Original Article

Immunohistochemical Study of Hydatidiform Mole in Ahmadu Bello University Teaching Hospital Zaria: A Ten-Year Review

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Abstract

Background: Morphological examination still forms the main diagnostic tool in the differential diagnosis of molar pregnancies. However, there may be inter and intra observer variability in differentiating partial from complete moles, and hydropic abortions from partial moles. The study was aimed at confirming the diagnoses of hydatidiform moles over a ten-year period using p57KIP2 and Ki-67 immunohistochemical markers. **Methods:** All morphologically diagnosed cases of hydatidiform moles from 1st January 2006 to 31st December 2015 formed the study materials. The total number of deliveries within the study period was obtained from the obstetrics and gynaecology department. All the relevant request cards, tissue blocks and slides stained with Haematoxylin and Eosin (H&E) were retrieved from the departmental records and stained with p57KIP2 and Ki-67 immunohistochemical stains. **Results**: There were one hundred (100) histologically diagnosed cases of hydatidiform moles during the period of the study, of these 71 (71 %) met inclusion criteria. The ages-ranged from 15 to 50 years with a mean age of 29.6 $\pm$ 1SD years. The highest prevalence was in the 2nd decade of life. The initial H&E diagnosis was 51 partial and 20 complete moles. Of the 71 cases of hydatidiform-moles analysed, 48 (67.6%) showed positivity for both p57 and Ki-67 immunostains and were classified as partial moles, whereas 23 (32.4%) were complete-moles for being negative for p57 immunohistochemical marker. No cases of hydropic abortions were seen. **Conclusion**: This study showed that hydatidiform-mole affected women more in their 2nd decade of life. Partial-mole is commoner than complete-mole, and with immunohistochemical markers such as p57 and Ki-67, it is easier to confirm the diagnosis of molar gestations and differentiate between partial-mole, hydropic-abortion, and complete-mole.

Keywords: Hydatidiform mole, Immunohistochemistry, Partial Mole, p57, Ki-67

INTRODUCTION

Gestational trophoblastic disease encompasses a range of pregnancy-related disorders, characterized by proliferation of pregnancy associated trophoblastic tissue of progressive malignant potential.[1, 2] They consist of the premalignant disorders, complete and partial hydatidiform moles, and the malignant

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disorders, invasive mole, choriocarcinoma, and the rare placental-site trophoblastic tumour. These malignant forms are termed gestational trophoblastic tumours or neoplasia.[1]

Hydatidiform mole could be partial or complete, both of which could be invasive.[3] Complete moles are diploid or tetraploid, and their genetic material is entirely paternally derived.[4] In most cases a single sperm fertilizes an "empty ovum" followed by chromosome duplication, leading to a homozygous 46XX genotype. Less commonly complete moles can be heterozygous resulting from dispermy and have a 46XY or 46XX genotype.[4, 5] Tetraploid complete moles have also been reported with varying incidence.[4] Complete moles are characterised by progressive oedematous enlargement of villi, marked trophoblastic hyperplasia and no ascertainable embryo.[3, 6] Virtually all partial moles are triploid, that is diandric monogynic, arising from two sperms fertilising an egg (dispermic, heterozygous partial moles, approximately 90% of cases) or one sperm fertilising an egg followed by reduplication of the paternal chromosome set (monospermic, homozygous partial moles). Thus, 70% of partial moles have a 69XXY karyotype, 27% are 69XXX and 3% 69XYY.[4, 5] Partial or incomplete moles are characterised by a slowly progressive and variably oedematous enlargement of villi, functioning villous capillaries, focal mild to moderate trophoblastic hyperplasia, and an ascertainable foetus.[3, 6]

Most women present with abnormal uterine bleeding that may begin early in the course of the pregnancy and this is accompanied by passage of thin watery fluid and bits of vesicles. The uterine size is larger than expected and ultrasound examination can be diagnostic in most cases.[7]

