Diagnosis of Partial Hydatidiform Mole: Histological Reassessment in Correlation with DNA Genotyping

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ABSTRACT

Background: Routine histological diagnosis of partial hydatidiform mole (PHM) continues to be challenging. DNA genotyping has recently become available to precisely separate PHM from its mimics, allowing an opportunity to reassess the histological criteria for the workup of PHM. We undertook a comprehensive reevaluation of histological parameters of PHM in correlation with DNA genotyping results.
Design: A total of 143 early abortion specimens (< 16 weeks gestational age) were included in the study. All cases have been subjected to short-tandem-repeat (STR) genotyping. Of the 143 cases, 60 were diagnosed as PHM, 52 had various chromosomal trisomies, and 31 cases were non-molar diploid gestations by genotyping. All available hematoxylin and eosin (H&E) stained slides were reviewed independently by two gynecologic pathologists blinded to the genotyping results, and the morphologic variables were evaluated in detail. Cases with major discrepancies in interpretation were re-reviewed by the two specialty pathologists together.
Results: Of the morphologic parameters assessed, the following emerged with diagnostic significance for PHM: villus size, presence of two villous populations, round or oval pseudoinclusions, at least moderate villous hydrops, cistern formation and trophoblastic hyperplasia. The average villus size of PHMs measured 3.2 mm, compared with 2.2 mm in trisomies and diploid abortions. The most sensitive (although non-specific) morphologic feature for PHM is villous hydrops (86%), or presence of at least one of the following three parameters: two villous populations, round or oval pseudoinclusions and cisterns (84%). The presence of cisterns and villous size equal to or larger than 2.5 mm had the highest positive predictive value for PHM (90%).
Conclusions: Confirming previous studies, significant histological overlaps exist among PHM, hydropic abortions and chromosomal trisomy syndromes. The presence of any one of the following histological findings should prompt DNA genotyping workup to rule out PHM: round or oval pseudoincludions, cistern formation, two populations of villi and villous size of 2.5 mm or larger. The presence of both cisterns and villous size of 2.5 mm or larger has a 90% positive predictive value for PHM.

BACKGROUND

- PHMs have a triploid – diandric monogynic – genome, arising from two sperms fertilizing an egg (90%) or from one sperm fertilizing an egg followed by reduplication of the paternal chromosome set.
- PHM is associated with ~0.5-5% risk for persistent gestational trophoblastic disease (GTD).
- Histopathological diagnosis of PHM is challenging, with poor inter- and intra-observer agreement and significant under- and overdiagnosis when using morphology alone.
- Morphologic mimics of PHM include complete hydatidiform mole (CHM), chromosomal trisomies, digynic triploid gestations, hydropic non-molar gestations and placental mesenchymal dysplasia.
- DNA ploidy analysis (by conventional karyotyping or flow cytometry) and p57 immunohistochemistry may be helpful, but are unable to reliably differentiate PHM from all of its mimics.
- DNA genotyping can accurately and reliably distinguish PHM and CHM from their mimics based on identification of parental haploid genetic contribution to the fetal tissues.

METHODS

A total of 143 early abortion specimens (<16 weeks gestational age) were selected from our departmental archives. All cases showed various degree of morphologic and/or clinical features suspicious for a molar gestation and had been subjected to short tandem repeat (STR) genotyping as part of the initial diagnostic work-up. Using genotyping results, the retrospective study cohort was specifically selected to include PHMs (60 cases), gestations with chromosomal abnormalities, suggestive of trisomies (52 cases) and non-molar diploid conceptuses (31 cases). Relevant clinical history was collected from the patients' medical records.
All available H&E slides were reviewed independently by two gynecologic pathologists blinded to the genotyping results and the morphological features were assessed in detail. Cases with significant disagreement in the morphologic features were re-reviewed by the two pathologists together (blinded to the genotyping results), until a consensus was reached. Adequacy of the specimens was defined as presence of at least 50 chorionic villi; cases with fewer villi were excluded from further analysis.
Genotyping was performed by the *AmpFlSTR Identifiler* PCR amplification system (Applied Biosystems, Inc., Foster City, CA): fifteen different STR loci are amplified in a single reaction and the allelic patterns of maternal decidua and chorionic villous tissue are then compared. The presence of two distinct paternal alleles in at least two loci is diagnostic of heterozygous (dispermic) PHM and paternal alleles in duplicate quantity in addition to a maternal allele indicate a homozygous (monospermic) PHM. Non-molar gestations show balanced biallelic profiles with both maternal and paternal alleles in the chorionic villi.

