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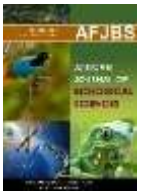

TITLE: A DIAGNOSTIC APPROACH FOR DIFFERENTIATING MOLAR GESTATION AND HYDROPIC ABORTUS USING p57 AND Ki-67 EXPRESSION

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ABSTRACT

Background: Molar pregnancy is the most common form of gestational trophoblastic disease with a high incidence also associated with a high risk of choriocarcinoma.There is interobserver and intraobserver variablilities in histopathologically classifying this disease. To overcome this difficulty, expression of p57 and Ki-67 are used to categorize the entities.

Materials and methods: Total of 80 cases were included in this study, out of which 27 (33.45%) were complete moles, 23 (28.75%) partial moles and 30 (37.5%) hydropic abortus on Hematoxylin and Eosin (H&E) stain, 4 cases (2 HA and 2 PHM cases) showed discordance on p57 and Ki-67 immunostaining, hence were not included in the statistical analysis.

Results: Immunoreactivity of p57 showed 100% sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) respectively in differentiating CHM from PHM. Immunoreactivity of Ki-67 at Grade 0 and grade 1 had a sensitivity of 100% and 92.86% in diagnosing HA respectively. At grade 2, the specificity was 98.18% in diagnosing PHM, but the sensitivity was low compared to grade 1. Grade 3 for diagnosing CHM has good sensitivity of 96.30%, specificity 100%, PPV 100% and NPV 100%.

Conclusion: The diagnostic validity of p57 in identifying CHM is most reliable. The diagnostic validity of Ki – 67 showed an overlapping between HA and PHM at grade – 1 level, but PHM cases graded 2 were more reliable, in case of CHM, grade 3 was most reliable, hence making CHM reliably distinct from PHM. Differentiation of molar gestation from hydropic abortus using
p57 and ki-67 expression can aid the clinician in planning the treatment strategies

Keywords: Molar pregnancy, Hydropic abortus, p57 and Ki-67

INTRODUCTION:

Gestational Trophoblastic Diseases (GTD) are a heterogenous group of diseases with distinct clinical, morphological and pathogenetic features.[1] World Health Organisation (WHO) has classified GTD into tumor like lesions that include exaggerated placental site nodule and placental site nodule and plaque, abnormal (Non – Molar) villous lesions, molar pregnancies that include complete molar pregnancy (CHM), partial molar pregnancy (PHM) and invasive hydatidiform mole and gestational trophoblastic neoplasms such as epithelioid trophoblastic tumor, placental site trophoblastic tumor, choriocarcinoma, NOS and choriocarcinoma combined with other germ cell elements.[2] Among which the topics of this study are partial and complete hydatidiform mole. Although the epidemiological data on incidence of hydatidiform mole and choriocarcinoma varies throughout the world, it has been reported the highest among Asians than North Americans, with an incidence rate among Asians ranging from 100 – 1000 in every 1,00,000 pregnancies when compared to 100 per 1,00,000 pregnancies among the North Americans.[1]

Hydatidiform moles are abnormal gestations caused as a result of abnormal fertilization that has occurred due to imbalance in parental genomes. These abnormal gestations could be the androgenetic diploid ones, such as the complete mole or biparental triploid, such as the partial mole.[3] These molar gestations usually undergo spontaneous abortion in the second trimester and when subjected to histopathological evaluation it was comparatively easily distinguished, but the routine use of ultrasound in pregnancy has led to early diagnosis and evacuation by the first trimester, has made it difficult for the histopathologists to distinguish between complete and partial mole. In addition to this hydropic changes of the villi due to various reasons such as genetic abnormalities also makes it difficult to differentiate molar gestations from hydropic abortus.[1] The clinical significance of distinguishing these entities from one another is that, the frequency of persisitant trophoblastic tumors are 15% - 20% and 0.2% - 4% in complete mole and partial mole respectively, while complete mole poses a threat of causing choriocarcinoma in 2.5% of the cases.[3,4]

