Utility of p57KIP2 and Her-2 Fluorescence in Situ Hybridization in Differentiating Partial from Complete Hydatidiform Mole

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ABSTRACT

Introduction. Hydatidiform mole (HM) is an abnormal gestation characterized by significant hydropic enlargement, trophoblastic proliferation and atypia involving part or all of the chorionic villi. The diagnosis and classification of hydatidiform moles is subject to great inter-observer variability due to significant morphologic overlaps. This study aims to evaluate the utility of p57KIP2 immunohistochemistry and ploidy by Her-2 FISH in refining the diagnosis of molar tissues.

Method. 113 and 78 molar cases were retrieved from the archives of the Histopathology Section of the Philippine General Hospital and Pathology Department of Seoul National University Hospital, respectively. TMA sections were submitted for immunohistochemical analysis for p57KIP2. Ploidy was determined by fluorescence in situ hybridization using Her-2 probe. An interrater reliability analysis was done using the Kappa statistics with 95% confidence interval.

Results. All 68 (100%) cases diagnosed as CH were negative for p57KIP2 staining and are diploid. Among the 54 cases of PH, only 1 (2%) is positive for p57KIP2 and is diploid. The interrater reliability between p57KIP2 and Her-2 FISH ploidy results is 0.66 (p <.0.001), 95% CI (0.02, 1.00) which is considered "fair to good." The kappa value between review diagnosis and p57KIP2 is 0.024 while the kappa between review diagnosis and Her-2 FISH ploidy is 0.050 both signifying poor agreement beyond chance.

Conclusion. Morphologic assessment alone may not be sufficient in problematic cases. p57KIP2 in conjunction with by Her-2 FISH are good adjuncts in the diagnosis and classification of hydatidiform mole.

Key Words: hydatidiform mole, p57KIP2, ploidy

Introduction

Hydatidiform mole (HM) is an abnormal gestation characterized by significant hydropic enlargement, trophoblastic proliferation and atypia involving part or all of the chorionic villi.[1,2] They carry significant risk for developing persistent gestational trophoblastic disease in the form of a persistent mole in the uterine cavity, an invasive mole or a choriocarcinoma.[1] Choriocarcinoma arises in 10% - 30% of complete hydatidiform moles and rarely, after a partial hydatidiform mole in 0.5 – 5% of cases.[3] Thus, as much as discriminating molar pregnancy from a non-molar gestation is essential, distinction between partial and complete hydatidiform mole is also significant and has great impact in the prognosis of these patients.[3,4,5] However, the diagnosis and classification of hydatidiform moles is subject to great inter-observer variability due to significant morphologic overlaps.[6]

Ancillary techniques such as immunohistochemistry and more sophisticated methods like electron microscopy, DNA flow or image cytometry, chromosome and fluorescence in situ hybridization, polymerase chain reaction-based genotyping and cytogenetics may be utilized to arrive at a more definitive diagnosis.[4,7-11] However, most, if not all of these are technically cumbersome, expensive and are unlikely to become available in most laboratories.

Several studies have been conducted to prove the utility of p57KIP2 immunohistochemistry in the sub-classification of molar pregnancy.[4,6,8,10-13] p57KIP2 gene (CDKN1C) is a cyclin-dependent kinase inhibitor and tumor suppressor gene located on chromosome 11p15.5.[11,14] It is paternally imprinted but maternally expressed.[11] Since CMs contain only paternal genes, p57KIP2 gene should be underexpressed, in contrast to PM and HA which have contributions from both maternal and paternal genomes. However, this may pose a problem when the diagnostic dilemma is between a PH and an HA because immunohistochemical staining for p57KIP2 cannot distinguish between the two as both are immunoreactive. In these instances, other ancillary methods that detect ploidy of gestational products should be helpful.

Ploidy studies take advantage of the fact that different pathologic conditions have different genetic features. A vast majority of CH are diploid gestations and of pure androgenetic origin. It develops when an empty egg is fertilized by one or two spermatozoa resulting in two complete male-derived haploid chromosomes thus resulting to diploid gestation. Most PH are triploid gestations and are formed from fertilization of an ovum by two spermatozoa

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but fertilization of an egg by a diploid sperm cannot be ruled out entirely.[15] HA, on the other hand, is also diploid.

