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The Clinical Value Of Ki-67/Mib-1 Proliferation Index In Hydatidiform Mole

Ki-67/Mib-1 Proliferasyon İndeksinin Hidatiform Mol Tanısındaki Klinik Değeri

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ABSTRACT

Aim: The significance of Ki-67 index in gestational trophoblastic diseases remains a controversial issue. This study was designed to determine whether proliferation marker, Ki-67, in trophoblastic tissue would have any clinical discriminatory value.

Material and Methods: The pathology reports of patients with diagnosis of complete hydatidiform mole (CHM) (n=32) or partial hydatidiform mole (PHM) (n=6) were included. All the patients in this study were followed up with clinical records for one year. Paraffin blocks were stained immune histochemically and computer-assisted image analysis system was used to detect Ki-67 antigen.

Results: None of the continuous variables including maternal age, gravidity, size of the uterus, hemoglobin, preevacuation serum β-hCG levels were significantly correlated with Ki-67 index (p>0.05). Ki-67 index was not discriminative for symptoms and signs including vaginal bleeding, hyperemesis, asymptomatic presentation, consistency with gestational age, theca lutein cysts, hyperthyroidism, development of GTN or remission (p>0.05). On the other hand, PHMs were found to have lower Ki-67 index [median=8 (IQR=11.00)] than CHMs [median=21 (IQR=43.00)] (Z=1.944; p=0.050).

Conclusion: It is our opinion that in the light of current data, it seems Ki-67 index has promising value in discriminating complete and partial HMs. Further prospective studies will help to determine the prognostic value for adverse clinical outcome.

Key Words: Hydatidiform mole, Ki-67, image analysis

ÖZET

Amaç: Gestasyonel trofoblastik hastalıklarda Ki-67 indeksinin önemi hala tartışılan bir konudur. Bu çalışmanın amacı proliferasyon markerlarından olan Ki-67'nin trofoblastik hastalıklarda ayırıcı tanıda herhangi bir değerinin olup olmadığını belirlemektir.

Gereç ve Yöntemler: Çalışmaya, komplet hidatidiform mol tanısı almış (n = 32) veya parsiyel mol tanısı almış (n = 6) hastaların patoloji raporları dahil edildi. Çalışmaya dahil edilen tüm hastaların kliniği 1 yıl boyunca izlendi. Parafin bloklar immünhistokimyasal olarak boyandı ve bilgisayarla asiste görüntü analiz sistemi ile Ki-67 antijeni açısından incelendi.

Bulgular: Maternal yaş, gravida, uterus büyüklüğü, hemoglobin, serum β-hCG seviyesi gibi devamlı değişkenlerin hiçbiri Ki-67 indeksi ile anlamlı korele değildi (p>0.05). Ki-67 indeksi, vajinal kanama, hiperemezis, asemptomatik prezentasyon, gebelik yaşı ile tutarlılık, teka lutein kisti, hipertiroidizm, GTN gelişimi ya da remisyonu gibi semptom ve şikayetlerle korele değildi. Fakat parsiyel molde Ki-67 indeksi [median=8 (IQR=11.00)] , komplet mole göre [median=21 (IQR=43.00)] daha düşük bulundu (Z=1.944; p=0.050).

Sonuç: Bizim çalışmamızdaki datalar, Ki-67 indeksinin komplet mol ile parsiyel mol ayırımında umut verici bir marker olduğunu göstermektedir. Bizim bulgularımızın prospektif çalışmalarla desteklenmesi gerekmektedir.

Anahtar Kelimeler: Hidatidiform mol, Ki-67, görüntü analizi

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Introduction

Molar pregnancy and gestational trophoblastic neoplasms (GTN) originate from placental tissue and are among the rare human tumors. In the United States, hydatidiform moles (HM) complicate approximately 1 in 1500 pregnancies (1). GTN include a spectrum of interrelated tumors including complete (CHM) and partial hydatidiform mole (PHM), invasive mole, choriocarcinoma and placental site trophoblastic tumor that have varying propensities for local invasion and spread (2). The latter three are true neoplasms, whereas CHM and PHM represent abnormal placentas prone to neoplastic transformation. The incidence of GTN after a CHM in the United States has been reported to be 18 to 29% and has not been affected by the earlier diagnosis and treatment of complete moles (3). With no alternative markers available, the human chorionic gonadotrophin (hCG) hormone titres remain the most reliable and specific prognostic indicator for the management of patients with molar pregnancies (4). The current classification of HMs has limitations in predicting prognosis and there is need for additional factors.