Various immunohistochemical markers help in distinguishing molar gestations and hydropic abortus. In this study, the immunohistochemical markers used are $p57^{kip2}$ and Ki – 67.

p57 is a paternally imprinted gene, hence its expression is absent or low in the cytotrophoblasts of complete mole, whereas it shows diffuse positivity in partial mole.[1] Ki – 67 gene encodes a nuclear protein that is expressed in all phases of cell cycle except the G0 phase. As cytotrophoblast is a germinating stem cell and also molar gestations are characterized by hyperplasia of trophoblasts including cytotrophoblasts, these cytotrophoblasts demonstrate proliferative activity with Ki-67.[1, 5]

This study was aimed at differentiating molar gestation and hydropic abortus using p57 and ki-67 expression, thereby aiding the clinician in planning the treatment strategies. Also early and accurate diagnosis of the molar pregnancy can help identify the high risk population that is most likely to develop choriocarcinoma.

MATERIALS AND METHODS:

This was a prospective and retrospective study with a total of 80 cases (27 complete moles, 23 partial moles and 30 hydropic abortus). These cases were obtained from the Department of Pathology, Vinayaka Mission's Kirupananda Variyar Medical College and Hospitals, Tamilnadu, India during the period of January 2020 to December 2022, after obtaining approval from the **Institutional Ethical Committee**. Hematoxylin and Eosin (H & E) stained slides were reviewed by 2 pathologists who were not aware of the original diagnosis and once the reviewing pathologists' diagnosis and the reporting pathologist's diagnosis were similar, the blocks were subjected for immunohistochemical analysis by p57 and Ki 67. The clinical data and pathological records were retrieved from the Medical Record Department. **Inclusion criteria** parameters were samples with adequate amount of villi, cases whose results were in agreement with the reviewing pathologists' diagnosis and after applying immnuhistochemical (IHC) markers, cases that were in agreement between the H & E diagnosis and IHC findings. **Exclusion criteria** parameters were cases with inadequate villi, blocks with excessive blood clots, cases with disagreement between the reporting and reviewing Pathologists and cases with discordant results between H & E diagnosis and IHC interpretations.

H & E slide interpretation criteria: The criteria for a diagnosis of CHM were edematous villi characterized by central acellular cisterns and circumferential trophoblastic proliferation. The criteria for a diagnosis of PHM were a mixture of normal villi, smaller villi and edematous villi, haphazard trophoblastic hyperplasia and trophoblastic infolding forming

inclusions or prominent villous scalloping. The histologic criteria of hydropic abortion (HA) included villous oedema with minimal to no cistern formation and mild trophoblastic hyperplasia, villi were found to contain residual vessels with nucleated fetal RBC, along with the presence of intermediate trophoblastic clumps and fibrin. Lack of cisterns larger than 3mm and presence of polar trophoblasts were essential features in differentiating PHM from simple hydropic abortion. [6]

Immunohistochemical Technique*:* For immunohistochemical assay, two sets of 4 μm thick sections were taken on two positively charged slides and incubated overnight at 37˚C and the next morning incubated at 60˚C to 70˚C. After which paraffin removal from the tissue was done by 2 changes in xylene for 10 minutes each and rehydrated in descending grades of alcohol. Antigen retrieval was achieved by heating slides at 95˚C for 10-20 minutes in retrieval buffer of TRIS EDTA (pH 8.5 – 9.0) using water bath. After cooling the sections at room temperature, endogenous peroxidases were blocked with 3 % H_2O_2 for 10 minutes. To each slides, one of the primary antibodies were added p57 (anti-p57kip2 mouse monoclonal antibody, 1:100 dilution), ki67(rabbit polyclonal at 1:100 dilution) to each slide and left overnight. Then Standard avidin-biotin-horseradish peroxidase complex was used to detect antigen-antibody reactions. Positive staining was seen with 0.3 % 3,3-diaminobenzidine chromogen and counterstained with Harry's hematoxylin.