RESULTS

- On histologic review, 138 of the 143 selected cases (56 PHMs, 51 chromosomal trisomies and 31 non-molar diploid gestations) contained adequate number of chorionic villi (i.e., 50 villi or more).
- Initial review showed significant discrepancies between the two study pathologists in 50 cases (36.2%) (16 PHMs (28.6%), 19 chromosomal trisomies (37.3%) and 15 diploid non-molar gestations (48.4%)) that have been re-reviewed by the two pathologists together until a consensus was reached.

CLINICAL FEATURES

	PHM (n=56)	Trisomy (n=51)	Non-molar diploid (n=31)
Maternal age (mean)	16-42 (29.4)	18-46 (35.2)	23-43 (32.2)
Gestational age (weeks)	6 – 15+6/7	6 – 15	6 – 14+5/7
Clinical history:			
Missed/incomplete abortion/IUFD	37	44	21
Rule out PHM/ suspicious for PHM	8	0	0
Fetal anomalies	2	1	0
Ectopic pregnancy	0	1	0
Elective termination	0	1	3

MORPHOLOGIC PARAMETERS

	PHM n=56	Trisomy n=51	Non-molar diploid n=31
Maximum size of chorionic villi: range (mean)	1-6 mm (3.2 mm)	0.9-4.5 mm (2.1 mm)	1-4 mm (2.0 mm)
Two villous populations	28 (50%)	15 (29.4%)	10 (32.2%)
Round or oval trophoblastic pseudo-inclusions	28 (50%)	15 (29.4%)	10 (32.2%)
Villous hydrops (at least moderate)	48 (85.7%)	41 (80.4%)	23 (74.2%)
Cistern formation	33 (58.9%)	7 (13.7%)	9 (29.0%)
Trophoblastic hyperplasia (at least moderate)	10 (17.8%)	4 (7.8%)	2 (6.4%)
Single trophoblast inclusions	9 (16.1%)	12 (23.5%)	7 (22.5%)
Nucleated fetal red blood cells	38 (67.8%)	32 (62.7%)	20 (64.5%)
Syncytiotrophoblast knuckles	52 (92.8%)	51 (100%)	27 (87.1%)
Syncytiotrophoblast lacunae	53 (94.6%)	47 (92.2%)	27 (87.1%)

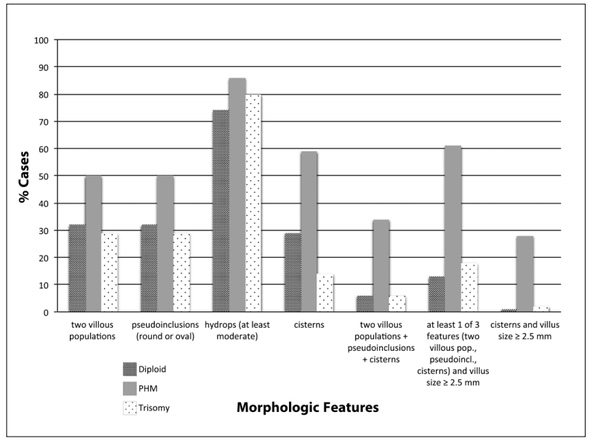

Figure 1. Morphologic features: Amongst the morphologic features assessed, the presence of two villous populations, pseudoinclusions and villous hydrops were relatively non-specific for PHM. However, cistern formation was significantly more common in PHM compared with trisomies and non-molar diploid cases. The combination of cisterns and villous size ≥ 2.5 mm emerged as the most specific feature for PHM (96% specificity), and had the highest positive predictive value (90%).

