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Original Research

Clinical application of short tandem repeat polymorphism analysis in the differential diagnosis of early hydatidiform mole and hydropic abortion

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Summary

Objective: To determine the clinical differential diagnosis value of short tandem repeat polymorphism (STR) analysis in early hydatidiform moles (HMs) and non-molar gestations. *Methods*: Four hundred eighty-one patients with suspected HMs were examined using traditional pathology methods and STR analysis. The value of the STR method in distinguishing HMs from non-molar gestations was evaluated. *Results*: Among 481 patients with suspected HMs, 177 were diagnosed with HMs and 304 with non-molar gestations based on histopathologic examination. The STR genotypic results show that 151 patients were diagnosed with HMs, including 63 complete HMs and 88 partial HMs. Three hundred thirty patients were diagnosed with non-molar gestations, including 43 patients with chromosome abnormalities. Among 63 complete HMs, 56 were monosyllabic and 7 were dispermic. All 88 partial HMs were dispermic. The histopathologic diagnoses in 84 patients were not in agreement with STR analyses. Among the 84 cases, 29 were incorrectly diagnosed with non-molar gestations and the others were incorrectly diagnosed with HMs by histopathologic examinations. The chi-test result demonstrated that the two methods were quite different. *Conclusion*: STR polymorphism analysis is a rapid, simple, and accurate method that can be utilized for accurate diagnosis of early HMs and hydropic abortions.

Key words: Short tandem repeat polymorphism analysis; Missed abortion; Hydatidiform mole; Hydropic abortion.

Introduction

The major causes of abortion in the first trimester of pregnancy are chromosome abnormalities. The incidence increases with the duration of pregnancy and childbirth, as well as the age of both men and women. In recent years, as China's "two-child policy" has been gradually liberalized, both the number of older pregnant women and the incidence of early abortion have increased. Among the pathologic tissues of patients with primary abortions, some had villous interstitial edema, mainly including hydatidiform moles (HMs) and non-molar gestations. Although HMs and non-molar gestations are similar with respect to gross pathology, HMs and non-molar gestations have abnormal pregnancy outcomes and prognosis. Indeed, accurate diagnoses of HMs and non-molar gestations affects the treatment plans and prognoses of patients.

Based on the histologic features, karyotype characteristics, and clinical histories, HMs can be subdivided into complete HMs (CHMs) and partial HMs (PHMs) [1]. Even though a HM is a benign gestational trophoblastic disease, HMs progress into the gestational trophoblastic tumors, which threaten health and quality of life.

Oncologists mainly focus on the treatment plan and the follow-up evaluation, while gynecologists pay more attention to the diagnosis of the disease. An accurate diagnosis of HMs facilitates making a decision on whether the patient requires close follow-up or whether it is necessary to take use reliable contraception during the follow-up period and to guide the childbearing plan at the end of the follow-up.

Clinical manifestations, laboratory testing, imaging examinations, and histopathologic examinations are utilized to perform comprehensive analyses and diagnoses of HMs. With the popularization of early ultrasound examinations and hug detection, HMs can be diagnosed earlier and the pregnancy can be terminated. Among tissue samples from premature abortions, both HMs and non-molar gestations have villous edema to varying degrees. For this reason, it is extremely difficult to make a morphologic feature-based diagnosis using traditional pathologic methods. Moreover, gynecologic pathologists are notoriously reluctant to distinguish between these two diseases. Two different diagnostic outcomes have changed the prognosis, which will directly affect treatment and follow-up [2, 3].

In this study, we relied on short-tandem repeat (STR) polymorphisms and histology for the diagnostic analysis of suspected HM specimens in early pregnancy. The STR polymorphism is a genetic analysis method which can assess the source of genetic material from a HM, diagnosing the HM precisely at the molecular level, and distinguishing

