Diagnostic Pathology: Open Access

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The Role of p57^{kip2} Immunohistochemistry in Differentiating Complete from Partial Hydatidiform Mole

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Abstract

Background: The examination of morphological features of gestational products accounts as a core diagnostic process, especially for the distinction of complete hydatidiform mole (CM) from Partial Mole (PM) cases. Nevertheless, subjective evaluation of the traditional histopathological criteria might occur with substantial inter-observer variability.

Objective: To assess the utility of p57^{kip2} immunohistochemical (IHC) expression in distinguishing the CM from PM cases.

Materials and methods: This study was a cross-sectional analysis conducted entirely among 34 patients, including those cases with molar pregnancies and product of conception after uterine evacuation. All cases were recruited between January-July 2018 into a Gynecology and Obstetrics Department. Together with the histomorphological assessment, we performed p57^{kip2} IHC staining in all the specimens.

Results: The histological diagnostic categories were as follows: CM (n=12), PM (n=8), and placenta and non-molar product of conception group (n=14), based on previously reported criteria and IHC. Accordingly, the morphological complete mole diagnosis was consistent with p57^{kip2} IHC, displaying cytotrophoblast and villous stromal cells with a negative stain in 9 out of 12 observed compelete specimens. However, one case had aberrant p57^{kip2} expression and two others were morphologically concerned, having a mild degree of villous edema and greater scalloping morphology. The later cases confirmed as PMs based on IHC p57^{kip2} positive staining. For PM, almost all cases histologically had consistent IHC findings with positive p57^{kip2} immunostaining in cytotrophoblast and villous stromal cells. Two cases were lacked p57^{kip2} marker positivity and considered as CM albeit with a milder degree of trophoblast hyperplasia. All products of conception and hydropic abortion showed fewer villi formation and positive p57^{kip2} immuno-reactivity.

Conclusion: This study further confirms the importance of p57^{kip2} immuno-staining as an ancillary test with the traditional histopathological criteria to distinguish complete mole from other mimic cases.

Keywords: Hydatidiform mole; Hydropic pregnancy; Immunohistochemistry; p57^{kip2}

Introduction

Hydatidiform mole is regarded as molar pregnancy that has a potential to form inside the uterus at the beginning of pregnancy, due to abnormal proliferation of placental villous trophoblasts. It classifies into either CM or PM based on clinical, morphologic, and genetic alterations [1]. The former is attributed to single or duplicate sperms joining a lost DNA egg, with an incidence of almost 90 or 10%, respectively [2]. By this way, the karyotype is 46, XX (diploid) owed to entry and fertilization of empty ova by two sperms or duplicated sperm but less common might be 46, XY (diploid) [1,3]. In contrast, PM arises once an egg is fertilized by two sperm or even by one sperm that reduplicates itself developing the triploid genotype (69, XXY or 69, XXX or 69, XYY) [4]. Prompt and appropriate management of cases of molar pregnancy will probably minimize such disease complications, highlighting the necessity of follow-up.

The persistence and development of malignant sequelae of gestational trophoblastic disease is a major concern. In Western countries, the risk of CM to be essentially progressed into choriocarcinoma is less than 5% whereas it increases to 10-15% in Asian inhabitants [5]. CMs account for 50% of all cases of choriocarcinoma [6]. Moreover, there is about 15% chance of CM to turn into an invasive mole [7]. Partial moles may also become invasive with less than 5% risk but rarely associated with choriocarcinoma [8,9]. This implies that the actual identification of the products of conception is crucially affecting the therapy responses.

Despite the existence of altered risk outcomes of molar pregnancy, post-evacuation recommendations are still identical for its persistent disease or metastasis. To great extent, regular examination of serum human chorionic gonadotropin levels occur till normalization and followed by monthly monitoring for up to 6 months [10,11]. In addition, core individual morphologies have been anticipated to identify samples of a product of conception consist of CMs, PMs, as well as hydropic abortion (HA). Nevertheless, these entities exhibit a considerable histo-pathological similarity in particular within cases of

Citation: Khalifa ES (2019) The Role of p57kip2 Immunohistochemistry in Differentiating Complete from Partial Hydatidiform Mole. Diagn Pathol Open 4: 153.