Interpretation of p57 Immunostain*:* p57 was a nuclear immunostain, where the decidua or intermediate trophoblasts nuclear expression of p57 was taken as internal control. p57 was interpreted positive when the villous cytotrophoblasts and villous stromal cells showed extensive or diffusely positive nuclear staining. It was considered negative when the villous cytotrophoblasts and stromal cells demonstrated minimal or no nuclear expression of p57, inspite of the internal control being positive.[6]

Scoring of Ki – 67 Immunostain*:* Ki67 is another nuclear immunostain, where a section from tonsil positive for Ki-67 was taken as a positive control. Ki67 staining was considered positive when nuclei of villous cytotrophoblasts and stromal cells were stained positive and was calculated using the formula Ki67 index = Number of positive tumor cells/total No. of tumor cells ×100 and graded as Negative (0) – Nuceli of villous cytotrophoblasts and stromal cells fail to express the nuclear stain (0%), weakly positive (grade 1) - (< 20%), moderately positive (grade 2) - (21-50%) and strongly positive (grade 3) - (>50%).[6,7]

Statistical Analysis*:* All the data collected were meticulously entered in Microsoft Excel and analyzed in SPSS 23. The continuous measures such as age and weeks of gestation were summarized as means and standard deviation. The immunoreactivity of Ki-67 and p57 in various types of HA and molar gestations were analysed and graded in proportions. The diagnostic validity of the immunohistochemical markers Ki-67 and p57 were expressed as sensitivity, specificity, positive and negative predictive values in percentage.

RESULTS:

Total of 80 cases included in this study, out of which 27 (33.45%) were identified as complete moles, 23 (28.75%) partial moles and 30 (37.5%) hydropic abortus using Hematoxylin and Eosin (H&E) stain, 4 among these cases (2 HA and 2 PHM cases) showed discordance on p57 (negative) and Ki-67 (grade 1 positive) immunostaining, hence these 4 cases were not included in the statistical analysis.

Demographic data:

Among the 76 cases studied, the age in years ranged between 19 and 45 with a mean age of 27.17years. Patients with HA ranged from 19 – 45(Mean - 27.96 years), PHM ranged between 20 – 33 (Mean - 26.52 years) and CHM ranged between 20 – 36(Mean - 26.85years). Among the 76 cases, the gestational age at the time of products submission to Pathology laboratory ranged between 3 weeks to 12 weeks (Mean – 7.84 weeks). Patients with HA ranged between 3weeks to 12 weeks (Mean – 7.46 weeks), PHM and CHM ranged between 6 weeks to 10 weeks with a Mean of 7.85 and 8.22 weeks each respectively.

Immunoreactivity:

p57 – All the 28 HA cases and 21 PHM cases showed cytotrophoblastic and stromal nuclear positivity when stained with p57. Among the 27 CHM cases all the cases were negative for p57.
Ki – 67 – Among the 28 HA cases, Ki-67 was graded 1 in 26 (92.8%) cases and 2 (7.1%) cases were completely negative (grade-0). Among the 21 PHM cases, graded 2 in 6 cases (28.5%) and grade 1 in 15 cases (71.4%). 27 CHM cases were graded 3 in 26 (96.3%) and graded 2 in (3.7%).

Statistical analysis:

Table:1 – Demographic details and Ki – 67 & p57 Immunoreactivity of the specimens

	HA (n = 28)	**PHM - 21**	**CHM - 27**	**Total (N = 76)**	**p - Value**
BACKGROUND					
a. Age	27.96 +/-5.68	26.52+/-4.27	26.85+/-4.99	27.17+/-5.11	0.63
b. Weeks of Gestation	7.46 +/-1.99	7.85+/-1.28	8.22+/-1.31	7.84+/-1.62	0.23
IMMUNO REACTIVITY					
a. Ki – 67					
i. Grade 0 - (0%)	2	0	0	2	<0.01
ii. Grade-1 (</=20%)	26	15	0	41	
iii. Grade-2 (21% - 50%)	0	6	1	7	
iv. Grade-3 (>50%)	0	0	26	26	
b. p - 57					
i. Positive	28	21	0	49	0.012
ii. Negative	0	0	27	27	

As per above results there is no statistically significant difference in the mean age and the week of gestation among the study participants presented with Hydropic Abortus, Partial Hydatidiform Mole and Complete Hydatidiform Mole. There is statistically significant differences in the immunoreactivity grading of Ki-67 and expression of p57 across the three types of abortus ($p<0.01$ and $p = 0.012$ respectively).

