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Detecting Mosaicism of Monosomy X Using FISH in Prenatal Samples: Post High Risk NIPT

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Abstract

Noninvasive prenatal testing (NIPT) is a highly specific and sensitive aneuploidy screening method with low false positive results. Sex chromosome aneuploidy (SCA) is not picked up in prenatal ultrasounds, as they may not have antenatally identifiable features, except for hydrops in monosomy X cases. Women with high risk NIPT results for SCAs are recommended to go for invasive prenatal diagnosis for confirmation by diagnostic tests like chromosome microarray, karyotyping, and/or fluorescence in situ hybridization (FISH). We present two cases that showed a high risk for monosomy X on NIPT. Chromosomal microarray was negative for SCA. Further, FISH was done to confirm the results and confirm the presence of low level mosaicism for monosomy X. FISH proves to be the test of choice to detect low level mosaicism in high risk NIPT cases with high positive predictive values.

Keywords
- cell free fetal DNA
- noninvasive prenatal testing
- mosaicism
- monosomy X

Introduction

Noninvasive prenatal testing (NIPT) is a highly specific and sensitive screening method with low false positive results.[1,2] Sex chromosome aneuploidy (SCA) is an abnormality of the number of X and Y chromosomes. The majority of the clinical features of SCA are observed only after birth and sometimes even after puberty. Monosomy X can present antenatally as hydrops fetalis, but mosaicism of SCAs does not present with any significant antenatal findings. Pregnant women with high risk NIPT results for SCAs are counseled for invasive diagnostic tests by amniocentesis or chorionic villus sampling[3] for confirmation with chromosome microarray (CMA), karyotyping, fluorescence in situ hybridization (FISH), and/or copy number variation by sequencing (CNV seq).[4] The sensitivity and specificity of cell free fetal DNA (cffDNA) for SCAs are 80 to 90% on average and more than 99%, respectively.[4,5] Structural rearrangements and mosaicism for sex chromosomes are estimated to be 3% and 3 to 20% of SCA cases, respectively.[6]

Mosaicism of monosomy X is present in 30 to 40% of Turner syndrome (TS) women and presents with a mild to moderate phenotype. Low levels of mosaicism are hard to detect and can be detected by FISH or high cell count on karyotype. The identification of low levels of mosaicism in TS by FISH was first documented in 2004 by Wiktor and Dyke.[7] The nondetection of low levels of mosaicism is a limitation of the methodology of low-resolution CMA.[8,9] The copy number, DNA quality, data quality, size of imbalance, and analytical methods all influence CMA's sensitivity to detecting

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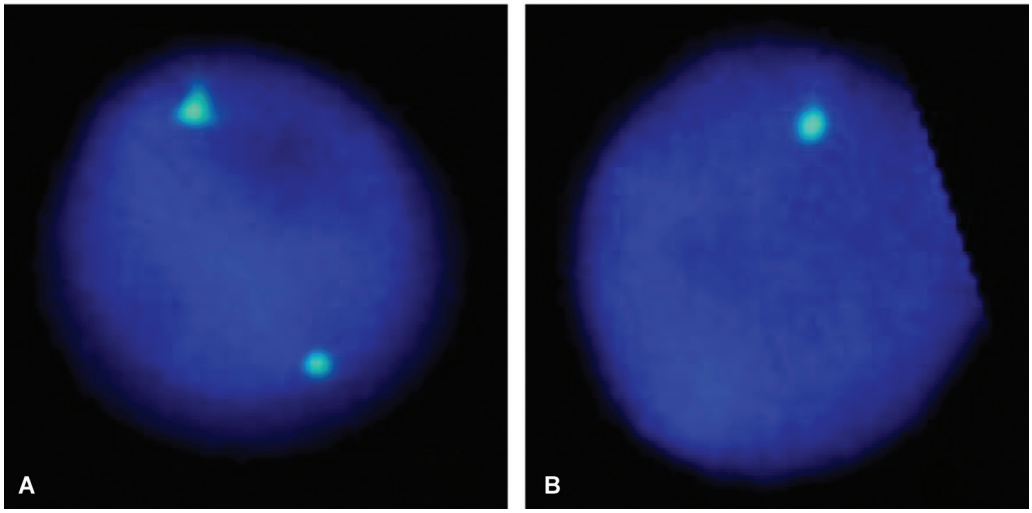

Fig. 1 Case 1: Fluorescence in situ hybridization analysis of amniotic fluid interphase cells showing (**A**) 62% normal cells (XX; 2 green signals) and (**B**) 38% monosomy X cells (XO; 1 green signal).

mosaicism. We present two cases with a high risk for monosomy X on NIPT, where FISH detected low level mosaicism but was not detected by CMA.

