Introduction

Molar pregnancy is defined as a non-viable pregnancy with trophoblastic proliferation and an imbalance in the number of haploid sets of paternal and maternal chromosomes. Molar pregnancies occur in about 1/5000 to 1/3000 pregnancies in the western hemisphere, and are more common in some Asian countries. Molar pregnancies typically abort in the first trimester, and they are associated with a risk for persistent trophoblastic disease. The most concerning trophoblastic disease is choriocarcinoma, which can occur in up to 7% of women with a history of complete molar pregnancy. Choriocarcinoma is an aggressive malignancy, but it is highly responsive to chemotherapy.

Complete molar pregnancy is characterized by florid trophoblastic proliferation in the context of greatly reduced or absent embryonic tissue. Clinically, complete molar pregnancy is characterized by greatly elevated hCG levels and by the absence of an embryo on ultrasonography. Complete moles are nearly always diploid and are considered complete unisexual disomy because they consist solely of maternal DNA (excluding mitochondrial DNA). Complete moles are most commonly associated with fertilization of an “empty ovum” (egg lacking nuclear DNA by one spermatozoon) that subsequently duplicates its DNA. Complete mole may also result from fertilization of an empty egg by two sperm. Since complete moles are diploid, cytogenetic studies and flow cytometry are of limited diagnostic utility. Currently, many laboratories use histopathologic features and clinical history to diagnose complete molar pregnancy. Immunohistochemical staining for p57 has also been utilized in some centers, with variable success.

Partial molar pregnancy is characterized by less florid trophoblastic proliferation, often in the context of an incompletely formed embryo or fetus. Ploidy analysis by flow cytometry can distinguish triploid populations. However, triploidy is a common occurrence (about 1 in 100 conceptions), and only a third of these cases are truly partial moles. STR analysis can identify triploidy and can also identify the parental origin of the extra haploid set of chromosomes. Paternally derived triploid conceptions do not result in partial molar pregnancies. A little over half of paternally derived triploid conceptions result in partial molar pregnancies.

The histopathologic features of products of conception from very early molar pregnancies may overlap considerably with those from non-molar pregnancies. Non-molar pregnancies may exhibit features of molar pregnancies, including hydropic villi. Genetic studies of short tandem repeat (STR) polymorphisms offer a robust and reliable method for definitively identifying complete molar pregnancy (see Table 1). In addition, STR analysis may be performed on blood, fresh tissue, or formalin-fixed paraffin-embedded tissue. Since the latter is often the only specimen available, STR analysis has become a useful ancillary technique for pathologic diagnosis of complete molar pregnancy.

Materials and Methods

20 cases of complete mole (n=6), partial mole (n=4), and villous hydropic change (n=2) were selected from archived formalin-fixed, paraffin embedded products of conception (Table 2). Areas of villi and decidua were microdissected from animal blue stained slides and extracted overnight with proteinase K.

DNA was amplified with the AmpFISTR Identifier kit (Applied Biosystems) and subsequently analyzed with GeneMapper (Applied Biosystems). Ploidy analysis by flow cytometry was performed on all 20 samples. Fluorescence in situ hybridization (FISH) for the RB1 locus on chromosome 13q14 was employed to investigate a triallelic pattern in one non-molar pregnancy sample (Case 4).

Results

STR analysis was successfully performed on 19 of the 20 cases (Table 2). In one sample of villous hydropic change, decidua could not be isolated from the sample. Maternal blood was not available to complete STR analysis on this case. 4 cases histologically classified as complete mole and 7 cases histologically classified as hydropic change were confirmed by STR analysis. 5 out of 9 cases histologically classified as partial mole or suspicious for partial mole were re-classified as non-molar pregnancy by STR analysis; the remaining 4 cases with STR results concordant with STR analysis. Flow cytometry results were concordant with STR analysis results for all cases of hydropic change or partial mole, but flow cytometry did not identify complete moles.

One case had a triallelic pattern at D13S317, and was demonstrated to have 3 copies of RB1 by FISH, consistent with triploidy (Figures 3 and 4). Complete moles were characterized by an increased 5 phase population by ploidy analysis, but this flow cytometry pattern was also observed in two partial moles and one non-molar pregnancy sample.

Conclusions

Short tandem repeat (STR) analysis of formalin-fixed, paraffin embedded products of conception shows high sensitivity and specificity in classifying complete and partial molar pregnancies. In addition, STR analysis reveals the different genetic mechanisms causing molar pregnancies. Ploidy analysis by flow cytometry does not detect complete molar pregnancies, and histologic classification of molar pregnancy is not as reliable as STR analysis. Autosomal trisomies and rare mutations of STR loci may be detected by STR analysis, but these entities do not result in misclassification with the use of a carefully constructed reporting algorithm. STR analysis is highly specific and is therefore a useful method for evaluating the possibility of molar pregnancy and the need for follow-up.

Acknowledgements

The authors wish to thank Carl Wittwer and Rae Lynn Wies (Flow Cytometry Laboratory, ARUP Laboratories, Maria Bettinson (Population Genetics Laboratory, Institute for Advanced Technology, ARUP) for facilitating this project.