CASE REPORT

Non-invasive prenatal testing for sex chromosome abnormalities: a source of confusion

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SUMMARY

Cell-free fetal DNA has received significant attention for the purposes of prenatal genetic testing in the past decade. Fetal DNA testing is a new method and promising for many applications such as aneuploidy screening, prenatal diagnosis, prediction of preeclampsia and more. A 37-year-old primigravida, with a pregnancy conceived by intracytoplasmic sperm injection (ICSI), was offered non-invasive prenatal testing (NIPT) due to advanced maternal age. NIPT performed at 23 weeks' gestation reported a diagnosis of monosomy X. She was offered an amniocentesis, which revealed a euploid fetus with no sex chromosome abnormalities. Even with single nucleotide polymorphism-based NIPT, positive predictive value for detection of sex chromosome abnormalities is around 50%. Positive results of NIPT should be heeded with caution and an invasive diagnostic procedure should be performed, especially for rare chromosomal abnormalities and sex chromosome abnormalities where NIPT performs subpar compared to its performance for detection of trisomy 21.

BACKGROUND

Cell-free fetal DNA (cffDNA) has received significant attention for the purposes of prenatal genetic testing in the past decade. Non-invasive prenatal testing (NIPT), based on isolation of cffDNA from maternal blood, is a new method of testing. NIPT is promising for many applications including but not limited to: aneuploidy screening, prenatal diagnosis and prediction of preeclampsia.

Depending on the method of isolation, replication and analysis, commercially available NIPT kits yield different sensitivity and specificity ratios for each disease. Some of the available kits for fetal chromosome abnormalities offer a combined detection rate of 99% for trisomies with 0.15% false-positive rates for aneuploidy detection.¹⁻⁵ However, positive predictive value (PPV) of NIPT testing for sex chromosome abnormalities lingers around 50% at best.⁶⁻⁷ NIPT offers a much better PPV compared to available non-invasive screening tests. However, there is a misconception about NIPT results being diagnostic. Dar *et al*⁶ reported that 6.2% of women receiving high-risk NIPT results opted for elective pregnancy termination without confirmatory testing. There are numerous factors including but not limited to: placental mosaicism and abnormal maternal karyotype, which influence testing performance of NIPT. PPVs of NIPT for rare chromosome abnormalities are lower than anticipated by clinicians, given reported sensitivities; we believe it is time to raise some

awareness about this misunderstanding. We present a case of false-positive result for monosomy X diagnosed with NIPT.

CASE PRESENTATION

A 37-year-old woman, gravida 1, para 1, at 23 weeks of pregnancy conceived by intracytoplasmic sperm injection (ICSI) was evaluated in our institution. Her previous obstetrical history revealed that she was suffering from unexplained infertility for 4 years before she became pregnant by ICSI. Her obstetrical follow-up care included a quadruple biochemical screening test, which was reported as lower than cut-off risk value for trisomy 21. However, due to her age, the test reported a higher than screening cut-off risk for age alone. She was offered Harmony Prenatal Test Ariosa Diagnostics, which were performed at 23w1d gestational age. Fetal DNA ratio in her blood was reported to be 9.2%. Results showed low risk (likelihood ratio less than 0.01%) for trisomy and a diagnosis for monosomy X (likelihood ratio more than 99%). After the test results the patient was advised pregnancy termination. Before undertaking the procedure she decided to get a second opinion and was evaluated at our institution. She presented in our clinic at 26 weeks of gestation and a detailed sonographic examination was performed. The fetus, at the gestational age of 26w1d (calculated from last menstrual period) was found to be appropriate for gestational age. Structural analysis of the fetus failed to show any of the abnormalities associated with Turner syndrome, such as coarctation of aorta, bicuspid valves, lymphedema, horseshoe kidneys or cystic hygroma. In light of these findings, she was offered a diagnostic amniocentesis. The procedure was carried out and 20 cc of amniotic fluid was collected. The analysis was performed applying the fluorescence in situ hybridisation (FISH) method using CEPX locus for sex chromosome analysis. The results reported a normal euploid fetus with no sex chromosome abnormalities, a finding that was confirmed 2 weeks later by chromosome culture analysis.