Although defined genetically, karyotyping is seldom practically performed on these placentas on a routine diagnostic basis. Histological examination forms the main tool in the diagnosis of molar pregnancies. However, there is considerable overlap in the histological features between molar and non-molar pregnancies and between complete and partial moles, resulting in significant inter-observer variability in the diagnosis.[8, 9] Pathologists now rely on molecular techniques that make use of DNA content differences between complete mole and partial mole, including DNA flow or image cytometry, chromosome in situ hybridisation, polymerase chain reaction–based genotyping or Human Leucocyte Antigen (HLA) typing, to help in the differential diagnoses of these hydropic placentas.[8, 9] However, these techniques are technically difficult and relatively expensive, and unlikely to become routine in all laboratories. In addition, ploidy analysis does not differentiate between complete mole and hydropic abortion.[8, 9]

Regarding this issue, therefore, the use of immunohistochemistry in the detection of expression of the product of some genes as p57KIP2, a paternally imprinted and maternally expressed gene by the trophoblastic cells should be highlighted in confirming the diagnosis of moles, since it is reactive for partial moles but negative for complete moles. Also, the fact that the Ki-67 protein is present during all active phases of the cell cycle (G1, S, G2 and mitosis), but is absent from quiescent or resting cells (G0) makes it an excellent marker for reflection of the tissue proliferation compartment and thus could be of value in studying the biological behaviour of molar gestations, differentiating them from hydropic abortions which are negative for this marker.[8, 9, 10, 11]

Hydatidiform mole carries a significant risk for developing persistent gestational trophoblastic disease and the most important reason for the correct recognition of true moles is that they may precede choriocarcinoma.[6] Moles have been found to occur at any age during active reproductive life, but the risk is higher in the teens or between the ages of 40 and 50 years.[7]

MATERIALS AND METHOD

This is a 10-year retrospective study. Ethical clearance for the study was obtained from the Ethics and Scientific Committee of the ABUTH, Shika-Zaria. The study included only those specimens submitted to the Pathology Department of the Hospital and diagnosed as hydatidiform moles, between 1st January 2006 and 31st December 2015. The hospital is a referral centre for the Northwestern region of the country, which serves Kaduna, Zamfara, Jigawa, Katsina and Niger States.

The sources of data included the total number of deliveries per year, obtained from the delivery register of the department of obstetrics and gynaecology of this hospital, pathology departmental records, comprising of bench books, request cards, paraffin-embedded tissue blocks as well as slides stained with haematoxylin and eosin (H&E). These were retrieved from the departmental archives.

Where slides were missing or broken, fresh sections were made from stored paraffin-embedded tissue blocks. Cases with missing request cards and tissue blocks were excluded from the study. Also, cases that did not fulfill the criteria for hydatidiform mole after the histological review were excluded. Patients'

biodata was extracted from the accompanying request cards and the H & E slides were reviewed.

Immunohistochemical stains comprising of p57KIP2 and Ki-67 were applied on paraffin-embedded tissue blocks to differentiate between partial and complete moles, and partial moles and non-molar gestations (hydropic abortions) respectively, using the staining protocol by Genemed™ California, USA. This protocol comprises of endogenous peroxidase blocking, primary antibody incubation, Linker for mouse (reagent A) addition, poly conjugate incubation (reagent B), chromogen incubation, counter staining with Haematoxylin, and mounting. In this study, immunoreactivity for the immunohistochemical stains were mainly confined to cytotrophoblasts. The decidual and villous stromal cells served as internal control. The immunohistochemistry slides were then reviewed, and the cases classified accordingly.

Analysis of the collected data was carried out using Statistical Program for Social Sciences (SPSS) version 20.0 and data was presented in frequency distribution tables and figures, and photomicrographs.