Case History

In both patients, pretest counseling for NIPT was done. The couple was counseled regarding the limitations of the test; the possibility of false positive and false negative as well as a need for an invasive procedure in case the result is high risk was explained. The chance of requiring a repeat sample in case of low fetal fraction was also explained. Before the invasive testing, they received a genetic consultation with a genetic counselor or clinical geneticist, after which a prenatal diagnosis was recommended. Informed consent was obtained from both patients.

Case 1

A 35 year old patient with a gestational age of 23 weeks was referred for NIPT in view of advanced maternal age. A first-trimester biochemical screen had not been done and the Nuchal Translucency (NT) scan was reported as normal. On analysis, the fetal fraction was found to be 8.04%. CHROME results showed a low risk for trisomy 13, 18, and 21, but a high risk of monosomy X (CHROME risk ratio of $>$ 90/100). Pretest counseling for the need of an invasive procedure for confirmation was done and CMA was performed on amniotic fluid (AF). The minimal risk of termination of pregnancy and infection was explained. No aneuploidy or CNVs for chromosomes 13,18, 21, or sex chromosomes were detected on low-resolution CMA. Being confident about the NIPT result, with a high positive predictive value based on internal sample validation, we performed FISH on the AF sample to reconfirm the NIPT results. No maternal cell contamination was detected by quantitative fluorescence polymerase chain reaction (QF-PCR).

FISH analysis was performed on amniocytes using the XA X/Y mix of specific probes from metasystems.[10] No aneuploidy of chromosomes 13, 18, and 21 was detected in the 150 cells analyzed. FISH done for sex chromosomes showed 62% of the cells (110/178) with XX status and 38% of cells (68/178) with monosomy X (►**Fig. 1**). This confirmed a mosaicism of disomy X and monosomy X.

Case 2

The second case was of a 28 year old female with a gestational age of 14 + 4 weeks, referred for routine NIPT. CHROME results detected a low risk for trisomy 13, 18, and 21, but a high risk for monosomy X ($>$ 90/100, CHROME risk ratio). The fetal fraction was 5.52%. The AF FISH did not detect aneuploidy of chromosomes 13, 18, and 21. But, FISH done for sex chromosomes showed 94.82% of cells (110/116) with XX status and 5.17% of the cells (6/116) had monosomy X (►**Fig. 2**), thus confirming low-level mosaicism for monosomy X.

Methodology

Eight milliliters of maternal peripheral blood were received in appropriate conditions. CHROME NIPT was executed with a validated methodology for the extraction of cffDNA from maternal blood, whole genome sequencing of cffDNA using the Illumina platform, calculation of the molecular mass of fetal DNA, and analysis with CHROME analysis pipeline version 2.1.2. Chromosomal microarray was done on AF using the Affymetrix CytoScan Optima platform and FISH analysis was performed on amniocytes using the XA X/Y mix of specific probes from metasystems 8. Maternal cell contamination was ruled out by QF-PCR in both cases.

