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The Role of Ki-67 Immunoexpression in Diagnosis of Molar Pregnancy and Differentiating its Subtypes

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Abstract

Objectives: The study is intended to evaluate the role of Ki-67 immunoexpression in the diagnosis of molar pregnancy & differential diagnosis of its subgroups from miscarriage.

Methods: Sixty eight formalin-fixed, paraffin- embedded specimens of products of conception , including 1st trimester miscarriage (n=15), partial hydatidiform mole PHM (n=24), complete hydatidiform CHM (n=24) and full term placenta (n=5), all were examined at Histopathology Department of Maternity Teaching Hospital in Erbil, Iraq during the period Sep.2012-Sep.2013. Ki-67 immunohistochemical staining was performed by using the monoclonal antibody MIB-1 and the standard streptavidin-biotin immunoperoxidase method. The labeling index (number of positive nuclei/total number of nuclei) for villous, cytotrophoblasts , syncytiotrophoblasts and stromal cells were evaluated separately. Statistical analysis was carried out by Fisher's exact test & statistical significance was determined at $p < 0.05$.

Results: The study shows that all villous trophoblastic lesions showed high Ki-67 in all villous components especially cytotrophoblasts, being the highest in CHM mole(>50%) followed by PHM (>20%). Also found a statistically significant differences in immunoexpressins of Ki-67 that was useful in separating miscarriage from CHM, $p<0.01$ (highly significant), and partial hydatidiform mole $p<0.05$, (significant).

Conclusion: Ki-67 labeling index of villous cells ,especially cytotrophoblasts, is valuable in diagnosis and differentiation of hydatidiform mole from 1st trimester miscarriage as well as between different subgroups of hydatidiformmoles (CHM & PHM).

Keywords: Partial Hydatidiform Mole, Complete Hydatidiform Mole, Miscarriage, Ki-67, Immunohistochemistry.

Introduction

Trophoblastic diseases constitute a spectrum of tumors and tumor- like conditions characterized by proliferation of pregnancy associated trophoblastic tissue of progressive malignant potential. The lesions include Hydatidiform mole HM (Complete Hydatidiform mole CHM and Partial Hydatidiform mole PHM), invasive mole, and malignant Choriocarcinoma [(1)]. Accurate diagnosis and classification of HM is important as the risk of persistent gestational trophoblastic disease, including the CC, is significantly high. The risk of CC in CHM is 10% – 30% and in PHM is 0.5% – 5% [(2)]. Despite well-described histopathologic criteria, the distinction

of spontaneous miscarriage from HM and CHM from PHM remains a problem because of interobserver and intraobserver variability [3]. Thus, development of new methods that allow differentiating these diseases in doubtful cases is important. A complementary method to the pathologic interpretation is immunohisto-chemistery (IHC). Among the immunohistochemical markers, proliferation markers such as Ki-67 have been established as a valuable reflection of the tissue proliferative compartment and thus could be of value in studying the biologic behavior of gestational tophoblastic diseases [4]. Ki-67 is a labile non histone nuclear protein that is tightly linked to the cell cycle, it is expressed in proliferating cells during mid G1 phase, increasing level through S and G2 phases and peaking in the M phase of the cell cycle. It is rapidly catabolized at the end of M phase and it's undetectable in resting cells and during DNA repair process, compared with other proliferative markers, Ki-67 staining is easy to perform, economical and more reproducible [5.6]. The *Ki-67* gene is present on the long arm of human chromosome 10 (10 q25) and its half life has been estimated approximately 60-90 minutes [7]. The aim of this study was to evaluate the value of Ki-67 immunoexpression in diagnosis of molar pregnancy & in the differential diagnosis of subgroups of HM from miscarriage.