Whereas multiple technologies are available to determine ploidy, they are expensive, time and labor intensive and are not catered by most laboratories.[16]

Several studies have evaluated the utility of FISH using different probes to determine ploidy of hydatidiform moles and other gestational trophoblastic neoplasms including a recent study that was conducted by Le Gallo et al that used Her-2 probe.[4] Her-2/neu is an oncogene located on chromosome 17q21 that is amplified in 20 – 30% of breast cancer cases. Since it is widely used nowadays in the management of breast cancer, Her-2 probe is readily available in most laboratories compared to other probes that were suggested in past studies. In developing countries where the availability of the different molecular methods is limited, Her-2 FISH is probably one of the most readily available due to its wide application in breast cancer. This method is a useful tool in cases of diagnostic dilemma especially between partial mole and hydropic abortus which cannot be differentiated by immunohistochemistry.

In light of this information, we set to evaluate the utility of p57KIP2 immunohistochemistry and ploidy by Her-2 FISH in refining the diagnosis of molar tissues.

Methods

Case Selection

113 and 78 molar cases were retrieved from the archives of the Histopathology Section of the University of the Philippines-Philippine General Hospital and Pathology Department of Seoul National University Hospital, respectively. Two pathologists reviewed all cases simultaneously using a multi-header microscope and rendered a review diagnosis based on established criteria.[1,15,16] Both pathologists were blinded with regard to the previous histopathologic diagnosis of the cases. Cases wherein there were complete agreement between the two pathologists 'diagnoses were included in the study. The study was approved by the institutional review board of Seoul National University.

Tissue Microarray

Three areas containing villous cytotrophoblasts and mesenchymal cells were marked on the hematoxylin and eosin stained slides. Two-millimeter cores were obtained from the respective paraffin-embedded, formalin-fixed blocks, using a manual tissue arrayer. These tissue cores were inserted into a recipient paraffin block.

Immunohistochemistry

TMA sections were submitted for immunohistochemical analysis for p57KIP2 (DAKO, Freemont, USA, dilution 1:200) Three-micrometer thick TMA sections were mounted on positively charged slides and dried inside the oven for one hour. Antigen retrieval was done using standard procedure. Slides were stained using a DAKO autostainer.

Immunoreactivity was assessed among the nuclei of cytotrophoblasts and villous mesenchymal cells using a two-tiered system (Negative and Positive). A case is considered positive if nuclear immunoreactivity is observed in at least 50% of the cells.

Her-2 Fluorescent-In-situ Hybridization for Ploidy Determination

Ploidy was determined by fluorescence in situ hybridization using Her-2 probe according to manufacturer's procedure (Pathvysion Her-2 DNA Probe Kit, Abbott-Vysis, Downers Grove, IL, USA). In each case, signals of 150 tumor nuclei of villous and cytotrophoblastic cells (50 nuclei for each core) were counted using a BX51TRF microscope (Olympus, Japan) equipped with DAPI, green, orange, aqua, and triple-pass (DAPI/Green/Orange) filters (Abbott-Vysis). Since not all cores have interpretable signals, the average number of signals per case was obtained.

Detection of two green signals was considered as diploid. Cases wherein there were more than 10% of the villous stromal and cytotrophoblastic cells with 3 or 4 intranuclear signals were interpreted as triploid and tetraploid, respectively. Tissues with very weak or without signals were reprocessed.

Statistical Analysis

An interrater reliability analysis using the Kappa statistic was computed using SPSS version 17 to determine consistency among raters. Kappa values cannot exceed the range -1.0 - +1.0 and a value of 0 is equivalent to chance. By arbitrary convention, k value of < 0.40 was considered "poor," 0.41 – 0.75 was "fair to good" and ≥ 0.75 was "excellent."[17] Ninety five percent confidence intervals (CIs) was also calculated using SAS version 9.0.

