ORIGINAL ARTICLE

Diagnostic Utility of P57 Immunomarker in Differentiating Complete and Partial Hydatidiform Mole

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ABSTRACT

Objective: To assess diagnostic utility of p57 in differentiating complete and partial hydatidiform mole.

Study Design: Cross-sectional descriptive study.

Place and Duration of Study: Histopathology department at Chugtai's Institute of Pathology, Lahore, Pakistan for a period of six months starting from 1st January 2022 to 30th June 2022.

Materials and Methods: A total of 50 cases of molar pregnancies were included in this study. Age, gross findings, and histomorphological features were noted. p57KIP2 immunostain was applied on diagnostic histology slide for definitive subtyping into complete and partial mole. The Chi-squared test and Fisher Exact test were used to assess associations between different variables, with statistical analysis conducted using SPSS version 26.

Results: The preliminary diagnosis based on morphology alone revealed 29 (58%) cases of complete mole while 21 (42%) cases of partial mole. However, when p57 antibody was interpreted in conjugation with histology the final diagnosis revealed 37 (74%) cases turned out to be complete mole and 13 (26%) cases were categorized as partial mole.

Conclusion: p57KIP2 immunostain is a useful and reliable ancillary test to reach final diagnosis which helps in accurate diagnosis in the best interest of patients.

Key Words*: Complete hydatidiform mole, Gestational trophoblastic disease, grape like vesicles, partial hydatidiform mole, p57 protein.*

Introduction

A kind of gestational trophoblastic disease (GTD) known as hydatidiform mole has been identified as a noteworthy pathological entity with a special capacity for both local invasion and distant metastasis. There are three types of this condition: invasive mole (IM), partial hydatidiform mole (PHM), and complete hydatidiform mole (CHM).[1] Since hydatidiform moles were first described as a disorder characterized by aberrant growth of trophoblastic tissue and distinctive hydropic degeneration of chorionic villi, our understanding of these entities, both clinically and pathologically, has changed significantly.[2] Only histomorphological evaluation could distinguish between complete and partial moles. Overlapping morphological traits, however, frequently present difficulties for this method, particularly when specimen preservation is inadequate or diagnostic expertise is lacking.[3] The genetic etiology of these entities has been clarified by recent developments in molecular genetics. CHM is usually the result of a single sperm fertilizing an enucleated ovum, giving rise to a diploid androgenetic origin. On the other hand, PHM, is the outcome of two sperm fertilizing an ovum producing diandric triploidy. Despite these developments, precise and dependable subtyping is still essential since it has a big impact on patient care and follow-up.[4]

The clinical significance of subtyping molar pregnancies for risk assessment and follow-up monitoring to stop the development of gestational trophoblastic neoplasia (GTN) is established by the literature now in publication. It is commonly

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recognized that CHM is more likely to progress into gestational trophoblastic neoplasia (GTN) than PHM.[5] To guarantee correct subtyping, there are still gaps in the standardization of diagnostic procedures, especially in areas with restricted access to sophisticated genetic testing. In this regard, immunohistochemical markers such as p57, a protein that is expressed by the mother and has a paternal imprint, have become useful adjuncts.[6] Although p57 antibody usefulness has been investigated, little information is available from South Asian communities, where the prevalence of molar pregnancies and the ensuing GTN is far greater than in Western cultures.

Our work sought to fill these gaps by assessing the diagnostic value of the p57 immunomarker in distinguishing between partial and complete hydatidiform moles in a Pakistani sample. We aimed to improve diagnostic precision and offer data to support clinical judgment by integrating histomorphological evaluation with p57 immunostaining. This study is also noteworthy since it offers insights specific to a certain location and emphasizes the need of integrating p57 staining into regular diagnostic procedures, which may standardize care and enhance results.

In this study, we studied molar pregnancies to improve patient care by guaranteeing proper management and follow-up plans, we postulated that combining p57 immunostaining with histological examination would greatly increase the accuracy of subtyping molar pregnancies.

The rationale for this study was its ability to bridge the gap in region-specific evidence on the usefulness of the p57 immunomarker and to solve the diagnostic difficulties presented by histology alone. This work adds to our understanding of GTD and highlights its importance in high-incidence communities by clarifying the role of p57 immunostaining in the subtyping of molar pregnancies.