Page 2 of 7

early recognition that probably leads to inter-observational and intra-observational variability during the diagnosis of the product of conception cases [12-14]. Accordingly, the appearance of hydropic villi in the product of conception continues to be a challenge encountering the pathologist; even for those experts, if they are based on the histological diagnosis alone, especially to discriminate PM from HA [14]. Collectively, both accurate classification of the molar pregnancy as partial or complete moles, along with differentiating these conditions from HA have often management and prognostic value. An adjuvant though related studies involving ploidy, cytogenetics, and IHC analyses may be particularly helpful in revealing the underlining genetics of such aspects; and also perhaps enable better diagnosis and classify of Hydatidiform mole [10].

P57^{kip2} is a potent cyclin-dependent kinase inhibitor member protein that has the ability to regulate definite cellular processes, and it is the product of paternally imprinted Cyclin-Dependent Kinase Inhibitor 1C (CDKN1C) gene located on short arm of chromosome 11. The p57^{kip2} was considered initially as tumor suppressor relied on its ability to inhibit cell growth cues of upstream signaling networks, albeit suggesting not so simple the situation [15]. Accordingly, it is enrolled in other cellular regulation mechanisms, namely apoptosis, transcription, and cytoskeletal dynamics. Together with inhibition of cyclin-cyclin dependent kinase complex, p57^{kip2} often modulates the apoptosis process through binding of its QT domain (aa 238-316) to the pathway of stress-signaling and thereafter block its kinase activity (c-Jun NH2-terminal kinase 1/Stress-activated protein kinase [JNK1/ SAPK] activity) [15,16]. Moreover, transcription factors regulation could be promoted *via* p57^{kip2} directly when an interaction of its N-termini region with MyoD occurs, thereby, stabilize MyoD and enhance transcription of its target genes [15,17]. Kip protein can also act as an indirect inhibitor of E2F-mediated transcription process by inhibiting complexes of cyclin-cyclin dependent kinases with subsequent hypophosphorylation of RB protein that sequestering E2F transcription factors [15,18]. To some extent, the cytoskeletal dynamics process may be regulated following binding of cytosolic p57^{kip2} to LIM-Kinase molecule, inducing its nuclear translocation that ultimately losing actin stress fibers formation [15,19,20]. Collectively, these findings have suggested robust evidence that the Kip family of cyclin-dependent kinase inhibitor is a multifunctional protein to regulate cellular process; not merely involved in restricting division of the cell, and perhaps has other unrevealed functions so far [15]. This denotes the need for more effort in understanding p57^{kip2} regulation and its inter-connection roles with other corresponding proteins. Recently, the CDKN1C gene, encoding the p57kip2 protein, has been considered as a strongly paternally imprinted gene though exclusive or preferential expresses from maternal allele at chromosome 11p15.5 location [10,12,21-23]. As the component of maternal genome is to be deficient in CMs, it is unlikely the imprinted gene which is normally derived from maternal allele has to be expressed; and this suggested the p57^{kip2} IHC analysis may be a critical implementation for the diagnosis of a CM [10,12,24-28]. Conversely, p57^{kip2} IHC staining could not recognize between PMs and HA [10].

Aim of the Study

In our study, we aimed to assess the role of p57^{kip2} IHC staining and histo-morphological features in order to differentiate CM from PM, especially at the early stage of gestation.

Materials and Methods

Sample collection and histopathological assessment

This study was a cross-sectional analysis conducted entirely among 34 patients, including those cases with molar pregnancies and product of conception (POC) after uterine evacuation. All cases were recruited during the period from January to July 2018 into a Gynecology and Obstetrics Department at Al-Yarmouk Teaching Hospital in Baghdad, Iraq. The histological samples, patients consent and the processing of immunohistochemical staining were approved by the local ethical committee at Pathology and Forensic Medicine department in College of Medicine-Al-Mustansiriya University, Baghdad, Iraq.