Ki-67 is an IgG1 class monoclonal antibody that was discovered by Gerdes et al. in 1983 (5). It recognizes a core antigen present in proliferating cells and absent in quiscent cells. It is recognized by the MIB-1 monoclonal antibody and expressed throughout the cell cycle in G1, S, G2 and M phases, but not the quiescent G0 phase. The prognostic value of Ki-67 immunostaining in neoplastic lesions has been well documented including non-Hodgkin's lymphoma, carcinomas of breast, kidney, lung and astrositoma (6,7,8,9,10). However, the significance of Ki-67 index in gestational trophoblastic diseases remains a controversial issue.

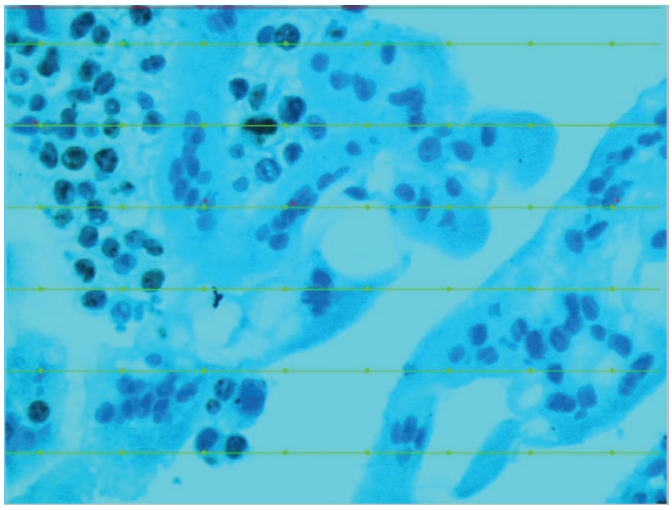
This study was designed to determine whether proliferation marker, Ki-67, in trophoblastic tissue would have any clinical discriminatory value.

Material and Methods

The pathology reports of patients with a diagnosis of CHM or PHM treated at the Etlik Zübeyde Hanım Women's Health Teaching and Research Hospital were reviewed. The diagnosis was confirmed in each case by review of hemotoxylene&eosin-stained sections. Specimens (n=44) included 36 CHM and 8 PHMs. All the patients in this study were followed up with clinical records for one year. After evacuation, patients were monitored with serial hCG levels in order to facilitate the early detection of persistent GTN. To ensure complete remission, hCG tests were performed weekly until levels had been undetectable (<5 mIU per milliliter), with subsequent monthly testing until levels had been undetectable for 6 months (3). Patients were strongly advised to use oral contraceptives during the entire interval of hCG monitoring because the occurrence of a new gestation would interfere with follow-up testing of hCG levels. The diagnosis of persistent tumor after molar pregnancy was done according to the following guidelines of International Federation of Gynecology and Obstetrics: four or more measurements of the hCG level that show a plateau in the values over a period of at least 3 week, an increase in the hCG level of 10% or more in three or more measurements over a period of at least 2 weeks and the persistence of detectable hCG levels 6 months after evacuation of a mole (11).