Results

Histopathology

After histopathologic review of the cases, only 122 out of the 191 cases were included. Of the 122 cases, 68 were diagnosed as complete mole and 54 were diagnosed as partial mole. Reasons for exclusion of cases were missing paraffin blocks, inadequate amount of tissue for immunohistochemistry and FISH and no consensus in the review diagnoses between the pathologists. Diagnosis was revised in 22 cases. Seven cases with original diagnosis of "hydatidiform mole" were revised to CH (case nos. 1, 2, 3, 4, 7, 8) and PH (case no. 16); one case diagnosed as "products of conception" was revised to PH (case no. 13); 10 cases diagnosed as CH were revised to PH (case nos. 714, 29, 31, 33, 38, 40, 54, 64, 72, 74); 4 cases diagnosed as PH were revised to CH (case nos. 15, 17, 26, 78).

p57KIP2 Expression

Table 1 shows the results immunohistochemistry and ploidy results of all cases. p57KIP2 expression was observed in only 1 (2%) of the 54 cases diagnosed as PH (Figure 1). All 68 (100%) cases diagnosed as CH were negative for p57KIP2 staining (Figure 2). Nuclear reactivity was seen in intermediate trophoblasts and served as internal positive control. Syncytiotrophoblasts were negative in all cases.

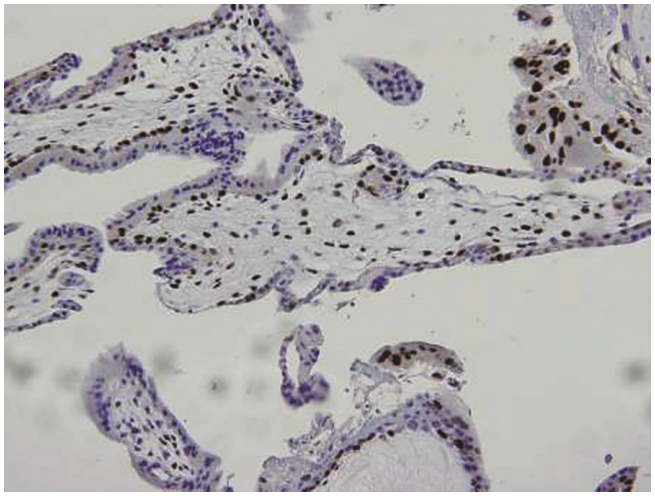

Figure 1. Partial hydatidiform mole. Positive for p57KIP2 immunostain in cytotrophoblasts and villous mesenchymal cells. (400x).

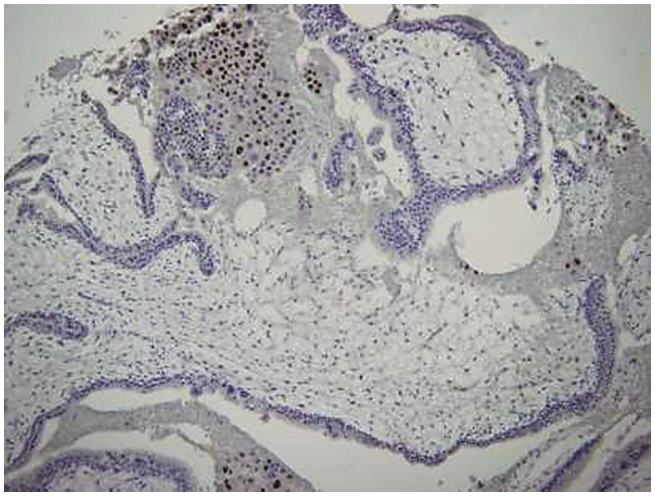

Figure 2. Complete hydatidiform mole. Negative to p57KIP2 immunostain in cytotrophoblasts and villous mesenchymal cells. Intermediate trophoblasts are positive. (40x).

Ploidy study by Her-2 FISH

FISH was not successful in 5 cases (case nos. 14, 21, 79, 98, 121). Diploid cases consisted of two green signals (Figure 3) in 32 – 96% of the cells counted and were seen in all 68 cases diagnosed as CH and 53 cases diagnosed as PH. Triploidy consisted of three green signals in at least 20% of the cells and was seen in only one case diagnosed as PH (Figure 4). There was no triploidy observed among cases diagnosed as CH.