From each case, serial specimen sections were performed with 4 µM thickness and then routinely stained using Hematoxylin-Eosin as representative samples. All sections were independently evaluated by two pathologists. Herein, The molar pregnancy was classified into CMs and PMs depending on histopathological criteria outlined by Szulman and Surti [27,29,30]. The diagnosis of the former was dependent on the following: presence of complete hydatidiform alteration range from edema to assembly of a central cistern, lack of embryo, and noticeable trophoblastic hyperplasia. However, for the PMs, the diagnosis was based on the occurrence of both normal and edematous villi simultaneously; that is mean, partial involvement of villi. There was also an embryo be existent, as well as slight to moderate focal trophoblastic hyperplasia and inclusion formation. The morphology of trophoblastic hyperplasia is considered as a fundamental finding to differentiate between hydatidiform moles and HA.

Immunohistochemistry

p57^{kip2} IHC was performed using a polymer-based method according to manufacturer's instructions. Briefly, formalin-fixed paraffin embedded sections were subjected to deparaffinization and rehydration steps prior to the addition of 3% hydrogen peroxide in order to quench an endogenous peroxidase activity. The slides were then boiled with citrate buffer, pH 6, for 20 minutes for antigen retrieval. Subsequently, Blocking reagent (hydrogen peroxide reagent ab64218 Abcam)was applied for 20 minutes at room temperature, and the section samples were next incubated overnight with rabbit monoclonal antibody against human p57^{kip2} from American abcam company (1:200 dilution) at 4°C in a humidified chamber. Polyclonal secondary antibodies were horseradish peroxidase polymer-conjugated from American abcam and colorimetric detection was performed using 3,3 Diaminobenzidine for 10 minutes at room temperature. To counterstain of nuclei, hematoxylin stain was also used. All tissue sections were viewed using Omax 40X-2000X Lab LED Binocular Compound Microscope.

The assessment of each specimen was reported blindly by two observers. p57^{kip2} nuclear staining was identified in both cytotrophoblast and stromal cells within villi, at which any pattern of distinct nuclear staining was interpreted as positive. Conversely, less than 10% with limited p57^{kip2} nuclear staining in such locations was considered negative. Strong expression of p57^{kip2} at the site of extravillous trophoblastic cells and maternal decidua served as an internal positive control for all obtained samples. The syncytiotrophoblastic cells, however, were always negatively stained. There was a review of cases to those inconsistent IHC staining pattern and/or different reported opinion, in order to provide the final diagnosis.

Results

In order to observe the morphological differences between CMs and PMs, we first assessed the histopathological features of thirty-four cases at first or early second stage of gestation. These diagnosed as twelve, eight and fourteen cases of CMs, PMs, and non-molar POC, respectively, based on previously reported criteria [27,29,30] as well as IHC (Table 1). For CMs, there was not unlikely to form oedematous villous with a cistern. In addition, mucous stromal degeneration and edema were found. To some extent, circumferential/marked trophoblastic hyperplasia was also observed with extravillous trophoblastic proliferation site and nuclear atypia (Table 2). However, PMs had two villi populations including avascular distended hydropic villi and normal smaller one. Moreover, mild trophoblastic hyperplasia was present as well as noticeable scalloping and central cistern formation (Table 2). Various degree of vascularised with slightly enlarged villi was seen in all cases of HA.

Initial diagnosis	Final diagnosis*					
	CM		PM		HA	
	n=12	IHC (p57kip2)	n=8	IHC (p57kip2)	n=14	IHC (p57kip2)
CM	10	9N, 1P	2	2P	0	0
PM	2	2N	6	6P	0	0
HA	0	0	0	0	14	14P

Table 1: Total number of cases that addressed Complete Mole (CM), Partial Mole (PM) and Hydropic Abortus (HA) are summarised as an initial and final diagnosis after p57^{kip2} IHC staining. *Includes the cases that reported Negative (N) or Positive (P) as assessed by p57^{kip2} immunohistochemistry (IHC) staining.