For immunohistochemistry, formalin-fixed and paraffin-embedded tissues were used. All slides were reexamined by an expert, and the most representative section was chosen for immune histochemical examination in each case. Standard sections 4-μm-thick sections obtained from paraffin blocks were stained immune histochemically using an automated system (Labvision autostainer 480). In order to detect Ki-67 antigen, we used a commercially available immune histochemical kit (Close MIB-1, mouse monoclonal antibody, Neomarkers, Union City, CA). The most representative area for Ki-67 was defined as the area with the most concentrated immune histochemically labeled nuclei consisting cytotrophoblastic cells. Computer-assisted image analysis system used in the antibody evaluation is a light microscope-based system that utilizes a macro program. This system is composed of the following components: a personal computer running Microsoft Windows NT 4.0 Service Pack 6a operating system (Microsoft, Redmond, Washington, U.S.A); a light microscope with motorized stage (Zeiss Axioscope, Zeiss, Göttingen, Germany); a frame grabber (Matrox Meteor, Quebec, Canada); a digital camera attached to the microscope (Sony AVT Horn 3 CCD); and a special image analysis software (Zeiss Vision KS 400 version 3.0 for Windows). The representative areas were analyzed, and in the first stage, the measurement procedure to be carried out was defined at low-power scanning magnification (25 x objective). In the second stage, this rectangular area was scanned sequentially in consecutive, smaller rectangular areas at high-power magnification (400 x objective). In every scanned field, a digital image was captured, and a randomly oriented parallel line-and-dot graph (grid) was superimposed on that image. Next, positive cells for nuclear staining for Ki-67 with the dots on the grid were counted (Figure 1). The measurement process was carried on until all the dots in the scanned field were counted. The proportion of positive-stained cells per total number of counted nuclei was calculated as a percentage by the software.

Figure 1: A screen capture taken during the interactive measurement of Ki-67 expression percentage. The procedure is based on the counting of the labelled and unlabelled cells by systematic randomized sampling with the aid of the computer assisted image analysis.



Patient data included, age, gravidity, size of the uterus, hemoglobin, preevacuation serum β-hCG levels, presenting symptoms, presence of theca lutein cysts and hyperthyroidism and outcome as remission or GTN. The correlation between proliferative index assessed by Ki-67 (MIB1) immunore activity and clinical parameters were investigated.

Statistical analysis was performed by using SPSS for Win. Ver. 15.00 (SPSS Inc., Chicago, IL., USA) statistical software. Shapiro-Wilk test was used to test the normality of data and it was found that only normally distributed data was hemoglobin. Descriptive data were expressed as mean ± standard deviation and skewed data were shown as median and interquartile range (IQR). The correlation between continuous variables and Ki-67 value were assessed by Spearman rank correlation coefficient. Clinical symptoms and signs were compared with Mann Whitney U test for Ki-67 indices. The levels of significance were set as $p \leq 0.05$.

Results

Of 44 specimens, 6 were too degenerated to allow interpretation of any immunohistochemical staining. Thirty-eight cases remained for analysis including 32 CHMs and 6 PHMs. The patients' ages ranged from 18 to 49 years. The data on maternal age, gravidity, hemoglobin, uterine size (as gestational weeks), preevacuation serum β-hCG levels and Ki-67 positivity were shown in Table 1. The most common presenting symptom was vaginal bleeding (71.1%). The symptoms and signs of patients were summarized in Table 2.

Table 1: Descriptive data of patients

Parameter	Minimum	Maximum	Median (IQR) Mean ± SD
Age (years)	18.00	49.00	28.50 (13.50)
Gravidity	0	10.00	1.00(2.00)
Hemoglobin (g/dl)	8.00	14.60	11.72 ± 1.62
Uterine size (gestational weeks)	4.00	20.00	12.00 (4.00)
bHCG (IU/L)	207.00	983.819.00	196.265.00 (464.695.00)
Ki-67 (percentage)	3.00	81.00	15.50 (41.25)

Table 2: Symptoms and signs of patients

Symptom	n	%
Vaginal bleeding	27	71.1
Hyperemesis	12	31.6
Abdominal pain	8	21.1
Asymptomatic	7	18.4
Consistent with gestational age	16	42.1
Theca lutein cyst	2	5.3
Hyperthyroidism	3	7.9
Development of gestational trophoblastic neoplasia	3	7.9
Remission	35	92.1

Three (7.9%) out of the 38 cases of HMs subsequently developed persistent GTN and required chemotherapy. Remaining 35 (92.1%) patients underwent remission. The times of remissions of the patients were as follows: 10 (28.6%) patients underwent before 2 months after evacuation, 21 (60.0%) patients underwent between 2-4 months and 4 (11.4%) patients after 4 months. Three (8.6%) of the patients had subsequent pregnancy after remission and all the pregnancies were normal in ultrasonographic examination.