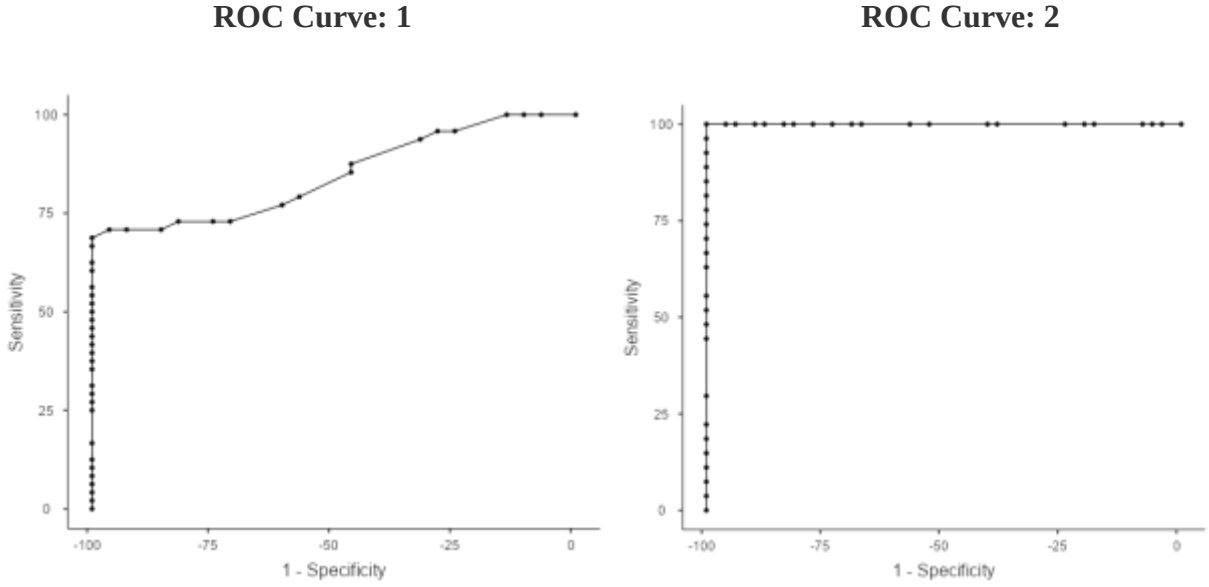

Figure – 1: ROC – 1: Shows that Immunoreactivity of Ki-67 equal to or more than 20% has 70.83% sensitivity and 96.43% specificity and hence is not diagnositic of HA

ROC – 2 shows that immunoreactivity expression of 43% showed the maximum sensitivity 100% in diagnosing CHM compared to immunoreactivity expression of 50% with sensitivity 96.3%.

Table:2– Diagnostic validity of p57 and Ki-67 in various gestations:

Ki-67	**Sensitivity %**	**Specificity %**	**PPV %**	**NPV %**
Grade 0 (0%) in diagnosing HA	7.14	100	100	64.86
Grade 1 (</=20%) in diagnosing HA	92.86	68.75	63.41	94.29
Grade 1 (</=20%) in diagnosing PHM	71.43	52.73	36.59	82.86
Grade 2 (21% - 50%) in diagnosing PHM	28.57	98.18	85.71	78.26
Grade 3 (>51%) in diagnosing CHM	96.30	100	100	98
p57				
p57 in identifying CHM	100	100	100	100

Immunoreactivity of Ki-67 at Grade 0 in diagnosing HA showed 100% specificity and PPV, while the sensitivity was low. At grade 1, the sensitivity was 92.86% in diagnosing HA compared to 71.43% for diagnosing PHM. At grade 2, the specificity was 98.18% in diagnosing PHM, but the sensitivity is low compared to immunoreactivity grade 1. Grade 3 for diagnosing CHM has good sensitivity of 96.30%, specificity 100%, PPV 100% and NPV 100%. Immunoreactivity of p57 showed 100% sensitivity, 100% specificity, 100% Positive Predictive Value (PPV) and 100% Negative Predictive Value (NPV) in differentiating CHM from PHM.