Morphological characteristics	CM		PM	
	Absent (-) or present (+)	Cases (n=12) (%)	Absent (-) or present (+)	Cases (n=8) (%)
Villous edema	-	2 (16.0)	-	4 (50.0)
	+	10 (84.0)	+	4 (50.0)
Cistern formation	-	1 (8.0)	-	4 (50.0)
	+	11 (92.0)	+	4 (50.0)
Scalloping	-	2 (16.0)	-	0 (0.0)
	+	10 (84.0)	+	8 (100.0)
Mild villous trophoplastic hyperplasia	-	10 (84.0)	-	0 (0.0)
	+	2 (16.0)	+	8 (100.0)
Marked villous trophoplastic hyperplasia	-	2 (16.0)	-	8 (100.0)
	+	10 (84.0)	+	0 (0.0)
Circumferential villous trophoplastic hyperplasia	-	1 (8.0)	-	4 (50.0)
	+	11 (92.0)	+	4 (50.0)
Polar villous trophoplastic hyperplasia	-	12 (100.0)	-	4 (50.0)
	+	0 (0.0)	+	4 (50.0)
Mild to moderate extravillous implantation site	-	7 (58.0)	-	2 (25.0)
	+	5 (41.0)	+	6 (75.0)
Marked extravillous implantation site	-	5 (41.0)	-	6 (75.0)
	+	7 (58.0)	+	2 (25.0)
Nuclear atypia	-	9 (76.0)	-	12 (100.0)
	+	3 (24.0)	+	0 (0.0)

Table 2: The principal histopathological findings of Complete Mole (CM) and partial mole (PM).
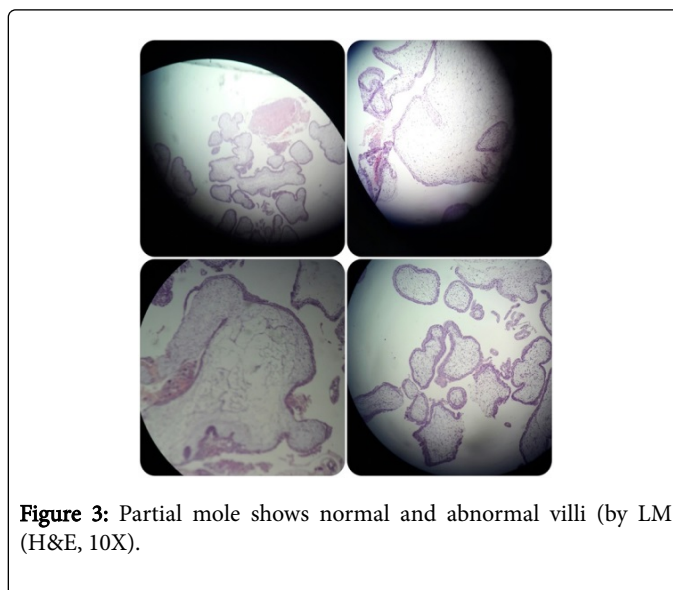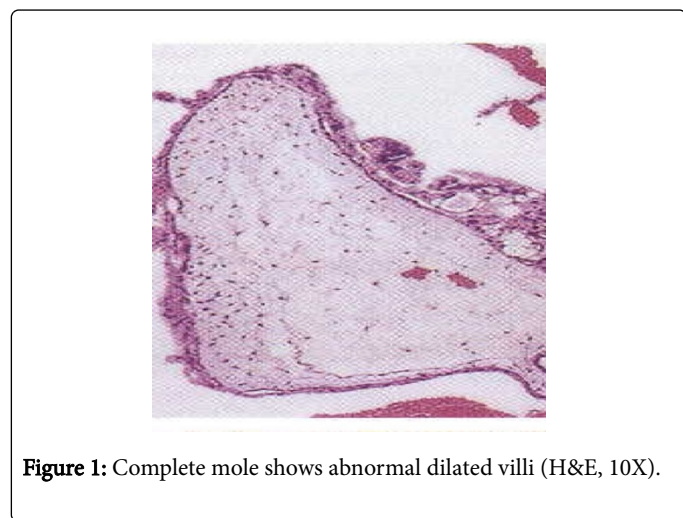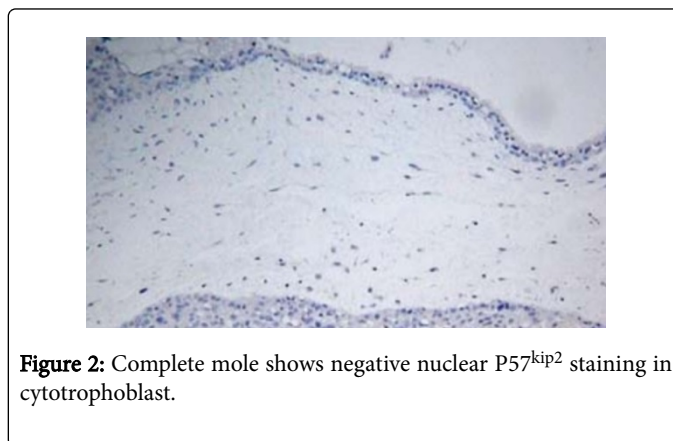
To assess whether the expression of p57^{kip2} was undetectable in cytotrophoblast and stromal cells within villi of CMs, we analysed the IHC staining of p57^{kip2} in all collected cases including CMs, PMs, and HA. As predicted, almost entire lack of p57^{kip2} positivity was seen in nuclei of cytotrophoblast and villous stromal cells in 9 out of 12 cases of CMs albeit present in the extravillous trophoblast and decidua sites (Table 3). One case of CMs, however, showed inconsistent IHC staining pattern (Table 1). In addition, there was a concern about the diagnosis of two cases of CMs due to having a mild degree of villous edema and greater scalloping morphology; but these were confirmed as PMs based on IHC p57^{kip2} positive staining.