Table 1. Morphologic Parameters used in evaluation of discordant cases

Size of villi in the population: small, large, mixed
Shape of villous borders: rounded, scalloped
Pattern of trophoblastic proliferation: circumferential, multifocal
Proliferating trophoblasts: cyto-, syncytio-, or extravillous intermediate trophoblasts
Presence of trophoblastic atypia
Presence of cisterns
Presence of vascular structures
Presence of trophoblastic inclusions

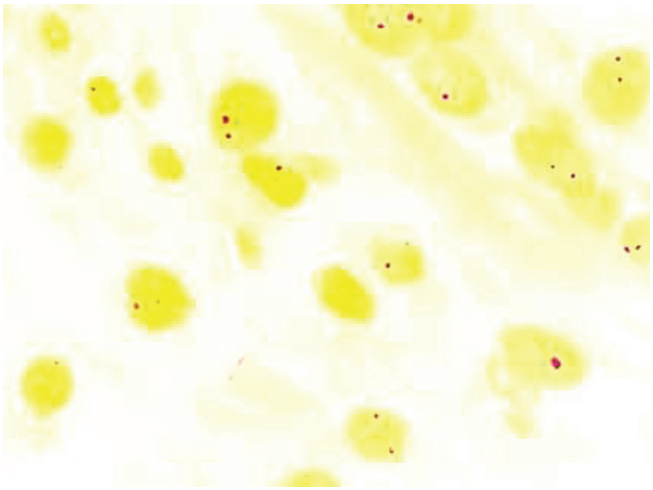

Figure 3. Complete hydatidiform mole. Her-2 FISH test showing two (CEP 17) green signals (diploid). (1000x).

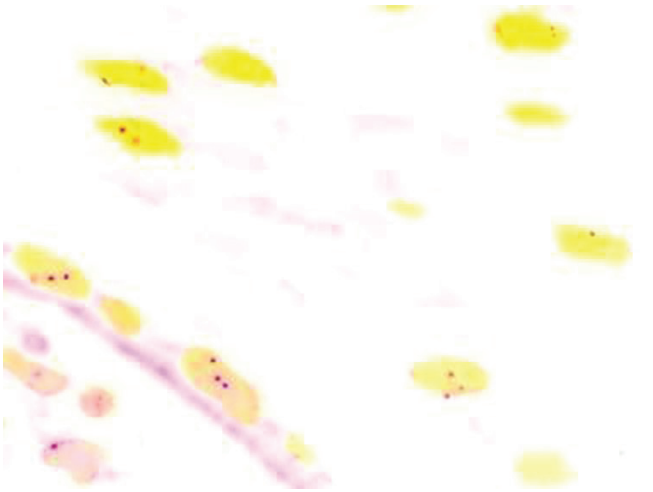

Figure 4. Partial hydatidiform mole. Her-2 FISH shows three (DEP 17) green signals (triploid). (1000x).

Table 2. Summary of the diagnostic utility of complementary use of p57KIP2 and Her-2 FISH ploidy analysis