The objective of this research was to evaluate the diagnostic potential of p57 immunostaining in distinguishing between complete and partial hydatidiform moles, thereby offering a dependable supplementary instrument for precise diagnosis and enhanced clinical handling of molar pregnancies.

Materials and Methods

This cross-sectional descriptive study was conducted over six months, from January 1, 2022, to June 30, 2022, at Chughtai's Institute of Pathology, Lahore, Pakistan. The permission to carry out this research was obtained from institutional review board at Chughtai's Institute of pathology under letter number (*CIP/IRB/1095A*). After approval data was actively collected for a period of six months. Approval for the waiver of informed consent was obtained from the Institutional Review Board prior to the commencement of this study. The waiver was granted because most of the data were sourced from electronic medical records, and there was no direct involvement of the study subjects. We receive specimens from different hospitals from all over country. Cases reported as molar pregnancy (complete or partial mole) were actively collected on daily basis and their processed formalin fixed paraffin embedded blocks were recut for subsequent immunostaining. Total 50 cases were included in this study. While non-molar products of conception, products of conception with hydropic change and invasive mole were excluded. One diagnostic Hematoxylin & Eosin (H&E) slide was kept from the archives. The type of antibody applied was Polyclonal rabbit anti-p57 KIP2 oncoprotein using Dako Link 48 auto stainer. External control was applied to each batch of cases for quality assurance. The IHC staining was carried out using the heat-induced epitope retrieval method followed by a standard streptavidin–biotin peroxidase complex technique (MRH534 L obtained from Biocare Medical, USA). Positive reactivity was interpreted only when distinct nuclear staining was identified. In at least 10% of villi nuclear positivity of p57 antibody in the cytotrophoblasts and stromal cells were considered positive. However internal control was considered positive when it stained maternal decidua and syncytiotrophoblasts.[1]

Two pathologists, both belonging to a different stratum of pathology with reporting experience of up to 5 years independently interpreted all the fifty slides of H&E and p57 antibody and gave their final diagnosis. Data was then analysed, quantitative variable like age was calculated as mean while qualitative variables like vesicle and cistern formation, fetal parts, p57 expression were calculated as frequency and percentage. The Chi-squared test and Fischer Exact test was used to

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assess associations between different variables, with statistical analysis conducted using SPSS version 26 (IBM, Armonk, NY, USA), and a p-value of < 0.05 was considered statistically significant.

Results

Total 50 cases of molar pregnancies were included in this study. Minimum age of patient was 17 and maximum age was 46. Mean age was 26 ± 5.74 standard deviation (SD). We did not find any significant correlation of age with the final diagnosis. The interpretation of histomorphological features has been shown in Table I.

The preliminary diagnosis based on morphology alone revealed 29 (58%) cases of complete mole while 21 (42%) cases of partial mole. However, when p57 antibody was interpreted in conjugation with histology the final diagnosis revealed 37 (74%) cases turned out to be complete mole and 13 (26%) cases were categorized as partial mole. (Figure 1)

When the gross feature of grape like vesicle was correlated with final diagnosis. We found that out of 37 (74%) cases diagnosed as complete mole in 15 (40.54%) cases grape like vesicle were absent and in 22 (59.46%) cases grape like vesicle were present grossly. While in case of partial mole out of 13 (26%) cases in 4(30.77%) cases grapes like vesicles were absent and in 9 (69.23%) cases grapes like vesicles were present grossly. According to Fisher Exact test, P value is 0.390 (statically insignificant).

When the histomorphological features were correlated with final diagnosis. We found that out of 37 (74%) cases diagnosed as complete mole 20 (54.05%) cases of complete mole showed circumferential trophoblastic proliferation on histology (Figure 2) and 17 (45.95%) cases showed polar proliferation on histology (Figure 3). While in case of partial mole out of 13 (26%) cases 3(23.08%) showed circumferential trophoblastic proliferation on histology and 10 (76.92%) cases showed polar proliferation on histology. According to Fisher Exact test, P value is 0.053 (statically insignificant).