OUTCOME AND FOLLOW-UP

The early FISH results reported a normal euploid fetus with no sex chromosome abnormalities, a finding that was confirmed 2 weeks later by chromosome culture analysis. Routine antenatal care was continued and a 3026 g healthy female baby was born at 39th gestational weeks.

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DISCUSSION

NIPT testing based on cfDNA is a promising new technology for early detection of genetic abnormalities. Initial research of commercially available NIPT kits reported sensitivity and specificity ratios exceeding 99%.⁸ Some physicians interpret these NIPT results as being diagnostic. However, later clinical trials have shown that PPV of NIPT is lower than anticipated by clinicians. Dar *et al*⁶ recently reported that PPV of single nucleotide polymorphism (SNP)-based NIPT for trisomy 21 is 90.9%. Although its clinical performance is well within expected performance parameters, NIPT testing performed alone cannot be used for diagnosis. Gil *et al*⁹ suggest that findings should be confirmed with an invasive procedure after an abnormal result is encountered.

Diagnosis of sex chromosome abnormalities is mostly secondary to invasive procedures performed for evaluation of autosomal chromosome abnormalities, and traditional serum screening is not aimed at detection of such aneuploidies. NIPT stands as a unique method for non-invasive screening of sex chromosome abnormalities. However, performance of NIPT for sex chromosome abnormalities is not as good as it is for trisomy 21. Despite the high-sensitivity numbers reported by some groups, for example, Hooks *et al*,¹⁰ these do not translate into high PPVs when used in a clinical setting where population incidences are much lower than in most published validation studies. PPV of such testing for sex chromosome abnormalities is around 50% at best. Placental mosaicism and maternal fetal chromosome copy numbers are confounding factors while testing for autosomal chromosome abnormalities. In addition, Wang *et al*⁷ reported maternal sex chromosome mosaicism is an important confounding factor while testing for sex chromosome abnormalities. In this study, 8.6% of all positive results of sex chromosome abnormalities were caused by abnormal maternal karyotype and the report actually calls for testing maternal karyotype to eliminate it as a confounding factor.¹¹ However, there is also the issue of low levels of X chromosome mosaicism being more common than reported by karyotype analysis due to age or technical artefacts.^{12–13} Most often, low levels of mosaicism are not reported in otherwise healthy women.

When encountered with a positive test result, clinicians must evaluate the case with caution. Using a second method such as FISH or QF-PCR chromosome analysis before taking any clinical decision is preferable. Before taking necessary steps for the confirmation of the diagnosis, interventions such as pregnancy termination may result in irreversible mistakes, which were avoided in the case presented. With this knowledge in mind, patients must be counselled on the need for high clinical suspicion or a very likely invasive procedure after a positive result from NIPT may be encountered. Similar cases have been published urging physicians to use chorionic villus sampling or amniocentesis when a positive result is obtained.^{14–15} Verweij *et al*¹⁴ claim NIPT may result in unnecessary procedures and follow-up when rare chromosome abnormalities are reported, pointing out the hypothetical lower than expected PPV of NIPT for low incidence abnormalities. Although exceedingly rare, false-negative results are also possible.

In summary, NIPT is a very strong candidate for being the future of fetal chromosome abnormality screening. The only aspect of traditional serum screening over NIPT is serum screening's cost. Neither of these testing modalities is diagnostic, and whichever is used still requires a diagnostic procedure for confirmation when a positive result is obtained. Even with SNP-based NIPT, performance of sex chromosome abnormality detection is not very high.

Learning points

- ▶ Cell-free fetal DNA is a reliable method when screening for common aneuploidies in high-risk patients.
- ▶ A positive test result should be heeded with caution and should always be confirmed by a diagnostic procedure.
- ▶ Considering the low prevalence of such abnormalities and inherent confounding factors unique to NIPT for detection of such abnormalities, results should be evaluated with caution.
- ▶ Considering the cost of the test and the cost of the diagnostic procedure that will follow in case of a positive result, the need for using this test in low-risk populations should be evaluated carefully.

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