

Association between Histomorphological Types of Hydatidiform Mole and the Expression of Ki67 Immunohistochemical Marker as well as β -hCG Level Status

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Abstract

Background: Hydatidiform mole (HM) is the most common type of gestational trophoblastic disease which is sub-classified into complete hydatidiform mole (CHM) and partial hydatidiform mole (PHM). Accurate subclassification of HM is very important as the development of choriocarcinoma and persistent trophoblastic diseases are more related to CHM than PHM. However, sometimes this subclassification is more challenging only based on histomorphological findings. Because there is considerable intraobserver and interobserver variability, histomorphological findings in conjunction with some complementary methods such as immunohistochemistry and biochemical markers could be helpful in the accurate interpretation of the HMs. The aim of this study was to evaluate the expression of Ki 67 immunostain along with the pretreatment β -hCG level and to identify their association with histomorphological types of hydatidiform mole.

Methods: This cross-sectional observational study was done at Department of Pathology, Dhaka Medical College. The cases were classified into CHM and PHM based on histopathological features. Pretreatment β -hCG level noted and Ki 67 immunohistochemistry was done. The results of the cases were collected and tabulated. Statistical analysis was performed on the tabulated data using Fisher Exact test, Chi-square test and Unpaired t-test.

Results: Out of 50 cases, based on histopathological criteria, 27(54.0%) cases were CHM and 23(46.0%) were PHM. After evaluating Ki67 immunohistochemical staining, among CHM, 23(85.2%) cases showed Ki67 score-3 and 4(14.8%) cases score-2. Out of 23 PHM, 22 (95.7%) cases showed Ki -67 score-2 and 1(4.3%) case showed score-3. These were statistically significant. On the other hand, among the CHM 22 (81.5%) had β -hCG level ≥ 100000 mIU/ml and 5 (18.5%) had <100000 mIU/ml. Among PHM 2 (8.7%) had β -hCG ≥ 100000 mIU/ml and 91.3% had β -hCG <100000 mIU/ml. It was also statistically significant.

Conclusion: Diagnosis of HM can be improved by Ki67 labeling index and evaluation of pretreatment β -hCG level, which will help in differentiating CHM from PHM. The correct diagnosis is crucial for future treatment and follow-up of the patients.

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Keywords: Hydatidiform mole, Complete hydatidiform mole, Partial hydatidiform mole, β -hCG, Ki67 Immunomarker

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Introduction

Hydatidiform mole or molar pregnancy results from genetic defects during conception.¹ It is an abnormal pregnancy characterized by varying degrees of trophoblastic proliferation (both cytotrophoblast and syncytiotrophoblast) and vesicular swelling of placental villi associated with an absent or abnormal fetus.² Hippocrates first reported the moles (470–410 BC) and explained how they were formed by pregnant women. However, William Smellie later used the names "mole" and "hydatidiform" (1752).³ In Southeast Asia, the incidence of HMs per 1000 pregnancies ranges from 13 in Indonesia, 8 in Taiwan, 5 in Philippines and 3.8 in Japan. According to hospital data-based studies, the incidence of molar pregnancy in India is 1.05/1000 deliveries.⁴ Shamima et al. have reported that the incidence of molar pregnancy in Bangladesh is 5.3 per 1000 births in their study and most patients are multigravida.⁵

HM has a potential for malignant transformation. Based on morphological, genetic and clinical characteristics, hydatidiform moles are further classified as a complete hydatidiform mole (CHM) and partial hydatidiform mole (PHM).⁶

According to multiple studies, CHM carries a 10–30% risk, whereas a PHM carries a 0.5–5% risk of developing persistent gestational trophoblastic disease.⁷ Research has shown that approximately 8% to 30% of individuals with CHMs require chemotherapy after evacuation for the development of persistent trophoblastic disease and 0.6% to 1.5% of patients have experienced recurrent molar pregnancy. Only 5% of patients with PHMs need treatment following evacuation.⁸ So, the clinical difference between CHMs and PHMs impacts the treatment and prognosis of the patients.⁹ Despite well described histopathologic criteria, differentiation

between CHM and PHM remain problematic because of intra-observer and interobserver variability.¹⁰ Especially in early pregnancy, the diagnostic criteria differ slightly from the classical pathological feature.

