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P57Kip2 immunostaining, a diagnostic marker in differentiating complete hydatidiform mole from its mimics

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Abstract

Background and objective: The distinction of hydatidiform mole from hydropic abortion remains a problem because of interobserver and intraobserver variability. This study aimed to determine the utility of p57Kip2 as a diagnostic marker in differentiating complete hydatidiform mole from its mimics.

Methods: A total of 97 formalin fixed paraffin embedded material including forty cases of complete hydatidiform mole, 36 cases of partial hydatidiform mole and 21 cases of hydropic abortion were selected randomly from the files of histopathology laboratory of Maternity Teaching Hospital in Erbil. The samples were reviewed by two pathologists, afterward; immunohistochemical staining was performed by using a p57Kip2 marker. We considered p57Kip2 positive only if nuclear p57Kip2 staining was identified in at least 10% or more of all in a tissue section.

Results: Negative immunostaining was seen in 77.5% of the complete hydatidiform mole in both villous cytotrophoblast and stromal cells. In contrast, 86.1% of partial hydatidiform mole showed positive immunostaining for p57Kip2. All cases of hydropic abortion 100% were positive for p57Kip2 immunostaining. In all gestations, p57Kip2 was strongly expressed in decidua which served as internal positive control. The concordance between the initial histological diagnosis and p57Kip2 immunostaining was statistically significant (P <0.001).

Conclusions: p57Kip2 immunostaining is a highly sensitive and specific marker for diagnosis and classification of hydatidiform mole. p57Kip2 staining has the advantage of differentiating hydropic abortuses from the complete hydatidiform mole.

Keywords: Hydatidiform mole; p57Kip2; Hydropic abortion; immunohistochemical study.

Introduction

Hydatidiform mole (HM) is an abnormal gestation characterized by trophoblast hyperplasia and overgrowth of placental villi. It is classified into the complete mole, partial mole and invasive mole based on clinical, morphologic, and genetic differences.[1,2] The most important reason for the correct recognition of mole is that they are associated with increased risk of persistent trophoblastic disease that develops after complete hydatidiform mole (CHM) in 10%-30% and 0.5%-5% after PHM;[3] thus differentiation of CHM from partial hydatidiform mole (PHM) and hydropic abortion (HA) in early gestation is very important for patient management.[4] CHM results from fertilization of an empty egg with single sperm and the genetic material are completely paternally derived. Ninety percent of CHM have 46 XX diploid pattern; the remaining 10% are from fertilization of an empty egg by two sperm (46 XX, 46 XY). While the PHM associated with the presence of abnormal embryo, it results from fertilization of an egg with two sperm. In PHMs the karyotype is triploid (69XXY) or occasionally (92 XXXY).[5] Detailed histopathological examination remains to be the basis for the diagnosis of HM, and in the last 20 years, the diagnosis of HM had become more difficult because of the widespread use of early uterine evacuation, and the pathologist has to separate the different entities on the basis of very subtle morphologic criteria.[4]

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In addition, the criteria for diagnosis are subjective and showed considerable inter-observer and intra-observer variation among pathologists.[4] Thus, development of a new method that allows differentiating these pathologies in doubtful cases is important. A complementary method to pathology interpretation is immunohistechemistry (IHC).[6] One of the advantages of this method is the ability to apply it retrospectively to sections of routinely formalin-fixed and paraffin embedded (FFPE) tissue. Another advantage is that there is no need for expensive or sophisticated equipment.[7-9] $P57^{Kip2}$ Cyclin-dependent kinase inhibitor (CDK1C) is a tight-binding inhibitor of several G1 cyclin /Cdk complexes and a negative regulator of cell proliferation and tumor suppressor gene. It is a protein product of the paternally imprinted but maternally expressed gene CDKNIC located on chromosome 11p15.5.[10,11] Because a CHM is the only type of conceptus lacking a maternal contribution, $p57^{KIP2}$ immunostaining is correspondingly absent. Whereas, it is present in CHM mimics (PHM and HA) thus IHC analysis of $P57^{Kip2}$ has been shown to be valuable tool in distinguishing CHMs from PHMs.[12,13] This study aimed to determine the utility of $p57^{Kip2}$ immunostaining as a diagnostic marker in differentiating CHM from PHM and HA.