Materials and Methods

During the period between Jan 2011-Jun 2013, the pathology archives of the Maternity Teaching Hospital & some private histopathological laboratories were searched for the pathological diagnosis of molar pregnancy & 1st trimester non molar miscarriage. A total of (68) formalin fixed & paraffin-embedded samples of products of conception were randomly selected and categorized into the following groups: 1st trimester miscarriage (n=15), PHM(n=24), CHM(n=24), in addition 5 samples of full term placenta were included as a negative control. All the blocks were examined and the one which represented the diagnosis best (no necrosis, no hemorrhage) was selected for the study and new section was made and stained with hematoxylin and eosin (H&E) for histological reevaluation, In order to differentiate PHM from CHM the histologic features of the specimen were assessed according to diagnostic agreed with the histopathological criteria [8], another thin 4 mm section was made and submitted for IHC. The study was approved by the Ethical Committee of the College of Medicine, Hawler Medical University, Erbil, Iraq.

Immunostaining: Immunohistochemistry was performed using the avidin-biotin-peroxidase complex in which primarily monoclonal antibodies raised against Ki-67 (Dako Cytomation, Denmark, clone MIB1) were used & according to Dako Cytomation EnVisionR+Dual link system-HRP(DAB+) staining protocol for immunostaining as the manufacturer's instructions described in the leaflet supplied with the antibody. Briefly, for antigen retrieval, deparaffinized sections were pretreated by being heating in a microwave oven in 10 mM citrate buffer, pH 6.0, for 20 minutes. After cooling, sections were immersed in phosphate buffered saline (PBS) containing 3% hydrogen peroxide for 10 min to block endogenous peroxidase activity. Sections were then incubated in a humid chamber overnight at 4°C with the following primary antibodies: Ki-67 (clone MIB-1; dilution 1:100; DakoCytomation R, Denmark). After rinsing with PBS,

slides were incubated with a secondary antibody followed by streptavidin-biotin-peroxidase complex, both for 30 min at room temperature with a PBS wash between each step (LSAB+ system; DakoCytomationR, USA Denmark). The slides were developed with diaminobenzidine-H2O2 (DAB+ system; DakoCytomationR, USA), counterstained with Harry's hematoxylin and mounted. Negative controls, in which N-universal negative control replaced the primary antibody, were run with each batch of stain and breast cancer sections known to stain strongly positive for Ki-67 was included with each run as positive controls.

Assessment of nuclear accumulation of Ki-67: For Ki-67 immunoexpression, the evaluation was conducted by using a semi quantitative scoring method by determining the proportion of +ve cells over total numbers of cells. All significantly stained cells were counted and the labeling index (LI) were determined by counting 1000 cells then divided by 10 to acquire the percentage ; at least 10 HPF were measured for each case for the purpose of scoring. The LI in miscarriage and molar pregnancy were evaluated separately for cytotrophoblasts (cyto.), syncytiotrophoblasts (syn.), and stromal cells by counting 1000 cells for each population .Also the distribution of Ki-67 immunoreactivity in trophoblastic diseases were quantitatively assessed as -ve (equal or less than 10% are positive cells) & +ve (more than 10% are positive cells) and the positive cases were graded as: + (10-20%) ; ++ (20-50 %); and +++ (more than 50%) are positive cells.

Statistical analysis:- Data were analyzed using the statistical package for social sciences (SPSS, version 19). The results obtained from each case groups were compared in pairs for the following parameters included in the study(Ki-67 LI and distribution of Ki-67 immunostaining) by means of Fischer's exact test. A p value of ≤ 0.05 was considered statistically significant, and p value <0.01 was considered statistically highly significant.

Results

The mean age (± SD) for miscarriage was 29.6 ±3.7yrs, range: (20 - 42) ; for PHM 29.03±2.1 yrs., range: (20 - 40) & for CHM 31.04±3.3 yrs., range: (17- 50). Specific staining of Ki-67 protein with monoclonal antibody MIB-1 was confined to the nuclei of cyto., syn. & stromal cells of placental villi. Figure (1).