IHC p57^{kip2} expression sites	CM		PM		Control	
	Absent (-) /Present (+)	Cases (n=12) (%)	Absent (-) /Present (+)	Cases (n=8) (%)	Absent (-) /Present (+)	Cases (n=14) (%)
Cytotophoblast	-	11 (92.0)	-	0 (0.0)	-	1 (7.0)
	+	1 (8.0)	+	8 (100.0)	+	13 (93.0)
Villous stromal cells	-	11 (92.0)	-	1 (12.0)	-	4 (28.0)
	+	1 (8.0)	+	7 (88.0)	+	10 (72.0)
Extravillous trophoblast	-	2 (16.0)	-	0 (0.0)	-	0 (0.0)
	+	10 (84.0)	+	8 (100.0)	+	14 (100.0)
Decidua	-	7 (56.0)	-	1 (12.0)	-	6 (42.0)
	+	5 (44.0)	+	7 (88.0)	+	8 (58.0)

Table 3: The main sites of p57^{kip2} Immunohistochemistry (IHC) staining in Complete Mole (CM), Partial Mole (PM) and controls.

For PMs, besides the involvement of extravillous trophoblast and decidua sites, the nuclear staining signals of p57^{kip2} were largely observed in cytotrophoblast and villous stromal cells (Table 3). However, two cases had an absence of IHC p57^{kip2} marker positivity thereby they considered as CMs (Table 1), irrespective of their morphology that showed a mild degree of trophoblast hyperplasia. All the control, including fourteen cases of HAs, had less villi formation as well as fewer backgrounds haemorrhage or infarcted regions (Figures 1 and 3). There was also a clear positivity for p57^{kip2} in nuclear cytotrophoblast cells, and decidua of HAs cases. Overall, the final categories of molar cases (CMs and PMs) in this study were twelve and eight cases, respectively (Figures 2 and 4). These data suggest that the p57^{kip2} IHC marker display a valuable role in the diagnosis of CM enhanced by the traditionally available histomorphological features.
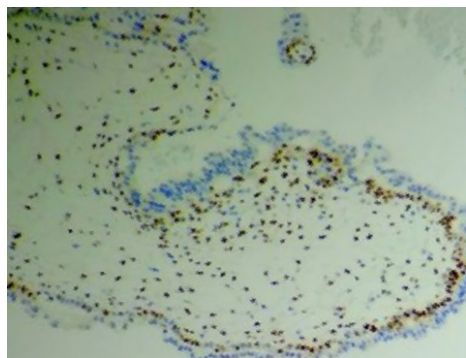

Figure 2: Complete mole shows negative nuclear P57^{kip2} staining in cytotrophoblast.

Figure 1: Complete mole shows abnormal dilated villi (H&E, 10X).

Figure 3: Partial mole shows normal and abnormal villi (by LM (H&E, 10X).

Figure 4: Positive nuclear staining by p57^{kip2} in cytotrophoblast in partial mole.

