Six-years' Experience of Pseudomosaicism and Maternal Cell Contamination in Cultured Amniocytes

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Purpose: To present our experiences in pseudomosaicism or maternal cell contamination in genetic mid-trimester amniocentesis confirmed through percutaneous umbilical blood sampling.
Methods: From 1992 to 1997, repeated cytogenetic evaluation with fetal cord blood was carried out in 14 cases showing mosaic patterns.
Results: We confirmed pseudomosaicism in 12 cases (85.7%) by repeated cytogenetic evaluation, and also maternal cell contamination in 2 cases.
Conclusions: Repeated cytogenetic evaluation via percutaneous umbilical blood sampling was a rapid and useful method for the confirmation of mosaicism resulted from genetic mid-trimester amniocentesis.

Key Words: pseudomosaicism, maternal cell contamination, percutaneous umbilical blood sampling, genetic amniocentesis

INTRODUCTION

Amniocentesis has been used as one of the most popular techniques to detect fetal chromosomal abnormalities since 1967 when Jacobson and Barter reported chromosomal anomalies in cultured amniocytes (Jacobson and Barter, 1967). Through karyotyping from cultured amniocytes, both numerical and structural abnormalities can be detected prenatally, but mosaic patterns, in which various cell lines exist, or maternal cell contamination are problematic in prenatal cytogenetic diagnosis.

Recently, accepted protocol regarding mosaicism has been developed at the Prenatal Diagnosis Laboratory of New York City (Hsu *et al.*, 1992). When a chromosome abnormality is found in one or more cell lines in one flask, another 20-40 cells must be examined from one or two additional flasks, and chromosome mosaicism is diagnosed only when an identical abnormality is detected in cells from two or more flasks.

But, the possibility of misdiagnosis still exists in the 'three culture flasks' method and impedes the identification of actual incidence of mosaicism. Our laboratory has adopted the policy of repeated cytogenetic evaluation with fetal blood in cases showing mosaic patterns at genetic mid-trimester amniocentesis. We will present our experiences in the pseudomosaicism or maternal cell contamination in genetic amniocentesis, which initially considered as mosaic patterns, and confirmed through percutaneous umbilical blood sampling.

MATERIALS AND METHODS

From 1992 to 1997, a total of 1446 cases of genetic amniocentesis was performed at our center. Among these, we repeated cytogenetic evaluation with fetal cord blood in fourteen cases showing mosaic patterns in amniotic fluid culture (incidence=1.0%).

The indications of fourteen genetic amniocenteses showing mosaic patterns were as follows: abnormal

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maternal serum alpha-fetoprotein(n=5), advanced maternal age (n=4), previous history of anomalous baby (n=1), history of chemotherapy due to lymphoma (n=1), known maternal reciprocal translocation between chromosome 9 and 12 (n=1). The mean maternal age was 32.9 years (range 27-40 years).

Amniocentesis was performed during the second trimester after 16 weeks of gestation. The amniocytes were cultured with Chang's media (Irvine Scientific) for 10-12 days, trypsinization (0.25%, GIBCOBRL), harvesting with 0.2 μg/ml Colcemid (GIBCOBRL) and hypotonic treatment with 75 mM KCl were performed as our standard protocol. After double fixation with Carnoy solution (3:1 v/v methanol : glacial acetic acid), slides were prepared and stained for GTG banding.

Umbilical blood sampling was carried out under direct sonographic guidance with 18 gauge needle from either the placental insertion of the cord or a free loop. A sample of 1.5-3 ml was aspirated into a pre-heparinized syringe. Fetal blood was confirmed by measurement of mean corpuscular volume. An aliquot was then incubated with Ham's F-10 media (GIBCOBRL) containing phytohemagglutinin (GIBCOBRL) for 72 h. Harvesting, hypotonic treatment, fixation, and then, GTG banding were performed as our standard protocol. At least 30 metaphases per specimen were scored for defining mosaicism. All cytogenetic description followed as ISCN (1995). All patients were given a thorough explanation about the procedure and its possible risks and they gave informed consent.

RESULTS

Fourteen cases, in which mosaicisms were found in amniocytes, involved four with numerical chromosomal abnormalities, seven with structural chromosomal abnormalities and three with different sex chromosomal constitution. Repeated cytogenetic evaluation via percutaneous umbilical blood sampling excluded mosaicism in 12 cases (No. 1~10, 13~14)(85.7%). Overall incidence of 0.83% pseudomosaicism was noted. We also confirmed maternal cell contamination in No. 13, 14, which finally showed normal chromosomal pattern of male. A different mosaic pattern was found in No. 12, in which translocation mosaicism was found initially between chromosome 3 and 21, but finally inversion 12 mosaicism. In cases with pseudomosicism and/or maternal cell contamination as a final interpretation, all babies were born without any phenotypical abnormalities, although preterm delivery occurred in No. 2, 8 and 13, and loss of follow-up in No. 9. Fetus with true mosaic 47,XXY/46,XY (No. 11) was terminated under the consent of parents, and No. 12 was lost to follow-up. Postnatal karyotyping was not carried out in any cases.