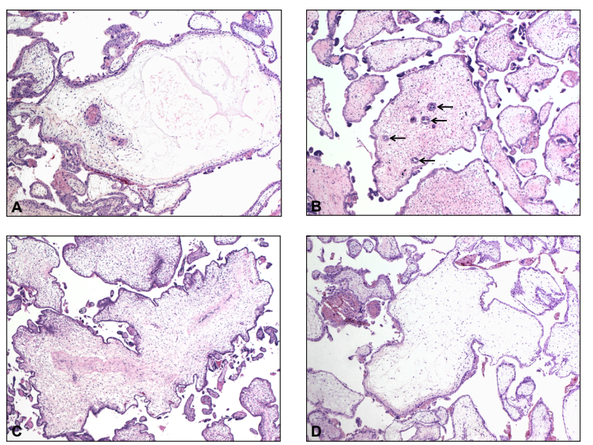

Figure 2. Microscopic features: The most characteristic microscopic features of PHM include villous size ≥ 2.5 mm, marked villous hydrops with cistern formation **(A)** and round or oval trophoblastic pseudo-inclusions (arrows) **(B)**. Chromosomal trisomies often have markedly irregular villi with trophoblastic hyperplasia, mimicking PHM – Trisomy 13, **(C)**. Non-molar diploid gestations may have significant villous hydrops, but typically lack cisterns and trophoblastic pseudo-inclusions **(D)**.

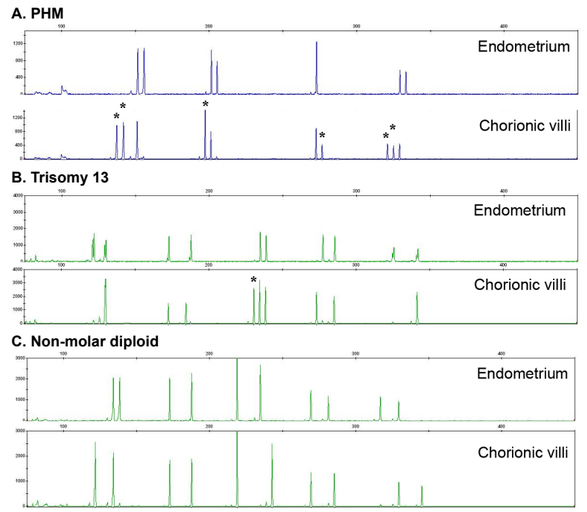

Figure 3. Genotyping results: (**A)** Dispermic (heterozygous) PHM showing two distinct paternal alleles in addition to a maternal allele. **(B)** Trisomy 13: three distinct alleles are present at D13S317 allele. All other loci showed a normal biallelic pattern. **(C)** Non-molar diploid allelic pattern with balanced biparental genetic composition.

CONCLUSIONS

- **Most morphologic features traditionally attributed to PHMs are non-specific**: e.g., villous hydrops, two villous populations, trophoblastic pseudo-inclusions, single trophoblast inclusions, syncytiotrophoblast knuckles and lacunae.
- Chromosomal trisomies and non-molar diploid gestations have significant overlapping histologic features with PHM.
- The most sensitive morphologic features for PHM are:
  - ***villous hydrops*** (86% sensitivity, 22% specificity);
  - presence of at least one of the following three parameters: ***two villous populations, round or oval pseudoinclusions and cisterns*** (84% sensitivity, 49% specificity).
- Presence of at least ***one of the above features and villous size ≥ 2.5 mm*** increases specificity to 84% (sensitivity 61%, positive predictive value (PPV) 72%).
- **Chorionic villous size is significantly larger in PHM** compared to non-molar diploid or trisomic gestations (p<0.0001).
- Presence of both ***cisterns and villous size ≥ 2.5 mm*** is the most specific and has the highest PPV for PHM (specificity 96%, PPV 90%).
- Gestations with any one of the following features: ***villous size ≥ 2.5 mm, cistern formation, two villous populations and round or oval pseudo*-inclusions,** should be subjected to molecular genotyping to confirm or rule out PHM.