MOLECULAR AND EXPERIMENTAL BIOLOGY IN MEDICINE

TWO SUCCESSFUL PREGNANCIES IN A WOMAN WITH MOSAIC FORM OF DOWN SYNDROME

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Abstract: We present two successful pregnancies in a patient with mosaic trisomy 21, diagnosed shortly after birth with a proportion of 20% trisomic cells. Amniocentesis was performed in both pregnancies and normal female karyotypes were obtained by classical cytogenetic analysis. Fluorescence in situ hybridization (FISH) showed trisomy 21 in 10% of patientôs peripheral blood cells and confirmed a normal fetal karyotype in amniotic fluid cell cultures from both pregnancies. Phenotypically normal female infants were delivered at 36 and 40 weeks gestation, respectively. Postnatal follow-up was uneventful. Our cases emphasize the importance of FISH as a very useful method for fast screening of large number of fetal cells within a short time for proper prenatal diagnosis.

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INTRODUCTION

About 95% of all patients with Down syndrome result from free trisomy 21, 2-4% have translocations involving chromosome 21, and only 1-2% are mosaics.[1, 2] decreased number of follicles and gonadal dysfunction have been described in females with non-mosaic Down syndrome. Despites that, a number of such patients have had normal menorrheal age and are fertile.[3] Data evaluation on pregnancies described in females with non-mosaic Down syndrome showed that they have an increased risk of delivering chromosomally abnormal offspring.[4] Beside a classical cytogenetic approach, the use of molecular cytogenetic technique, fluorescence in situ hybridization (FISH), enables the detection of a large number of nuclei in a short time, which is of great importance in mosaic cases.[5, 6]

We report two successful pregnancies in a patient with mosaic Down syndrome. Fluorescence in situ hybridization was used to provide a correct diagnosis, as accurate genetic counseling is fully dependent of it.

CASE REPORT

A 25-year gravida 2 para 0 was referred to our hospital because she was diagnosed to be mosaic for trisomy 21 in proportion of 20% for aneuploid cell line (Figure 1). The diagnosis was made shortly after birth based on the classical phenotypic signs of Down syndrome. Her mother was aged 33 at the time of her birth. The patientôs husband has a normal male karyotype. In her medical history, she had one spontaneous abortion where no additional tests were performed. The couple was counseled in the presence of the patientôs mother and they agreed on prenatal diagnosis.

Vaginal bleeding and supracervical hematoma measuring 33x17 mm were ascertained at 12 weeks of pregnancy. Fetal nuchal translucency was measured, and was 0.6 mm. The ultrasound scan at 16 weeks of gestation revealed a eutrophic fetus with moderate