Case No.	Review Diagnosis	p57KIP2 staining	Her-2 FISH	Final Diagnosis
10*	PH	-	D	CH
14*	PH	-	No Signal	CH
16*	PH	-	D	CH
21*	PH	-	No signal	
27*	PH	-	D	CH
29*	PH	-	D	CH
31*	PH	-	D	CH
33*	PH	-	D	CH
38*	PH	-	D	CH
40*	PH	-	D	CH
41*	PH	-	D	CH
54*	PH	-	D	CH
64*	PH	-	D	CH
72*	PH	-	D	CH
74*	PH	-	D	CH
79*	PH	**(+) CT (-) VM**	No Signal	CH
80*	PH	-	D	CH
81*	PH	-	D	CH
82*	PH	-	D	CH
83*	PH	-	D	CH
86*	PH	-	D	CH
88*	PH	-	D	CH
89*	PH	-	D	CH
90*	PH	-	D	CH
91*	PH	-	D	CH
93*	PH	-	D	CH
94*	PH	-	D	CH
95*	PH	-	D	CH
97*	PH	-	D	CH
98*	PH	-	No Signal	CH
99*	PH	-	D	CH
100*	PH	-	D	CH
101*	PH	-	D	CH
102*	PH	-	D	CH
103*	PH	-	D	CH
104*	PH	-	D	CH
105*	PH	-	D	CH
106*	PH	-	D	CH
107*	PH	-	D	CH
108*	PH	-	D	CH
109*	PH	-	D	CH
110*	PH	-	D	CH
111*	PH	-	D	CH
112*	PH	-	D	CH
113*	PH	(+) CT (-) VM	D	CH
114*	PH	-	D	CH
115*	PH	-	D	CH
116*	PH	-	D	CH
117*	PH	-	D	CH
118*	PH	-	D	CH
119*	PH	-	D	CH
120*	PH	-	D	CH
121*	PH	-	No Signal	CH
122*	PH	(+) CT (-) VM	D	CH

(Legend: PH – Partial hydatidiform mole, CH – Complete hydatidiform mole, D-Diploid, CT- Cytotrophoblasts, VM – Villous Mesenchymal cells)

Of the 54 cases that were diagnosed as partial mole on review, 49 (90%) were negative for p57KIP2 and were diploid. In the remaining five cases, two were negative for p57KIP2 and have no signals on FISH (case nos. 14, 21). Three cases expressed nuclear positivity only on cytotrophoblasts with no staining of the villous mesenchymal cells (case nos. 79,113, 122). Two of these cases were diploid and one showed no signals.

The interrater reliability between p57KIP2 and Her-2 FISH ploidy results is 0.66 ($p < 0.001$), 95% CI (0.02, 1.00) which is considered "fair to good."

The kappa value between review diagnosis and p57KIP2 is 0.024 while the kappa between review diagnosis and Her-2 FISH ploidy is 0.050 both signifying poor agreement beyond chance.

Discordant Cases

Comparing the review histologic diagnosis with the results obtained in immunohistochemistry and FISH, 53 cases were found to have discordant results. H and E slides of these cases were reviewed using the morphologic parameters presented in Table 1 and correlated them with p57KIP2 and Her-2 ploidy status. The histologic diagnosis was revised in all 53 cases (Table 2).

Discussion

In the present study, we examined the usability of Her-2 FISH in determining the ploidy of hydatidiform mole cases by comparing it with immunohistochemical examination with p57KIP2. Histologic features of each type of hydatidiform mole as well as its most important differential diagnosis, hydropic placenta have been defined extensively and repeatedly. [10,15,18-24] However, due to some overlaps in their morphology, discrimination among the three entities still continues to be a problem among pathologists, even to experts in the field.[1,2,14,22] With the emergence of ultrasonography, detection and evacuation of molar pregnancy may be done at an even earlier phase giving rise to another entity designated as early complete mole (eCH) – an addition to the list of differential diagnoses.[14] Early CH has more subtle features than CH with few morphologic overlaps with PH to which it can easily be mistaken for. Thus, recognition may oftentimes be challenging.[18]

Because of the dilemma that pathologists constantly face when presented with hydropic placental products, they have scoured for different ways and means to increase accuracy in the diagnosis -- from re-evaluation of the traditional morphologic criteria to evaluation and validation of different complex methods at the molecular level. Among those which gave gained popularity among investigators are the combination of p57KIP2 immunohistochemistry and ploidy analysis by FISH. [4,5,13]

p57KIP2 has been found to be helpful in many published studies.[1-6,8,9,10,13,14,24,25] It can very well differentiate CH from PH but requires proper interpretation in terms of the type of trophoblasts to which it should be present and the level of expression.[25] However, one of its drawbacks is that it cannot discriminate between PH and HA. Villi in both conditions will be positive for the test. An additional test such as a ploidy study is necessary to distinguished between the two.

