, and subsequently of placental-derived RNA, the foundation for non-invasive prenatal testing was established. Following this, all efforts were concentrated on improving this technique.[2-4]

Fetal circulating DNA constitutes between 3 and 15% of the cell-free circulating maternal DNA. Fetal DNA is stable and disappears within a maximum of 2 days after birth, thus ensuring that there cannot be fetal DNA fragments from a previous pregnancy.[2,5]

Studies indicate that the origin of fetal DNA in maternal circulation is attributed to placental apoptotic processes, while the source of maternal DNA is hematopoietic cells. Cff DNA can be detected in maternal serum starting from the 5th week of gestation.[6]

Since its inception in 2011 in Hong Kong, non-invasive prenatal testing (NIPT) has established itself as an intermediary diagnostic modality, bridging the gap between traditional serological marker-based screening tests and ultrasound indices, and the more invasive procedures such as amniocentesis and chorionic villus sampling (CVS). The invasive methodologies boast exceptionally low rates of false-positive outcomes, with the attendant risk of procedure-related miscarriage hovering between 1% and 2%. In a conventional prenatal diagnostic timeline, amniocentesis is typically administered at the 15th gestational week, whereas CVS is generally conducted between the 10th to 13th weeks of gestation.[6,7]

Historically, the American College of Obstetricians and Gynecologists (ACOG) delineated five distinct categories of women perceived to be at elevated risk for fetal aneuploidies, for whom Non-Invasive Prenatal Testing (NIPT) was deemed advantageous. These categories encompassed: women of advanced maternal age, specifically those exceeding 35 years; the presence of ultrasonographic markers indicative of aneuploidy; a history of pregnancies impacted by trisomies; the existence of parental Robertsonian translocations heightening the risk for trisomies 13 or 21; and the acquisition of positive outcomes from serological screening methodologies.[2,8]

Currently, in developed countries, Non-Invasive Prenatal Testing (NIPT) is broadly utilized for all pregnancies, irrespective of the stratification of risks for aneuploidies, owing to its specificity and sensitivity. Recent research has demonstrated the utility of NIPT in identifying subchromosomal abnormalities, such as microdeletions and microduplication.[9,10] Microdeletions and microduplications represent numerical variations of chromosomes and have recently become a major focus in the development of NIPT. CNVs are responsible for a range of genetic syndromes that can lead to various clinical or subclinical forms of disease.[3] Globally, the incidence of CNVs is higher

than the incidence of Trisomy 21.[11] Currently, there are no screening tests specifically targeted at detecting microdeletions, but ultrasound anomalies, such as increased nuchal translucency, may suggest an elevated risk of microdeletions. Most often, these syndromes occur sporadically, but there are also some specific cases where a recurrent pattern has been observed, namely DiGeorge Syndrome 22q11.2 deletion, Cri du Chat syndrome (5p deletion), and Charcot-Marie-Tooth type 1A disease (17p11.2 duplication).[11-13]

DiGeorge syndrome, or 22q11.2 deletion syndrome, is a condition characterized by varying degrees of immunodeficiency associated with cardiac, renal, palatal defects, hearing loss, spinal anomalies, and ophthalmological abnormalities, with each phenotype presenting a different clinical spectrum. Its birth prevalence is 1 in 4000, and its incidence is increasing.[14]

CASE REPORT

We present the case of a 32-year-old woman, without associated pathologies, without a family history of genetic diseases, and without previous pregnancies, who presented to the Department of Obstetrics and Gynecology of University Emergency Hospital of Bucharest for investigations and pregnancy assessment. The first trimester morphology ultrasound only revealed non-pathological aspects, such as a nuchal translucency of 1.4 mm, the presence of the nasal bone, an intact abdominal wall, a heart with its axis to the left, with 4 chambers, and a stomach visibly positioned on the left. The first trimester morphology ultrasound was performed at a gestational age of 12 weeks and 5 days, calculated from the date of the last menstrual period, and on the same day, the patient opted for the Panorama type non-invasive prenatal test. Panorama analyzed DNA from the placenta, with a fetal fraction 4.6% and the result showed a suspected maternal deletion of the 22q11.2 region with recommendation of genetic counseling and diagnostic testing with microarray, considering the fetal risk for 22q11.2 deletion syndrome is 50%, fetal diagnostic with microarray may be considered.