DISCUSSION

Our data clearly show that repeated cytogenetic evaluation with fetal blood is very useful for the verification of pseudomosaicism, and such policy enables us to identify the actual incidence of this phenomenon and to prevent inadvertent termination of normal pregnancies.

We were able to exclude mosaicism by repeated cytogenetic evaluation of cord blood in 12 cases, which were initially considered as mosaic (85.7%). This figure was not significantly different with the other reports; 87.0% (20/23) (Shalev *et al.*, 1994), 75.6% (31/41) (Gosden *et al.*, 1988).

We experienced two cases of maternal cell contamination, in which mosaic 46,XX/46,XY at initial amniocentesis was finally confirmed to normal male. In case of No. 9, in which showed different sex chromosomal constitution, contamination of 46,XY cell line was a very curious event, but it could occur occasionally from unknown source as described in other report (Worton *et al.*, 1984).

It was depicted that mosaicism found in amniotic fluid culture may indicate one of the followings; 1) the presence of true mosaicism of a major chromosomal abnormality in the fetus, 2) a contamination from maternal tissue, 3) non-disjunctional or post-zygotic error restricted entirely to extra embryonic membrane and trophoblast, 4) in vitro changes in cultured amniotic fluid cells of unrecognized dizygotic twin pregnancy with early death of an abnormal twin and persistence of its trophoblast (Shalev *et al.*, 1994).

In 'three culture flasks' method, level 1 mosaicism was defined by single abnormal cell, level 2 by multiple cells with the same abnormality in a single flask or colony, and level 3 by multiple abnormal cells in multiple flasks or colonies. Level 3 is considered as a strong indication of true mosaicism, but it was reported that more than 40% of this level are actually normal, and in level 2 mosaicism, 80% are found to be normal (Bui *et al.*, 1984 ; Hsu *et al.*, 1984 ; Worton *et al.*, 1984).

It is interesting to note that all cases with pseudomosaicism (No. 1~10) had abnormal cell constitution of 20% or below. No. 11 showing true mosaicism had 47,XXY cell line in 28.0% at initial amniocentesis, and subsequent 22.0% at cordocentesis.

Table 1. Initial results of genetic amniocentesis and final results from repeated cytogenetic evaluation through fetal cord blood

No. of Case	Indication of genetic amniocentesis	Initial result from genetic amniocentesis	Final result from fetal cord blood
1	high AFP	46,XY,18q-[5]/46,XY[25]	46,XY
2	high AFP	46,XY,t(5;15)[3]/46,XY[70]	46,XY
3	high AFP	47,XY,+13[1]/46,XY[69]	46,XY
4	high AFP	46,XY,t(5;9)[4]/46,XY[40]	46,XY
5	low AFP	45,X[1]/46,XX[49]	46,XX
6	previous baby anomaly	46,XY,t(6;7)[6]/46,XY[24]	46,XY
7	chemotherapy	46,XY,t(1;18)[5]/46,XY[55]	46,XY
8	old age	46,XY,t(1;11)[4]/46,XY[25]	46,XY
9	old age	46,XY[1]/46,XX[49]	46,XX
10	maternal 46,XX,t(9;12)	45,X[10]/46.XY[50]	46,XY
11	old age	47,XXY[14]/46,XY[36]	47,XXY[11]/46,XY[39]
12	NA	46,XY,t(3;21)[8]/46,XY[30]	46,XY,inv(12)[7]/46,XY[60]
13	NA	46,XX[10]/46,XY[22]	46,XY
14	old age	46,XX[8]/46,XY[30]	46,XY

AFP : alpha-fetoprotein, NA : Not available

In a recent series of 12,000 cases studied according to protocol developed at Prenatal Diagnosis Laboratory of New York City, 927 cases (7.7%) of pseudomosaicism and 24 cases (0.2%) of true mosaicism were diagnosed (Hsu *et al.*, 1992). There was a considerable variation between reports with regard to the frequencies, although our incidence of 0.83% pseudomosaicism seems to be somewhat smaller. But, most importantly, the possibility of misdiagnosis still exists in the 'three culture flasks' method, and the actual incidence of mosaicism is to be confused.

In conclusion, repeated cytogenetic evaluation via percutaneous umbilical blood sampling is a rapid and useful method for the confirmation of mosaicism resulted from genetic mid-trimester amniocentesis.

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