DOI: https://doi.org/10.53350/pjmhs2115102619

ORIGINAL ARTICLE

To Determine the Degree of Agreement between Ki67 and Histopathology to Differentiate between Hydatidiform Mole and Hydropic Abortus

MARIYA MANZOOR[1], HASEEB AHMED KHAN[2], SABIHA RIAZ[3], IMRANA TANVIR[4], ATIYA BATOOL GARDEZI[5], ABDUR REHMAN[6]
[1,2,5]Pathology Department, FMH College of Medicine & Dentistry, Lahore
[3]Professor of Pathology, FMH College of Medicine & Dentistry, Lahore
[4]Department of Pathology, King Abdul Aziz University, Jeddah
[6]Medicine Department, CMH, Kharian
Correspondence to Dr. Mariya Manzoor, Email: mariyafmh@gmail.com tel. 03336539919

ABSTRACT

Aim: To determine the degree of agreement between Ki67 and histopathology to differentiate between hydatidiform mole and hydropic abortus.
Methods: Descriptive, Cross Sectional Survey was conducted in the Department of Pathology, Fatima Memorial Hospital, Lahore during 6 months (May 11, 2016 to Nov 11, 2016). Using non-probability consecutive sampling, 105 cases were included in this study as per the inclusion and exclusion criteria. 10% buffered formalin was used to fix the sepecimens. Gross examination and staining with Hematoxylin and Eosin was done. The cases were diagnosed by a histopathologist. IHC staining Ki67 was performed, assessed by histopathologist and the data was recorded on the proforma.
Results: The mean age was 27.79±5.81 years with minimum and maximum ages of 18 years and 42 years respectively. The histopathological findings showed hydatidiform mole in 41 (39%) women and hydropic abortus in 64(61%) women. The Ki67 was reported to be >25 in 41(39%) patients and less than or equal to 25 in 64(61%) patients. The agreement of differentiation in the two types for histopathology and Ki67 was found in 101(96.2%) patients. Kappa statistics showed 92% (p-value= 0.000) strength of agreement between histopathology and Ki67.
Conclusion: There is high degree of agreement between Ki67 and histopathology for differentiation of hydatidiform mole and hydropic abortus. So Ki67 can be used as an adjacent in histopathologic diagnosis of hydatidiform mole in difficult cases.
MeSh words: Hydatidiform Mole, Immunohistochemistry, Ki-67 Antigen

INTRODUCTION

Hydatidiform mole (HM) is a gestational trophoblastic disease (GTD) characterized by proliferation of trophoblastic cells and enlarged, edematous placental villi. HM is subdivided into complete hydatidiform mole (CHM) and partial hydatidiform mole (PHM). Their main differential diagnosis is with early non molar hydropic abortus (HA). Their diagnosis has been conventionally based on histopathology[1-3]. HM should be correctly diagnosed and subclassified, as persistent GTD and choriocarcinoma is a significantly higher risk in these conditions[4-6]. The risk of persistent GTD in CHM is 15% – 25% and in is 0.2%–5% in PHM. The risk of choriocarcinoma in CHM is 3-5%. The risk is even higher in women less than 20 years of age.[7] HA has no such risk at all, therefore the differentiation between these conditions is very significant.[8] The occurrence of GTD varies worldwide. It is more frequent in South East Asia compared to the western countries. GTD occurs in approximately 1:3000 in U.K and USA. The reported incidence of GTD in Pakistan is 28/1000 live births.[9] Similarly, the rate of complications in HM is reported more frequently in Asian countries as compared to the western countries. The complications were noted in 1:100 in Indonesia, compared to 1: 1500 pregnancies in USA[9].

The histologic features differences between CHM and PHM are very subtle[10,11] and often result in difference of opinion between even the experienced pathologists[4]. In addition, this difficulty in diagnosis is also compounded by the fact that HM are nowadays evacuated in early pregnancy, when the histologic features are not fully developed. Hydropic abortus can also have similarity of microscopic features with enlarged villi and focal trophoblastic proliferation. This leads to increased diagnostic difficulties and interobserver variability[12,13].

Ancillary techniques like immunohistochemistry have been proved useful in accurately diagnosing different pathologic conditions. This technique may also help to confidently sub-classify complete mole, partial mole and hydropic abortus. Ki67, an immunohistochemical stain, is a proliferation marker which is used to differentiate lesions with different malignant potentials[14,15]. Previous literature suggests that Ki67 can be used to differentiate hydropic abortus from HM[15]. However, data regarding Ki67 immunostaining in HA and HM has not been studied in our population.

The purpose of my study was to determine the degree of agreement between Ki67 and histopathology for diagnosis of HM and HA. This can help us in accurate diagnosis in difficult cases and will also help in accurate assessment of risk of persistent GTD and choriocarcinoma for these patients.