Ki 67 is a labile non-histone nuclear protein gene located on the long arm of human chromosome 10 (10 q25). High levels of Ki67 immunoexpression indicate a poor prognosis.¹¹ Ki67 labeling index of villous cells, especially cytotrophoblasts, is valuable in diagnosis and differentiation between different subgroups of molar pregnancy. It is highest in CHM (More than 40%), followed by PHM (20% - 40%).¹² The risk of the development of Choriocarcinoma in CHM is higher than in PHM.¹³ It may need chemotherapy after evacuation and long-term follow-up. Molecular genotyping is the gold standard method for definite diagnosis.¹⁴

Objective

The present study aimed to define the biological activities of molar pregnancy. It may be helpful for patient follow-up plans, guiding therapeutic strategies and reducing the total treatment cost of the patients.

Methods

It was an observational type of cross-sectional study done at Department of Pathology at Dhaka Medical College from March, 2021 to February, 2023. Total 50 cases were enrolled in this study by non-probability type purposive sampling technique. After considering all the inclusion and exclusion criteria, all the relevant information and demographic data were collected from the patient. Pretreatment β -hCG value from the patient file was collected. Hematoxylin & Eosin (H&E) stained sections of each case were evaluated elaborately for defined morphological features like trophoblastic hyperplasia, intensity and location, cytologic atypia of trophoblastic cells, pseudo

inclusions of trophoblast, cistern formation, scalloping, fetal vessels in the villous stroma. According to histopathological criteria cases were divided into two groups - CHM and PHM. One section from the representative paraffin block for each case was selected for immunohistochemical stain with Ki67 which was done at Department of Pathology, Bangabandhu Sheikh Mujib Medical University, Dhaka. Evaluation of immunostaining was performed by light microscopy. Primary antibody was - Monoclonal Mouse Anti-Human Ki 67 antigen Clone MIB-1 Ready to use (LINK). Secondary Antibody was DAKO REALTM EnVision TM (HRP RABBIT/MOUSE) (ENV)

Positive control was normal lymphocytes in the lymphoid follicle of an appendix. Brownish nuclear staining of proliferating trophoblastic cells was considered Ki 67 scoring. Score 2(++): 21-40% representing cases of partial mole. Score 3(+++): Above

40% representing cases of complete mole.¹²

All the collected data were checked, compiled and statistically assessed to search any association between Ki 67 and β -hCG level with HM. Statistical analysis was done by SPSS (version 21) and statistical significance was set at $p < 0.05$.

Results

In this cross sectional observational study total 50 cases were selected. Among them 20 cases were below 20 years of age, 19 were within the age range of 21-30 years and only 4 cases were more than 40 years of age. Most (62%) of the cases were multigravida whereas in 66% cases gestational age were 12-16 weeks. In this study it was observed that molar pregnancy is mostly prevalent in blood group A-Positive (40%) and B-Positive (37%) and least in O-negative (2%). After careful histological evaluation 46% cases were PHM and rest (54%) were CHM.

Table I: Socio demographic characteristics of the respondents

Character	Distribution	Frequency	Percentage
Age	≤ 20 years	20	40%
	21- 30 years	19	38%
	31-40 years	7	13.7%
	> 40 years	4	8%
Gestational age	< 12 weeks	17	34%
	12-16 weeks	33	66%
Parity	Primigravida	19	38%
	Multigravida	31	62%
Blood group	A-Positive	20	40%
	B-Positive	17	34%
	B-Negative	2	4 %
	AB - Positive	2	4%
	O-Positive	8	16%
	O -Negative	1	2%
Histopathological type	CHM	27	54%
	PHM	23	46%