RESULTS

A total of twenty-seven thousand six hundred and forty-six (27,646) specimens were received in the Department of Pathology, Ahmadu Bello University Teaching Hospital Zaria during the study period, out of which 922 (3.3%) were products of conception. Of the total number of products of conception, 100 cases were hydatidiform moles. Twenty-nine (29) (29%) of the hydatidiform moles were excluded. The 71 cases included 7.7% of all products of conception and 0.3% of the entire biopsies received in the department within the period under review. One (1) (1%) of the cases was a tubal hydatidiform mole. There was a total number of 13,385 deliveries in Ahmadu Bello University Teaching Hospital (ABUTH) complex during the period under review, thereby translating into a ratio of 1 hydatidiform mole for every 189 deliveries. The year with the highest number of cases of hydatidiform mole was 2012 with 13 (18.3%) cases, followed by 2009 with 12 (16.9%) cases, with an annual average of 7.1 cases. The sharp decline in the number of cases in 2010 was due to the highest number of missing tissue blocks and cards from that year. (Figure 1)

Age Distribution of Hydatidiform Mole

The ages ranged from 15 years to 50 years with a mean age of 29.6 years and a peak age incidence in the 2nd decade of life. (Table 1)

Table 1: Age Distribution of Cases of Hydatidiform Mole (n=71).

Age (years)	Frequency (%)
15-19	4 (5.6)
20-24	16 (22.5)
25-29	18 (25.4)
30-34	14 (19.8)
35-39	11 (15.5)
40-44	4 (5.6)
45-49	3 (4.2)
>49	1 (1.4)
Total	**71 (100.0)**

Table 2: Frequency distribution and immunohistochemical profile of hydatidiform mole seen.

Type of mole	H & E (%)	Immuno-histochemistry		Total (%)
		P57 (+)	Ki-67 (-)	
Partial mole	51 (71.8)	48	48	48 (67.6)
Complete mole	20 (28.2)	-	23	23 (32.4)
Total	71 (100.0)	48	71	71 (100.0)

Frequency Distribution and Immunohistochemical Profile of Hydatidiform Mole

The initial H & E diagnosis was however, 51 partial moles and 20 complete moles. (Table 2). Two immunohistochemical stains were used in this study, p57 and Ki-67. Of the 71 cases of hydatidiform moles analysed, 48 (67.6%) showed positivity to both p57 and Ki-67 immunostains and were classified as partial moles, whereas 23 (32.4%) were complete moles for being negative for p57 immunohistochemical marker. No cases of hydropic abortions were seen.

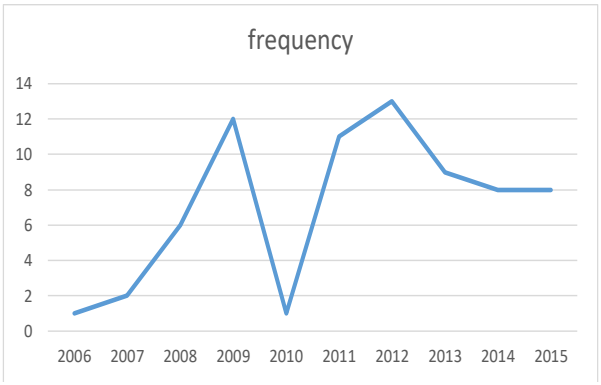

Figure 1: Yearly distribution of hydatidiform mole

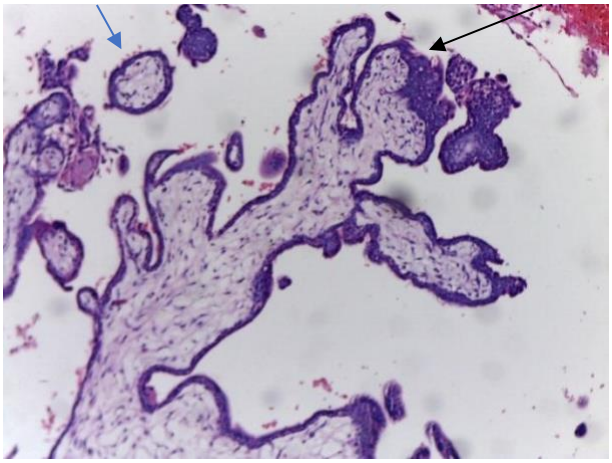

Figure 2: Photomicrograph of a partial mole showing an oedematous chorionic villus with focal trophoblastic hyperplasia (black arrow), and a normal chorionic villus (blue arrow). H & E. x100.