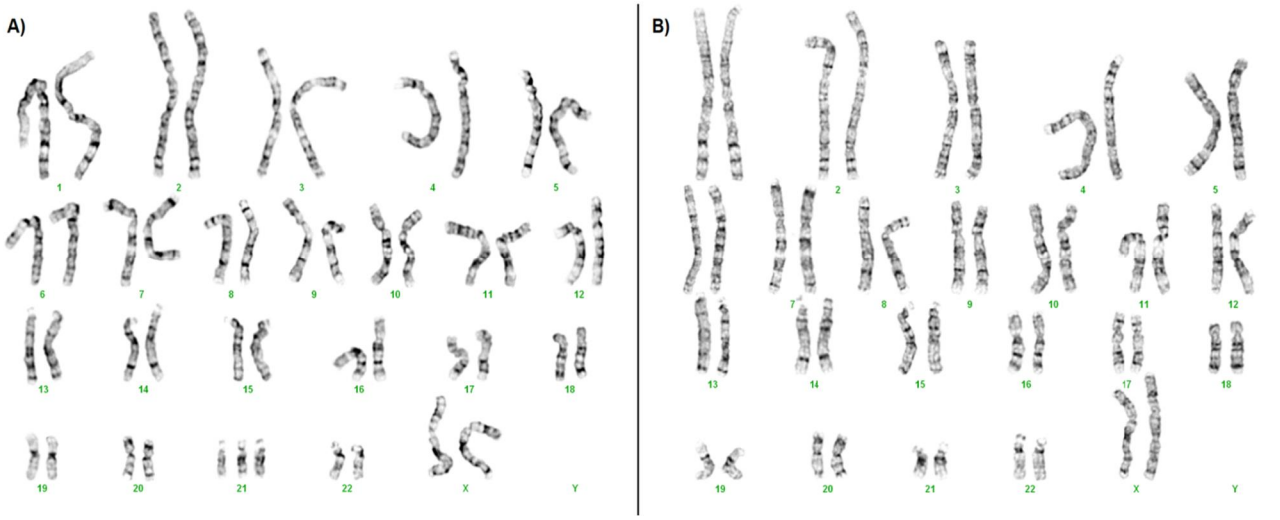

Figure 1. Cytogenetic analysis of maternal peripheral blood showing mosaicism with two cell lines: one with trisomy 21 (A), and other with normal female karyotype (B).

hyperechogenic bowel. Amniocentesis was performed at 17 weeks of pregnancy and 18 ml of dark brown-stained amniotic fluid was obtained, indicating an earlier intrauterine bleeding. Trypsin-Giemsa-banded chromosomes from maternal short-term phytohemagglutinin-stimulated whole blood culture and amniotic fluid culture were analyzed. A normal fetal female karyotype was obtained. FISH was also performed as described by Pinkel et al. using a centromere-specific 13/21 DNA probe (D13Z1/D21S55, Oncor) and a single-copy 21q22.2 (DSCR, Oncor) probe specific for Down syndrome critical region on cultivated amniotic fluid cells.[5] FISH evaluation of the native sample was not used because of maternal bleeding into amniotic fluid. At least 200 interphase nuclei per sample were analyzed. FISH studies showed trisomy 21 in 10% of the patientôs peripheral blood cells (Figure 2) and confirmed a normal female karyotype from an amniotic fluid culture sample. Fetal echocardiography showed normal heart morphology. A phenotypically normal female was vaginally delivered at 36 weeks of pregnancy. The preterm born infant weighed 2120 g and was 44 cm long (head circumference 29.5). Apgar scores were 9 and 10 at 1 and 5 minutes, respectively. Postnatal development was uneventful.

Her third and fourth pregnancies also ended in spontaneous miscarriages.

In her fifth pregnancy, amniocentesis was performed at 16 weeks of gestation, and 18 mL of clear, non-stained amniotic fluid was obtained. Chromosomal analysis on cultured amniotic fluid cells and FISH studies on native amniocytes, both revealed a normal female karyotype (100+300 cells). Fetal ultrasonography and echocardiography showed normal morphological appearance. A phenotypically normal female was delivered at 40 weeks of gestation. Postnatal development in both cases was uneventful.