Different histopathological parameters were used to sub classify the HM. It was observed that diffuse hydropic degeneration of villi was present in 24 (88.9%) cases of CHM and one (4.3%) PHM case. One of the histological

parameters was trophoblastic hyperplasia, intensity and location. Among them, 20 cases (74.1%) of CHM had marked circumferential trophoblastic hyperplasia, and 7 cases (25.9%) of CHM had marked polar

hyperplasia. Almost all cases (100%) in PHM had mild polar trophoblastic hyperplasia. In CHM, 17 cases (63.0%) had marked cytologic atypia of trophoblastic cells. This finding was not found in PHM. Pseudo-inclusions of trophoblasts were found in 8 (29.6%) cases of CHM and 19(82.6%) cases of PHM. Cistern

formation was found in 26 (96.3%) cases of CHM and 6 cases (26.1%) of PHM. Scalloping was seen in 5 cases (18.5%) of CHM and 22 cases (95.7%) of PHM. All (100%) PHM cases had fetal blood vessels in the villous stroma, which was not found in CHM.

Table II: Different histopathological findings in Hydatidiform mole (n=50)

Histopathological findings	CHM(n=27)		PHM(n=23)	
	n	%	n	%
Hydropic degeneration of villi				
Diffuse	24	88.9	1	4.3
Focal	3	11.1	22	95.7
Trophoblastic hyperplasia intensity and location				
Circumferential	20	74.1	0	0.0
Polar	7	25.9	23	100.0
Cytologic atypia of trophoblastic cell				
Mild	10	37.0	23	100.0
Marked	17	63.0	0	0.0
Pseudo inclusions of trophoblast	8	29.6	19	82.6
Cistern formation	26	96.3	6	26.1
Scalloping	5	18.5	22	95.7
Fetal vessels in villous stroma	0	0.0	23	100.0

The observation was that out of 27 CHM cases, 22 (81.5%) of patients belonged to pretreatment β -hCG level ≥ 100000 mIU/ml, and 5(18.5%) cases had <100000 IU/ml. Out of 23 PHM cases, 21 (91.3%) cases of pretreatment β -hCG level had <100000 IU/ml, and 2 (8.7%) cases of pretreatment β -hCG level had ≥ 100000 mIU/ml. Out of 27 CHM cases 23(85.2%) patients had(score-3) Ki67 immunoexpression and 4(14.8%) patients had (score-2). Out of 23 PHM cases, 22 (95.7%) patients had Ki67 Score-2, and 1 (4.3%) had score-3. Pretreatment β -hCG level and Ki67 expression were significantly ($p<0.05$) associated with histopathological types of hydatidiform mole.

Table III: Association between histopathological types of hydatidiform mole and Ki67 immunoexpression along with pretreatment β -hCG level (n=50)

Histopathological types Of hydatidiform mole	CHM(n=27)		PHM (n=23)		P value
	n	%	n	%	
Pretreatment β -hCG (mIU/ml)					
<100000	5	18.5	21	91.3	0.001 ^s
≥ 100000	22	81.5	2	8.7	
Mean $\pm$ SD	153486.4 $\pm$ 170139.9		58989.3 $\pm$ 103130.2		0.001 ^s
Range (Min-Max)	20755-749400		618-500500		
Ki67 immunoexpression					
Score-2 (PHM)	4	14.8	22	95.7	0.001 ^s
Score-3 (CHM)	23	85.2	1	4.3	

s=significant

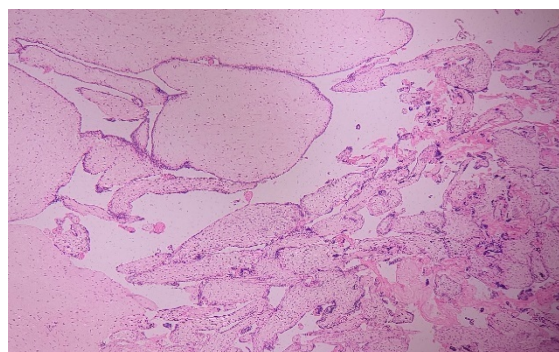
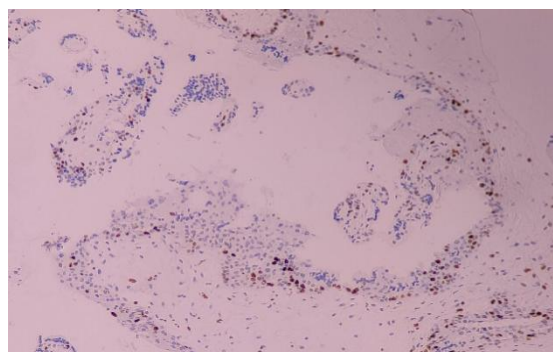
**A****B**