Discussion

The presence of overlap in findings of histomorphological identity is remarkable among a discipline of hydatidiform moles; especially CMs and PMs, and from non-molar pregnancies with hydropic changes. Accordingly, this does indeed have an intra and inter-observer variation when gross and microscopical features alone were used for diagnosis [10,14,31]. Earlier established and commonly accepted diagnostic criteria for molar pregnancy are still based on those studies at which evacuation of molar tissue occur at late gestational age [14,24]. Thus, the distinctive features of either villous edema and/or trophoblastic hyperplasia may not entirely be developed during the first trimester of gestation (5-9 weeks of pregnancy), resulting in a frequent problem for differentiating CMs from PMs cases [10,31]. In addition, the diagnosis of PMs may perhaps not be relied on the existence of associated fetal tissues, as they might have androgenetic origin in relation to initial embryonic development arise in CMs [31]. There is also a substantial difficulty in distinguishing PMs from HA when depends only on histological features [14]. Therefore, it has become critically important to use other techniques to better diagnose those atypical cases.

Sophisticated molecular diagnostic tests have addressed this concern by examining the alterations in DNA content between molar cases. These include cellular DNA analyses by flow cytometry, chromosome *in situ* hybridization, genotype by PCR, or HLA type. Nevertheless, difficult issues exist in their technical performance, as well as relative cost effect and unavailability in most pathological laboratories [32,33]. There is a functional role for both cytogenetic and ploidy analysis by flow cytometry in the distinction of CMs from PMs in most settings, but a degree of uncertainty would have remained in essence for those atypical cases. Moreover, such tests could not discriminate CMs from HA [10]. It has been considered that p57^{kip2} IHC, as a promising method could be used in addition to its cost-effectiveness to allow for definite classify of most troubling cases.

In this study, we identified 9 cases of CM by demonstrating loss of the p57^{kip2} stain in IHC; all HA and PM specimens showed diffuse p57^{kip2} staining. It was revealed that the majority of CMs were almost always p57^{kip2} negative expression. However, there was morphological features conflict in actual subtyping of four molar pregnancy cases at that point they seem to be determined correctly by applying IHC. Herein, despite one of the cases morphologically denoted as CM, it was still exhibited positive stain for p57^{kip2} IHC. One possibility for this could have been that other pathogenesis was considered; in particular when recurrent hydatidiform moles were previously reported as diploid by FISH and finally diagnosed as CM albeit with discrepant positive p57^{kip2} immuno-reactivity [10], suggesting the likelihood of other underlying mechanisms for such recurrent mole cases. In an earlier study, however, it was observed the lack of p57^{kip2} staining during the assessment of seven patients who were all known as with recurrent moles, including two sisters, and biparental in genotype [34]. Patients with recurrent molar pregnancies are a rare condition, inherited in an autosomal recessive pattern and considered familial disease in some cases [35,36]. Despite the genetic heterogeneity, the major locus has been mapped to the long arm of chromosome 19 (Chr 19q13.42) [36]. It recently has attributed to mutations in NALP like receptor family Pyrin Domain Containing 7 (NLPR7) or Chromosome 6 Open Reading Frame 221 (C6orf221) genes that run in families affected by recurrent molar pregnancies, lead to an epigenetic defect in multiple loci and imprinted genes express abnormally [37-40]. This disorder could present as multiple or recurrent CM, and also much the same as the conventional CM in regards to morphology, immunophenotype and clinical status. Consequently, it has negative p57^{kip2} stain and likewise the conventional case display comparable risk of persistent gestational trophoblastic disease to some extent [33,35]. In contrast, this recurrent CM is genotypically different, being biparental diploidy with the contribution of both maternal and paternal haploids instead of androgenetic diploidy of the typical situation [36].