Methods

This observational study involved a total of 97 formalin fixed paraffin embedded samples of the product of conception that were selected randomly from the files of histopathology laboratory of Maternity Teaching Hospital in Erbil / Kurdistan region of Iraq, during the period from January 2012 to July 2013. The cases studied were categorized into the following groups: 1^{st}-trimester abortion (n=21), PHM (n=36) and CHM (n=40). For each case, all hematoxylin and eosin stained sections were reviewed by two pathologists, to differentiate PHM from CHM; the histological features of the specimen were assessed according to histopathological diagnostic criteria. Another thin 4 mm sections were taken and submitted for $p57^{Kip2}$. CDKN1C antibody was produced in rabbit, and it is a synthetic peptide was raised against human. The antibody supplier was LIFESPAN BIOSCIENCES, Cataloge ID/Lot ID- LS-C138762/ 43194) from the USA, immunohistochemistry was performed according to manufacturer instructions, by using the avidin-biotin-peroxidase complex in which primarily monoclonal antibodies raised against CDKN1C/Kip2 was used and according to Dako Cytomation EnVision®+Dual link system-HRP(DAB+) staining protocol for immunostaining. Briefly, for antigen retrieval, deparaffinized sections were prepared by heating in a microwave oven in 10 mμ citrate buffer, PH 6.0, for 20 minutes. After cooling, sections were immersed in phosphate buffer saline (PBS) containing 3% hydrogen peroxide for 10 min to block endogenous peroxidase activity. Sections were then incubated in a hummed chamber overnight at 4^{o}_{C} with the fallowing primary antibody: CDKNC1 (was produced in rabbit, clone EP2718 (2), dilution 1:200) after rinsing with PBS slides were incubated with secondary antibody followed by streptavidin-biotin-peroxidase complex, both for 30 min at room temperature with PBS wash in between each step (LSAB+ system; Dako Cytomation®, USA), the slides were developed with diaminobenzidin- H_2O_2 (DAB+ SYSTEM; Dako Cytomation®, USA), counter stains by Mayer's hematoxylin and mounted, Positive and negative control slides were involved in each run of staining. Negative control slides were prepared from the same tissue block, but incubated with Tris Buffer Saline (TBS) instead of the primary antibody. While the maternal decidua and intervillous proliferating tro-phoblasts islands served as an internal positive control for $p57^{Kip2}$ and staining was necessary for the results to be considered valid.

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Immunohistochemical Staining Interpretation

After CDCN1C/ p57^{Kip2} staining, positive expressions in a light microscope gives distinct brown color nuclear staining in villous cytotrophoblasts and stromal cells, intervillous trophoblastic hyperplasia, intermediate trophoblast and decidual cells but it was absent in syncytiotrophoblast. More than 1000 tumor cells in multiple high power fields (HPF) have been counted for assessing the percentage of positive cells. Cells with questionable nuclear staining were discounted. Also, necrotic or thick areas and severely degenerated areas were avoided during evaluation. We considered p57^{Kip2} positive only if nuclear p57^{Kip2} staining was identified in at least 10% or more of all in a tissue section. P57^{Kip2} staining was considered negative when cytotrophoblasts and villous stromal cells showed less than 10% nuclear immunoreactivity.

Statistical Analysis

The relationship between histopathological diagnosis of molar pregnancy and p57^{Kip2} expression was analyzed by the Chi-square test and student's t- test. The results were considered statistically significant if the *P* value was ≤0.05.