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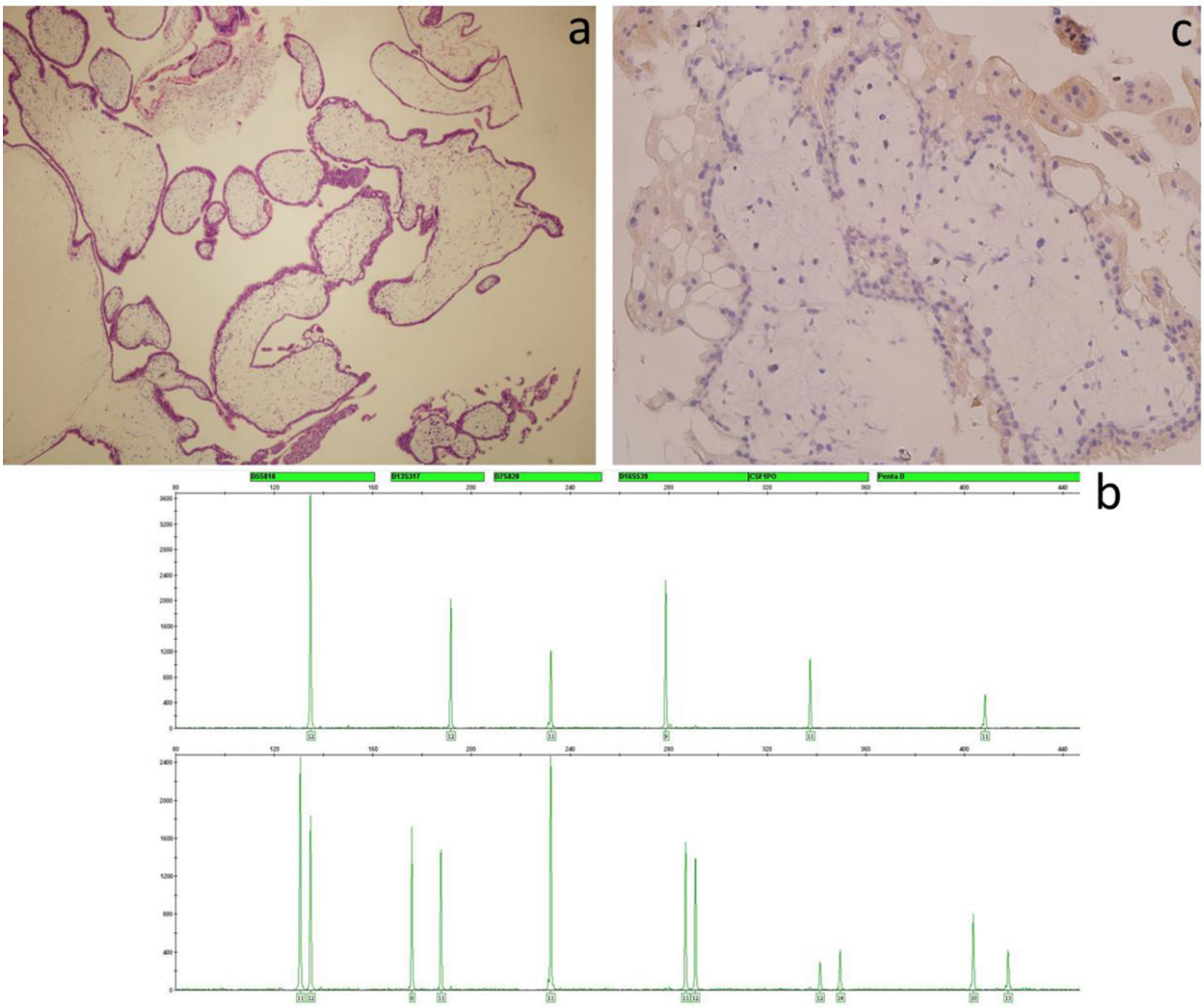

Figure 1. — Case 1: Complete Hydatidiform Mole. The patient was 22 years old, first pregnancy, 9 menopause weeks, serum β-HCG $> 10^5$ U/L before surgery, and vaginal bleeding absent. It had been incorrectly diagnosed as a non-molar gestation by the pathologic features (a). It is demonstrating exclusively paternal alleles in the villous tissue [top]. Normal biallelic profiles are seen in the maternal endometrium [bottom] (b). p57 immunohistochemical stain shows absence of staining in the villous stroma and cytotrophoblast (c).

non-molar gestations from HMs. At the same time, STR polymorphism analysis provides strong evidence for the accurate diagnosis and prognosis of early HMs, which will be of great help in the clinic setting.

Materials and Methods

Clinical information

A total of 481 missed abortion specimens, among which the pathologic diagnosis was suspected to be HMs, were collected between July 2015 and September 2017 from the Department of Birth Control (Beijing Obstetrics and Gynecology Hospital, Capital Medical University). All cases were painless abortions. Additionally, heart, lung, liver, and kidney functions were normal before surgery. Paired tissue samples of chorionic villi and maternal gestational endometrium were selected from abortion tissue specimens obtained during surgery, then rinsed with saline to eliminate the blood.