DISCUSSION:

In this study, the age in years ranged between 19 and 45 with a mean age of 27.17years and this was comparable with a study conducted by Salah-el-din Sayed Semary where the age range was between 17 to 51 years with a mean of 29.2 years.[6] Patients with HA ranged from 19 – 45(Mean - 27.96 years), PHM ranged between 20 – 33(Mean - 26.52 years) and CHM ranged between 20 – 36(Mean - 26.85years), the same was comparable with a study conducted by Mahi Balci where the age distribution was 31.6, 27.2 and 25.3 for HA, PHM and CHM respectively.

The gestational age at the time of products submission to Pathology laboratory for all the 76 cases ranged between 3 weeks to 12 weeks (Mean – 7.84 weeks). Patients with HA ranged between 3weeks to 12 weeks (Mean – 7.46 weeks), PHM and CHM ranged between 6 weeks to 10 weeks with a Mean of 7.85 and 8.22 weeks each respectively, whereas in a study by Mahi Balci it was 9.7, 9.4 and 8.4 for HA, PHM and CHM respectively.[8]

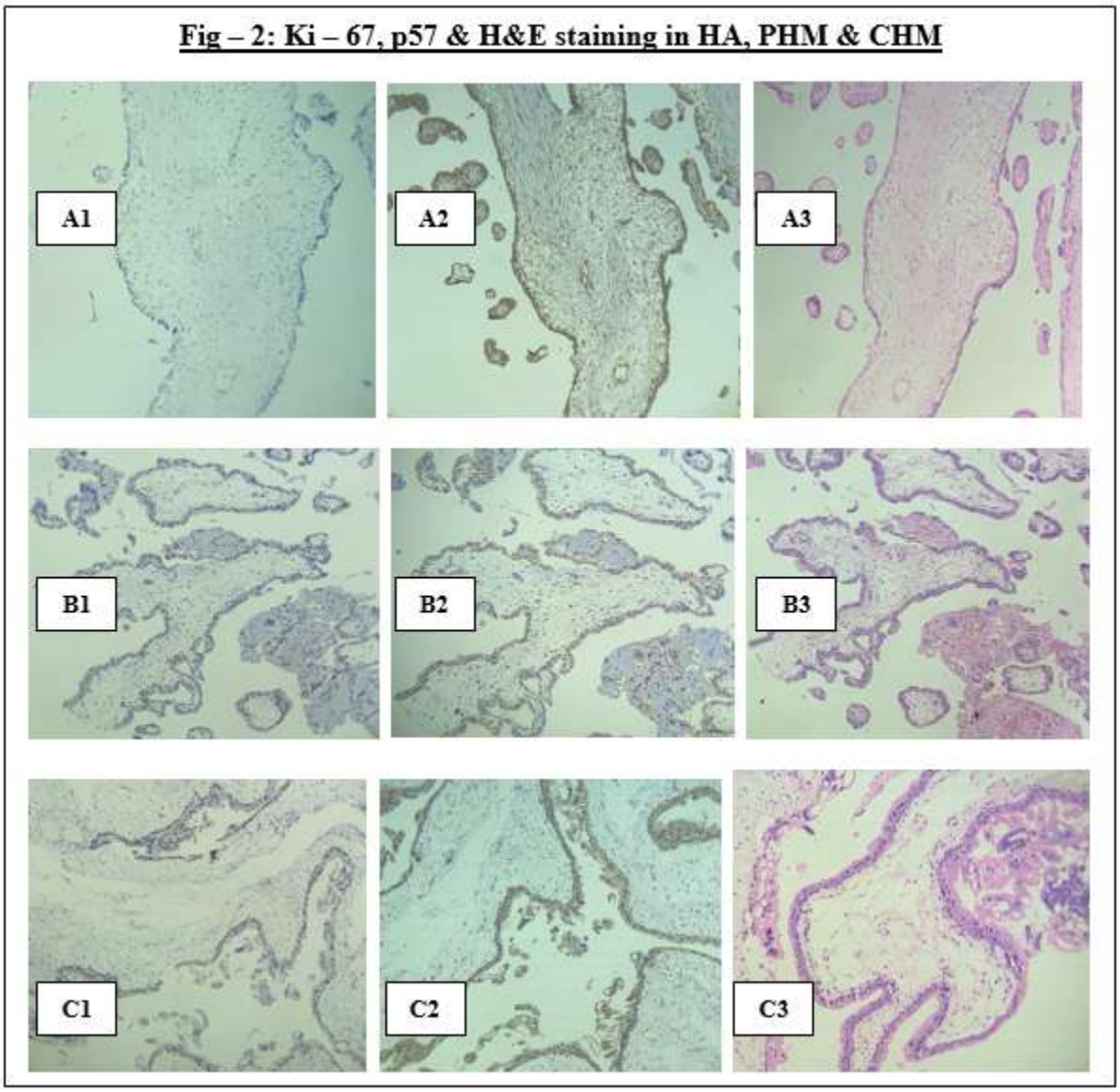

Figure – 2:

A1: HA with Ki-67 weak positivity; A2: HA with p57 diffuse nuclear positivity; A3: HA with H&E. B1: PHM with Ki-67 moderate positivity; B2: PHM with p57 diffuse nuclear positivity; B3: PHM with H&E. C1: CHM with Ki-67 strong positivity; C2: CHM with p57 nuclear negativity; C3: CHM with H&E

In this study, p57 immunoreactivity among the 30 HA cases and 23 PHM cases, 2 cases from each entity were negative and were poorly stained with Ki-67 as well and hence were considered discordant cases and were not included in further statistical analysis. Discordance in

the 4 cases could have been because of androgenetic/biparental mosaicism and such cases pose a great diagnostic threat to the pathologists and also require molecular genotyping for definite diagnosis.[9]