Results

The immunohistochemical assessment of p57^{Kip2} in 97 cases revealed a statistically significant correlation between complete molar pregnancy and its mimics. Nine out of 40 (22.5%) cases of CHM showed positive immunostaing in villous cytotrophoblast and stromal cells, while 31/40 (77.5%) cases showed negative immunostaining in both villous cytotrophoblast and stromal cells, as shown in Figure 1. In contrast, 31/36 (86.1%) cases of PHM showed positive immunostainiy for p57^{Kip2}, as shown in Figure 2,

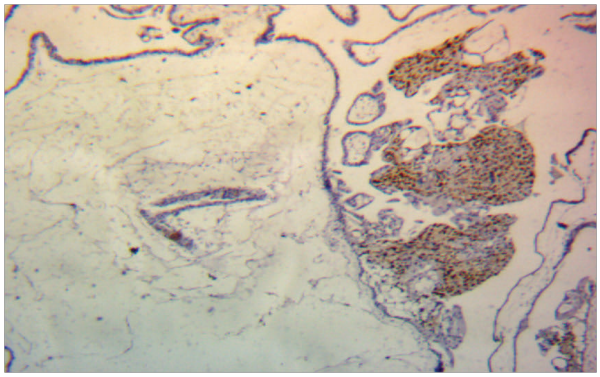

Figure 1: P57^{Kip2} immunostaining in a complete Hydatidiform mole shows a lack of staining in the villous cytotrophoblast and stromal cells, with positive staining of the intervillous trophpblastic hyperplasia (IP-Mayer's Hx. counterstain x100).

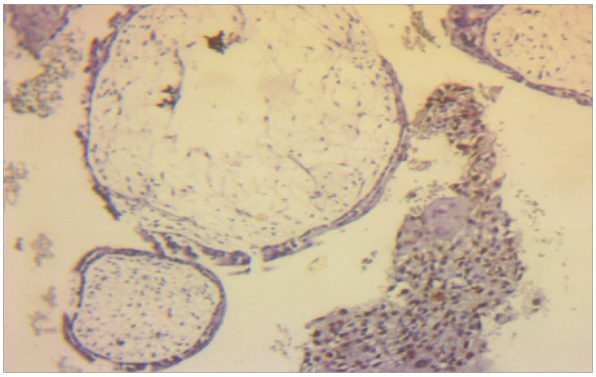

Figure 2: P57^{Kip2} immunostaining in a partial Hydatidiform mole shows strong brown color nuclear staining in the villous cytotrophoblast and stromal cells (IP-Mayer's Hx. counterstain x100).

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While only 5/36 (13.9%) cases with negative immunostaining. All cases of hydropic abortion 21(100%) were positive for p57^{Kip2} immunostaining, as shown in Table 1 and Figure 3. In all gestations, p57^{Kip2} was strongly expressed in decidua which served as internal positive control. The concordance between the initial histological diagnosis and p57^{Kip2} immunostaining was statistically significant (*P* <0.001).

Table 1: The frequency of p57^{Kip2} immunostaining in CHM, PHM and HA.

Type of lesion	P57Kip2 +ve No. (%)	P57Kip2 –ve No. (%)	Total No. (%)
Complete hydatidiform mole	9(22.5%)	31(77.5%)	40 (100%)
Partial hydatidiform mole	31(86.1%)	5(13.9%)	36(100%)
Hydropic abortion	21(100%)	0(0%)	21(100%)