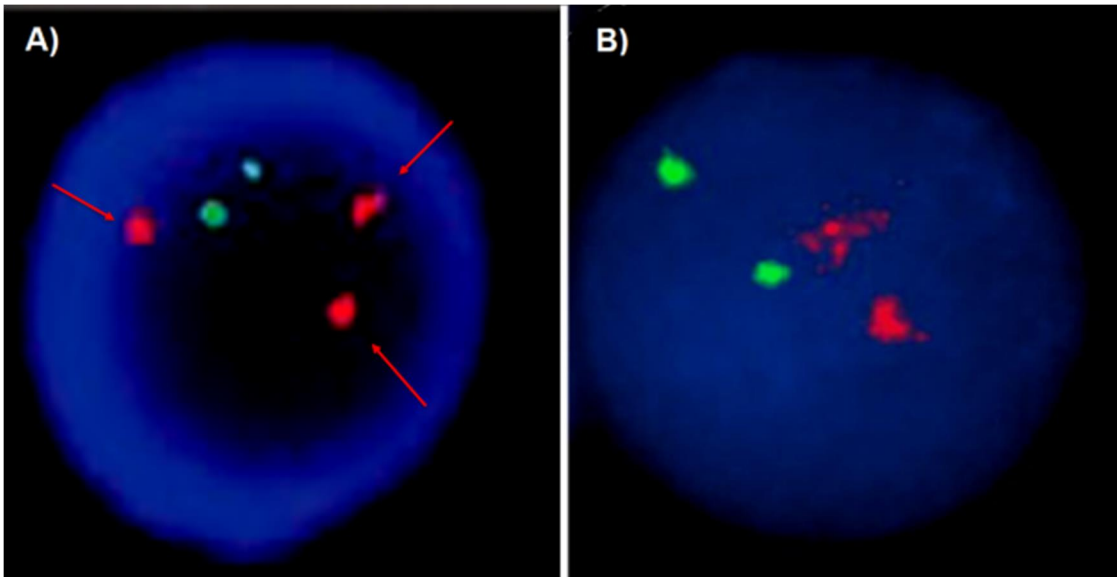

Figure 2. FISH analysis on interphase cells form maternal peripheral blood culture, using a 13q14 (green signals) and 21q22.1 specific regions probe (red signals). A) Three red signals indicate the presence of trisomy 21. B) Two red signals delineate an interphase with normal chromosomal constitution.

DISCUSSION

Fluorescence *in situ* hybridization represents a powerful technique for the investigation of a great number of cells within a short time, especially in individuals exhibiting low-level mosaicism.[5] An increase in low-level mosaicism for disomy 21 was observed in older individuals with Down syndrome. [7, 8] In their familial segregation study of Down syndrome and Alzheimer disease, Jenkins et al. noted a 15.2% increase of disomy 21 mosaicism in Down syndrome individuals older than 45 years.[7] Percentages of disomic cells ranged from 2 to 30%. A comparison of cytogenetic findings at birth and 25 years later in our patient confirms the postulation about the increase in the percentage of disomic cells with age. Shi et al. used FISH on binucleated lymphocytes of trisomic patients to simultaneously determine the percentage of mosaicism and chromosome 21 malsegregation frequency.[8] Their study on trisomy 21 patients and matched controls showed an increased frequency of cells with non-disjunction rather than with chromosome loss both in normal and trisomy 21 patients. The non-disjunction occurred more frequently in trisomic cells than in normal cells. Their work strongly suggested on the process of *in vivo* cell selection, which could explain the increase of diploid cells in older trisomy 21 patients. The same mechanism should be used to explain the decrease of trisomic cell proportion in mosaic Down syndrome individuals, which was also demonstrated in our patient.

It has been estimated that the risk of having chromosomally abnormal offspring for non-mosaic Down syndrome females is between 35 and 50%.[9, 10] On the contrary, data regarding risk estimation for mosaic form Down syndrome are not available, causing difficulties for genetic counseling for those individuals. Obstacles in setting up risk estimation arise for several reasons. One is the inability to predict a trisomic cell proportion within gonadal cells.[11] The other could be misdiagnosed low-level mosaicisms in phenotypically normal individuals. Performing FISH analysis on a large number of peripheral blood lymphocytes within phenotypically normal parents, Frias et al. noted a significantly higher proportion of trisomic cells in couples who have one or more Down syndrome children.[12] Furthermore, a low-level trisomy 21 mosaicism was found in 2/22 and 2/3 of couples who have one child and recurrent Down syndrome pregnancies, respectively. However, in their study on social conditions for people with Down syndrome Zhu et al. recorded that 7% of persons with mosaic Down syndrome had children, in comparison to only 1% of those with regular form.[13]

In conclusion, we report a successful pregnancy in a patient with mosaic Down syndrome. An increase of diploid cell proportion in mosaic trisomy 21 patients should be investigated by both standard and molecular cytogenetic techniques. FISH represents a very useful method for fast screening of large number of fetal cells within a short time, providing a correct prenatal diagnosis in such cases.

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