Table (1):-Ki-67 LI in miscarriage & molar pregnancy in all villous components

Type of lesion	Ki-67 LI in Cyto.	Ki-67 LI in Syn.	Ki-67 LI in stromal cells
Miscarriage	7±4.971	3.3733±3.615	2.867±3.159
PHM	22.5±16.611	17.833±15.228	11.292±10.732
CHM	68.542±11.275	53.958±9.778	39.375±11.545

All full term placenta specimens showed negative Ki-67 immunostaining in all components of villi, while 1st trimester miscarriage showed positive Ki-67 immunostaining of villi with a LI =7±4.971, 3.3733±3.615, 2.867±3.159 in cyto., syn. & stromal cells respectively . In comparison

with Ki-67 LI of 1st trimester miscarriage (control group), molar pregnancy (PHM &CHM) showed a higher Ki-67 LI of all villous components especially of cyto. ; being the highest in CHM (68.542±11.275) then PHM (22.5±16.611). The lowest Ki-67 LI observed in stromal cells of villi in both molar pregnancy & miscarriage, while Ki-67 LI of syn. was in between them. As shown in Table (1).

Table(2):- Distribution of Ki-67 immunoreactivity in miscarriage and HM

Type of lesion	**Cytotrophoblasts. No. (%)**				**Syncytiotrophoblasts. No. (%)**				**Stromal cells No. (%)**			
	+	++	+++	-ve	+	++	+++	-ve	+	++	+++	-ve
Miscarriage	1 (6.7)	0	0	14 (93.3)	1 (6.7)	0	0	14 (93.3)	1 (6.7)	0	0	14 (93.3)
PHM	0	12 (50)	1 4.2	11 (45.8)	6 (25)	5 (21)	0	13 (54)	4 (16.6)	4 (16.6)	0	16 (66.8)
CHM	0	0	24 (100)	0	0	20 (83.3)	4 (16.6)	0	0	20 (83.3)	4 (16.6)	0

+ 10-20%; ++ 20-50; +++ > 50%; -ve < 10%

Regarding the distribution of Ki-67 LI in miscarriage and villous lesions; fourteen out of fifteen cases (93.3%) of first-trimester miscarriage had a LI ≤10% in all cell populations (cyto. ,syn. & stromal cells) of villi . All 24 cases (100%) of CHM showed positive Ki-67 immunoexpression in all villous components with a LI above 50% in all cases. while 12/24 cases (50%) of PHM had Ki-67 LI above 20% and below 50% in cyto. Only one case of PHM had a LI above 50% in cyto. Table (2).

In order to study the role of Ki-67 immunoexpression in differentiating these trophoblastic lesions, the data of these three groups (miscarriage, PHM & CHM) were analyzed, matched in pairs & evaluated statistically according to their Ki-67 expressions(Table 3). A significant difference was found between Ki-67 immunoexpression in cyto. of miscarriage & PHM ($p< 0.05$) and there was a highly significant difference between Ki-67 immunoexpression of all villous components of miscarriage & CHM and those of PHM & CHM ($p< 0.01$).

Table (3): Statistic comparison of Ki-67 immunoexpression between miscarriage and hydatidiform mole in all villous components

Type of lesion	Cyto.		P value	Syn.		P value	Stromal cells		P value
	+ve	-ve		+ve	-ve		+ve	-ve	
Miscarriage	2	13		1	14		1	14	
PHM	13	11	0.0173**	11	13	0.0131**	8	16	0.1152*
Miscarriage	2	13		1	14		1	14	
CHM	24	0	<0.0001***	24	0	<0.0001***	24	0	<0.0001***
PHM	13	11		11	13		8	16	
CHM	24	0	0.0003***	24	0	<0.0001***	24	0	<0.0001***

*Non significant (p>0.05), ** significant (p≤0.05), *** Highly significant(p≤0.01)

Discussion

Gestational trophoblastic diseases are defined as a spectrum of abnormal gestations and neoplasms arising from villous or extravillous trophoblasts that are associated with pregnancy. They takes several forms, each with its own risk of mortality and responsiveness to chemotherapy. Differential diagnosis of these diseases by routine histopathologic examination can be challenging. Studies have recently shown that IHC for various markers is useful for confirming the diagnosis & it is a complementary method to pathologic interpretation [(4)].One of the advantages of this method is the ability to apply them retrospectively to sections of routinely formalin-fixed and paraffin-embedded tissue and there is no need for expensive or sophisticated equipments. In this study we investigated the immunoexpression of a proliferation marker (Ki-67) protein in various trophoblastic diseases.