Sample preparation

All abortion tissues were formalin-fixed and paraffin-embedded (FFPE). The tissue sections were stained with hematoxylin-eosin and viewed under a light microscope to determine the existence of villous and normal maternal tissues. Furthermore, 5-8 serial section (8-μm thick) were reduced from FFPE tissue blocks stained with hematoxylin-eosin and dissected to separate the two tissue types with dissection blades under the light microscope. DNA was extracted using Simplex OUP® FFPE DNA according to the manufacturer's instructions. Furthermore, the quality of the extracted DNA was assessed by the optical density ratio (260/280).

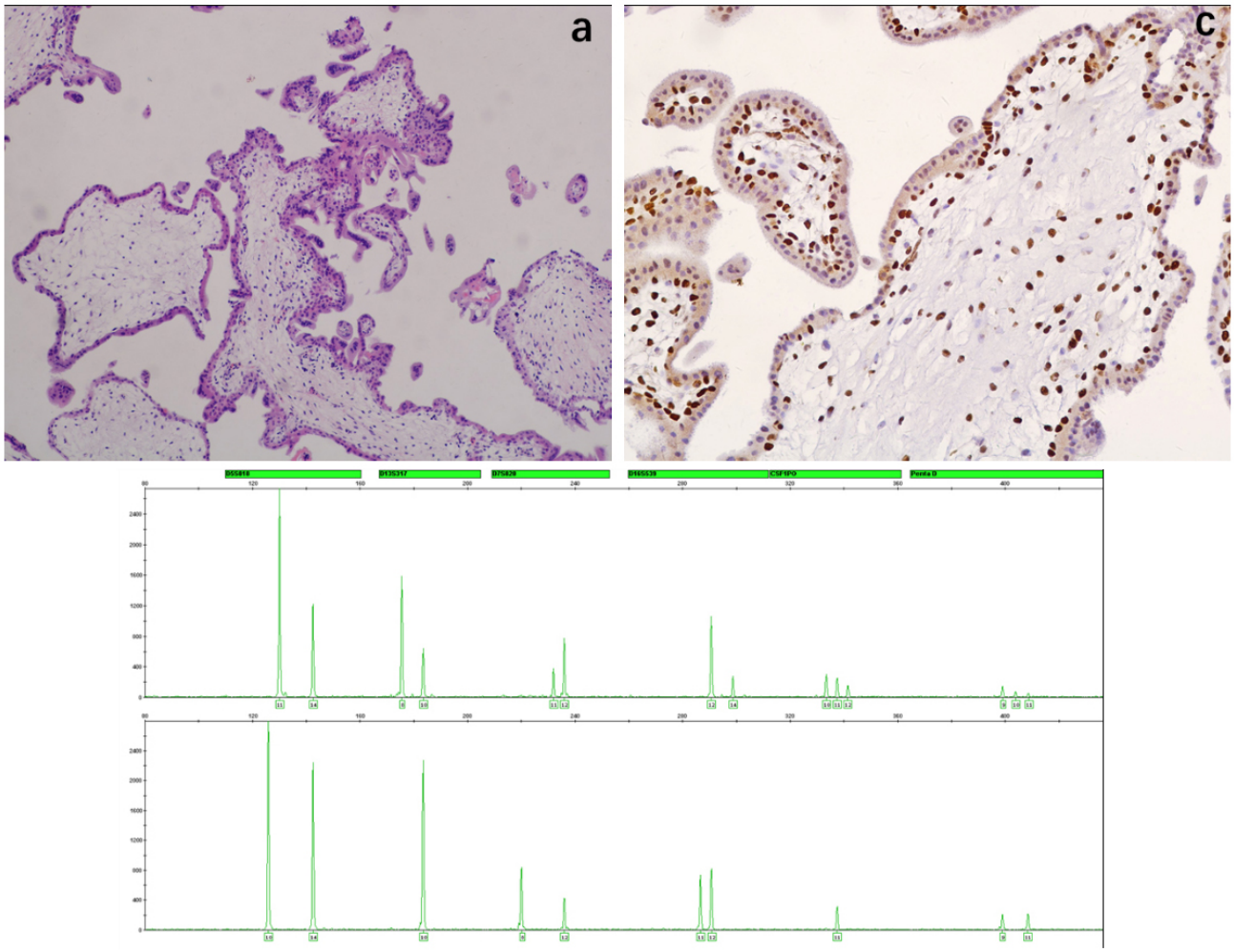

Figure 2. — Case 2: Partial hydatidiform mole. The patient was 32 years old, first pregnancy, 8 menopause weeks, serum β-HCG = 55307.5 U/L before surgery, and vaginal bleeding absent. It had been incorrectly diagnosed as a non-molar gestation by the pathologic features (a). It harbors diandric heterozygous paternal alleles in addition to one maternal allele at every locus [top]. Normal biallelic profiles seen in the maternal endometrium [bottom] (b). p57 immunohistochemical stain is positive (c).

Multiplex polymerase chain reaction

Genotyping was performed by a multiplex polymerase chain reaction (PCR) assay, which amplified 15 STR polymorphic loci (D3S1358, TH01, D21S11, D18S51, Penta E, D5S818, D13S317, D7S820, D16S539, CSF1PO, Penta D, vWA, D8S1179, TPOX, and FGA) and the sex-determining marker (amelogenin) in a single reaction. One nanogram of genomic DNA was amplified in a 25-μL reaction, which contained 5.0-μL of reaction premix, 2.5-μL of primer mix, and 17.5-μL of ddH_2O. The PCR amplification consisted of 2 min at 96 ^{o}C, followed by 10 cycles at 94 ^{o}C for 30 sec, 60 ^{o}C for 30 sec and 72 ^{o}C for 45 sec, 20 cycles at 90 ^{o}C for 30 sec, 60 ^{o}C for 30 sec and 70 ^{o}C at 35 sec, and 60 ^{o}C for 60 min. PCR products were identified by capillary electrophoresis on an ABI3500 platform. Furthermore, data collection and analysis were performed using GeneMapper® ID-X (version 1.2).

Genotyping of HMs