Figure 1. Photomicrograph showing a case of partial hydatidiform mole, (A) H&E stain revealed, two populations of villi - small fibrotic and hydropic villi (40x), (B) Immunohistoexpression of Ki67 in cytotrophoblastic cells and stromal cells (100x).

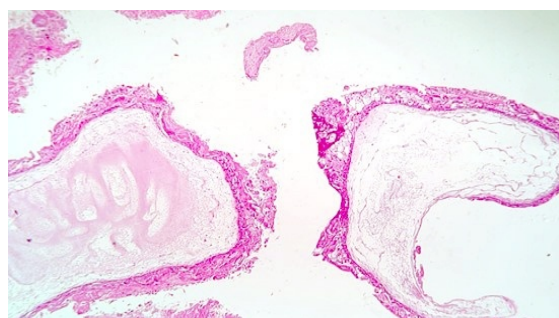


Figure 2. Photomicrograph showing a case of complete hydatidiform mole, H&E stain revealed diffuse hydropic degeneration of villi with cistern formation (100x).

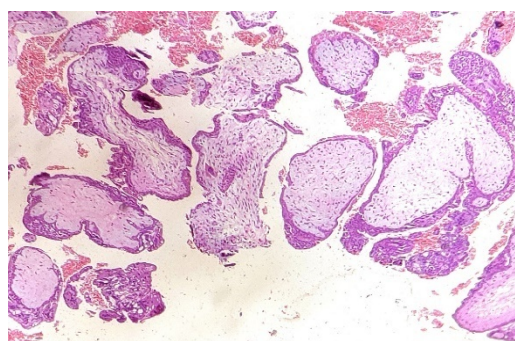
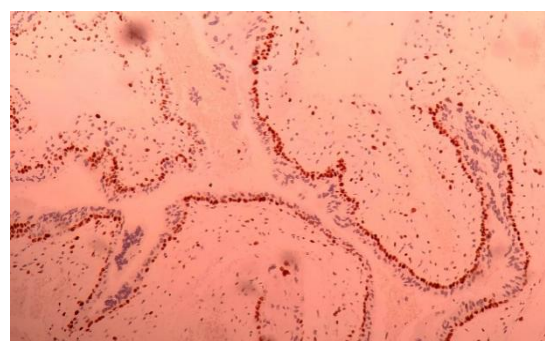
**A****B**

Figure 3: Photomicrograph showing a case of Complete hydatidiform mole, (A) H&E stain revealed marked circumferential trophoblastic hyperplasia (40x), (B) Immunohistoexpression of Ki67 in cytotrophoblastic cells and stromal cells (100x)

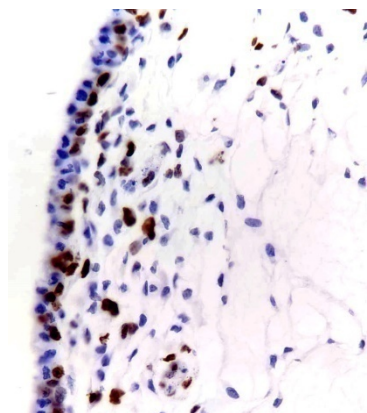


Figure 4: Photomicrograph showing a case of complete hydatidiform mole revealed Immunoexpression of Ki67 in stromal cells (100x).

Discussion

The diagnosis of molar pregnancies into a CHM and PHM is usually achieved by histomorphological features alone. Ki 67 immunostaining techniques have been reported as a known diagnostic technique complementary to histopathological diagnosis. This cross-sectional study was carried out to observe the association among Ki 67 immunoexpression and β -hCG level in CHMs and PHMs.