Received on 23-03-2021
Accepted on 03-08-2021

METHODS

This cross sectional study was carried out in the Department of Pathology, Fatima Memorial Hospital Lahore after taking permission from the hospital ethical committee. We included a total of 105 cases of endometrial curettings diagnosed as HM and HA on histopathology. A case number was assigned to each case. Demographic details were collected. As per the departmental protocols, 10% formaline was used to preserve the specimens, which were then examined grossly and microscopically after staining with the routine Hematoxylin and Eosin (H&E) stains. The cases were diagnosed by a consultant histopathologist. IHC staining of Ki67 was performed on all of these cases, according to the procedure specified by the manufacturer. IHC staining expression was assessed by consultant histopathologist and the acquired data was recorded.

The collected information was entered and analyzed by using computer software SPSS version 18. The quantitative variables like age was presented as mean ± standard deviation. The qualitative variables like HM and HA on histopathology and Ki67 and their agreement was presented in the form of frequencies and percentage. Kappa statistics was calculated to see the strength of agreement between histopathology and Ki67 in differentiating HM and HA.

RESULTS

The mean age in our study was 27.79±5.81 years with minimum and maximum ages of 18 years and 42 years respectively. There were 77(73.3%) women in age category of 18-30 years and 28 (26.7%) in 31-42 years age category. The histopathological

findings showed HM in 41 (39%) women and HA in 64 (61%) women. The Ki67 was reported to be >25 in 41 (39%) patients and less than or equal to 25 in 64 (61%) patients (Table-1). The agreement of differentiation in the two types for histopathology and Ki67 was found in 101 (96.2%) patients (Table-2). Kappa statistics showed 92.0% (p-value= 0.000) strength of agreement between histopathology and Ki67 (Table-3).

Table 1: Findings of Ki 67 staining

	Frequency	Percent
>25%	41	39.0
≤ 25%	64	61.0
Total	105	100.0

Table 2: Diagnostic agreement in Findings of Ki 67 staining and histopathology findings

	Frequency	Percent
Yes	101	96.2
No	4	3.8
Total	105	100.0

Table 3: Comparison of Ki 67 staining and histopathology findings

Ki 67 staining	Histopathology results		Total
	Hydatidiform mole	Hydropic abortus	
>25%	39(95.1%)	2(3.1%)	41(39%)
≤ 25%	2(4.9%)	62(96.9%)	64(61%)
Total	41(100%%)	64(100%)	105(100%)

Kappa statistics = 0.92 , p-value < 0.001

DISCUSSION

GTD include a variety of morphologically and prognostically different conditions ranging from malignant (e.g. choriocarcinoma) and premalignant (e.g. HM) to non-neoplastic conditions (e.g. exaggerated placental site). [16,17] CHM represents abnormal placentation due to abnormal fertilization of an empty egg by either a single spermatozoon (in most cases) or by two spermatozoa (less commonly). Therefore, it results predominantly into 46XX karyotype.[1, 12] PHM on the other hand, is associated with an ascertainable embryo/fetus, dead or alive, alongwith a triploid karyotype, as its usual pathophysiology is abnormal fertilization of a normal ovum by two spermatozoa[1,15].

South Asia has a higher incidence of HM as compared to the western countries. In Pakistan, this incidence has been reported as 28 per 1000 pregnancies in one study[9] and 5 per 1000 pregnancies in another study.[18] Marked variation of incidence is seen in different areas of the world and different ethnic populations. Maternal age, previous history of HM, environmental, reproductive and socioeconomic factors have been linked to increased risk of development of HM[9,18,19]. The incidence has been rising even among the developed countries. The risk of development of HM is considerably higher in females over 50 or under 15, as compared to those between the ages of 25 and 29. This risk is also increased in later gestations, being 1 in 76 for second HM and 1 in 65 for third HM[20].

Histologically, HM are characterized by enlargement of villi and trophoblastic proliferation. The microscopic differentiation between CHM and PHM is aided by specific features, including villous size heterogeneity, extent of trophoblastic proliferation, presence or absence of cistern formation, scalloping of borders and fetal parts. Hydropic abortus (HA) is a benign entity, which is also a differential diagnosis of HM. HA also shows edematous, dilated villi and can have focal trophoblastic hyperplasia[1,21].

Ultrasound is commonly used to diagnose HM, but it is not reliable and many cases of CHM and PHM can be missed on ultrasound. Histologic examination remains the key to accurate diagnosis in all cases of miscarriage.[21,22] Accuracy of diagnosis of HM versus HA is very important for correct treatment and prognosis determination. Although there are differentiating histologic features reported in literature to distinguish between these entities, however, these features often overlap and practically the diagnosis is not as straight forward on histology alone. It often leads to confusion and difference of opinion between pathologists[23,24]. Molar pregnancies are often evacuated early nowadays, when the histologic features are not fully developed, which adds to the diagnostic difficulty[23].