For another potential, It was also recently proposed that aberrant expression of p57^{kip2} whether for CM or PM is probably attributed to retained maternal allele from chromosome 11 trisomy [41-43], or loss of such maternal chromosome 11 copy [44], respectively. Rare PM sample has also been reported in a previous study with the divergent expression of P57^{kip2}, showing positive stain in villous stromal cells but negative for several cytotrophoblastic cells that have seen within the single villi; and it recognized as triandric tetraploid [45]. Nevertheless, the later pattern of PM discordant p57^{kip2} expression has not been the case here, as all our specimens of PM were virtually positive stain for p57^{kip2} in IHC. Overall, these data, therefore, highlights the complexity of genomic nature that might be encountered in the sampling of hydatidiform mole; and likely suggests the use of short tandem repeat genotyping for such hydatidiform mole specimens with aberrant expression of p57^{kip2} as an ancillary technique to define whether maternal or paternal source of chromosomal components has involved.

The importance of differentiating PM from CM is highly considered in clinical practice in order to manage the conditions accurately and to assess the sequential risk of the persistent gestational trophoblastic disease that might arise. Given that the risk of CM (15-20%) is substantially more than that of PM (0.2-4%) [46,47], Nevertheless, aberrant p57^{kip2} expression of CM that under diagnosis as PM by p57^{kip2} IHC alone in the absence of molecular genotype would be undervalued the standing risk potential of persistent gestational trophoblastic disease, resulting in inadequate clinical estimation and further monitoring action [35].

Our findings were in accordance with others, and have also confirmed the proposition that p57^{kip2} IHC can act as an adjunct in practice to identify CM and its mimics in accurate manner, since it is sensibly reliable, simple and cost-effectiveness method. There was an absence or marked decline in p57^{kip2} expression of almost all CM samples in cytotrophoblastic and villous mesenchyme [25]. It was also

previously reported that 96 percent of CM specimens were negative for p57^{kip2} IHC reactivity, while one case had an aberrant IHC pattern [21]. All the CM cases were lacked the p57^{kip2} expression in villous cytotrophoblasts and stromal cells irrespective of gestational age [48]. Similarly, in another study, the positivity of p57^{kip2} IHC staining was agreed as a highly sensitive and specific marker to exclude CM from PM and also HA specimens, although the later distinction could not be performed by p57^{kip2} [24]. The equivocal cases with polar trophoblastic hyperplasia that observed in both PM and HA cases, however, were likely distinguished using ploidy study; resulting in a reduction of inter/intra-observer variability [14]. The combination of histomorphology and P57^{kip2} immuno-staining technique was validated in earlier work on conception products in order to triage cases designed for molecular genotype. Accordingly, several inspected specimens had consistent results of p57^{kip2} expression and molecular genotyping, being negative in nearly all CM except one case was confirmed by molecular test, together, diffuse p57^{kip2} expression was noticed in both PM and non-molar samples and merely three cases confirmed as PM in molecular genotype with aberrant or equivocal p57^{kip2} expression states [41]. The molecular genotype revealed the diagnosis of CM with validation into androgenetic diploidy and also determined those positive p57^{kip2} samples as PM diandric triploidy, biparental diploidy of non-molar cases and those rare cases of CM displaying aberrant p57^{kip2} expression [41]. The high correlation of p57^{kip2} IHC staining and molecular genotype was also mentioned previously with 0.8 percent of hydatidiform moles samples had aberrant expression of p57^{kip2} [35]. Interestingly, the p57^{kip2} IHC analysis has also confirmed its usefulness in distinguishing androgenetic/biparental mosaic/chimeric conceptions, including those uniform androgenetic/biparental mosaic samples in the absence of molar characteristics, androgenetic/biparental mosaic specimens in the presence of a molar constituent, and twin gestations consisted of CM and non-molar sample components [35]. The identification of unsatisfactory and discrepant staining pattern in such specimens is required for correct interpretation of these complex cases, as well as, its necessity for particular microdissection of different components in order to permit molecular genotyping accurately [35,49]. Collectively, the best way for an attempt to proper classification of molar and/or non-molar pregnancy specimens is, thereby, by applying a reciprocal approach. This comprises the assembly of morphological criteria, IHC of p57^{kip2} expression, as well as molecular genotype test [35]. The combined approach could be essential to evaluate those problematic and challenging cases at which molecular methods are still requisite especially when discordance stain as positive p57^{kip2} occurs.

Conclusion

Our work further confirms the role of p57^{kip2} IHC negative expression as a reliable diagnostic way in association with the traditional histopathological features to improve the identity of CM from other mimic cases.

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