None of the continuous variables including maternal age, gravidity, size of the uterus, hemoglobin, preevacuation serum β-hCG levels were significantly correlated with Ki-67 index ($p > 0.05$). On the other hand, the patients presented with abdominal pain were all diagnosed as CHM and had significantly lower Ki-67 index [median= 6.5 (IQR=11.00)] comparing with patients without pain [median= 21.00 (IQR=44.00)] (Z=2.635; p=0.007). Likewise, PHMs were found to have lower Ki-67 index [median=8 (IQR=11.00)] than CHMs [median=21 (IQR=43.00)] (Z=1.944; p=0.050) (Figure 2). Prominent Ki-67 labeling was observed in CHMs while a few trophoblastic cells highlighted by Ki-67 were seen for most of the PHMs (Figure 3 and 4).

Figure 2: The mean Ki-67 indices of complete (CHM) and partial (PHM) hydatidiform moles. The values were expressed as mean±SD.

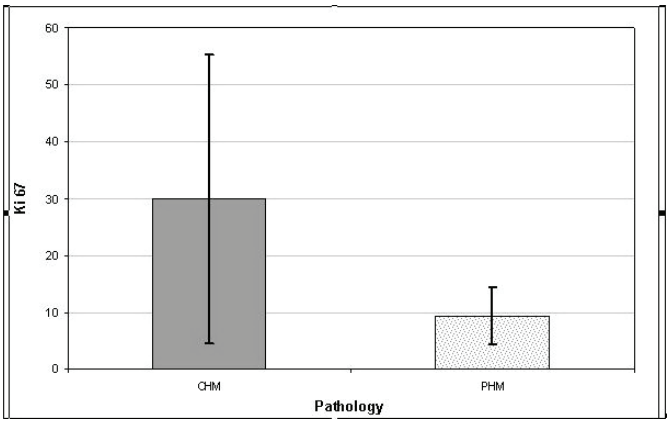


Figure 3: Prominent Ki-67 labelling in one of the complete mole hydatiform cases (x25 magnification, immunoperoxidase).

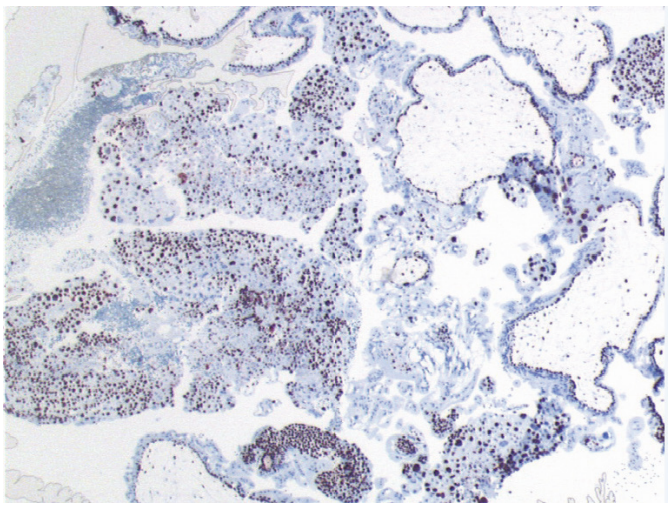
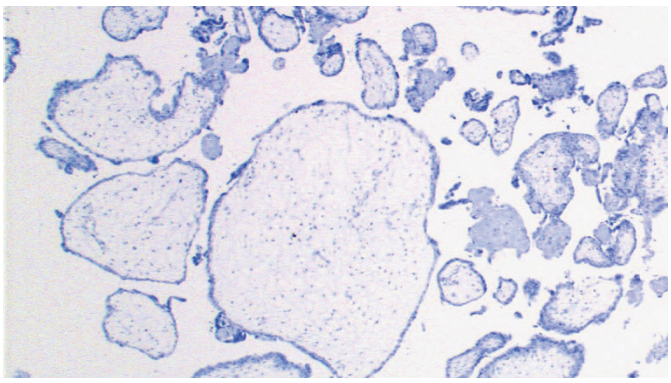


Figure 4: A few trophoblastic cells highlighted by Ki-67 were observed for most of the partial hydatidiform mole cases. This picture was taken from among them in which Ki-67 labelling index was measured less than 5 % (x25 magnification, immunoperoxidase).