When the presence of fetal parts was correlated with final diagnosis. We found that out of 37 (74%) cases diagnosed as complete mole in all 37 (100%) cases fetal parts were absent grossly. While in case of partial mole out of 13 (26%) cases in 8 (61.54%) cases fetal parts were absent and in 5 (38.46%) cases fetal parts were present. According to Fisher Exact test, P value was 0.001 (statically significant).

When the presence of nucleated RBC's was correlated with final diagnosis. We found that out of 37 (74%) cases diagnosed as complete mole in 33 (89.19%) cases nucleated RBCs were absent on histology and 4 (10.81%) cases showed nucleated RBCs on histology. While in case of partial mole out of 13 (26%) cases in 11 (84.62%) cases nucleated RBCs were absent and 2 (15.38%) cases showed presence of nucleated RBCs. According to Fisher, Exact test, P value was 0.049 (statically insignificant).

When the presence of cistern formation (Figure 4) was correlated with final diagnosis. We found that out of 37 (74%) cases diagnosed as complete mole in 12 (32.43%) cases cistern formation were absent on histology and 25 (67.57%) cases showed cistern formation on histology. While in case of partial mole out of 13 (26%) cases in 8 (61.54%) cases cistern formation were absent and 5 (38.46%) cases showed presence of cistern formation. According to Fisher Exact test, P value was 0.066 (statically insignificant).

Table I: The interpretation of Histomorphological features (n=50)

Histomorphological features	Interpretation	Cases (n)	Percentage (%)
Grape like vesicle	Absent	19	38.0
	Present	31	62.0
Cistern formation	Absent	20	40.0
	Present	30	60.0
Nucleated RBC's	Absent	44	88.0
	Present	6	12.0
Fetal parts	Absent	45	90.0
	Present	5	10.0

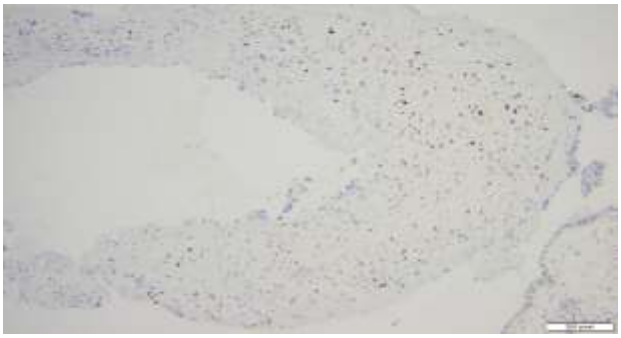

Figure 1: Positive Staining of p57 in Partial Mole at 400x Magnification

Discussion

The diagnostic value of the p57 immunomarker in distinction between partial and complete hydatidiform moles was assessed in this study. 74%

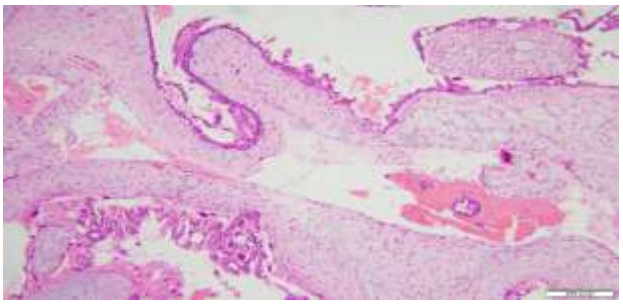

Figure 2: Circumferential Trophoblastic Proliferation at 400X Magnification

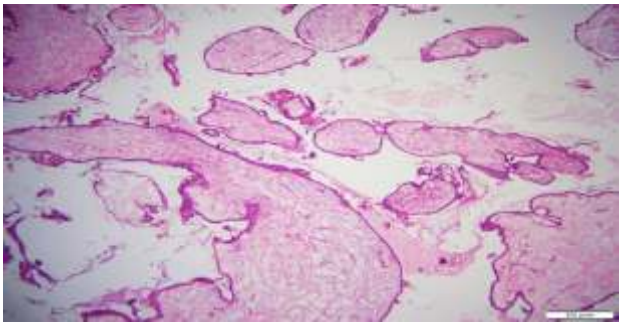

Figure 3: Polar Trophoblastic Proliferation and Admixture Of Variable Sized Villi At 200X Magnification