STR genotyping was performing using a PowerPlex 16 HS kit. Genotyping of HMs was established according to molecular diagnostic criteria [4]. Complete HMs existed when the genotypic profile of the villous tissue exclusively demonstrated paternal alleles with monophonic (homozygous paternal alleles) or dispermic patterns (heterozygous paternal alleles). Partial HMs existed when the genotypic profile of the villous tissue showed two distinct paternal alleles in at least two loci, but other alleles consisted of a duplicate quantity of homozygous paternal and maternal alleles. If genotypic profiles of the villous tissue showed a balanced ballistic profile, a non-polar gestation was diagnosed.

Immunohistochemical

Immunohistochemical p57 staining was performed using the streptavidin-peroxidase three-step method to detect p57 protein expression. Cell trophoblast and villous mes-

enchyme cells showed no nuclear expression, suggesting that there was no maternal p57 protein expression. The results were negative for trophoblast cells, intermediate trophoblast cells, villous interstitial cells, and decidua. The cytoplasmic cells showed nuclear p57 expression and the results were positive [5, 6].

Statistics analysis

Data are expressed as the mean $\pm$ S.E.M., and the differences between groups were evaluated using SPSS 13.0 software. The significance test for differences between groups was performed using multivariate analysis of variance. The mean of the two samples was compared using a chi-squared test. Statistical significance was set at a $p < 0.05$.

Results

Clinical information

The age of 481 patients was 19-51 years (average age, 31.48 $\pm$ 7.47 years). Among the 481 patients, 177 were primigravidas, 161 had two pregnancies, and 143 had $\geq$ 3 pregnancies. All patients were menopausal before surgery, including 374 patients with vaginal bleeding after menopause, 6 with abnormal uterine enlargement, 53 with a serum β-HCG $>$ 105 U/L, 12 with hyperemia, and 3 with hyperthyroidism. The clinical symptoms resolved in all patients pot-operatively, and the serum β-HCG returned to normal within 8 weeks post-operatively.

Comparison of STR diagnostic results and traditional histopathologic methods

DNA was successfully extracted from all 481 patients. The diagnostic results of STR and traditional histopathologic methods are presented in Table 1.