Ki-67 index was not discriminative for none of the other symptoms and signs including vaginal bleeding, hyperemesis, asymptomatic presentation, consistency with gestational age, theca lutein cysts, hyperthyroidism, development of GTN or remission ($p>0.05$). Three patients, including 1 PHM and 2 CHM, who progressed to GTN, had the Ki-67 index values as follows, respectively: 5%, 7% and 16%. Although these patients had lower Ki-67 index, there was no statistically significant difference with patients who underwent remission [median=19.5 (IQR=42)].

Discussion

The pathogenesis and factors predicting the progress of HMs remain uncertain. This study evaluates the correlation of clinical parameters with proliferative activity in HMs assessed by Ki-67 index.

The past 25 years has witnessed the advent of diagnostic immunohistochemistry. One of the most widely used reagents in this field is Ki-67 index (12). It is relatively a simple alternative to the more complex techniques. Initially it was a practical problem that the Ki-67 antibody could not be used on fresh or frozen tissue, as fixation greatly reduced the immunostaining. The discovery of MIB-1 antibody, however, that could recognize the Ki-67 antigen in formalin fixed and paraffin-embedded tissue sections, greatly improved the value of the detection of Ki-67 antigen. So, retrospective studies could be performed to investigate the clinical value as a marker of proliferation for prognostic purposes (10,13).

The main finding of our study was none of the clinical parameters and symptoms except abdominal pain had any discriminative value for Ki-67 index. Secondly, the expression of Ki-67 was significantly higher in CHMs. It is very difficult to explain why the patients presenting with abdominal pain had lower Ki-67 expressions.

About 5-20% of CHMs progress to invasive moles or choriocarcinoma. The genetic compositions of partial and complete HMs are well known but the key steps in the molecular pathogenesis and progression of gestational trophoblastic disease remain unknown (14). Cheung et al. assessed the role of Ki-67 immunoreactivity in predicting the clinical progress of HMs and concluded that HMs which give rise to persistent trophoblastic disease did not have a higher proliferative rate that those which do not (15). In their study, the rate of persistent GTN was 22.9%. Similarly, Jeffers et al. performed immunohistochemistry for Ki-67 on cases of CHM complicated by persistent GTN and cases that resolved spontaneously after evacuation. They found no significant difference in Ki-67 index between them (16). Likewise, in our study the Ki-67 index of those who developed persistent disease was not significantly different from that of who had spontaneous remission of the disease. However, our results should be interpreted carefully because we had only 3 (8.6%) patients who developed persistent GTN and this was a limitation of our study. On the other hand, Kale et al. evaluated 22 PHMs, 17 CHMs, 6 invasive HMs and 20 nonhydropic spontaneous abortions for Ki-67 expression and reported that invasive HM showed the highest Ki-67 expressions, followed by CHM and PHM (17). Further studies investigating the association of developing GTN and Ki-67 index with larger sample sizes are necessary to establish.

The accurate diagnosis of CHM and its reliable distinction from PHM is essential because of differing clinical behavior. No single histological feature permits the distinction between complete and partial HM (12). Thus, additional biomarkers are needed and have been investigated. In our study, Ki-67 indices of CHMs showed a wide range of variation. This might reflect an intrinsic variation in the proliferative potential of trophoblasts. Also, trophoblastic proliferation in PHM was less marked than in CHM. Although PHMs are less in number than CHMs, it seems that expression of Ki-67 may not be equivalent in the two conditions (16). Ostrzega and et al. investigated whether Ki-67 could help distinction between complete HM, partial HM, abortus with hydropic changes and found that Ki-67 was useful for distinguishing complete and partial HMs (17). Cheville et al. also demonstrated that Ki-67 could be used to distinguish partial from complete HMs (18). Likewise Kale et al. also found that Ki-67 index was a discriminating variable for complete and partial HMs, and was lower in partial HMs (19). In this study, we also found lower expressions of Ki-6 in partial HMs. On the other hand, Schammel and Bocklage found that Ki-67 was not discriminatory between complete and partial HMs (20).

In conclusion, it is our opinion that in the light of current data, it seems Ki-67 index has promising value in discriminating complete and partial HMs. Further prospective studies will help to determine the prognostic value for adverse clinical outcome.

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