DNA Flow Cytometric Quantification and DNA Polymorphism Analysis in the Case of a Complete Mole with a Coexisting Fetus

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Purpose: Our purpose was to investigate whether DNA flow cytometric quantification and DNA polymorphism analysis are useful for cytogenetic diagnosis in the case of a complete hydatidiform mole that coexists with a living fetus.

Methods: Flow cytometric analysis of the nuclear DNA content and polymerase chain reaction (PCR) amplification of the minisatellite locus with the MCT118 probe were performed on the tissues (fetus, placenta and mole) obtained at the initial evacuation.

Results: DNA histograms of placental, fetal, and molar tissues showed diploid peaks. PCR products demonstrated that the allele of the mole was homozygous and inherited solely from the husband and that the mole differed genetically from the fetus and the placenta.

Conclusions: These results suggested that DNA flow cytometry and DNA polymorphism analysis may be useful for the cytogenetic diagnosis of a complete hydatidiform mole and a coexisting fetus.

KEY WORDS: complete mole; twin pregnancy; flow cytometry; DNA polymorphism.

INTRODUCTION

A twin pregnancy consisting of a complete hydatidiform mole and a coexisting fetus occurs in only 1 per 22,000 to 100,000 pregnancies (1). Because the incidence of persistent gestational trophoblastic disease is higher in patients with a complete hydatidiform mole than in patients with a partial hydatidiform mole (2), it is clinically important to distinguish between the different types of molar pregnancies.

Cytogenetic studies have shown that most complete hydatidiform moles have a diploid karyotype composed solely of paternal chromosomes (3); the majority of partial hydatidiform moles have a triploid karyotype with a maternal haploid and a paternal diploid contribution (4). Studies have suggested that cytogenetic analysis may be capable of distinguishing between complete and partial moles (5) and confirming that a pregnancy with a complete hydatidiform mole and a coexisting fetus originally involved dizygotic twins (6).

In this paper, we investigate the usefulness of DNA flow cytometry and polymorphism analysis for the cytogenetic diagnosis of a complete hydatidiform mole and a coexisting fetus.

MATERIALS AND METHODS

Materials

A 23-year-old Japanese woman, gravida 0, para 0, was referred to our hospital for further evaluation at 13 weeks' gestation because she had been experiencing a small amount of vaginal bleeding for a few days and because an echogram showed the presence of multiple intrauterine cysts. She had not previously undergone induction of ovulation. The marriage was not consanguineous. Physical examination on admission showed that the size of the uterus was consistent with 15 to 16 weeks' gestation. There was a brownish vaginal discharge. She had no overt signs of preeclampsia and hyperthyroidism. The results of laboratory tests showed no proteinuria, mild hypoproteinemia (serum albumin, 3.3 g/dl), mild anemia (hematocrit, 29.6%), mild liver dysfunction [aspartate aminotransferase, 126 IU/L (normal, 5 to 30)], and hyperthyroidism (thyroid stimulating hormone, <0.1 μ IU/L; free thyroxine, 4.92 ng/dl).

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Ultrasonography showed a live fetus with an apparently normal placenta and a separate multicystic mass with a diameter of 10 cm (Fig. 1). The multicystic mass was located in the lower portion of the uterine cavity, and the fetus and placenta in the upper portion. There was a clear demarcation between the multicystic mass and the placenta. The fetus had a crown-rump length of 5.5 cm, which was appropriate for gestational age, and showed no anomalies.

These findings suggested a twin pregnancy with a complete hydatidiform mole and a coexisting fetus. The urinary human chorionic gonadotropin (hCG) level was 1,024,000 mIU/ml, which was abnormally high for the gestational week. The serum level of α -fetoprotein was 26.9 ng/ml, compatible with 13 weeks' gestation. X-rays and computed tomography showed no lung or liver metastasis. Magnetic resonance imaging revealed no myometrial invasion.

Termination of pregnancy was recommended due to the presence of hyperthyroidism and liver dysfunction. Evacuation was performed guided by transabdominal ultrasonography. After the cervix was dilated, most of the multicystic mass was removed with forceps. The tissue had grape-like villi and a macroscopic appearance consistent with a hydatidiform mole. The fetus and placenta were also removed. Although the fetus was too fragmented to examine, the placenta appeared to be macroscopically normal. Final suction curettage was performed to remove residual tissue. Reevacuation was performed a week later, and no residual chorionic tissue was present. The patient's hypoproteinemia, anemia, liver dysfunction, and hyperthyroidism were resolved within 3 weeks of the initial evacuation. The urinary hCG level was undetectable (cutoff level, 0.2 mIU/ml) 14 weeks after the evacuation. The patient has shown no evidence of persistent gestational tropho-

blastic disease for 2 years since the urinary hCG level became undetectable.

Light microscopy of the placenta showed normal chorionic villi with vessels containing erythroblasts and without swollen stromata (Fig. 2A). The molar tissue showed swollen stromata in the chorionic villi and prominent trophoblastic proliferation, which was compatible with the diagnosis of a complete hydatidiform mole (Fig. 2B).

Methods

Flow cytometric analysis of the nuclear DNA content was performed on the tissues (fetus, placenta, and mole) obtained at the initial evacuation. About 200 mg of tissue was minced into 1-mm² specimens. The minced tissues were mixed with 0.1% RNase and 0.1% Triton X-100 in calcium-free Dulbecco's phosphate-buffered saline and stirred in a vortex mixer for 5 min. The preparation was filtered through a nylon mesh with 40- μ m pores to remove cell clusters. Nuclei were stained with 0.005% propidium iodine in Dulbecco's phosphate-buffered saline. After the preparation was again filtered through the nylon mesh, the DNA content was determined by flow cytometry (FACScan; Becton Dickinson Co., San Jose, CA) (5).