Pathologic examinations revealed 177 cases of villous tissue edema with uncommon degrees of trophoblastic proliferation, which was consistent with the diagnosis of HM, and 304 cases of villi and decidua tissues, which was in agreement with a diagnosis of non-molar gestation. STR molecular genotypic analysis identified 63 complete HMs (CHMs, including 56 monospermic and 7 dispermic CHMs, 88 partial HMs (PHMs), all of which were dispermic PHMs, and 330 non-molar gestations, including 43 chromosome abnormalities. The 43 chromosomal abnormalities included twenty 16-trisomies, four 8-trisomies, four 3-trisomies, four 13-trisomies, three 18-trisomies, three 21-trisomies, two 7-trisomies, one 2-trisomy, one 4-trisomy, and one comprehensive binovular and monosperm triploidy.

A p57 IHC stain examination showed that 418 cases were 2-3 + positive and 63 cases were negative, all of which were consistent with the STR genotypic results. However, the pathologic examination results of 84 cases did not agree with the STR genotypic results. As shown in Table2, the missed diagnosis rate of HMs using traditional histopathologic methods was 19.21% and the HM misdiagnosis rate was 31.08%. Twenty-nine cases diagnosed with non-molar gestations by histopathologic methods were suggested to be HMs by STR genotyping and p57 IHC stain examination, resulting in insufficient diagnoses for HMs. However, 55 cases thought to be HMs by histopathologic methods were diagnosed as HMs by STR genotyping, leading to overdiagnosis of HM.

The 29 missed diagnosis cases included 2 cases of monospermic CHMs (Figures 1 and 2) and 27 cases of dispermic PHMs. The misdirected 55 cases included 11 chromosome abnormalities (four 16-trisomies, two 3-trisomies, one 8-trisomy, one 4- trisomy, one 13-trisomy, one 18-trisomy, and one 21-trisomy). Of the 29 patients with missed diagnoses, the length of menopause diagnosed by traditional histopathologic methods was 8-14 weeks (average, 9.97 weeks), the pre-operative serum β-HCG in 6 of the 29 missed diagnosed cases was $>$ 105U/L, and the clinical examinations showed no abnormal uterine enlargement, symptoms of hyperthyroidism, or localized cysts. The results of histopathologic hematoxylin-eosin staining, p57 IHC staining, and STR genotyping results of one missed diagnosis case are shown below.

Discussion

During the pathologic pregnancy period, it is difficult to distinguish HM, especially PHM, from hydropic abortion. In the initial stage of pregnancy, the villi are immature and often lack characteristic histopathologic changes. As a result, ultrasound examinations before surgery have no distinctive signs, which are often mistaken for normal early pregnancy, and thus neglected. There were only atypical villous edema in the tissue specimens achieved during surgery. Therefore, establishing a clinical diagnosis is difficult [7].

STR

STR, known as microsatellite DNA, is a DNA repeat sequence composed of 27 nucleotides. STR is widely distributed in the non-coding region of the human genome. Because of the different repeat number in the core sequence, STR has rich polymorphisms and genetic stability [8]. By utilizing this characteristic, we not only amplified the specific DNA sequences by PCR, but also analyzed the polymorphisms. Furthermore, we quickly and accurately identified the source of DNA and chromosome ploidy in HMs and non-molar gestations. Therefore, STR can accurately distinguish between monospermic PHMs, triploids consisting of a diploid egg and haploid sperm, dispermic complete HMs, and diploid non-molar gestations. In fact, accurate diagnosis can provide dependable laboratory evidence for the determination of the causes of abortion, the length of time of follow-up, and determination of the pre-pregnancy schedule.

The advantage of STR polymorphism analysis for the identification of HMs and non-molar gestations involves the clinical application; it is of absolute necessity to distinguish HMs from non-molar gestations. Because HMs are not evenly divided into CHMs and PHMs based on histopathologic methods, the missed diagnosis and misdiagnosis rates of each type of HM are not stratified at the

Table 1. — *Summary of results.*

HM(n)					Non-molar gestation(n)									
Histologic Diagnosis	177				304									
	151				330									
Molecular	CHM(n)		PHM(n)		Normal	Trisomy (n)								
Diagnosis(STR)	MCM	DCM	MPM	DPM	diploid (n)	Trisomy 16	Trisomy 8	Trisomy 3	Trisomy 13	Trisomy 18	Trisomy 21	Trisomy 7	Trisomy 2	Trisomy 4	Triploid
	56	7	0	88	287	20	4	4	4	3	3	2	1	1	1

n: The cases of HM and Non-molar gestation.