Ploidy analysis (either by FISH, CISH or flow cytometry) can differentiate between PH and CH. PH will be triploid and CH will be diploid. It cannot however, discriminate CM from HA since both are diploid. FISH, the method we chose for this study, has three of powerful advantages: first, they do not require fresh specimen and can be used in archived material (FFPE and aspirate smears); second, it allows for correlation of FISH results with tissue morphology thus, the pathologist can easily identify which region of the section to be counted; third, it has a relatively fast turn-around time (2 days).[16,26] But FISH analysis on FFPE is not always as simple. Preanalytical and analytical difficulties factors may be encountered that may have considerable effects in the quality of signals and thus the interpretation. Suboptimal fixation, longer storage time, inadequate masking of DNA and poor hybridization may result to weak or absent signals.[4,16,26] Loss of signals were observed in 5 of our cases and this is attributed to suboptimal fixation.

In our study, there is good concordance between immunohistochemistry and FISH results in all our cases of CH. p57KIP2 was positive in only one case of PH which was also triploid by Her-2 FISH. One case may not be enough for us to derive conclusions regarding the utility of both tests in PH.

Discordant cases

Consistent with the experience of other pathologists, we also encountered difficulty in differentiating partial from complete mole based on morphology alone.[5,14,21,22] Almost all of our cases with original and review diagnosis of PH were non-reactive to p57KIP2 and were diploid on Her-2 FISH except for one case. This means that all those cases, except for one, are CH. They were diagnosed as PH because the following morphologic features of PH were observed: 1. mixed population of large and small villi (83%); 2. presence of trophoblastic inclusions (91%); 3. presence of blood vessels (47%); 4. scalloped borders (68%); 5. mild to moderate trophoblastic proliferation. These are commonly used and published criteria for PH.[15,17,18,23,27,28,29] Unfortunately, these features were also observed in CH.[12,17,22,30,31] Upon reviewing these cases, we noted that there were also features of CH that were present such as: 1. trophoblastic atypia (94%); 2. marked hydropic changes in villi (89%); 3. circumferential and/or multifocal trophoblastic proliferation in at least 2 villi (92%); 4. proliferation of the three types of trophoblasts: cyto-, syncytio-, and extravillous intermediate trophoblasts (81%); 5. cistern formation (94%).

Trophoblastic atypia

In Paradinas' paper, he included trophoblastic atypia as a finding in both PH and CH but he qualified further that it is a rarer finding in PH than in CH.[32,33] Thus, trophoblastic atypia alone is not a distinct feature of CH. Other morphologic findings mentioned above must be present.

Marked hydropic change

Marked hydropic change was also observed by Fukunaga et al in PH. In his study, he measured the size of individual villi the greatest dimension reaching up to 6.5 mm with mean of 3.3 mm.[28] In our study, all but 6 cases (11%) have at least one villi exhibiting marked hydropic change. Two of these cases were eCH (by AOG and morphology), two cases were possibly eCH exhibiting its classic features but information on AOG was not available for confirmation. In two cases, four features (#1, 3, 4, 5) of CH were present despite the absence of marked hydropic change. This would still warrant diagnosis of CH. Adequacy of sampling is one factor to be looked at.

Trophoblastic proliferation

There are several contrasting views regarding pattern of trophoblastic proliferation. Howat used circumferential trophoblastic proliferation as one of the criteria for PH.[19] Shih et al[17] and Chew et al,[34] on the other hand, considered circumferential trophoblastic proliferation as a feature of PH further qualifying that the degree of proliferation should be mild. On the other hand, Fukunaga recommended that circumferential trophoblastic proliferation observed in at least two villi warrants diagnosis of CH even if the degree of trophoblastic proliferation is mild.[28] In this study, circumferential and/or multifocal trophoblastic proliferation in at least two villi were seen in 92% of cases regardless of the degree of trophoblastic proliferation and 81% have proliferation of the three types of trophoblasts.