As not many local studies establish status of any of these two tools for diagnosis, we aimed to determine the degree of agreement between Ki67 and histopathology for diagnosis of HM and HA. In our study the mean age of patients was 27.79±5.81 years with minimum and maximum ages of 18 years and 42 years respectively. There were 77(73.3%) women in age category of 18-30 years and 28 (26.7%) in 31-42 years age category.

A previous local study and many international studies have evaluated the use of p57 immunohistochemical stain and found it useful to distinguish between CHM and PHM.[3,11,25] However, this stain cannot be used to differentiate between PHM and HA as it is positive in both of these entities.[25] Genotyping can also be used to classify HM into CHM and PHM, and to distinguish it from HA, but it is a costly technique and not easily available to our population in Pakistan[1,25].

Previous studies on Ki67 staining in CHM, PHM and HA have yielded variable results. They have used different percentages of cells as positive cut offs. The difference in positivity of Ki67 was found to be better than p63 and p53 immunohistochemistry in order to differentiate between PHM and PA in a previous study[26]. Another immunohistochemical study compared Ki67 with proliferating cell nuclear antigen (PCNA) and found Ki67 to be better to differentiate PA from PHM and CHM.[27] Another recent study found difference of Ki67 expression between CHM (72.1%) and HA (46.2%)[28]. The findings in these studies is similar to our study, however, these studies used different cut-offs for positive and negative staining, and the staining was performed on archival tissues which often yields different results as compared to routine specimens in which the tissue are recently processed.

In our study the histopathological findings showed HM in 41(39%) women and HA in 64(61%) women. The Ki67 was reported to be >25 in 41(39%) patients and less than or equal to 25 in 64 (61%) patients. The agreement of differentiation in the two types for histopathology and Ki67 was found in 101(96.2%) patients. Kappa statistics showed 92% (p-value= 0.000) strength of agreement between histopathology and Ki67. The agreement of diagnosis was observed in 73 (72.2%) patients in age group of 18-30 years and 28(27.7%) in 31-42 years of age. There was no significant association between the age groups and agreement of diagnosis.

CONCLUSION

There is high degree of agreement between Ki67 and histopathology for differentiation of HM and HA. So Ki67 can be used as an adjunct in differentiating HM from HA in difficult to diagnose cases.

Conflict of interest: Nil

REFERENCES

1. Lipata F, Parkash V, Talmor M, Bell S, Chen S, Maric V, et al. Precise DNA genotyping diagnosis of hydatidiform mole. Obstetr & Gynecol 2010;115(4):784-94.
2. Soper JT, Mutch DG, Schink JC, ACOG. Diagnosis and treatment of gestational trophoblastic disease: ACOG Practice Bulletin No. 53. Gynecol Oncol 2004;93(3):575-85.
3. Sarmadi S, Izadi-Mood N, Abbasi A, Sanii S. p57KIP2 immunohistochemical expression: a useful diagnostic tool in discrimination between complete hydatidiform mole and its mimics. Archives of gynecology and obstetrics 2011;283(4):743-8.
4. Ozalp SS, Telli E, Oge T, Tulunay G, Boran N, Turan T, et al. Multicenter analysis of gestational trophoblastic neoplasia in Turkey. Asian Pacific journal of cancer prevention: APJCP 2013;15(8):3625-8.
5. Shazly SA-EM, Ali MK, Badee AYA, Alsokkary A-bA, Khodary MM, Mostafa NA-E. Twin pregnancy with complete hydatidiform mole and