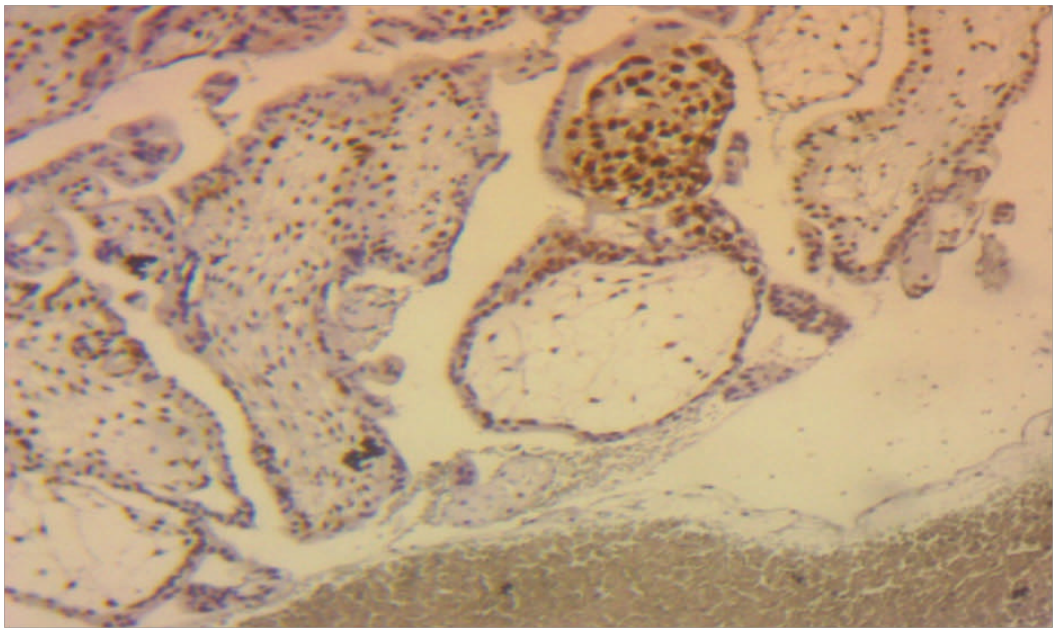

Figure 3: P57^{Kip2} immunostaining in a hydropic abortion shows strong brown color nuclear staining in the villous cytotrophoblast and stromal cells (IP-Mayer's Hx. counterstain x100).

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Discussion

The diagnosis of molar pregnancy remains problematic, particularly when the pathologist is faced with cases of early abortion (less than 10-week gestation). The challenge is to discriminate it from early hydropic miscarriage and early partial moles.[3] Since the consequences of molar pregnancy and risk of persistent trophoblast disease (most especially with CHM), the accurate diagnosis is essential in the categorization of complete mole from its mimics. $P57^{Kip2}$ is paternally imprinted maternally expressed gene.[14] In normal placenta, nuclear $P57^{Kip2}$ expression occurs at high frequency (up to 100%) in villous cytotrophoblast and implantation site interstitial trophoblast, but absent in syncytiotrophoblast.[12,13] Villous stromal cells and maternal decidual cells also express $p57^{Kip2}$, in contrast, $P57^{Kip2}$ has been shown to be absent in CHM but present in PHM.[5,12,13] Since CHM lacks a copy of maternal genome it's negative for $P57^{Kip2}$ while partial moles gives a positive immunstaining for $P57^{Kip2}$ because it has a haploid copy of maternal genome and two haploid copies of the paternal genome.[14-18] The present study showed that $p57^{Kip2}$ expression was absent in 31/40 (77.5%) of cases previously diagnosed as CHM by histopathology, while it was expressed in 9/40 (22.5 %) of CHM cases. This result is relatively different from that reported by Fukunga et al.[4] who detected $p57^{Kip2}$ expression in 7/44 (15.9%) of the cases with CHM, while it was absent in 37/44 (84.1%) of CHM cases. Robin et al.[1] found $p57^{Kip2}$ expression in 2/13 (15.4%) of CHM cases, while it was absent in 11/13 (84.6%) of cases. Crisp et al.[5] found $p57^{Kip2}$ expression in 2/22 (9.1%) of CHM cases while it was absent in 20/22 (90.9%). Castrillon et al.[7] detected $p57^{Kip2}$ in 1/59 (1.7%) of CHM cases and it was absent in 58/59 (98.3%). Jun et al.[8] reported $p57^{Kip2}$ expression in 1/52 (1.9%) of cases while it was absent in 51/52 (98.1 %). Both Chen et al.[10] and Maggiori et al.[9] reported absent of $p57^{Kip2}$ in all cases of CHM 13/13, 20/20 (100%) respectively. Landolsi et al.[11] found 132/132 (100%) cases of initially diagnosed as CHM were negative immuostaining for $p57^{Kip2}$ and he confirmed this diagnosis by microsatellite DNA genotyping analysis of these cases. This variation in detection of $p57^{Kip2}$ in different studies may be attributed to the number of cases studies, IHC methodology used including tissue fixation and antigen preservation, because prolonged tissue fixation (more than 24 hours) can cause masking of the antigenic epitope and results in strong non-specific background staining, choice of antibody, sensitivity of the detection system and the determination of criteria for positive results used. In addition, this variation may reflect differences in subjective evaluation of $p57^{Kip2}$ status. It could also be related to differences in population groups, diversity of risk habits and variation of genetic predisposition may contribute to that wide variation in the expression of $p57^{Kip2}$ that reported in different countries.[1,4,5,7-11,18] Moreover, McConnell et al.[14] and DeScipio et al.[15] in their studies were called the positive $p57^{Kip2}$ in CHM as aberrant $p57^{Kip2}$ expression and explained that by demonstration trisomy of chromosome 11 or retention of maternal allele. The findings of Masoumeh et al.[16] in his study support the hypothesis that misexpression of $p57^{Kip2}$ is involved in the abnormal development of androgenetic CMs. Thus immunohistochemical analysis is a useful tool for the differential diagnosis of CMs. Banet et al.[18] had reported in his study of 14 CHM androgenetic / biparental mosaics with discordant $p57^{Kip2}$ expression, 6 were uniformly mosaic, and 8 had a $p57^{Kip2}$ - negative androgenetic molar component, and he found that $p57^{Kip2}$ expression is highly correlated with genotyping, which serves as a reliable marker for diagnosis of complete hydatidiform moles, and identifies androgenetic cell lines in mosaic conceptions. He concluded that cases with aberrant and discordant $p57^{Kip2}$ expression could be correctly classified by genotyping.