Table 2. — *Rate of missed diagnosis and Misdiagnosis rate.*

	HM	Non-molar gestation
Histologic Diagnosis (n)	177	304
Molecular Diagnosis (STR)(n)	151	330
Consistent(n)	122	275
Rate of missed diagnosis(n, %)	29, 19.21	55, 16.67
Misdiagnosis rate (n, %)	55, 31.08	29, 9.54
p-value		0.00024

CHM and PHM level. If non-molar gestations are over-diagnosed as HMs, it will lead to more rigorous follow-up, longer contraception, preventive chemotherapy, delay to subsequent pregnancy, and give birth to miss of reproductive age. If a HM is misdiagnosed as a non-molar gestation, a lower level of diagnosis will develop, the disease process will develop further, the disease will deteriorate, and death may result. At the molecular level, according to the respective genetic characteristics, we can identify HMs and non-molar gestations accurately to help clinically-targeted follow-up of patients and detect malignant transformation as early as possible. However, because the early histopathologic characteristics of HMs are atypical and accurate identification methods are insufficient, it is difficult to distinguish HMs from non-molar gestations in clinical practice. Specifically, diagnosis of PHMs are most difficult, which has resulted in missed diagnosis and over-diagnosis [9]. Because HMs are not clearly separated into CHMs and PHMs by histopathologic method, the missed diagnosis and misdiagnosis rates of each type of HM are not stratified at the CHM and PHM level. As a result, different diagnoses outcomes have different prognoses, which will directly affect the arrangement of treatment and follow-up. In this study we compared the accuracy of the STR detection method with the traditional pathologic detection method to determine the clinical significance of the STR detection method.

The diagnostic results were comprised of STR and traditional histopathologic methods. Pathologic examinations revealed 177 cases of villous tissue edema with different degrees of trophoblastic proliferation, which was in agreement with HM diagnosis, and 304 cases of villi and decidua tissues, which was consistent with a diagnosis of non-molar gestation. STR molecular genotypic analysis identified 63 CHMs, including 56 monospermic CHMs and 7 dispermic CHMs, 88 partial HMs, all of which were dispermic PHMs, and 330 non-molar gestations. The missed diagnosis and misdiagnosis rates of HMs using the traditional histopathologic method were 30.77% and 57.14%, respectively. The missed diagnosis and misdiagnosis rates of non-molar gestations were 19.23% and 4.55%, respectively. In addition, these data were reported based on chi-squared testing; the P value was < 0.01, demonstrating that the two methods were significantly different. These results were consistent with other studies on this subject [10]. These results demonstrated that the STR method is more sensitive than the traditional histopathologic method, which can help distinguish the causes of chronic edema in early pregnancy.

Reproductive guidance for patients with different causes

Generally speaking, patients who have experienced abortions have anxiety, depression, fear, self-accusation, self-guilty, and other emotions. Patient concerns are primarily focused on physical injury and post-operative infertility [11]. In addition, a diagnosis of HM in doubt aggravates the aforementioned mood. It is thus urgent to determine the cause of abortion in a timely fashion and gives proper guidance in regenerative education. Malignant transformation of HA is rare. After the exclusion of a maternal factor, a subsequent pregnancy can be scheduled within 6 months of surgery. However, patients with a HM need increased follow-up time based on the different pathologic types. CHM has a greater chance of developing gestational trophoblastic tumors. Therefore, close follow-up is essential. Therefore, the follow-up period should be at least 1 year after the serum β-HCG returns to normal. In the case of PHMs, follow-up should not be scheduled until the serum β-HCG returns to normal or 3 months after the serum β-HCG returns to normal. Targeted contraception and birth guidance for patients with different causes of disease, as well as scientific guidance for the next pregnancy, cannot only significantly improve fertility outcomes, but also effectively protect the fertility, and physical and mental health of patients.

Conclusion

STR molecular typing technology is useful to the diagnosis of HMs, especially PHM diagnosis and typing. STR technology is significant in establishing a differential diagnosis for HMs and non-molar gestations in early pregnancy.

Acknowledgments

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Ethics Approval and Consent to Participate

This study was subject to approval by the Ethics Committee of Beijing Obstetrics and Gynecology Hospital, Capital Medical University. Written informed consent was obtained.

Conflict of Interest

The authors declare no competing interests.

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