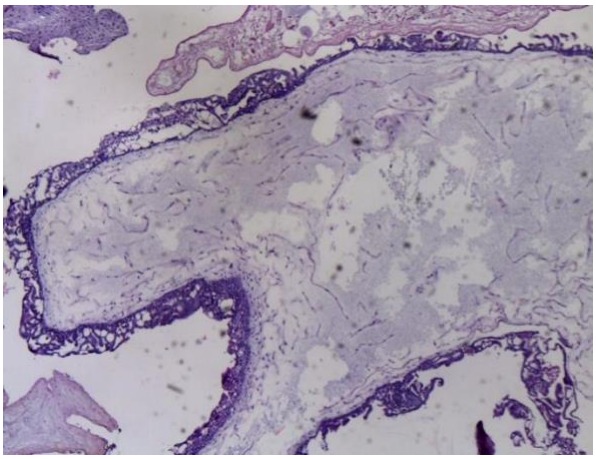

Figure 3: Photomicrograph of a complete mole showing a markedly oedematous chorionic villus with circumferential trophoblastic hyperplasia. H & E. x200.

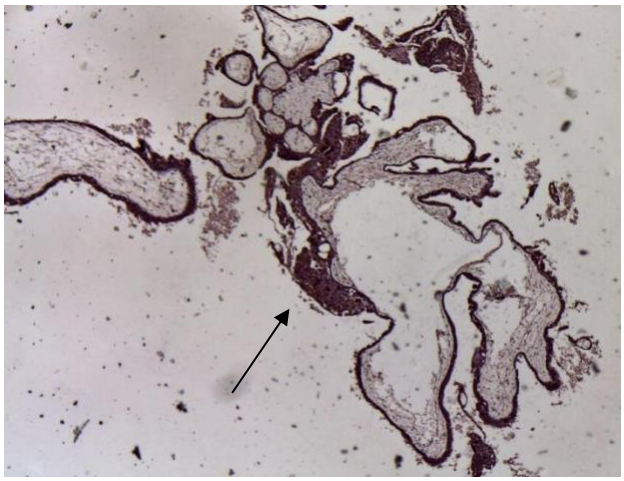

Figure 4: Photomicrograph of a partial mole showing the positive p57 immunostaining cytotrophoblastic cells (arrow). Immunoperoxidase stain. x100.

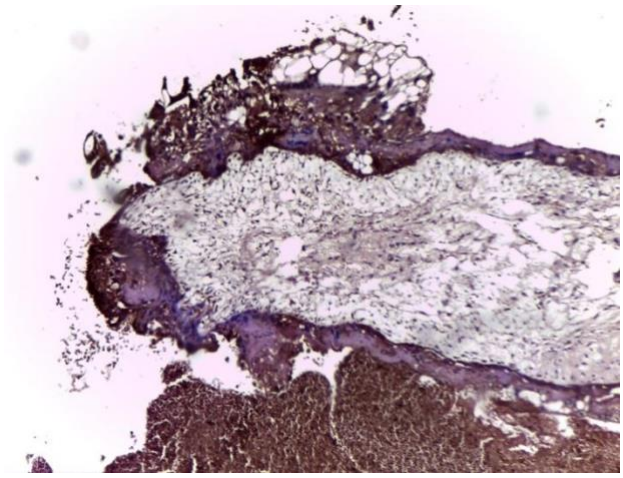

Figure 5: Photomicrograph showing positive Ki-67 immunostaining. Immunoperoxidase stain. X200

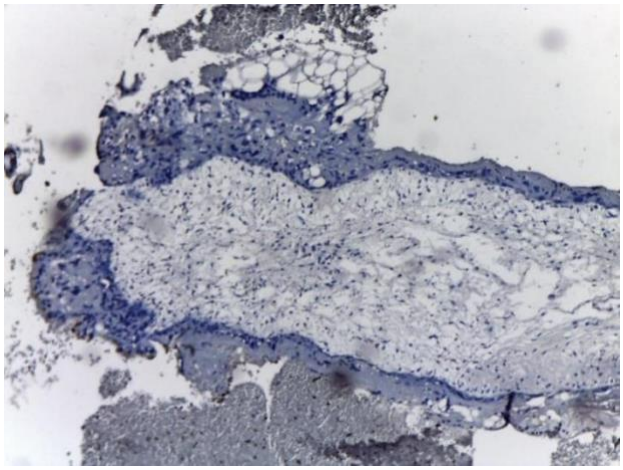

Figure 6: Photomicrograph of a complete mole showing negative p57 immunostaining. Immunoperoxidase stain. x200.