Therefore, at a gestational age of 16 weeks and 3 days, amniocentesis was performed under ultrasound guidance, with the aspiration of 20 ml of amniotic fluid. Subsequently, cityarray testing was conducted, which did not detect the 22q11.2 additionally a deletion in cytoband 2q13 has been detected, this heterozygous encompasses the NPHP1 gene, which could be associated with Joubert syndrome, juvenile nephronophthisis and Senior-Loken syndrome, taking into account its autosomal recessive nature, this heterozygous deletion alone cannot be considered pathological. Investigations were continued, and Next Generation Sequencing (NGS) for NPHP1 was performed, with a negative result for a pathogenic variant. The pregnancy continued to progress physiologically, and the patient underwent fetal morphology ultrasounds in the second and third trimesters, both with favorable results. The patient gave birth at a gestational age of 38 weeks and 3 days gestational age, via cesarean section due to metrorrhagia at the onset of labor. The patient gave birth to a female newborn weighing 3100 grams with an Apgar score of 9 at 1 minute and 10 at 5 minutes. The mother's postpartum recovery was favorable, while the newborn exhibited biological leukopenia, which spontaneously resolved within 7 days.

DISCUSSION

Since its discovery, NIPT has experienced rapid progress in its development and widespread implementation. Currently, the areas of maximum focus are represented by monogenic diseases and copy number variants, such as microdeletions and duplications. However, by far the most important application of these screening tests is the detection of aneuploidies and fetal sex, the latter having particular importance in the transmission of X-linked diseases.[15]

Currently, the utility of Non-Invasive Prenatal Testing (NIPT) in the context of diagnosing microdeletions or microduplications is subject to ongoing investigation. While findings in the literature appear promising, a more comprehensive inquiry is necessary to establish precise insights into the performance of NIPT in detecting Copy Number Variations (CNVs).

Regarding copy number variants, deep sequencing is needed to reduce the number of false-positive tests. Two major factors that influence the accuracy of the test are the fetal fraction[6] and the size of the copy number variants, the larger these are, the better the resolution of the test.[2,16]

A study conducted in China by Hua Hu et al. analyzed samples from 8141 eligible patients, focusing on both aneuploidies and syndromes of microdeletions/microduplications. Among these, 51 cases with positive results for subchromosomal anomalies were recorded, of which 13 (36.11%) were true positives. Among the false positive cases, four of them were represented by parental mutations. These results, however, underscore a relatively high rate of false positive outcomes, thereby

emphasizing the need for intensified and refined utilization of NIPT for subchromosomal anomalies.[17]

Another study conducted in China at the Ningbo Women and Children Hospital between 2015 and 2018 analyzed a cohort of 42,910 patients aged between 18 and 49 years, with gestational ages between 12 and 16 weeks. Their results revealed a total of 109 patients with positive results for subchromosomal anomalies, among which 20 cases were true positives, 49 were false positives, and 50 cases remained unconfirmed.[18]

In our case, we aimed to underline the available prenatal screening modalities and the subsequent approach adopted for a positive result. The positive result of the NIPT test for maternal del 22q11.2 prompted further testing and specialized genetic counseling to stratify the fetal risk of inheriting this microdeletion. However, due to strictly financial reasons for the patient, the investigative process to confirm the diagnosis was not pursued. Nonetheless, in the future, with the development of these testing capabilities in our country and the hope for future reimbursement of genetic testing, we aspire to conduct a follow-up for the patient.

CONCLUSION

The identification of circulating fetal DNA within maternal blood marked the inception of non-invasive prenatal testing (NIPT), which currently exhibits remarkable fidelity in detecting aneuploidies. Subsequent investigations engender optimism for the advancement of NIPT methodologies towards the detection of microdeletion/microduplication syndromes and monogenic disorders. With the continual refinement and optimization of techniques, coupled with advancements in bioinformatic algorithms, it is foreseeable that non-invasive prenatal testing (NIPT) will attain a level of precision and efficacy in detecting copy number variations (CNVs) comparable to its current proficiency in identifying trisomies. The sustained efforts dedicated to the development of these tests are justified by the numerous advantages inherent to NIPT. Consequently, it is reasonable to anticipate remarkable outcomes in the forthcoming decade.

Disclosure: None of the authors have a conflict of interest. All authors have participated equally in developing this study.

The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from the patient included in the study.

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