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The results of this study differ (as shown in Table 1) from that found by Chen et al[10], Robin et al.[1], Maggiori et al.[9], Fukunga et al.[4], Crisp et al.[5], Jun et al.[8], and Castrillon et al.[7] who reported that all cases (100%) with PHM expressed p57Kip2. Our results agree with that reported by Landolsi et al. who found 8/49 (16.3%) cases of initially diagnosed as PHM by histopathology has negative immuostaining for p57Kip2. Landolsi et al. analyzed these negative cases by microsatellite DNA genotyping which agreed with the results of p57Kip2 staining, confirming the diagnosis of CHM in these cases rather than PHM, and indicated the absence of maternal contribution and the homozygosity for a single paternal allele in concordance with the androgenetic and monospermic origin of CHM in these cases. In this study, all cases of HA 21/21 (100%) expressed p5 Kip2, which is in agreement with that reported by Chen et al.[10], Robin et al.[1], Maggiori et al.[9], Fukunga et al.[4], Jun et al.[8] and Castrillon et al.[7]. However, it disagrees with that reported by Landolsi et al.[11] who found 1/30 (3.3%) cases of initially diagnosed as HA by histopathology were negative immuostaining for p57Kip2. Later on, he analyzed this single negative case by microsatellite DNA genotyping, that agreed with the results of p57Kip2 staining, confirming the diagnosis of CHM rather than HA. In the current study, the concordance between the initial histological diagnosis and p57Kip2 immunstaining was statistically significant. This result is in agreement with that reported by others.[1,4,5,7,9,10,16]

Conclusions

p57Kip2 immunostaining is a marker for diagnosis and classification of HM. Besides, p57Kip2 staining has the advantage of differentiating hydropic abortuses from CHM. This differentiation is clinically relevant since patients with hydropic abortions do not need to be followed up while patients with molar gestations do. P57kip2 immunostaining results were Helpful in determining histologically equivocal cases. However, molecular techniques are still required for the evaluation of some difficult cases with discordant or aberrant positive p57Kip2 staining.

Conflicts of interest

The author reports no conflicts of interest.

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