coexisting fetus following ovulation induction with a non-prescribed clomiphene citrate regimen: a case report. Journal of medical case reports 2012;6(1):1.
6. Niemann I, Vejerslev LO, Frøding L, Blaakær J, Maroun LL, Hansen ES, et al. Gestational trophoblastic diseases-clinical guidelines for diagnosis, treatment, follow-up, and counselling. Dan Med J. 2015;62(11):A5082.
7. Salehi S, Eloranta S, Johansson AL, Bergström M, Lambe M. Reporting and incidence trends of hydatidiform mole in Sweden 1973–2004. Acta Oncologica 2011;50(3):367-72.
8. Kipp BR, Ketterling RP, Oberg TN, Cousin MA, Plagge AM, Wiktor AE, et al. Comparison of fluorescence in situ hybridization, p57 immunostaining, flow cytometry, and digital image analysis for diagnosing molar and nonmolar products of conception. Am J Clinic Pathol 2010;133(2):196-204.
9. Nizam K, Haider G, Memon N, Haider A. Gestational Trophoblastic Disease: Experience at Nawabshah hospital. J Ayub Med Coll 2009;21(1):94–97.
10. van Trommel N, Lybol C, Thomas C, Sweep F, Massuger L. Diagnosis and treatment of gestational trophoblastic disease. Europ Obstetr &Gynaecol 2011;6:28-32.
11. Kalsoom R, Jaffar R, Qureshi N, Aziz F. A Study of p57KIP2 expression and morphological findings of complete and partial hydatidiform moles. Biomedica 2015;31(1):11-4.
12. Soares P, Maestá I, Costa O, Charry RC, Dias A, Rudge M. Geographical distribution and demographic characteristics of gestational trophoblastic disease. J Reproduc Med 2009;55(7):305-10.
13. Biscaro A, Silveira SK, Locks GdF, Mileo LR, Silva Júnior JPd, Pretto P. Frequency of hydatidiform mole in tissue obtained by curettage. Revista Brasileira de Ginecologia e Obstetrícia 2012;34(6):254-8.
14. Chen Y, Shen D, Gu Y, Zhong P, Xie J, Song Q. The diagnostic value of Ki-67, P53 and P63 in distinguishing partial Hydatidiform mole from hydropic abortion. Wiener klinische Wochenschrift 2012;124(5-6):184-7.
15. Khooei A, Pasdar FA, Fazel A, Mahmoudi M, Nikravesh MR, Delui MK, et al. Ki-67 expression in hydatidiform moles and hydropic abortions. Iranian Red Crescent Medical Journal 2013;15(7):590.
16. Shih I-M, Mazur MT, Kurman RJ. Gestational trophoblastic tumors and related tumor-like lesions. Blaustein's Pathology of the Female Genital Tract: Springer; 2011. p. 1075-135.
17. Berkowitz RS, Goldstein DP. Molar pregnancy. New England journal of medicine 2009;360(16):1639-45.
18. Fatima M, Kasi PM, Baloch SN, Kassi M, Marri SM, Kassi M. Incidence, management, and outcome of molar pregnancies at a tertiary care hospital in quetta, pakistan. ISRN Obstet Gynecol. 2011;2011:925316.
19. Altieri A, Franceschi S, Ferlay J, Smith J, La Vecchia C. Epidemiology and aetiology of gestational trophoblastic diseases. Lancet Oncol. 2003;4(11):670-8.
20. Bagshawe K, Dent J, Webb J. Hydatidiform mole in England and Wales 1973-83. The Lancet 1986;328(8508):673-7.
21. Szulman A, Surti U. The syndromes of hydatidiform mole: II. Morphologic evolution of the complete and partial mole. American journal of obstetrics and gynecology 1978;132(1):20-7.
22. Memtsa M, Johns J, Jurkovic D, Ross JA, Sebire NJ, Jauniaux E. Diagnosis and outcome of hydatidiform moles in missed-miscarriage: a cohort-study, systematic review and meta-analysis. Eur J Obstet Gynecol Reprod Biol. 2020;253:206-212.
23. Dong Y, Zhang S, Wang ML, Guo H, Zhang Y, Wang Y, et al. Histopathological features of hydatidiform moles and placenta changes of nonmolar miscarriage in early stage of pregnancy. Beijing Da Xue Xue Bao Yi Xue Ban. 2011;43(2):194-8.
24. Ostrzega N, Phillipson J, Liu P. Proliferative activity in placentas with hydropic change and hydatidiform mole as detected by Ki-67 and proliferating cell nuclear antigen immunostaining. American journal of clinical pathology 1998;110(6):776-81.
25. Ronnett BM. Hydatidiform Moles: Ancillary Techniques to Refine Diagnosis. Arch Pathol Lab Med. 2018;142(12):1485-502.
26. Chen Y, Shen D, Gu Y, Zhong P, Xie J, Song Q. The diagnostic value of Ki-67, P53 and P63 in distinguishing partial Hydatidiform mole from hydropic abortion. Wien Klin Wochenschr 2012;124, 184–7.
27. Ostrzega N, Phillipson J, Liu P. Proliferative activity in placentas with hydropic change and hydatidiform mole as detected by Ki-67 and proliferating cell nuclear antigen immunostaining. Am J Clin Pathol. 1998;110(6):776-81.
28. Missaoui N, Landolsi H, Mestiri S, Essakly A, Abdessayed N, Hmissa S, et al. Immunohistochemical analysis of c-erbB-2, Bcl-2, p53, $p21^{WAF1/Cip1}$, p63 and Ki-67 expression in hydatidiform moles. Pathol Res Pract. 2019;215(3):446-452.