Cistern formation

Cistern formation, in our opinion, was a non-specific finding as it may be observed in CH and PH and HA.[31] One study claimed to have observed it in 98% of its cases of PH.[28]

It is quite taxing to pinpoint which among these criteria can reliably discriminate CH from PH. It seems that the appropriateness of the criteria is not only the sole problem for the discordance but also pathologist's interpretation of the criteria and how he/she applies them. The adequacy of sampling is an important factor as well.[21] During our initial review of all the cases, we have given more weight on the heterogeneity of the villus population and mild to moderate trophoblastic proliferation especially if they were accompanied by trophoblastic inclusions and blood vessels. We have underestimated the presence of important features of CH especially if the extent of involvement was just focal. In retrospect, based from our observations, the combination of trophoblastic atypia, marked hydropic villous change and circumferential trophoblastic proliferation in as few as two villi even in a specimen with heterogenous population is most likely a CH. eCH should be suspected if one criterion is not observed. Look for the characteristic myxoid hypercellular stroma and confirm the age of gestation. Based on our experience, CH appears to occur at a higher rate than

PH; hence, the possibility of a CH or an eCH should be ruled out first before rendering a diagnosis of PH.

P57kip2 staining pattern

We also encountered variations in p57KIP2 in three cases. Nuclear reactivity was observed only on the cytotrophoblasts and not on villous mesenchymal cells. Similar cases have been encountered in other studies and were regarded as possible cases of placental mosaicism.[12,35] Cytogenetic studies, however, are required for confirmation. Two of the cases were diploid and exhibited marked hydropic change, circumferential trophoblastic proliferation of cyto- and syncytiotrophoblasts, trophoblastic atypia and cistern formation. Thus, both cases were classified under CH. The other case has no readable signals on Her-2 FISH but reevaluation of morphology revealed it has multifocal and circumferential proliferation of the three types of trophoblasts, trophoblastic atypia and cistern formation. The morphologic features are compatible with CH.

There were two cases that were negative for p57KIP2 and no signals were seen on Her-2 FISH. Morphologic features include predominantly large villi with marked hydropic changes, circumferential trophoblastic proliferation of three types of trophoblasts, trophoblastic atypia and cistern formation. The final diagnosis for both cases was CH.

Conclusion

As anatomic pathologists, we believe that morphologic examination of a tumor is still the best method to render a diagnosis. Hence, one should try to the best of his abilities to render an accurate histologic diagnosis. However, in this study, we have once again proven the limitations of morphologic assessment alone. The use of ancillary techniques for problematic cases is highly recommended. There is no single ancillary technique that can discriminate between HA, PH, eCH and CH. Two complementary methods such as p57KIP2 immunohistochemistry and ploidy study (using Her-2 or other probes as well) should be used.

We have shown in our study that DNA ploidy analysis using Her-2 FISH probe is practical and accurate adjunct to immunohistochemical staining with p57KIP2 immunohistochemistry in the diagnosis of complete hydatidiform mole. However, further studies with more number of PH cases should be done to confirm its utility in PH. Her-2 FISH in conjunction with p57KIP2 immunohistochemistry and morphology creates a synergistic effect in clinching the diagnosis.

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Appendix
Histologic diagnosis, immunohistochemical and ploidy analyses of all cases