p57 immunoreactivity was positive in 28 HA and 21 PHM cases and negative in all the 27 CHM cases. The same findings were almost in conjunction with other studies for p57 immunoreactivity.[10-13] In this study the staining characteristics of p57 among the HA and PHM groups were similar whereas in a study by Rita.L HA showed diffuse p57 and PHM with focal staining.[14] Even though p57 was positive in both HA and PHM, its diagnostic validity in identifying CHM is most reliable with a PPV of 100% therefore helps distinguish CHM from its other mimics and the same is accepted in other literatures.[9, 15]

In this study, for Ki-67 immunostaining, HA cases were predominantly grade 1(92.8%) overlapping with PHM grade 1(71.4%) and PHM graded 2 (28.5%). CHM was predominantly graded 3 (96.3%) and graded 2 in 3.7% and the same pattern of immunostaining was found in other literatures.[16 - 19] The diagnostic validity of Ki – 67 used in this study showed an overlapping between HA and PHM at grade – 1 level, but PHM cases graded 2 were more reliable, in case of CHM grade 3 was most reliable, hence making CHM reliably distinct from PHM and the same was found in other studies.[20 - 24]

Limitations in this study were small sample size as this was a single institutional study. Also cause for discordance was not identified in 4 cases due to the cost effectiveness of the molecular genotyping studies.

CONCLUSION:

CHM needs to be undoubtedly diagnosed and reliably differentiated from PHM as both entities have different clinical behavior. To establish this, p57 immunohistochemical marker not only helps diagnoses CHM but also helps distinguish CHM from its mimics, however it cannot distinguish HA from PHM, whereas Ki-67 shows an overlap of staining characteristics of PHM with HA and CHM. Hence in this study a clear cut diagnosis of CHM was made using p57 and this early detection will definitely pave way for stringent follow up and prevention or early detection of the malignant potential of the condition.

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CONFLICTS OF INTEREST:

Nil

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