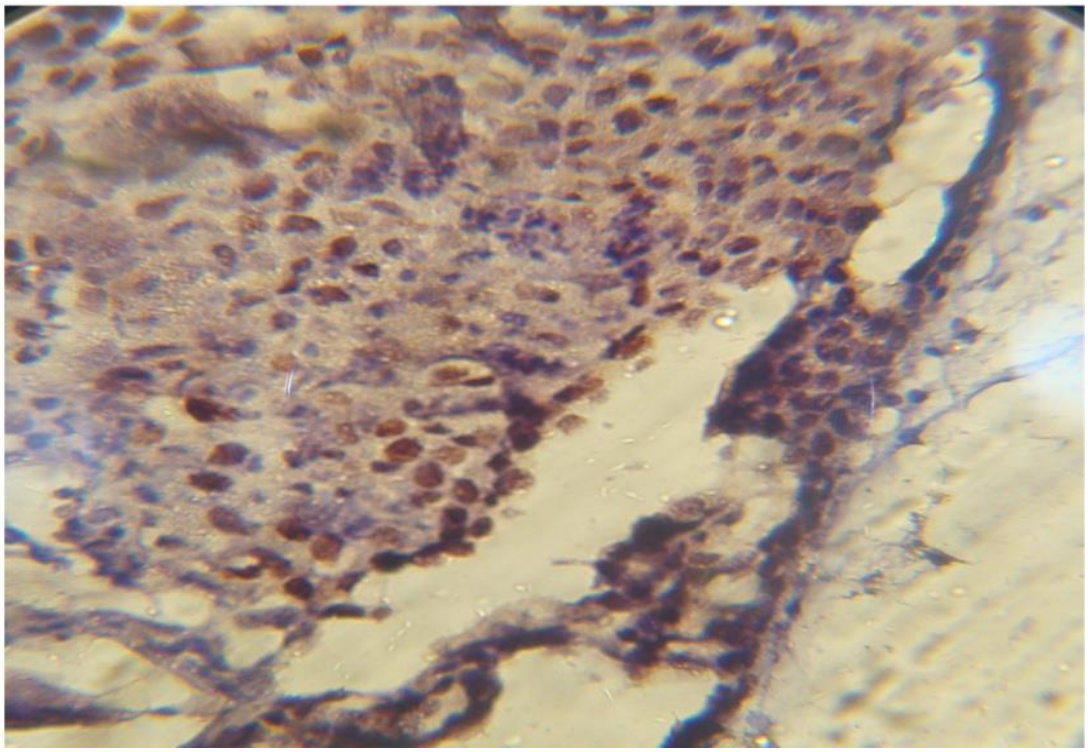

Figure (1): Complet hydatidiform mole with positive nuclear Ki-67 immunoexpression.

All full term placenta specimens showed negative Ki-67 immunostaining in all components of villi; while 1st trimester miscarriage showed positive nuclear Ki-67 immunostaining of all villous components with a LI =7±4.971, 3.3733±3.615, 2.867±3.159 in cyto. , syn. & stromal cells respectively. This indicated that full term placenta did not have any proliferative activity while 1st trimester villi had some proliferative activity especially the cyto. ; But in the majority of miscarriage cases (93.3%) the value did not exceed 10%, In hydatidiform mole, Ki-67 LI was higher than that of miscarriage (i.e ≥ 10%), many other studies showed the same results[9,10,11]. i.e Ki-67 immunoexpression in miscarriage was less than in molar pregnancy.