Case No.	Original Diagnosis	Review Diagnosis	p57KIP2 staining	Her-2 FISH	Final Diagnosis
1	H. mole	CH	-	D	CH
2	Consistent with H. mole	CH	-	D	CH
3	H. mole	CH	-	D	CH
4	H. mole	CH	-	D	CH
5	CH	CH	-	D	CH
6	CH	CH	-	D	CH
7	H. mole	CH	-	D	CH
8	H. mole	CH	-	D	CH
9	CH	CH	-	D	CH
10*	PH	PH	-	D	CH
11	CH	CH	-	D	CH
12	CH	CH	-	D	CH
13	Products of conception	PH	+	T	PH
14*	CH	PH	-	No signal	CH
15	PH	CH	-	D	CH
16*	H. mole	PH	-	D	CH
17	PH	CH	-	D	CH
18	CH	CH	-	D	CH
19	CH	CH	-	D	CH
20	CH	CH	-	D	CH
21	CH	CH	-	No signal	CH
22	CH	CH	-	D	CH
23	CH	CH	-	D	CH
24	CH	CH	-	D	CH
25	CH	CH	-	D	CH
26	PH	CH	-	D	CH
27*	PH	PH	-	D	CH
28	CH	CH	-	D	CH
29*	CH	PH	-	D	CH
30	CH	CH	-	D	CH
31*	CH	PH	-	D	CH
32	CH	CH	-	D	CH
33*	CH	PH	-	D	CH
34	CH	CH	-	D	CH
35	CH	CH	-	D	CH
36	CH	CH	-	D	CH
37	CH	CH	-	D	CH
38*	CH	PH	-	D	CH
39	CH	CH	-	D	CH
40*	CH	PH	-	D	CH
41*	CH	PH	-	D	CH
42	CH	CH	-	D	CH
43	CH	CH	-	D	CH
44	CH	CH	-	D	CH
45	CH	CH	-	D	CH
46	CH	CH	-	D	CH
47	CH	CH	-	D	CH
48	CH	CH	-	D	CH
49	CH	CH	-	D	CH
50	CH	CH	-	D	CH
51	CH	CH	-	D	CH
52	CH	CH	-	D	CH
53	CH	CH	-	D	CH
54*	CH	PH	-	D	CH
55	CH	CH	-	D	CH
56	CH	CH	-	D	CH
57	CH	CH	-	D	CH
58	CH	CH	-	D	CH
59	CH	CH	-	D	CH
60	CH	CH	-	D	CH
61	CH	CH	-	D	CH
62	CH	CH	-	D	CH
63	CH	CH	-	D	CH
64*	CH	PH	-	D	CH
65	CH	CH	-	D	CH
66	CH	CH	-	D	CH
67	CH	CH	-	D	CH
68	CH	CH	-	D	CH
69	CH	CH	-	D	CH
70	CH	CH	-	D	CH
71	CH	CH	-	D	CH
72*	CH	PH	-	D	CH
73	CH	CH	-	D	CH
74*	CH	PH	-	D	CH
75	CH	CH	-	D	CH
76	CH	CH	-	D	CH
77	CH	CH	-	D	CH
78	PH	CH	-	D	CH
79*	PH	PH	(+) CT (-) VM	No Signal	CH
80*	PH	PH	-	D	CH

81*	PH	PH	-	D	CH
82*	PH	PH	-	D	CH
83*	PH	PH	-	D	CH
84	PH	CH	-	D	CH
85	PH	CH	-	D	CH
86*	PH	PH	-	D	CH
87	PH	CH	-	D	CH
88*	PH	PH	-	D	CH
89*	PH	PH	-	D	CH
90*	PH	PH	-	D	CH
91*	PH	PH	-	D	CH
92	PH	PH	-	D	CH
93*	PH	PH	-	D	CH
94*	PH	PH	-	D	CH
95*	PH	PH	-	D	CH
96	PH	CH	-	D	CH
97*	PH	PH	-	D	CH
98*	PH	PH	-	No signal	CH
99*	PH	PH	-	D	CH
100*	PH	PH	-	D	CH
101*	PH	PH	-	D	CH
102*	PH	PH	-	D	CH
103*	PH	PH	-	D	CH
104*	PH	PH	-	D	CH
105*	PH	PH	-	D	CH
106*	PH	PH	-	D	CH
107*	PH	PH	-	D	CH
108*	PH	PH	-	D	CH
109*	PH	PH	-	D	CH
110*	PH	PH	-	D	CH
111*	PH	PH	-	D	CH
112*	PH	PH	-	D	CH
113*	PH	PH	(+) CT (-) VM	D	CH
114*	PH	PH	-	D	CH
115*	PH	PH	-	D	CH
116*	PH	PH	-	D	CH
117*	PH	PH	-	D	CH
118*	PH	PH	-	D	CH
119*	PH	PH	-	D	CH
120*	PH	PH	-	D	CH
121*	PH	PH	-	No Signal	CH
122*	PH	PH	(+) CT (-) VM	D	CH

* Discordant cases.