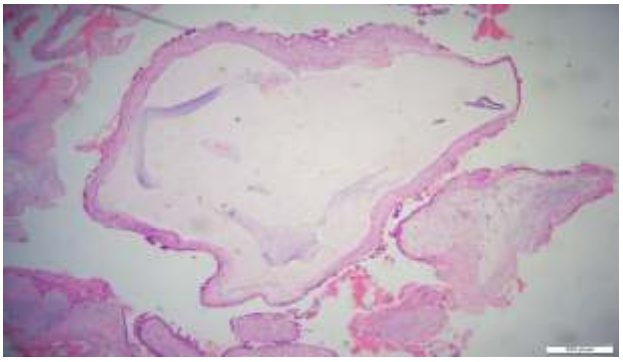

Figure 4: Cistern Formation is Seen in a Dilated Villi Seen in a Diagnosed Case of CHM at 200X Magnification

of cases were found to be complete moles and 26% to be partial moles when p57 immunostaining and histological examination were combined, highlighting the crucial role that p57 plays in enhancing diagnostic precision.

Although grape-like vesicles were found in 62% of cases, 40.54% of complete moles did not have them, which emphasizes the necessity of further diagnostic methods. Histologically, polar trophoblastic proliferation dominated partial moles (76.92%), whereas circumferential trophoblastic proliferation was seen in 54.05% of complete moles. Despite their use, these histological characteristics were not statistically significant (p = 0.053), which emphasizes the relevance of p57 immunostaining.

Complete moles had no fetal components at all, while 38.46% of partial moles had them, which is a statistically significant difference (p = 0.001). Despite being more prevalent in complete moles, nucleated RBCs and cistern development did not significantly differ across the groups, indicating their poor diagnostic accuracy on the basis of histology alone.

There is significant interobserver variability in diagnosing molar pregnancies based on histomorphological features alone as per previous studies as well as in current study.[5,6,7] This variation in morphological features is well established which is related to several factors such as flawed histologic criteria as well as its subjective application for diagnosis and variation in histological features dependent on gestational age of the sample. Since the features are less developed on histological examination, the early diagnosis of molar pregnancies in first trimester scans emphasizes the necessity of a well-established diagnostic criteria.[8]

Like our study research carried out in Iran, CHM was diagnosed on the presence of variable size of chorionic villi from miniscule to very large with central cistern formation and circumferential proliferation of both cytotrophoblasts and syncytiotrophoblasts on histology. PHM was diagnosed based on two types of chorionic villi, focal trophoblastic proliferation, and presence of pseudo-inclusions. However, our study showed significant variable histological features in both entities.[9]

In addition, diagnostic criteria of early CHM can overlap with PHM showing minimal cistern formation, mild hydropic change, fetal blood vessels, irregular villous hyperplasia and pseudo-inclusions in the villi further adding to variable histologic picture.[10]

Classic morphologic features that are diagnostic of molar and non-molar pregnancies on histology are well defined in literature. Although in early gestation the typical morphology is vague making it even difficult to differentiate between hydropic and molar pregnancies, however whenever molecular methods are applied there is considerable discordance between initial and final diagnosis.[11]

We only found presence of fetal parts to be statically significant as presence of abnormal fetal parts in partial mole is due to Di spermic fertilization

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eventually producing a triploid set of chromosomes.[12]

The use of p57KIP2 immunostain to distinguish between partial and full hydatidiform moles is recommended by several national, regional, and worldwide studies in order to prevent interobserver variability and provide a definitive diagnosis.[13, 14, 15] By confirming the use of p57 immunostaining in regional contexts with disparate diagnostic resources and proficiency, our study adds even more value by emphasizing how it might enhance diagnostic precision and lessen interobserver variability in these kinds of situations. A study carried out by Erol O et. al.,[16] used four immunomarkers that are p57, c-erbB-2, CD117 and Bcl-2 in differential diagnosis of hydatidiform mole and hydropic abortion. All four immunostains proved to be efficient, cost effective and simple methods for differentiation among them.