Discussion

In both these cases, CHROME NIPT was successful in detecting a low level mosaicism for monosomy X. We wish to highlight the sensitivity of NIPT in predicting SC mosaicism. In a recent systemic review, the pooled positive predictive value was 32.0% (27.0–37.3%, 95% confidence interval) for

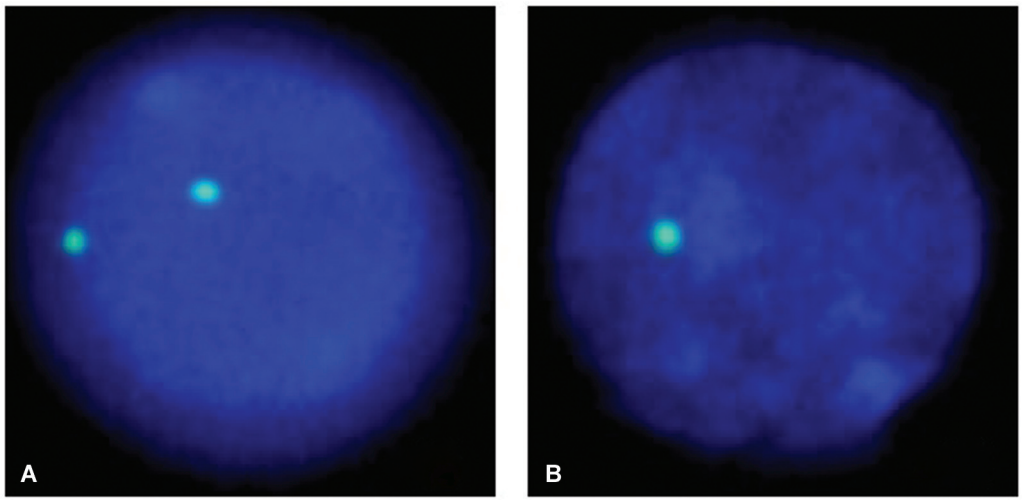

Fig. 2 Case 2: Fluorescence in situ hybridization analysis of amniotic fluid interphase cells showing (**A**) 94.82% normal cells (XX; 2 green signals) and (**B**) 5.17% monosomy X cells (XO; 1 green signal).

monosomy X, 67.6% (62.5–72.5%, 95% confidence interval) for XXY, 57.5% (5.7–63.1%, 95% confidence interval) for XXX, and 70.9% (63.9–77.1%, 95% confidence interval) for XYY.[5] We could not find any report regarding the predictive positive value for mosaicism in SCA. Though CMA is recommended for high risk NIPT cases, it did not detect any sex aneuploidy. FISH analysis detected 38% mosaicism for monosomy X in case 1 and low level mosaicism (5.17%) for monosomy X in case 2. Hence, NIPT proved to be highly sensitive and specific even in detecting low level mosaicism of monosomy X.

Though CMA is a high resolution technique that allows the detection of aneuploidies as well as CNVs in the genome, it fails to detect mosaicism of less than 30 to 40%. Traditional karyotyping remains a test of choice to detect SC aneuploidies and structural anomalies that could be associated with TS.[11] But for low level mosaicism, high cell count ($>$ 50 cells) may be required. The laboratory guidelines for TS also mention that microarrays should not be used for the initial screening of SCA.[12] Thus, cytogenetic techniques like karyotype and FISH are the tests of choice to detect mosaicism.[13]

Several studies have shown that FISH is better at detecting low level mosaicism.[7,10,14,15] However, the clinical impact of low level mosaicism has to be carefully discussed with the parents. In comparison to nonmosaic monosomy X, the phenotypic symptoms in cases of mosaic monosomy X may be mild, and many cases may even remain undiagnosed. Except for short stature and infertility in a few cases, most symptoms may not lead to any long-term consequences. It becomes difficult to predict the postnatal phenotype in low level SCA and hence, post-test counseling of parents could be emotionally challenging. An irreversible reproductive decision based on such reports may not be recommended till a wider, well studied cohort can help in creating guidelines.

Conclusion

FISH, in adjunct with karyotyping, is a powerful tool to identify monosomy X mosaicism. Since NIPT is now being widely used to screen SCA in the prenatal period, it becomes imperative to follow it up with the most conclusive follow up diagnostic test. Based on the positive predictive value, the choice of karyotype, microarray, QF-PCR, or FISH needs to be discussed for the final reproductive decision. Keeping in mind the mild clinical presentation postnatally, the post-test counseling becomes very important to help the couple make appropriate reproductive decisions. Counseling for low levels of mosaicism, especially in SCA, can be emotionally challenging and should be addressed appropriately.

Conflict of Interest

None declared.

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