It seemed that among these three groups of villous cells (cyto., syn ., and stromal cells), the highest Ki-67 LI was in cyto. of all villous trophoblastic lesions. This comes in agreement with two other studies done [4,12]. The highest Ki-67 LI was in cyto. of CHM (68.542±11.275) followed by PHM (22.5±16.611).The lowest Ki-67 LI observed in stromal cells of villi in all villous lesions while Ki-67 LI of syn. was in between. This indicates that Ki-67 LI of cyto. is the best index in separating these three entities, three other studies showed the same results .[4,13,14]

The distribution of Ki-67 immunoexpression in miscarriage and villous trophoblastic lesions (table 2) comfirmed that 10% is a good cutoff value in reading & separating positive Ki-67 immunoexpression (≥10%) from negative ones (< 10%). Majority of miscarriage cases (93.3%) showed –ve Ki-67 immunoreactivity in all villous cella while all CHM cases (100%) had +ve Ki-67 immunoexpression with a value above 50% in all villous cells. For PHM, more than half of

cases (54.2%) had +ve Ki-67 immunoexpression in cyto. cells. Other studies as Hasanzadeh [13] prefered to use 12.5% & 6% as a cut off value for reading Ki-67 immunoexpression in cyto. & syn. cells respectively.

There was a statistically significant differences in Ki-67 immunoexpression in both cyto. and syn. between miscarriage and PM ($p<0.05$); there also were extremely significant differences in Ki-67 immunoexpression found between miscarriage and CHM and between PHM and CHM, in all villous cells($p<0.01$).This indicated that expression of Ki-67 is useful in separating miscarriage from CHM & PHM, and in differentiation between CHM & PHM, some other studies showed similar findings [9-11] while others as Jeffers [15] did not detect any significant difference in Ki-67 expression between patients with CHM complicated by gestational trophoblastic neoplasia and molar pregnancy that resolved spontaneously.

Conclusion

Ki-67 labeling index of villous cells ,especially cytotrophoblasts, is valuable in diagnosis and differentiation of hydatidiform mole from 1st trimester miscarriage as well as between different subgroups of hydatidiformmoles (CHM & PHM).

CONFLICT OF INTERESTS.

There are non-conflicts of interest.

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الخلاصة

الهدف:- لدراسة دور التعبير المناعي ل Ki-67 في تشخيص الحمل العنقودي وتمييزها عن الانواع الأخرى لأنواع اجهاض الحمل.

العمليات:- ثمان وستون نموذج لنواتج الحمل المثبتة بالفورمالين والمطمورة بالفورمالين ، والتي شملت أجهاض الحمل في الأشهر الأولى (15 نموذج)، حمل عنقودي جزئي (24 نموذج)، حمل عنقودي كامل (24 نموذج) ومشيمة الحمل الكامل (5 نموذج). جميع النماذج تم جمعها من مختبرات فحص الأنسجة في مستشفى الولادة في اربيل / العراق خلال الفترة من ايلول 2012- ايلول 2013. تم استخدام التصبيغ المناعي الهيستوكيمياوي ل Ki-67 وباستعمال المضاد الأحادي MIB-1 وباستخدام الطريقة المعتادة . المؤشر التعريفي لKi-67 (عدد الأنوية المصبوغة/عدد الأنوية الكلي) لكل من الزغابات المشيمة والجذعة الغذائية الخلوية والجذعة الغذائية المخلاوية والخلايا السدوية تم حسابها بصورة منفصلة. الحسابات الأحصائية تم اجراءها بأستخدام مؤشر Fisher واذا كانت قيمة الp أقل من 0.05 تم اعتباره ذو قيمة أحصائية.

النتائج:- أظهرت الدراسة أن مؤشر ال Ki-67 في كل امراض الزغابات المشيمية كان عالي وبصورة خاصة في الزغابات المشيمية للحمل العنقودي الكامل (50%<) ثم في الحمل العنقودي الجزئي (20%<). كذلك تم ايجاد علاقة ذو قيمة احصائية للتعبير المناعي لل Ki-67 والتي كانت مفيدة في التمييز بين الأجهاض والحمل العنقودي الكامل ($p<0.01$) والحمل العنقودي الجزئي ($p<0.05$).

الأستنتاج:- المؤشر التعريفي ل Ki-67 في الزغابات المشيمية كان ذو قيمة في تشخيص وتمييز الحمل العنقودي من الأجهاض في الأشهر الأولى للحمل وكذلك في تمييز الحمل العنقودي الكامل من الحمل العنقودي الجزئي .

الكلمات الدالة: حمل عنقودي جزئي، حمل عنقودي كامل ، اجهاض، التعبير المناعيKi-67 .