Genomic DNA was prepared from fetal, placental, and molar tissues and blood samples obtained from the parents with DNAzol reagent (Life Technologies Inc., Gaithersburg, MD). The samples were stored at -20°C until use. Samples (80 to 135 mg) of each tissue were homogenized with a handheld glass homogenizer in 2 ml of DNAzol reagent. Blood samples (5 ml) were centrifuged at 700g for 10 min, and the pellets were dissolved in 1 ml of the reagent. Genomic DNA was extracted according to the manufacturer's instructions. The DNA content was quantified spectrophotometrically.

Genetic polymorphisms resulting from a variable number of tandem repeats have been identified using various probes (7). The MCT118 (D1S80) probe is a highly polymorphic minisatellite marker. Polymerase chain reaction (PCR) amplification of the minisatellite locus was performed as described by Kasai *et al.* (8), with modifications. The sequences of the primers used were 5'-GAACTGGCCTCCAAACACTGCCCCG-CCG-3' and 5'-GTCTTGTTGGAGATGCACGTGCCCTTGC-3'. The PCR mixture contained a 2 μ M concentration of each primer, 1 mM deoxynucleoside triphosphate, 40 ng of genomic sample DNA, and 50 mU/ μ l Taq DNA polymerase (Takara, Ohtsu, Japan) in 40 μ l of the buffer supplied with the Taq polymerase.

Fig. 1. Transabdominal ultrasonography revealed a placenta and a live fetus with a normal size and shape in the upper portion of the uterine cavity and a multicystic molar lesion in the lower portion.

Fig. 2. Microscopic observation of tissue obtained at evacuation. (A) The placenta was composed of normal chorionic tissues. (B) Chorionic villi with swollen stromata and prominent trophoblastic proliferation were observed in the molar tissue, which is consistent with a complete hydatidiform mole. Hematoxylin/eosin stain. Original magnification, $\times 100$.

The PCR mixture was first incubated at 94°C for 7 min and then subjected to 30 cycles of PCR as follows: 94°C for 1 min, 64°C for 1 min, and 72°C for 8 min, followed by 10 min of extension. Amplified DNA was electrophoresed on a 4% polyacrylamide gel at 200 V for 3 hr. The amplification products were visualized directly with ethidium bromide.

RESULTS

DNA histograms of flow cytometry showed that nuclear DNA contents of placental, fetal, and molar tissues were diploid, not triploid (Fig. 3).

Amplification products from the fetal and the placental tissue showed two distinct bands with a similar pattern (Fig. 4). The molar product showed only one band. The MCT118 probe identified three alleles that differed with respect to the length of the amplified gene region. The largest fragment was present in the patient's husband, fetus, placenta, and mole but not in the patient herself. This genetic variability was inherited in an autosomal codominant manner, suggesting that the allele of the mole was inherited from the husband. The present results suggest that DNA flow cytometry and DNA polymorphism analysis may be useful for the cytogenetic diagnosis of a complete hydatidiform mole and a coexisting fetus.

DISCUSSION

A hydatidiform mole is characterized by hydropic changes and trophoblastic proliferation in the chorionic tissue of the conceptus and may occur in a complete or partial form. Complete hydatidiform moles consist entirely of hydropic villi and no fetal parts (2) and have a diploid karyotype composed solely of paternal chromosomes (5). Partial hydatidiform moles have a triploid karyotype composed of maternal haploid and paternal diploid contributions (4) and consist of partial or diffuse hydropic changes with fetal parts. Thus, the presence of fetal parts distinguishes a partial mole from a complete mole. Although rare, a complete hydatidiform mole may coexist with a fetus in a twin pregnancy (1,9), which was the case in our patient. It is important to distinguish between complete and partial moles because the risk of malignant trophoblastic sequelae is four times higher in patients with a complete hydatidiform mole than in patients with a partial hydatidiform mole (2) and because, although a triploid fetus in a partial hydatidiform mole has no chance of survival, a fetus coexisting with a complete hydatidiform mole in a twin pregnancy may survive (1).

DNA flow cytometry showed that fetal, placental, and molar tissues had diploid karyotypes in this case. The MCT118 gene contains a hypervariable region consisting of a variable number of tandemly repeated DNA sequences. Alleles differ in the overall length of

Fig. 3. DNA flow cytometric analysis. All tissues showed diploid peaks (arrows): (A) fetal tissue; (B) placental tissue; (C) molar tissues.

the segment, with a range of 368 to 850 bp. PCR analysis of the MCT118 variable number of tandem repeats in this case showed that the amplified fragments were of the expected size range, based on the position of the synthetic oligonucleotide primers and on the published sequence data, and that the mole was homozygous for the typed alleles, indicating that the mole differed genetically from the fetus and the placenta. The present results suggest that DNA flow

Fig. 4. DNA polymorphism analysis of a variable number of tandem repeats in the MCT118 region. The products of the PCR amplification from five samples were electrophoresed on a polyacrylamide gel, with size markers shown at the left. H, leukocytes from the patient's husband; P, leukocytes from the patient; Pla, placental tissue; F, fetal tissue; M, molar tissue.

cytometry and DNA polymorphism analysis may be useful for the cytogenetic diagnosis of a complete hydatidiform mole and a coexisting fetus as reported previously (9–11).

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