In this study, it was observed that almost half (40.0%) of patients belonged to the age ≤ 20 years. The mean age of the study cases was 25.2 ± 7.71 years, consistent with those mentioned by Mungan et al. (1995).¹⁵ They reported that the age range in HM mean age was 25.29 ± 7.40 years. In Bangladesh, a study was done at Dhaka Medical College Hospital (DMCH), showing that 63.75% of patients were in the 21-30 age group.¹⁶

In this study the mean gestational age was 12 ± 2.42 weeks ranging from 7 to 16 weeks. Moussa et al. (2018) found that the mean gestational age at presentation was 7-17 weeks, which is consistent with current study.⁹ Regarding the parity, 62.0% of patients were multigravida and 38.0% were primigravida. Shamima et al. (2018) reported

that 81.2% of patients were multigravida and 18.8% were primigravida.⁵ Another study was done by Agrawal (2015), where most patients were multigravida.¹⁷ Almost half (40%) of patients had A-Positive blood groups, 34% had B-Positive, 2(4%) had B-Negative, 2(4%) had AB-Positive, 8 (16%) had O-Positive and 1(2.0%) had O-Negative. Similar fact observed in previous study done by Jagtap et al.¹⁸

In this study, there were also some overlapping histopathologic features. Though most of the cases of CHM in the study had marked circumferential trophoblastic hyperplasia (74.1%), a significant number case of CHM (25.9%) had marked polar trophoblastic hyperplasia. Cistern formation is an important finding of CHM, but 6 cases (26.1%) of PHM showed this finding. Like that, scalloping and pseudo inclusion of trophoblasts were associated with PHM, but 5 (18.5%) CHM cases showed scalloping. These were consistent with the study carried out by Samadder and Kar.¹⁹

Regarding the association between histopathological types of hydatidiform mole and β -hCG level, it was observed that the majority (81.5%) of CHM patients belonged to β -hCG level ≥ 100000 mIU/ml and 2(8.7%)

in PHM. Most (91.3%) of patients with PHMs belonged to β -hCG level <100000 mIU/ml. Only in 5 cases (18.5%) of CHM β -hCG level was <100000 mIU/ml. The differences were statistically significant ($p<0.05$) between the two groups.

This study investigated the association between Ki67 expression and the pretreatment β -hCG level. The findings were that out of 24 patients who belonged to Ki67 score-3, 21 (87.5%) had pretreatment β -hCG level ≥ 100000 mIU/ml, and 3 (12.5%) had pretreatment β -hCG level <100000 mIU/ml. Out of 26 patients who had Ki67 score- 2, twenty-three (88.4%) patients had pretreatment β -hCG level <100000 mIU/ml and 3 (11.6%) patients had >100000 mIU/ml. The differences were statistically significant ($p<0.05$) between the two groups. Thus, in histomorphologically and immunohistochemically diagnosed cases, pretreatment β -hCG levels were above 1 lac in the case of CHM and below 1 lac in PHM. This study is consistent previous one.²⁰

The present study exhibited the association between Ki67 expression and histopathological diagnosis. Out of 27 histopathologically diagnosed CHM cases, 23 (85.4%) cases had score-3, and 4 (14.5%) had score-2. Among twenty-three histopathologically diagnosed PHM cases, 1 (4.3%) case had score-3 and 22 (95.2%) had score-2. The differences were statistically significant ($p<0.05$) between the two groups. Deka et al. (2019) stated, "Ki67 has been established as a valuable reflection of the tissue proliferative compartment and thus could be of value in studying the biologic behavior of the HM."¹²

Conclusion

Expression of Ki67 and pretreatment β -hCG level showed statistically significant association with histopathological subtypes of

hydatidiform mole (HM). So, using Ki 67 immunostain, pretreatment β -hCG level and histomorphological examination of hydatidiform mole (HM) for identifying high-risk patients could be done for accurate diagnosis.

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