METAPHASE OBSERVED IN DIRECT AMNIOTIC FLUID ANALYSIS BY FISH; AN UNEXPECTED FINDING

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ABSTRACT

To report an unexpected finding of XY metaphases in uncultured amniotic fluid leading to an unusual case of XXYO mosaicism detected at amniocentesis following a positive prenatal screening. To detect chromosomal abnormalities, fluorescent in situ hybridization technique (FISH) was performed on uncultured amniocytes followed by conventional karyotype and FISH with X and Y probes on cultured amniocytes. FISH with 13 and 21 chromosome probes gave normal results, with 18, X and Y probes showed a signal for X chromosome in 87% of cells and 116% of cells showed one signal for X chromosome and one signal for Y chromosome. Amniotic fluid cell culture gave an XX karyotype and FISH with X and Y probes on cultured cells showed two signals for chromosome X and no signal for chromosome Y. The patient delivered a healthy baby girl. The presence of metaphases in direct amniotic fluid analysis has never been described neither observed. The potential presence of XY cells in this analysis may be explained by an undetected degenerating dizygotic twin.

INTRODUCTION

Amniocentesis for chromosome analysis is routinely offered to women at risk for carrying a child with chromosome abnormalities. This technique is being rapidly but may be helped by FISH.

However, uncultured amniotic fluid cells have higher chance of maternal cells contamination and it is regularly observed in pregnancies with an anterior placenta. So, finding XY/XX cells in uncultured amniotic cells is not a rare event and most often results in maternal contamination of a XY sample. Other rarer events can explain the presence of two cell lines: chromianer and vanishing twin. This finding is always worrying because of the variable phenotypic spectrum of 46,XXXY/XXY chimeric patient. It ranges from normal male or female genitalia to different degrees of ambiguous genitalia.

Presence of metaphases in uncultured amniotic fluid has never been reported. Here, we describe a case of XY metaphase cells in uncultured amniotic cells.

MATERIAL AND METHOD

A 23 years old woman, grade 3 para 1, one previous female baby, has been referred at amniocentesis after a positive maternal screening of 1:50 for having a child with Down syndrome, PAPP-A and free β-hCGs are normal but nuchal translucency is 3.0 mm (D.D. Mm) in MiM. Amniocentesis was done under ultrasound by an experienced clinician. The placenta is anterior, 20 ml of amniotic fluid has been dispatched in three tubes: two for cell culture and one for FISH.

RESULTS

FISH on uncultured amniotic fluid gave 97.99% of cells showing 2 signals for chromosomes 13, 18 and 21. 87% of cells showing 2 signals for X chromosome and 116% of cells showed 1 signal for X and 1 signal for Y chromosome. From 116% of cells, 3% are metaphase cells (Fig 1). On cultured amniotic fluid, 500 cells and metaphase have been analysed: 2 signals corresponding to X chromosome and no signal for Y chromosome have been observed (Fig 2). Karyotype analysis confirmed female gender. Patient gave birth to a normal female baby as predicted by the complete chromosome analysis.

DISCUSSION

Presence of XXXY cells in uncultured amniocytes can be explained by maternal contamination, by mosaicism (melois error in a single zygote) or by chromianer: true chromianer as true hemiaphrodism or confirmed chromianer as confirmed bilateral chromianer (CBC) and confirmed placental chromianer (CPC). It seems that confirmed placental chromianer is more frequent than observed, may interfere with prenatal diagnosis with OVS but this discordance cannot extend to amniotic fluid where presence of XXXY cells is explained as maternal contamination which may be wrong in some situations. Finally, two different cell populations, XY/XX may be the result of differentiated cells from a residual shrunken trophoblast belonging to a “vanished” dizygotic twin. At the start point, independently from where these cells come from, in each case, even if we want to be reassuring, there is always a risk or a doubt for something wrong and that needs more investigation, as ultrasound examination for external genitalia, placental analysis and sometimes cord blood chromosome analysis. In fact, that leads to an anxious situation for patient and clinician because this can be a chromosome abnormality leading to a variable and unpredictable sexual phenotype of XYXX.

In this analysis, it seemed evident that the finding of XY cells is not a maternal contamination: majority of XX cells, XY metaphases and female genitalia and finally, only XX cells on cultured amniotic cells. Cultured amniotic cells have a lower chance of maternal contamination because during culturing processes and finding XX cells in cultured amniotic cells gives a good idea of the gender. However, this finding could be a true chromianer with female genitalia but this hypothesis can be eliminated because we found XY metaphases in direct amniotic fluid analysis not in cultured amniotic fluid analysis. There are only few tissues they are spontaneously dividing bone marrow, gonadal tissues and trophoblastic cells. So, amniotic fluid is not one of them.

So the most probably explanation for the presence of metaphases in direct amniotic fluid examination comes from some viable residual trophoblastic cells from an unsupervised and undetected developing twin. The exact time of the disappearance of twins cannot be determined but it may be expected between 6-8 weeks as Noyy’s cystography around 6-8 weeks of gestation can reveal the majority of vanishing twin as echodensities suggestive of a degenerating twin sac or sometimes an embryo cystic degeneration. At 11 weeks of pregnancy the sac may no longer visible. This patient had her first ultrasound at 12 weeks of pregnancy, no echodensities and no visible sac has been observed. Another point explaining the time of demise is the normal maternal amniotic level of PAPP-A and β-hCG. Spontaneous reduction in twin pregnancies, within 4 weeks of biochemical measurement, is associated with higher levels of PAPP-A and β-hCG. Increased nuchal translucency of the viable fetus may be probably explained by the fact of a higher prevalence of increased nuchal translucency in dizygotic twin pregnancies compared to singleton pregnancies (5.4% vs 2.4%).

We report, for the first time, the presence of XY metaphases cells in an uncultured amniotic fluid and we propose an undetected degenerating dizygotic twin for explaining this finding.

REFERENCES