DISCUSSION

From our study, one hydatidiform mole occurs in 189 deliveries, confirming that it is a common condition in our environment. This is higher than 1 in 239 deliveries seen in Ilorin,[19] 1 in 623 seen in Calabar,[21] 1 in 357 seen in Jos[20] and 1 in 797 deliveries seen in Zaria in an earlier study.[7] This is likely due to the emergence of newer healthcare centres and migration of ABUTH from the city centre to a more peripheral area, and as such mostly the problematic pregnancies present to or are referred to the hospital, and also probably due to the cases that were excluded from the study. The is however similar to 1 in 166 deliveries seen in Gombe.[28]

The age range for all the cases of hydatidiform moles was 15-50 years, with a mean age of 29.8 years. This is closer to what was obtained by Jimoh et al[19] and Fukunaga et al[29] in Ilorin and Tokyo respectively. The peak incidence of hydatidiform mole occurred in the 2nd decade of life. This is in keeping with previous findings by Jimoh et al from Ilorin.[29] This however differs from earlier studies in Zaria[7] and Calabar,[21] where patients between the ages of 30 and 39 years were more prone to developing hydatidiform mole.

Partial type occurred more frequently (67.6%) than the complete type (32.4%). This is in keeping with studies from Yemen by Ali et al,[30] where the frequency of partial and complete hydatidiform moles were found to be 50% and 43.75% respectively. However, findings in earlier studies from Zaria,[7] Ilorin,[19] Tokyo[29] and Saudi Arabia[16] are not in support of this as complete mole was found to be more prevalent than partial mole. This may be due to inter-observer and intra-observer variability using the H & E diagnosis. Also, the previous studies did not use immunohistochemistry but relied on H&E.

In this study, immunoreactivity for the immunohistochemical stains were mainly confined to cytotrophoblasts. The decidual and villous stromal cells served as internal control. This is in concordance with a previous study that has recognized cytotrophoblasts as the sensitive germinative zones based on DNA analysis, flow cytometry and FISH.[5]

Our study demonstrated that majority of the cases were partial moles, after utilizing immunohistochemistry. This, however, differs with a study carried out by Landolsi et al in Tunisia, where out of the 220 cases that were analysed using the p57 immunostain, 140 did not express p57 and were definitely classified as complete moles.[9] In their initial H & E diagnosis however, there were 131 cases of complete moles, eight cases of partial moles and one case only of hydropic abortion.[9] Sharifi et al in Iran[6] and LeGallo et al in Virginia[25] found that p57 expression in cytotrophoblasts and villous mesenchyme was absent in complete mole, compared to strong expression in both partial mole and hydropic abortions, and may be useful where the main differential diagnosis is between complete and partial molar gestations. Also, in contrast with this study, Ali et al reported that out of the 15 hydropic abortions analysed, 93.3% showed negative Ki-67 immunoreactivity in all villous cells while all the 24 complete moles (100%) had positive Ki-67 immunoexpression.[2] For partial moles, more than half of the 24 cases (54.2%) had positive Ki-67 immunoexpression in the cells, and this was attributed to the loss of antigenicity in some of the cells.[2]

CONCLUSION

In conclusion, this study showed that hydatidiform mole is a common problem in our environment and it affects women more in their 2nd decade of life. Partial mole is commoner than complete mole, and with immunohistochemical markers such as p57 and Ki-67, it is easier to confirm the diagnosis of molar gestations and differentiate between partial mole, hydropic abortion and complete mole.

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