Triratanachat S et. al., concluded in their study that histomorphology has clear cut limitations in accurately diagnosing partial and complete mole with sensitivity of 89.7% and 95.0% respectively. While its specificity turned out to be 95% and 89.7% respectively. They also showed that P57KIP2 immunostain is a helpful cost-effective method to subtype PHM and CHM for their definitive diagnosis.[7] In 2018 Madi JM et. al.,[17] carried out a systemic review & bivariate meta-analysis to determine accuracy of p57kip2 antibody by comparing it with genotyping. Results showed sensitivity of 0.984 (95% confidence interval [CI]: 0.916–1.000) and specificity of 0.625 (95% CI: 0.503–0.736) with significant heterogeneity for specificity (I2 = 71.8, Chi-square P = 0.029) proving high diagnostic accuracy with an area under the curve of 0980.

A recent large study carried out in 2021 of 2017 potentially molar products of conception specimens at john Hopkins further supports that utility of p57 immunohistochemistry and DNA genotyping should be encouraged in routine practice to accurately diagnose and assess the risk of persistent gestational trophoblastic disease. Precise diagnosis of molar conceptus guides the clinician in specific and well-aimed management.[18,19]

Currently molecular genotyping is considered gold standard for subtyping molar pregnancies as it determines ploidy and the paternal origin of molar tissue. Furthermore, it helps in differentiating complete and partial moles from mimics of gestational trophoblastic disease. To establish accurate diagnosis in all cases morphology, p57 immunostain and genotyping must be correlated. Use of genotyping has significantly improved the diagnostic accuracy of GTD which is of clinical significance.[20]

Uterine evacuation and histopathology of the products of conception followed by HCG monitoring are part of obstetric care of molar pregnancy. HCG is an ideal biochemical marker as it precisely reflects the disease burden. Follow up of patients with human chorionic gonadotrophin (HCG) is essential following a diagnosis of molar pregnancy to rule in/out gestational trophoblastic neoplasia. Two normal HCG levels with a one-month gap are recommended for PHM. Follow-up with monthly HCG level for six months is recommended for CHM. The risk of gestational trophoblastic neoplasia following beta HCG normalization was 0.25% for CHM and 0.03% for PHM, according to a large retrospective study of 20,000 women carried out in women who had HCG monitoring following a molar pregnancy. However, even after normalization of HCG levels in complete hydatidiform mole, there was critically increased risk of GTN (0.35%), according to systemic analysis of 19 independent trials. Given the rarity of GTN, another study concluded that extended HCG surveillance, especially following PHM, is not cost-effective. Treatment of GTD entirely depends on FIGO staging system and guidelines.[21] Gestational trophoblastic neoplasia is treated by chemotherapy.[22]

Conclusion

Based on results of this study we concluded that morphological features alone are not reliable in subcategorization of complete and partial molar pregnancies. The only reliable histological parameter is presence of fetal parts that can distinguish between CHM and PHM, yet those might not be present in very early gestations leading to incorrect diagnosis. We recommend application of p57 immunomarker in every case of molar pregnancy for accurate diagnosis and further management of patient. In future reflex genetic testing should be encouraged as it is considered gold standard for establishing ploidy and to differentiate between

CHM and PHM and all the mimics of gestational trophoblastic disease.

Limitation and Future Suggestions

Molecular genotyping unavailability limited our study for a valuable correlation with ploidy. Another study on a larger sample for a longer duration with corresponding Fluorescence in situ hybridization (FISH) studies can provide improved results. Our study's capacity to correlate with ploidy was hindered by the lack of molecular genotyping. Larger sample size, longer study periods, and the use of molecular genotyping methods like (FISH) should improve diagnostic precision and offer a better understanding of hydatidiform moles in the future. Clinical therapy of (GTD) may be enhanced due to accurate diagnosis refined by combining p57 immunostaining with molecular diagnostics.

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CONFLICT OF INTEREST

Authors declared no conflicts of Interest.

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DATA SHARING STATEMENT

The data that support the findings of this study are available from the corresponding author upon request.